Handbook of Experimental Pharmacology 243

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Heart Failure

Springer

Handbook of Experimental Pharmacology

Volume 243

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ISSN 0171-2004 ISSN 1865-0325 (electronic)
Handbook of Experimental Pharmacology
ISBN 978-3-319-59658-7 ISBN 978-3-319-59659-4 (eBook)
DOI 10.1007/978-3-319-59659-4

Library of Congress Control Number: 2017942786

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Heart Failure and Heart Failure Drug Therapy: Preface

Chronic heart failure (HF) remains a worsening global problem and represents the end sequelae of a variety of cardiovascular (CV) diseases. With the worldwide aging of the population and an increasing burden of comorbidities, it is projected that the increasing prevalence of HF will pose an even greater challenge to future healthcare systems than at present. Thus, identifying effective pharmacologic therapies for patients with HF, to reduce the burden of disease and to develop effective preventive strategies, is a call to action for researchers, for the pharmaceutical industry, and health care providers and systems across the world.

HF is broadly categorized as HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF), with approximately equal proportions of patients in each category. Morbidity and mortality in patients with HFrEF have improved in recent decades through modulation of the renin–angiotensin–aldosterone system (RAAS), β-adrenergic blockade, use of mineralocorticoid receptor antagonists (MRA), and most recently neprilysin inhibition (Packer et al. 2014). Despite these advances, there remains a significant residual risk of further hospitalization and death in patients with HFrEF. Importantly, no clinical trials to date have been successful in demonstrating improved outcomes for patients with HFpEF, and thus no therapies are approved for these patients. Similarly, no specific therapies exist for patients with worsening HF who are hospitalized. Comorbidities play a major role in determining outcomes in patients with HF. Recent data on the use of sodium/glucose cotransporter-2 inhibitors in patients with diabetes mellitus, and effects on CV outcomes, especially HF, have raised new possibilities for management of comorbidities in these patients (Zinman et al. 2015).

The focus of this volume of the Handbook of Experimental Pharmacology on HF is to review and highlight the pharmacologic advances made in HF research and to discuss promising targets for future treatments. Besides signaling pathways and pharmacological targets, this handbook will also cover epidemiology and comorbidities, clinical trial design, biomarkers, and current guideline-based therapy, allowing a complete overview of chronic HF. Given the high incidence and prevalence of HF, and the high morbidity and mortality associated with this disease, continuing intensive research and development efforts are essential to address the

unmet needs of these patients. This book, authored by outstanding experts in the field, will summarize existing knowledge and will also describe future treatment approaches, with the hope that this will stimulate further research, ultimately leading to new, effective therapies and improved outcomes in patients with HF.

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The Three-Decade Long Journey in Heart Failure Drug Development

Kelly S. Lewis, Javed Butler, Johann Bauersachs, and Peter Sandner

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Abstract

Heart failure is a global disease with increasing prevalence due to an aging worldwide population with increasing co-morbidities. Despite several therapeutic options available to treat HFrEF, morbidity and mortality remain high. Importantly, no approved therapies are available to treat HFpEF. This paper will briefly summarize the burden of disease, HF classification and definitions and the landmark clinical trials in both HFrEF and HFpEF. Given the increasing incidence and prevalence of HF and the high morbidity and mortality associated

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_101

with this disease, continued development efforts are essential to address the unmet needs of these patients.

Keywords
Heart failure • Heart failure statistics • Heart failure trials • HFpEF • HFrEF

1 Heart Failure: Disease, Definitions and Treatments

The importance of HF cannot be overemphasized due to its high prevalence, the severity of its clinical manifestations and related poor outcomes, and extraordinarily high societal costs. This paper will briefly summarize the burden of the disease, HF classification, guidelines, and the landmark HF trials to date, in both HFrEF and HFpEF.

2 The Global Burden of Heart Failure

Statistics related to HF are alarming: The global prevalence of HF is estimated to be about 26 million people, with more than one million hospitalizations annually in the USA and Europe (Ambrosy et al. 2014). In the USA alone, there were an estimated 5.7 million patients living with HF in 2012 (Writing Group et al. 2016), with this figure expected to increase by 46% from 2012 to 2030, resulting in more than eight million adults with HF (Writing Group et al. 2016; Heidenreich et al. 2013). In the countries represented by the European Society of Cardiology, there are 15 million patients living with HF (Dickstein et al. 2008; Ponikowski et al. 2014). The disease is more common with increasing age: in the USA, more than 80% of patients are 65 years of age or older (Bui et al. 2011) and the incidence of HF approaches 10 per 1000 population after 65 years of age (Lloyd-Jones et al. 2002). In countries such as Japan with aging populations, the number of patients with HF is predicted to increase considerably (Mosterd and Hoes 2007; Shiba and Shimokawa 2008). In Asia, the increased prevalence of HF has been attributed to the adoption of a Western lifestyle and its associated comorbidities (Sakata and Shimokawa 2013; Sasayama 2008). Additionally, with improved treatment of myocardial infarction and other CV diseases, those surviving CV events are at high risk of developing HF (Ambrosy et al. 2014). In economically developed countries, one in five people are expected to develop HF at some point in their lifetime (Lloyd-Jones et al. 2002).

Heart failure is the leading cause of hospitalization in elderly people in the USA and Europe, representing 1–2% of all hospitalizations (Blecker et al. 2013; Zannad et al. 2009; Braunwald 2013; Centers for Disease Control and Prevention 2016). In 2012, the total cost of HF in the USA was estimated to be approximately $40 billion, of which 68% was attributable to direct medical costs; these costs are expected to more than double by 2030 (Heidenreich et al. 2013).

Despite advances in therapy and management, HF remains a deadly disease. Across the globe, 17–45% of patients admitted to the hospital with HF die within 1 year of admission and the majority die within 5 years of admission. In-hospital mortality ranges from 2 to 17% (Maggioni et al. 2013). Survival rates are better for those treated in outpatient clinics, who typically have less severe symptoms than those in the hospital setting (Maggioni et al. 2013; Yancy et al. 2006). Approximately 50% of patients diagnosed with HF will die within 5 years (Go et al. 2013), a statistic worse than for bowel, breast, or prostate cancer (Brenner et al. 2012; Coleman et al. 2011; Siegel et al. 2012).

3 Definitions and Classifications

Several guidelines for the management and treatment of HF have been written in recent years by the European Society of Cardiology (ESC), as well as by the American Heart Association (AHA)/American College of Cardiology (ACC) (Ponikowski et al. 2016; Writing Committee et al. 2013; Yancy et al. 2016). These guidelines define HF as a "'clinical syndrome characterized by typical symptoms (e.g., shortness of breath, ankle swelling, and fatigue) and signs (e.g., elevated jugular venous pressure, pulmonary crackles, and peripheral edema) caused by a structural and/or functional cardiac abnormality, resulting in decreased cardiac output and/or elevated intra-cardiac pressures at rest or with stress (Ponikowski et al. 2016; Writing Committee et al. 2013; Yancy et al. 2016)."

Generally the left ventricular ejection fraction (LVEF) determines how HF is categorized, which treatments are given, and what the prognosis may be. Ejection fraction (EF) is considered important in the classification of patients with HF because of differing patient demographics, comorbid conditions, prognoses, and response to therapies, and because most clinical trials selected patients based on EF (Fonarow et al. 2007). Until recently, guidelines for the management of HF divided patients with HF into two categories: those with reduced ejection fraction (EF $\leq$40%; HFrEF) and those with preserved ejection fraction (>40%; HFpEF) (Writing Committee et al. 2013). In the present ESC and AHA guidelines, HFrEF is defined as the clinical diagnosis of HF and LVEF $\leq$40% (Ponikowski et al. 2016; Yancy et al. 2016). Patients with HFpEF may not have entirely normal contractility but also do not have a major reduction in systolic function, and therefore the term "preserved ejection fraction" has been used. HFpEF has traditionally been defined as LVEF >40%, although it has been classified as EF from >40% to $\geq$55% across study types and by hospitalization status. Clinical studies of patients with HFpEF tended to use thresholds of 40–45%, while community-based studies and registries used more variable thresholds (Vaduganathan et al. 2016). Patients with HFpEF are usually older women with a history of hypertension, and share a similar comorbidity profile with patients with HFrEF (Adams et al. 2005). Hypertension is the most important cause of HFpEF, with a prevalence of 60–89% in patients with HFpEF (Sanderson 2007). Associated CV risk factors such as obesity, coronary artery disease (CAD), diabetes mellitus, atrial fibrillation (AF), chronic kidney disease,

Table 1 ESC and AHA guideline definitions of heart failure

Type of HF	HFrEF	HFmrEF	HFpEF
ESC criteria (Ponikowski et al. 2016)	• LVEF <40% • Symptoms ± signs	• LVEF 40–49% • Symptoms ± signs • Elevated levels of natriuretic peptides; BNP >35 or NT-proBNP ≥125 • Relevant structural heart disease (LVH and/or LAE) or diastolic dysfunction	• LVEF ≥50% • Symptoms ± signs • Elevated levels of natriuretic peptides; BNP >35 or NT-proBNP ≥125 • Relevant structural heart disease (LVH and/or LAE) or diastolic dysfunction
AHA/ ACCFCriteria (Writing Committee et al. 2013)	• LVEF ≤40%	• 41–49%[a]	• LVEF ≥50%

ACC American College of Cardiology, *AHA* American Heart Association, *BNP* B-type Natriuretic Peptide, *ESC* European Society of Cardiology, *HFmrEF* heart failure with mid-range ejection fraction, *HFpEF* heart failure with preserved ejection fraction, *HFrEF* heart failure with reduced ejection fraction, *LAE* left atrial enlargement, *LVEF* left ventricular ejection fraction, *LVH* left ventricular hypertrophy, *NT-proBNP* N-terminal-pro-B-type Natriuretic Peptide
[a]ACC/AHA distinguishes LVEF 41–49% as "HFpEF, borderline (or intermediate)"

and hyperlipidemia are also highly prevalent in patients with HFpEF (Adams et al. 2005; Sanderson 2007).

Recently, the ESC guidelines added a new definition and third class of HF, described as HFmrEF (LVEF 41–49%) (Table 1). The ACC/AHA guidelines call this group borderline (or intermediate) (Writing Committee et al. 2013). Classifying HFmrEF as a separate entity may stimulate research into the underlying characteristics, pathophysiology, and treatment of this group of patients. HF with recovered or improved EF (HFiEF) has recently been proposed as a further new category (Ponikowski et al. 2016; Yancy et al. 2016). In the valsartan Heart Failure Trial (Val-HeFT), of those patients who had a baseline LVEF of < 35% and a follow-up echocardiographic assessment of EF at 12 months, 9.1% had a 12-month EF that improved to >40%. Recovery of the EF to >40% was associated with better survival than persistently reduced EF (Florea et al. 2016). Classifying HFmrEF and HFiEF as separate entities may stimulate research into the underlying characteristics, pathophysiology, and treatment of these patients and further distinguish whether they are a distinct clinical entity.

4 Historical Aspects Treatment Guidelines and Pivotal Trials in HFrEF

Until the 1980s, treatment for HFrEF was limited to digoxin and diuretics. Although effective for symptoms, there was no evidence of mortality benefits with this treatment regimen, and it was an inadequate option for many patients

with advanced symptoms. The evolution of treatment over the past 30 years has been extensive, beginning with the introduction of vasodilator therapy (hydralazine/isosorbide dinitrate combination) in 1986 and culminating in 2015 with the approvals in the USA of ivabradine and LCZ696 (valsartan/sacubitril), an angiotensin-receptor blocker/neprilysin inhibitor combination that reduced CV morbidity and mortality in the PARADIGM-HF trial (Packer et al. 2014). Key trials of therapies for HFrEF and HFpEF are depicted in Figs. 1 and 2.

The main goals of HF treatment are to reduce symptoms, prolong survival, improve quality of life, and prevent disease progression. Before the Veterans Administration Cooperative trial, the first outcomes trial in CV disease in the late 1960s, clinicians based their practice on prior experience, tradition, or observational studies. Prior to 1980, during the "non-pharmacologic era," treatments focused on lifestyle changes such as bed rest, reduced activity, and fluid restriction.

The 1980s marked the beginning of the "pharmacologic era," heralded by the first Vasodilator Heart Failure Trial (V-HeFT) (Cohn et al. 1986). Data from this trial suggested that the combination of hydralazine and isosorbide dinitrate, given in addition to diuretics and digoxin, had a favorable effect on left ventricular function, exercise capacity, and clinical outcomes in patients with HF. The two Vasodilator Heart Failure Trials, V-HFT-I and V-HFT-II were among the first large randomized, placebo controlled trials in CV medicine (Cole et al. 2011). The subsequent V-HeFT-II trial was undertaken to compare isosorbide dinitrate/

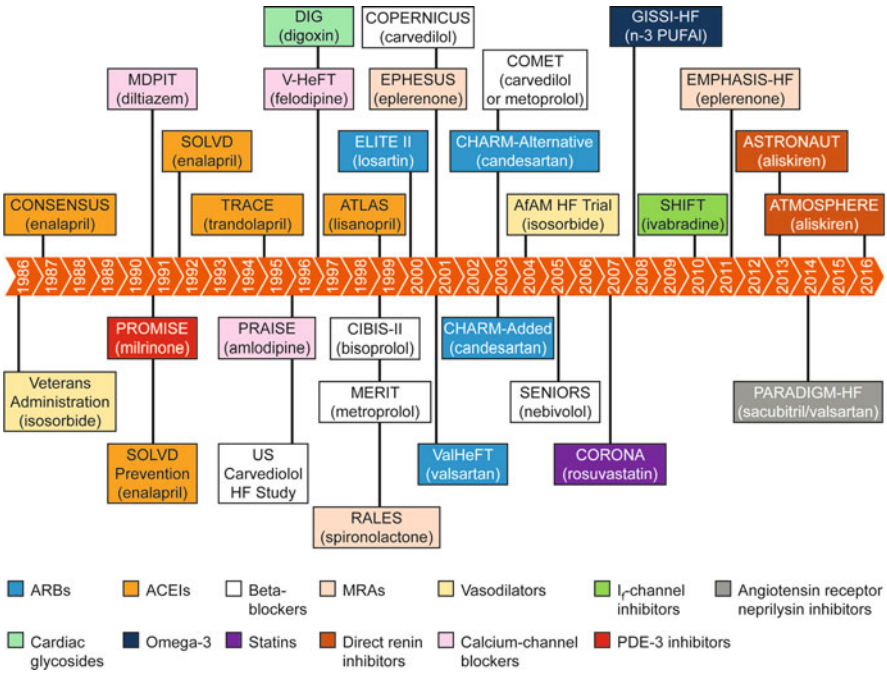

Fig. 1 30 years of development efforts in heart failure: Pivotal HFrEF trials

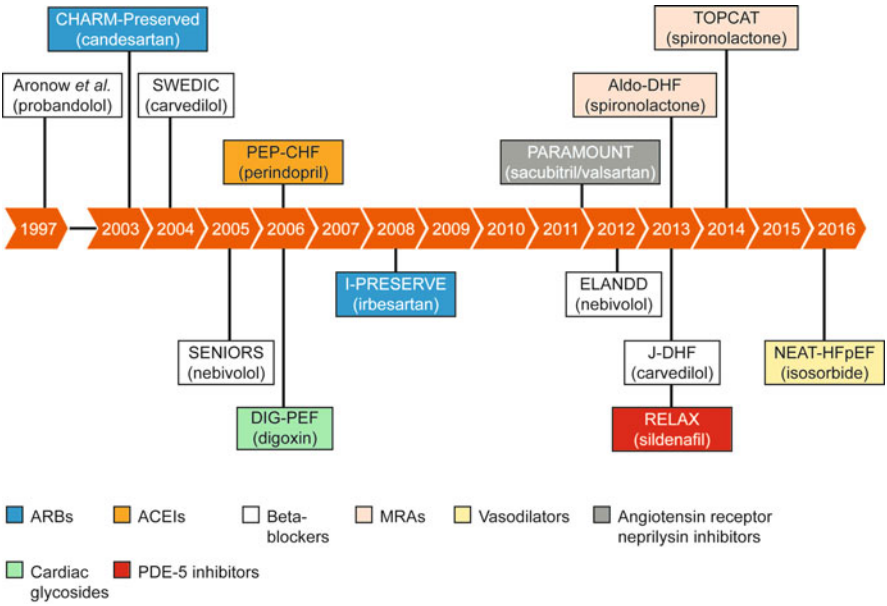

Fig. 2 30 years of development efforts in heart failure: Pivotal HFpEF trials

hydralazine with the angiotensin-converting enzyme (ACE) inhibitor, enalapril, and showed that enalapril conferred a survival benefit over isosorbide dinitrate/hydralazine (Cohn et al. 1991). A post hoc subgroup analysis suggested improved survival with isosorbide dinitrate/hydralazine among black patients (Carson et al. 1999), prompting the subsequently positive A-HeFT trial. The study was terminated early, owing to significantly higher mortality in the placebo group than in the isosorbide dinitrate/hydralazine group (Taylor et al. 2004).

The 1990s was a decade that brought neuro-hormonal interventions to the forefront of treatment pathways. Targeting the renal–angiotensin–aldosterone system (RAAS) provided evidence that ACE inhibitors, angiotensin II-receptor blockers (ARBs), and MRAs alter the natural history of heart failure.

There is considerable evidence to support the use of ACE inhibitors in symptomatic and asymptomatic patients with HFrEF and an EF of <40% (Ponikowski et al. 2016; Writing Committee et al. 2013; Yancy et al. 2016). Randomized trials have shown that therapy with ACE inhibitors leads to symptomatic improvement, reduced hospitalization, and enhanced survival in patients with HFrEF (Cohn et al. 1991; Cleland et al. 1985; Sharpe et al. 1984; Pfeffer et al. 1992; The SOLVD Investigators 1991; The CONSENSUS Trial Study Group 1987; Erhardt et al. 1995). ARBs were developed with the rationale that angiotensin II production continues in the presence of ACE inhibition, and are associated with a lower incidence of cough and angioedema than ACE inhibitors. In trials, long-term therapy with ARBs has been shown to reduce morbidity and mortality, especially in patients who are intolerant to ACE inhibitors (Cohn and Tognoni 2001; Pfeffer

et al. 2003a; Konstam et al. 2009; Pfeffer et al. 2003b). As such, guidelines recommend that initial therapy for patients with symptomatic HFrEF should comprise an ACE inhibitor or an ARB, along with β-blockers and an MRA, unless these drugs are contraindicated or not tolerated (Ponikowski et al. 2016; Writing Committee et al. 2013; Yancy et al. 2016).

In the placebo-controlled Randomized Aldactone Evaluation Study (RALES), adding spironolactone to baseline therapy in patients with HFrEF and moderate-to-severe symptoms decreased mortality and the risk of hospitalization for CV events (Pitt et al. 1999). Spironolactone has anti-androgenic and progesterone-like effects, which may cause gynecomastia or impotence in men, and menstrual irregularities in women. To avoid these side effects, eplerenone was developed. In the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), eplerenone reduced all-cause mortality and hospitalization for CV events in patients with myocardial infarction complicated by left ventricular systolic dysfunction and HF (Pitt et al. 2001). In the more recent EMPHASIS-HF study, eplerenone when added to standard therapy reduced mortality and hospitalization in patients with HFrEF (New York Heart Association [NYHA] class II) and mild symptoms (Zannad et al. 2011). Consequently, current guidelines recommend the use of an MRA in HFrEF. Recently finerenone, a non-steroidal MRA with a potentially more favorable cardiac-to-renal activity ratio, has shown benefit over eplerenone in a population of patients with HFrEF (Filippatos et al. 2016; Bauersachs et al. 2015).

In angiotensin receptor-neprilysin inhibition (ARNI) therapy, an ARB is combined with an inhibitor of neprilysin, an enzyme that degrades the natriuretic peptides, bradykinin and adrenomedullin, as well as other vasoactive peptides. In the PARADIGM-HF trial, the ARNI valsartan/sacubitril significantly reduced the composite endpoint of CV death or HF hospitalization by 20%, compared with enalapril, in symptomatic patients with HFrEF tolerating an adequate dose of either ACE inhibitor or ARB. A similar benefit was seen for both all-cause mortality and HF hospitalization (Packer et al. 2015). Sacubitril/valsartan should not be given in combination with an ACE inhibitor as this is associated with an increased risk of angioedema.

Treatment with β-blockers, in addition to ACE inhibitors and digoxin, reduces the risk of death and hospitalization in patients with HF. The Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) study demonstrated that carvedilol reduced 12-month mortality in patients with severe HF by 38% and the relative risk of death or HF hospitalization by 33%. The favorable effects of carvedilol were apparent even in patients at highest risk (i.e., those with recent or recurrent cardiac decompensation or very depressed cardiac function), who had a 33% decrease in the combined risk of death or hospitalization for a CV reason (95% CI, 14% to 48%, $P=0.002$) and a 33% decrease in the combined risk of death or hospitalization for heart failure (95% CI, 13% to 49%, $P=0.002$) when treated with carvedilol (Packer et al. 2002). In the Carvedilol or Metoprolol European Trial (COMET) study, carvedilol (25 mg twice daily) was compared with immediate-release metoprolol tartrate (50 mg twice daily). Carvedilol was associated with an

all-cause mortality of 34%, compared with 40% for metoprolol (Poole-Wilson et al. 2003). Based on these results, short-acting metoprolol tartrate is not recommended for use in the treatment of HF. In the DIG trial, digoxin had no effect on overall mortality in patients receiving diuretics and ACE inhibitors, but it did reduce the overall number of hospitalizations and the combined outcome of death or hospitalization attributable to worsening HF (Digitalis Investigation 1997). As participants in the DIG trial were not systematically treated with β-blockers and MRAs, the results of the ongoing DIGIT HF study (EudraCT DIGIT-HF) comparing the effects of digoxin and placebo in patients with advanced HFrEF on current standard therapy will be of major interest.

Elevated resting heart rate is associated with increased CV morbidity and mortality (Pocock et al. 2006; Lechat et al. 2001), independently of other established CV risk factors. The beneficial effects of β-blockers in HF have been thought to be related in part to heart-rate lowering effects. Ivabradine acts by selective inhibition of the pacemaker I_f channel, which is responsible for the autonomic capacity of the sinoatrial node. I_f channels are up-regulated in atrial tissue of patients with HF. In the SHIFT (Systolic Heart failure treatment with the I (f) inhibitor ivabradine Trial) study, ivabradine significantly reduced the composite primary endpoint of CV death and hospitalization for worsening HF by 18%, driven mainly by a reduction in hospitalization and deaths attributable to HF. CV deaths and all-cause mortality were not significantly reduced with ivabradine (Swedberg et al. 2012).

5 HFpEF

Although many treatments have been tested in HFpEF, all have returned neutral or negative results in randomized clinical trials. Treating the underlying comorbidities is the current mainstay of therapy. Guidelines recommend diuretics to control water retention, and to relieve breathlessness and edema. It is also recommended that hypertension is optimally managed and myocardial ischemia is assessed and treated, and in patients with AF heart rate is controlled.

The nitric oxide (NO) pathway is a key regulator of many physiological processes, and modulates vascular tone and myocardial performance. Numerous lines of evidence indicate that abnormalities in NO–cyclic guanosine monophosphate (cGMP) signaling play a central role in limiting exercise capacity in patients with HF. In HFPEF, comorbidities contribute to a systemic inflammatory state, which induces oxidative stress in the coronary microvascular endothelium and reduced myocardial NO bioavailability (Paulus and Tschope 2013). In the NEAT-HF trial, isosorbide mononitrate did not improve daily activity level, 6 min walk distance, dyspnea, quality-of-life scores, or NT-proBNP levels in patients with HFpEF. Indeed, dose-dependent decreases in daily activity levels were seen with isosorbide mononitrate (Redfield et al. 2015). Another means of targeting the NO-cGMP pathway is via phosphodiesterase inhibition. However, in the RELAX trial, the use of sildenafil in patients with HFpEF did not result in any improvement in

exercise capacity or clinical status over 24 weeks of treatment (Redfield et al. 2013). Reduced NO levels in HF leads to a decrease in the stimulation of an important enzyme called soluble guanylate cyclase (sGC). A lack of sGC stimulation leads to reduced activity of the NO-sGC-cGMP pathway, causing coronary dysfunction and progressive myocardial damage (Greene et al. 2013). More recently, a novel class of drug has been discovered which modulates cGMP production by targeting and stimulating the sGC enzyme (Gheorghiade et al. 2013). These compounds, sGC stimulators, have a dual mechanism of action: they have the ability both to stimulate sGC directly and independently of NO, and also to increase its sensitivity, thus reactivating the vital cardiovascular NO–sGC–cGMP pathway, even in the presence of the low NO levels seen in patients with HF. These compounds are now being studied in HF to target this critical pathway. A phase III clinical study (VICTORIA) is ongoing to study the once daily sGC stimulator, vericiguat, on outcomes in patients with HFrEF.

Use of β-blockers and RAAS blockers in patients with HFpEF has not produced positive results; there is no evidence from randomized trials of a clinical benefit of ACE inhibitors or ARBs in patients with HFpEF (Yusuf et al. 2003; Massie et al. 2008; Cleland et al. 2006; McKelvie et al. 2010). In the CHARM-PRESERVED trial of candesartan versus placebo in addition to background therapy (except ARBs), CV death did not differ between groups, but fewer patients in the candesartan group were admitted to hospital for HF (Yusuf et al. 2003). In the PEP-CHF study, perindopril did not improve the composite of all-cause mortality and unplanned HF-related hospitalization at 1 year, but did improve exercise capacity and NYHA functional class (Cleland et al. 2006). In the I-PRESERVE trial, treatment with irbesartan did not reduce the risk of death or hospitalization for CV causes in patients with HFpEF, nor did it improve any of the secondary outcomes, including quality of life (Massie et al. 2008). Trials of β-blockers have failed to provide conclusive results in HFpEF. A small-scale trial with carvedilol suggested that long-term therapy could improve diastolic function, with prevention or partial reversal of progressive left ventricular dilatation (Capomolla et al. 2000). Analysis of data from the SENIORS trial reported that nebivolol had similar efficacy in preventing all-cause and CV death in a subgroup of patients with HFpEF compared with those with HFrEF (van Veldhuisen et al. 2009).

Some argue that the findings from the TOPCAT study were inconclusive rather than neutral. Although spironolactone failed to demonstrate a benefit for the primary endpoint of CV death, cardiac arrest, or hospitalization for HF (Pitt et al. 2014), the overall neutral results may have been related to stratification by enrollment criteria and regional variations. Patients enrolled on the basis of hospitalization criteria had a lower event rate than those enrolled on the basis of natriuretic peptide level. In a post hoc analysis, spironolactone significantly reduced the rates of CV death and hospitalization for HF in patients enrolled from the Americas but not in those enrolled from Russia or Georgia (Pfeffer et al. 2015).

As in HFrEF, optimal heart rate is becoming an important target in the management of HFpEF. An analysis of patients with HFpEF in the I-PRESERVE database showed that every 12.4 bpm increase in heart rate was associated with a 13%

increase in the risk of a composite of CV death or hospitalization for HF (Bohm et al. 2014). Preliminary and experimental results with ivabradine indicated potential for heart-rate reduction in HFpEF. Ivabradine is currently undergoing further phase 2 testing in HFpEF in the ongoing EDIFY trial (EudraCT 2012).

Taken together, optimal medical therapy in patients with HFrEF modifies the clinical course of the disease. When patients are treated according to current guidelines, annual mortality is much lower than previously. However, in many patients commonly prescribed drug regimens are inadequate and more effort is necessary to achieve optimal medical therapy at evidence-based target doses (Packer 2016).

6 Conclusion

Despite 30 years of clinical research in HF and the approval of many effective therapies for patients with HFrEF, rates of CV events including hospitalizations, emergency department and office visits, the need for acute interventions (e.g., intravenous diuretics), and even death remain unacceptably high. Morbidity and mortality in patients with HFpEF are similar to those in patients with HFrEF, and there are no approved therapies. Understanding the molecular pathophysiology of HFpEF might serve as a key to identifying new pharmacologic targets for this disease. Clearly, additional innovative and more effective therapies that target new pathways are needed in all patients with HF.

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Epidemiology of Heart Failure

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Abstract

Heart failure (HF) is a major public health problem affecting more than 23 million patients worldwide. Incidence and prevalence rates vary significantly according to the source of data, but both increase with advancing age reaching, in the very elderly, prevalence rates that represent a challenge for the organization of medical care systems. Even if evidence-based treatments have improved

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_74

prognosis in some patients with HF, patients with HF still need to be carefully characterized, described, and treated. Hospitalizations for acute HF are frequent and costly accounting for the vast majority of HF-related costs.

Keywords
Epidemiology • Heart failure • Prognosis • Registries

1 Introduction

Heart failure (HF) is a major clinical and public health problem with a prevalence of more than 23 million worldwide, associated with significant mortality, morbidity, and health-care expenditures; direct costs of HF account for almost ~2% of the total health-care budget in many European countries. Significant changes have occurred in the outcomes of patients with HF in the past 20 years mainly due to the development of pharmacological and non-pharmacological treatments that have improved survival in at least a group of patients with HF, specifically those with reduced ejection fraction (EF). Understanding the evolving epidemiology of HF is important in order to target interventions and for health-care planning.

In this chapter HF epidemiology will be discussed from two different and complementary approaches: the first part will describe general epidemiological data of the HF syndrome (e.g., incidence, prevalence, etiology, and outcomes), whereas in the second part, a picture of current cardiology clinical management of HF will be carried out by describing characteristics of patients with HF included in large European registries in patients with HF: the Italian Registry on Heart Failure Outcome (IN-HF Outcome; Tavazzi et al. 2013) and the Heart Failure Registry of the European Society of Cardiology (ESC-EORP-HF Pilot and Long Term) Maggioni et al. 2010; Maggioni et al. 2013a, b).

2 Incidence and Prevalence

In the past decades, the prevalence of HF has grown, particularly in the elderly, and the expression HF epidemic is frequently used to describe this phenomenon. This epidemic is the result of several factors, some of which may be related to the increased incidence (e.g., demographic changes with longer life expectancy in the general population or improved survival in patients with ischemic heart disease) and other to the increased survival due to the use of drugs and devices tested in several successful randomized clinical trials (RCTs) conducted in the past 30 years and able to improve the outcomes of ambulatory patients. Studies report an incidence of HF of 1–4/1,000 person-years (Levy et al. 2002; Roger et al. 2004; McMurray and Stewart 2000; Zarrinkoub et al. 2013). The prevalence has increased over time due to improved survival after diagnosis of HF and aging of the population, accounting for 1–3% in the adult population in developed countries, rising to

more than 10% and 30% among people >70 and >85% years of age, respectively (Dunlay and Roger 2014; Mosterd and Hoes 2007). Estimation of the lifetime risk for the development of HF is important for population health planning. Projections show that the prevalence of HF will increase 46% from 2012 to 2030, resulting in >8 million people ≥18 years of age with HF (Heidenreich et al. 2013). Data regarding incidence and prevalence of HF derives from several studies, the most important of which are reported in Table 1. Notably there are significant differences across studies which might have several explanations:

- Type of studies. It should be noted that epidemiological studies considered were of different nature, some being based on administrative data (Curtis et al. 2008; Yeung et al. 2012; Zarrinkoub et al. 2013; Maggioni et al. 2016) and some others on clinical data derived from population-based observational studies (Levy et al. 2002; Roger et al. 2004; McCullough et al. 2002; Bleumink et al. 2004; Gottdiener et al. 2000).
- Different populations of HF patients. Some studies included patients with self-reported diagnosis of HF, others used hospital discharge diagnosis which could be administrative (ICD, International Classification of Diseases), with potential risk for up-coding of discharge diagnoses due to reimbursement incentives, or clinical.
- Different definitions and different diagnostic criteria of HF, which have changed significantly in the past years, have been used for the diagnosis of HF in these studies. Some studies used guideline diagnostic criteria, whereas others used Gothenburg, Boston, or Framingham diagnostic criteria (Eriksson et al. 1987; Carlson et al. 1985; McKee et al. 1971).

3 Hospitalizations and Mortality

Acute heart failure (AHF) is a complex, heterogeneous, clinical syndrome, often life threatening and requiring hospitalization for urgent therapy (Rosamond et al. 2007; Gheorghiade et al. 2005).

Despite the relevant burden of this clinical condition, therapeutic developments have been scarce in the last couple of decades; for this reason patients with HF remain at substantial risk for recurrent acute exacerbations and death (Fonarow et al. 2005; Abraham et al. 2008; Rudiger et al. 2005). Further, local conditions leading to hospitalization of patients with HF, as well as their in-hospital care, may be hugely different in various countries and can change over time (Maggioni et al. 2013a, b).

In total, there are more than 1 million hospitalizations for HF each year in the USA (Blecker et al. 2013). Heart failure is the leading cause of hospitalization among Medicare beneficiaries in the USA. Patients hospitalized with HF have the highest 30-day readmission rate (~25%) of any diagnosis (Jencks 2009); over half of patients are readmitted within 1 year, and multiple readmissions are common (Chun et al. 2012; Dunlay et al. 2009a). Many of these readmissions are due to

Table 1 Incidence, prevalence, and mortality of heart failure (Modified from Roger 2013)

Population (years)	Type of study	Incidence	Prevalence	Mortality
NHANES (2012) (Mozaffarian et al. 2015)	Interview-based survey	–	2.2% (≥20 years)	–
Framingham Heart Study (1950–1999) (Levy et al. 2002)	Population-based observational study	≈5/1,000 person-years	–	At 1-year age adjusted 1950–1969 Men: 30% Women: 28% 1990–1999 Men: 28% Women: 24%
Olmsted County (1979–2000) (Roger et al. 2004)	Population-based observational study	≈3/1,000	–	At 1 year (75 years old) 1979–1984 Men: 30% Women: 20% 1996–2000 Men: 21% Women: 17%
REACH Study (1989–1999) (McCullough et al. 2002)	Population-based observational study	Women: 3.7–4.2/1,000 Men: 4.0–3.7/1,000	Women: 0.4%–1.4% Men: 0.4%–1.5%	Per year: 17%
The Rotterdam Study (1989–2000) (Bleumink et al. 2004)	Population-based observational study	Women: 12.5/1,000 person-years Men: 17.6/1,000 person-years	1998: 7%	At 1 year: 37%
Cardiovascular Health Study (1990–1996) (Gottdiener et al. 2000)	Population-based observational study	Nonblack: 19/1,000 person-years Black: 19/1,000 person-years Women: 15/1,000 person-years Men: 26/1,000 person-years	–	–

(continued)

Table 1 (continued)

Population (years)	Type of study	Incidence	Prevalence	Mortality
Medicare beneficiaries (1994; 2003) (Curtis et al. 2008)	Administrative database	1994: 32/1,000/ person-years 2003: 29/1,000 person-years	1994: 9% 2003: 12%	1-year risk adjusted 1994: 29% 2002: 28%
Ontario, Canada (1997–2007) (Yeung et al. 2012)	Administrative database	1997: 4.5/ 1,000 persons 2007: 3.1/ 1,000 persons	–	1-year risk adjusted 1997 Outpatients: 18% Inpatients: 36% 2007 Outpatients: 16% Inpatients: 34%
Sweden (1990–2007) (Zarrinkoub et al. 2013)	Administrative database	2010: 3.1/ 1,000 persons	Crude: 1.8% Adjusted for demographic: 2.2%	Women 3.2/1,000 person-years Men 3.0/1,000 person-years 5-year survival rate was 48%
ARNO (2008–2012) (Maggioni et al. 2016)	Administrative database	–	2.2	28% at 1 year

non-cardiovascular causes (Maggioni et al. 2016; Carson et al. 2015; Desai et al. 2014).

This vulnerability to a diversity of illnesses may explain why interventions to prevent them should be delivered by a multidisciplinary team. A multidisciplinary strategy of intervention has been demonstrated to be more likely to reduce readmissions, specifically in the HF clinical area (Hansen et al. 2011; Rich et al. 1995).

Furthermore, annual total direct medical costs for patients with HF are $21 billion and expected to increase to $53 billion by 2030 (Heidenreich et al. 2013), and hospitalizations account for up to three-quarters of those costs (Dunlay et al. 2011).

Numerous studies have consistently shown that mortality from HF has steadily declined in recent decades (Barasa et al. 2014; Levy et al. 2002; Chen et al. 2011; Yeung et al. 2012), largely reflecting the introduction of medications (e.g., angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, mineral-ocorticoid receptor antagonists, beta-blockers, and more recently ARNI) and

devices (e.g., implantable defibrillators and biventricular PM) which improve survival in patients with HFrEF (Guidelines ESC 2016). Trends in mortality from the time of initial diagnosis of HF are summarized in Table 1. However, despite these improvements, HF remains associated with poor outcomes. After initial diagnosis of HF, the estimated survival is 72–75% at 1 year and 35–52% at 5 years (Barasa et al. 2014; Levy et al. 2002). Most studies have suggested that women have better survival than men after diagnosis, adjusting for age (Levy et al. 2002; Roger et al. 2004).

Only very few studies examined the cause of death in patients with HF. In Olmsted County, 43% of deaths were due to non-cardiovascular causes, and the proportion was higher in patients with HFpEF (Henkel et al. 2008). In TIME-HF study causes and modes of death were specifically analyzed in elderly patients with HF: cause of death was more often non-cardiovascular in HFpEF patients than in HFrEF patients (33% vs. 16%, $P < 0.05$), and cardiac mode of death were more frequent in HFrEF patients (75% vs. 56%, $P < 0.05$), mainly due to more sudden deaths (25% vs. 7%, $P < 0.05$) Rickenbacher et al. 2012).

4 Etiology of Heart Failure

Several studies have examined the contribution of risk factors to the development of HF (Dunlay et al. 2009b; Folsom et al. 2009; Levy et al. 1996; Loehr et al. 2010; He et al. 2001). Different factors that predispose to HF in the general population have been identified, and, among these, coronary artery disease, hypertension, hypercholesterolemia, diabetes, smoking, arrhythmias, and obesity are the most important. These risk factors may coexist and interact with each other in an individual patient; nevertheless, their contribution to the development of HF varies significantly according to the type of HF. Patients with HFpEF are more frequently obese, with a history of hypertension and arrhythmias (particularly atrial fibrillation), whereas patients with HFrEF have more frequently a history of coronary artery disease, diabetes, and smoking (Senni et al. 2014). On the contrary, in the Physicians' Health Study, healthy lifestyle factors (normal weight, not smoking, regular exercise, moderate alcohol intake, consumption of breakfast cereals, and consumption of fruits and vegetables) were related to lower risk of HF (Djoussé et al. 2009).

Interesting and under intensive study is the role of chronic comorbidities that are frequent, particularly in the elderly (Saczynski et al. 2013), and have strong prognostic implications not only by summing their independent prognostic burden but also by limiting the use of evidence-based treatments and conditioning the eligibility for advanced heart failure therapies. Patients with HF are also affected by five or more concomitant chronic conditions in more than 50% of cases (Wong et al. 2011). Particular attention deserves noncardiac comorbidities that are highly prevalent in older patients with HF and strongly associated with adverse clinical

outcomes. The risk of hospitalization and potentially preventable hospitalization strongly increases with the number of chronic conditions (Braunstein et al. 2003), and non-cardiac comorbidities are responsible for almost half of the hospitalizations that characterize and frequently worsen the clinical history of HF patients (Maggioni et al. 2016). Cardiologists and other physicians routinely caring for older patients with HF may improve outcomes in this high-risk population by better recognizing non-cardiovascular conditions in a comprehensive multidisciplinary approach. Furthermore, in HFpEF it is now clear that comorbidities and, to a greater extent, noncardiac comorbidities, are not only responsible for worsening clinical conditions and causing hospitalizations but also have a fundamental pathophysiological role in the evolution of preclinical diastolic dysfunction into of overt HFpEF (Paulus and Tschöpe 2013; Wan et al. 2014).

5 Heart Failure with Different Levels of Ejection Fraction (EF)

Among the several HF classifications that have been proposed, beside the distinction in chronic and acute HF (which comprises both patients with new-onset HF or *de novo* HF and worsening chronic heart failure), the differentiation more frequently used in clinical practice (because associated with important differences concerning demographics, underlying etiologies, comorbidities, and response to therapies) is based on EF measurement considering HF with preserved EF (HFpEF) vs. reduced EF (HFrEF). The 2016 ESC Guidelines (Ponikowski et al. 2016) have recently defined EF cutoff for HFpEF and for HFrEF as $\geq$50% and $<$40%, respectively. These guidelines have also introduced a third group, midrange (HFmrEF), that includes patients in the range 40–49%, the socalled gray area that seems to have different and still unexplored characteristics compared to patients with HFrEF and HFpEF, and on which epidemiological data is lacking. EF thresholds for the definition of HFpEF have varied significantly according to different study population and therefore it is difficult to give precise data on its prevalence, but several studies suggest that this form of HF accounts for almost 50% of all forms of HF. As previously reported in this chapter, patients with HFpEF are more likely to be older, female, a specific pattern of cardiac and noncardiac comorbidities.

In the second part of this chapter, a picture of current cardiology will be described; clinical management of HF will be carried out by describing characteristics of HF patients included in important European registries: the Italian Registry on Heart Failure Outcome (IN-HF Outcome; Oliva et al. 2012; Tavazzi et al. 2013) and the Heart Failure Registry of the European Society of Cardiology (ESC-HF) (Maggioni et al. 2010; Maggioni et al. 2013a, b).

6 Patients Hospitalized for Acute HF

6.1 IN-HF Outcome Findings

The registry included 5,610 HF patients: 1,855 patients were admitted for acute HF and were classified as new de novo HF in 43.0% of patients and worsening HF in 57.0%.

According to the ESC Guidelines utilized at the time of data collection (Dickstein et al. 2008), the acute HF profiles at entry were classified as acute pulmonary edema in 27.0% of patients, cardiogenic shock in 2.3%, decompensated HF in 43.9%, right failure in 8.8%, and HF in the context of acute coronary syndrome in 12.9%; hypertension was interpreted as the cause of decompensation in 5.1% of the patients.

The mean age was 72 ± 12 (range 21–98) years and 40% were women. Ischemic etiology was significantly higher in the worsening HF group. Comorbidities such as chronic obstructive pulmonary disease (COPD), diabetes, and history of renal failure or atrial fibrillation (AF) were more frequent in worsening HF; in contrast, entry systolic blood pressure (SBP), heart rate, and left ventricular ejection fraction (LVEF) were significantly higher in the de novo HF patients. An implantable cardioverter defibrillator (ICD) and/or cardiac resynchronization therapy (CRT) were present in 13.3% of cases (21.1% in worsening HF compared with 2.9% in de novo HF, $p < 0.0001$). The large majority of patients had signs of peripheral and/or pulmonary congestion.

Anemia was observed in 38.7% of the patients. Almost 55% showed an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2, and 13.1% of the patients had severe renal dysfunction (eGFR <30 mL/min/1.73m^2), mainly in the worsening HF group. When measured, the median values of N-terminal pro brain natriuretic peptide (NT-proBNP) or brain natriuretic peptide (BNP) were very elevated, and they were similar in the worsening and de novo HF groups. In the de novo group, high-sensitivity C-reactive protein (hsCRP), as a possible index of inflammation, was higher than in the worsening HF patients. Atrial fibrillation was present in one-third of the subjects (31.5%) that had an available ECG (>95%). ECG was defined as normal in only 2.3% of the entire population. When considering echocardiographic parameters, worsening HF patients showed a larger proportion of patients with a severely reduced LVEF (<30%) and with severe mitral regurgitation. In this registry, the median time spent in hospital was 10 days (IQR 7–15); 51.9% of patients (46.5% and 59.1% of those with worsening and de novo HF, respectively, $p < 0.0001$) were admitted to the intensive coronary care unit (ICCU) for a median time of 4 days (IQR 3–7). Despite (or as consequence of) a greater number of admissions to ICCU, de novo HF patients spent less time in hospital [9 (IQR 6–14) vs. 10 (IQR 7–16) days, $p = 0.0001$]. The all-cause in-hospital death rate was 6.4% (almost 90% cardiac) and was not different in

worsening and de novo HF groups (6.0 vs. 6.9, respectively, $p = 0.41$). Patients with cardiogenic shock had the highest mortality rate (23.8%), followed by those with acute coronary syndrome (13.0%), while patients with hypertensive HF had the lowest death rate (3.2%).

6.2 ESC-HF Pilot Survey

The ESC-HF Pilot Survey enrolled 5,118 patients from October 2009 to May 2010. Table 2 shows the characteristics of in-hospital patients also compared with those of ambulatory patients with chronic HF. In-hospital patients were generally older than ambulatory patients with chronic HF and were more often female. As expected, comorbidities were more frequent in patients admitted for acute HF, whereas the rate of implanted devices was more common in patients with chronic HF. More than half of the patients with acute HF had an ischemic etiology, confirmed by coronary angiography in 64% of the cases. In patients with chronic HF, an ischemic etiology accounted for just 40% of the cases; angiographic confirmation was available for 85% of the cases. At hospital entry, clinical signs of pulmonary congestion were detected in 62% of the cases, peripheral congestion was detected in 65%, and either pulmonary or peripheral congestion was detected in 82% of the cases. Clinical signs of peripheral hypoperfusion were reported in 8.6% of the patients; 10.5% of admitted patients were described as somnolent or confused. At the ECG performed at hospital entry, AF was diagnosed in 35% of the cases, and a large QRS ($\geq$120 ms) was reported in 35.5% of the patients. An echocardiographic examination was performed in 75% of the patients. The median ejection fraction was 38% (IQR

Table 2 ESC-HF Pilot Registry: comparison between acute and chronic heart failure – baseline characteristics (Maggioni et al. 2010)

	AHF pts (n. 1892)	CHF pts (n. 3226)
Age (years), mean ± SD	70 ± 13	67 ± 13
Females, %	37.3	29.7
Ischemic etiology, % *Documented by coronary angiography, %*	50.7 *64.0*	40.4 *84.9*
SBP (mmHg), mean ± SD	133 ± 29	125 ± 20
HR (bpm), mean ± SD	88 ± 24	72 ± 14
Treated hypertension, %	61.8	58.3
Diabetes mellitus, %	35.1	29.0
History of atrial fibrillation, %	43.7	38.6
Chronic kidney dysfunction, %	26.0	18.5
ICD, %	6.0	13.3
CRT-P, %	0.4	1.1
CRT-D, %	2.9	8.7

AHF acute heart failure, *CHF* chronic heart failure, *SD* standard deviation, *SBP* systolic blood pressure, *HR* heart rate, *ICD* implantable cardioverter, *CRT-P* cardiac resynchronization therapy, *CRT-D* cardiac resynchronization therapy-defibrillator

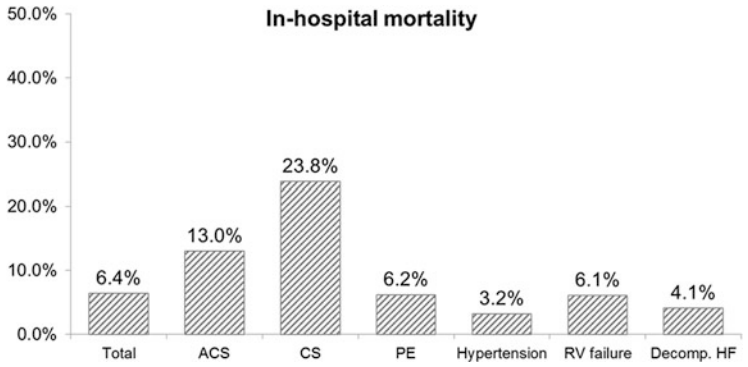

Fig. 1 IN-HF Outcome Registry: All-cause in-hospital mortality according to the acute heart failure profile at entry (Tavazzi et al. 2013). *ACS* acute coronary syndrome, *CS* cardiogenic shock, *PE* pulmonary edema, *RV* right ventricular, *HF* heart failure

27–52); 39.1% of the patients had a preserved ejection fraction, defined at the time of the study as >40%. A moderate-to-severe mitral regurgitation was diagnosed in 43.4% of the patients.

Anemia, defined as a hemoglobin level inferior to 12 g/dL, was detected in 31.4% of the patients; an eGFR <50 and <30 mL/min/1.73 m^2 was reported, respectively, in 32.9 and 9.8% of the patients. NT-proBNP and BNP were measured at entry in 489 and 204 patients only, respectively. The median values were 4007 pg/mL (IQR 2043–9487) and 870 pg/mL (IQR 423–1950), documenting the severity of the clinical conditions at hospital admission.

Figure 1 shows the overall rate of in-hospital mortality and that stratified by clinical profiles. Overall, 71 patients died during the hospital stay; the highest mortality rate being observed in patients with cardiogenic shock and the lowest in those with hypertensive HF. The cause of death was cardiovascular in 90.1% of the cases.

6.3 ESC-HF Long-Term Registry

From May 2011 to April 2013, of the 12,785 patients screened for the study, 12,440 gave their informed consent and therefore are part of this analysis (Maggioni et al. 2013a, b). Of these patients, 5,039 (40.5%) were patients hospitalized for acute HF, while 7,401 (59.5%) were ambulatory patients with chronic HF. Patients included in the ESC Long-Term Registry present with baseline characteristics, clinical history, and comorbidities which largely overlap the observations of other European or US registries (Maggioni et al. 2010; Tavazzi et al. 2006; Adams et al. 2005; O'Connor et al. 2005).

Patients hospitalized for acute HF show a more severe clinical profile, as well as a higher rate of comorbidities than patients with chronic HF, showing the substantial similarity of this population of patients with respect to previous reports.

To have a summary of the findings presented so far, Table 3 reports some selected clinical characteristics of the IN-HF Outcome and the ESC-HF Pilot Survey patients compared with subjects enrolled in other US and European registries (Abraham et al. 2008; Nieminem et al. 2006; Adams et al. 2005; Tavazzi et al. 2006; Tavazzi et al. 2013; Maggioni et al. 2010).

The in-hospital all-cause mortality rate of the IN-HF Outcome Registry was slightly lower than reported in a previous Italian survey (6.4% vs. 7.3%) but still higher as compared with the ESC-HF Pilot Survey (Maggioni et al. 2010) and with US registries (4%) reported in Table 3. However, considering the differences in length of stay and the rapid discharge home in US hospitals, some deaths may have occurred some days later. This is supported by the analysis of the 3-month mortality which seems similar in European and US registries (about 13%).

7 Ambulatory Patients with Chronic HF

7.1 IN-HF Outcome Findings

The all-cause mortality rate at 1 year of chronic stable HF patients was 5.9%, relevantly reduced with respect to the mortality rates observed in the previous decades. The rate of death is much higher, as expected, in patients in NYHA classes III–IV than in those in class I–II (14.5% vs. 4.1%, $p < 0.0001$). Etiology did not seem to have a remarkable impact on outcome. The death rate was higher, but not statistically significant, in patients with ischemic etiology (6.5%) than in those with nonischemic etiology (5.4%) ($p = 0.17$). Of the 222 patients with chronic HF who died at 1 year, 65.3% of deaths were due to cardiovascular reasons, 16.2% were due to noncardiovascular reasons, and in 18.5% of cases the cause of death remained unknown. Among the cardiovascular causes of death, HF was the most frequent cause (62.1%) followed by arrhythmic deaths (15.2%).

The 1-year admission rate was 22.7%, one-third (8.8%) only was due to HF, and 13.9% was related to other causes, in 8.4% these were CV causes. The proportion of HF hospitalizations was higher in patients with severe HF, while in patients with ischemic etiology, the prevalent cause of hospitalization was cardiovascular but not due to worsening HF. The rate of use of the pharmacological treatments recommended by the current guidelines was high at the entry visit and did not change during the follow-up period.

The IN-HF Outcome study shows two encouraging findings. First, the recommended pharmacological treatments are incorporated in the clinical practice of cardiologists in Italy and maintained over time. Second, the evolutionary nature of chronic HF looks relatively controlled with an annual mortality rate lower than

Table 3 Patients' characteristics in previous AHF registries and in IN-HF outcome

	Adhere ($N = 105,388$) (Adams et al. 2005)	EHFS II ($N = 3,580$) (Nieminen et al. 2006)	Italian S ($N = 2,807$) (Tavazzi et al. 2006)	OPTIMIZE-HF ($N = 48,612$) (Abraham et al. 2008)	ESC-HF pilot ($N = 1,892$) (Maggioni et al. 2010)	IN-HF outcome ($N = 1,855$) (Tavazzi et al. 2013)
Age (years), mean ± SD	72.4 ± 14	69.9 ± 12	73 ± 11	73 ± 14	70 ± 13	72 ± 12
Females (%)	52	38.7	39.5	51.6	37.3	39.8
Ischemic etiology (%)	65	53.6	46	45.7	50.7	42.3
Medical history						
Hypertension (%)	73	62.5	65.6	70.9	61.8	57.8
Diabetes mellitus (%)	44	32.8	38.4	41.5	35.1	40.4
Renal insufficiency (%)	30	16.8	24.7	19.6	26	32.5
Atrial fibrillation (%)	31	38.7	28.4	30.8	43.7	37.7
Baseline medications						
ACE-I/ARBs (%)	53	63	72.5	51.3	60	59
Diuretics (%)	70	71	81	65.7	68	64
Beta-blockers (%)	48	43	32	53.1	62	41
Digoxin (%)	28	27	NA	23.4	21	16

De novo HF (%)	35	37.1	44	11.7	NA	43
Cardiogenic shock at entry (%)	NA	3.9	7.7	NA	2.3	2.3
Hypertensive AHF at entry (%)	NA	11.4	NA	NA	4.7	5.1
Physical and laboratory findings						
SBP (mmHg) (mean ± SD)	144 ± 33	135 ± NA	141 ± 37	143 ± 33	133 ± 29	134 ± 33
HR (bpm) (mean ± SD)	NA	95 ± NA	97 ± 22	87 ± 21	88 ± 24	93 ± 26
Creatinine (mg/mL) (mean ± SD)	1.8 ± 1.6	NA	1.7 ± 1	1.8 ± 1.6	NA	1.5 ± 1.0
LVEF (%) (mean ± SD)	34.4 ± 16	38.0 ± 15	37 ± 13	39 ± 18	38 ± NA	38 ± 14
HFpEF (%) (LVEF cutoff)	46 (>40%)	34.3 (>45%)	34 (>40%)	51.2 (>40%)	36.1 (>40%)	35.0 (>40%)
IV therapy and intervention						
Diuretic (%)	NA	84.4	95.3	NA	84.6	99.0
Vasodilators (%)	21	38.7	51.3	14.3	18.5	29.9
Inotropes (%)	15	29.8	24.6	10.9	10.5	19.4

(continued)

Table 3 (continued)

	Adhere ($N = 105,388$) (Adams et al. 2005)	EHFS II ($N = 3,580$) (Nieminen et al. 2006)	Italian S ($N = 2,807$) (Tavazzi et al. 2006)	OPTIMIZE-HF ($N = 48,612$) (Abraham et al. 2008)	ESC-HF pilot ($N = 1,892$) (Maggioni et al. 2010)	IN-HF outcome ($N = 1,855$) (Tavazzi et al. 2013)
Outcome						
ICU admission (%)	18.7	50	69	NA	48	51.9
Length of stay (days)	4.3	9.0	9.0	5.7	NA	10
In hospital mortality (%)	4.0	9.0	7.3	3.8	3.8	6.4

SBP systolic blood pressure, *HR* heart rate, *LVEF* left ventricular ejection fraction, *ICU* intensive care unit

6% (2/3 for cardiovascular causes) in a population representative of the Western world with a proportion of NYHA III–IV patients of 17.9% and a median age of 71 years. Among the prognostic predictors of mortality, low systolic blood pressure and low body weight are confirmed as alarming signals in chronic HF patients, together with severe mitral regurgitation, anemia, renal dysfunction, and large QRS. High heart rate also emerges as an important marker of risk with a 2% increase in probability of dying within 1 year per one incremental heart beat.

7.2 ESC-HF Pilot and Long-Term Registries

The rate of chronic HF patients with moderate (NYHA class I–II) or severe HF (NYHA class III or IV) was 72% and 28%, respectively, of the total population of ambulatory patients. An ejection fraction value was available in 2,857 patients (89% of outpatients): the median ejection fraction was 36% (IQR 30-46), a preserved ejection fraction was reported in 36.1% of the cases. A hemoglobin level lower than 12 g/dL was reported in 18.8% of cases, an eGFR lower than 60 or lower than 30 mL/min/1.73m^2 was reported, respectively, in 40.7 and 5.1% of patients. NT-pro BNP or BNP was measured in a minority of cases (747 and 285 patients). Median values were 1387 (IQR 485–3381) and 390 pg/mL (IQR 133–870), respectively.

A blocker of the renin-angiotensin system, a beta-blocker, and an aldosterone blocker were prescribed, respectively, in 89, 87, and 44% of the cases. The combination of a renin-angiotensin system blocker, a beta-blocker, and an aldosterone blocker was prescribed in 35% of patients, while the association of a betablocker, an ACE inhibitor, and an angiotensin receptor blockers was reported in just 3% of the patients. Ramipril and enalapril were the most prescribed ACE inhibitors; the target dose of these drugs was used, respectively, in 38.2 and 46.2% of the cases. With respect to angiotensin receptor blockers, the target dose of candesartan, losartan, and valsartan was reached in 28.0, 19.7, and 16.7% in the cases. The target dosage of carvedilol, bisoprolol, and metoprolol was reached in 37.3, 20.7, and 21.4% of patients, while target dosage of spironolactone, canrenone, or eplerenone was prescribed in 22.2, 61.3, and 32.7% of patients.

The all-cause mortality rate at 1 year of chronic stable HF patients was 7.2%. Patients in NYHA classes III–IV showed a much higher mortality than in those in class I–II (13.5% vs. 4.8%, $p < 0.0001$). Of the 233 patients with chronic HF who died at 1 year, 54.5% of deaths were due to cardiovascular reasons, 16.3% to non-cardiovascular reasons, and in 29.2% of cases the cause of death remained unknown. Among the cardiac causes of death, sudden death occurred in 40.2% of the cases.

The 1-year admission rate was 31.9%, 24.0% for cardiovascular reasons, and 11.4% for non-cardiovascular reasons. Of all admissions, 41.7% was due to HF.

8 Conclusions

Patients with HF remain a major health-care problem. Prevalence and incidence are still increasing due to the aging of the population and the improvement of care in acute cardiovascular conditions. Regarding patients' outcomes, while some improvement was observed in patients with chronic HFrEF, due to the favorable results of several trials on drugs and devices, no substantial changes were observed in patients with chronic HFpEF, even if the outcomes of these patients are generally more favorable. The risk of early and long-term death of patients hospitalized for acute HF is still unacceptably high, and for this reason, studies on new therapeutic approaches are necessary.

Disclosure Statement The authors have nothing to disclose.

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Comorbidities in Heart Failure

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2017_27

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Abstract

Comorbidities frequently accompany chronic heart failure (HF), contributing to increased morbidity and mortality, and an impaired quality of life. We describe the prevalence of several high-impact comorbidities in chronic HF patients and their impact on morbidity and mortality. Furthermore, we try to explain the underlying pathophysiological processes and the complex interaction between chronic HF and specific comorbidities. Although common risk factors are likely to contribute, it is reasonable to believe that factors associated with HF might cause other comorbidities and vice versa. Potential factors are inflammation, neurohormonal activation, and hemodynamic changes.

Keywords

Anemia • Cognitive dysfunction • Comorbidities • Diabetes mellitus • Heart failure • Hematinic deficiencies • Hyperkalemia • Iron deficiency • Renal failure • Sleep apnea • Sleep-disordered breathing

1 Introduction

Comorbidities frequently accompany chronic heart failure (HF), contributing to increased morbidity and mortality, and an impaired quality of life. The detrimental consequences of comorbidities in HF patients have been acknowledged for several years. We describe the prevalence of several high-impact comorbidities in chronic HF patients being higher compared to age-matched controls. We specifically focused on the impact of comorbidities on survival as well as pathophysiologic processes underlying the interaction between HF and comorbidities, which are

complex and remain largely unknown. Although common risk factors are likely to contribute, it is reasonable to believe that factors associated with HF might cause other comorbidities. Potential factors are inflammation, neurohumoral activation, and hemodynamic changes.

Although chronic heart failure (HF) is primarily a cardiac problem, its pathophysiology also has characteristics of systemic and metabolic derangements. By means of a discrepancy between cardiac function and systemic metabolic requirements, virtually every chronic HF patient has at least one comorbidity. For example, van Deursen et al. showed that renal dysfunction, anemia, diabetes mellitus, and COPD are particularly prevalent in chronic HF patients (van Deursen et al. 2014).

In the next part, several high-impact comorbidities will be discussed systematically. For different reasons, these comorbidities are of significant importance. First, comorbidities may lead to worsening of HF symptoms and quality of life (e.g., iron deficiency, anemia, sleep-disordered breathing, renal dysfunction, and cognitive dysfunction). Second, comorbidities might inhibit optimal HF treatment (e.g., contraindication of beta-blocking therapy in patients with asthma or hyperkalemia for uptitration of RAAS inhibitors). Third, some comorbidities might constitute a therapeutic target with possible beneficial effects on HF-related symptoms and health-related quality of life (e.g., iron deficiency and diabetes mellitus). Fourth, HF-targeting therapy and comorbidity-targeting therapy leads to polypharmacy and might interact with each other (e.g., beta-blockers for HF and beta-adrenergic agonists for respiratory diseases). Finally, as many comorbidities are an exclusion criterion in many trials on HF treatment, less data on the safety and efficacy of HF treatment in chronic HF patients with comorbidities is available (Ponikowski et al. 2016). We chose to discuss the following comorbidities as of their high prevalence, significant clinical and prognostic consequences, and novel data on therapeutic strategies: anemia, cognitive dysfunction, chronic obstructive pulmonary disease, diabetes mellitus, hypercholesterolemia, iron deficiency, potassium disorders, renal dysfunction, and sleep-disordered breathing.

2 Anemia

2.1 Epidemiology

Anemia has been acknowledged as a significant comorbidity during the past decade. The most commonly used definition for the diagnosis of anemia is provided by the World Health Organization criteria (i.e., Hb <13 g/dL in men and <12 g/dL in women). Due to definition differences, prevalence indices vary between 4 and 61% (Tang and Katz 2006; Groenveld et al. 2008). Anemia in chronic HF patients is independently associated with impaired cardiac function, worse clinical state, and increased mortality. Klip et al. recently showed that the association between Hb levels and the risk of new-onset HF in a general Dutch population is U-shaped, indicating that also higher

Hb levels are associated with an increase in HF incidence. Only severe anemia was associated with an increased HF incidence (Klip et al. 2015).

2.2 Pathophysiology

The pathophysiology of anemia in chronic HF is multifactorial in most patients. Iron deficiency (both absolute and functional) is a significant contributor to the development of anemia, with a prevalence of ~50% in chronic HF patients. Iron deficiency and its treatment will be discussed in a separate paragraph. Other major etiological factors related to anemia comprise hemodilution due to fluid retention in decompensated HF patients (Westenbrink et al. 2007), renal dysfunction with a consequent decrease in erythropoietin (EPO) synthesis (van der Meer et al. 2005), and bone marrow dysfunction due to endogenous EPO resistance (Westenbrink et al. 2010).

2.3 Treatment

Erythropoiesis-stimulating agents (ESA) have successfully been used for the treatment of anemia in patients with chronic kidney disease and can be used in a selected group of patients after correctable causes of anemia have been addressed (Work Group Membership 2012). ESA therapy for anemic chronic HF patients has been evaluated in several small studies with varying effects on outcome (Silverberg et al. 2000, 2001; van der Meer et al. 2004; van Veldhuisen et al. 2007; Ponikowski et al. 2007). The largest randomized clinical trial assessing ESA therapy in chronic HF patients is the "Reduction of Events by Darbepoetin alfa in Heart Failure" (RED-HF). More than 2000 anemic chronic HFrEF patients received either darbepoetin alfa (ESA) or placebo. Despite a significant increase in hemoglobin levels in the darbepoetin alfa group (median levels 13.0 g/dL [12.4–13.4] versus 11.5 [10.7–12.2]; $P < 0.001$), more thromboembolic adverse events were observed in the darbepoetin alfa group versus placebo (13.5% versus 10.0%; $P = 0.01$) (Swedberg et al. 2013).

2.3.1 Future Perspectives

New therapeutic strategies for the treatment of anemia are currently being explored, as ESA therapy seems harmful in chronic HF patients. These strategies comprise EPO receptor targeting agents (gene therapy, fusion proteins, mimetic peptides, and receptor antibodies) and activin traps (involved in the regulation of erythropoiesis) (Bonomini et al. 2015). All agents, except for fusion proteins, have been shown to increase Hb levels. Moreover, sotatercept (activin trap) additionally inhibits hepcidin expression, making this drugs particularly interesting for the treatment of ID in patients with chronic low-grade inflammation, such as in HF (Bonomini et al. 2015; Finberg et al. 2010). On the other hand, mimetic peptides seemed an interesting drug group, until one agent (peginesatide) showed harmful effects in patients with chronic kidney disease and patients with EPO-related pure red cell

aplasia (Macdougall et al. 2013; Affymax and Takeda Announce a Nationwide Voluntary Recall of All Lots of OMONTYS® (peginesatide) Injection 2013). The most promising agents so far seem hypoxia-inducible factor (HIF) stabilizers. This drug type is able to upregulate hypoxia-induced pathways, among others EPO, thereby increasing Hb levels (Semenza et al. 1997). Therefore, treatment with HIF stabilizers can be considered as hypoxia mimicking. Attractive advantages of this strategy compared to ESA therapy are oral administration, more stable EPO level increase and possible improvement of iron metabolism (Ganz 2013). Currently, the safety and efficacy of several HIF stabilizers are being evaluated in phase-2 and -3 studies. For example, roxadustat (FG-4592) has been evaluated in 144 anemic end-stage renal disease patients, showing beneficial effects on Hb, cholesterol and hepcidin levels compared to epoetin alfa after a 19-week treatment with roxadustat (Provenzano et al. 2016). Other prominent HIF stabilizers are molidustat and daprodustat (Schmid and Jelkmann 2016). Because of their pleiotropic effects, HIF stabilizers interfere with numerous metabolic pathways (e.g., glucose and lipid metabolism), which might increase the risk of off-target and adverse side effects. Until now, HIF stabilizers have not been evaluated in chronic HF patients, nor have long-term safety and efficacy been addressed. Therefore, there is currently no place for these agents for the treatment of anemia in chronic HF patients.

2.4 Conclusion

Unfortunately, treatment of anemia using ESA therapy is associated with neutral prognostic consequences and higher risk of adverse thromboembolic events. This therapy is therefore currently not recommended in anemic HF patients. Novel agents are intervening at EPO-regulating pathways, thereby increasing endogenous EPO levels. Whether these promising new agents are safe and effective in chronic HF patients is currently unclear. Until such data are available, current therapy of anemia should focus on the underlying cause of anemia (e.g., ID).

3 Cognitive Dysfunction

3.1 Epidemiology

Up to half of all chronic HF patients have cognitive dysfunction, varying from mild cognitive impairment to vascular dementia and Alzheimer's disease, which are generally poorly recognized by physicians (Vogels et al. 2007; Pressler 2008; van den Hurk et al. 2011; Dodson et al. 2013). Unfortunately, a clear definition of cognitive dysfunction is currently lacking, causing a broad range of prevalence indices. Cognitive function is usually assessed using nonspecific questionnaires such as the Mini-Mental State Examination or the Montreal Cognitive Assessment.

3.2 Pathophysiology

Chronic HF seems a strong risk factor for the development of cognitive dysfunction with a correlation between HF severity and the degree of cognitive dysfunction (Cohen and Mather 2007). Important risk factors for the development of cognitive dysfunction in chronic HF patients are atrial fibrillation, obesity, COPD, depression, age, renal failure, and reduced exercise tolerance (Chong et al. 2015). The etiology of cognitive dysfunction in chronic HF is largely unknown, as only few studies have performed systematical cognitive tests in these patients. Several hypotheses for cerebral and cognitive dysfunction have been proposed, including ischemia due to cerebrovascular disease and cardiac thromboembolism (Pullicino et al. 2001). Another hypothesis is cerebral hypoperfusion, as significant regional blood flow abnormalities were observed in chronic HF patients, which might be a risk factor for the development of cognitive dysfunction (Choi et al. 2006; Roher et al. 2012). However, it should be noted that the brain is relatively protected by strongly developed autoregulatory mechanisms. Hypothetically, with further deterioration in cerebral blood flow, autoregulation will eventually fail in patients with severe chronic HF, resulting in the development of cerebral dysfunction. This hypothesis was evaluated in a recent study by Erkelens et al., focusing on symptomatic chronic HF patients. This study included 15 chronic HF patients and 15 healthy controls. All participants underwent full cardiologic examination including LVEF measurement, MRI scanning to assess cerebral blood flow using arterial spin labeling, cerebrovascular function testing focusing on cerebral dynamic autoregulation, and full neuropsychological testing. No difference in global and regional cerebral perfusion was observed between chronic HF patients and healthy controls (44.3 mL/100 g brain tissue/min versus 42.1; $P = 0.68$), although basilar blood flow was impaired in chronic HF patients (1.95 mL/s versus 2.51 mL/s; $P = 0.009$). More interestingly, cerebral autoregulation, regional cerebral oxygen saturation, and vasomotor reactivity were significantly reduced in chronic HF patients (all $P < 0.05$), whereas no differences in overall cognitive function were observed (Erkelens et al. 2016). This mechanism might be comparable to the autoregulatory mechanisms of the kidneys, which are able to maintain glomerular filtration rate over a broad range of systemic arterial pressure (Damman et al. 2007, 2009). In progressive chronic HF, these mechanisms may fail ultimately and renal dysfunction may occur (i.e., cardiorenal syndrome). The study by Erkelens et al. suggests that cerebral autoregulation is already impaired in mild chronic HF. Future studies should focus on cerebral perfusion and autoregulation in more severe chronic HF patients.

As cognitive dysfunction seems severely underdiagnosed in chronic HF patients, systematic screening for this comorbidity is of significant importance to detect this comorbidity. Chronic HF patients with cognitive dysfunction show an increased hospitalization rates, reduced therapy adherence, and physical inactivity, further deteriorating prognosis.

3.3 Conclusion

Cognitive dysfunction varies from mild cognitive impairment to vascular dementia and Alzheimer's disease and this whole spectrum is present in almost half of all chronic HF patients. Cognitive dysfunction is highly underrecognized due to varying presentation and infrequent screening of cognitive function. While cerebrovascular disease and thromboembolic events are obvious etiological factors for the development of cognitive dysfunction, cerebral hypoperfusion has been studied less thoroughly in chronic HF. Recent data suggest that cerebral perfusion is preserved in mild chronic HF, but with already impaired cerebral autoregulation. This mechanism is comparable to the autoregulation of renal perfusion. Whether this mechanism and preservation of cerebral perfusion is also true for more severe HF is currently unclear.

4 Chronic Obstructive Pulmonary Disease

4.1 Epidemiology

Chronic obstructive pulmonary disease (COPD) and HF are likely to occur simultaneously. The diagnosis of the individual diseases is challenging, as both diseases can result in symptoms of obstructive and restrictive pulmonary abnormalities, together with muscular changes (Gosker et al. 2000). Up to 50% of all chronic HF patients have COPD, which is up to seven times higher compared to the general population (van Deursen et al. 2014; Hawkins et al. 2010). Prevalence indices are frequently based on self-reported COPD, whereas spirometry testing would be desirable. Not even half of all HF patients meeting the spirometric criteria of COPD self-reported the presence of COPD, emphasizing the need for more structured screening for this comorbidity. Unfortunately, spirometry testing is not always conclusive in chronic HF patients due to pulmonary congestion, especially in unstable patients, making the diagnosis even more challenging (Hawkins et al. 2009; Brenner et al. 2013; Guder et al. 2014; Iversen et al. 2010).

4.2 Pathophysiology

Due to its high prevalence in chronic HF patients, COPD and HF are likely to have common risk factors. Possible pathophysiological mechanisms are smoking, chronic low-grade inflammation, hypoxia, oxidative stress, systemic inflammation, and sympathetic nerve system dysfunction. Moreover, reduced long volume due to cardiomegaly, pulmonary edema, interstitial fibrosis, and respiratory muscle weakness are also likely pathophysiological and common determinants of both diseases (Hawkins et al. 2013; Wouters 2005; Dimopoulou et al. 1998). Furthermore, HF might be caused or worsened by an increase in pulmonary blood pressure, as observed in patients with COPD (Sabit et al. 2010).

4.3 Treatment

Although beta-blockers are contraindicated for some pulmonary diseases, beta-blockers can be used in patients with HF and COPD with a preference for selective beta-blockers (e.g., nebivolol, bisoprolol, and metoprolol). The long-term safety and prognostic consequences of inhaled bronchodilators and corticosteroids in HF patients with COPD are currently unclear. While oral corticosteroids may cause sodium and water retention with possible adverse consequences in HF patients, inhaled corticosteroids are not likely to have these consequences (Ponikowski et al. 2016). COPD has significant prognostic consequences in chronic HF patients with a clear and independent prognostic value of spirometry parameters, making the diagnosis of COPD using spirometry even more important (van Deursen et al. 2014; Iversen et al. 2010).

4.4 Conclusion

Chronic HF and COPD are likely to occur simultaneously and have common pathophysiological mechanisms. Prevalence indices vary widely due to suboptimal screening for this comorbidity. Even if adequate spirometric screening is performed, results are not always conclusive in chronic HF patients. Although beta-blockers are relatively contraindicated in some pulmonary diseases, selective beta-blockers can be used in chronic HF patients with COPD. While systemic corticosteroids may cause sodium and water retention and thereby possibly aggravating HF symptoms, inhaled corticosteroids seem safe to use in chronic HF patients.

5 Diabetes Mellitus

5.1 Epidemiology

Diabetes mellitus (DM) is present in up to half of all chronic HF patients (van Deursen et al. 2014; Mentz et al. 2014). There are only small differences in the prevalence of DM between HFpEF and HFrEF patients. Van Deursen et al. report a 28% prevalence for HFpEF and 30% for HFrEF patients, whereas Mentz et al. show slightly higher prevalence indices (45% for HFpEF versus 40% for HFrEF patients) (van Deursen et al. 2014; Mentz et al. 2014).

5.2 Pathophysiology

While chronic HF patients with DM seem to have an increased risk of short-term rehospitalization and short-term mortality (especially in patients with ischemic heart disease), the long-term prognostic consequences of DM are less clear (Sarma et al. 2013; Parissis et al. 2012; MacDonald et al. 2008; De Groote et al.

2004; Harjola et al. 2010; Greenberg et al. 2007). Pathophysiological consequences of DM are mainly based on glycemic dysregulation and comprise mitochondrial dysfunction, (micro)vascular disease, and oxidative stress (Lam 2015; Seferovic and Paulus 2015). It is believed that a bidirectional association exists between DM and HF, as HF also predisposes to the development of DM (Amato et al. 1997; Tenenbaum et al. 2003).

5.3 Treatment

Although numerous traditional and novel drugs are available for the treatment of diabetes mellitus, only few have been evaluated in HF populations. An overview of traditional and novel glucose-lowering agents has recently been provided by van der Wal et al. (2016). In the following paragraphs, we will mention several glucose-lowering agents together with their cardiovascular consequences. While the optimal treatment for diabetic HF patients is currently unclear, only metformin therapy is currently recommended as first-line treatment in recent HF treatment guidelines, as this drug is safe and effective in chronic HF populations (Ponikowski et al. 2016; MacDonald et al. 2010). Thiazolidinediones show increased risk of adverse HF-related events and are contraindicated in chronic HF patients (Komajda et al. 2010). Although observational data show increased risk of HF development compared to other glucose-lowering agents, a randomized clinical trial focusing on insulin therapy in patients with cardiovascular risk factors showed neutral results with respect to cardiovascular mortality and HF hospitalization (Gerstein et al. 2012; Nichols et al. 2005; Karter et al. 2005).

5.3.1 Dipeptidyl Peptidase 4 (DPP-4) Inhibitors

By inhibiting incretin hydrolysis (glucagon-like peptide 1 and gastric inhibitory polypeptide), DPP-4 inhibitors are able to increase endogenous insulin synthesis and decrease glucagon release. DPP-4 inhibitors have not been studied specifically in HF populations and the long-term prognostic consequences of these agents are currently unknown. However, DPP-4 inhibitors and their cardiovascular effects have been evaluated in diabetic subjects with and without established cardiovascular disease. Current data on the cardiovascular effects of DPP-4 inhibitors are conflicting. Recent meta-analyses including randomized clinical trials showed that some DPP-4 inhibitors (saxagliptin) may be related to HF-related events, whereas other DPP-4 inhibitors (vildagliptin, linagliptin, alogliptin, and sitagliptin) showed more neutral results regarding cardiovascular safety (Li et al. 2016; Kongwatcharapong et al. 2016). The uncertainty regarding DPP-4 inhibitor use for the treatment of DM in HF patients makes this drug group less attractive for this patient group.

5.3.2 Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists

GLP-1 receptor agonists stimulate endogenous insulin release, while glucagon secretion is inhibited. Although several GLP-1 receptor agonists are currently

available now, studies on the cardiovascular effects of these agents are scarce. In a large randomized, double blind trial with more than 9,000 diabetic patients and established cardiovascular disease, the cardiovascular effects of the GLP-1 agonist liraglutide were assessed. The primary outcome was the first occurrence of either death from cardiovascular cause, nonfatal myocardial infarction or nonfatal stroke. Patients receiving liraglutide had less cardiovascular events compared to the placebo group (13.0% versus 14.9%; hazard ratio, 0.87; 95% CI, 0.78–0.97; $P < 0.001$ for superiority). Both all-cause death rate and cardiovascular death rate were lower in the liraglutide group, whereas HF rehospitalization rate was not significantly lower (Marso et al. 2016a). The cardiac effects of liraglutide were evaluated very recently in a cohort of stable chronic HFrEF patients with and without diabetes mellitus. In this study, patients ($n = 241$) were randomized to either liraglutide 1.8 mg once daily or placebo. After 24 months of treatment, no significant change in LVEF between the treatment group and placebo group was observed. However, more importantly, heart rate was significantly higher in the liraglutide group [mean increase, 7 (5–9); $P < 0.0001$] and more serious cardiac events were observed in patients receiving liraglutide (10% versus 3%; $P = 0.04$) (Jorsal et al. 2017). Although left ventricular function was not affected by liraglutide, this study raises issues regarding the safety of liraglutide use in chronic HFrEF patients. Other trials on different GLP-1 receptor agonists (i.e., lixisenatide and semaglutide) showed more neutral and noninferior results on cardiovascular outcome (Pfeffer et al. 2015; Marso et al. 2016b). It is however important to note that these studies are designed as noninferiority studies with relatively short follow-up. Due to this design, current studies cannot be used to quantify beneficial cardiovascular consequences of GLP-1 receptor agonists (Chawla and Tandon 2017). Long-term studies focusing on beneficial cardiovascular effects should therefore be conducted before these agents should be used in chronic HF patients.

5.3.3 Sodium-Glucose Cotransporter 2 (SGLT2) Inhibitors

SGLT2 inhibitors promote glucosuria by inhibiting glucose reabsorption in the renal proximal tubules. Besides beneficial effects on serum glucose levels, SGLT2 inhibitors seem to have additional beneficial cardiovascular effects, among others increased diuresis, and blood pressure and weight reduction) (Zinman et al. 2015; Ferrannini et al. 2014). The cardiovascular effects and prognostic consequences of the SGLT2 inhibitor empagliflozin were evaluated recently in >7,000 diabetic patients with established cardiovascular disease in the EMPA-REG trial. Of all patients, 10.1% had HF at baseline. Patients received either empagliflozin (10 or 25 mg once daily) or placebo. The primary endpoint of the study (i.e., composite of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke) was observed in 10.5% of all patients receiving empagliflozin versus 12.1% in controls (HR, 0.86; 95% CI 0.74–0.99; P for superiority, 0.04) (Zinman et al. 2015). More interestingly, HF hospitalizations and cardiovascular mortality were significantly lower in the empagliflozin group compared to controls (5.7% versus 8.5%; HR, 0.66; 95% CI, 0.55–0.79; $P < 0.001$). These effects were consistent across patients with and without HF at baseline (Fitchett et al. 2016).

Limitations of this study were, among others, the lack of cardiac biomarkers (e.g., NT-proBNP) and LVEF. It is therefore currently not possible to determine the differential impact of empagliflozin on HF-related outcomes in HFrEF and HFpEF patients.

Empagliflozin is the first glucose-lowering agent able to improve HF-related outcome in diabetic patients. Trials on other SGLT2 inhibitors (i.e., dapagliflozin and canagliflozin) are currently ongoing. A recent meta-analysis on the cardiovascular outcomes of SGLT-2 inhibitors included 81 trials with >37,000 patients showed beneficial effects of this drug group on HF-related outcomes (i.e. all-cause mortality, cardiovascular mortality, and heart failure). However, these effects were only observed for empagliflozin, and dapagliflozin was linked to an increased cardiovascular mortality rate (OR, 2.15; 95% CI, 0.92–5.04; $P = 0.08$) (Saad et al. 2017). In another meta-analysis however, dapagliflozin was not associated with cardiovascular events (Tang et al. 2016). These ambiguous results make it even more important to study the long-term safety and efficacy of SGLT2 inhibitors in diabetic patients. Several large randomized clinical trials on the safety of SGLT2 inhibitors other than empagliflozin are currently conducted, of which the DECLARE-TIMI 58 trial (dapagliflozin versus placebo; NCT01730534) and the CREDENCE trial (canagliflozin versus placebo; NCT02065791), because of pre-specified adjudicated HF-related endpoints (HF rehospitalization).

5.4 Conclusion

While several new glucose-lowering agents have recently become available, metformin remains the first-line treatment in diabetic chronic HF patients. The safety and effectivity of new glucose-lowering agents in chronic HF patients is largely unclear. Thiazolidinediones are associated with worsening HF and HF rehospitalization and should therefore be avoided in chronic HF patients. Both DPP4 inhibitors and GLP1 receptor agonists are not evaluated in chronic HF populations, making these drug groups currently less attractive for these patients. Although the SGLT2 inhibitors empagliflozin seems to have beneficial effects in diabetic chronic HF patients, it is currently unclear whether this is true for other SGTL2 inhibitors. Current ongoing trials will provide effectivity and safety data on these and other new glucose-lowering agents.

6 Hypercholesterolemia

Although hypercholesterolemia is a well-known risk factor for cardiovascular disease, the prevalence of hypercholesterolemia in patients with HF is relatively low. Especially patients with low levels of low-density lipoprotein have a higher risk of death. Nonetheless, statins have several possibly beneficial properties (e.g., inhibition of platelet aggregation, improvement of myocardial remodeling and endothelial function, and anti-inflammatory, antihypertrophic and antifibrotic

effects) (Luo et al. 1999; Zheng et al. 2013; Jain and Ridker 2005; Brili et al. 2012), and are clearly effective in improving prognosis in patients with atherosclerosis. The effects of statins in HF are less clear and two randomized clinical trials assessing the prognostic effects of statins in HFrEF patients did not show any beneficial effects (Kjekshus et al. 2007; Tavazzi et al. 2008). However, in a substudy of one of these trials, repeated HF-hospitalization rate dropped significantly by 15–20% in the rosuvastatin group (Rogers et al. 2014). The association between statin use and clinical outcome of HFrEF and HFpEF patients has recently been elaborated by Alehagen et al. in two unselected propensity score matched cohort studies (21,864 and 9,140 subjects, respectively) (Alehagen et al. 2015a, b). In the HFrEF population, statin use was associated with reduced all-cause and cardiovascular 1-year mortality, and HF hospitalization (HR, 0.81; 95%-CI, 0.76–0.86; HR, 0.80; 95%-CI, 0.75–0.87; HR, 0.92; 95%-CI, 0.86–0.97; all $P < 0.005$, respectively) (Alehagen et al. 2015b). There was a strong interaction between statin use and the presence of ischemic heart disease (IHD) for all-cause mortality (HR IHD, 0.76; 95%-CI, 0.70–0.82; $P < 0.001$; HR no IHD, 0.95; 95%-CI, 0.85–1.07; $P < 0.430$). Comparable effects were observed in the HFpEF population (HR all-cause 1-year mortality, 0.80; 95%-CI, 0.72–0.89; HR cardiovascular death, 0.86; 95%-CI, 0.75–0.98; HR cardiovascular hospitalizations, 0.89; 95%-CI, 0.82–0.96; all $P < 0.05$) (Alehagen et al. 2015a). No interaction between statin use and IHD was observed in the HFpEF cohort. The proposed mechanism of the beneficial effects of statins in HFpEF is unclear, but may comprise an anti-inflammatory effect rather or in addition to a direct effect on atherosclerosis (Alehagen et al. 2015a). These data would definitely warrant a propensity matched meta-analysis or randomized clinical trials with more liberal inclusion, especially focusing on IHD and HFpEF, as the latter has been barely studied in earlier trials (<10% of study population in the GISSI-HF trial) (Tavazzi et al. 2008). Currently, HF guidelines do not recommend initiation of treatment with statins in symptomatic HFrEF patients, as statins have no clear benefits in these patients. Continuation of statin therapy can be considered in HF patients who already receive statins and experience no significant side effects. There are no recommendations for HFpEF patients (Ponikowski et al. 2016; Yancy et al. 2013). However, in view of the possible beneficial effects of statins in HFpEF patients, statin use should be studied more thoroughly in these patients, especially when cardiovascular risk factors are present (diabetes mellitus, IHD).

6.1 Conclusion

Hypercholesterolemia is an important risk factor for cardiovascular disease. However, the prevalence of hypercholesterolemia is relatively low in chronic HF patients. Although randomized trials on statin therapy in chronic HF did not show any beneficial effects, observational data in unselected patient cohorts showed a reduced mortality risk in HF patients receiving statin therapy, especially in patients

with ischemic heart disease. These findings should definitely be studied more thoroughly in patients with HFpEF, as this patient group has barely been studied.

7 Iron Deficiency

7.1 Epidemiology

Iron deficiency (ID) is very prevalent in chronic HF patients. Depending on the definition, ID is present in up to half of all chronic HF patients, even in patients without anemia (Cohen-Solal et al. 2014). Although bone marrow staining on iron is the gold standard for evaluating iron status, the definition of ID is based on circulating markers reflecting iron status (i.e., serum iron, ferritin, and transferrin saturation) (Jankowska et al. 2013a). However, these markers have some significant disadvantages in clinical practice. For example, besides its role as iron storage protein, ferritin is also an acute phase protein. In chronic HF patients, circulating ferritin levels might be higher due to chronic low-grade inflammation. On the other hand, transferrin saturation can be artificially increased due to impaired hepatic transferrin synthesis when cardiac decompensation is present (Jankowska et al. 2013a). As long as more accurate definitions of ID for HF patients are lacking, ID is diagnosed based on the definition used in several randomized clinical trials focusing on intravenous iron therapy in HF patients (i.e., serum ferritin <100 μg/L, or serum ferritin 100–300 μg/L with transferrin saturation $<20\%$) (Anker et al. 2009; Ponikowski et al. 2015).

7.2 Pathophysiology

Two types of ID can be distinguished: absolute and functional ID. In absolute ID, body iron stores are depleted, thereby impairing hemoglobin synthesis and eventually causing anemia. Common causes of absolute ID are drug interactions, poor dietary iron intake, gastro-intestinal malabsorption, and gastro-intestinal blood loss. Functional ID is characterized by unavailability of iron for cellular processes with sufficient iron stores. In functional ID, iron is trapped inside the reticuloendothelial system, making it unavailable for cellular and metabolic processes. Functional ID is associated with pro-inflammatory cytokines, including hepcidin, which traps iron in the reticuloendothelial system and prohibits dietary iron uptake (Jankowska et al. 2013a). Numerous studies have elaborated on the role of ID (both with and without anemia) in chronic HF patients, showing a strong and independent association between ID and a broad range of prognostic and surrogate endpoints (i.e., exercise tolerance, and health-related quality of life) (Klip et al. 2013; Enjuanes et al. 2014; Jankowska et al. 2010, 2011, 2014; Klip et al. 2014; Comin-Colet et al. 2013). For example, Klip et al. showed that ID is a strong predictor of all-cause mortality in chronic HF patients, independent of anemia, left ventricular function, and HF severity (HR, 1.42; 95% CI 1.14–1.77; $P = 0.002$) (Klip et al. 2013).

7.3 Treatment

7.3.1 Intravenous Iron Therapy

Intravenous iron therapy for the treatment of ID in chronic HF patients has been evaluated in several randomized clinical trials. Of these, the Ferinject Assessment in Patients with Iron Deficiency and Chronic HF (FAIR-HF) trial was the first to assess whether intravenous iron therapy would lead to improvement of symptoms in these patients. In this study, 459 chronic HF patients were randomly assigned to either ferric carboxymaltose or placebo. Patients receiving intravenous iron had significantly less symptoms, and improvement in NYHA functional class and quality of life. This improvement was independent of baseline Hb levels (Anker et al. 2009). The 1-year effects of intravenous iron therapy were further elaborated in the CONFIRM-HF trial, comprising 304 symptomatic HFrEF patients with ID. Significant improvements in six-minute walking test distance, NYHA functional class, and health-related quality of life were observed in the intravenous iron arm. Although not powered for this endpoint, the risk of HF rehospitalization was significantly reduced in the intravenous iron group (HR, 0.39; 95% CI, 0.19–0.82; $P = 0.009$). Finally, the results of the Effect of Ferric Carboxymaltose on Exercise Capacity in Patients With Iron Deficiency and Chronic Heart Failure (EFFECT-HF) have been published only very recently. This study enrolled 174 stable, symptomatic chronic HF patients, who randomly received either ferric carboxymaltose or standard care. After 24 weeks, exercise capacity (i.e., change in peak VO_2) improved significantly in patients receiving intravenous iron therapy compared to standard care (Van Veldhuisen et al. 2016).

Although intravenous iron supplementation showed some promising clinical and biochemical improvements in chronic HF patients, larger and longer trials with more clinical endpoints (such as HF hospitalizations) are needed to elaborate on the definite role of intravenous iron in the treatment of ID in chronic HF patients. Until such trials have been performed, intravenous iron therapy should at least be considered in iron-deficient HF patients to improve HF-related symptoms, quality of life, and exercise tolerance (Ponikowski et al. 2016). Whether intravenous iron supplements have also beneficial effects on objective endpoints (i.e., all-cause mortality, cardiovascular-related mortality, HF hospitalizations) is currently unknown. The Intravenous Iron Treatment in Patients with Heart Failure and Iron Deficiency (IRONMAN) trial will hopefully provide more insight in these endpoints (NCT02642562). This randomized, open-label trial aims to enroll 1,300 chronic HF patients, which will receive either iron (III) isomaltoside or standard care. The primary outcome measure of this study is a composite of cardiovascular mortality and HF (re)hospitalization. This study with long-term follow-up (2.5 years) only includes HFrEF patients (i.e., LVEF <45%) and is estimated to be completed in the first quarter of 2021. A second future study will focus on the prognostic consequences (i.e., composite of cardiovascular mortality and HF rehospitalizations) of ferric carboxymaltose in patients admitted for acute HF (Study to Compare Ferric Carboxymaltose With Placebo in Patients With Acute Heart Failure and Iron Deficiency; Affirm-AHF; NCT02937454). This study aims

to enroll 1,100 iron-deficient HF patients with an LVEF <50% and iron deficiency, admitted for acute HF. Patients will randomly receive either ferric carboxymaltose or saline. The estimated study completion date is June 2019.

7.3.2 Oral Iron Therapy

Although oral iron therapy is readily available, inexpensive, and does not require in-hospital administration, very little data regarding the clinical and biochemical effects of oral iron therapy in chronic HF are available. Only one clinical trial prospectively assessed the clinical and biochemical effects of oral iron compared to intravenous iron. This study One retrospective study comprising 105 chronic HFrEF patients receiving oral iron therapy showed a significant increase in hemoglobin levels, TSAT and serum ferritin levels after a median treatment duration of 164 days. However, the increase in median serum ferritin level was not clinically relevant (40–72 μg/L; $P < 0.0001$) and definitely not comparable to the biochemical effects observed in the intravenous iron trials (Niehaus et al. 2015). Nevertheless, the authors suggested that oral iron supplementation might be useful for the treatment of ID in HFrEF patients. This hypothesis was further tested in the "Oral Iron Repletion Effects on Oxygen UpTake in Heart Failure" (IRONOUT-HF) trial (Lewis et al. 2016). This randomized, double-blind, placebo-controlled clinical trial included 225 iron-deficient HFrEF patients, who randomly received either oral iron polysaccharide (150 mg twice daily) or placebo for 16 weeks. Both clinical and biochemical effects of oral iron therapy were assessed. Iron parameters (serum ferritin levels, TSAT) only marginally increased in patients receiving oral iron. This minor improvement in iron status did not show any effects on the primary study endpoint (exercise capacity, measured as peak oxygen uptake) and health-related quality of life (unpublished data). Very interestingly, high hepcidin levels were correlated to poor repletion effects of oral iron. As chronic HF is frequently accompanied by chronic low-grade inflammation and thereby increased pro-inflammatory cytokine and hepcidin levels (Cohen-Solal et al. 2014; Jankowska et al. 2013b), oral iron therapy might be less attractive compared to intravenous iron therapy in HF patients with high hepcidin levels.

7.4 Conclusion

Iron deficiency is comorbidity of significant importance in chronic HF due to its high prevalence and detrimental prognostic consequences, independent of anemia. Whereas therapy with intravenous iron improves exercise capacity, HF-related symptoms and health-related quality of life, the prognostic consequences of intravenous iron therapy still need to be elucidated. Until such endpoints have been studied, intravenous iron therapy should be considered in symptomatic HFrEF patients with ID. Compared to intravenous iron therapy, oral iron therapy might be less attractive in chronic HF patients.

8 Potassium Disorders

8.1 Pathophysiology

Although data on the prevalence of either hypo- or hyperkalemia are scarce, potassium disorders can have serious consequences in chronic HF patients. Both hypo- and hyperkalemia can have pro-arrhythmogenic effects varying from ventricular fibrillation to asystole. Potassium disorders are associated with the use of HF-targeting drugs (Anker et al. 2015). Thiazide and loop diuretics have the ability to induce hypokalemia, whereas treatment with angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, and mineralocorticoids (RAAS inhibitors) might cause hyperkalemia, possibly inhibiting optimal uptitration of these drugs. Only a minority of HF patients receive these drugs at their target dose (Epstein et al. 2015; Albert et al. 2009; Maggioni et al. 2013).

8.2 Treatment

Currently, there is no evidence for acute correction of a hyperkalemic state in emergency situations with respect to clinically significant outcomes (Mahoney et al. 2005). However, long-term and chronic lowering of potassium levels might have beneficial effects on RAAS inhibitor uptitration by preventing recurrent hyperkalemia. Two potassium-binding agents (patiromer and sodium zirconium cyclosilicate) have recently become available (Packham et al. 2015; Weir et al. 2015). Both agents have also been evaluated in HF patients.

8.2.1 Patiromer

Patiromer was evaluated in 105 normokalemic chronic HF patients with an indication for spironolactone treatment (PEARL-HF trial). Patients randomly received either patiromer (15 mg twice daily) or placebo. After 28 days of treatment, patients receiving patiromer had significantly lower potassium levels compared to the control group (between group difference, −0.45; $P < 0.001$). More interestingly, patients in the patiromer group were more likely to receive higher spironolactone doses compared to placebo (uptitration rate from 25 to 50 mg, 91% versus 74%, $P = 0.019$). In general, patiromer was well tolerated with no serious adverse events (Pitt et al. 2011). These results were comparable to a substudy of the OPAL-HF trial, focusing on 102 HF patients with renal insufficiency and receiving RAAS-inhibiting therapy. After an initiation phase of 4 weeks, patients were randomized either to continuation of patiromer therapy or to withdrawal (placebo). In this randomized withdrawal phase, recurrent hyperkalemia was observed in 8% of all patients who continued patiromer and in 52% of patients receiving placebo. Again, patiromer was generally well tolerated with only mild-to-moderate gastrointestinal side effects (Pitt et al. 2015).

8.2.2 Sodium Zirconium Cyclosilicate

Sodium zirconium cyclosilicate (ZS-9) was evaluated for the treatment of hyperkalemia in 94 HF patients receiving RAAS-inhibiting therapy from the HARMONIZE trial (Kosiborod et al. 2014). All patients received open-label ZS-9 for 48 h, which significantly lowered serum potassium levels (from 5.6 mmol/L; 95% CI, 5.5–5.7; to 4.4 mmol/L; 95% CI, 4.3–4.5). Patients who were normokalemic after 48 of ZS-9 treatment were randomized between ZS-9 (5, 10, or 15 g once daily) or placebo. Patients receiving ZS-9 showed persistent normokalemia more frequently compared to placebo (40% for placebo versus 83, 89, and 92% for different ZS-9 doses), despite continuation of RAAS-inhibiting therapy. Although no treatment-related serious adverse events were observed, edema was more frequently observed in the high-dose ZS-9 group (Anker et al. 2015).

8.3 Conclusion

As hyperkalemia inhibits optimal uptitration of RAAS-inhibiting therapy, long-term and chronic lowering of potassium levels might have beneficial effects on uptitration of these drugs. Recently, two potassium-binding agents have become available, which significantly reduce serum potassium levels and maintain normokalemia. Additionally, patiromer allows HF patients to receive higher spironolactone doses. However, these drugs have only been studied in short-term trials. It is conceivable that potassium-lowering therapy might be used chronically to prevent recurrent hyperkalemia. Although both patiromer and ZS-9 show promising results in chronic HF patients, future trials should assess the long-term effectivity and safety of these two potassium-lowering agents, especially focusing on the uptitration of RAAS-inhibiting therapy in patients receiving these agents and, consequently, the effects on prognosis.

9 Renal Dysfunction

9.1 Epidemiology

Renal dysfunction is the most important comorbidity in chronic HF, both in terms of prevalence and its association with clinical outcome (van Deursen et al. 2014; Ponikowski et al. 2016; Damman et al. 2014). Moreover, chronic HF and renal dysfunction have many risk factors in common (e.g., diabetes mellitus, dyslipidemia, and hypertension). The strong relationship between chronic HF and renal dysfunction has been called the cardiorenal syndrome (Shlipak and Massie 2004). A meta-analysis by Damman et al. studied more than 1.1 million HF patients with either chronic kidney disease (CKD) or worsening renal function (WRF). CKD was present in almost a third of all patients (32%), whereas WRF was observed in almost a quarter (23%). Both CKD and WRF were independently associated with

all-cause mortality (Damman et al. 2014). The prevalence of renal dysfunction seems similar between HFpEF and HFrEF (van Deursen et al. 2014; Mentz et al. 2014). The current definition of renal dysfunction (or chronic kidney disease) is based on the estimated glomerular filtration rate and/or the presence of albuminuria (i.e., eGFR <60 mL/min/1.73 m^2). Worsening renal function is defined by an increase in serum creatinine level of 0.3 mg/dL and/or a 20% drop in eGFR (Ponikowski et al. 2016). It has been recognized that even a slight increase in serum creatinine levels is associated with worse outcome (Damman et al. 2014; Gottlieb et al. 2002).

9.2 Pathophysiology

The development of renal dysfunction in chronic HF patients is multifactorial. The role of reduced renal perfusion and venous congestion has been acknowledged for several decades. Renal function is protected over a wide range of renal blood flow by means of renal autoregulation. However, when renal perfusion drops below a certain level, GFR will eventually decrease despite maximum autoregulatory compensation (Smilde et al. 2009; Ljungman et al. 1990). On the other hand, the role of venous congestion and central venous pressure is more ambiguous, with evidence that both high and low central venous pressure are associated with worsening renal function, independent of renal perfusion (Damman and Testani 2015). Several other factors are associated or related to the cardiorenal syndrome, including neuroendocrine activation, RAAS-inhibiting therapy, chronic low-grade inflammation, and endothelial dysfunction (Damman and Testani 2015).

9.3 Implications of Renal Dysfunction

Unfortunately, no tailored evidence-based therapy is available for chronic HF patients with renal dysfunction. The presence of renal dysfunction can have significant consequences for HF treatment and the clinical status and prognosis of HF patients. For example, renal dysfunction is common when receiving RAAS-inhibiting therapy, which is used in a great majority of chronic HF patients. A recent meta-analysis by Beldhuis et al. demonstrated that in HFpEF patients, RAAS inhibitor-induced renal dysfunction is associated with worse prognosis, in contrast to HFrEF patients (Beldhuis et al. 2017). Discontinuation of RAAS-inhibiting therapy is however not advised, as there is usually a slight reduction in renal function when initiating and uptitrating RAAS inhibitors (Ponikowski et al. 2016). Second, diuretics might be less effective in chronic HF patients with renal dysfunction and higher diuretic doses are usually necessary (Ponikowski et al. 2016). Third, renal dysfunction might predispose to anemia due to insufficient EPO production, thereby increasing morbidity and mortality in HF patients even further (Westenbrink et al. 2007).

9.4 Conclusion

Renal dysfunction is a frequent occurring comorbidity in chronic HF patients with significant prognostic consequences. Important pathophysiological factors in the development of renal dysfunction include renal hypoperfusion and venous congestion. As no tailored evidence-based therapy for renal dysfunction in chronic HF is available, morbidity and mortality in HF patients with renal dysfunction remains significant.

10 Sleep-Disordered Breathing

10.1 Epidemiology

Sleep-disordered breathing (SDB) is one of the most common high-impact comorbidities in patients with chronic HF and is of emerging interest in this patient group. Depending on the definition, up to 70% of all chronic HF patients have SDB, compared to <1% in the general population (Lyons and Bradley 2015; Bradley and Floras 2003a; b; Arzt et al. 2016). The current definition for SDB is based on the number of apneas (i.e., complete cessation of breathing for ≥10 s) and hypopneas (i.e., ≥30% reduction in respiratory flow for ≥10 s, including ≥4% decrease in oxygen saturation) per hour (i.e., apnea-hypopnea index, AHI). Nevertheless, the prevalence depends on the degree of screening, due to overlapping symptoms with HF. In other words, without screening for SDB, clinicians might interpret SDB-related symptoms as being HF-related symptoms, due to the nonspecific clinical signs and symptoms of SDB. The gold standard diagnostic test for SDB is poly(somno)graphy. Due to its limited availability and relatively high costs, screening every chronic HF patient using this test is not feasible and desirable. A clinical risk score for the presence of SDB in chronic HF patients was proposed recently by Parisot et al. By combining age, gender, BMI, and NYHA functional class, this clinical risk score had a sensitivity and specificity of 78.9 and 61.5%, respectively, to predict the presence of SDB in these patients, with only fair discriminating capacity (area under the curve [AUC], 0.737; 95% CI, 0.663–0.810) (Parisot et al. 2015). Using this proposed risk score would imply that a significant number of SDB patients would be missed, and even more patients would be overscreened. Thus, a valid and easy-to-use screening tool is desirable. One such screening device (nasal airflow and pulse oximetry) was assessed in an observational study comprising 90 stable HF patients. Simultaneous standard home-based polysomnography and screening device analysis was performed in each patient. Using an AHI cutoff of 15 events per hour, sensitivity and specificity for the presence of SDB compared to polysomnography were 92.9 and 91.9%, respectively, and an excellent diagnostic accuracy (AUC, 0.94; 95% CI 0.89–1.00) (de Vries et al. 2015).

10.2 Pathophysiology

Each SDB subtype is characterized by its own pathophysiological mechanisms, although the phenotypes share common pathophysiological pathways and can be present simultaneously. For example, both obstructive (OSA) and central (CSA) are characterized by a proinflammatory state and an increased sympathetic tone (Lyons and Bradley 2015). SDB seems highly underdiagnosed in patients with chronic HF, as excessive daytime sleepiness is frequently absent and symptoms of chronic HF often resemble the clinical course of SDB (Javaheri et al. 1998; Oldenburg et al. 2007; Sin et al. 1999). Note that both OSA and CSA are frequently present at the same time (i.e., "mixed" SDB, either with predominantly central or obstructive apneic events).

OSA is caused by a combination of abnormalities in pharyngeal anatomy, pharyngeal function, and ventilatory control during sleep, causing recurrent upper airway collapse, compromised ventilation, oxygen desaturation, and arousal from sleep. Cardiac consequences of OSA comprise a decreased preload and increased afterload, caused by a more negative intrathoracic pressure observed in OSA patients, an increased sympathetic tone and adrenergic activity, intermittent hypoxemia, possibly leading to cardiac myocyte apoptosis and necrosis, and oxidative stress (Lyons and Bradley 2015; Bradley and Floras 2003a). Although OSA is considered a risk factor for both hypertension and cardiovascular disease (including HF), it is likely that OSA and HF have similar risk factors (Lyons and Bradley 2015; Bradley and Floras 2003a; Sin et al. 1999). Although all-cause mortality seems to be twofold increased in chronic HF patients with untreated OSA compared to no or mild OSA, the independent prognostic value of OSA in chronic HF patients is still unclear (Lyons and Bradley 2015).

On the other hand, the pathophysiology of CSA is more complex. CSA may be considered a consequence of chronic HF, by means of a deranged hemodynamic state and an altered respiratory response. In short, chronic HF is characterized by an increase in venous filling pressures due to fluid-retaining. Especially in supine position, this may lead to pulmonary congestion, which triggers a pulmonary irritant receptor reflex (Lyons and Bradley 2015). In most subjects with HF, this leads to chronic hyperventilation and, consequently, to chronic hypocapnia. Additionally, both central and peripheral chemosensitivity is increased in chronic HF, which further worsens chronic hypocapnia (Lyons and Bradley 2015; Bradley and Floras 2003b). Central apneic or hypopneic events will occur when the already low $PaCO_2$ further drops below the apneic threshold, for example due to trivial arousal from sleep. CSA is frequently characterized by a typical waxing-and-waning breathing pattern (Cheyne-Stokes respiration), which might be considered a protective response to cardiac dysfunction (Naughton 2012). Major risk factors for CSA include HFrEF, presence of atrial fibrillation, older age, and male sex. The clinical diagnosis of CSA in chronic HF patients is challenging, as most patients do not exhibit clear SDB-related symptoms. A very recent prospective, longitudinal community-based study in almost 3,000 male subjects showed that an increased

central apnea index and the presence of Cheyne-Stokes were significant predictors of new-onset HF and risk of decompensated HF (Javaheri et al. 2016).

10.3 Treatment

Currently, there are several pharmacological and non-pharmacological options for treatment of SDB, although non-pharmacological therapy has been studied more extensively. Multiple studies have demonstrated that optimization of HF therapy also significantly improves SDB severity (mainly CSA), including the use of beta-blockers, angiotensin-converting enzyme inhibitors, aldosterone antagonists, and cardiac resynchronization therapy (Lamba et al. 2011). SDB-specific treatment modalities are discussed in the next paragraphs.

10.3.1 Positive Airway Pressure Therapy

Continuous positive airway pressure (CPAP) therapy as a treatment modality for SDB (both OSA and CSA) in chronic HF has been evaluated in several clinical trials, of which the "Canadian Continuous Positive Airway Pressure for Patients with Central Sleep Apnea and Heart Failure" (CANPAP) trial is the largest. This randomized controlled clinical trial included 258 chronic HF patients with a reduced LVEF and severe CSA. Patients were assigned to either CPAP (including standard HF care) or standard HF care alone. After 3 months of treatment, significant improvement was observed with respect to SDB severity (i.e., AHI and oxygen saturation), catecholamine levels, LVEF, and six-minute walking test. CPAP therapy did not affect survival and hospitalization rates (Bradley et al. 2005). Interestingly, heart transplant-free survival improved significantly in patients with adequately suppressed CSA (i.e., AHI <15 per hour) compared to the control group (hazard ratio [HR], 0.37; 95% CI, 0.14–0.967; $P = 0.043$). Such improvement was not observed in patients with refractory significant CSA, which was present in half of all patients. Moreover, only in patients responding to treatment, a significant improvement in LV function was observed (Arzt et al. 2007). A recent meta-analysis of PAP therapy in HF patients with SDB showed a significant improvement of BNP levels in the PAP group (standardized mean difference -0.517; 95% CI, -0.764 to -0.270; $P < 0.001$) (Zhang et al. 2015).

A more recent study evaluated the safety and efficacy of adaptive servo-ventilation (ASV) in chronic HF patients. This study (Treatment of Sleep-Disordered Breathing with Predominant Central Sleep Apnoea by Adaptive Servo Ventilation in Patients with Heart Failure, SERVE-HF) randomized 1,325 symptomatic chronic HF patients with reduced ejection fraction and moderate CSA (i.e., AHI $\geq$15/h with predominantly central events) between ASV therapy including guideline-based HF therapy, or guideline-based HF therapy only. Although the AHI improved drastically after 12 months of treatment (from median 31.2 [IQR 10.3–115.3] to 6.6 [0.0–50.8]), all-cause mortality and cardiovascular mortality were significantly higher in the actively treated group (HR all-cause mortality, 1.28; 95% CI 1.06–1.55; HR cardiovascular mortality, 1.34; 95% CI 1.09–1.65; all

$P < 0.01$) (Cowie et al. 2015). Therefore, ASV therapy is not recommended in HFrEF patients with CSA (Ponikowski et al. 2016).

10.3.2 Acetazolamide

Acetazolamide is a carbonic anhydrase inhibitor, and also has mild diuretic and respiratory-stimulating properties. By inducing mild acidosis and increasing pCO_2, acetazolamide affects both central and peripheral chemosensitivity to carbon dioxide and oxygen, thereby decreasing the number of central apneic events in subjects with idiopathic CSA (White et al. 1982; DeBacker et al. 1995). Two small studies addressed the role of acetazolamide in the pharmacological treatment of CSA in chronic HF, all showing a significant decrease in central AHI, oxygen desaturation, and improvement in sleep quality and daytime sleepiness (Javaheri 2006; Fontana et al. 2011). The effect of acetazolamide on SDB severity in chronic HF patients will be more elaborated in the Predicting Successful Sleep Apnea Treatment With Acetazolamide in Heart Failure Patients (HF-ACZ) trial (NCT01377987). This randomized, double-blinded cross-over trial aims to enroll 85 subjects including HF patients and healthy controls. Moreover, this study will provide more insight in the mechanism of action of acetazolamide. Researchers hypothesize that acetazolamide will reduce SDB severity, improve markers of HF severity (i.e., echocardiographic parameters, NT-proBNP, troponin I), and that patients with the severest dysregulation will have the most benefit from treatment.

10.3.3 Future Perspectives

A novel and promising new treatment modality, especially for chronic HF patients with CSA, might be phrenic nerve stimulation. This treatment modality consists of a neurostimulation device, providing rhythmical stimulation to the diaphragm using a transvenous phrenic nerve pacing lead (Abraham et al. 2015). Phrenic nerve stimulation restores the normal (nocturnal) breathing pattern without requiring non-invasive ventilation with possible high ventilator pressures. A very recent pilot study by Jagielski et al. evaluated the 1-year efficacy of transvenous phrenic nerve stimulation in 41 patients with moderate-to-severe CSA, of which 78% had HF. After 12 months of treatment, both overall AHI and central apnea index markedly improved (central apnea index from 28.2 ± 15.0 at baseline to 6.0 ± 9.2 after 12 months; $P < 0.001$). Moreover, a significant improvement in other sleep-related parameters (e.g., oxygen desaturation index, sleep quality, rapid eye movement sleep) and quality of life was observed (Jagielski et al. 2016). Although authors report a relatively low serious adverse event rate, both safety and efficacy of this new and innovative treatment modality should be confirmed in adequately powered randomized controlled trials.

Another non-ventilation treatment modality which might be suitable for HF patients with OSA might be positional therapy. By preventing patients from sleeping in a supine position, positional therapy is able to decrease SDB severity, mainly by reducing obstructive apneic and hypopneic events (van Maanen and de Vries 2014; Jackson et al. 2015). This treatment modality was evaluated recently in a stable symptomatic HF population with significant SDB. SDB severity decreased

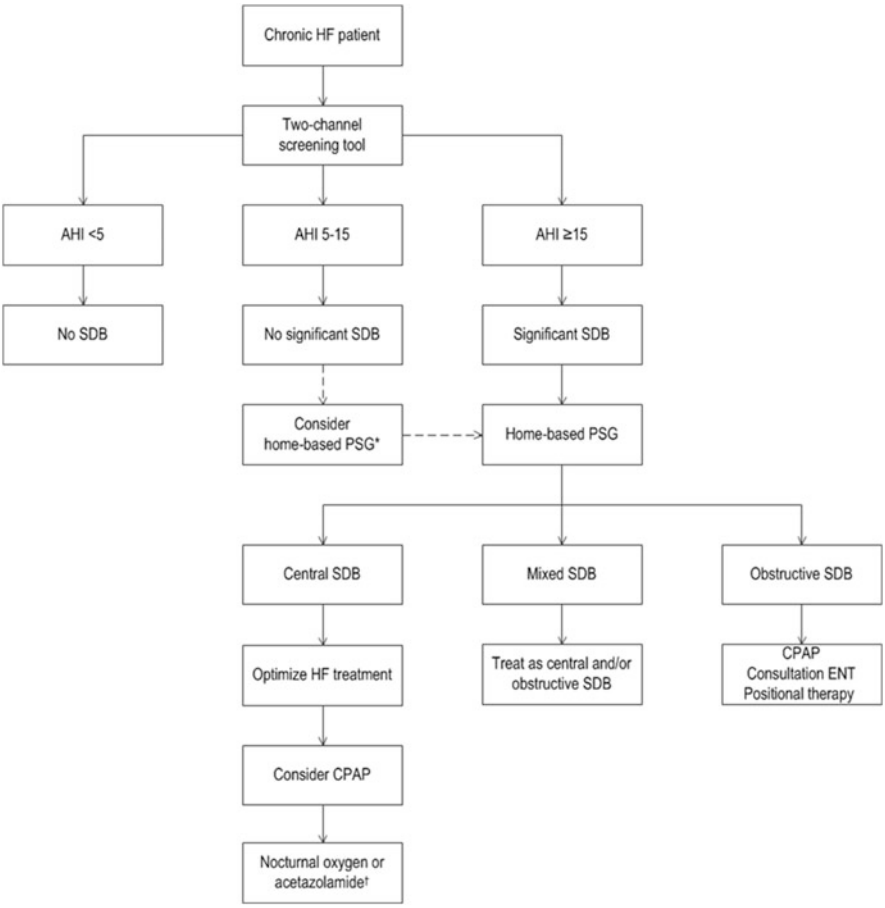

Fig. 1 Proposed diagnostic and treatment protocol for sleep-disordered breathing in chronic HF patients. *AHI* apnea-hypopnea index, *CPAP* continuous positive airway pressure, *ENT* otorhinolaryngology, *PSG* polysomnography, *SDB* sleep-disordered breathing. * PSG can be considered in patients with high clinical suspicion of SDB. † A trial of acetazolamide might be considered in refractory CSA patients with HF

considerably, especially in OSA, but also in CSA patients (AHI_{OSA} median [+ interquartile range] from 50.3 [36.9–67.6] to 10.4 [7.0–18.5]; AHI_{CSA} from 47.4 [37.6–56.0] to 19.3 [11.9–33.3]; both $P < 0.0001$) (Pinna et al. 2015). Given the high prevalence of positional SDB in chronic HF patients, a trial with positional therapy could be considered in these patients, especially in patients with intolerance to CPAP therapy.

10.4 Conclusion

Both OSA and CSA are very prevalent in chronic HF and have serious prognostic consequences in these patients. In general, SDB treatment should be based on the SDB (main) subtype. For CSA, treatment with CPAP seems safe and effective with improvement in LVEF and exercise tolerance in approximately 50% of chronic HF patients. It might even improve survival in responsive patients. Other ventilation modalities should not be used in chronic HF patients until more data on the prognostic consequences of these modalities are available. CPAP should also be used in HF patients with OSA; referral to the otorhinolaryngology department can be considered in these patients. For selected patients with positional OSA and/or intolerance to CPAP, a trial with positional therapy can be considered. As many chronic HF patients with SDB cannot be easily diagnosed based on clinical characteristics, we believe it would be reasonable to screen every chronic HF patient for SDB using a nasal flow meter, either with or without pulse oximetry. Our proposed diagnostic and treatment protocol is depicted in Fig. 1.

11 Conclusion

In this chapter, we discussed several high-impact comorbidities in chronic HF patients, with a focus on epidemiology, pathophysiology, and treatment. We tried to stress the importance of comorbidities in chronic HF patients, as many comorbidities have adverse effects on clinical status and prognosis in these patients. More importantly, some comorbidities even constitute a therapeutic target for the treatment of HF. As many studies on comorbidities in HF focus on HFrEF patients, it is urgently needed to perform studies focusing on this patient group with at least comparable burden of comorbidities and their clinical and prognostic consequences.

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Clinical Trial Design, Endpoints, and Regulatory Requirements

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_75

Abstract

A new therapeutic agent for heart failure can be approved if it improves survival and/or reduces hospitalisations or if it safely improves functional capacity. Therefore its clinical development program must demonstrate clinically relevant improvement in a robust clinical end point and adequate safety to justify regulatory approval and clinical use. Mortality and hospitalisations are now combined with new composite end points in order to improve trial efficiency and adequate assessment of efficacy of newer molecules, biologicals and cell therapies developed for the treatment of heart failure. Newer regulatory practices have been developed in the past few years and they will require design of innovative study designs able to demonstrate a sound clinical benefit alongside with adequate safety profile.

Keywords

Drug-therapy • Drug safety • Heart failure • Prognosis • Regulatory • Therapy

The value of a new therapeutic intervention is dependent upon its effect on mortality, morbidity, and functional capacity. A new therapy can be approved if it improves survival and/or reduce hospitalizations or if it safely improves functional capacity. Therefore, the clinical development program of new therapies must demonstrate clinically relevant improvement in a robust clinical end point to justify regulatory approval and clinical use. Traditional endpoints are now combined with new composite endpoints in order to improve trial efficiency and adequate assessment of efficacy of newer molecules, biologicals, and cell therapies developed for the treatment of heart failure (Anker et al. 2016; Schüssler-Lenz et al. 2016; Pani et al. 2015).

Heart failure encompasses heterogeneous groups of patients with a wide spectrum of symptoms, different causes, and different left ventricular ejection fraction (LVEF). Within this spectrum, patients may either have heart failure with reduced ejection fraction (HFrEF) or heart failure with a normal or relatively preserved ejection fraction (HFpEF, HFmrEF) (Ponikowski et al. 2015). The distinction between patients with HFrEF from those with HFpEF is important because they represent groups with different underlying pathophysiologic, hemodynamic, and neurohormonal abnormalities, distinctly different clinical characteristics, varying risks for adverse outcomes, and dissimilar efficacy of existing therapies. The identification of patients with HFmrEF is also important, but its real pathophysiological and prognostic relevance is not adequately known (ESC Committee for Practice Guidelines 2016).

Patients may also oscillate between periods of stability, where they are generally well managed as outpatients (i.e., chronic heart failure) and periods of decompensation requiring hospitalization [i.e., hospitalized heart failure (HFH)] (Greene et al. 2014; Butler et al. 2014; Gheorghiade et al. 2013). Available therapies for patients with chronic heart failure do not curb the high mortality and

rehospitalization rates following a hospital discharge for an episode of decompensation. The 90-day mortality of patients discharged after an acute episode of heart failure is approximately 10–15% and reaches 20–35% at 1 year. Clinical trials for the development of new therapeutic agents traditionally include patients who have just been hospitalized (within few days or hours) for heart failure or patients that have been stable for at least few months. Patients who are still hospitalized and waiting for discharge and those who have just been discharged from hospital are not included in clinical trials. Therefore, there is the need to adequately define HFH patients in order to allow their inclusion in heart failure trials.

HFH is frequently included as a co-primary endpoint or as part of a composite primary endpoint in heart failure trials. Despite the clinical relevance of HFH, its use as an endpoint in heart failure trials is controversial since the decision to hospitalize and the threshold for hospitalization are variable across regions of the world and may affect the interpretability and applicability of study results in different regions. Local standards of care (such as length of stay or availability of out-of-hospital treatment resources) may differ substantially by country. Although there is a relatively robust consensus on using heart failure hospitalization as an endpoint in heart failure trials, there is a need to better define this endpoint and to harmonize the definitions for related endpoints. Repeat hospitalizations are a common occurrence in patients with heart failure, but, despite their importance, repeated events are ignored in the majority of clinical trials and "time to first event" analyses are usually preferred. Accounting for repeated events may increase statistical power with smaller sample sizes and better characterize and quantify the occurrence of morbid events throughout the follow-up period particularly in scenarios where mortality is low.

In the past decades, despite promising results of phase II clinical trials based on surrogate endpoints in patients with heart failure, most drugs tested have failed to show efficacy or have even shown detrimental effect on long-term outcomes (Vaduganathan et al. 2013; Zannad et al. 2013). Therefore, in surrogate endpoints, there is a need to identify endpoints that may represent an adequate surrogate for cardiovascular mortality and HFH. Endpoints that may reflect manifestations of disease pathophysiology (e.g., biomarkers or parameters for remodeling) are often applied in earlier phases of drug development. Available biomarkers and changes in left ventricular remodeling, although associated with prognostic benefit, have not been shown to predict the clinical efficacy of drug therapies in heart failure. In the past, significant effects on surrogate endpoints have been associated with unfavorable outcome, and for this reason, there has been the need to request mortality data for approvability of new therapeutic agents for the treatment of heart failure. However, a mortality study in chronic heart failure is time consuming and may significantly delay the approval of effective therapies. Therefore, if a new agent shows a sizeable effect on a relevant clinical trial, this has to be associated with a favorable prognostic trend and absence of safety warning signals in order to obtain to an early approvability.

1 Efficacy Criteria for Developing Agents for the Treatment of Chronic Heart Failure

The primary aims of new agents developed for the treatment of chronic heart failure are to improve survival and prevent hospitalizations. However, functional capacity is also of importance in the treatment of patients with heart failure, but the approval based on these endpoints must take into account that in the past, some treatments effective in improving exercise capacity have also increased mortality.

Efficacy endpoints must be clinically relevant and should be divided into three groups according to their hierarchical relevance. Endpoints of the first group may represent per se a primary endpoint, endpoints of the second group can be used as primary endpoints only if group 1 endpoints are concurrently used as a safety measure in the development program, and endpoints of the third group can be used only as supportive evidence.

Group 1: all-cause mortality, cardiovascular mortality, hospitalization for heart failure, and recurrent morbid events (HFH, worsening heart failure without hospitalization, hospitalization for cardiac causes, cardiovascular mortality)
Group 2: functional capacity
Group 3: hemodynamic changes (e.g., LVEF, left ventricular remodeling), biomarkers, symptoms, and quality of life

Since the effects on functional capacity do not always correlate with prognosis, positive effects on these surrogate endpoints must be supported by favorable effects on mortality and morbidity before or after an initial approval. A positive trend toward a reduction in mortality along with a sound positive clinical effect should be demonstrated.

2 Primary Endpoints

Primary endpoints of studies in patients with chronic heart failure should include all-cause mortality, hospitalization for heart failure and/or the clinical composite endpoint of cardiovascular mortality, hospitalization for heart failure, worsening heart failure without hospitalization, and hospitalization for cardiac causes.

2.1 Mortality

Some drug classes (ACE inhibitors, beta-blockers, mineralocorticoid antagonists, the drugs are If-channel blockers, NEP inhibitors) have shown to improve mortality in patients with chronic heart failure, while other drug classes have increased mortality despite a positive effect on surrogate endpoints (e.g., flosequinan, ibopamine). Therefore, many drugs will require at least a demonstration of a trend toward a mortality benefit. Specifically, if the new investigational agent

belongs to a class that has been shown to be associated with detrimental effects on survival, an adequately designed randomized controlled survival study must be performed.

3 Heart Failure Hospitalization

Hospitalization for heart failure can be included as a co-primary endpoint, as part of a composite primary endpoint or as a secondary endpoint in clinical trials in patients with heart failure. The threshold for hospitalization is highly variable across (and within) regions of the world, and this may affect the interpretability and applicability of study results. Furthermore, since patients with heart failure may be often hospitalized for reasons unrelated to heart failure, objective evidence of cardiovascular decompensation as cause of hospitalization should always be provided. In this case a central adjudication of the events may be necessary.

4 Composite Endpoints and Recurrent Morbid Events

Prespecified composite endpoints may be appropriate for the prognostic assessment of an agent developed for the treatment of heart failure. Primary composite endpoints must include mortality and hospitalization for heart failure and for cardiovascular causes. It may be appropriate to include in the composite endpoint aspects of functional status and validated patient-related outcomes. However, these latter two endpoints must be adequately weighted against mortality and morbidity, and appropriate scientific advice should be sought before trial initiation. Composite endpoints that include mortality and repeat hospitalizations have been used as primary composite endpoints in several studies assessing the effect of new treatments in heart failure.

Most often repeat events are ignored in the majority of studies while their assessment may be useful in shortening and simplifying the studies, especially when low event rates are expected. Several methodologies have been suggested for the assessment of recurrent events in clinical trials. However, the preference of one over the other should be assessed case by case according to the population under study and to the expected mortality and morbidity.

5 Worsening Heart Failure Without Hospitalization

Patients are often managed for worsening heart failure in outpatient settings (e.g., emergency departments, observation units, other outpatient settings). The capture of events of worsening heart failure is warranted since the presence of non-hospitalization events indicates a high-risk population. However, strict criteria should be used to define these events, and clear evidence of decompensation should be obtained. Furthermore, these events should be always centrally adjudicated.

6 Functional Capacity

Exercise capacity is a reliable marker of functional status, and it is a more reliable endpoint than subjective assessment of clinical status. Measurement of maximal oxygen consumption during bicycle or treadmill exercise (MVO2) and supervised 6-minute walking test (6MWT) are both reliable methods for the assessment of functional capacity. However, limitations exist in the applicability of MVO2 tests because of patient adherence (e.g., inability to ride a bicycle, intolerance to the mouthpiece, etc.). The 6MWT correlates better with the effect of the pharmacological agent on daily clinical symptoms and functional capacity (Fishbein et al. 2014). Repeated measurements of exercise capacity throughout a study enable a better repeatability of the results. In frail patients other measures of functional capacity like handgrip test or short physical performance battery (SPPB) may be appropriate.

7 Hemodynamic Parameters

Hemodynamic parameters are not adequate to demonstrate benefit of a new therapeutic agent, but changes in hemodynamic parameters are useful to elucidate the mode of action of a therapeutic agent and should be demonstrated during the clinical development program. A correlation between hemodynamic parameters and clinical and functional status must be shown with the therapeutic agent if the approval is to be granted on the basis of an effect on functional parameters.

7.1 Biomarkers

Apart from heart rate, no biomarker has been shown to be a reliable surrogate for clinical outcomes in patients with heart failure. To this end biomarkers other than heart rate (only for drugs with a specific direct effect on it) cannot be considered as primary endpoints in clinical trials in heart failure. Biomarker data can be used together with hemodynamic data to elucidate the mechanism of action of a new therapeutic agent.

8 Patient-Reported Outcomes

Patient-reported outcomes (PROs) may be used as secondary endpoints in heart failure studies. Any study showing a clinically meaningful effect on PROs must show a trend toward a beneficial effect of the investigational agent on mortality/morbidity. However, in patients with advanced disease where there is a need for palliative care (end-stage HF, HF cachexia), PROs may be relevant endpoints.

9 Assessments of Efficacy

Some methods available for studying patients with heart failure when investigating the efficacy and the safety of new therapeutic interventions are subject to placebo effects, and therefore the imputable placebo effect should always be assessed in comparative studies.

10 Survival

Although overall mortality should be the preferred mortality endpoint in chronic heart failure studies, both total death and cause-specific death must be quantified. Since non-cardiovascular death is increasing among patients with heart failure, the assessment of cardiovascular death may represent an adequate endpoint if the therapeutic effect on overall mortality is at least neutral. Therefore, it is of importance to demonstrate a directionally similar effect on overall and cardiovascular mortality. Excess in non-cardiovascular mortality could be acceptable if the overall mortality is reduced or unchanged.

Regarding cardiac mortality, it is important to define the mode of cardiac death occurring in the studies, and, therefore, efforts should be made in order to define the specific mode of cardiac death. A central adjudication of the causes of death may be warranted (Seltzer et al. 2015).

The effect of a new pharmacological agent for the treatment of heart failure on mortality can only be assessed definitively in the context of a randomized placebo- or treatment-controlled trial in an adequately large number of patients. Survival studies using positive control drug(s) may be acceptable but should be limited to drugs that have consistently shown efficacy on survival.

In all trials the analysis should be conducted according to the "intention-to-treat" principle. Therefore, all patients must be followed up for the whole study duration. Analyses should also consider data gathered during the run-in as well as during the dose-titration periods. This is important also to assess the external validity of the study.

11 Hospitalization for Heart Failure

Evidence of hospitalization for worsening heart failure severe enough to require a therapeutic intervention may be used as endpoint for efficacy. It is well known that a sizeable proportion of hospitalizations labeled as due to heart failure are instead elated to other comorbid causes. Therefore, when assessing hospitalizations for heart failure, it is important to define what is required for a hospitalization to be labeled as due to heart failure. These hospitalizations should be centrally adjudicated.

Hospitalization for heart failure can be defined by the one associated with signs and symptoms of deteriorating clinical conditions along with increased plasma

levels of BNP/proBNP and the need for acute treatments for heart failure (e.g., increase in diuretic dose, intravenous diuretics, or intravenous vasodilators/inotropes). The use of the increase in the need for treatments for heart failure may be not appropriate to define worsening heart failure when therapies with hemodynamic effects are not tested against an appropriate comparator.

12 Worsening Heart Failure Without Hospitalization

Moderate worsening heart failure is often managed in outpatient settings (e.g., emergency departments, observation units, other outpatient settings). In order to define an episode of decompensation as related to worsening heart failure in the outpatient settings, it is required to demonstrate a cardiac cause for the worsening of symptoms. Therefore a chest X-ray along with elevated BNP/proBNP levels and the need of a treatment component to define the event (e.g., the use of intravenous therapies or augmentation of existing therapies) are required (Collins et al. 2013).

13 Composite Endpoints End Recurrent Morbid Events

Mortality, either overall or cardiovascular, can be combined with heart failure hospitalizations in a composite primary endpoint. When cardiovascular mortality is used in the primary endpoint, overall mortality should be assessed as a secondary safety endpoint.

Furthermore, composite hierarchical endpoints can be applied to heart failure studies providing that mortality (overall and cardiovascular) and hospitalizations for heart failure are, respectively, the first two hierarchical endpoints. These endpoints may be followed in order of relevance by measures of functional status (6MWT, MVO2), left ventricular remodeling, and PROs.

Recurrent morbid events are becoming a popular and acceptable endpoint in heart failure trials. They should be assessed with appropriate statistical methods. Usually hospitalizations for heart failure represent the recurrent event to include in the analysis alongside the terminal event. Time-to-recurrent HF-related hospitalizations may therefore be adjusted for correlated terminal CV events (all-cause death, heart transplant, etc.).

The analysis of recurrent events can be performed after study patients have been observed for an adequate follow-up or when an adequate number of adjudicated events have occurred (counting multiple events per subject).

Different statistical methods can be applied to assess recurrent morbid events, among these the marginal approach by joint parametric modeling of repeated in-patient episodes (via a Poisson model) and survival time or by a nonparametric penalized likelihood method for estimating hazard functions.

The “joint frailty method,” “days alive and out of hospital,” and the “patient journeys” are adequate methods to assess recurrent morbid events. The preference

of one method over the other should be assessed according to the study patients and to the expected rate of deaths and hospitalizations.

14 Assessment of Functional Status

Exercise testing allows objective evaluation of functional capacity and exertional symptoms, such as dyspnea and fatigue in patients with heart failure. Exercise testing should be performed using appropriate protocols specifically designed for the functional assessment of patients with cardiac failure.

Submaximal protocols should specify a priori the reasons for termination of the tests. Naïve patients to exercise protocols (bicycle, treadmill, measurement oxygen consumption) should first be made familiar with the technique before they are included in the trial. In case the familiarization tests are performed in the run-in phase of the study, the variability in exercise capacity between repeated tests should be $<5\%$. Repeated baseline and repeated follow-up testing may reduce variability of the results and increase statistical power.

Because of the variability in the execution and settings of the 6MWT, multicenter studies must use standardized length corridors with appropriate markings for the execution of the test. The test should be supervised by a physical therapist, patients should be asked to walk on their own, and no phrases of encouragement should be told. Patients may be allowed to stop walking if signs or symptoms of significant distress occur during the test, and they should be instructed to resume walking as soon as possible.

15 Hemodynamic Studies and Studies of Left Ventricular Function

Hemodynamic data are valuable in defining dose-response relationships; the value of these measures in evaluating the efficacy of the drug in patients with cardiac failure during long-term treatment is very limited. Therefore, hemodynamic data that may include ventricular dimensions, ejection fraction, and indices of systolic and diastolic functions (e.g., LVedp) should be regarded as supportive only.

The use of these newer techniques used to study the hemodynamic effect of a new agent in heart failure must be validated and justified.

Noninvasive techniques that show inter-operator variability should take into account this variability in the statistical planning. Although centralized readings are usually used in order to reduce the interobserver variability, they add significant costs and most often hamper significantly the recruitment rates.

16 Quality of Life

Almost all the components of the life quality may be influenced by an intervention for heart failure. Various quality-of-life questionnaires are used in practice for the assessment of patients with chronic heart failure. Questionnaires must be fully validated in all the languages of the countries where they are applied in order to be used in a multicenter clinical trial. Evidence of efficacy derived from quality-of-life questionnaires can be used only as supportive evidence of efficacy.

17 Pharmacodynamics/Pharmacokinetics

Pharmacokinetic of new compounds should be adequately assessed before proceeding to phase II studies. Pharmacodynamic studies should assess tolerability and duration of the effect and the action of the new agent on hemodynamic parameters (e.g., stroke volume, PCWP), heart rate, neurohormonal parameters (e.g., sympathetic nervous system), and renal function. Studies on the effect on impulse formation, its conduction, and on the effect of the agent on repolarisation (i.e., QT/QTc intervals) and cardiac rhythm should be performed. If the new agent has a significant effect on cardiac electrophysiology, the potential for pro-arrhythmic effect should be investigated.

Several dosages should be tested, and it would be preferable to select more than one dosage to be tested in confirmatory clinical trials. In phase II studies, patients with degrees of heart failure ranging from mild to severe must be studied.

18 Confirmatory Studies

These studies should be randomized and double blind. If an appropriate blinding of the study cannot be guaranteed, a PROBE design can be accepted. A run-in period of appropriate duration to verify the clinical stability is recommended. A control group on placebo is preferable if it is ethically feasible. When the new agent is proposed as an add-on treatment to an existing therapy, a placebo group is mandatory. Pretrial registries can be appropriate to adequately select sites with adequate patient volumes and quality of care (Harinstein et al. 2015; Pelliccia and Rosano 2015).

Although in principle, one large well-controlled trial of adequate statistical power and unequivocal results may be sufficient to confirm the efficacy of a new drug in practice; this is difficult to achieve in order to demonstrate an adequate external validity. Therefore, for drugs to be developed for the treatment of chronic cardiac failure, it is preferable to plan at least two trials to demonstrate either the superiority of the new agent over a placebo/active comparator or the undeniable equivalence of the new agent to an active comparator. In this case the non-inferiority margins to the active comparator and the imputable placebo effect should be clearly defined.

19 Safety

Patients with chronic heart failure require lifelong treatments. Therefore, the effect of the new agent on adverse effects and drug-to-drug interactions should always be investigated.

Potential adverse effects that are dependent upon the pharmacodynamics mode of action of the drug should be investigated. In general attention should be paid to cardiac rhythm, pro-ischemic effects, hypotension, bradycardia, renal function, and electrolyte homeostasis.

20 Conclusion

Newer regulatory practices will require design of innovative study designs (Rosano et al. 2015). However, the clinical studies to be conducted for the approval of new agents in the treatment of heart failure should show a sound clinical benefit alongside with adequate safety profile. For this reason all intervention studies conducted in heart failure should be reported and published (Capuano et al. 2015).

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Biomarkers of Heart Failure with Preserved and Reduced Ejection Fraction

Michele Senni, Emilia D'Elia, Michele Emdin, and Giuseppe Vergaro

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Abstract

Biomarkers are increaingly being used in the management of heart failure not only for the purpose of screening, diagnosis, and risk stratification, but also as a guide to evaluate the response to treatment in the individual patient and as an

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_86

entry criterion and/or a surrogate marker of efficacy in clinical trials testing novel drugs. In this chapter, we review the role of established biomarkers for heart failure management, according to the main classification of HF phenotypes, based on the measurement of left ventricular ejection fraction, including heart failure with reduced (<40%), preserved (≥50%), and, as recently proposed, mid-range (40-49%) ejection fraction.

Keywords
Biomarkers • Diagnosis • Heart failure management • Prognosis • Screening

1 Introduction

Early diagnostic and therapeutic interventions counteracting pathophysiological mechanisms of heart failure (HF) progression, such as drugs inhibiting the renin-angiotensin-aldosterone system (RAAS) and adrenergic system activation, as well as device implantation in selected patients positively influence its clinical course, impacting on the arrhythmic burden and hemodynamics. Notwithstanding, outcome is still characterized by a high morbidity and mortality. Interest is therefore growing in the optimization of HF management and in the identification and validation of novel therapeutical tools. With these premises, research has focused on the use of biomarkers not only for the purpose of screening, diagnosis, and risk stratification, but also as a guide to the assessment of response to treatment in the individual patient, and finally as an entry criterion and/or a surrogate marker of efficacy in clinical trials testing novel drugs (Braunwald 2008; Braunwald 2013; Emdin et al. 2009).

In 2001, a National Institute of Health working group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" and identified different classes of biomarkers according to their role and potential utility in disease management. In particular, biomarkers were classified as antecedent biomarkers (i.e., those identifying the risk of developing a disease), screening biomarkers (those able to screen for subclinical disease), diagnostic biomarkers (recognizing clinically overt disease), staging biomarkers (those reflecting disease severity), or prognostic biomarkers (predicting the future course of the disease and response to pharmacological and non-pharmacological approaches) (Biomarkers Definitions Working Group 2001; Vasan 2006).

A number of biomarkers have been proposed for HF management purposes in the last years; nonetheless only a very few of them (namely B-type natriuretic peptides) are routinely used in current clinical practice. Indeed, several characteristics are requested for a circulating biomarker to be of real clinical value, related to (pre)analytical, biological, feasibility, and cost-effectiveness issues. In particular, the *ideal* biomarker for HF management should reflect the cardiovascular response to a specific pathogenic *noxa*; provide early information on

Table 1 Effects of currently recommended and previously proposed drugs for heart failure treatment on circulating levels of biomarkers

Drug class	BNP	NT-proBNP	PRC	PRA	Ang I	Ang II	Aldosterone	Gal-3	sST2	CRP	GDF-15
Beta-blockers	↑ > ↓	↑ > ↓	↓	↓	↓	↓	↓	=	↓	↓	↓?
ACEi	↓	↓	↑	↑	↑	↓	↓ / ↑	=	↓	↓	↓?
ARB	↓	↓	↑	↑	↑	↑	↓ / ↑	=	↓ / =	↓	=
MRA	↓	↓	?	↑	?	↑	↓ / ↑	=	?	=	=?
DRI	↓	↓	↑	↓	↓	↓	↓ / =	?	?	?	?
ARNI	↑	↓	↑	↑	?	↑	↓	=	=	=	?

Ang angiotensin, *ACEi* Angiotensin converting enzyme inhibitors, *ARB* angiotensin receptor blockers, *ARNI* angiotensin receptor/neprilysin, *BNP* brain natriuretic peptide, *CRP* C-reactive protein, *DRI* direct renin inhibitor, *Gal-3* galectin-3, *GDF-15* growth differentiation factor 15, *MRA* mineralocorticoid receptor antagonists, *NT-proBNP* amino-terminal fragment of proBNP, *PRA* plasma renin activity, *PRC* Plasma renin concentration, *sST2* soluble suppression of tumorigenicity protein 2

cardiac involvement in preclinical stages (screening in HF stages A–B) and/or guide HF diagnosis when cardiac origin of symptoms is doubtful (HF stage C); be influenced by clinical modification in the disease history; and stratify patients' risk and help in therapeutic decision making (Table 1). Still, novel biomarkers are clearly required to improve risk prediction already offered by existing models, with the mere demonstration of a statistically significant association with a prespecified end-point not being enough (Pencina et al. 2008). Further to c-statistic and receiver-operating-characteristic curve (AUC) measure (Pencina et al. 2010), providing estimate of the discriminative ability of the model (i.e., the capacity to separate subject developing/not developing the outcome), and measures of calibration (indicating how close are predicted and observed risks in different groups of observations, e.g., by means of the Hosmer-Lemeshow test) and of reclassification (indicating the ability of the model to reclassify individuals into a different risk category, e.g., by calculating the net reclassification improvement, NRI) are required to adequately assess the clinical utility of novel biomarkers (McGeechan et al. 2008; Parikh and Vasan 2007).

In this chapter, current evidences on the role of established and candidate biomarkers for HF management are discussed, according to the main classification of HF phenotypes, based on the measurement of left ventricular ejection fraction (LVEF), including HF with reduced EF (HFrEF, LVEF <40%), HF with preserved EF (HFpEF, LVEF ≥50%), and the recently proposed category HF with mid-range EF (HFmrEF, LVEF 40–49%) (Ponikowski et al. 2016).

2 Biomarkers of Heart Failure with Reduced Ejection Fraction

2.1 B-type Natriuretic Peptides in Heart Failure with Reduced Ejection Fraction (HFrEF): Standing on the Shoulders of Giants

Human brain natriuretic peptide (BNP) and the amino-terminal fragment of proBNP (NT-proBNP) are produced in equimolar fashion from the cleavage of their 108-amino acid precursor proBNP by proprotein convertases, such as corin and furin. The biologically active BNP is rapidly degraded *in vivo* by several peptidases, such as dipeptidyl peptidase IV and neutral endopeptidases (NEP, neprilysin) (Clerico and Emdin 2004; Pankow et al. 2007; Vanderheyden et al. 2009). BNP, together with NT-proBNP, is mainly product of ventricular myocytes in response to increased myocardial wall stress due to volume or pressure overload states and plays a major role in HF pathophysiology, given its diuretic, natriuretic, vasodilator, and anti-hypertrophic properties (Motiwala and Januzzi 2013).

Since the beginning of the twenty-first century, several studies have accumulated showing the clinical utility of B-type natriuretic peptide testing, supporting the use of a circulating biomarker to diagnose and assess severity of HF (Januzzi et al. 2005; Maisel et al. 2002; Troughton et al. 2000). At present, BNP and NT-proBNP are routinely used for HF management in a large variety of clinical settings. Strong evidence exists supporting the use of natriuretic peptide testing to diagnose (and rule out) HF in patients presenting with dyspnea. The Breathing Not Properly and the ProBNP Investigation of Dyspnea (PRIDE) trials have shown that BNP and NT-proBNP, respectively, can diagnose HF in patients admitted to the emergency department for breathlessness, with a high accuracy and a significant negative predictive value for levels of BNP <100 ng/L (Januzzi et al. 2005; Maisel et al. 2002). Although with lower cut points, the diagnostic value of natriuretic peptides has been confirmed in ambulatory settings (Wright et al. 2003). Moreover, in a cohort of around 800 patients with chronic HF, they have been shown to be associated with the presence of LV systolic dysfunction (defined as LVEF <40%) with an AUC of 0.803 (95% CI 0.757–0.849) and 0.730 (0.681–0.778) for BNP and NT-proBNP, respectively (Moertl et al. 2009). In stable patients with HFrEF, BNP circulating levels reflect disease severity, and increase with worsening symptoms (NYHA class) (Emdin et al. 2004). The assay of natriuretic peptides is currently recommended for diagnostic purposes by all major scientific societies, including the American College of Cardiology, the American Heart Association, the Heart Failure Society of America, and the European Society of Cardiology (Lindenfeld et al. 2010; Ponikowski et al. 2016; Yancy et al. 2013); however, no single diagnostic cutoff exists and natriuretic peptide levels often fall into a "grey zone." Further, both BNP and NT-proBNP are influenced by gender, age, and comorbidities (in particular by renal function); therefore their interpretation must take into account the overall clinical assessment (Passino et al. 2008; van Kimmenade et al. 2008).

In adjunct to diagnostic properties, there is overwhelming evidence that natriuretic peptides hold prognostic value in HFrEF in both acute and chronic settings over other clinical factors. In >19,000 patients with HFrEF form the ADHERE (Acute Decompensated Heart Failure National Registry), admission BNP levels were near-linearly associated with in-hospital mortality, independently from other major clinical and laboratory risk factors (Fonarow et al. 2007). In a systematic review of studies performed in different clinical settings, Doust and colleagues have demonstrated that each 100 ng/L increase in BNP is associated with a relative 35% increase in risk (Doust et al. 2005). NT-proBNP predicts as well both short- and long-term prognosis after hospitalization for acute HF, with values >986 ng/L predicting 1-year death (Januzzi et al. 2006a, b). Although most studies have investigated the prognostic role of admission natriuretic peptide levels, there is evidence that discharge BNP and NT-proBNP, and their change during hospitalization, also predict outcome of patients with HFrEF. Data from the Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure (OPTIMIZE-HF) show that the addition of discharge BNP to a clinical model significantly improved risk classification for 1-year mortality with an NRI of 5.5% (Kociol et al. 2011), thus suggesting that serial natriuretic peptide testing may be useful in predischarge clinical decision making (Januzzi et al. 2012). A post hoc analyses of the Valsartan Heart Failure Trial (Val-HeFT), enrolling patients with chronic symptomatic HF with LVEF <40% and LV diameter in diastole adjusted for body surface area ≥2.9 cm/m^2, demonstrated that BNP and NT-proBNP similarly predicted all-cause mortality, while NT-proBNP outperformed BNP for the prediction of mortality and morbidity or hospitalization for HF (Masson et al. 2006). Moreover, experience from the Val-HeFT indicates that changes over time in natriuretic peptides, too, hold a prognostic value in stable HF, envisaging a role for serial BNP and NT-proBNP testing in patient monitoring and in the evaluation of response to therapeutical strategies (Masson et al. 2008; Richards 2008).

2.2 B-type Natriuretic Peptide-Guided Therapy

Circulating levels of B-type natriuretic peptides are influenced by most of the pharmacological and non-pharmacological therapeutical approaches in acute, acutely decompensated, and chronic HF. Indeed, in the acute setting, treatment with furosemide and the inodilator levosimendan decreases BNP (Cohen-Solal et al. 2009; Farmakis et al. 2010; Palazzuoli et al. 2014). Moreover, a reduction >30% from baseline values following levosimendan administration has been associated with an improvement in either short- (31 days) or long-term (180 days) mortality (Cohen-Solal et al. 2009). Other therapeutic strategies acting on hemodynamics and cardiac remodeling have been demonstrated to elicit similar effects on natriuretic peptides in stable HFrEF, including angiotensin-converting enzyme inhibitors (Rosenberg et al. 2008), angiotensin-receptor blockers (Anand et al. 2003), mineralocorticoid receptor antagonists (Berry et al. 2007), and, at a lower extent, β-blockers, whose initiation may also cause a transient increase in

BNP and NT-proBNP, which subsequently decrease (Davis et al. 2006; Rosenberg et al. 2008). Evidence for a beneficial effect on natriuretic peptides also exists for other non-pharmacological tools, such as aerobic training and cardiac resynchronization therapy (Fruhwald et al. 2007; Passino et al. 2006). More recently, the first-in-class neprilysin/angiotensin receptor inhibitor sacubitril valsartan (LCZ696) has been proved effective in reducing the risks of death and of hospitalization in patients with HFrEF compared to enalapril in the PARADIGM-HF trial (McMurray et al. 2014). Such beneficial effects are possibly due, at least in part, to the inhibition of BNP degradation exerted by sacubitril, while its precursor and NT-proBNP are virtually resistant to degradation (Semenov and Katrukha 2016), as also supported by the observation that after LCZ696 initiation, circulating levels of NT-proBNP decrease, while BNP slightly increases (Packer et al. 2015). It has been therefore questioned that assessing the clinical significance of BNP in the era of LCZ696 may be challenging and that NT-proBNP may be preferred over BNP for biochemical monitoring of patients with HF, in order to assess its effectiveness on hemodynamic status (Lippi and Sanchis-Gomar 2016).

As discussed before, natriuretic peptides perform well for diagnostic and prognostic purposes and are associated with disease severity and, importantly, their circulating levels change in response to evidence-based therapies for HF. Based on these premises, several studies have tested the (cost)effectiveness of strategies of treatment titration guided by natriuretic peptide circulating levels, the so-called *biomarker-guided therapy*. In the BATTLESCARED, the first of these studies, 364 HF patients were randomly allocated to therapy guided by NT-proBNP levels (with adjustments in medications and additional follow-up visits triggered by an NT-proBNP level >150 pmol/l) or by intensive clinical management, or according to usual care (Lainchbury et al. 2009). NT-proBNP-guided treatment was associated with a significant reduction in a primary end-point of death and/or readmission with heart failure in younger population (age ≤75 years), who also presented more often with HFrEF (61% vs. 47% in patients aged >75 years). In the same year results from the TIME-CHF trial, enrolling only patients with LVEF ≤45%, were published, showing that patients with therapy titration based on NT-proBNP (target: <400 ng/L in patients aged <75 years and <800 ng/L in patients ≥75 years) had improved 18-month survival free of hospitalizations for HF (Pfisterer et al. 2009). Following these pivotal trials, other studies using either BNP or NT-proBNP have provided mixed outcomes (Kim and Januzzi 2010). Some systematic reviews have addressed the issue of the effects on hard end-points, such as all-cause mortality, demonstrating a benefit from natriuretic peptide-guided therapy (Don-Wauchope and McKelvie 2015), and a meta-analysis by Felker has demonstrated a 30% improvement in survival, without an increase in therapy-related adverse events (Felker et al. 2009). As a general view, the use of natriuretic peptide to tailor HF therapy is likely to be more effective when lower target values are applied (i.e., <100 ng/L and <1,000 ng/L for BNP and NT-proBNP, respectively) (Januzzi et al. 2011; Jourdain et al. 2007; Troughton et al. 2000). Nonetheless, evidence is still scarce to support a general recommendation and some issues

still need to be addressed, including the most appropriate use of biomarker with this respect (disease management vs. monitoring), the choice of the end-point, and the cost-effectiveness of such a strategy (Pruett et al. 2015).

2.3 Back to the de Bold's "Atrial Natriuretic Factor": MR-proANP

Although B-type natriuretic peptides represent the most widely studied cardiac hormones, both in the experimental and clinical field, atrial natriuretic peptide (ANP) was first described in early 1980s, as a substance secreted from atrial granules with endocrine functions, by Adolfo de Bold (de Bold 1981). ANP assay has with time been substituted by assays of B-type natriuretic peptides both for analytical and clinical reasons (Clerico 2006). More recently, the potential clinical applications of the mid-regional pro-atrial natriuretic peptide (MR-proANP), with a longer half-life than ANP, have been tested. Data from the Biomarker in the Acute Heart Failure (BACH) and the PRIDE study have demonstrated a good performance of MR-proANP for diagnostic and prognostic purposes in acute HF, even in addition to NT-proBNP (Maisel et al. 2011a; b, c; Shah et al. 2012). MR-proANP also revealed a significant prognostic value in 1237 patients with chronic HF enrolled in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza Cardiaca-Heart Failure (GISSI-HF) study (proposed cutoff 278 pmol/L, AUC 0.74, 0.71–0.77), outperforming other established and candidate biomarkers (including copeptin and mid-regional proadrenomedullin, MR-proADM, and C-terminal proendothelin-1) and beyond NT-proBNP and a set of clinical variables (NRI = 0.06) (Masson et al. 2010a).

2.4 Cardiac Troponins

Cardiac troponins are released into the circulation following the disruption of cardiomyocyte membrane due to cardiac injury, namely after cardiac necrosis, and have become the standard of care biomarker for diagnosis of myocardial infarction. Nonetheless, elevation of circulating troponins has been reported also in non-acute settings, including chronic HF, due to mechanisms still to be fully elucidated, possibly involving inflammation, neurohormonal activation, myocardial stretch, hypoxia, and cytotoxicity (Gaggin and Januzzi 2013). With the improvement in troponin assays and the rise of high-sensitivity troponins (hsTn), detectable circulating troponin is now observed in a large proportion of HF patients and several studies have explored its use in clinical practice as an additional biomarker for disease management. In 140 patients with acute HF, TnI was found to be increased in about 1/3 of cases and to independently predict 90-day mortality and readmission. Further, increase in TnI during hospitalization was also associated with a worse outcome (Xue et al. 2011). In the RELAX-AHF trial, hs-TnT was elevated above the 99% upper reference limit in most of the AHF patients. Baseline, peak, and peak change hs-cTnT were associated with worse

outcomes, in particular with 180-day cardiovascular mortality (Felker et al. 2015). In the ADHERE study, patient with TnI elevation had significantly higher in-hospital mortality when treated with intravenous inotropic therapy as compared with intravenous vasodilator therapy (Peacock et al. 2008).

As in acute settings, circulating Tn is associated with prognosis also in chronic HF. In an analysis performed in >4000 patients enrolled in the Val-HeFT, TnT was detectable in about 10% of patients using a conventional assay, while 92% showed detectable Tn when the hsTnT assay was used. In the same cohort, hsTnT was associated with the risk of death and LV remodeling, and improved prognostic discrimination when added to a baseline model including BNP (Latini et al. 2007). The prognostic utility of serial Tn measurement in chronic HF has been investigated in patients from both the Val-HeFT and the GISSI-HF study. In this cohort, although increase in hsTnT over 3 to 4 months was strongly associated with all-cause mortality after adjustment for clinical risk factors, baseline levels, and NT-proBNP, it only modestly improved prognostic discrimination (Masson et al. 2012).

Previous reports show an association between the use of ACEi and beta-blockers with lower circulating Tn levels (Horwich et al. 2003; Masson et al. 2012), thus suggesting that guideline-recommended therapy may mitigate the risk in subsets with elevated Tn. More recently, in the PARADIGM-HF trial, treatment with LCZ696 led to a significant and sustained reduction in hsTnT that was not observed in the enalapril arm (Packer et al. 2015). Still, the issue of a Tn-guided therapy in chronic HF remains to be explored.

2.5 Candidate Biomarkers of HFrEF

Natriuretic peptides represent nowadays a fundamental aid to the clinical management of HF; nonetheless circulating levels of either BNP or NT-proBNP increase following each generic insult to the cardiovascular system. Therefore, while they are highly sensitive in the detection of ongoing damage, they are not able to provide clinically significant information concerning the nature of the *noxa*. The development and progression of the HF syndrome result from a complex interplay between different pathogenic determinants sustaining the ongoing myocardial damage and (mal)adaptive mechanisms. As a consequence, biomarkers providing insights into the extent of activation of specific axes of disease progression may help the clinician in the process of disease (and patient) characterization and of treatment tailoring.

A huge number of biomarkers have been tested or are currently under investigation for HF which may be classified according to he pathophysiological mechanism of damage they are considered to reflect. As previously suggested, biomarkers of sympathetic and RAAS activation, inflammation, fibrosis, and comorbidities can be distinguished (Braunwald 2013; Emdin et al. 2009).

2.5.1 Sympathetic Activation

Activation of the sympathetic, and inhibition of the parasympathetic system, represents one of the first (mal)adaptive mechanisms in disease onset and progression and one of the bases of the neuroendocrine model of HF, given its vasoconstrictive, profibrotic, and arrhythmogenic effects (Leimbach et al. 1986). Circulating norepinephrine increases with disease severity (Emdin et al. 2004), and, in a seminal paper by Cohn, it was the only independent predictor of mortality in 106 patients with moderate to severe HF, although such finding was not confirmed later on in more contemporary series (Cabassi et al. 2013; Cohn et al. 1984). Data from the Val-HeFT have shown that treatment with valsartan can blunt the increase in norepinephrine compared to placebo, while it was not affected by the mineralocorticoid receptor antagonist (MRA) spironolactone (Latini et al. 2002; Rousseau et al. 2002).

Together with catecholamines, chromogranin A is a component of chromaffin granules in the adrenal glands and, although its biological effects on the cardiovascular system remain to be elucidated, it seems to be involved in the regulation of adrenergic system (Tota et al. 2014). Limited evidence exists that circulating chromogranin A is increased in either acute or chronic HF and that may have some prognostic value (Dieplinger et al. 2009a; Røsjø et al. 2010).

2.5.2 Renin-Angiotensin-Aldosterone System Activation

RAAS is a complex endocrine system participated by the kidney, liver, vascular endothelium, and adrenal cortex regulating salt/water homeostasis and vasomotion. Either systemic or tissue RAAS are involved in tissue remodeling after damage, and can promote fibrosis, hypertrophy, and apoptosis. All the effectors of RAAS are increased in chronic HF (Emdin et al. 2004) and RAAS activation is an indirect or direct target of most effective pharmacological treatments in heart failure, such as beta-blockers, inhibitors of angiotensin-converting enzyme, angiotensin receptor blockers, direct renin inhibitors, mineralocorticoid receptor blockers, and angiotensin receptor/neprilysin inhibitors. Circulating biomarkers of RAAS activation are currently available, such as plasma renin activity (PRA), renin, angiotensin II, and aldosterone, although with different feasibility and accuracy, and some of them are well-recognized prognostic factors, even in patients with optimal therapy. Notably, chronic use of drugs acting on RAAS induces, per se, neurohormonal reactivation (Lee et al. 1999; Rousseau et al. 2002).

PRA has been demonstrated to hold an independent prognostic value in a systolic HF cohort on evidence-weighted treatment, with and without significant renal comorbidity (Poletti et al. 2013; Vergaro et al. 2011), thus potentially qualifying as a tool to identify patients with persistent RAAS activation despite adequate neurohormonal antagonism and to select subsets at higher risk for cardiovascular events. Further, aldosterone was shown to be a predictor of all-cause mortality in ≈300 patients with HF (most of them with systolic dysfunction), independently from other clinical and biohumoral variable including NT-proBNP (Güder et al. 2007). Specifically, the increase in circulating aldosterone following the initiation of RAAS acting therapy, a phenomenon termed “aldosterone

breakthrough," may hold clinical relevance, given the growing evidence on non-mineralocorticoid receptor-mediated effects of aldosterone. Further, in a small group of patients with systolic HF, increased PRA predicted ACE inhibitor-induced natriuresis (Lim et al. 2000).

The above-mentioned data support the rationale for the use of biomarkers of RAAS activation as a guide for treatment monitoring and tailoring, as well as a rule-in variable for trials in HF setting, a role up to now discarded as for the case of trials with the direct renin inhibitor aliskiren.

2.5.3 Inflammation and Immunity

There is growing information on the role of inflammatory cells and pathways during acute cardiovascular injury and in the reparative process that is subsequently activated. Elevation of inflammatory biomarkers, including C-reactive protein (CRP), members of the interleukin family (e.g., IL-1, IL-6, and IL-18), and TNF-α, is a hallmark feature of chronic ischemic and non-ischemic HF, although whether inflammation is causative to disease progression is not yet clear (Mann 2015). Moreover, viral infection is thought to participate in the development of dilated cardiomyopathy, sustaining acute and chronic inflammation (Kühl et al. 1996).

The Val-HeFT study demonstrated a direct correlation between elevated CRP level and HF severity (Anand et al. 2005); further, CRP predicts the risk of death and early readmission in acutely decompensated HF (Lourenço et al. 2010). Anti-inflammatory properties have been described for statins and their effect has been tested in HF settings. Data from the CORONA study show that subjects with HF of ischemic etiology and elevated baseline high sensitivity-CRP ($\geq$2 mg/L) exhibited a greater benefit from statin therapy in terms of reduction of the primary end-point of cardiovascular death, myocardial infarction, and stroke (McMurray et al. 2009), while controversial results come from studies performed on patients with HF of non-ischemic origin (Bleske et al. 2006; Sola et al. 2006).

Some trials have addressed TNF-α elevation in HF, which is associated with worsened prognosis. Two large clinical studies, the Randomized Etanercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE) and the Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction (RECOVER), were stopped because of lack of clinical benefit, and patients receiving the highest dose had increased adverse cardiac outcomes (Mann et al. 2004). Similar results were observed in the Anti-TNF-α in Congestive Heart Failure (ATTACH) trial, testing humanized neutralizing antibodies (infliximab) instead of the soluble receptor etanercept (Chung et al. 2003). The negative results of such trials may be, at least in part, explained by the inappropriate blockade of "physiological" inflammation that may be required for tissue-reparative processes.

2.5.4 Fibrosis

Galectin-3 In recent years galectin-3 (Gal-3), a soluble β-galactoside-binding lectin, has been found to play an important mechanistic role in the development

of cardiac fibrosis and remodeling and to identify high-risk subsets in HF cohorts, thus qualifying both as a risk marker and a risk factor (de Boer et al. 2009). There is a growing amount of evidence that Gal-3 is essential for migration and phagocytic activity of macrophages. Macrophage-derived Gal-3 may then act on fibroblast proliferation and on collagen synthesis, by increasing collagen I and reversing the collagen I-to-collagen III ratio (Henderson and Sethi 2009). Together with liver and kidney fibrosis, Gal-3 has been strictly linked to the development of cardiac fibrosis, a key determinant of cardiac remodeling and HF progression, possibly by interacting with mechanisms of aldosterone-mediated damage (Vergaro et al. 2016). Further, circulating levels of Gal-3 are associated with biomarkers of extracellular matrix turnover (including PIIINP, TIMP-1, and MMP-2), after adjustment for several clinical variables in a population 106 patients with systolic HF (Lin et al. 2009).

The experimental demonstration of a mechanistic involvement of Gal-3 in fibrotic, inflammatory, and remodeling processes in heart disease led to a novel interest in the potential use of Gal-3 assayed in plasma, as a biomarker. van Kimmenade and colleagues in 2006 first compared NT-proBNP, apelin, and Gal-3 in the management of acute HF patients (van Kimmenade et al. 2006). Out of 599 acutely dyspneic patients, 209 were later diagnosed with HF. Although Gal-3 showed a limited diagnostic accuracy in identifying acute HF, it was the strongest predictor of early events (60-day re-hospitalization for HF or all-cause mortality). Later on, Gal-3 was also demonstrated to predict long-term outcome (4-year mortality) in another cohort of acute HF patients (proposed cutoff: 14.97 ng/mL), independently of echocardiographic indices of cardiac structure and function (LV end-diastolic/systolic diameter, EF and right ventricular pressure) (Shah et al. 2010). In the following years the prognostic role of Gal-3 in HF settings has been investigated in some substudies from larger clinical trials. Gal-3 levels were determined in 232 NYHA III–IV chronic HF patients enrolled in the Deventer-Alkmaar HF (DEAL-HF) study, who were then followed up for a period of 6.5 years (Lok et al. 2010). Baseline Gal-3 (cutoff: 17.6 ng/ml) predicted all-cause mortality after adjustment for age, gender, creatinine clearance, and NT-proBNP. By dichotomizing the population according to NT-proBNP and Gal-3 levels, the authors also demonstrated an additive prognostic power for Gal-3, since patients with elevation of both biomarkers had a 1.5- to 2-fold higher mortality rate compared to patients in other subgroups. In a larger population of 895 chronic HF patients from the HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise TraiNing) study with LVEF <35%, Gal-3 lost its univariate prognostic value in predicting the composite outcome of all-cause death or re-hospitalization, when corrected for peak oxygen consumption at cardiopulmonary test or NT-proBNP (Felker et al. 2012).

Despite this wide amount of data confirming its prognostic role in HF, there is still limited information on whether Gal-3 assay may help in adjusting therapeutical strategies. Current knowledge is indeed limited to a benefit from statin therapy in patients with chronic HF of ischemic cause and low Gal-3 level (Gullestad et al.

2012) and to the lack of power in predicting response to mineralocorticoid receptor antagonists or to cardiac resynchronization therapy (Gandhi et al. 2015; Lopez-Andrès et al. 2012).

Recently, measurement of Gal-3 has received a class IIb recommendation in acute decompensated (level of evidence A) and in chronic (level of evidence B) HF for risk stratification purposes in the 2013 ACCF/AHA Guideline for the Management of Heart Failure (Yancy et al. 2013).

sST2 Suppression of tumorigenicity 2 protein (ST2) is a member of the Toll-like/interleukin-1 receptor superfamily, which is expressed together with its ligand interleukin-33 (IL-33) following myocardial stretch and cardiovascular injury. The IL-33/ST2 interaction exerts positive effects in terms of blunting prohypertrophic and profibrotic signals (Kakkar and Lee 2008; Pascual-Figal and Januzzi 2015; Yancy et al. 2013). Indeed, the transmembrane receptor for IL-33 (ST2 ligand, ST2L) is one of ST2 isoforms. Among others, a soluble isoform of ST2 exists, sST2, arising from a dual-promoter system driving differential mRNA expression. There is evidence that sST2 may act as a decoy receptor competing with ST2L for IL-33 binding. For example, administration of IL-33 to cultured rat neonatal cardiomyocytes inhibited the prohypertrophic signals of angiotensin II or phenylephrine, these effects being reversed by sST2 (Sanada et al. 2007). Reflecting these experimental evidences, serum levels of sST2 have been associated to LV systolic function and remodeling, as well as to a more decompensated hemodynamic profile (Shah et al. 2009). Of interest, sST2 concentration does not appear to be influenced by age, kidney function, or body mass index, unlike natriuretic peptides (Dieplinger et al. 2009b).

sST2 assay has been tested for diagnostic and prognostic purposes in either acute or chronic HF populations. Data from the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) study have shown higher sST2 concentration in subjects with acute HF compared to those with dyspnea of non-cardiac origin (Januzzi et al. 2007). In the same cohort, sST2 was reported to be the best predictor of 1-year death, outperforming NT-proBNP (hazard ratios equal to 4.6 and 2.3, respectively) (Januzzi et al. 2010). Interestingly, in a population of acute HF patients, most of them with LV systolic dysfunction, sST2 provided additional prognostic value over NT-proBNP in the prediction of death, even in subset with lower NT-proBNP (Rehman et al. 2008). Fewer studies have explored the prognostic value of sST2 in chronic HF. In a well-treated subset of patients enrolled in the HF-ACTION trial, with LVEF <35%, ST2 was only modestly associated with functional capacity while it was significantly associated with outcomes (death or hospitalization, cardiovascular death or HF hospitalization, and all-cause mortality). In the larger study in chronic HF patients, a post hoc analysis of the Val-HeFT, baseline sST2 levels added significant information to the Cox regression model of first morbid events composed of 23 clinical variables including NT-proBNP and the treatment allocation (Anand et al. 2014). In the same study, sST2 values were also available at 4 and 12 months and patients randomized to the valsartan arm showed a

milder increase in sST2 over time compared to those assigned to placebo. Further, sST2 was the only circulating biomarker, in an analysis including also growth differentiation factor-15 (GDF-15) and hs-TnT to add prognostic information to baseline concentration and to predict the occurrence of reverse remodeling when serially tested in chronic systolic HF patients (Gaggin et al. 2014). Based on these data, and on the association of neurohormonal antagonism therapy with lower sST2 concentrations, sST2 is particularly promising as a tool for patient monitoring and therapeutic optimization (Januzzi et al. 2015). Further, as for Gal-3, sST2 measurement has received a class IIb recommendation in acute decompensated (level of evidence A) and in chronic (level of evidence B) HF for risk stratification purposes in the latest American guidelines (Yancy et al. 2013).

2.5.5 Cardiorenal Syndrome

Renal impairment and worsening renal function are very common in HF, and represent a key element in disease pathophysiology, thus constituting an aspect of the clinical spectrum of HF – configuring the so-called cardiorenal syndrome – rather than a mere comorbidity. In this view, the clinical utility of both markers of renal function and of renal damage has been tested in HF patients. Although kidney dysfunction generally reflects a loss of glomerular filtration rate (GFR), it is participated by alteration in renal hemodynamics, sodium and water retention, and glomerular and tubulo-interstitial function. Biomarkers have been proposed (and tested) in HF settings to investigate each of these aspects.

Chronic kidney disease, as assessed by either GFR or serum creatinine, is strongly associated with a worse outcome in HF patients (Dries et al. 2000; Hillege et al. 2000). Worsening renal function is also frequent in HF, sometimes associated with the initiation of RAAS-acting therapy. This phenomenon, termed "pseudo-worsening renal function," is likely to have scarce effects on prognosis and may be associated with beneficial effects of ACE inhibitors in patients with LV systolic dysfunction (Clark et al. 2014; van Veldhuisen et al. 2015). Cystatin C is a small (13 kDa) protein produced by all nucleated cells, filtered and then catabolized by tubular cells, that can be considered an alternative tool to estimate renal function. A prognostic value has been demonstrated for cystatin C in chronic HF population (Damman et al. 2012) but it has never been used as a guide for HF treatment yet.

Albuminuria and proteinuria both reflect loss of integrity and damage to the glomerular membrane. Analyses from >2000 patients with chronic HF enrolled in the GISSI-HF study have shown an increasing mortality rate in subjects with normal albumin excretion, microalbuminuria or albuminuria, even after adjustment for other clinical risk factors (Masson et al. 2010b), with additive prognostic properties over GFR (Damman et al. 2011).

Some markers of tubular damage, including neutrophil-gelatinase-associated lipocalin (NGAL), kidney injury molecule (KIM)-1, and *N*-acetyl β-(d)-glucosaminidase (NAG), have been used for (early) detection of acute kidney injury. NGAL is a siderophore-binding protein expressed by various epithelial cells which is over-expressed in kidney injury and can be dosed in either serum or urine. Serum NGAL may be of help for prediction of acute kidney injury and in

risk stratification in acute HF (Aghel et al. 2010; Maisel et al. 2011b). In patients with chronic HFrEF enrolled in the CORONA study, plasma NGAL did not add significant prognostic information when included in a model with NT-proBNP and GFR (Nymo et al. 2012). Also KIM-1 and NAG are increased in chronic HFrEF and may provide clinical aid in the prediction of worsening renal function in HF, while solid evidences on their prognostic value are still lacking (Jungbauer et al. 2011).

2.5.6 Comorbidities and Cachexia

HF is a systemic syndrome and peripheral organ dysfunction may either contribute to the disease onset and progression or represent a consequence of primitive cardiac abnormalities.

Thyroid hormones are crucial for cardiovascular homeostasis and even subclinical alteration of thyroid function is associated with worsened outcome in HF patients, independently from natriuretic peptides (Passino et al. 2009). Low-triiodothyronine syndrome is a recognized entity in HFrEF but, despite some promising results from animal studies and small-scale supplementation trials, general recommendation is still lacking (Gerdes and Iervasi 2010). Among hematologic conditions, anemia is prevalent in acute and chronic HF and low hemoglobin levels hold significant prognostic value, additive to NT-proBNP (Baggish et al. 2007). Further, ferritin and transferrin saturation assessment is of key importance to identify subgroups of patients with iron deficiency, which is coming up as a therapeutical target in patients with HFrEF, as confirmed in a recent meta-analysis (Qian et al. 2016).

Cachexia and wasting frequently develop in later stages of HF, as a consequence of a complex interplay among (neuro)hormonal, immune, inflammatory dysregulation and anabolic/catabolic imbalance. Ghrelin and adiponectin are peptide hormones involved in energy balance, and are both increased in cachectic chronic HF patients. Some evidence exists that adiponectin predicts mortality in systolic HF and that treatment with the beta-blocker carvedilol decreases circulating levels of adiponectin (Tsutamoto et al. 2007; Yamaji et al. 2009).

2.5.7 Adrenomedullin

Adrenomedullin (ADM) is a peptide hormone acting as a potent vasodilator expressed by all human tissues. Circulating ADM is increased in HF and correlates with disease severity. Nonetheless, ADM is highly unstable and, recently, a novel assay measuring the mid-region of the more stable prohormone (MR-proADM) has been developed, with similar behavior in HF patients (Shah et al. 2012). Prognostic value of ADM has been tested in a cohort of 297 patients with HFrEF of ischemic origin. In this study ADM predicted the risk of mortality and of HF hospitalization independently from other clinical variables (Richards et al. 2001). Interestingly, an interaction with carvedilol therapy was reported in the same paper.

2.5.8 GDF-15

Growth differentiation factor 15 (GDF15) is a member of the TGF-ß cytokine superfamily which is highly expressed during inflammatory stress. GDF circulating

levels are increased in HF patients and correlate with the extent of myocardial fibrosis (Lok et al. 2012). Both baseline and repeated measurements of GDF-15 have additional prognostic value over hsTnT and NT-proBNP in patients with HFrEF (Chan et al. 2016). In an analysis from the Val-HeFT study, an independent, although weak, predictive value of GDF-15 for all-cause mortality was also shown, with GDF-15 levels increasing over time independently from the assignment to the placebo or the valsartan arm (Anand et al. 2010).

2.5.9 Copeptin

Arginine vasopressin (AVP), also known as antidiuretic hormone, is involved in the regulation of free water clearance, plasma osmolality, and vasomotricity. It is known since a long time that circulating AVP is elevated in HF settings (Goldsmith et al. 1983), but its assay is challenging. Copeptin, the c-terminal segment of the precursor of provasopressin, is a reliable surrogate marker for AVP and has been proved to independently predict mortality in acute HF, especially in subsets with hyponatremia (Maisel et al. 2011c). In 195 patients with chronic HFrEF, copeptin predicted 5-year all-cause mortality, although with poor additive prognostic accuracy over NT-proBNP (IDI 9.3%, NRI 8.2%) (Pozsonyi et al. 2015).

2.5.10 miRNAs

MicroRNAs (miRNAs) are non-coding RNAs which are involved in different cell processes by repressing messenger RNA translation. miRNAs have been shown to participate in several pathophysiological processes related to heart failure, including cardiac fibrosis and hypertrophy. Several circulating miRNAs have been tested for diagnostic and prognostic purposes either in acute or in chronic HF (Vegter et al. 2016). For example, Tijsen has reported that miR-423-5p is differentially expressed between HF patients, healthy controls, and patients with dyspnea of non-cardiac origin (Tijsen et al. 2010). In a small cohort of patients with chronic HF, miR-126 and miR-508-5p are associated with cardiovascular death in subsets with ischemic and non-ischemic HF, respectively (Qiang et al. 2013). Interestingly, there is experimental evidence that circulating miRNAs fluctuate in response to pharmacological HF treatment (e.g., by ACE inhibitors) and that miRNAs may represent, themselves, a therapeutical target (Vegter et al. 2016).

3 Biomarkers of Heart Failure with Mid-Range and Preserved Ejection Fraction

Among HF patients, a significant proportion exists presenting with preserved or only slightly impaired EF, especially in the elderly population. As mentioned above, these clinical entities have been defined as HFpEF and as HFmrEF in the latest European guidelines for the management of acute and chronic HF. Although specific EF cutoff has been proposed in these recommendations, in previous studies different definitions of “preserved” EF have been considered, ranging from 40 to

55%. There is therefore consistent overlap among HFpEF and HFmrEF; thus we will consider them on a whole in the following section.

Independent from its definition, HFpEF is associated with high rates of morbidity and mortality both in ambulatory and in-hospital settings, comparable to HFrEF (Senni et al. 2014). Nonetheless, while in the last few decades, several pharmacological and non-pharmacological approaches have been validated for HFrEF, up to now no single intervention has been demonstrated to modify the clinical course of HFpEF, possibly due to a consistent phenotypic variability and to the enrolment of heterogeneous population in large clinical trials (Butler et al. 2014). Different pathophysiological mechanisms indeed underlie the development of the clinical syndrome of HFpEF and the role of comorbidities is likely more relevant than in HFrEF. In the effort toward individualization that is therefore required for the management of HFpEF, circulating biomarkers – tracking specific damage pathways – may represent fundamental tools.

3.1 Natriuretic Peptides

As discussed above, natriuretic peptides are the cornerstone biomarkers in HFrEF. Nonetheless, their clinical value has been demonstrated across the whole spectrum of LV systolic function. Although to a lower extent as compared to patients with more severe reduction in EF, both BNP and NT-proBNP are increased and are key elements in the diagnosis of HFpEF. Moreover, their levels increase with more severe cardiac morphological and functional abnormalities (including hypertrophy, fibrosis, and diastolic dysfunction) (Tschope et al. 2005). Still, a clear cutoff does not exist to distinguish HFpEF from HFrEF (Parekh and Maisel 2009; van Veldhuisen et al. 2013). Further, prognostic properties of BNP are maintained independently from the extent of LV dysfunction, since, for a given BNP level, the risk of death and HF hospitalization of patients with HFpEF is as poor as in those with reduced LVEF (van Veldhuisen et al. 2013). The prognostic value of NT-proBNP has also emerged in HFpEF patients (defined as LVEF $\geq$45%) from the i-PRESERVE study, showing that baseline (proposed cutoff 339 pg/mL) and changes in NT-proBNP improve the prediction of mortality and HF re-hospitalization (Anand et al. 2011; Jhund et al. 2015).

As for the case of HFrEF, circulating levels of natriuretic peptides are influenced by several cardiac and extra-cardiac conditions (including female sex and advanced age), which is even more important in HFpEF, given the high prevalence of comorbidities. For example, a fivefold increase in NT-proBNP has been reported in subjects with HFpEF and atrial fibrillation, compared to those in sinus rhythm (McKelvie et al. 2010); likewise, renal function has been reported to significantly influence natriuretic peptide concentration (McCullough et al. 2003).

A few studies have specifically explored the effectiveness of a natriuretic peptide-guided therapy in HFpEF. Maeder and colleagues enrolled 123 patients with HF and LVEF >45%, who were randomized to medical therapy titrated to reduce symptoms to NYHA $\leq$ II or also to reduce NT-proBNP below the inclusion

threshold (>400 or >800 ng/L depending on age). Differently from patients with HFrEF, NT-proBNP-guided management tended to worsen 18-month outcomes in HFpEF (Maeder et al. 2013), thus questioning the clinical benefit of such approach, as later confirmed in a recent individual patient meta-analysis (Brunner-La Rocca et al. 2015).

3.2 Other Biomarkers in HFpEF

As mentioned before, HFrEF and HFpEF are likely sustained by a different pathophysiology, thus explaining the variable response to pharmacological and non-pharmacological treatment. Analyses of data from the Trial of Intensified versus standard medical therapy in elderly patients with congestive heart failure (TIME-CHF) have shown a different circulating biomarker profile in patients with LVEF $\leq$40% ($n = 458$) vs. those with LVEF $\geq$50% ($n = 112$). After adjustment for other clinical variables accounting for heterogeneity between populations, patients with HFpEF displayed significantly higher levels of sST2, hs-CRP, and cystatin-C. On the other hand, HFrEF patients presented higher NT-proBNP, hs-TnT, and hemoglobin (Sanders-van Wijk et al. 2015). These results suggest that markers of myocardial damage and loading are activated in HFrEF, while elevation of biomarkers of inflammation and fibrosis characterizes HFpEF.

3.2.1 sST2

Among markers of inflammation and fibrosis, sST2 seems to be promising in the clinical management of HFpEF. First data came from a prognostic study showing that sST2, although not correlating with echocardiographic indices of diastolic function, was the only biohumoral marker predicting mortality in 200 patients with dyspnea and normal LV systolic function (Shah et al. 2011). These data have been confirmed in further studies, demonstrating that prognostic value of sST2 in HFpEF was comparable to that in HFrEF, especially in acute settings (Manzano-Fernández et al. 2011).

3.2.2 Gal-3

Gal-3 has also been mechanistically involved in the proceses of cardiovascular inflammation and fibroblast proliferation and fibrosis, which are thought to be at play in the development of HFpEF. In 2011 de Boer has shown that in HFpEF patients from the Coordinating study evaluating Outcomes of Advising and Counseling in Heart Failure (COACH) (with LVEF >40%), Gal-3 appeared to have a particularly strong predictive value, compared to HFrEF patients (de Boer et al. 2011). Gal-3 also yielded significant reclassification indices in one of the largest biomarker studies in HFpEF so far, conducted in a cohort of 419 HF patients with LVEF >45% (Carrasco-Sánchez et al. 2013). The Aldo-DHF trial explored the effects of spironolactone 25 mg vs. placebo in chronic HFpEF patients. Gal-3 was associated with functional performance and quality of life, and its increase in serial measurements predicted all-cause death or hospitalization independently from

NT-proBNP. However, no specific interaction between treatment arm and Gal-3 levels could be observed (Edelmann et al. 2015).

3.2.3 GDF-15

As previously discussed, GDF-15 is a marker of cell injury and inflammation that has been shown to circulate in higher concentrations in patients with HFpEF compared to controls (Stahrenberg et al. 2010). Diagnostic and prognostic properties have been advocated for GDF-15 in HFpEF. For example, the NT-proBNP/GDF-15 ratio has been shown to properly distinguish between HFpEF and HFrEF (AUC 0.709) (Santhanakrishnan et al. 2012); further, higher GDF-15 is associated with increased risk of death and HF hospitalization in HFpEF patients, providing additional prognostic information over hsTnT and NT-proBNP (Chan et al. 2016). Although not conclusive, these data strongly support a putative role of GDF-15 – both a determinant and marker of risk – as a useful tool for HFpEF management, as well as a possible therapeutical target (Putko et al. 2014a, b).

3.2.4 Inflammatory Biomarkers

Activation of pro-inflammatory pathways is a fundamental element in HFpEF pathophysiology (Paulus 2000). Elevated levels of inflammatory cytokines, such as TNF-α, IL1, IL6, IL8, and CRP, are often observed in HFpEF patient; interestingly, circulating levels of TNF-α receptors (TNFR1 and TNFR2) are associated with the severity of diastolic dysfunction and of symptoms (Putko et al. 2014a, b). Still, scarce evidence on their prognostic role is currently available.

3.2.5 Directions

Other biomarkers have been tested for the management of HFpEF in the past years, yielding different (in some case promising) results. For example, ADM levels have been correlated to diastolic dysfunction (Yu et al. 2001), and MR-proADM, which is also emerging in HFrEF, may help in identifying new-onset HFpEF (Brouwers et al. 2014). Von Willebrand factor has been shown to additive prognostic value over NT-proBNP in 457 patients with HFpEF enrolled in the LUdwigshafen Risk and Cardiovascular Health (LURIC) study (Kleber et al. 2015). In a small study, no significant difference in microRNA levels was identified between HFrEF and HFpEF.

RAAS activation is associated with most of the pathophysiological mechanisms leading to cardiac phenotypes commonly observed in HFpEF, including hypertrophy, apoptosis, and fibrosis; therefore, from a theoretical standpoint, biomarkers of RAAS should provide useful information for disease management and, possibly, for therapeutic optimization. Two trials, Aldo-DHF and TOPCAT, have investigated the effects of spironolactone in HFpEF patients showing no significant effect on outcome, despite a beneficial effect on diastolic function (Edelmann et al. 2013; Pitt et al. 2014). Also LCZ696 has been demonstrated to reduce left atrial volume in HFpEF (Solomon et al. 2012), but its prognostic effect is still under investigation in the phase III PARAGON-HF trial.

4 Conclusions

BNP and NT-proBNP have first demonstrated how biomarkers can dramatically influence the everyday management of HF patients, providing information to the clinician far exceeding initial expectations. Despite recent advances, HF still remains a syndrome with unacceptable morbidity and mortality, possibly reflecting a complex pathophysiology and an extremely heterogeneous presentation which is only partly counterbalanced by a "one-size-fits-all" therapeutic strategy. Novel biomarkers addressing specific disease phenotypes and paths of damage will be of help for treatment tailoring and to move toward a more rational use of drugs and devices in HF. Research has recently focused on the clinical value of Gal-3 and sST2, which are both markers and effectors of cardiovascular damage, but currently, still there is no clear indication for therapeutic driving or optimization based on circulating levels of such biomarkers and, more generally, on a single HF biomarker.

For prognostic purposes, it seems reasonable that the use of multiple markers reflecting the activation of different pathophysiological pathways may more accurately identify high-risk subjects. This hypothesis has been recently tested, for example, in a large cohort of decompensated HF patients who were tested for several (novel) HF biomarkers. Elevation of at least three among MR-proADM, hs-TnT, combined free light chains, hsCRP, and sST2 provided incremental prognostic value when added to a multivariable model including BNP (NRI 32.5%) (Jackson et al. 2016). Nonetheless, the feasibility and the cost-effectiveness of multimarker strategies remain to be elucidated.

During the last years, a growing number of biomarkers have been proposed as potentially useful in HF patients, but no one of them still resembles the characteristics of the "ideal biomarker." A single marker will hardly perform for screening, diagnostic, prognostic, and therapeutic management purposes; therefore, a rational use of biomarkers will likely contemplate different analytes for different purposes. Moreover, the pathophysiological and clinical significance of biomarkers may depend on the presentation, stage, and severity of the disease (e.g., on etiology, presence/extent of LV systolic dysfunction, comorbidities). In this view, one could envisage specific sets of biomarker with different performances in HFpEF, HFmrEF, and HFrEF, especially as concerns prediction of the future course of the disease and of LV adverse/reverse remodeling.

Biomarkers are likely the most promising tools to make a step toward individualized therapy in HF. In the next future, a lot will depend on the quality (even more than on results) of clinical studies aimed at improving current diagnostic and prediction models and of studies using biomarkers to tailor therapeutics.

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Heart Failure Guidelines on Pharmacotherapy

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2017_24

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Abstract

Heart Failure (HF) is a serious emerging Public Health issue mainly in the high-income countries. In the USA, more than 6 million adults are affected. Despite the latest advances in device and pharmacological therapeutics, it still carries a huge burden, partially reflected in the annual healthcare cost of approximately $30 billion (2012) and the 5 year mortality rate of 50%. In this article, we review the medications, proven to significantly reduce mortality and morbidity in HF patients with structural myocardial disease and past or current symptoms, based on the latest North American HF guidelines. We, finally, perform a brief comparison between the former recommendations and the published 2016 HF guidelines by European Society of Cardiology.

Keywords

Guidelines • Heart failure • HFrEF • Stage C

1 Introduction

Heart Failure (HF) is a global epidemic. The prevalence of HF in the USA has increased from 5.7 million (2009–2012) to 6.5 million (2011–2014), in subjects ≥20 years old (Benjamin et al. 2017). Despite all of the advances in pharmacologic and device therapy, the 1 and 5 year mortality remains relatively high at approximately 30 and 50%, respectively. The scope of this chapter is to describe the guideline-directed medical treatment (GDMT) for subjects with stage C HF. More specifically we are going to analyze the pharmacological regimens for HF subjects with established myocardial structural disease and current or prior symptomatology, with reduced ejection fraction (EF) of ≤40% (HFrEF) or preserved EF of ≥50% (HFpEF). Both types have different pathophysiologic mechanisms and myocardial phenotypes, however, they trigger the same pathophysiologic cascade, initiated by the reduced supply of blood and oxygen to the human body.

HFpEF represents almost 50% of the total HF population, with similar morbidity and mortality rates compared to HFrEF. However, up to date there are no evidence-based therapies shown to benefit survival in HFpEF subjects. Additionally, the European Society of Cardiology (ESC) recently published the latest HF recommendations, where they introduced the novel concept of HF with a middle range EF, between 40 and 49%

(HFmrEF). Due to the recent introduction of the latter, there is scarce evidence regarding treatment, thus we are not going to make particular references to this classification.

Finally, we are going to depict the similarities and differences between the HF guidelines of ESC 2016 (Ponikowski et al. 2016) and the joint recommendations for HF treatment from the American College of Cardiology (ACC), American Heart Association (AHA) (Yancy et al. 2013a), in conjunction with the respective latest focused pharmacologic update of 2016 (Writing Committee M and Acc/Aha Task Force M 2016).

2 Role of Renin Angiotensin Aldosterone System and Sympathetic System Activity in Heart Failure

The detrimental effects of HF on exercise capacity, symptoms, and mortality can be attributed to the overactivation of Renin Angiotensin Aldosterone System (RAAS) and Sympathetic System Activity (SSA). Low cardiac output activates the sympathetic system (SS) by desensitizing the aortic arch and carotid baroreceptors and stimulating the cardiovascular low threshold polymodal receptors and peripheral chemoreceptors (Triposkiadis et al. 2009), resulting in secretion of nor-epinephrine (NE) and epinephrine (EPI) in the cardiovascular system.

The overactivation of RAAS in HF is mediated by renal hypoperfusion and increased SSA. As a result, renin is secreted by the juxtaglomerular cells, catalyzing the transformation of angiotensinogen to angiotensin I and through ACE to angiotensin II. Angiotensin II is the main concluding product of this cascade, which by stimulating specific receptors and the secretion of aldosterone, leads to vasoconstriction, oxidative stress, fibrosis, salt and water retention, and further activation of the SS (Orsborne et al. 2017).

There is ample evidence that agents targeting RAAS and SSA improve mortality, morbidity, and symptomatology in HF. Of these, angiotensin-converting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARB), beta-blockers (BB), mineralocorticoid receptor antagonists (MRA), and angiotensin receptor/neprilysin inhibitors (ARNIs) benefit survival.

From the other types of HF medications, which act through other pathways, such as diuretics, antiarrhythmic medications, vasodilators, calcium channel blockers (CCB), and statins, only a novel blocker of the funny channel, ivabradine, and the combination of hydralazine/isosorbide dinitrate improved survival, while the others only improved signs and symptoms.

In this chapter, we are initially going to describe the guideline recommendations and details about the agents that act through the RAAS and SSA pathophysiologic mechanisms, and then the other types of agents, all in subjects with stage C HF.

3 HFrEF Stage C Pharmacological Therapy

3.1 Beta-Blockers

3.1.1 Recommendations

According to the latest 2013 American HF guidelines the use of one of the three BB proven to reduce mortality (bisoprolol, carvedilol, metoprolol succinate) is recommended for all subjects with HFrEF, unless contraindicated, to reduce mortality and morbidity with a class I and level of recommendation A, which is in line with the latest ESC 2016 guidelines.

3.1.2 Mechanism of Action

Bisoprolol, like metoprolol, is a cardioselective BB, which means that it targets only the b1 receptors. It has a half-life of 9–12 h, low plasma binding and is excreted by both the liver and kidneys. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II) established the use of bisoprolol in HF. This was a multicenter trial of 2,647 patients with an EF $\leq$35%, already receiving ACE-I, randomized to bisoprolol vs placebo, with a primary endpoint of all cause mortality. CIBIS-II was stopped early, because it demonstrated that bisoprolol decreased mortality by 34% (hazard ratio (HR): 0.66, 95% confidence interval (CI) 0.54–0.81, $p < 0.0001$) (CIBIS-II Investigators and Committees 1999). The starting dose of bisoprolol is 1.25 mg, and should be uptitrated to 10 mg, if tolerable.

Sustained-release metoprolol (succinate) and carvedilol are both non-selective blockers of b1, b2 adrenergic receptors, while carvedilol also blocks a1 receptors. In the Effect of Metoprolol CR/XL in Chronic Heart Failure: Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure (MERIT-HF), almost 4,000 subjects, with EF $\leq$40% were randomized to metoprolol CR/XL vs placebo. The study was stopped early after a mean follow-up of 1 year, demonstrating a reduction in all cause mortality by 34% (HR: 0.66, 95%, CI 0.53–0.81, $p = 0.0009$). Metoprolol has a half-life of 3–7 h, low plasma protein binding and is excreted through the liver. In HF, the starting dose could be between 12.5 and 25 mg daily, and uptitrated to a target dose of 200 mg as tolerated. The BB with the most ample evidence supporting the reduction of mortality in HFrEF is carvedilol. In the Effect of Carvedilol on the Morbidity of Patients With Severe Chronic Heart Failure (COPERNICUS) study (Packer et al. 2002) 2,289 subjects with chronic stable HF but with an EF $\leq$25% were randomized to carvedilol vs placebo. After a mean follow-up of 10.4 months, it was shown that carvedilol reduced the primary endpoint of all cause mortality by 35% ($p = 0.00013$). Similarly, carvedilol reduced the combined endpoint of death from cardiovascular (CVD) hospitalization by 27% ($p = 0.00002$). This effect was consistent across several subgroups, based on ischemic or non-ischemic etiology, age, and gender. In another trial with carvedilol vs placebo in 1,959 patients with EF <40% post MI, there was an absolute risk reduction of 3.4% for total mortality in the carvedilol subgroup (Dargie 2001).

The three key trials (CIBIS II, MERIT-HF, COPERNICUS) reduced mortality and hospital admissions for HF by 34 and 28–36%, respectively. BB have proven their efficacy in patients with mild, moderate, and severe (NYHA class IV) HF. However, the beneficial effect of BB cannot be applied as a class effect. This is supported by the direct comparison of metoprolol vs carvedilol in 3,029 patients with an EF <30%, with a mean follow-up of 58 months. All cause mortality was significantly lower in the carvedilol vs the metoprolol group (34 vs 40%, respectively), though there was no statistically significant difference between the composite endpoint of mortality and all cause admission (HR: 0.94, 95% CI: 0.86–1.02, $p = 0.122$) (Poole-Wilson et al. 2003). In this study carvedilol extended survival over metoprolol, however, it should be noted that the short acting metoprolol tartrate was used and not metoprolol succinate, which was used in MERIT-HF.

BB should be started at low doses in stable patients, in addition to an ACE-I or ARB. It should also be noted that the addition of a BB to a small dose of ACE-I or ARB, rather than a single uptitration of the latter is preferred, and that in the presence or recent history of fluid overload, they should not be used without diuretics (Yancy et al. 2013b). During the early stages of use, there should be close monitoring, since hypotension and fluid retention may occur requiring pharmacotherapy adjustments. The final goal is the careful uptitration to the target doses that were effective in the major clinical studies, or the maximum tolerated doses.

3.1.3 Adverse Effects

The main side effects of BB in HF are: (1) allergic reactions to the active/non-active ingredients, (2) fatigue: this symptom could be attributed to decreased availability of oxygen to the muscles, the co-existence of skeletal muscle inflammation, anemia, and mood disorders, (3) bradycardia or advanced heart block: as a direct effect of the *b*-adrenergic receptor blockade, heart rate (HR) will decrease, and some times 2nd or 3rd degree heart block may develop. In those cases the BB should be discontinued, otherwise if there are symptoms of lightheadedness and dizziness a decrease in dose would be of some benefit, (4) hypotension: in the occurrence of hypotensive episodes, the administration of a BB or ACE-I/ARB at different times during the day, and the decrease in the diuretic dose in volume depleted subjects would be the first therapeutic steps, (5) worsening of HF and fluid retention: increase in diuretic dose and/or decrease in the BB dose may be warranted. In general, abrupt withdrawal of BB should be avoided because it can lead to acute decompensation.

Absolute contraindications to the use of BB are: (1) presence of 2nd or 3rd degree heart block (in the absence of a pacemaker), (2) known allergic reaction or other adverse reaction, (3) critical limb ischemia, (4) asthma: this is a relative contraindication, but cardioselective BB (metoprolol, bisoprolol) could be used under close monitoring. Chronic Obstructive Pulmonary Disease (COPD) is not a contraindication.

4 ACE Inhibitors

4.1 Recommendations

In the latest 2016 pharmacologic update of the HF guidelines (Writing Committee M and Acc/Aha Task Force M 2016) it is stated that all patients with HFrEF should be prescribed an ACE-I or ARB or ARNI and a BB, if tolerated as a class I level of evidence A, which is in line with the recent ESC HF guidelines.

The use of ACE-I is beneficial for patients with prior or current symptoms to reduce morbidity and mortality. Though not all ACE-I were tested in HF, it appears that there is a class effect. In a large meta-analysis of approximately 7,100 patients participating in clinical trials in HF with various ACE-I, it was shown that ACE-I significantly reduced total mortality (HR: 0.77, 95% CI: 0.67–0.88, $p < 0.001$) and the combined endpoint of mortality and HF hospitalization (HR: 0.65, 95% CI: 0.57–0.74, $p < 0.001$) (Garg and Yusuf 1995). The reductions in mortality and the combined endpoint were similar among the various subpopulations based on age, sex, race, etiology, and severity of symptoms.

The key trials, which established the use of ACE-I in HF, were CONSENSUS and SOLVD, both with enalapril. The Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) (The CONSENSUS Trial Study Group 1987) included 253 patients with NYHA class IV HF randomized to placebo vs enalapril with a target daily dose of 20 mg. By the end of the study, enalapril resulted in a 27% reduction in all cause mortality ($p = 0.003$), and significant improvement in NYHA classification, and heart size. In Studies of Left Ventricular Dysfunction (SOLVD) (The SOLVD Investigators 1991) approximately 2,500 subjects with an EF $\leq$35% were randomized to placebo vs enalapril, with a target daily dose of 20 mg. After a mean follow-up of 41 months, it was shown that enalapril significantly reduced total mortality by 16% ($p = 0.003$) and HF hospitalizations by 26% ($p = 0.0001$).

Based on these two trials, and others with ACE-I mostly in left ventricular dysfunction after myocardial infarction (MI), it is considered that the mortality and morbidity benefit of ACE-I is a class effect. However, the beneficial effect seems to occur at the higher target doses used in the trials. In Assessment of Treatment with Lisinopril and Survival (ATLAS) (Packer et al. 1999) the use of an average dose of 33.2 $\pm$ 5.4 vs 4.5 $\pm$ 1.1 mg lisinopril failed to meet the primary endpoint of mortality, in 3,164 patients with an EF $\leq$30% and a median follow-up of 45 months. However, the higher doses of lisinopril did reduce the composite secondary endpoint of death or hospitalization by 12% ($p = 0.002$), and HF hospitalizations by 24% ($p = 0.002$). Interestingly, another study assessing the effect of a daily dose of 5 vs 10 vs 20 mg enalapril on 1,500 HF subjects with NYHA class II–IV found that there was no difference between the three groups on survival and morbidity (The NETWORK Investigators 1998).

4.2 Mechanism of Action

ACE-I diminish the production of angiotensin II, and produce a subsequent reduction in the levels of aldosterone and catecholamines and an increase in renin, angiotensin I, and bradykinin, by inhibiting its degradation. ACE-I trigger a reduction in cardiac and vascular hypertrophy and extracellular matrix proliferation, and improve ventricular remodeling after an MI. Production of bradykinin and subsequently of nitric oxide (NO) have also been related with the antiatherogenic effects of ACE-I. In the kidneys, they maximize renal blood flow by decreasing renal vascular resistance, thus enhancing sodium and water excretion.

ACE-I should be started at low doses and gradually uptitrated, with a target of the clinical trials' dose if tolerated. Renal function and potassium should be checked within 1–2 weeks from initiation, and periodically thereafter. Abrupt discontinuation is strongly discouraged since it can lead to clinical deterioration.

4.3 Adverse Effects

Specific attention should be given along with close follow-up in patients with baseline hypotension, renal dysfunction, high serum potassium levels, hyponatremia, and diabetes mellitus (DM). History of angioedema or of any other allergic reaction and bilateral renal artery stenosis are absolute contraindications for the use of ACE-I. ACE-I are contraindicated during pregnancy, since they are shown to exert teratogenic effects on fetuses, such as oligohydramnios, renal dysgenesis, pulmonary hypoplasia, and neonatal death.

The main adverse reactions of ACE-I can be attributed to the suppression of angiotensin II and the accumulation of kinin. Hypotension, renal dysfunction, and hyperkalemia can occur not only because of the former, but also because of the increase in kinin and angiotensin I, which have established vasodilatory properties. Potentiation is to blame for the up to 20% rate of ACE-I induced cough and the less frequent angioedema. However, they are well tolerated by almost 90% of HF patients.

The concomitant use of NSAIDs could blunt the vasodilatory effects, while antacids reduce ACE-I availability. The use of low salt substitutes, potassium supplements, potassium sparing diuretics, MRAs, renin inhibitors with ACE-I should be done under close monitoring. When ACE-I induced cough occurs, substitution with an ARB is recommended.

5 ARBs

5.1 Recommendations

Regarding the use of ARBs in HF both sides of the Atlantic are in agreement that ARBs are recommended as class I, in patients with stage C chronic HF who cannot tolerate an ACE-I because of cough or angioedema to reduce morbidity and mortality. Both ESC and American Cardiology Associations hold a class IIb recommendation for the use of ARB in persistently symptomatic subjects with HFrEF, already on ACE-I and BB, but who cannot tolerate an aldosterone antagonist, under close observation in order to reduce morbidity and mortality. The ACC slightly differentiate from the Europeans, since in their 2013 HF guidelines recommend ARB as a class IIa to reduce morbidity and mortality in patients with HFrEF, as first line therapy (alternatives to ACE-I), especially for those already on ARB for other indications (i.e., hypertension) unless contraindicated. Finally, both guidelines do not recommend (class III: harm) the combined use of ACE-I, ARB, and MRA, due to the renal and electrolyte hazards.

5.2 Mechanism of Action

ARBs were developed, due to the belief that many of the adverse reactions of ACE-I could be attributed to the production of bradykinin. ARBs block the angiotensin receptor type I, thus blocking the harmful effects of angiotensin II from the conversion of angiotensin I through converting enzyme or other "escape" pathways. However, the production of kinins, except for the pathogenesis of angioedema and coughing, is believed to exert cardioprotective effects through vasodilating prostaglandins and NO.

The main ARBs that were tested and proved to prolong survival and reduce morbidity in HF are candesartan, valsartan, and losartan. In a study with over 5,000 subjects with HF NYHA class II–IV and an EF $\leq$40%, the subgroup randomized to a valsartan target daily dose of 320 mg had the same overall mortality, but lower mortality and morbidity (combined primary endpoint) compared to placebo (HR = 0.87, 95% CI: 0.77–0.97, $p = 0.009$) (Cohn and Tognoni 2001). The beneficial effect was consistent regardless of age, gender, cause of HF and EF. However, a post hoc analysis revealed that in subjects that were already on ACE-I and BB, the addition of valsartan caused a significant adverse increase in mortality and a trend towards higher rates of the combined endpoint of death, cardiac arrest with resuscitation, HF hospitalization or therapy with intravenous inotropes/vasodilators. The Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity (CHARM) trial with HF patients with EF $\leq$40% showed that subjects randomized to a candesartan daily target dose of 32 mg vs placebo had reduced mortality (covariate adjusted, HR: 0.90, 95% CI: 0.82–0.99, $p = 0.032$). Interestingly, in a subpart of the study CHARM-added (McMurray et al. 2003) when candesartan was added on top of ACE-I, a significant reduction in the composite primary endpoint of CVD death and HF hospitalization was

found (HR: 0.77, 95% CI: 0.67–0.89, $p = 0.0004$). However, when valsartan was added to captopril inpatients after an acute MI and left ventricular systolic dysfunction (LVSD), survival was not prolonged when compared to the captopril subpopulation ($p = 0.73$), while there was a substantial increase in adverse events (Pfeffer et al. 2003). Thus the combined use of ARB and ACE-I in HF holds a class IIb recommendation per 2013 ACC HF guidelines.

5.3 Adverse Effects

Prescription strategy is very similar to that of ACE-I with initiation at a low dose and uptitration after careful monitoring of laboratory tests and vital signs. ARBs have the same adverse reactions and contraindications to ACE-I, except for cough. Though ARBs are considered an alternative therapeutic strategy for subjects who experienced angioedema with an ACE-I, there have been reports of ARB induced angioedema, thus extreme caution should be applied to subjects with a suspicious medical history. As mentioned before, the combined use of an ACE-I, ARB, and MRA is contraindicated.

6 ARNIs

6.1 Recommendations

As mentioned earlier, in the 2016 pharmacological update, valsartan/sacubitril received a class I level A recommendation for HFrEF patients with NYHA symptoms II–III. The ACC/AHA recommended replacement with an ARNI for all patients with HFrEF, who tolerated an ACE-I or ARB and had class II–III symptoms with acceptable blood pressure. On the contrary, ESC opted to stay more attached to Prospective Comparison of ARNI with ACE-I to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM HF) trial inclusion criteria. Thus, they recommend the use of ARNI in HFrEF patients who remain symptomatic despite optimal treatment with an ACE-I, BB, and MRA, with a class IB, but only to those who fulfill the basic inclusion criteria of PARADIGM HF, which was a double blind randomized trial of 8,442 subjects with NYHA class II–IV symptoms and an EF $\leq$40% initially, which later changed to $\leq$35%, randomized to a total daily target dose of 400 mg vs placebo. The trial was terminated early, after a median follow-up of 27 months, showing a 20% reduction in the composite endpoint of death from cardiovascular causes and HF hospitalization. Furthermore, valsartan/sacubitril decreased all cause mortality, compared to enalapril by 16% (p-value <0.01). Regarding the safety of this new drug combination, the ACC/AHA issued a class III recommendation, not to use valsartan/sacubitril because of potential harm (a) concomitantly with an ACE-I, and not to start the ARNI within 36 h from the last dose of the latter and (b) in subjects with a past medical history of angioedema.

6.2 Mechanism of Action

Valsartan/sacubitril is a member of a novel pharmacologic category called ARNI consisting of the ARB valsartan and the neprilysin inhibitor sacubitril. Neprilysin is a membrane metalloendopeptidase involved in the degradation of natriuretic peptide (NP) among several endogenous molecules, expressed in several tissues especially in the kidney. By blocking neprilysin, and subsequently the catabolism of NPs, the plasma levels of Atrial Natriuretic Peptide (ANP) and BNP increase, which serves as a restorative mechanism against the increased atrial and ventricular pressures, respectively, by enhancing systemic and kidney vasodilation and natriuresis, thus decreasing preload, afterload, and the body's sodium and fluid content.

6.3 Adverse Effects

Valsartan/sacubitril should be started (1) at a low dose of 24/26 mg twice daily in patients (a) not already on ACE-I or ARB (b) with severe kidney or/and hepatic dysfunction or (2) at a dose of 49/51 mg. After 2–4 weeks and, after checking for electrolyte/kidney abnormalities, and excluding hypotensive episodes, it can be uptitrated to a target dose of 97/103 mg twice daily.

Since neprilysin is involved in the degradation pathway of beta-amyloid peptides, there have been concerns about a potential interaction with Alzheimer disease and macular degeneration. There were no warning signals in PARADIGM-HF, but there are ongoing studies exploring further the safety profile of valsartan/sacubitril. However, the most common side effects were hypotension, hyperkalemia, renal dysfunction (which could be handled with adjustment of the dose or discontinuation), and dizziness or cough.

Contraindications to its use are hypersensitivity to any of the components, prior history of angioedema, and concomitant use of ACE-I or aliskiren in patients with DM. Valsartan/sacubitril is contraindicated in pregnancy.

7 Mineralocorticoid Receptor Antagonists

7.1 Recommendations

The ACC 2013 HF guidelines recommend the use of MRA as a class I in (1) patients with HFrEF NYHA class II–IV with EF $\leq$35%. Specifically the class II patients should also have a recent cardiovascular hospitalization or an elevated BNP (2) all patients after an acute MI who have an EF $\leq$40% with symptoms of HF or who have DM in order to reduce morbidity and mortality.

Regarding the contraindications to the use of MRAs, ACC does not recommend its use (class III: harm), because of life threatening hyperkalemia or renal dysfunction, to patients with (a) creatinine levels $>$2.5 mg/dL in men/ $>$2.0 mg/dL in women (or estimated glomerular filtration rate (GFR) $<$30 mL/min/1.73 m^2) and/or

(b) potassium level >5.0 mmeq/L. The latest 2016 ESC guidelines are based on the same studies, suggesting a staged therapy, recommending as class I the use of MRA in HFrEF subjects who remain symptomatic, though already on ACE-I (or ARB) and a BB.

The recommendation for the prescription of MRA was based on two landmark trials: The Effect of Spironolactone on Morbidity and Mortality in Patients with Severe Heart Failure (RALES) and Eplerenone in Patients with Systolic Heart Failure and Mild Symptoms (EMPASIS-HF). In RALES, patients with severe (NYHA class IV) HF symptoms and EF ≤35% were randomized to a target dose of spironolactone 25 mg vs placebo. After almost 2 years of follow-up the study was terminated early because the spironolactone group had significantly lower mortality which was the primary endpoint (HR: 0.70, 95% CI: 0.60–0.82, $p < 0.001$) (Pitt et al. 1999). Accordingly, in EMPHASIS-HF, almost 2,800 patients with HFrEF, EF ≤35%, but milder symptoms (NYHA class II), were randomized to a target daily dose of eplerenone 50 mg vs placebo. The composite primary endpoint of CVD death and HF hospitalization was lower in the eplerenone arm (HR: 0.63, 95% CI: 0.54–0.74, $p < 0.001$) (Zannad et al. 2011).

7.2 Mechanism of Action

The use of MRA in HF was mandated by the finding that aldosterone levels were negatively associated with outcomes. Their beneficial effects in HF could be attributed not only to the additional diuresis caused by the blockage of aldosterone specific receptors in the collecting tubule (excreting sodium and water) but also to the antifibrotic, anti-catecholaminergic, and even vasodilatory properties of "anti-aldosteronism."

The prescription of MRA should be done under close follow-up, especially in patients who are already on ACE-I (or ARB). When subjects meet the aforementioned "renal and electrolyte" criteria, they should be started at a dose of 12.5 or 25 mg for spironolactone and 25 mg for eplerenone. In patients with borderline real function an every-other-day plan can be adopted. Concurrent use of potassium supplements or NSAIDs should be discontinued or diminished.

Potassium levels and renal laboratory tests should be done in 2–3 days and then 1 week after the initiation. The same follow-up plan should be implemented in the cases of combined ACE-I (or ARB) introduction or uptitration. When potassium levels rise (1) >5.5 meq/L a reduction in dose or discontinuation is recommended, (2) >6.0 meq/L discontinuation and immediate medical attention is required.

7.3 Adverse Effects

Beside the allergic reactions, the main side effects MRA consist of electrolyte abnormalities and renal insufficiency. Between the two approved MRAs spironolactone has pronounced antiandrogenic properties, potentially causing gynecomastia, based on its

similarity to progesterone and its non-selectiveness to aldosterone, androgen, and glucocorticoid receptors. On the other hand, eplerenone is more selective to the mineralocorticoid receptors, and thus lacks this category of side effect.

8 Hydralazine/Nitrates

8.1 Recommendations

The combination of hydralazine and isosorbide dinitrate is recommended for patients self-described as African American with NYHA class II–IV, under optimal medical treatment with ACE-I and BB, to reduce morbidity and mortality as class I by the 2013 ACC HF guidelines. The ESC is more meticulous in the selection of patients, since hydralazine-isosorbide dinitrate is recommended as a class IIa for self-identified African-American subjects with either an EF $\leq$35% or with an EF $\leq$45% combined with a dilated left ventricle.

Furthermore while the 2013 American HF guidelines recommend the use of the above combination in subjects who cannot tolerate an ACE-I (or ARB), in order to reduce mortality and morbidity as a class IIb, European guidelines recommend hydralazine-isosorbide dinitrate as a class IIb and only for a reduction in mortality.

The African American Heart Failure Trial (A-HeFT) consisted of 1,050 black subjects randomized to a total daily dose of 225 mg hydralazine and 120 mg of isosorbide dinitrate vs placebo. Participants had HF with NYHA class II–IV symptoms, and had either an EF $\leq$35% or EF $\leq$45% and a left ventricular diameter $>$6.5 cm or $>$2.9 cm/body surface area. The study was terminated early because the group under the active combination drug had a significantly lower mortality (HR: 0.57, $p = 0.02$) and HF hospitalization ($p = 0.001$) compared to placebo (Taylor et al. 2004). However, the beneficial effects of this combination cannot be applied to other ethnic groups, even more so after the publication of controversial results in HF. An additional problem with hydralazine-isosorbide dinitrate was that the studies were done during the late 1980s–early 1990s, when the adoption of ACE-I and BB was relatively low, as was the diversity of the study designs.

8.2 Mechanism of Action

Isosorbide dinitrate exerts vasodilatory effects, by providing a source of NO, but has also been found to inhibit platelets aggregation, decrease afterload and preload (by dilating the lower large veins of the human body), and reduce the myocardial oxygen demand. The actual mechanism of action of hydralazine is not completely understood, but is believed to control the release of calcium from the myocardial sarcoplasmatic reticulum, however, its co-administration with isosorbide dinitrate diminishes the development of nitrate tolerance.

The starting dose should be 1 tablet of 37.5/20 mg of hydralazine/isosorbide dinitrate, respectively, 3 times daily and it can be increased to 2 tablets 3 times daily. The two active drugs can also be used separately.

8.3 Adverse Effects

Frequent adverse effects are hypotension, headache, dizziness, and gastrointestinal complaints. Additionally, hydralazine hydrochloride has been reported to cause drug induced systemic lupus erythematosus and peripheral neuritis. Absolute contraindication to their use is only the presence of allergic reaction to one of the two ingredients. Regarding pregnancy, hydralazine/isosorbide dinitrate belongs to category C.

9 Ivabradine

9.1 Recommendations

In the recent joint ACC/AHA HF guidelines update, ivabradine was given a class IIa recommendation for symptomatic HFrEF subjects with NYHA class II–III, who are already receiving GDMT, including a BB at maximum tolerated dose and a HR $>$70 bpm at rest, while in sinus rhythm. In 2016 the ESC HF guidelines gave ivabradine the same level of recommendation IIa. ESC recommends the use of ivabradine (and Valsartan/sacubitril), in HFrEF subjects irrespective of their HF symptoms classification. However, since the relevant landmark trials for ivabradine and Valsartan/sacubitril had a modest representation of advanced HF patients, their American counterparts chose to demarcate the use of these two novel compounds only to patients with HF symptoms NYHA class II–III.

9.2 Mechanism of Action

Ivabradine is a molecule blocking the funny (f) ion channels. The current through this channel is a mixed inward sodium/potassium current, activated in hyperpolarization. Ivabradine decreases the HR by targeting the repetitive activity of the myocardial pacemaker cells, and by delaying diastolic depolarization of the sinoatrial node cells. Their main advantage over the classic BB in managing HR is the lack of negative inotropism of the former (DiFrancesco and Borer 2007).

The results of the Effects on Outcomes of Heart Rate Reduction by Ivabradine in Patients with Congestive Heart Failure: Is There an Influence of Beta-Blocker Dose? (SHIFT) Trial (Swedberg et al. 2010), which came out in 2010, constitute the basis for the updated recommendation. The SHIFT trial was a randomized double blind worldwide study with 6,558 HFrEF subjects and a median follow-up period of 22.9 months. The primary endpoint, a composite of cardiovascular death or HF hospitalization, was met with a HR of 0.82, 95% CI 0.75–0.90, $p < 0.0001$, by the subgroup of patients who were randomized to a total daily dose of 15 mg

ivabradine (compared to placebo). Death from HF was significantly lower in the ivabradine subgroup (HR = 0.74, 95% CI 0.58–0.94, $p = 0.014$), however, all cause and cardiovascular mortality did not differ between the treated and placebo subjects.

Ivabradine should be started with a dose of 5 mg twice daily and uptitrated to a target dose of 7.5 mg twice daily if well tolerated, after 2 weeks of vital signs monitoring. In subjects older than 75 years old a starting dose of 2.5 mg may be warranted. If the HR drops less than 50 bpm or if symptoms of bradycardia occur (i.e., dizziness, fatigue) ivabradine can either be downtitrated or stopped. If atrial fibrillation occurs, the medication has to be discontinued.

9.3 Adverse Effects

Ivabradine has a satisfactory safety profile. The most common side effects were bradycardia, hypertension, and atrial fibrillation. Phosphenes, which are a transient enhanced brightness in a restricted area of the visual field, have also been reported and the patient and doctor should be aware of this effect. Contraindications to the use of ivabradine consist of acute decompensation of HF, systolic blood pressure <90 mmHg, severe hepatic impairment, resting HR less than 60 bpm at baseline, pacemaker dependence, bradyarrhythmias, and the co-administration of strong cytochrome CYP3A4 inhibitors. It cannot be used in pregnancy.

10 Diuretics

10.1 Recommendations

Diuretics are the cornerstone of therapy for HF patients who present with fluid overload. Even though, for ethical reasons, there are no randomized trials investigating the mortality benefit of diuretics, the 2013 American guidelines recommend as a class I the use of diuretics in patients with HFrEF, with evidence of fluid retention to improve symptoms. The 2016 European HF guidelines have extended the class I use of diuretics by the same group of patients to improve exercise capacity, while they added a class IIa for the reduction of HF hospitalizations.

10.2 Mechanism of Action

There are several categories of diuretics, acting on different sites of the renal tubule. We are not going to describe the mechanism of action and the specific adverse effects of the various diuretics, since it is not within the scope of this manuscript and they have already been analyzed elsewhere. However, loop diuretics (furosemide, bumetanide, torsemide, and ethacrynic acid) have emerged as the diuretic of choice in HF patients.

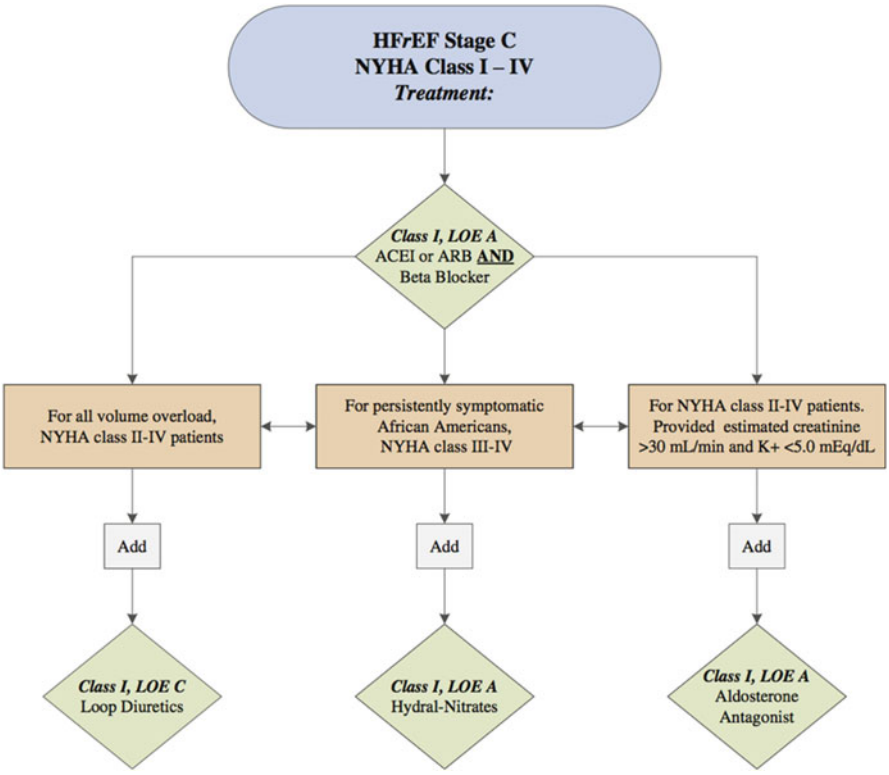

Fig. 1 Stage C HFrEF: evidence-based, guideline-directed medical therapy. Reproduced with permission by Yancy et al. (2013a). Abbreviations: *ACEI* angiotensin-converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *HFrEF* Heart Failure with reduced ejection fraction, *Hydral-Nitrates* hydralazine and isosorbide dinitrate, *LOE* level of evidence, and *NYHA* New York Heart Association

The most common loop diuretic is furosemide, but some patients may respond better to another one (i.e., torsemide, bumetanide) because of their better bioavailability. Furosemide in outpatient HF should be started in low doses and should be uptitrated accordingly in order to increase or sustain diuresis and weight reduction. Patients may become unresponsive in the case of development of diuretic resistance, use of NSAIDs, consumption of large amounts of sodium, and a substantial reduction in renal function. In cases of diuretic resistance, an increase in the dose or the addition of thiazide/metolazone, causing sequential blockage of the renal tubule electrolyte receptors could be of some value.

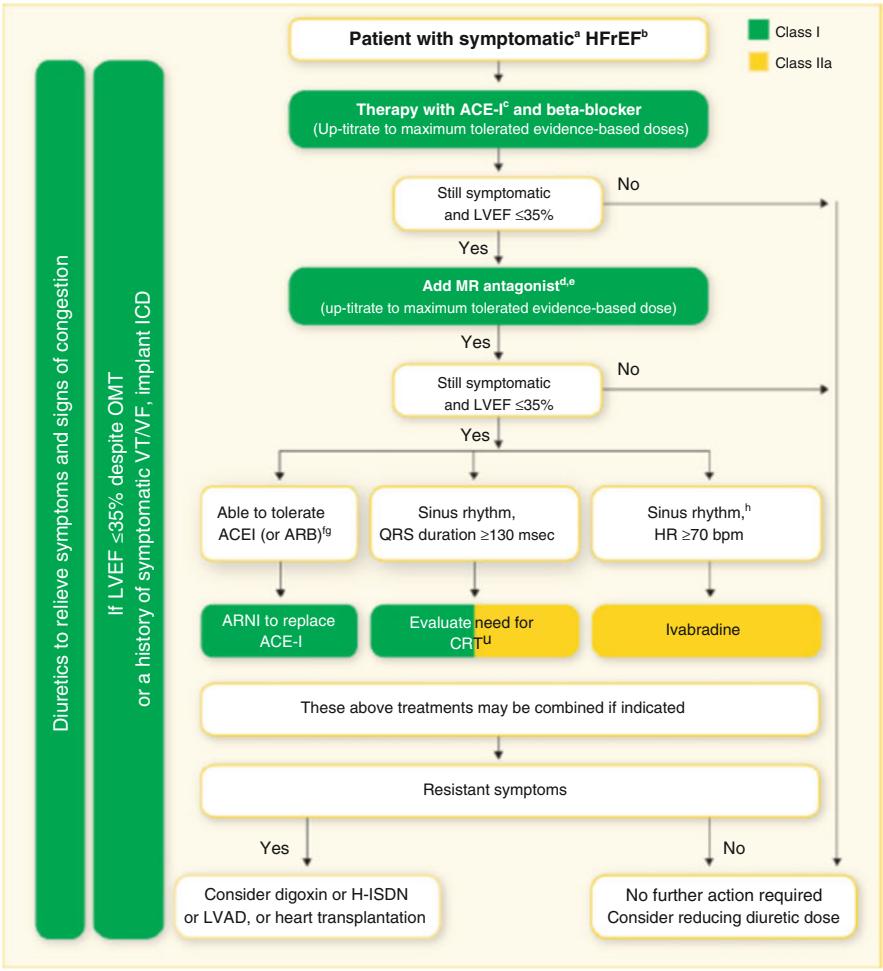

Fig. 2 Therapeutic algorithm for a patient with symptomatic Heart Failure with reduced ejection fraction. Reprinted with permission of Oxford University Press on behalf of the European Society of Cardiology, https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines/Acute-and-Chronic-Heart-Failure. *Green* indicates a class I recommendation; *yellow* indicates a class IIa recommendation. Abbreviations: *ACEI* angiotensin-converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *ARNI* angiotensin receptor neprilysin inhibitor, *BNP* B-type natriuretic peptide, *CRT* cardiac resynchronization therapy, *HF* Heart Failure, *HFrEF* Heart Failure with reduced ejection fraction, *H-ISDN* hydralazine and isosorbide dinitrate, *HR* heart rate, *ICD* implantable cardioverter defibrillator, *LBBB* left bundle branch block, *LVAD* left ventricular assist device, *LVEF* left ventricular ejection fraction, *MR* mineralocorticoid receptor, *NT-proBNP* N-terminal pro-B type natriuretic peptide, *NYHA* New York Heart Association, *OMT* optimal medical therapy, *VF* ventricular fibrillation, *VT* ventricular tachycardia. [a]Symptomatic = NYHA class II–IV. [b]HFrEF = LVEF, 40%. [c]If ACE inhibitor not tolerated/contraindicated, use ARB. [d]If MR antagonist not tolerated/contraindicated, use ARB. [e]With a hospital admission for HF within the

10.3 Adverse Effects

The main side effects include (1) electrolyte abnormalities, such as hyponatremia, low potassium, and magnesium levels which could be potentially arrhythmogenic, and (2) volume depletion which can lead to hyperuricemia/gout, hypotension, and renal insufficiency.

Diuretics are contraindicated in patients who have had an allergic reaction, or have never had symptoms or signs of congestion.

11 Other Recommended Medications for Treatment of HFrEF Stage C

Finally, (1) digoxin could be considered in order to decrease morbidity/HF hospitalization, in subjects with HFrEF as a class IIa in American guidelines and IIb in the European counterparts.

(2) Omega-3 polyunsaturated fatty acids (PUFAs) could be considered as adjunctive therapy in HFrEF or HFpEF patients with NYHA class II–IV, in order to reduce mortality and CVD hospitalizations as classes IIa and IIb according to the 2013 American and ESC HF guidelines, respectively.

Figures 1 and 2 depict the therapeutic strategy for stage C HFrEF based on 2013 North American and 2016 ESC HF guidelines, respectively.

The recommended starting and maximum doses of the most commonly used medications in HFrEF stage C are shown in Table 1.

12 HFpEF Stage C Pharmacological Therapy

Both 2013 North American and 2016 ESC HF guidelines recommend the use of diuretics in congested patients to relieve symptomatology as a class I.

The 2013 ACC HF guidelines differentiate from their European counterparts by granting a IIb recommendation for the use of ARB in HFpEF, resulting in the decrease of HF hospitalizations.

Up to date there is no pharmacological treatment proven to benefit survival in subjects with current or past symptomatology and HFpEF. All HFpEF trials, with

Fig. 2 (continued) last 6 months or with elevated natriuretic peptides (BNP 250 pg/mL or NTproBNP 500 pg/mL in men and 750 pg/mL in women). [f]With an elevated plasma natriuretic peptide level (BNP $\geq$150 pg/mL or plasma NT-proBNP $\geq$600 pg/mL, or if HF hospitalization within recent 12 months plasma BNP $\geq$100 pg/mL or plasma NT-proBNP $\geq$400 pg/mL). [g]In doses equivalent to enalapril 10 mg b.i.d. [h]With a hospital admission for HF within the previous year. [i]CRT is recommended if QRS $\geq$130 ms and LBBB (in sinus rhythm). [j]CRT should/may be considered if QRS $\geq$130 ms with non-LBBB (in a sinus rhythm) or for patients in AF provided a strategy to ensure bi-ventricular capture in place (individualized decision)

Table 1 Medications most commonly used in stage C HFrEF

	Initial daily dose(s) (mg)	Maximum dose(s) (mg)
ACE-I		
Captopril	6.25 TID	50 TID
Enalapril	2.5 BID	10–20 BID
Lisinopril	2.5–5.0 QD	20–35 QD
Ramipril	2.5 QD	10 QD
Trandolapril	0.5 QD	4 QD
Beta-blockers		
Bisoprolol	1.25 QD	10 QD
Carvedilol	3.125 BID	25 BID
Metoprolol succinate (CR/XL)	12.5–25 QD	200 QD
Nebivolol	1.25 QD	10 QD
ARBs		
Candesartan	4–8 QD	32 QD
Valsartan	40 BID	160 BID
Losartan	50 QD	150 QD
MRAs		
Spironolactone	12.5–25 QD	50–150 QD or BID
Eplerenone	25 QD	50 QD
ARNI		
Sacubitril/valsartan	49/51 BID	97/103 BID
If-channel blocker		
Ivabradine	5 BID	7.5 BID
Loop diuretics		
Furosemide	20–40 QD or BID	600
Bumetanide	0.5–1.0 QD or BID	10
Torsemide	10–20 QD	200
Thiazides		
Chlorothiazide	250–500 QD or BID	1,000
Chlorothalidone	12.5–25 QD	100
Hydrochlorothiazide	25 QD or BID	200
Metolazone	2.5 QD	20
Indapamide	2.5 QD	5
Potassium sparing diuretics		
Amiloride	2.5 QD	5
Triamterene	50–75 BID	200
Hydralazine hydrochloride and isosorbide dinitrate		F
Fixed dose combination	37.5 mg hydralazine/20 mg isosorbide dinitrate TID	75 mg hydralazine/40 mg isosorbide dinitrate TID
Hydralazine hydrochloride	25–50 mg 3–4 times daily	300 mg daily in divided doses
Isosorbide dinitrate	20–30 mg 3–4 times daily	120 mg in divided doses

Abbreviations: *ACE-I* angiotensin-converting enzyme inhibitors, *ARBs* angiotensin receptor blockers, *ARNI* angiotensin receptor-neprilysin inhibitor, *MRAs* mineralocorticoid receptor antagonists, *QD* once daily, *BID* twice daily, *TID* three times daily

BB, ACEI, ARB, MRA, ARNI, demonstrated inconsistent results. The only medication that has shown to decrease mortality, in an elderly population, 36% of which had an EF >35%, thus including HFpEF, was nebivolol.

In this study, 2,128 HF subjects ≥70 years old were randomized to a target daily dose of 10 mg nebivolol vs placebo. In the nebivolol group there was a significant reduction in the composite endpoint of all cause mortality and cardiovascular readmissions (HR: 0.86, 95% CI 0.74–0.99, $p = 0.039$) (Flather et al. 2005). However, the subanalysis in subjects with EF >35% showed the same trend, but didn't reach statistical significance. However, due to the design and the results of the former, both guidelines didn't include nebivolol in their official recommendations.

Candesartan, in a population of HF patients with NYHA II–IV and EF>40%, failed to meet the primary endpoint, but statistically reduced the secondary composite endpoint of CVD death and hospitalizations due to HF, MI or stroke (HR: 0.86, 95% CI: 0.75–0.99, $p = 0.037$), and HF hospitalizations (HR: 0.84, 95% CI: 0.84–1.00, $p = 0.047$), which was one of the two components of the primary endpoint (Yusuf et al. 2003).

Spironolactone is another medication with some evidence in reducing rehospitalizations in HFpEF. "Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT)" was a randomized double blind clinical study of 1,723 symptomatic patients with EF ≥45%, to spironolactone vs placebo. The trial, after a mean follow-up of 3.3 years failed to meet the composite primary endpoint of CVD death, aborted cardiac arrest, or HF hospitalization ($p = 0.14$). However, it was noted that the active group had less HF hospitalizations compared to placebo (HR: 0.83, 95% CI: 0.69–0.99, $p = 0.04$) (Pitt et al. 2014).

Currently, there is a consensus from both sides of the Atlantic, reflected in the recommendations, that the treatment of HFpEF should be targeting the underlying comorbidities, considered to play a critical role in the increased morbidity and mortality burden, such as hypertension and coronary artery disease.

Disclosures

LP, CEH report no disclosures. JB reports receiving research support from the National Institutes of Health and European Union, and serves as a consultant to Amgen, Astra Zeneca, Bayer, Boehringer Ingelheim, Cardiocell, Gilead, Merck, Novartis, Relypsa, Z Pharma.

Support

None.

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Sacubitril/Valsartan (LCZ696) in Heart Failure

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Abstract

It has been known since the 1990s that long-term morbidity and mortality is improved in patients with heart failure with reduced ejection fraction (HFrEF) by treatments that target the renin–angiotensin–aldosterone system (RAAS). It has also long been thought that enhancement of the activity of natriuretic

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_77

peptides (NPs) could potentially benefit patients with HFrEF, but multiple attempts to realize this benefit had failed over the years – until 2014, when a large, phase III, randomized, controlled clinical trial (PARADIGM-HF) was completed comparing sacubitril/valsartan with enalapril, a well-established treatment for HFrEF. Sacubitril/valsartan (formerly known as LCZ696) is a first-in-class angiotensin receptor neprilysin inhibitor (ARNI) that simultaneously suppresses RAAS activation through blockade of angiotensin II type 1 receptors and enhances vasoactive peptides including NPs through inhibition of neprilysin, the enzyme responsible for their degradation. In PARADIGM-HF, patients with HFrEF treated with sacubitril/valsartan had 20% less risk for cardiovascular death or hospitalization for heart failure (the primary endpoint), 20% less risk for cardiovascular death, 21% less risk for first hospitalization for heart failure, and 16% less risk for death from any cause, compared with enalapril (all $p < 0.001$). Concerning tolerability, the sacubitril/valsartan group had higher proportions of patients with hypotension and nonserious angioedema but lower proportions with renal impairment, hyperkalemia, and cough, compared with the enalapril group. The use of sacubitril/valsartan has been endorsed by the latest heart failure treatment guidelines in Europe and the USA. This chapter reviews the discoveries, scientific reasoning, and clinical evidence that led to the development of sacubitril/valsartan, the first novel therapy in a new drug class to improve survival in HFrEF in the last 15 years.

Keywords

HFrEF • Natriuretic peptides • Neprilysin • PARADIGM-HF • Sacubitril/valsartan

Abbreviations

ACE	Angiotensin-converting enzyme
ACEI	Angiotensin-converting enzyme inhibitor
AE	Adverse event
ANP	Atrial natriuretic peptide
ARB	Angiotensin receptor blocker
ARNI	Angiotensin receptor neprilysin inhibitor
AT_1	Angiotensin II type 1
AUC	Area under the plasma concentration curve
bid	Twice daily
BNP	B-type natriuretic peptide
BP	Blood pressure
cGMP	Cyclic guanosine monophosphate

CI	Confidence interval
C_{max}	Maximum plasma concentration
CNP	C-type natriuretic peptide
CSF	Cerebrospinal fluid
CV	Cardiovascular
ED	Emergency department
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
EMPHASIS-HF	Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure
HbA1c	Glycated hemoglobin
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Hazard ratio
hsTnT	High sensitivity troponin T
KCCQ	Kansas City Cardiomyopathy Questionnaire
LVEF	Left ventricular ejection fraction
MAGGIC	Meta-analysis Global Group in Chronic Heart Failure
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial infarction
NEP	Neprilysin
NEPI	Neprilysin inhibitor
NNT	Number needed to treat
NP	Natriuretic peptide
NPR	Natriuretic peptide receptor
NT-proBNP	N-terminal pro B-type natriuretic peptide
NYHA	New York Heart Association
OATP	Organic anion transporter protein
PARADIGM-HF	Prospective comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure
PARAMOUNT	Prospective comparison of ARNI with ARB on Management Of heart failUre with preserved ejectioN fraction
PP	Pulse pressure
PRA	Plasma renin activity
PRC	Plasma renin concentration
RAAS	Renin–angiotensin–aldosterone system
SNS	Sympathetic nervous system
SOLVD	Studies of Left Ventricular Dysfunction
T_{max}	Time to peak concentration

1 Introduction

Over the past several decades, our understanding of the complex pathophysiology of heart failure (HF) has progressed considerably, leading to the development of new treatments that have significantly reduced morbidity and improved survival in patients with heart failure with reduced ejection fraction (HFrEF) (Katz 2008). There has remained, nonetheless, a high residual burden of morbidity and mortality in these patients, and an even greater burden in patients with heart failure with preserved ejection fraction (HFpEF). There has continued to be an urgent need for new therapies that improve clinical outcomes in patients with HF (Mozaffarian et al. 2015).

In 2014, a large ($N = 8{,}442$ patients) phase III clinical trial (PARADIGM-HF) provided compelling evidence for improved outcome in patients with HFrEF treated with a new class of therapeutic agent, an orally active angiotensin receptor neprilysin inhibitor (ARNI). In this trial, sacubitril/valsartan (formerly known as LCZ696), a first-in-class ARNI, was compared with enalapril, an angiotensin-converting enzyme inhibitor (ACEI) that has long been established as a treatment that reduces mortality and morbidity in patients with HFrEF (SOLVD 1991). It was found that patients treated with sacubitril/valsartan had significantly fewer deaths from cardiovascular (CV) causes ($p < 0.001$) and a significantly better outcome ($p < 0.001$) for the primary endpoint (CV death or hospitalization for HF) compared with those who received enalapril (McMurray et al. 2014b). In a population of randomized patients with HFrEF followed for a median interval of 27 months, the primary outcome occurred in 21.8% of patients treated with sacubitril/valsartan compared with 26.5% of patients treated with enalapril (hazard ratio, HR: 0.80; 95% confidence interval, CI: 0.73–0.87; $p < 0.001$).

This novel addition to the pharmacological armamentarium for the treatment of HFrEF was the fruition of decades of prior study and learning from unsatisfactory outcomes. The aims of this chapter are to review the discoveries, trials, and scientific reasoning that led to the initial development of sacubitril/valsartan as a novel treatment for HFrEF and the evidence from clinical trials that established its efficacy and safety profile.

2 Historical Background

Prior to the development of sacubitril/valsartan, HF outcomes had been improved primarily through the use of pharmacological interventions that targeted the renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system (SNS) activation, and implantable devices for defibrillation and resynchronization. The pathological roles played by persistent activation of RAAS and SNS had been well-recognized for several decades. Over the same epoch, there developed a substantial body of evidence regarding natriuretic peptide (NP) systems and potential benefits that might derive from interventions that enhanced their activity. Even

so, realization of these benefits had remained out of reach for several reasons outlined later in this chapter.

2.1 Therapies Targeting the Renin–Angiotensin–Aldosterone System

Understanding the pathological role of RAAS activation in HF advanced significantly in the 1970s when intravenous teprotide, one of the earliest synthetic ACEIs, was shown to reduce renal vasoconstriction, lower arterial pressure in patients with hypertension, and improve disordered hemodynamics in patients with HF. This was followed by the development of the orally active ACEI captopril, and later, enalapril. In a phase III randomized clinical trial (SOLVD-T), enalapril was found to reduce the risk of all-cause mortality by 16% and death or rehospitalization for HF by 26% in patients with HFrEF, compared with placebo (SOLVD 1991). By the mid-1990s, ACEIs were well-established as the standard of care in the management of HF (Cohn 1996; Williams et al. 1995) and hypertension (Unger and Gohlke 1994; Waeber and Brunner 1990).

The demonstrated benefits of treatment with ACEIs spurred the development of a second class of drugs that also suppressed RAAS overactivation but did so by blocking the angiotensin II type 1 (AT_1) receptor (Kang et al. 1994). Interest in angiotensin receptor blockers (ARBs) derived primarily from tolerability issues associated with ACEIs such as persistent dry cough (Sebastian et al. 1991) and angioedema, a rare but potentially life-threatening adverse event (AE) (Makani et al. 2012). These events are probably a consequence of increased levels of bradykinin and other mediators of inflammation that are metabolized by angiotensin-converting enzyme (ACE) (Sanchez-Borges and Gonzalez-Aveledo 2010). Because ARBs do not block the degradation of kinins, persistent cough and angioedema are uncommon with their use. Clinical use of ARBs began with losartan as a therapy for hypertension (Carr and Prisant 1996). Subsequent investigations established that treatment with the ARBs valsartan and candesartan significantly improved CV outcomes in patients with HFrEF, although ARBs did not consistently reduce mortality (Baguet et al. 2009; Cohn et al. 2001; McMurray et al. 2003).

2.2 Natriuretic Peptides

2.2.1 Discovery and Biological Roles

In ground-breaking studies performed in the 1970s, de Bold and colleagues described granules in mammalian atrial cardiocytes that contained a protein or polypeptide and hypothesized that atrial tissue had secretory as well as contractile function (de Bold et al. 1978). They showed that infusion of atrial extracts into the rat elicited a >30-fold increase in sodium excretion, a tenfold increase in urine volume, and a reduction in blood pressure (BP) (de Bold et al. 1981). The active

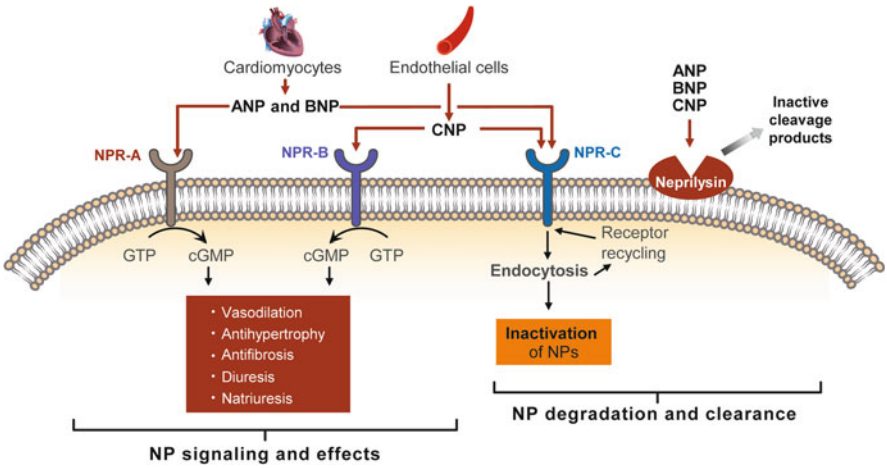

Fig. 1 Physiological effects and clearance mechanisms of natriuretic peptides. *ANP* atrial natriuretic peptide, *BNP* B-type natriuretic peptide, *CNP* C-type natriuretic peptide, *cGMP* cyclic guanosine monophosphate, *GTP* guanosine triphosphate, *NP* natriuretic peptide, *NPR* natriuretic peptide receptor. Adapted from Bayes-Genis et al. (2016) with permission

substance in these atrial extracts was subsequently identified as atrial natriuretic peptide (ANP).

Closely thereafter, two related NPs were isolated from porcine brain: brain or B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). These were later found to be released predominantly from the ventricles of heart and endothelial cells, respectively (Mukoyama et al. 1991; Sudoh et al. 1988; Sudoh et al. 1990). Subsequently, it was established that ANP and BNP are endocrine hormones released physiologically from cardiomyocytes in response to cardiac stretch and regulate fluid homeostasis and BP (Ogawa and de Bold 2014). Natriuretic Peptides act on target organs via interaction with NP receptors (NPR-A and NPR-B) coupled to cyclic guanosine monophosphate (cGMP) signaling pathways. They have a direct dilatory effect on the vascular system that decreases systemic vascular resistance, arterial pressure, and ventricular preload (Fig. 1). In addition, they have diuretic and natriuretic actions that contribute to the regulation of sodium and water balance, blood volume, and arterial pressure. They also suppress sympathetic input to the heart and increase vagal afferent activity, and they have growth-suppressing, antiproliferative, and anti-fibrotic activities that suppress interstitial remodeling in myocardium. Natriuretic peptides thus have multiple actions that protect the CV system from the negative effects of volume and pressure overload (Bayes-Genis et al. 2016; Langenickel and Dole 2012).

Under physiological conditions, NPs are rapidly degraded by enzymatic hydrolysis and deactivated by receptor-mediated clearance via NPR-C (Fig. 1) (Nakao et al. 1986; Potter 2011; Stephenson and Kenny 1987). The enzymatic degradation is primarily mediated by a neutral glycosylated zinc endopeptidase that also degrades other peptides including adrenomedullin, bradykinin, vasoconstrictors

such as angiotensin I and II, endothelin-1, as well as oxytocin, opioid peptides, substance P, gastrin, vasoactive intestinal peptide, apelin, and amyloid beta (Aβ) (Lisy et al. 1998; Liu et al. 2010; McKinnie et al. 2016). This enzyme has been identified by many names, including atriopeptidase, neutral endopeptidase, EC 3.4.24.11, enkephalinase, common acute lymphoblastic leukemia antigen, CD10, but it is commonly known as neprilysin (NEP) (Potter 2011). This enzyme is present in abundance in the renal cortex.

In addition to their physiological roles, NPs also emerged as important biomarkers for use in diagnosis and risk stratification of patients with HF because their levels are substantially elevated in these patients (Daniels and Maisel 2007). It was initially thought that NPs are upregulated in HF, but some recent studies have brought this into question. Circulating levels of mature BNP (BNP1-32) may actually be reduced in patients with HF due to altered processing of proBNP, resulting in the formation of precursors and degradation products of BNP1-32 that cross-react with current clinical assays but are nonetheless less biologically active (Hawkridge et al. 2005; Miller et al. 2011; Niederkofler et al. 2008).

2.2.2 Therapeutic Enhancement of Natriuretic Peptide Activity in Patients with HF

The growth in understanding of the homeostatic function of NPs stimulated the interest in finding interventions that would therapeutically enhance NP activity in patients with HF. An early approach was acute parenteral infusion of recombinant human BNP (nesiritide) in patients hospitalized with acute decompensated HF – a form of therapy that would not be applicable over the long term in patients with chronic HF. Initial results with nesiritide suggested favorable hemodynamic, neurohormonal, and renal actions, along with a reduction in dyspnea in patients with acute HF (Colucci et al. 2000; Publication Committee and for the VMAC Investigators 2002). However, no benefit was observed in reducing death or hospitalization in a large follow-up study (O'Connor et al. 2011). Carperitide is a synthetic form of human ANP that has been widely used in Japan as a treatment for acute HF, although retrospective analysis has challenged the effectiveness of the drug (Matsue et al. 2015). Ularitide – a synthetic form of the human natriuretic peptide urodilatin – failed to meet the primary endpoint in phase III trial in acute HF (Packer et al. 2016).

Inhibition of NEP has long been of interest as an approach to therapeutic intervention. Early studies of the nonselective neprilysin inhibitor (NEPI), thiorphan, investigated its potential as an antinociceptive agent (Roques et al. 1980). The NEPIs acetorphan (later known as racecadotril) and candoxatrilat were found to increase NP levels in the plasma and enhance diuresis (Gros et al. 1989; Northridge et al. 1989). Subsequent study suggested that candoxatrilat had the ability to reduce right atrial pressure and pulmonary capillary wedge pressure in patients with mild HF (Northridge et al. 1992). Similar effects were reported with the NEPI sinorphan (the s-enantiomer of the racemate acetorphan, also known as ecadotril) (Kahn et al. 1990). A number of novel NEPIs (including sacubitril) were synthesized by Ksander and colleagues in the early 1990s and shown in animal

models to have pharmacological actions that could be useful in treating hypertension or HF (Ksander et al. 1995).

Despite these encouraging early observations, subsequent investigations revealed only modest efficacy with NEPIs in patients with hypertension or HF (Ando et al. 1995; Bevan et al. 1992; Cleland and Swedberg 1998). This can possibly be attributed to simultaneous enhancement of physiologically antagonistic neurohumoral systems (RAAS) with NEP inhibition, resulting in increased levels of vasoconstrictor peptides including angiotensin II that neutralize the vasodilatory and natriuretic actions of NPs (Gafford et al. 1983; Richards et al. 1993; Stephenson and Kenny 1987).

2.2.3 Natriuretic Peptide Enhancement Combined with Inhibition of Angiotensin-Converting Enzyme

In the 1990s, investigators began to explore the potential benefits of combining RAAS inhibition with enhancement of NPs as a therapeutic strategy. Seymour and colleagues found that simultaneous administration of the ACEI captopril and the selective NEPIs (SQ 29,072 and SQ 28,603) elicited greater reductions in arterial pressure in rat and dog models of hypertension than that observed with each agent given separately (Seymour et al. 1993; Seymour et al. 1991). This was followed by similar observations in animal studies conducted in monkeys (Seymour et al. 1995), sheep with pacing-induced HF (Rademaker et al. 1998), and in studies in patients with essential hypertension (Favrat et al. 1995). These results provided impetus for the development of orally active dual inhibitors of ACE and NEP, sometimes referred to as "vasopeptidase" inhibitors (Burnett 1999).

Omapatrilat was the most extensively studied vasopeptidase inhibitor (Weber 2001). In the cardiomyopathic hamster, an experimental model of HF, omapatrilat improved hemodynamic function and prolonged survival more than an ACEI alone (Trippodo et al. 1999). Results from an early clinical trial (IMPRESS, $N = 573$) suggested a favorable trend for omapatrilat for the combined endpoint of death or hospitalization for worsening HF, as well as a greater renal benefit, compared with lisinopril (Rouleau et al. 2000). In the subsequent phase III OVERTURE trial ($n = 5{,}770$), omapatrilat 40 mg once daily was non-inferior to the ACEI enalapril 10 mg twice daily in reducing the primary endpoint of all-cause mortality or hospitalization for HF and was superior to enalapril in reducing the secondary endpoint of CV death or CV hospitalization (Packer et al. 2002). A very large trial (OCTAVE, $N = 25{,}302$) was also conducted in order to characterize the risk–benefit profile of omapatrilat in patients with hypertension. Compared with enalapril, omapatrilat had significantly greater reduction in systolic and diastolic BP and was associated with less use of adjunctive antihypertensive therapy by the end of trial ($p < 0.001$ for all comparisons). However, there was a higher incidence of angioedema in omapatrilat-treated patients compared with patients receiving enalapril (2.17 vs. 0.68%, relative risk, 3.17; 95% confidence interval, CI: 2.52–4.12) (Kostis et al. 2004). Further, the angioedema events in the omapatrilat group were of greater severity grade compared with enalapril ($p < 0.005$). The incidence of angioedema was higher in black patients (5.54 and 1.62%, respectively) and in

smokers (3.93 and 0.81%, respectively). The increased incidence of angioedema with omapatrilat may have been due to the simultaneous inhibition of three enzymes (ACE, NEP, and aminopeptidase P) involved in the metabolism of bradykinin and other vasodilator peptides (Gu et al. 2010). In view of these safety concerns and the limited efficacy of omapatrilat with respect to HF endpoints, further development of this drug was halted.

It was postulated that the use of a once-daily dosing regimen may have been a factor in the relatively limited benefit observed in studies of omapatrilat, as there was evidence that this regimen failed to maintain continuous suppression of RAAS and inhibition of NEP throughout dose cycle (Massien et al. 1999; Packer et al. 2002). With regard to risk for angioedema, the experience with omapatrilat emphasized a need to avoid inhibiting ACE and NEP at the same time. Nonetheless, the goal of harnessing the complementary benefits of enhancing NP activity and suppressing RAAS activation while minimizing the risk of adverse events could be achieved in principle, by combining NEP inhibitor with an ARB, rather than an ACEI. These considerations led to the development of sacubitril/valsartan – a first-in-class ARNI – as a novel treatment for HF.

3 Sacubitril/Valsartan

Sacubitril/valsartan is a sodium salt complex of sacubitril (an NEPI prodrug previously known as AHU-377) and valsartan (an ARB) in a 1:1 molar ratio. Following oral administration, sacubitril is rapidly hydrolyzed in vivo by carboxyl esterase 1 to the active NEPI, sacubitrilat (previously known as LBQ657) (Shi et al. 2016). Until recently, sacubitril/valsartan was known as LCZ696 and it was administered in clinical trials in dose formulations of 50, 100, and 200 mg. These same dose formulations are now referred to, respectively, as sacubitril/valsartan 24/26, 49/51, and 97/103 mg.

3.1 Pharmacokinetics

Following oral administration of 200–1,200 mg sacubitril/valsartan, systemic concentrations of sacubitril and valsartan increase rapidly with times to peak concentration (T_{max}) of 0.5–1.1 h and 1.7–2.2 h, respectively (Gu et al. 2010). Sacubitril is rapidly converted to sacubitrilat, the peak concentrations of which appear within 1.9–3.5 h after oral administration. Increases in exposure to sacubitril, sacubitrilat, and valsartan are dose-linear over the dose range of 20–600 mg (Akahori et al. 2016; Han et al. 2016). It is estimated that the oral absolute bioavailability for sacubitril is $\geq$60%. The bioavailability of valsartan administered as sacubitril/valsartan is 60% greater than it is when valsartan is administered in single-agent formulations. Thus, the 103 mg of valsartan present in a 200 mg dose of sacubitril/valsartan provides systemic exposure similar to the 160 mg dose of valsartan that is the maximum twice-daily dose approved for

treatment of HF (Gu et al. 2010; Mistry et al. 2006). Food decreases the maximum plasma concentration (C_{max}) and systemic exposure to sacubitril but has little impact on systemic exposure to sacubitrilat. The C_{max} and systemic exposure to valsartan are also decreased by food (Ayalasomayajula et al. 2016b); however, this is not considered clinically relevant, and valsartan is recommended to be administered regardless of meals (PI 2015). Potential drug–drug interactions with sacubitril/valsartan are discussed in a later section.

Following twice-daily dosing, steady-state levels for both sacubitrilat and valsartan are reached in 3 days (Ayalasomayajula et al. 2015). At steady state, there is a 1.6-fold accumulation of sacubitrilat and no significant accumulation of valsartan (PI 2015). Sacubitril, sacubitrilat, and valsartan are highly (94–97%) bound to plasma proteins and undergo substantial distribution to tissues. Average apparent volumes of distribution for sacubitril and valsartan are 103 and 75 L, respectively. Sacubitril, sacubitrilat, and valsartan are eliminated with estimated half-lives of ~1.4, ~11.5, and ~9.9 h, respectively. The primary route of elimination is renal for sacubitrilat (52–68% is eliminated unmetabolized in urine) and hepatic for valsartan (86% is excreted via the feces) (PI 2015).

In elderly (≥65 years) healthy subjects, exposure to sacubitrilat and valsartan was 42 and 30% greater, respectively, than in the younger (18–45 years) subjects, presumably due to decreased renal and hepatic function (Gan et al. 2016). However, these increases are not regarded as clinically relevant and the same dose is recommended in both age groups. Sex has no effect on the pharmacokinetics of sacubitrilat or valsartan (Gan et al. 2016). Exposure to sacubitrilat and valsartan was increased by approximately 110 and 132%, respectively in patients with HFrEF, compared with healthy subjects (Kobalava et al. 2016).

Mild-to-severe renal impairment was found to have no impact on the pharmacokinetics of valsartan. However, the C_{max} and exposure to sacubitrilat were significantly increased in these patients (Fig. 2) (Ayalasomayajula et al. 2016c). A lower starting dose of sacubitril/valsartan is thus recommended in patients with severe renal impairment (PI 2015). In patients with mild-to-moderate hepatic impairment, the exposure to sacubitrilat and valsartan was increased (Fig. 2) (Kulmatycki et al. 2014), suggesting the need for a lower starting dose in patients with moderate hepatic impairment (PI 2015). The impact of severe hepatic impairment was not studied as valsartan is not recommended in this population.

3.2 Pharmacodynamics

Sacubitril/valsartan enhances circulating NP levels and this leads to increased plasma and urine cGMP levels (Holmes et al. 1993). Plasma cGMP therefore serves as a useful biomarker of NEP inhibition in healthy subjects and patients. Changes in plasma renin concentration (PRC), plasma renin activity (PRA), and plasma angiotensin II concentration are indicators of pharmacodynamic response to valsartan (Muller et al. 1994) and lowering of blood pressure is a commonly measured pharmacodynamic response to both sacubitrilat and valsartan.

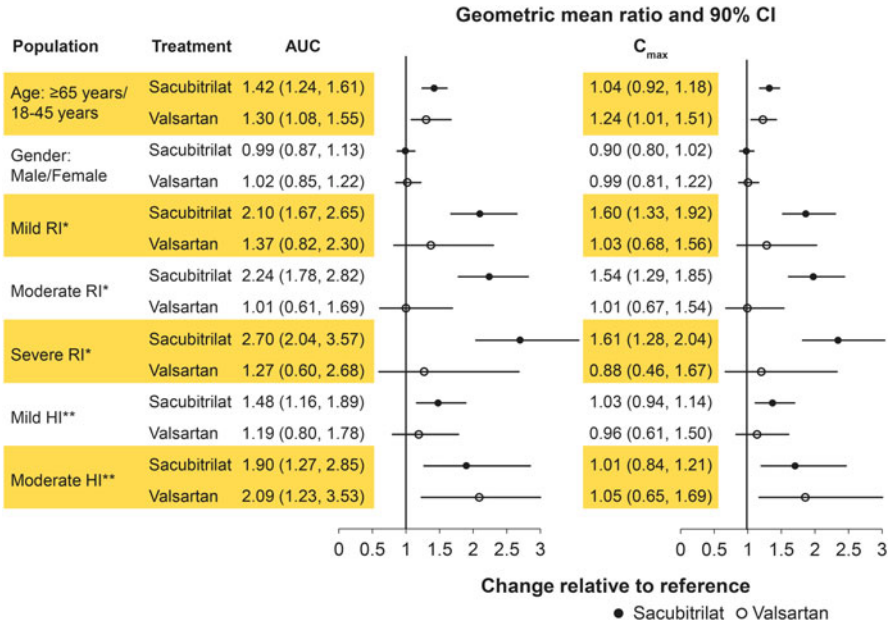

Fig. 2 Pharmacokinetic parameters (AUC and C_{max}) of sacubitrilat and valsartan in special populations. Values for RI (mild, moderate, and severe) and HI (mild and moderate) are relative to normal. *Mild RI (60–89 mL/min per 1.73 m^2), moderate RI (30–59 mL/min per 1.73 m^2), and severe RI (15–29 mL/min per 1.73 m^2); **mild HI (Child–Pugh A scores of 5–6) and moderate HI (Child–Pugh A scores of 7–9) *AUC* area under the plasma concentration curve, *CI* confidence interval, C_{max} maximum plasma concentration, *HI* hepatic impairment, *HR* hazard ratio, *RI* renal impairment

3.2.1 Changes in Plasma Cyclic Guanosine Monophosphate

In healthy subjects given once-daily oral doses of 50–900 mg sacubitril/valsartan for 14 days, all doses significantly increased mean plasma cGMP levels (Gu et al. 2010). All doses provided significantly elevated plasma cGMP levels at 4 h postdose and the levels of plasma cGMP remained significantly elevated at 12 h following 600 and 900 mg doses of sacubitril/valsartan. cGMP levels returned to baseline by 24 h after administration of all doses. The maximum increase in plasma cGMP was 40%, which was observed with the 200 mg dose (Gu et al. 2010).

3.2.2 Biomarkers of Renin–Angiotensin–Aldosterone System Inhibition by AT_1 Receptor Blockade

Once-daily dosing with 50–900 mg sacubitril/valsartan resulted in significant, dose-dependent increases in PRC, PRA, and angiotensin II, compared with placebo (Gu et al. 2010), indicating AT_1 receptor blockade. These biomarkers were maximally increased at 4 h postdose and remained elevated at 24 h.

3.2.3 Blood Pressure Lowering by Sacubitril/Valsartan

In a phase II dose-ranging study in patients with mild-to-moderate hypertension, the effects of sacubitril/valsartan and valsartan alone on BP were compared over a range of bioequivalent doses (100 mg sacubitril/valsartan vs. 80 mg valsartan, 200 vs. 160 mg, and 400 vs. 320 mg, respectively) (Ruilope et al. 2010). In this proof-of-concept study, the goal was to determine whether treatment with an ARNI elicits greater reductions in BP than dose-equivalent treatment with an ARB. This was indeed observed: at the doses of 200 and 400 mg sacubitril/valsartan, treatment with the ARNI resulted in significantly greater reductions in mean sitting BP, pulse pressure (PP), and 24-hour mean ambulatory systolic BP and PP than did treatment with equivalent doses of the ARB alone. This study also included an arm in which patients were treated with sacubitril alone and patients in this arm realized less lowering of BP than did patients treated with an equivalent dose of sacubitril/valsartan. The results of this study indicated that treatment with sacubitril/valsartan results in greater BP lowering than treatment of equivalent doses of either valsartan or sacubitril alone.

3.2.4 Selection of Recommended Clinical Dose

The dose of 97/103 mg bid was selected as the target dose for sacubitril/valsartan for the treatment of patients with HFrEF (McMurray et al. 2013). This dose provides an amount of valsartan bioequivalent to the dose of 160 mg bid that has been shown to be efficacious in patients with chronic HF and after myocardial infarction (MI) (Cohn et al. 2001; Pfeffer et al. 2003). In addition, this dose was known to provide nearly 90% maximal inhibition of NEP in healthy subjects (Gu et al. 2010). Twice-daily dosing was chosen in order to elicit sustained inhibition of NEP (as indicated by plasma cGMP data) and minimize potential risk for hypotension that might result if a higher dose were to be given once daily.

3.2.5 Effect of Sacubitril/valsartan on Cardiac Repolarization

Drug-induced delayed cardiac repolarization may lead to increased susceptibility to cardiac arrhythmias (Cubeddu 2009). Sacubitril/valsartan at single therapeutic (194/206 mg) and supratherapeutic (582/618 mg) doses showed no effect on cardiac repolarization in healthy subjects as evidenced by lack of effect on the QTc interval (Langenickel et al. 2016a).

3.3 Pharmacokinetic and Pharmacodynamic Drug–Drug Interactions

Sacubitril, sacubitrilat, and valsartan are not significantly metabolized by CYP450 enzymes (Flarakos et al. 2016). Hence, coadministration of drugs that induce or inhibit CYP450 enzymes is not likely to influence the pharmacokinetics of sacubitril/valsartan. Furthermore, CYP450 enzymes are neither induced nor inhibited by the sacubitril, sacubitrilat, or valsartan at clinically relevant concentrations. Therefore, the potential for drug interactions involving sacubitril/valsartan as an inhibitor or inducer of CYP450 enzymes is low. Consistent with this, no clinically relevant pharmacokinetic interactions have been observed upon

coadministration of sacubitril/valsartan and hydrochlorothiazide, warfarin, carvedilol, omeprazole, amlodipine, metformin, or levonorgestrel–ethinyl estradiol (PI 2015). Although sacubitril and digoxin are both P-glycoprotein substrates, no clinically relevant pharmacokinetic drug interaction was observed between sacubitril/valsartan and digoxin (Ayalasomayajula et al. 2015).

In vitro data show that sacubitril inhibits organic anion transporter proteins (OATPs) 1B1, OATP1B3 (Ayalasomayajula et al. 2016a), and OAT3 at clinically relevant concentrations, indicating a potential drug–drug interaction with statins that undergo hepatobiliary elimination through OATP transporters and furosemide, a loop diuretic that is excreted via OAT3 transporter at proximal renal tubule (Niemi 2007). Atorvastatin is an OATP1B1 and OATP1B3 substrate; coadministration with sacubitril/valsartan increased the C_{max} of atorvastatin and its metabolites by two-fold and AUC by <1.3-fold (Ayalasomayajula et al. 2016d). These increases are unlikely to be of clinical significance given that in PARADIGM-HF, coadministration of sacubitril/valsartan with statins did not result in an increased risk for rhabdomyolysis or statin-related muscle toxicity. No interaction was observed when sacubitril/valsartan was coadministered with simvastatin (Ayalasomayajula et al. 2016a). It is hypothesized that interaction of sacubitril/valsartan with atorvastatin but not simvastatin can be attributed to the rapid clearance of sacubitril and the differences in the rates of absorption between simvastatin/simvastatin acid and atorvastatin. Caution is recommended when coadministering sacubitril/valsartan with statins (PI 2015).

Coadministration with sacubitril/valsartan decreased the C_{max} of furosemide by 50%, AUC by 28%, and its urinary excretion by 26% (unpublished data). Sacubitril/valsartan had no impact on mean urinary excretion of potassium, creatinine, and overall urine volume over 24 h. However, it reduced natriuresis by ~30 mmol over 24 h (an effect that was most pronounced in the first 4 h). This decrease in natriuresis is possibly due to the reduction in systemic exposure and urinary excretion of furosemide when coadministered with sacubitril/valsartan (unpublished data). However, these observed changes do not mandate any dosage adjustment of furosemide when coadministered with sacubitril/valsartan (PI 2015).

No clinically relevant pharmacodynamic interaction is observed upon coadministration of sacubitril/valsartan and nitroglycerin (unpublished data). Caution is recommended when administering sildenafil or any other phosphodiesterase-5 inhibitor to patients treated with sacubitril/valsartan. These agents also increase cGMP levels and may have a greater than additive effect on BP when combined with sacubitril/valsartan (unpublished data).

4 Overview of Clinical Trials of Sacubitril/Valsartan in Heart Failure

The efficacy and safety of sacubitril/valsartan has been evaluated in three key clinical trials to date (Table 1). These include PARADIGM-HF, a phase III trial in patients with HFrEF (McMurray et al. 2014b), TITRATION, a phase II study

Table 1 Phase II and III clinical trials of sacubitril/valsartan in HF

Study	Treatments	Primary assessments	Study population	Key findings
PARAMOUNT (Phase II) $N = 301$	Sacubitril/valsartan 97/103 mg bid versus valsartan 160 mg bid	Change in NT-proBNP from baseline to 12 weeks	Symptomatic HFpEF, LVEF $\geq$45% , NT-proBNP >400 pg/mL	Significant reduction of NT-proBNP at Week 12 with sacubitril/valsartan vs. valsartan (relative difference of 23% between two groups)
PARADIGM-HF (Phase III) $N = 8,442$	Sacubitril/valsartan 97/103 mg bid versus enalapril 10 mg bid	Composite primary outcome of CV death or HF hospitalization	Symptomatic HFrEF, LVEF $\leq$40%[a], elevated plasma BNP levels ($\geq$150 pg/mL), or BNP $\geq$100 pg/mL, if hospitalization for HF within the previous 12 months	Sacubitril/valsartan superior to enalapril in reducing the risk of CV death or hospitalization for HF (HR 0.80, 95% CI: 0.73–0.87, $p < 0.001$)
TITRATION (Phase II) $N = 538$	Sacubitril/valsartan uptitration regimens (3-week condensed vs. 6-week conservative regimen)	Safety and tolerability	HFrEF, LVEF $\leq$35%	Overall, 76% of patients achieved and maintained sacubitril/valsartan 97/103 mg bid (77.8% in condensed regimen and 84.3% in conservative regimen)

bid twice daily, *HFpEF* heart failure with preserved ejection fraction, *HFrEF* heart failure with reduced ejection fraction, *HR* hazard ratio, *LVEF* left ventricular ejection fraction, *NT-proBNP* N-terminal pro-brain natriuretic peptide, *PARADIGM-HF* Prospective comparison of ARNi with ACEi to Determine Impact on Global Mortality and morbidity in Heart Failure, *PARAMOUNT* Prospective comparison of ARNI with ARB on Management Of heart failUre with preserved ejectioN fraction

[a]LVEF was later changed to $\leq$35% after a protocol amendment

(Senni et al. 2016) in patients with HFrEF, and PARAMOUNT, a phase II study in patients with HFpEF (Solomon et al. 2012).

4.1 PARADIGM-HF

The phase III PARADIGM-HF trial, the largest HF study to date, was designed to test the superiority of sacubitril/valsartan over an ACEI (standard of care) in reducing the risk of mortality and morbidity in patients with HFrEF (McMurray et al. 2013). The typical phase II development stage for sacubitril/valsartan was bypassed based on substantial and reassuring safety experience in hypertension studies, prior experiences with omapatrilat, and the fact that phase II studies often do not predict success in phase III trials (McMurray et al. 2013).

In the PARADIGM-HF trial, enalapril was chosen as an active comparator because of its established mortality and morbidity benefits in patients with HFrEF (CONSENSUS 1987; SOLVD 1991) and because it is the most widely prescribed ACEI (McMurray et al. 2012). The dose of enalapril 10 mg bid was chosen because it was demonstrated to provide a mortality benefit when compared with placebo in the SOLVD-T trial and was used in subsequent HF outcome trials (SOLVD 1991; Cohn et al. 1991; McMurray et al. 2013; Packer et al. 2002).

This randomized, double-blind, active-controlled, event-driven trial enrolled patients aged $\geq$18 years with New York Heart Association (NYHA) class II–IV symptoms, an ejection fraction (EF) $\leq$40% (later amended to $\leq$35%) and a plasma BNP $\geq$150 pg/mL (or N-terminal pro B-type brain natriuretic peptide, NT-proBNP $\geq$600 pg/mL) or a BNP $\geq$100 pg/mL (or NT-proBNP $\geq$400 pg/mL), if there was a history of hospitalization for HF within the last 12 months. Patients with an estimated glomerular filtration rate (eGFR) $<$30 mL/min/1.73 m^2, serum potassium $>$5.4 mmol/L, systolic BP $<$95 mmHg, or history of angioedema were excluded (McMurray et al. 2013).

The trial had a study design with two sequential single-blind run-in periods followed by a randomized, double-blind treatment period. In the first run-in period, patients received enalapril 10 mg bid for 2 weeks. In the second run-in period, patients were administered sacubitril/valsartan for 4–6 weeks, starting at a dose of 49/51 mg bid (formerly referred to as 100 mg bid) and then uptitrated to 97/103 mg bid (formerly 200 mg bid). Patients who were able to tolerate target doses of both treatments were then randomized in a double-blind manner to receive either enalapril 10 mg bid or sacubitril/valsartan 97/103 mg bid. Two 36-hour washout periods were included after each run-in period to minimize the chances of concomitant NEP and ACE inhibition, which could increase the risk of angioedema (McMurray et al. 2013).

The active run-in periods ensured the attainment of target doses of both drugs that was critical to test for superiority of sacubitril/valsartan over enalapril. For example, the enalapril run-in period was designed to ensure that a minimal mean daily dose of 16.6 mg of enalapril was achieved, as that was the average dose attained in the SOLVD-T trial, which demonstrated the mortality benefit of this

ACEI over placebo. Further, the active run-in periods provided an opportunity to assess investigator-unblinded short-term safety and tolerability of sacubitril/valsartan. This was particularly important in the absence of prior phase II safety experience with this drug in HFrEF (McMurray et al. 2013).

A total of 8,399 patients were randomized in PARADIGM-HF at 985 sites across 47 countries. Patients had mean age of 64 years. The majority were males and had either NYHA class II (70%) or class III (24%) symptoms of HF. The mean EF was 29%, and most of the patients received optimized background therapy for HF. The patient demographics and baseline disease characteristics were comparable between the sacubitril/valsartan ($n = 4,187$) and enalapril ($n = 4,212$) groups (McMurray et al. 2014b).

The trial was stopped early by the data monitoring committee at the third interim analysis because of statistically compelling evidence for a benefit with sacubitril/valsartan compared with enalapril for both the primary endpoint (time to CV death or HF hospitalization) and time to CV death alone (McMurray et al. 2014b). The median duration of follow-up was 27 months and most patients were able to tolerate the targeted sacubitril/valsartan dose of 97/103 mg bid. Expressed in the terminology used for LCZ696, the mean daily dose at the end of trial was 375 mg. The average enalapril dose was higher in PARADIGM-HF than in the SOLVD-T trial (18.9 mg vs. 16.6 mg) (SOLVD 1991).

4.1.1 Outcomes

Sacubitril/valsartan provided superior benefits over enalapril in reducing the risk of the primary composite endpoint (CV death or HF hospitalization) by 20%, risk of CV death by 20%, risk of first HF hospitalization by 21%, and risk of all-cause mortality by 16% (Fig. 3) (McMurray et al. 2014b). The benefits of sacubitril/valsartan over enalapril in reducing the risk of primary composite endpoint and CV death were consistent across the 18 prespecified subgroups (McMurray et al. 2014b) except that a nominally significant interaction was observed between NYHA class at randomization and treatment effect on the primary endpoint ($p = 0.03$, without adjustment for multiple comparisons). No significant interaction was observed between NYHA class and CV death ($p = 0.76$). Further, the superior outcome benefits observed with sacubitril/valsartan over enalapril were independent of the BP lowering effect of sacubitril/valsartan (McMurray et al. 2014b).

The number of patients needed to treat (NNT) with sacubitril/valsartan in order to prevent one primary endpoint event was 21. The NNT to prevent one death due to CV causes was 32.

There were no significant differences between sacubitril/valsartan- and enalapril-treated patients for new-onset atrial fibrillation (3.1 vs. 3.1%) or decline in renal function, defined as end-stage renal disease or a decrease of $\geq$50% in the eGFR from the value at randomization or a decrease in the eGFR of >30 mL/min per 1.73 m^2, to less than 60 mL/min per 1.73 m^2 (2.2 vs. 2.6%) (McMurray et al. 2014b).

Ethical considerations would preclude direct comparison of sacubitril/valsartan with placebo in a clinical trial, but a putative placebo analysis has been done for

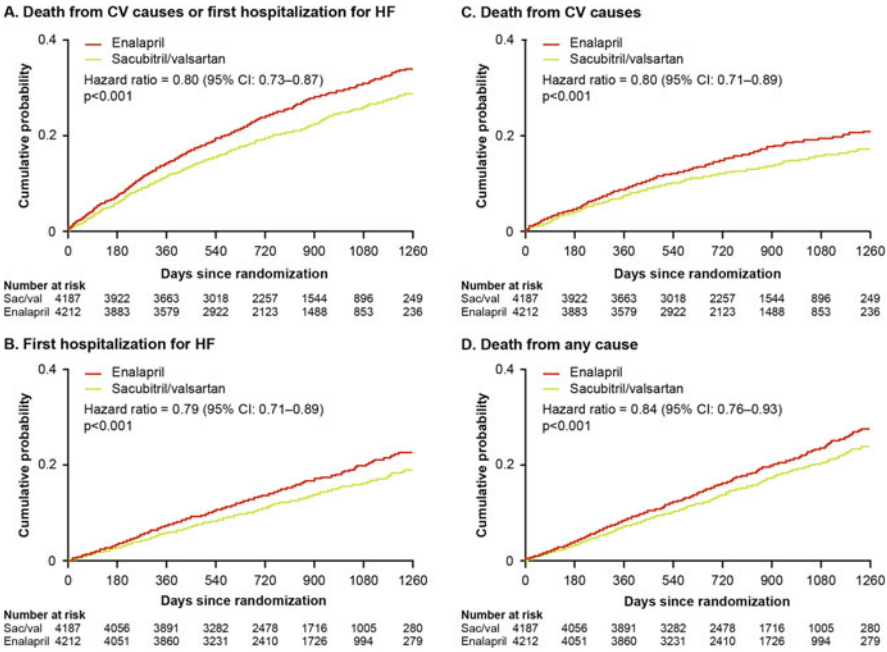

Fig. 3 Kaplan–Meier curves for key PARADIGM-HF study outcomes (**A**) death from CV causes or first hospitalization for HF; (**B**) first hospitalization for HF; (**C**) death from CV causes; (**D**) death from any cause. *CI* confidence interval, *CV* cardiovascular, *HF* heart failure, *Sac/val* sacubitril/valsartan. Source: McMurray et al. (2014b)

PARADIGM-HF using the results of SOLVD-T (ACEI vs. placebo) and CHARM-Alternative (ARB vs. placebo) trials as references. Compared with putative placebos, sacubitril/valsartan showed a significant reduction in risk for the primary endpoint by 43% (SOLVD-T) and 39% (CHARM-Alternative). Similar effects were observed for CV death (34% for SOLVD-T and 32% for CHARM-Alternative), HF hospitalization (49% for SOLVD-T and 46% for CHARM-Alternative), and all-cause mortality (28% for SOLVD-T and 26% for CHARM-Alternative, $p < 0.0001$ for all comparisons) (McMurray et al. 2015).

4.1.2 Mode of Death

The majority of deaths in the PARADIGM-HF trial were attributed to CV causes (80.9%), of which 45% were due to sudden cardiac death and 27% were due to worsening HF. Sacubitril/valsartan significantly reduced the risk of both sudden cardiac death (HR: 0.80; 95% CI: 0.68–0.94; $p = 0.008$) and death due to worsening HF (HR: 0.79; 95% CI: 0.64–0.98; $p = 0.034$) compared with enalapril. Deaths due to other CV causes (including MI and stroke) were infrequent and distributed evenly between treatment groups, as were non-CV deaths (Desai et al. 2015).

4.1.3 Clinical Progression

Nonfatal worsening of symptoms is frequent in HF, necessitating treatment intensification or emergency treatment that may include hospitalization, intensive care, or surgical intervention (Butler et al. 2014). Prevention of worsening of HF and maintaining clinical stability are major goals of HF therapy, in addition to improving survival. In addition to the mortality benefit provided by sacubitril/valsartan in patients with HFrEF, the treatment provided significant improvements with regard to the multiple manifestations of clinical progression commonly observed in surviving patients. These included significant reductions, compared with enalapril, in the numbers of patients requiring treatment intensification, emergency department visits, and hospitalizations (Table 2). Compared with enalapril, sacubitril/valsartan reduced the risk of first hospitalization for any cause by 12%, overall hospitalizations for any cause (regardless of whether they were first or recurrent hospitalizations) by 16%, and overall hospitalizations for HF by 23% (Packer et al. 2015). The reduction in the rate of hospitalization for HF with sacubitril/valsartan was significant at 30 days after randomization (Packer et al. 2015). Sacubitril/valsartan also significantly reduced the rate of hospital readmissions for any cause within 30 days of discharge (OR, 0.74; 95% CI: 0.56–0.97; $p = 0.031$) (Desai et al. 2016a).

Irrespective of background therapy, fewer patients treated with sacubitril/valsartan vs. enalapril experienced an expanded composite outcome of death from CV causes, hospitalization for HF, an emergency department visit for HF, or therapy intensification for worsening HF (1,038 vs. 1,275 patients; HR 0.79; 95% CI 0.73–0.86; $p < 0.0001$) (Okumura et al. 2016b).

Based on survival analysis, it has been estimated that treatment with sacubitril/valsartan compared with enalapril would eventually lead to a gain of 2.1 years of HF-free survival and 1.4 years of overall survival in an average 55-year-old patient (Claggett et al. 2015).

4.1.4 Quality of Life

In the PARADIGM-HF trial, treatment with sacubitril/valsartan was associated with a significantly less deterioration of health-related quality of life than enalapril. Worsening of HF symptoms and physical limitations as measured by the Kansas City Cardiomyopathy Questionnaire (KCCQ) scores were significantly lower with sacubitril/valsartan compared with enalapril (reduction in KCCQ score from baseline to 8 months: −2.99 in sacubitril/valsartan group vs. −4.63 in enalapril group; between-group difference 1.64 points; $p = 0.001$) (McMurray et al. 2014b). Fewer patients in the sacubitril/valsartan group had ≥5-point deterioration in the KCCQ scores compared with enalapril group; the between-group difference for this endpoint was significant at 4, 8, and 12 months after randomization ($p = 0.002$, $p = 0.001$ and $p = 0.03$, respectively). The benefit of sacubitril/valsartan over enalapril in preventing the worsening of KCCQ scores was consistent across the different age groups (Jhund et al. 2015b) and in patients with or without diabetes (Kristensen et al. 2016a).

Table 2 Measures of nonfatal worsening heart failure (clinical progression) in the enalapril and sacubitril/valsartan groups

Criteria for clinical progression in patients with HF	Enalapril ($n = 4{,}212$)	Sacubitril/valsartan ($n = 4{,}187$)	HR/rate ratio (95% CI); p value
Patients requiring intensification of outpatient therapy	604 (14.3)	520 (12.4)	0.84 (0.74–0.94); $p = 0.003$
Patients with ED visit for HF	150 (3.6)	102 (2.4)	0.66 (0.52–0.85); $p = 0.001$
Total number of stays in intensive care, *n*	879	768	0.82 (0.72–0.94)[a]; $p = 0.005$
Patients receiving IV positive inotropic drugs	229 (5.4)	161 (3.9)	0.69 (0.57–0.85); $p < 0.001$
Patients requiring cardiac resynchronization, ventricular assist device implantation, or cardiac transplantation	119 (2.8)	94 (2.3)	0.78 (0.60–1.02); $p = 0.07$
Patients hospitalized for HF, *n*	658 (15.6)	537 (12.8)	0.79 (0.71–0.89); $p < 0.001$
Patients hospitalized for CV reason	1,344 (31.9)	1,210 (28.9)	0.88 (0.81–0.95); $p < 0.001$
Patients hospitalized for any reason	1,827 (43.4)	1,660 (39.7)	0.88 (0.82–0.94); $p < 0.001$
Total number of hospitalizations for HF	1,079	851	0.77 (0.67–0.89)[a]; $p < 0.001$
Patients with worsening NYHA functional class (≥1 class) and surviving at			
4 months	218 (5.5)	186 (4.7)	0.113
8 months	266 (7.0)	205 (5.4)	0.004
12 months	271 (7.4)	225 (6.1)	0.023
Patients with worsening KCCQ total symptoms score (≥5 points) and surviving at			
4 months	1,012 (28.3)	899 (25.1)	0.002
8 months	1,087 (31.8)	974 (28.2)	0.001
12 months	1,029 (31.5)	964 (29.0)	0.03

CV cardiovascular, *ED* emergency department, *HF* heart failure, *HR* hazard ratio, *IV* intravenous, *KCCQ* Kansas City Cardiomyopathy Questionnaire, *NYHA* New York Heart Association
[a]Rate ratio estimated from a negative binomial model; ratios without [a] are hazard ratios derived by using the Cox proportional hazards model; Data are presented as *n* (%) unless indicated

4.1.5 Biomarkers

The biomarkers BNP and NT-proBNP are indicative of cardiac wall stress and are elevated in patients with HF. They are also used as diagnostic and prognostic tools in these patients. Measurements of BNP are subject to error, however, because conventional assay methods do not adequately distinguish between BNP itself and related inactive peptides that may be present at substantial levels in the plasma of patients with HF (Hawkridge et al. 2005; Miller et al. 2011; Niederkofler et al. 2008). Moreover, BNP is a substrate of NEP and, thus, its levels are expected to increase in patients treated with sacubitril/valsartan, given its mechanism of action. In contrast, NT-proBNP (a large N-terminal peptide fragment generated by cleavage of the BNP prohormone) is biologically inert and is not a substrate for NEP (Heeschen et al. 2004). Hence, NT-proBNP can be used an indicator of the cardioprotective effects of sacubitril/valsartan, with a decline in plasma NT-proBNP indicating less cardiac wall stress (Bayes-Genis et al. 2016) whereas, monitoring BNP levels in patients who receive treatment with sacubitril/valsartan would likely be less informative.

In PARADIGM-HF trial, patients in the sacubitril/valsartan arm had significantly lower circulating levels of NT-proBNP after 4 weeks of treatment than patients in the enalapril arm and this difference ($p < 0.0001$) was sustained over 8 months (Packer et al. 2015). Treatment with sacubitril/valsartan was twice as likely as enalapril to reduce NT-proBNP to <1,000 pg/mL and this reduction was associated with a significantly lower rate of subsequent CV death or HF hospitalization (Zile et al. 2016). In contrast, plasma BNP levels were significantly ($p < 0.0001$) higher in the sacubitril/valsartan arm than in the enalapril arm at 4 weeks and 8 months, consistent with the mechanism of action of sacubitril/valsartan. High sensitivity troponin T (hsTnT), a marker of cardiomyocyte injury, was elevated in majority of the patients in both treatment arms at baseline (median hsTnT 0.015 μg/L). Plasma levels of hsTnT were significantly ($p < 0.0001$) lower in sacubitril/valsartan arm than in the enalapril arm at 1 and 8 months post-randomization. The proportion of patients having hsTnT levels in the range indicative of myocyte injury (>0.014 μg/L) was also significantly reduced in sacubitril/valsartan arm (Jhund et al. 2015a).

4.1.6 Additional Subgroup Analyses and Other Analyses

Several additional analyses of the PARADIGM-HF trial were conducted to assess the effects of sacubitril/valsartan across various subgroups (Fig. 4). The benefits of sacubitril/valsartan over enalapril were consistent across all age subgroups assessed (<55, 55–64, 65–74, and ≥75 years). In addition, sacubitril/valsartan was well-tolerated across these age groups; intolerance leading to discontinuation of sacubitril/valsartan was less common even in the most elderly patients (Jhund et al. 2015b). The superiority of sacubitril/valsartan over enalapril was consistently observed across patients with left ventricular ejection fraction from 5 to 42% (Solomon et al. 2016a), baseline systolic BP values from 90 to 189 mmHg (Bohm et al. 2015), glycated hemoglobin (HbA1c) levels from normoglycemia (HbA1c < 6.0%) to diabetics (HbA1c ≥ 6.5%) (Kristensen et al. 2016a), and across

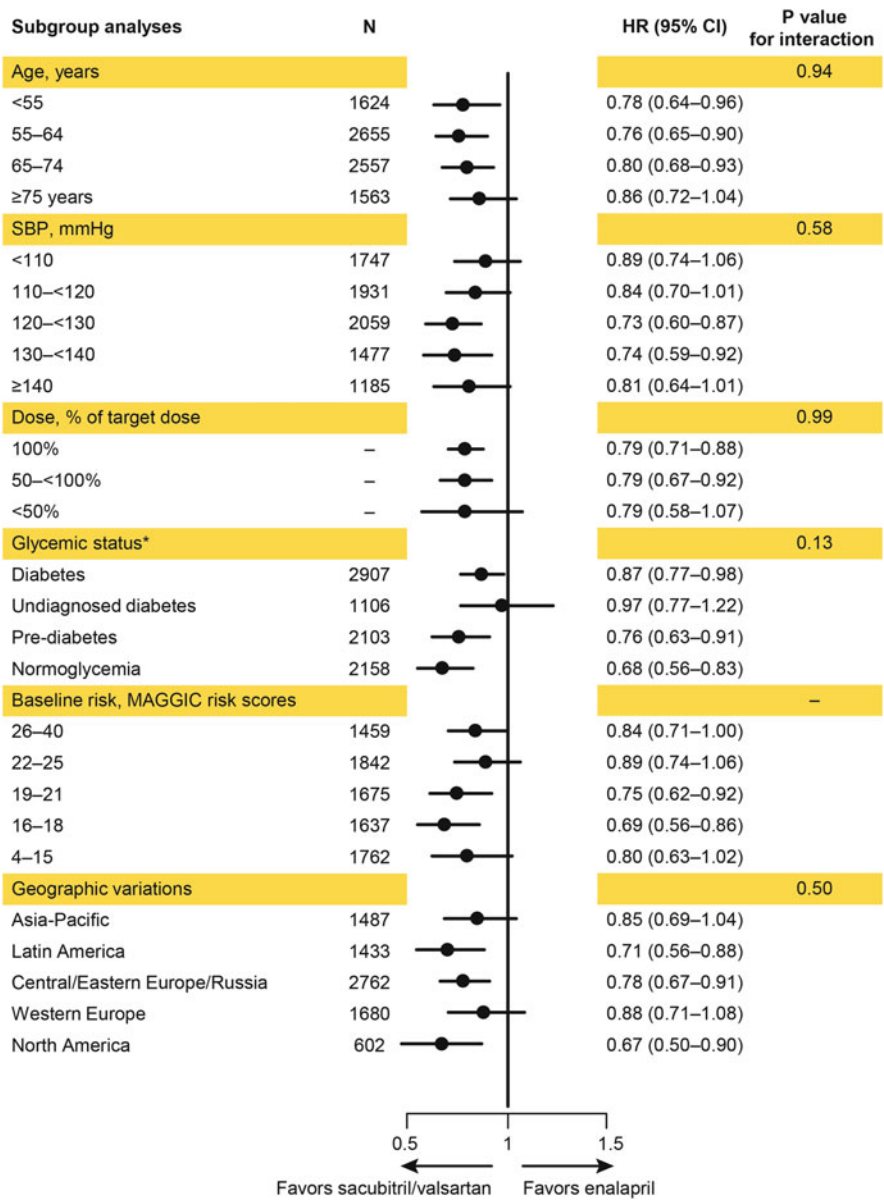

Fig. 4 Additional subgroup and other analyses in PARADIGM-HF trial. *Glycemic status: Patients without a previous diagnosis of diabetes mellitus were divided into three categories according to the hemoglobin A1c (HbA1c) levels: normal, <6.0% (<42 mmol/mol); prediabetes mellitus, 6.0–6.4% (42–47 mmol/mol); and undiagnosed diabetes mellitus, ≥6.5% (≥48 mmol/mol). Patients with a previous diagnosis of diabetes mellitus (irrespective of HbA1c level) were considered to have diabetes mellitus. *CI* confidence interval, *HR* hazard ratio, *MAGGIC* Meta-analysis Global Group in Chronic Heart Failure, *SBP* systolic blood pressure

a wide range of baseline MAGGIC and EMPHASIS-HF risk scores (Simpson et al. 2015). In addition, the outcomes of comparisons between sacubitril/valsartan and enalapril were similar across different geographic regions despite significant regional differences in the distributions of patients' ages, symptoms, comorbidities, background therapies, and event rates (Kristensen et al. 2016b).

Patients in the PARADIGM-HF were optimally treated with recommended pharmacologic therapies for HF prior to enrolment in the study, including ACEIs or ARBs (100%), β-blockers (93%), mineralocorticoid receptors antagonists (56%), and diuretics (80%) (McMurray et al. 2014b). In addition, post-randomization use of β-blockers, mineralocorticoid receptors antagonists, and diuretics remained consistently high throughout the follow-up period. Rates of use of implantable cardioverter defibrillator and cardiac resynchronization therapy devices were low in PARADIGM-HF (15 and 7%, respectively) but consistent with other recently completed HF trials and with real-world observational studies (McMurray et al. 2014a). Irrespective of background pharmacological therapies, surgical interventions (coronary revascularization), or devices used concomitantly, sacubitril/valsartan was more effective than enalapril in reducing the event rate of the primary composite endpoint and the rate of CV death alone. The benefits of sacubitril/valsartan over enalapril were incremental to those of other therapies. The HRs for the primary endpoint were 0.84 and 0.79, respectively, in patients with and without an implantable cardiac resynchronization device and they were 0.85 and 0.74, respectively, in patients with and without concomitant use of a mineralocorticoid receptor antagonist (Okumura et al. 2016a).

As in all trials, dose reductions of study medications were frequent in PARADIGM-HF; 42 and 43% of patients randomized to sacubitril/valsartan and enalapril, respectively, experienced a down-titration of dose at least once during the study. However, the treatment benefit of sacubitril/valsartan over enalapril following dose reductions was similar (for the primary endpoint event, HR 0.80, 95% CI 0.70–0.93, $p < 0.001$) to that observed in patients without any dose reductions (HR 0.79, 95% CI 0.71–0.88, $p < 0.001$). Patients taking lower mean daily doses of sacubitril/valsartan had fewer events of CV death or HF hospitalization than patients taking lower mean doses of enalapril, thus implying that the greater benefits of sacubitril/valsartan compared with enalapril were maintained even in patients who could not tolerate the target doses of sacubitril/valsartan (Vardeny et al. 2016).

In another analysis of PARADIGM-HF trial, the impact of sacubitril/valsartan was assessed in clinically stable patients to determine if an episode of clinical decompensation or instability was required to justify switching patients from an RAAS inhibitor to sacubitril/valsartan. For this analysis, patients were subgrouped by presence or absence of a history of prior hospitalization for HF and the length of time since that hospitalization ($\leq$3 months, $>$3 and $\leq$6 months, $>$6 and $\leq$12 months, $>$12 months, or no history of prior hospitalization for HF). The treatment effect of sacubitril/valsartan did not differ across these subgroups; patients who were clinically stable, as shown by a remote HF hospitalization or no prior HF hospitalization, were as likely to benefit from sacubitril/valsartan

therapy as were patients with a recent history of clinical decompensation (Solomon et al. 2016a, b).

Further, post hoc analyses of PARADIGM-HF trial were conducted to assess the effects of sacubitril/valsartan compared with enalapril in patients with prior history of MI or atrial fibrillation (AF). For analysis in patients with or without prior MI, clinical outcomes included primary composite endpoint (CV death or HF hospitalization), a prespecified adjudicated composite of CV death, HF hospitalization, MI, stroke, or resuscitated sudden death and a post hoc coronary composite of CV death, MI, hospitalization for unstable angina, hospitalization for any angina or coronary revascularization. The beneficial effects of sacubitril/valsartan over enalapril were independent of a prior history of MI for all the composite endpoints (p values for interaction 0.99, 0.51, and 0.36 for the primary, prespecified composite, and post hoc coronary composite endpoints, respectively) (Mogensen et al. 2016a). Similarly, a history of AF did not modify the beneficial effects of sacubitril/valsartan compared with enalapril on the risk of either the primary endpoint, HF hospitalization, or all-cause mortality (p value for interaction 0.25–0.95) (Mogensen et al. 2016b).

4.1.7 Safety and Tolerability

Sacubitril/valsartan was generally well-tolerated in PARADIGM-HF (McMurray et al. 2014b). Overall, 19.8% of subjects discontinued the study during the run-in periods, of which 10.5% of patients discontinued during the enalapril run-in phase and 9.3% discontinued during the sacubitril/valsartan run-in phase. Nearly two thirds of these discontinuations were due to AEs and abnormal laboratory tests; hypotension, hyperkalemia, and renal dysfunction were the AEs most often responsible for withdrawal during the run-in periods (Desai et al. 2016b). During the double-blind period, the incidence of serious AEs was lower in the sacubitril/valsartan arm than in the enalapril arm (46.1 vs. 50.7%, respectively). The overall incidence of AEs was similar in both treatment arms but fewer patients discontinued due to an AE in the sacubitril/valsartan arm than in the enalapril arm (10.7 vs. 12.2%).

With regard to protocol-specified AEs of special interest reported in the double-blind period, there was a higher incidence of hypotension in both the treatment arms. When comparing between the two groups, hypotension and dizziness were more frequent in the sacubitril/valsartan arm while hyperkalemia (serum potassium >6 mmol/L), renal impairment (serum creatinine ≥ 2.5 mg/dL), and cough were more frequent in the enalapril arm. The incidence of symptomatic hypotension was higher in the sacubitril/valsartan arm than in the enalapril arm (14.0 vs. 9.2%; $p < 0.001$) but the rate of discontinuation due to hypotension-related AEs did not differ significantly between the two treatment arms (0.9 vs. 0.7% for sacubitril/valsartan vs. enalapril, respectively; $p = 0.38$) (McMurray et al. 2014b).

The progression of renal dysfunction was slower in the sacubitril/valsartan arm than in the enalapril arm (change in eGFR, -0.11 vs. -0.14 mL/min per 1.73 m^2/month, respectively; $p = 0.01$). Compared with enalapril, sacubitril/valsartan showed a trend towards reduction in worsening renal function and improved

clinical outcomes in patients with or without chronic kidney disease at baseline (Damman et al. 2015). Within a subset of patients for whom urinary albumin/creatinine ratios were available at screening and at 1 and 8 months after randomization, sacubitril/valsartan was associated with a modest increase in this ratio compared with enalapril (median difference 0.30 mg/mmol). The increase was observed at 30 days post-randomization and remained stable over 8 months. Despite the increase, sacubitril/valsartan reduced the subsequent occurrence of the primary outcome by 21% compared with enalapril in this cohort, consistent with the reductions seen in the overall population in PARADIGM-HF (Gori et al. 2015).

Given that sacubitril/valsartan inhibits NEP, one of the enzymes involved in the metabolism of bradykinin, special attention was given to the evaluation of angioedema or angioedema-like events in the PARADIGM-HF trial. All suspected cases of angioedema were adjudicated by an independent angioedema adjudication committee (McMurray et al. 2014b). The overall incidence of angioedema events was low in both treatment arms. There were 25 confirmed angioedema events during the run-in periods – 15 during the enalapril run-in period and 10 during the sacubitril/valsartan run-in period. None of the events resulted in death or a need for mechanical ventilation.

During the double-blind period, angioedema was confirmed by blinded adjudication in 19 patients (0.45%) in the sacubitril/valsartan arm and in 10 patients (0.24%) in the enalapril arm ($p = 0.13$) (McMurray et al. 2014b). In neither treatment arm did any patient have an airway compromised or require mechanical airway protection because of angioedema. Angioedema led to the study drug discontinuation in 7 patients (0.17%) and 4 patients (0.09%) in sacubitril/valsartan and enalapril arms, respectively. The incidence of confirmed angioedema events was higher in black patients than others, irrespective of treatment arm. Angioedema was reported in 5 of 213 and 1 of 214 black patients in the sacubitril/valsartan and enalapril arms, respectively, and in 14 of 3,990 and 9 of 4,015 nonblack patients, respectively. However, none of these events were severe.

Given that NEP is one of many enzymes involved in the clearance of Aβ, there is a theoretical risk of accumulation of Aβ peptides in patients treated with sacubitril/valsartan (Liu et al. 2010). In a 14-day study in healthy subjects randomized to receive sacubitril/valsartan 194/206 mg once daily ($n = 21$) or placebo ($n = 22$), sacubitril/valsartan did not result in changes in cerebrospinal fluid (CSF) levels of aggregable Aβ isoforms (1–42 and 1–40) compared with placebo, despite achieving CSF concentrations of sacubitrilat sufficient to inhibit NEP. Though sacubitril/valsartan was associated with a 42% increase in soluble CSF Aβ 1–38 levels, its clinical relevance is currently unknown (Langenickel et al. 2016b). Furthermore, a post hoc analysis using Standardized Medical Dictionary for Regulatory Activities (MedDRA) Queries with "broad" and "narrow" preferred terms for dementia-related events found no significant difference in the incidence of these events between the sacubitril/valsartan and enalapril arms (broad search: HR, 1.01, 95% CI: 0.75–1.37; narrow search: HR, 0.73, 95% CI: 0.33–1.59) (Cannon et al. 2016).

4.2 TITRATION

TITRATION was a 12-week, multicenter, randomized, double-blind, parallel-group study that compared the safety and tolerability of uptitrating to a target dose of sacubitril/valsartan 97/103 mg bid (formerly 200 mg bid) over intervals of 3 and 6 weeks (Senni et al. 2016). It included a broader range of patients than PARADIGM-HF, the goal being to include a population more representative of daily clinical practice. Hospitalized and ambulatory patients were both included. There were no exclusions based on NT-proBNP level or histories of prior treatment with ACEIs or ARBs. Patients were further stratified according to the dose of ACEI/ARB at screening: low-dose RAAS stratum (patients who had been receiving a total daily dose of $\leq$10 mg enalapril or equivalent at screening, this group included ACEI/ARB naïve patients) and high-dose RAAS stratum (patients who were receiving >10 mg/day enalapril or equivalent at screening).

Treatments began with a short, open-label run-in period of 5 days during which patients received sacubitril/valsartan 24/26 mg bid. This was followed by double-blind randomization into either a "condensed" 3-week uptitration arm or a "conservative" 6-week uptitration arm. In both arms, patients were followed up for 11 weeks post-randomization.

The incidences of hypotension and renal dysfunction were comparable with the two regimens but there was a numerically higher incidence of hyperkalemia with the "condensed" regimen (7.7%) than with the "conservative" regimen (4.4%). Irrespective of uptitration regimen, hypotension, renal dysfunction, and hyperkalemia were more common in low-dose RAS stratum than in high-dose RAS stratum (Senni et al. 2016).

The majority of patients in the study achieved and maintained the target dose of sacubitril/valsartan 97/103 mg bid over the 12-week study duration regardless of baseline ACEI/ARB exposure (70.3% overall and 76.2% if discontinuations due to non-AE reasons were excluded). Among patients in the low-dose stratum, the proportion able to achieve and maintain a dose of 97/103 mg bid sacubitril/valsartan was greater with the conservative regimen than with the condensed regimen (84.9 vs. 73.6%). This was mainly due to lower incidences of hypotension, hyperkalemia, and renal dysfunction in patients in the conservative regimen. The proportions of patients who achieved and maintained the targeted dose in the high-dose stratum was similar irrespective of the uptitration regimen (82.6% for condensed and 83.8% for conservative) (Senni et al. 2016).

4.3 PARAMOUNT-HF

PARAMOUNT-HF was a phase II multicenter, randomized, double-blind, parallel-group, and active-controlled proof-of-concept study in which sacubitril/valsartan 97/103 mg bid (formerly 200 mg bid) was compared with valsartan 160 mg bid in patients with HFpEF ($N = 301$). The primary endpoint was change in NT-proBNP from baseline to 12 weeks and secondary endpoints included change in

echocardiographic measures, BP, NYHA class, clinical composite assessment, and KCCQ scores (Solomon et al. 2012).

There was a significantly greater reduction in NT-proBNP from baseline to Week 12 for patients in the sacubitril/valsartan arm, compared with the valsartan arm. The relative difference between the two arms was 23% (ratio for sacubitril/valsartan to valsartan: 0.77; 95% CI: 0.64–0.92; $p = 0.005$). The reductions in NT-proBNP continued throughout 36 weeks of observation, although at 36 weeks the difference between the treatment arms was no longer significant. The reduction in NT-proBNP at Week 12 was consistently observed in all prespecified subgroups (defined by age, gender, EF, presence or absence of AF, previous hospital admission for HF, baseline NYHA class, and baseline NT-proBNP) except that within the sacubitril/valsartan treatment arm, patients with diabetes had a greater reduction in NT-proBNP than patients without diabetes (interaction $p = 0.02$). A post hoc analysis showed that the greater reduction in NT-proBNP observed at Week 12 in the sacubitril/valsartan arm was independent of the extent of BP lowering (Jhund et al. 2014). At Week 36, there was evidence of cardiac reverse remodeling indicated by significantly greater reductions in left atrial volume ($p = 0.003$) and size ($p = 0.034$) in the sacubitril/valsartan arm than in the valsartan arm. Patients in the sacubitril/valsartan arm showed significantly greater improvement in NYHA class ($p = 0.05$) at Week 36 than patients in the valsartan arm, although there was no difference between the arms in KCCQ scores (Solomon et al. 2012). Both treatments were generally well-tolerated and AE profiles were similar in the two treatment arms. There was, however, a higher incidence of serious AEs in the valsartan arm than in the sacubitril/valsartan arm (20 vs. 15%).

5 Current Status of Sacubitril/Valsartan

Sacubitril/valsartan is approved in more than 60 countries worldwide (including the USA and the EU) and is indicated to reduce the risk of CV death and hospitalization for patients with chronic HFrEF (PI 2015). European Society of Cardiology guidelines and a joint statement issued by the American College of Cardiology/American Heart Association/Heart Failure Society of America recommend sacubitril/valsartan (Class Ib) for HFrEF in conjunction with evidence-based therapies such as beta-blockers and mineralocorticoid receptor antagonists (Ponikowski et al. 2016; Yancy et al. 2016).

5.1 Ongoing Outcome Studies

Several outcome studies are ongoing to evaluate the efficacy and safety of sacubitril/valsartan in different patient populations. These include PARAGON-HF (Prospective comparison of ARni with Arb Global Outcomes in heart failure with preserved ejectioN fraction) and PARADISE-MI (Prospective ARni vs. ACE inhibitor trial to DetermIne Superiority in reducing heart failure Events after

Myocardial Infarction). PARAGON-HF is a phase III study undertaken to evaluate the potential benefits of sacubitril/valsartan regarding long-term morbidity and mortality in patients with HFpEF, following up on the promising findings in patients with HFpEF in PARAMOUNT-HF trial (Solomon et al. 2012). PARADISE-MI is a phase III study that will assess the effects of sacubitril/valsartan on outcomes in post-MI patients. In addition, a phase III study, PANORMA-HF, to assess the safety and efficacy of sacubitril/valsartan in children with HFrEF is also underway.

Acknowledgments/Disclosures Y. Khder, M. P. Lefkowitz, and V. Shi are employees of Novartis, the manufacturer of sacubitril/valsartan. The authors thank Roohi Chopra, Sreedevi Boggarapu, and Ronald B. Langdon (all of Novartis) for the writing and editorial support.

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Ivabradine

Michel Komajda

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Abstract

Ivabradine is a blocker of the funny current channels in the sinoatrial node cells. This results in pure heart rate reduction when elevated without direct effect on contractility or on the vessels. It was tested in a large outcome clinical trial in stable chronic heart failure (CHF) with low ejection fraction, in sinus rhythm, on a contemporary background therapy including betablockers (SHIFT: Systolic Heart Failure Treatment with the If inhibitor Trial).

The primary composite endpoint (cardiovascular mortality or heart failure hospitalization) was reduced by 18% whereas the first occurrence of heart failure hospitalizations was reduced by 26%. The effect was of greater magnitude in

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_55

patients with baseline heart rate ≥75 beats per minute. Ivabradine improved also the quality of life and induced a reverse remodelling.

The safety was overall good with an increase in (a)symptomatic bradycardia and visual side effects.

The efficacy and tolerability were similar to those observed in the overall trial in subgroups with diabetes mellitus, low systolic blood pressure (SBP), renal dysfunction or chronic obstructive pulmonary disease (COPD).

Ivabradine is indicated in CHF with systolic dysfunction, in patients in sinus rhythm with a heart rate ≥75 bpm in combination with standard therapy including betablocker therapy or when betablocker therapy is contraindicated or not tolerated (European Medicine Agency).

Keywords

Cardiovascular mortality • Chronic heart failure • Funny channel • Heart failure • Heart rate • Hospitalizations • Sinoatrial node

1 The SHIFT trial

Ivabradine is a heart rate slowing agent which acts through blockade of the If ("funny current") channels within the sinoatrial node cells (Di Francesco 2006; Vilaine 2006). The drug binds to the inner part of the channel in the pacemaker cells. Blockade of the *f* channels reduces the Na^+ and K^+ flow and flattens the spontaneous depolarization slope of the pacemaker cells leading to heart rate reduction. The effect is "use dependent", i.e. the magnitude of the heart rate reduction observed with ivabradine is all the more important as the initial heart rate is high.

Since the pharmacological effect of ivabradine is only related to the blockade of *f* channels which are located in the sinoatrial node, this drug has no effect on contractility, relaxation, atrioventricular or intraventricular conduction or on the vessels.

Ivabradine was evaluated in heart failure in the SHIFT (Systolic Heart Failure Treatment with the If inhibitor ivabradine Trial) (Swedberg et al. 2010). 6,505 patients with stable symptomatic heart failure with an ejection fraction ≤35%, in sinus rhythm with a heart rate ≥70 bpm who experienced a heart failure hospitalization within 1 year were randomized to ivabradine or matching placebo. Patients had to be treated by guidelines recommended therapy. Ivabradine was started at 5 mg bid and uptitrated to 7.5 mg bid when tolerated.

The primary composite endpoint (cardiovascular mortality or heart failure hospitalization) was significantly reduced by 18% after a median follow-up of 22.9 months (Fig. 1). This beneficial effect was driven mainly by a reduction of heart failure hospitalizations (−26%) whereas, there was a non-significant trend for a reduction in cardiovascular mortality (Table 1). The effect on cardiovascular mortality, heart failure hospitalization or both was of greater magnitude when baseline heart rate was ≥75 bpm.

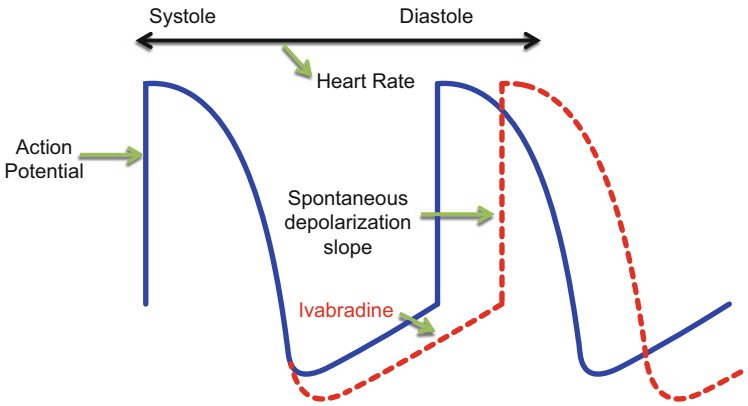

Fig. 1 Mechanism of action of ivabradine in the sino atrial node

Table 1 Effect of ivabradine on outcomes

Endpoints	Hazard ratio	95% CI	*P* value
Primary composite endpoint (CV death or hospital admission for worsening HF)	0.82	[0.75; 0.90]	$p < 0.0001$
All-cause mortality	0.90	[0.80; 1.02]	$p = 0.092$
Death from heart failure	0.74	[0.58; 0.94]	$p = 0.014$
All-cause hospital admission	0.89	[0.82; 0.96]	$p = 0.003$
Any CV hospital admission	0.85	[0.78; 0.92]	$p = 0.0002$
CV death/hospital admission for HF or non-fatal MI	0.82	[0.74; 0.89]	$p < 0.0001$

There was also a favourable effect on all-cause and cardiovascular hospitalizations but no effect on sudden death was observed. Patients enrolled in SHIFT were relatively young, predominantly male (76%) with a mean age of 60 years and the underlying aetiology was ischaemic in 68%. The mean ejection fraction was 29% and most patients were in NYHA class II or III.

The beneficial effect was observed in a well-treated population: 93% of the patients were taking an angiotensin-converting enzyme inhibitor or an angiotensin-II type 1 receptor blocker and 89% were treated by betablockers (56% were receiving at least 50% of the target dose of betablockers recommended by the European Society of Cardiology whereas 26% were at target dose).

The reduction in the occurrence of the primary composite endpoint was consistent in all pre-specified subgroups including sex, age, underlying aetiology, with one exception: the magnitude of the benefit derived from ivabradine was significantly greater in the subgroup with baseline heart rate above the median value (77 bpm in the SHIFT population).

Overall, the tolerability of ivabradine was good. However, symptomatic and asymptomatic bradycardia were reported in 5% and 6% of patients, respectively, in

the ivabradine arm, but led to treatment discontinuation in only 1% of cases for each of these two adverse effects. Visual side effects such as blurring vision or flashes related to blockade of *f* channels in the retina were uncommon, usually transient and led to treatment discontinuation in only a few cases.

A Holter substudy was performed at baseline and 8 months after randomization to ivabradine or matching placebo (Böhm et al. 2015). At 8 months, patients on ivabradine had more episodes of bradycardia <40 bpm than those on placebo but no episode of severe bradycardia <30 bpm was recorded. The number of pauses >2.5 s was similar in the two groups whereas the number of second or high degree atrioventricular blocks was numerically smaller in ivabradine treated patients. No episode of third degree AV block was observed. Atrial fibrillation was observed in a limited number of patients and was similar in the two groups. This substudy did not confirm the observation of a slight but significant increase in atrial fibrillation in the overall SHIFT population treated by ivabradine. These results suggest that ivabradine on top of betablocker does not induce clinically significant bradycardia or a pro-arrhythmic effect in patients with chronic heart failure (CHF) and low ejection fraction.

2 Hospitalizations for Heart Failure and Quality of Life

Hospitalization for heart failure is lengthy and recurrent and the risk is more important in the early phase after hospitalization named the "vulnerable period" (Abrahamsson et al. 2013). This accounts for 2/3 of the overall costs (Stewart et al. 2002). The effect of ivabradine on recurrent hospitalizations for heart failure was tested in SHIFT (Borer et al. 2012). Ivabradine was associated with a significant 25% reduction in the total number of HF hospitalizations and with a reduced risk for recurrent HF hospitalizations.

Another recent study looked at the risk of early readmission within 90 days in patients who experienced a heart failure hospitalization during the trial. Ivabradine treated patients had a 25–30% relative risk reduction of being rehospitalized for any cause compared to the placebo group (Komajda et al. 2016).

This important finding suggests that patients treated with ivabradine are less likely to be rehospitalized during the vulnerable phase post-discharge.

A pre-specified analysis of health related quality of life (HQoL) was made in # 2,000 patients enrolled in SHIFT. It used the self-reported disease specific Kansas City Cardiomyopathy Questionnaire (KCCQ) containing an overall summary score (including the social limitation induced by the disease and a clinical summary score) (Green et al. 2000). Ivabradine improved significantly the two dimensions of KCCQ, the clinical summary score and the overall summary score compared to placebo (Erkman et al. 2011).

3 Efficacy and Safety of Ivabradine in Populations with Important Comorbidities

3.1 Diabetes

Diabetes mellitus is a frequent comorbidity in patients with heart failure and the combination is associated with poor outcomes. An analysis compared the efficacy and the tolerability of ivabradine in 1,979 patients enrolled in SHIFT with diabetes mellitus vs patients without diabetes (Komajda et al. 2015).

The effect of ivabradine was similar on the relative risk reduction of the primary composite endpoint (20% and 16%, respectively, in patients with/without diabetes) and the benefit was mainly driven by a reduction in hospitalizations for worsening heart failure. The incidence of serious adverse events was not significantly different between patients with/without diabetes.

3.2 Patients with Low Systolic Blood Pressure

The presence of low systolic blood pressure (SBP) can be challenging for the management of heart failure and especially for the uptitration of recommended medications which lower blood pressure (Angiotensin-Converting Enzyme inhibitors, betablockers and angiotensin-II receptor blockers).

The efficacy and the safety of ivabradine was assessed in the SHIFT population divided by tertiles of baseline SBP: patients with SBP < 115 mmHg; patients with $115 \leq$ SBP < 130 mmHg and patients with SBP ≥ 130 mmHg (Komajda et al. 2014).

Ivabradine was associated with a similar relative risk reduction of the composite primary endpoint and its two components in the three SBP groups. There was no evidence of different safety issues in the three blood pressure groups.

This finding is in line with the mechanism of action of the f current inhibitor which does not affect the vascular bed and has therefore no vasodilatory effect. This may have implications for the management of HF with low SBP and elevated heart rate, a condition where uptitration of betablockers is often difficult due to hypotension.

3.3 Renal Dysfunction

Renal dysfunction is a frequent comorbidity in heart failure and is associated with poor outcomes. The efficacy of ivabradine was assessed in patients with low estimated glomerular filtration rate (eGFR < 60 ml/mn) compared to those with preserved renal function (eGFR ≥ 60 ml/mn) (Voors et al. 2014). Ivabradine use was associated with a similar reduction of the primary composite endpoint in patients with and without moderate renal dysfunction at baseline and the tolerability of ivabradine was comparable in the two groups.

Importantly, no differences in changes in renal function over time were found between ivabradine and placebo-treated patients.

3.4 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) was reported in # 12% of the patients enrolled in SHIFT. They were on average older and had an overall increased risk of death, all-cause and heart failure hospitalization than patients without COPD (Tavazzi et al. 2013).

Ivabradine reduced similarly the relative risk of the primary composite endpoint in COPD and in non-COPD patients. Adverse events were more frequent in COPD patients than in non-COPD patients but were not significantly different in the ivabradine and placebo arms of either group. This suggests that ivabradine is safe and efficient in this subgroup of patients who are difficult to treat with betablockers.

4 Mechanism of Action of Ivabradine in Heart Failure

4.1 Acute/Subacute Haemodynamic Effects

The force/frequency relationship is positive in the normal heart (i.e. force increases proportionally to the increase in heart rate) whereas in the falling myocardium force decreases with heart rate increase (Mulieri et al. 1992; Hasenfuss et al. 1994).

Thus, increasing heart rate in heart failure results in a decrease in cardiac output and an increase in cardiac dimensions. This unfavourable effect can be reverted by ivabradine as shown both in the experimental setting and in patients with advanced heart failure (Mulder et al. 2004; De Ferrari et al. 2008). A recent small size study evaluated the impact of early in hospital administration of ivabradine + betablocker versus betablocker only (Hidalgo et al. 2016). It concluded that the early co-administration is associated with an improvement in functional status and cardiac function. We however lack a large outcome study assessing the potential benefit of early administration of ivabradine during a heart failure decompensation.

4.2 Chronic Haemodynamic Effects

The impact of the pure heart rate lowering effect of ivabradine on cardiac function was assessed in an echocardiographic substudy (Tardif et al. 2011). Treatment with ivabradine reduced significantly left ventricular (LV) end systolic volume index compared to placebo. This reduction was also observed on LV end diastolic index whereas ejection fraction was slightly improved. This study suggests that ivabradine has an anti-remodelling effect which is associated with improved outcomes.

One potential explanation for the beneficial effect of heart rate reduction on cardiac dimensions is the reduction of afterload (Reil et al. 2013). In an echocardiographic substudy, it was observed that after 8 months of treatment, ivabradine induced a significant reduction in effective arterial elastance and improved total arterial elastance compared with placebo. Contractility remained unchanged and ventricular arterial coupling was improved, resulting in a higher stroke volume. This second mechanistic study therefore suggests that pure heart rate reduction with ivabradine unloads the failing left ventricle.

5 Indication

The current indication provided by the European Medicine Agency is "management of chronic heart failure NYHA Class I to IV with systolic dysfunction in patients in sinus rhythm and whose heart rate is $\geq$ 75 bpm in combination with standard therapy including betablocker therapy or when betablocker therapy is contra-indicated or not tolerated".

This drug is also approved by the Food and Drug Administration for the reduction in heart failure hospitalizations from worsening heart failure.

Finally, ivabradine is listed in the latest version of the heart failure guidelines of the European Society of Cardiology as a recommended medication in patients who remain symptomatic despite optimal therapy (including ACE inhibitors, betablockers and mineralocorticoid receptor antagonists) are in sinus rhythm and have an increased heart rate $\geq$70 bpm (Ponikowski et al. 2016).

6 Contraindications/Non-indications

Some important contraindications/non-indications and drug interactions are listed in Table 2.

Table 2 Important contra-indications and interactions

Contra-indications	*Non indications*
• Sick sinus syndrome	• Permanent atrial fibrillation
• Pace Maker dependent patients	• Heart failure with preserved ejection fraction
• High degree AV block	• Acute heart failure
Important interactions	
• QT prolonging drugs (quinidine, amiodarone, disopyramide, sotalol. . ..)	
• Cytochrome P450 3A4 inhibitors	
– Ketoconazole	
– Macrolides	
– VIH protease inhibitors	
– Grapefruit juice	

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Partial Adenosine A1 Agonist in Heart Failure

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_83

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Abstract

Adenosine exerts a variety of physiological effects by binding to cell surface G-protein-coupled receptor subtypes, namely, A1, A2a, A2b, and A3. The central physiological role of adenosine is to preclude tissue injury and promote repair in response to stress. In the heart, adenosine acts as a cytoprotective modulator, linking cardiac function to metabolic demand predominantly via activation of adenosine A1 receptors (A1Rs), which leads to inhibition of adenylate cyclase activity, modulation of protein kinase C, and opening of ATP-sensitive potassium channels. Activation of myocardial adenosine A1Rs has been shown to modulate a variety of pathologies associated with ischemic cardiac injury, including arrhythmogenesis, coronary and ventricular dysfunction, apoptosis, mitochondrial dysfunction, and ventricular remodeling. Partial A1R agonists are agents that are likely to elicit favorable pharmacological responses in heart failure (HF) without giving rise to the undesirable cardiac and extra-cardiac effects observed with full A1R agonism. Preclinical data have shown that partial adenosine A1R agonists protect and improve cardiac function at doses that do not result in undesirable effects on heart rate, atrioventricular conduction, and blood pressure, suggesting that these compounds may constitute a valuable new therapy for chronic HF. Neladenoson bialanate (BAY1067197) is the first oral partial and highly selective A1R agonist that has entered clinical development for the treatment of HF. This review provides an overview of adenosine A1R-mediated signaling in the heart, summarizes the results from preclinical and clinical studies of partial A1R agonists in HF, and discusses the potential benefits of these drugs in the clinical setting.

Keywords
Adenosine • Adenosine A1 receptor • Adenylate cyclase • Heart failure • Neladenoson

Abbreviations

A1R	A1 receptor
ATP	Adenosine triphosphate
AV	Atrioventricular
CAD	Coronary artery disease
cAMP	Cyclic adenosine monophosphate
FFA	Free fatty acid
GLUT	Glucose transporter
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
IS	Infarct size
K_{ATP}	ATP-sensitive potassium channel
LV	Left ventricular
LVEF	Left ventricular ejection fraction
MPTP	Mitochondrial permeability transition pore
ROS	Reactive oxygen species
SERCA2a	Sarcoplasmic reticulum calcium-ATPase 2a
SR	Sarcoplasmic reticulum
TAL	Medullary thick ascending limb
TG	Triglyceride
UPC	Uncoupling protein

1 Adenosine A1 Receptor Physiology

Adenosine is a purine nucleoside that acts as a cytoprotective modulator in response to stress to an organ or tissue (Fredholm et al. 2001; Jacobson et al. 1992). It is present in almost every cell of the body, and its cytoplasmic concentration is tightly regulated. The synthesis and facilitated diffusion of adenosine from cells via nucleoside transporters are increased mainly in response to unfavorable metabolic states, such as tissue hypoxia and stress. The concentration of adenosine in the extracellular compartment is the consequence of many biological processes, including extracellular adenosine production, exogenous and endogenous transport, and

formation from intracellular sources. The cellular effects of adenosine are mediated by four subtypes of G-protein-coupled adenosine plasma membrane receptors (A1, A2a, A2b, and A3). The A1 and A3 receptors are Gi-protein coupled and inhibit the production of cyclic adenosine monophosphate (cAMP), whereas the A2a and A2b subtypes are coupled to Gs or Go to stimulate adenylate cyclase; the A2b subtype is also coupled to Gq. In the heart, adenosine-induced protective and regenerative effects are mediated predominately by adenosine A1 receptors (A1Rs) (Mustafa et al. 2009). This review will focus on the A1R and its potential as a therapeutic target for heart failure (HF).

The A1R is the most conserved adenosine receptor subtype among species (Fredholm et al. 2001), and it is widely expressed throughout the body in adipose tissue, brain (especially excitatory nerves), kidney, and heart (Dixon et al. 1996). In the heart, A1Rs are expressed in cardiomyocytes (Hussain and Mustafa 1995), in both supraventricular tissue (atria) and ventricles (Hussain and Mustafa 1995; Musser et al. 1993).

Activation of A1R has minimal effects on adenylate cyclase activity in the absence of stimulation by catecholamines. However, in the presence of catecholamines (e.g., when the sympathetic nervous system is activated and cAMP is elevated), activation of A1Rs results in notable inhibition of adenylate cyclase (Bott-Flugel et al. 2011). Modulation of cAMP levels influences calcium handling and L-type calcium channel opening (Mustafa et al. 2009). In addition to inhibition of adenylate cyclase, adenosine A1R activation leads to stimulation of phospholipase C and modulation of mitochondrial adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP}) and pertussis-toxin-sensitive potassium channels resulting in reduced mitochondrial permeability transition pore (MPTP) opening, thereby improving mitochondrial function under hypoxic conditions (Kirsch et al. 1990; Xiang et al. 2010). Collectively, via these processes, A1R activation affords protection of the failing myocardium by limiting the triggering of cell death and injury (Fig. 1).

1.1 Limitations of Full Adenosine Receptor Agonists as a Therapeutic Option

A1Rs mediate multiple pharmacological effects due to their widespread distribution and diverse function in different organs and tissues. The primary limitations of full A1R agonists in HF, while offering potential therapeutic benefits, are related to undesirable cardiac effects including negative inotropic, chronotropic, and dromotropic effects that cause bradycardia and higher-degree atrioventricular (AV) block. Furthermore, A1R activation by a full agonist may lead to extra-cardiac effects, including reduction of excitatory neurotransmitter release and locomotor activity in the central nervous system and antidiuretic effects caused by vasoconstriction of renal afferent arterioles (Stone et al. 2009). Receptor desensitization is also a potential problem when considering the chronic use of full A1

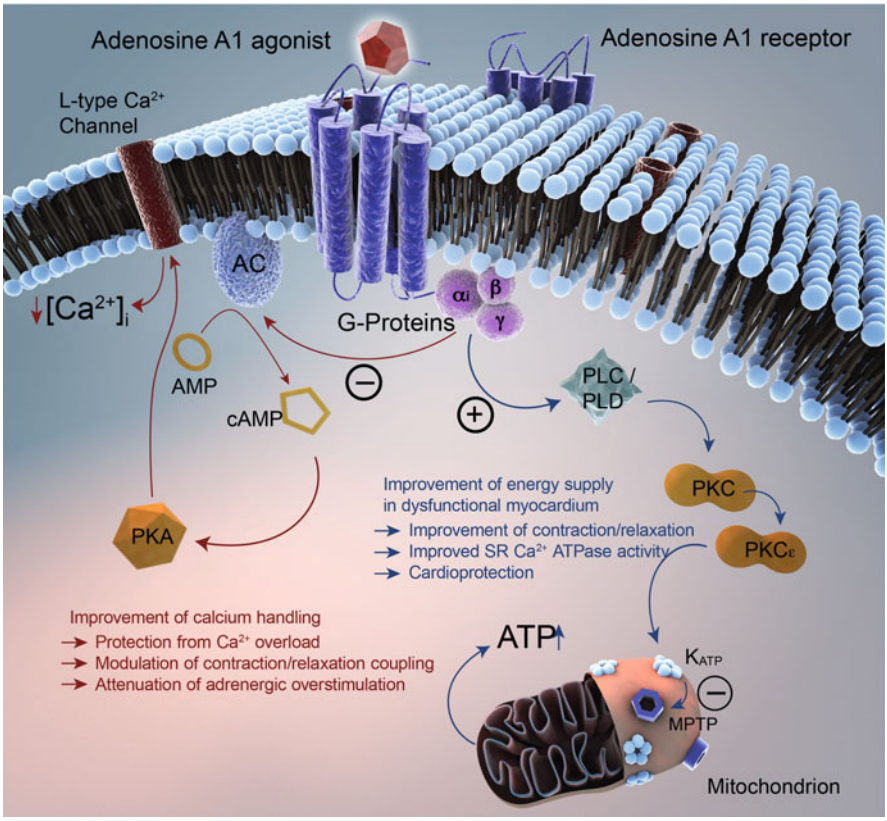

Fig. 1 Adenosine A1 receptor signaling pathways in the failing heart. *AC* adenylate cyclase, *AMP* adenosine monophosphate, *cAMP* cyclic adenosine monophosphate, *ATP* adenosine triphosphate, K_{ATP} ATP-dependent potassium channel, *MPTP* mitochondrial permeability transition pore, *PKA* protein kinase A, *PKC* protein kinase C, *PLC* phospholipase C, *PLD* phospholipase D, *SR* sarcoplasmic reticulum

receptor agonists (Roman et al. 2008), a phenomenon also evident with chronic administration of β-adrenergic agonists.

1.2 Partial Adenosine A1 Receptor Agonists

To overcome the limitations associated with the use of full A1R agonists, efforts were recently focused on designing compounds with better tissue specificity and selectivity. In this context, partial A1R agonists have been developed, which are likely to elicit only the therapeutic effects in the target tissues without giving rise to undesirable effects.

A partial adenosine A1 receptor agonist is a lower efficacy ligand compared with a full agonist, which elicits a submaximal response of the signaling cascade because it can only occupy certain activity states of receptors (Fig. 2).

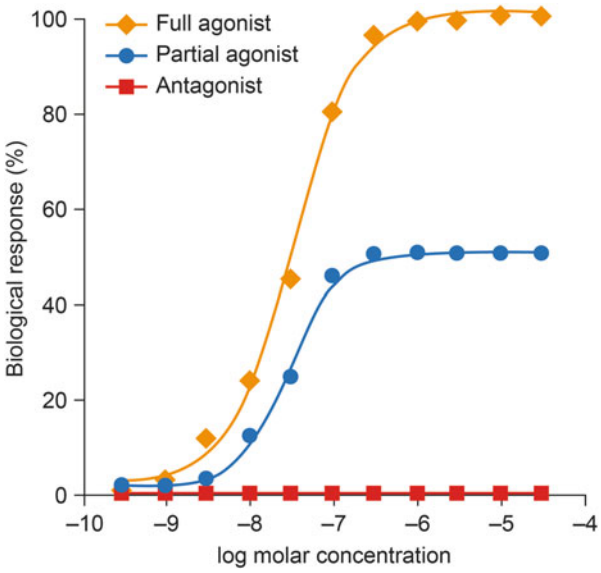

Fig. 2 Adenosine A1 receptor activation by full or partial agonists and blockade by an antagonist. Reprinted with permission from Greene et al. (2016)

A partial A1R agonist is expected to result in a robust pharmacological response only in tissues with a relatively high receptor reserve, whereas a full agonist may elicit a robust signal in all tissues expressing A1Rs. A partial receptor agonist is also less effective than a full agonist in evoking a response in a tissue with low amplification of the signal transduction pathway. For these reasons, tissue selectivity is more likely to be achieved with a partial agonist than with a full agonist. In HF, for example, partial A1R agonists are likely to be useful for achieving high selectivity for the heart, leading to beneficial effects such as improvement of cardiac energetics and function, cardioprotection, and reverse ventricular remodeling. Furthermore, a partial A1R agonist can act as semi-effective agonist or as a weak antagonist, depending on endogenous adenosine concentrations in the organ or tissue, thereby leading to further functional selectivity (Srinivas et al. 1997). When both a full (endogenous) agonist and a partial agonist are competing for the same receptors, the partial agonist acts as a competitive antagonist producing a net decrease in receptor activation. In HF, partial A1R agonists may therefore minimize undesired pharmacological effects and effects in nontarget tissues (e.g., neurological effects, antidiuretic effects, AV conduction abnormalities, bradycardia, or hypotension). Finally, a partial A1R agonist is also less likely to induce receptor desensitization compared with full agonists (Parsons and Stiles 1987). Partial A1R agonists are therefore likely to be more suitable for long-term treatment, offering broader dose ranges and higher selectivity toward a therapeutically desired pharmacology.

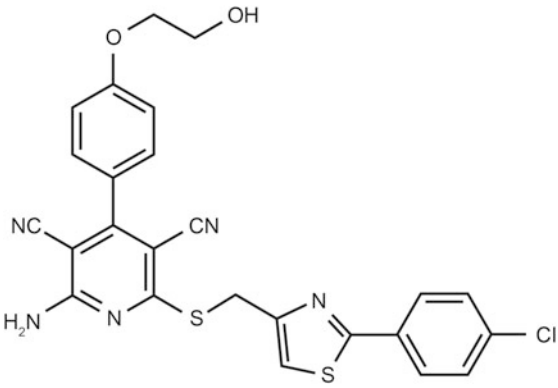

Fig. 3 Chemical structure of the partial adenosine A1 receptor agonist capadenoson. Reprinted with permission from Albrecht-Küpper et al. (2012)

1.3 Pharmacology of Adenosine A1 Receptor Agonists

Almost all A1R agonists developed are adenosine derivatives; however, adenosine-like A1R agonists often have the drawback of a short half-life and low bioavailability, making them unsuitable for chronic oral therapy. Thus, efforts were recently placed on developing non-adenosine-like partial A1R agonists with pharmacokinetics optimized for oral treatment. A new class of heterocyclic compounds, devoid of the ribose moiety found in adenosine, was identified as highly potent and selective full and partial A1R agonists. Here, we provide chemical and pharmacological data for partial A1R agonists with the example of capadenoson (BAY 68-4986) (Fig. 3). Optimization of partial A1 agonists has been described in detail by Nell and Albrecht-Küpper (2009).

Capadenoson belongs to the dicyanopyridine class of A1R agonists discovered via high throughput screening and hit optimization of the Bayer collection. In addition to being a potent and selective partial A1R agonist, capadenoson also possesses favorable metabolism and pharmacokinetic properties (Nell and Albrecht-Kupper 2009). In phase 2 clinical studies, capadenoson improved total exercise time in patients with stable angina compared with placebo without inducing AV block (Tendera et al. 2012). Capadenoson also showed beneficial effects on left ventricular ejection fraction (LVEF) in a canine HF model without any effects on blood pressure or heart rate (Sabbah et al. 2013). Thus, capadenoson is an example of a partial A1R agonist with selective pharmacological activity, providing further evidence that partial agonists can achieve desired pharmacological effects while avoiding undesired pharmacological activity (Nell and Albrecht-Kupper 2009; Albrecht-Küpper et al. 2012). However, the low solubility of capadenoson made tablet formulation difficult, therefore a soluble derivative, neladenoson bialanate was developed, which is currently in clinical development for the treatment of HF.

2 Selective A1R Agonists: Preclinical and Clinical Evidence in Cardiovascular Disorders and Clinical Implications

2.1 Intravenous Antiarrhythmic Agents for the Treatment of Supraventricular Tachycardia

With the exception of adenosine, the first A1R agonists to enter clinical development were the intravenous antiarrhythmic agents tecadenoson and selodenoson (Bayes et al. 2003), which were developed for the treatment of paroxysmal supraventricular tachycardia (Ellenbogen et al. 2005). By selectively targeting the A1R, tecadenoson was associated with fewer adverse effects such as flushing, dyspnea, chest discomfort, and hypotension compared with adenosine, which is a nonselective agonist of all four adenosine receptors. Both compounds showed a significant reduction in heart rate but resulted in higher-degree AV block at high doses (Peterman and Sanoski 2005). Of note, tecadenoson had no significant effects on blood pressure (Ellenbogen et al. 2005). In a phase 3 clinical trial, there were no reports of worsening pulmonary symptoms following administration of tecadenoson, which may be explained by lack of activity of tecadenoson on the A2b or A3 receptors (Ellenbogen et al. 2005).

2.2 Angina Pectoris

Coronary heart disease is the most significant cause of death in industrial countries and a major risk factor for the development of HF. Myocardial ischemia leads to an imbalance between supply and demand of myocardial oxygen and energy and results primarily from an epicardial coronary artery stenosis most often due to an atherosclerotic plaque. Activation of A1Rs by a partial A1R agonist can result in a reduction in oxygen consumption and improved energy supply by cardioprotective mechanisms, especially during exercise when most angina attacks occur. Evidence for the efficacy of partial A1R agonists in the treatment of angina pectoris was initially demonstrated in a clinical trial with capadenoson. In patients with stable angina, compared with placebo, capadenoson lowered exercise heart rate at comparable maximum workload and improved total exercise time while prolonging the time to ischemia (Tendera et al. 2012).

2.3 Antilipolytic Effects on Free Fatty Acids

Diabetes mellitus is one of the most common diseases worldwide and is associated with considerable morbidity and mortality caused primarily by the development of cardiovascular diseases, including coronary artery disease (CAD) and HF. Type 2 diabetes mellitus is characterized by insulin resistance in peripheral tissues (i.e., liver, skeletal muscle, and adipose tissue). Although the mechanisms that account for insulin resistance are not completely understood, elevated levels of circulating

free fatty acids (FFAs), derived from the hydrolysis of triglycerides (TGs) in adipocytes, are thought to play a role, with several studies demonstrating that an increase in FFA supply promotes insulin resistance and is linked to diabetes (Johnson et al. 1992). Chronically elevated plasma FFA levels lead to increased glucose production in the liver, impaired glucose uptake in skeletal muscle, and decreased pancreatic β-cell insulin secretion (Bajaj and Defronzo 2003; Boden 2001; Boden and Shulman 2002). Activation of the A1R has been shown to suppress lipolysis in adipose tissue, through inhibition of adenylate cyclase and downstream cAMP formation, resulting in a reduction of plasma FFA levels (Heseltine et al. 1995). An increase in FFA supply decreases insulin-mediated glucose metabolism and thus insulin sensitivity, whereas decreased lipolysis promotes insulin resistance (Johnson et al. 1992). Thus, a possible involvement of the A1R in the development of type 2 diabetes mellitus has been postulated. Therefore, reducing FFA availability and tissue utilization by A1R activation is a plausible pharmacological approach for the treatment of insulin resistance.

The therapeutic use of A1R agonists as antilipolytic and antidiabetic agents was investigated in early clinical trials, but their potential use for this indication was limited by the secondary adverse effects of full A1R agonism. Another limitation was the development of treatment tolerance to the antilipolytic effects due to receptor desensitization.

In order to overcome these limitations, partial A1R agonists have been developed (Dhalla et al. 2003, 2007a). One example is CVT-3619, a partial adenosine A1R agonist that has antilipolytic effects at concentrations that are not accompanied by significant cardiovascular effects. Studies have shown that CVT-3619 can lower circulating FFA levels and improve insulin sensitivity (Dhalla et al. 2007a). A study by Shearer et al. has shown that acute administration of CVT-3619 lowers circulating FFA levels and improves cardiac glucose clearance, providing evidence that partial A1R agonists may be a treatment option for the restoration of substrate balance in the insulin-resistant and diabetic heart (Shearer et al. 2009). Excess FFAs can also cause abnormalities of mitochondrial function, including the formation of reactive oxygen species (ROS), oxygen wastage, and increased mitochondrial and cytosolic calcium (Mjos 1971a, b), all of which may further impair myocardial function in HF (Tuunanen et al. 2006). Furthermore, increased FFA oxidation results in increased oxygen consumption and reduced cardiac energy efficiency. Indeed, observational studies have demonstrated a link between elevated plasma FFA levels and cardiovascular outcomes. In a study by Djoussé et al. including 4,248 patients, increased plasma FFA levels were associated with a high risk of HF development. In patients without HF at baseline, elevated plasma FFA levels were associated with a 12% (95% confidence interval: 6–19%) higher risk of HF than patients with normal FFA levels during a mean follow-up of 10.5 years (Djoussé et al. 2013).

The effects of partial A1R agonists on plasma FFA levels have also been tested in phase 1 clinical trials. The partial A1R agonist GS-9667 reduced plasma FFA levels and was well tolerated in healthy non-obese and obese volunteers (Staehr et al. 2013), further supporting the concept that partial A1R agonist therapy can

provide the desired biological responses and avoid undesired physiological effects such as higher-degree AV block.

As insulin resistance and diabetes are epidemics of the twenty-first century, it is highly likely that the increasing prevalence of these metabolic disorders will greatly affect the cardiovascular disease burden in the future. It may be hypothesized that activation of A1Rs may improve insulin resistance and restore the substrate balance in the failing human heart and skeletal muscle. Considering the magnitude of the global problem of diabetes and the strong unmet clinical need in patients with diabetes and HF, this may present an opportunity for treatment in the growing group of patients with HF and diabetes.

2.4 Preconditioning and Cardioprotection

The adenosine A1R has been of great interest to researchers in the field of cardioprotection. Extensive research involving transgenic mice has shown that overexpression of the adenosine A1R is associated with a decrease in ischemic damage to the heart (Mubagwa and Flameng 2001). It was reported that the increased expression of cardiac adenosine A1Rs in transgenic mouse models generated an ischemic-tolerant phenotype characterized by reduced contractile dysfunction, necrosis, and infarct size (IS) and by improved myocardial energetics (Headrick et al. 1998; Matherne et al. 1997). Both full and partial agonists of cardiac adenosine A1Rs have been proven to reduce ischemic damage to the heart. These cardioprotective effects have also been evoked with capadenoson in a model of acute myocardial infarction. Transient occlusion of the left anterior descending artery resulted in the induction of myocardial infarction. In animals receiving placebo, the IS reached $29 \pm 2\%$ of the area at risk (Fig. 4). Pre-ischemic treatment of rats with capadenoson dose dependently and significantly decreased IS by up to 30% (to $21 \pm 3\%$ with a dose of 0.3 mg/kg; Fig. 4) (Albrecht-Küpper et al. 2012). Furthermore, it has been shown that pharmacological preconditioning with a non-adenosine analogue A1R agonist improves cardiac function recovery as well as endothelial function after cardiopulmonary bypass surgery in dogs (Veres et al. 2010). In a study by Yao et al., stimulation of myocardial A1Rs was cardioprotective during repetitive and brief periods of coronary artery occlusion in anesthetized dogs (Yao and Gross 1993). The protective effects of activation of A1Rs are most likely mediated by inhibition of adenylate cyclase and a subsequent reduction in cAMP levels, as well as by activation of protein kinase C, which leads to the opening of mitochondrial K_{ATP} channels and a reduction in the opening probability of MPTP and thereby an improvement in mitochondrial function under hypoxic conditions (Xiang et al. 2010).

Emerging evidence has shown that patients with HF are susceptible to progressive myocardial failure because of the accelerated loss of cardiomyocytes. Potential principal mechanisms by which cardiomyocytes may be lost are apoptosis and necrosis. Necrosis is an episodic process by which myocytes undergo loss of membrane integrity and total disruption of cellular function, followed by cell

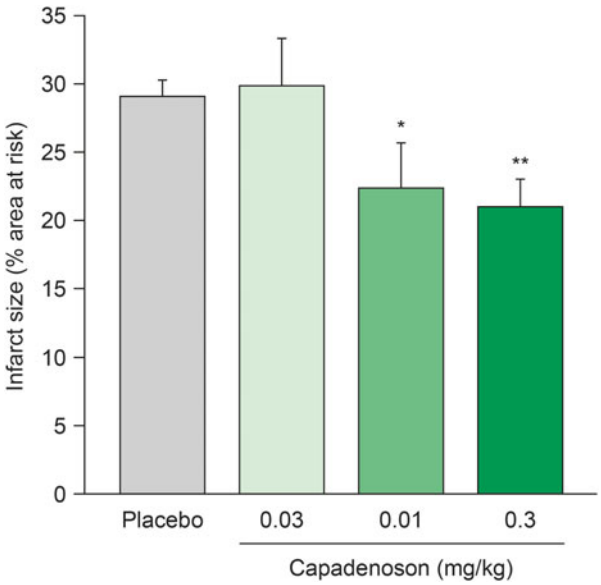

Fig. 4 Dose-dependent reduction of infarct size by capadenoson in a rat model of acute myocardial infarction. Capadenoson (mg/kg) was administered intravenously. Data are shown as mean ± standard error of the mean. $n = 9–18$. * denotes $P < 0.05$; ** denotes $P < 0.01$ versus placebo. Reprinted with permission from Albrecht-Küpper et al. (2012)

death and fibrosis. Clinically, the most common cause of necrosis is ischemia. In contrast, apoptosis typically involves smaller numbers of cells, follows a more prolonged time course, and may occur chronically, resulting in a substantial cumulative loss of cells (Mann 1999; Olivetti et al. 1997). CAD is the major cause of HF with reduced ejection fraction (HFrEF) in contemporary Western populations (Gheorghiade et al. 2006) and leaves patients at high risk of ongoing ischemic events and subsequent myocardial injury. This is supported by the observation that a significant proportion of patients with HF have detectable troponin levels, a marker of ongoing myocardial injury. It has been shown that cardiac troponin levels are associated with an increased risk of morbidity and mortality in both acute and chronic HF (Motiwala et al. 2015). In patients with HF with preserved ejection fraction (HFpEF), the reported prevalence of CAD or myocardial ischemia is approximately 50%. Patients with HFpEF and concomitant CAD are at higher risk of all-cause mortality and sudden death compared with those without CAD (Badar et al. 2015; Rusinaru et al. 2014).

During the clinical course of HF, myocyte loss caused by either necrosis or apoptosis may occur via several potential pathological mechanisms. In patients with CAD or decreased coronary reserve, cardiac decompensation may lead to increased myocardial oxygen demand causing flow-limited ischemia and myocyte death. Even in the absence of CAD, the hemodynamic stresses associated with acute decompensation may lead to subendocardial ischemia, microvascular hypoperfusion, and subsequent apoptosis and/or necrosis. During episodes of physical exercise, in patients with HF and CAD, coronary flow cannot be increased to match the oxygen requirements and metabolic needs of the heart, facilitating

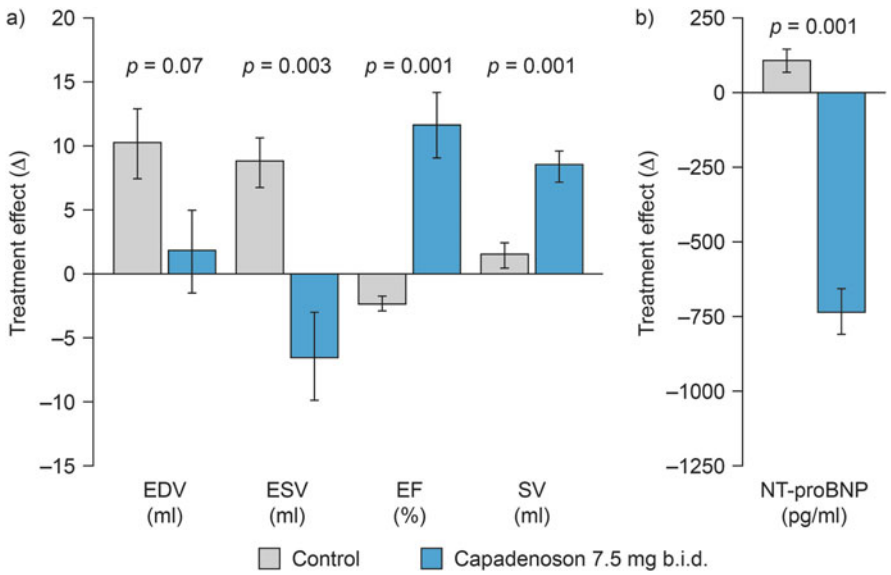

Fig. 5 The effects of 12 weeks of treatment with capadenoson (7.5 mg b.i.d.) in a canine heart failure model. *EDV* left ventricular end-diastolic volume, *EF* left ventricular ejection fraction, *ESV* left ventricular end-systolic volume, *NT-proBNP* N-terminal pro-brain natriuretic peptide, *SV* stroke volume. Data are shown as mean ± standard error of the mean. *P* values are comparisons between untreated dogs with heart failure and capadenoson-treated dogs with heart failure. Reprinted with permission from Sabbah et al. (2013)

myocardial loss and accelerating myocardial failure and deterioration of cardiac function.

2.5 Heart Failure

To date, there has been only one published preclinical study exploring the impact of partial A1R agonism in HF (Sabbah et al. 2013). In a canine HF model, the partial A1R agonist capadenoson produced a rapid and sustained improvement of LVEF (Fig. 5) and prevented progressive remodeling. In the same animal model, a selective β1-receptor blocker was also shown to increase LVEF significantly after 3 months of treatment, although to a lesser extent compared with capadenoson (Sabbah et al. 1994). Interestingly, with capadenoson treatment, LVEF increased early (1 week) after initiating treatment, whereas β-blockers reduced left ventricular (LV) systolic function early in the course of therapy owing to negative inotropic properties, before improvement took hold later in the course of therapy. These differences suggest there are additional mechanisms, unique to partial A1R agonism, that partly drive the observed improvement in LV systolic performance. The early improvement of LV systolic function with capadenoson may at least partly be the result of improved myocardial energetics resulting from selective

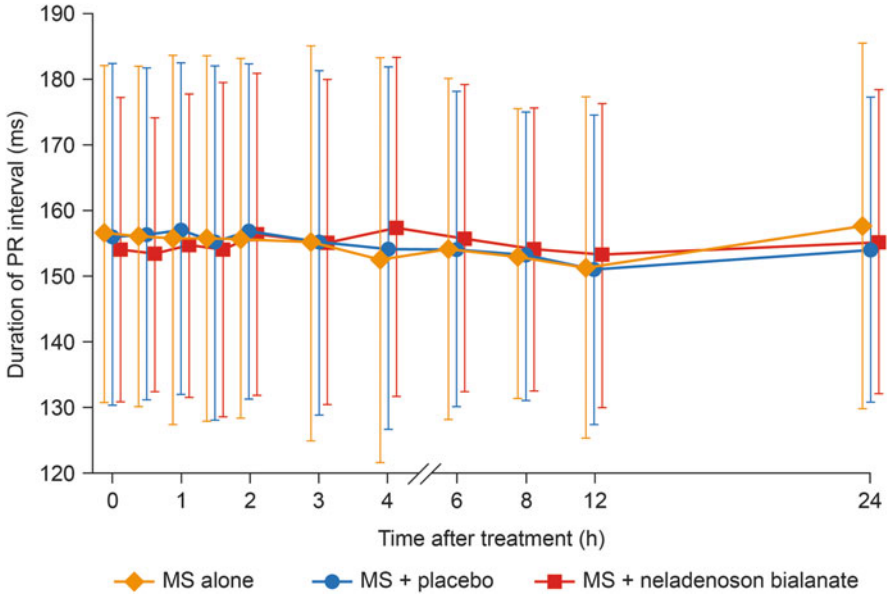

Fig. 6 Effects on PR interval of a single 50 mg dose of neladenoson bialanate administered together with metaprolol succinate in healthy volunteers who had received pretreatment with metaprolol succinate. Data are presented as mean ± standard deviation. *MS* metoprolol succinate. Reprinted with permission from Dinh et al. (2016)

activation of the A1R in cardiomyocytes. Capadenoson had no effects on heart rate or systemic blood pressure and did not trigger negative dromotropic effects, AV block, or sedation at any time during the study. There were no detectable increases in creatinine or blood urea nitrogen, indicating that renal function had not deteriorated as a result of partial A1R agonist therapy (Nell and Albrecht-Kupper 2009).

Recently, neladenoson bialanate (BAY 1067197), a novel highly selective oral non-adenosine-like partial A1R agonist, entered clinical development for the treatment of HF. Of particular importance in this clinical development is the potential influence of concomitant use of β-blockers as they have the potential to increase the risk of AV conduction abnormalities with A1R agonist treatment. Two small phase 2a pilot studies evaluating the short-term safety of neladenoson bialanate in patients with HFrEF pretreated with β-blockers have recently completed, and the results are pending (ClinicalTrials.gov identifiers: NCT02040233 and NCT01945606). In a small phase 1 trial in healthy volunteers pretreated with the β-blocker metoprolol succinate, a single 50 mg oral dose of neladenoson bialanate administered together with metaprolol succinate was found to be well tolerated. Notably, no higher-degree AV block, prolongation of the PR interval (Fig. 6), or relevant decreases in heart rate or systemic blood pressure were observed (Dinh et al. 2016).

3 Rationale for Partial Adenosine A1 Receptor Agonism for Heart Failure Treatment

The current treatment paradigm for patients with HFrEF aims to provide systemic blockade of the maladaptive neurohumoral response, notably the enhanced and sustained activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system (RAAS). Antagonism of the neurohumoral axis for the treatment of HF is achieved using "hemodynamically active medications" that decrease cardiac workload by reducing heart rate, preload, and afterload often with a reduction of systemic blood pressure. However, the repeated incremental addition of hemodynamically active medications raises tolerability and safety concerns (e.g., hypotension and bradycardia), specifically in patients with HFrEF (Vaduganathan et al. 2015). Indeed, low systolic blood pressure, which may reflect the extent of myocardial reserve, is one of the strongest determinants of outcome in ambulatory and hospitalized patients with HFrEF (Ambrosy et al. 2013). Hypotension, especially in high-risk patients with advanced HF, presents a major hurdle to drug development for HF and may pose an even greater obstacle once sacubitril/valsartan (Entresto™) achieves widespread clinical use. Moreover, upon hospitalization for HF, mortality and the rates of rehospitalization for HF remain high despite optimal use of the standard of care, indicating that there is an unmet clinical need in this patient population. This difficult landscape for HF drug development is particularly troubling considering the complete absence of effective therapy for HFpEF, a condition that affects nearly 40–50% of all patients with chronic HF (Steinberg et al. 2012). Attempts to reproduce the benefits of neurohormonal antagonism in patients with HFpEF have thus far been unsuccessful (Shah et al. 2010).

Given the limitations of current therapies for HF, refocusing attention on the failing heart and on restoring function to viable but nonfunctional cardiac units without significantly influencing blood pressure or heart rate is an alternative approach for the development of novel drugs for the treatment of HF. The primary goal of treating patients with HF is the restoration or protection of cardiac function. Recent studies show that cardiac function can be successfully recovered in patients with HF, even after structural alterations have occurred, suggesting that the damage to the myocardium is reversible (Bello et al. 2003). These observations pave the way for rational therapeutic design based on targeting the cardiomyocyte itself and thus complementing existing therapies that suppress the neurohumoral axis or induce vasodilation. The classes of agents that fit well within this paradigm include those that improve the balance between energy supply and demand (e.g., mitochondrial function), substrate utilization, and intracellular calcium handling, reduce interstitial fibrosis, and improve blood flow through the coronary microcirculation.

3.1 Mitochondrial Function

The past two decades of research have provided convincing evidence that altered energetics and mitochondrial dysfunction play an important role in the progressive deterioration of HF. It is a firmly established concept that the failing heart is "energetically starved," with an inability to generate sufficient ATP commensurate with myocardial energy demands (Neubauer 2007). These abnormalities of mitochondrial function include hyperplasia and reduced organelle size, poor respiration, reduced mitochondrial membrane potential, increased opening of MPTP, and reduced rates of ATP synthesis. The obvious consequence of these abnormalities is a reduction in the availability of cardiomyocyte ATP (energy) in patients with HFrEF and HFpEF (Sabbah et al. 1992, 2013; Neubauer 2007; Bayeva et al. 2013, 2014; Sharov et al. 1998, 2000). In addition, mitochondrial dysfunction is associated with derangements in the electron transport chain and excessive production of ROS (Bayeva et al. 2013). Excessive generation of ROS can exert multiple adverse effects through damage to cellular and subcellular components including those that form part of key signaling pathways, membrane lipid sublayers, and the extracellular matrix (Siwik and Colucci 2004). Mitochondrial uncoupling proteins (UPCs), particularly UCP-2 and UCP-3, are necessary for preserving appropriate mitochondrial membrane potential and regulating the production of ATP and ROS (Bugger et al. 2011; Laskowski and Russell 2008). Thus, restoring the integrity of mitochondrial membranes and optimizing their number and function represent emerging therapeutic targets in the treatment of HF. Studies of the effects of the partial A1R agonist capadenoson in a canine model of HF provide strong evidence that modulation of this pathway is a viable strategy for treating mitochondrial dysfunction in HF (Sabbah et al. 2013). In untreated dogs with HF, expression of UCP-2 and UCP-3 was reduced compared with healthy dogs, whereas in animals with HF that were treated with capadenoson, a near normalization of UCP-2 and UCP-3 protein levels was observed. Likewise, treatment with capadenoson appeared to regulate MPTP. In HF, these pores showed increased opening rates, facilitating increased rates of apoptosis (Sabbah 2000). Preclinical data suggest adenosine agonists are capable of preventing this increased rate of MPTP opening and thus maintaining mitochondrial and cell viability (Xiang et al. 2010). These results are in line with findings from the canine HF model study, where the partial A1R agonist capadenoson normalized expression of citrate synthase, an established marker of intact mitochondria (Sabbah et al. 2013).

3.2 Cardiac Energetics and Substrate Utilization

The demands on the heart in the form of cardiac output are in a constant state of flux to accommodate multiple changing bodily needs such as during rest and exercise. This variable workload necessitates a rapid and efficient source of energy that can meet the demands imposed on the heart. The processes of excitation–contraction coupling consume large amounts of free energy in the form of ATP. The ATP

necessary for this must be generated on demand, because ATP cannot be stored. The total cardiac ATP pool is turned over within a minute and thus needs to be replenished rapidly and efficiently (Mootha et al. 1997).

Aside from mitochondrial dysfunction, the failing heart may undergo other maladaptive changes that can adversely influence cardiomyocyte energetics. Under normal resting conditions, approximately 70% of the cardiac energy requirement is met by FFA oxidation and 30% from glucose oxidation. While oxidation of FFAs provides the highest energy yield per molecule of substrate metabolized through β-oxidation, the process is less efficient than glucose with regard to ATP production per molecule of oxygen consumed (Rosano et al. 2008). To generate comparable amounts of ATP, FFA oxidation requires about 15% more oxygen compared with glucose oxidation, indicating a higher energetic efficiency of glucose over FFAs (Abozguia et al. 2009). Specific metabolic changes in HF include a relative increase in cardiac FFA oxidation rates and an uncoupling of glycolysis from glucose oxidation. Increases in the amount of FFA oxidized in relation to carbohydrate oxidation have the potential to decrease cardiac efficiency and can further contribute to impaired heart function. These changes characterize the transition of the failing heart to a fetal metabolic phenotype and gene profile, an adaptation that can further promote HF progression (Staehr et al. 2013; Razeghi et al. 2001; Thum et al. 2007). Animal studies suggest that partial A1R agonists, such as capadenoson, can augment expression of glucose transporter (GLUT)-1 and GLUT-4 to near-normal levels (Sabbah et al. 2013).

Increased plasma levels of FFAs have been observed in patients with HF (Djoussé et al. 2013; Capurso and Capurso 2012), further contributing to insulin resistance, diabetes, and alterations in myocardial substrate utilization. This further shifts toward a greater percentage of cardiac energy being derived from FFA oxidation. As detailed previously, A1R agonists can reduce plasma levels of FFAs (Staehr et al. 2013) and improve insulin resistance (Dhalla et al. 2007b, 2009).

It has also recently been shown that altered myocardial energetics underlie diastolic function abnormalities in HFpEF, especially during exercise (Phan et al. 2009, 2010). Thus, the normalization of myocardial energetics through improvement of mitochondrial function supported by restoring a physiological metabolic profile may represent a novel therapeutic target in patients with HFrEF and HFpEF.

3.3 Adrenergic Over-Activation

Enhanced and sustained sympathetic drive can lead to marked cardiotoxicity. In HF, excessive activation of the sympathetic nervous system leads to systemic vasoconstriction with increasing cardiac workload, sodium and water retention, and ventricular remodeling, all of which contribute to disease progression. Adenosine possesses anti-adrenergic properties via activation of A1Rs that are coupled to Gi proteins, which inhibit adenylate cyclase and reduce cAMP levels in

cardiomyocytes. Activation of the A1R may also inhibit norepinephrine release from cardiac presynaptic nerves, as shown with the partial A1R agonist capadenoson (Bott-Flugel et al. 2011), therefore further counteracting catecholaminergic overstimulation. Notably, this study demonstrated that capadenoson modulates noradrenaline release and stress-induced heart rate changes in spontaneously hypertensive rats (a model of high sympathetic tone), but had no effect on heart rate in Wistar rats. Thus, the A1R appears to exert minimal effects on adenylate cyclase activity in the absence of catecholamines, at least in rodents (Shryock and Belardinelli 1997). Thus, partial A1R agonism might offer a unique opportunity to selectively modulate the sympathetic control of cardiac function via presynaptic A1 receptor activation and cAMP inhibition.

These observations suggest that partial A1R agonists in the setting of HF might act, at least in part, to inhibit adenylate cyclase in a similar manner to β-blockers. However, the hemodynamic response to β-blockade differs from that seen with the partial A1R agonist. As described previously, it is well known that in patients with HF, β-blockers reduce heart rate and blood pressure and induce a negative inotropic effect early in the course of therapy before improvement takes place later.

Considering the complementary effects of β-blockers and A1R agonists on adenylate cyclase inhibition, hypothetically, the combination of partial A1R agonists and β-blockers may offer a therapeutic opportunity to counteract sympathetic over-activation more efficiently than existing treatment strategies for HF (that may involve, e.g., a β-blocker, a diuretic, and an angiotensin-converting enzyme inhibitor), particularly in patients who cannot tolerate high-dose β-blockers. This combination approach of β-blocker and partial A1R agonist will be interesting to watch in the clinic.

In addition, it has been shown that activation of adenosine A1Rs attenuates the phenylephrine-induced hypertrophic response in vivo and counteracts the phenylephrine-induced pro-fibrotic response in preclinical models (Puhl et al. 2016). Furthermore, activation of the A1R reduces α1-adrenoceptor-induced oxidative stress in vivo. Inhibition of maladaptive ROS production would add to the effects of adenosine A1Rs in mediating cardioprotection.

These results suggest that targeting the adenosine A1R may be an appropriate therapeutic strategy to prevent transition from compensated myocardial hypertrophy to overt HFrEF or HFpEF.

3.4 Calcium Handling

Calcium is critical in both the electrical and mechanical properties of cardiomyocytes. The excitation–contraction coupling process in cardiomyocytes begins with the action potential. The initial depolarization triggered by sodium influx activates the L-type calcium current, which triggers even greater calcium release from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyRs) (Bers 2006). This elevates cytosolic calcium, which initiates the contraction process as a result of calcium binding to troponin C. For myocardial relaxation in diastole,

calcium must be transported out of the cytosol, resulting in calcium dissociation from troponin C and detachment of the myofilaments. The calcium decline is mediated by the SR calcium-ATPase 2a (SERCA2a), sodium–calcium exchange, the sarcolemmal calcium-ATPase, and the mitochondrial calcium uniporter. Under normal conditions, SERCA2a is responsible for the removal of 70% of cytosolic calcium during diastole.

In HF, there are significant alterations in how intracellular calcium is regulated. The principal defect of excitation–contraction coupling in HF is a decreased calcium load of the SR, which is caused by reduced SERCA2a activity and a calcium leak of the SR via RyRs (Bers 2006). Furthermore, ventricular relaxation during diastole is an energy-consuming process dependent on ATP hydrolysis, which is required for myosin detachment from actin myofilaments, calcium dissociation from troponin C, as well as calcium reuptake into the SR via SERCA2a. The failing heart is "energetically starved" with ATP levels approximately 30% lower than in the healthy heart. SERCA2a activity and SERCA2a protein expression are also decreased in HF (Sabbah et al. 2013; Hasenfuss et al. 1994). As SERCA2a is the first ATPase in the cardiomyocyte to respond to a decrease in the available free energy, it is reasonable to assume that an energetic deficit as well as reduced SERCA2a activity and expression in the failing heart could contribute to impaired excitation–contraction coupling and impaired relaxation in cardiomyocytes.

In preclinical studies, treatment with the partial adenosine A1R agonist capadenoson prevented the decline in SERCA2a activity (V_{max}, measured as nmol/mg protein) that was observed in the untreated HF control group. Furthermore, treatment with capadenoson resulted in a normalization of SERCA2a affinity (K_a) compared with the untreated HF control group (Fig. 7). The improved ATP synthesis by mitochondria may at least partly account for the increased calcium-ATPase activity (Sabbah et al. 2013). In addition, compared with untreated dogs, treatment with capadenoson was associated with a significant increase of SERCA2a protein expression to near-normal levels (Fig. 8).

3.5 Comorbidities of HF

Common comorbidities such as diabetes, renal insufficiency, CAD, and microvascular dysfunction contribute to the onset and progression of HF and drive LV remodeling and dysfunction. In addition, several studies indicate that non-cardiac factors such as skeletal muscle deconditioning as well as underlying cardiac dysfunction contribute to severe exercise intolerance in HF, which is typically the primary manifestation of chronic stable HF. The potential impact of any novel therapy for HF on these important comorbidities and "peripheral factors" merits consideration during drug development.

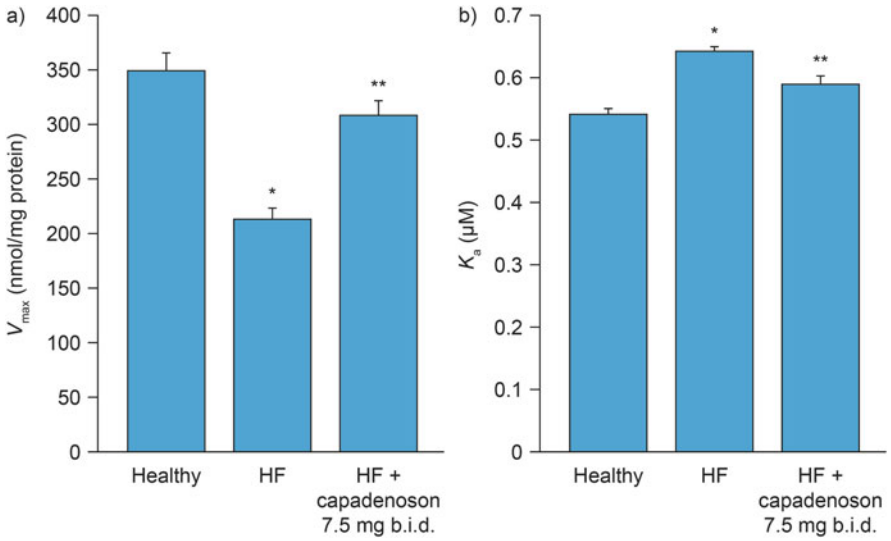

Fig. 7 The effects of capadenoson (7.5 mg b.i.d.) in a canine model of heart failure on: (**a**) left ventricular calcium-ATPase (SERCA2a) activity (V_{max}) and (**b**) calcium affinity (K_a). Data are shown as mean ± standard error of the mean. * denotes $P < 0.05$ versus healthy dogs; ** denotes $P < 0.05$ versus untreated dogs with heart failure. *Healthy* healthy dogs, *HF* dogs with heart failure. Reprinted with permission from Sabbah et al. (2013)

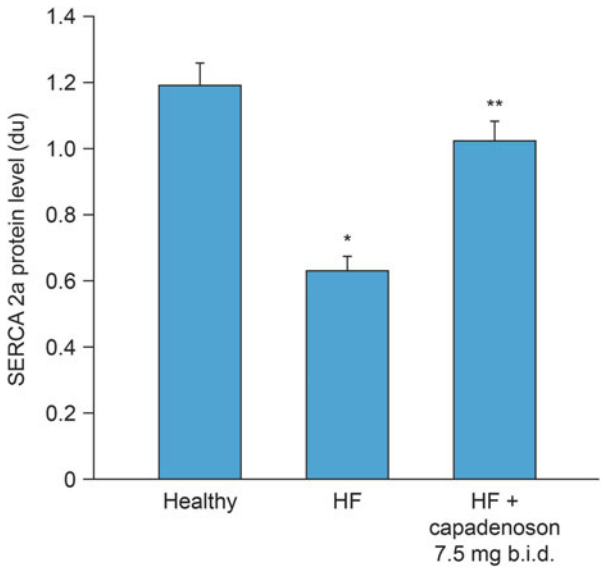

Fig. 8 The effects of capadenoson (7.5 mg b.i.d.) on left ventricular SERCA2a protein levels in a canine model of heart failure. Data are shown as mean ± standard error of the mean. * denotes $P < 0.05$ versus healthy dogs; ** denotes $P < 0.05$ versus untreated dogs with heart failure. SERCA2a, sarcoplasmic reticulum calcium-ATPase 2a. Reprinted with permission from Sabbah et al. (2013)

3.6 Insulin Resistance and Diabetes

Taking into account existing evidence that adenosine A1R agonists possess antilipolytic properties resulting in potentially beneficial effects on insulin resistance and diabetes, A1R activation may present an opportunity for the concomitant treatment of HF and metabolic abnormalities. However, further studies are needed to determine the efficacy of partial A1R agonists in the long-term treatment of insulin resistance, diabetes, and HF in humans. Further details on the pharmacological mechanisms and published literature on this topic are discussed in the previous chapter.

3.7 Coronary Artery Disease and Microvascular Dysfunction

Given that ischemia causing myocardial necrosis occurs frequently in the failing heart, it is reasonable to assume that partial A1R agonists may protect and potentially improve cardiac function via the anti-ischemic cardioprotective effects of adenosine A1R activation and subsequent reduced cellular injury. For details please refer to the previous chapter.

3.8 Renal Dysfunction

The progression of HF and renal insufficiency is inextricably linked. Renal dysfunction promotes HF by, for example, increasing preload, worsening systemic inflammation and endothelial function, and leading to accumulation of uremic toxins. HF promotes renal dysfunction, for example, as a result of insufficient cardiac output resulting in elevated central venous pressures and reduced renal blood flow, leading to reduced sodium excretion and renal insufficiency.

A full A1R agonist may cause antidiuretic effects by vasoconstriction of renal afferent arterioles and reduction of the glomerular filtration rate (Vallon et al. 2006, 2008). When the macula densa of the kidney senses salt loss, it produces adenosine which causes afferent vasoconstriction through activation of the adenosine A1 receptor in the afferent arteriole. Thus, renal blood flow decreases, and the glomerular filtration rate decreases. The potential effects of adenosine antagonism on the kidney led to large-scale drug development programs with adenosine A1R antagonists, such as rolofylline, that included multiple phase 2 and phase 3 trials. Phase 2 data were generally positive, suggesting rolofylline could be capable of augmenting urine output and glomerular filtration rate in patients with HFrEF and moderate renal insufficiency (Dittrich et al. 2007; Givertz et al. 2007; Vaduganathan et al. 2013). However, the subsequent large phase 3 study did not demonstrate a benefit on symptoms, renal function, mortality, or rehospitalization in patients with HFrEF and moderate renal insufficiency (Massie et al. 2010). Furthermore, higher rates of persistent renal impairment, seizure, and stroke were noted in the rolofylline group (Massie et al. 2010; Teerlink et al. 2012).

The potential effects of adenosine A1R agonists on renal function remain to be established. In a canine HF model, there was no evidence of worsening renal function after treatment with the partial A1R agonist capadenoson (Sabbah et al. 2013). This can partly be explained by the fact that a partial agonist can also act as a weak antagonist if endogenous adenosine levels are high. Thus, a partial agonist may counteract the effects of endogenous adenosine on the afferent arterioles in HF. However, it should be noted that renal function in the canine HF model was normal at treatment initiation, thus, the results may not translate into models of renal failure.

Nevertheless, in addition to its hemodynamic effects on afferent arterioles, the A1R has been shown to be involved in regulating renin release from juxtaglomerular cells. It has been shown that A1R activation tonically inhibits renin secretion and is critical for proper functioning of the intrarenal baroreceptor system (Schweda et al. 2005). In the medullary thick ascending limb (TAL), A1R activation inhibits sodium chloride reabsorption (Beach and Good 1992), which is of relevance as the renal medulla, already in normoxia, has a low partial oxygen pressure. It has been demonstrated that the isolated rat TAL releases adenosine, which is increased significantly during hypoxia. Thus, adenosine released into the renal medulla during hypoxia, for example, in acute or worsening HF, may protect the TAL from ischemic injury by directly inhibiting sodium chloride absorption and reducing transport-related oxygen consumption. These findings support the concept that adenosine acts as a metabolic mediator and may couple energy metabolism (ATP hydrolysis for tubular sodium transport) with the control of renin secretion and glomerular filtration rate. The A1R-mediated effects on the TAL together with the renal medullary vasodilation mediated by adenosine A2 receptors may serve to maintain balance in the renal medulla. This is supported by data from mouse models, in which a transient activation of renal A1R led to acute as well as delayed protective effects against renal ischemia-reperfusion injury (Joo et al. 2007).

In addition, a partial adenosine A1 agonist may act as a semi-quantitative antagonist counteracting the vasoconstriction of the afferent arteriole induced by endogenous adenosine in the setting of acute or exacerbated HF.

3.9 Muscular Deconditioning

Chronic HF is characterized not only by hemodynamic adaptation in the heart and circulation but also by changes in the skeletal muscle, a condition called “muscular deconditioning.” In fact, several studies have indicated that reduced exercise tolerance, the primary symptom in HFpEF, is at least partly due to peripheral factors such as skeletal muscle abnormalities, physical deconditioning, and deficient skeletal muscle oxygen extraction (Sabbah 2000). The extent of these changes is related to the severity of HF and disease progression. Furthermore, physical deconditioning per se may be at least partly responsible for some of the neurohumoral adaptation observed in HF, particularly in HFpEF. Changes to skeletal muscle may amplify neurohormonal hyperactivity independently of blood pressure

Table 1 Potential mechanisms of benefit from a (partial) A1 receptor agonist in heart failure

Improve mitochondrial function
• Increase levels of mitochondrial uncoupling proteins
• Decrease rate of opening of mitochondrial permeability transition pores leading to reduced apoptosis[a]
• Decrease reactive oxygen species production
• Improve efficiency of electron transport chain and ATP production/energy supply
• Protection from calcium overload and resultant mitochondrial damage
Energy substrate utilization
• Increase expression of GLUT-1 and GLUT-4
• Decrease levels of fatty acid oxidation and increase energy efficacy
Reverse ventricular remodeling
• Reduce interstitial fibrosis
• Prevent myocyte hypertrophy
• Preserve myocardial capillary density and oxygen diffusion distances
Anti-ischemic and cardioprotective effects
• Normalizes SERCA2a expression • Improved calcium handling and protection from calcium overload
• Attenuate mechanical and metabolic responses to excessive adrenergic stimulation
• Decrease catecholamine release and counteracting sympathetic overstimulation

ATP adenosine triphosphate, *GLUT* glucose transporter, *SERCA2a* sarcoplasmic reticulum calcium-ATPase 2a
Reprinted and modified with permission from Greene et al. (2016)
[a]Shown for full A1 receptor agonists (Xiang et al. 2010)

reflex effects. It has been shown that chronic HF causes enzymatic and mitochondrial abnormalities in skeletal muscles (Mootha et al. 1997), which may therefore be potential targets for novel therapeutic strategies. Preclinical data have shown that A1R activation increases the synthesis of nitric oxide by a process initiated by opening of K_{ATP} channels. Adenosine released from skeletal muscle fibers also contributes indirectly to vasodilation by activating A1Rs on muscle fibers leading to opening of K_{ATP} channels and efflux of potassium, which is a vasodilator. Thus, by acting on endothelial A1Rs, adenosine attenuates the vasoconstrictor effects of constant or bursting patterns of sympathetic activity in the skeletal muscle. This limits the extent to which the sympathetic nervous system can reduce oxygen delivery to muscle when it is already compromised by inadequate tissue supply in HF (Rosano et al. 2008; Abozguia et al. 2009)

4 Summary and Future Directions

HF remains a growing public health problem with unacceptably poor long-term outcomes despite the use of guideline-directed medical and device therapies. To date, almost all disease-modifying therapies in HF cause blood pressure lowering either as a therapeutic effect or an unintended consequence of treatment. Continued

development of more potent hemodynamically active medications for stepwise addition or replacement of existing therapy carries an inherent risk of poor tolerability and safety, and incremental benefit with the addition of such agents may be difficult or unattainable. It is under this framework in which a partial A1R agonist for HF holds promise. As a potentially hemodynamically neutral therapy, a partial A1R agonist could simultaneously improve cardiomyocyte energetics, cardiac structure, and function and potentially prevent further tissue injury and thereby cardiac function deterioration (Table 1). Although drug development of a partial A1R agonist remains at an early stage, compelling biologic rationale and encouraging preclinical evidence support the continued attention being given to ongoing and future trials with these agents in patients with HF. Partial agonists of adenosine A1Rs may offer a more favorable benefit–risk profile compared with full agonists due to more specific effects on the target tissue and fewer undesired effects on the ubiquitously expressed receptor.

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New and Emerging Therapies and Targets: Beta-3 Agonists

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Abstract

While crucial for the acute physiologic response to stress, the adrenergic system may become maladaptive upon prolonged stimulation in the course of development of heart failure. This has been the basis for the development of beta-

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_88

blocking therapies, targeting mainly beta1-2 adrenoreceptors (B1-2AR). The third isotype, B3AR, was more recently identified in cardiac myocytes and endothelial cells from human (and many other animal species), where its distinctive coupling to nitric oxide and antioxidant pathways suggested potential protective properties that were unexploited so far. The observation of beneficial effects of B3AR expression/activation on myocardial remodeling and the availability of specific agonists for clinical use now open the way for directly testing the hypothesis in heart failure patients. We will briefly review the specificities of B3AR signaling in the context of the cardiovascular adrenergic system, the evidence supporting its beneficial effects and outline an ongoing clinical trial using the B3AR agonist, mirabegron in patients with/at risk of developing heart failure with preserved ejection fraction (HFpEF).

Keywords
Adrenergic receptor • Catecholamines • Heart failure • Hypertrophy • Nitric oxide • Remodeling

1 Catecholamines and the Failing Heart

1.1 Catecholamines Synthesis and Circulation

Catecholamines, secreted in response to the stimulation of the sympathetic division of the autonomous nervous system, greatly impact cardiac function. In short, catecholamines synthesis in the chromaffin cells of the adrenal medulla is under the control of the corticotropin releasing hormone (CRH)–adrenocorticotropic hormone (ACTH)–cortisol axis. Therefore, the stress sensed and propagated by the CRH–ACTH–cortisol axis triggers the stimulation of norepinephrine synthesis by cortisol and upregulation of PNMT (phenylethanolamine N-methyl transferase) enabling epinephrine synthesis from norepinephrine. Chromaffin cells secrete predominantly epinephrine and to a lesser extent norepinephrine. Exocytotic release of catecholamines is initiated by acetylcholine stimulation of nicotinic Ach receptor that depolarizes the postganglionic chromaffin cells and triggers Ca^{2+}-dependent exocytotic release. In the bloodstream, catecholamines have a short half-life of few minutes (Porta et al. 1985) and, therefore, mediate a fast response to external stress by associating with alpha and beta-adrenoceptors expressed at the cell membrane. In this regard, the fight-or-flight response represents a major physiological reaction to severe external threat inducing the centrally driven release of adrenal hormones and activation of peripheral sympathetic nerves leading to an increase in heart rate, contractility, and the mobilization of fuel stores from muscles and fat. Due to the quick action of catecholamines after reaching their target tissues, all responses are activated within seconds with a brief biological action of about 10 s. Importantly, unlike other hormones the adrenal hormones do not have a negative

feedback system based on circulating levels, but their effect is limited in time due to catecholamines reuptake, degradation, and adrenergic receptors homologous desensitization.

1.2 Catecholamines in Heart Failure

A complex series of changes occurring in the cardiac sympathetic nervous system of patients suffering from heart failure leads to a pronounced increase in catecholamines release from sympathetic nerves. Additionally, activation of the adrenal glands elevates significantly the circulating catecholamines levels (Mahata et al. 2016). Recent studies showed that the activation of the adrenal medulla and its enhanced release of catecholamines in chronic heart failure result from the increased transsynaptic activation of the chromaffin cells. In such conditions, both metabolism and physiology of the chromaffin cells undergo an important remodeling to accommodate the accrued production of catecholamines, which include an increase in the mitochondrial content and in the endoplasmic reticulum (ER) and Golgi width (Mahata et al. 2016). Although the initial action of catecholamines is beneficial for the function of the cardiovascular system, the untimely activation of adrenergic receptors by elevated levels of agonists in the failing heart causes tremendous detrimental effects on cardiac physiology. In fact, circulating levels of catecholamines correlate with the degree of cardiac dysfunction (Bristow et al. 1990; Swedberg et al. 1990), a parameter inextricably linked to the physiology of the different members of the beta-adrenergic receptors family.

2 Beta-Adrenergic Receptors in the Normal and Failing Heart

2.1 Beta-Adrenergic Signaling in the Cardiovascular System

The orthosympathetic system is a key regulator of the cardiovascular function through the adrenoreceptors. The multiple roles of the adrenoceptors first arise from the observation that catecholamines can stimulate both vasodilation and vasoconstriction unraveling the diversity in cell response (Longhurst 1990; Saeed et al. 1982). In cardiac tissues, both alpha and beta-adrenoceptors are expressed although the beta-adrenoceptors are highly predominant. Under physiological conditions, beta-adrenoceptors and their signaling pathways regulate both force and rate of myocardial contraction and relaxation, while alpha-adrenoceptors are mostly implicated in the control of vascular tone due to their high density at the plasma membrane of the vascular smooth muscle cells. Among the three types of beta-adrenergic receptors implicated in cardiovascular processes beta1 and beta2 adrenergic receptors (B1-2AR) are the most extensively studied, due to their early identification (Lefkowitz et al. 1972); they share a common positive inotropic effect, but also mediate vasodilatation in peripheral vessels.

Despite belonging to the same subtypes of receptors, the signaling pathways of the two receptors are far from equivalent and include important differences, highly connected to their implications in the cardiac physiology; one of the most striking example being the marked cardiomyopathy developed in mice with low-level (fivefold) overexpression of B1AR while 100-fold overexpression of B2AR led solely to an increased cardiac contractile force (Ahmet et al. 2004; Patterson et al. 2004). Additionally, in B1AR knockout (KO) mouse models, a loss of inotropic effect is measured and, depending on the genetic background, embryonic lethality is observed (Rohrer et al. 1996; Susulic et al. 1995) while in B2AR KO mice, functional impairments were restricted to the stress of exercise (Chruscinski et al. 1999).

Upon binding of epinephrine to the B1AR, activation of G-alpha-s promotes the increase of intracellular cyclic AMP (cAMP) levels via the stimulation of the adenylyl cyclase (AC) and thus enhances the protein kinase A (PKA) activity promoting the phosphorylation, among others, of phospholamban, troponin I (TnI) and increasing the probability of opening of Ca^{2+} transporters such as $Ca_{v1.2}$, RyR2. Altogether these phosphorylations contribute to enhance cardiac contractility and reduce the duration of contraction, as it is the case during fight-or-flight response. As for B2AR, that comprises 20–25% of beta-ARs, the receptor contributes to both chronotropic and inotropic responses in the heart (Brodde 1991; Xiao et al. 1995) through its capacity to couple to both G-alpha-s and G inhibitory protein G-alpha-i. The switch of signaling pathways is governed by the direct phosphorylation of the receptor by PKA (Daaka et al. 1997). Therefore, B2AR can either participate to the elevation of intracellular cAMP via binding of G-alpha-s or bind G-alpha-i, pathway that in turn activates phospholipase 2 (PLA2), PI3K, and Akt providing an anti-apoptotic signal to cardiac myocytes (Chesley et al. 2000; Zhu et al. 2001).

2.2 Beta-Adrenergic Receptors in the Failing Heart

In the failing heart, overstimulation of the orthosympathetic system leads to cardiac cell damage through a sustained activation of B1-2AR. In patients suffering from heart failure, beta-adrenoceptors exhibit a lower sensitivity to catecholamines, mostly linked to a decrease in density of beta-adrenoceptors at the plasma membrane (Bristow et al. 1982). As for the B1AR subtype, this process includes a reduction of the subtype expression and its mRNA abundance by up to 50%, in correlation with the disease diversity (Brodde 1993; Engelhardt et al. 1996; Kiuchi et al. 1993). The remaining B1AR are desensitized through GRKs (e.g., GRK2 or beta-adrenoceptor kinase, BARK), whose expression is increased in the failing human heart (Pitcher et al. 1998). Furthermore a downregulation of G-alpha-s (Longabaugh et al. 1988) and adenylyl cyclase (Ishikawa et al. 1994) is measured in several animal models. Altogether the loss of response to catecholamines reduces intracellular cAMP levels as it is observed in denervated preparations of human heart (Danielsen et al. 1989). In addition, phosphatase-1 activated in the context of heart failure impacts the phosphorylation status of phospholamban. In its

dephosphorylated state, phospholamban inhibits the apparent affinity of SERCA 2A for Ca^{2+} (Chu et al. 2000; Dash et al. 2001; Rona 1985; Schwinger et al. 1999) which, in turn, causes major myocardial damage and contributes significantly to myocardial dysfunction and remodeling of the failing heart (Bristow 2000).

While B2AR expression levels remain unchanged following cardiac injury, an up to twofold increase of G-alpha-i is observed in early heart failure (Eschenhagen 1993; Feldman et al. 1988; Neumann et al. 1988). These observations are consistent with an activation of the antiapoptotic pathway of B2AR in the failing heart. In addition, in B1AR expressing cardiac cells of B2AR KO mice, the overstimulation of B1AR leads to apoptotic death of cardiomyocytes. Therefore, the coupling of B2AR with G-alpha-i would constitute an attempt to compensate and protect the heart against detrimental effects caused by the overstimulation of beta-adrenergic receptors, including arrhythmias, energy dysbalance, hypertrophy, and apoptosis, through activation of anti-apoptosis pathway and inhibition of the G-alpha-s pathway. Alternatively, the primary beneficial effect of B2AR may come at the cost of compromised contractile support since the pathway disrupts the beta-adrenergic inotropic response and may prevent the heart from meeting its demands, leading to further deterioration of heart failure (He et al. 2005). Nevertheless, the mechanisms of protection afforded by the last identified beta-adrenoreceptor expressed in the heart, the B3AR, are of particular interest to prevent cardiac remodeling. Recent evidence revealed the B3AR as a promising candidate for drug target in the treatment of the failing heart.

3 The Beta-3 Adrenergic Receptor

3.1 Structure of B3AR

Until recently, little was known on the role of the beta-3 adrenoceptor in the cardiovascular system. After its first cloning in 1989 (Emorine et al. 1989), B3AR was originally thought to have a restricted expression to adipocytes in brown and white adipose tissues where it mediates the adrenergic beta-oxidation of fatty acids. B3AR was then detected in the smooth muscle cells of the urinary bladder, where it is implicated in relaxation of the detrusor muscle, thereby improving the filling capacity of the bladder. This led to the development of specific B3AR agonists, one of which (mirabegron) is currently used in the clinic in the treatment of overactive bladder disease (Vij and Drake 2015). As for cardiac tissue, the expression of transcripts of B3AR was first reported as early as 1996 in human biopsies (Gauthier et al. 1996). Thereafter, an antibody with validated specificity for the human B3AR enabled the immunodetection of the receptor in human atrial and ventricular cardiac myocytes as well as in endothelial cells of the small coronary resistance vessels (Massion et al. 2004). The expression of the receptor varies, however, from species to species, with a low expression in mice, but higher in rats, rabbits, and dogs (Gauthier et al. 1999). Importantly, our group observed that B3AR expression is increased in human cardiac tissue from patients suffering

from heart failure (Moniotte et al. 2001b) and evidenced for the first time a difference in expression of the three adrenergic isotypes. The upregulation has been since then described also in diabetic hearts by several other groups (Amour et al. 2007; Dincer et al. 2001) and led to growing attention for the receptor and its implications in cardiac physio- and pathophysiology.

The B3AR belongs to the R7G superfamily of G protein coupled receptor and shares multiple structural characteristics with the other beta-adrenergic isotypes, in addition to the 40–50% similarity in primary sequence (Gauthier et al. 2000; Liggett et al. 1993). Similarly to other GPCRs, the beta-receptors contain seven transmembrane domains of about 22–28 residues involved in ligand binding with an intracellular C-terminus and extracellular N-terminus usually glycosylated. The C-terminus region is of great importance in B1-2AR as it contains consensus sequences for PKA and GRK phosphorylations, which are notably absent in B3AR. In addition, a polymorphism at position 64 is observed in 8–10% of Europeans and North Americans for which Trp^{64} is replaced by Arg^{64}. Several early studies ruled out a link of this polymorphism with type II diabetes or changes in ligand binding. Interestingly, a reduction in basal and agonist stimulated AC was observed (Candelore et al. 1996; Manning et al. 1996). Furthermore, recent studies suggested an implication in obesity and metabolic syndrome (de Luis et al. 2008; Malik et al. 2011) while an association with increased serum urate and risk of gout was recently suggested (Fatima et al. 2016).

3.2 Coupling of B3AR in Cardiovascular Tissues

Contrary to B1- and B2AR, B3AR is not subjected to short-term homologous desensitization, due to the absence of GRK phosphorylation sites at the C terminal tail of the receptor. Therefore, unlike other beta-isotypes, B3AR is less prone to the progressive loss of coupling and efficacy linked with the recruitment of beta-arrestin and the subsequent internalization of the receptor. The absence of agonist-induced desensitization linked with GRK phosphorylation was further demonstrated in the context of cardiac myocytes using chimeric receptors (Liggett et al. 1993). Unlike the B1 isotype, B3AR has a higher affinity for norepinephrine than for epinephrine. The coupling of B3AR to intracellular signaling effectors varies between tissues and cell types. In the vasculature, the expression of B3AR itself is different between vascular beds, the size of vessels, and species. B3AR vasodilating effect is coupled to an increase in cAMP levels in canine pulmonary arteries (Tagaya et al. 1999), akin to the classical cAMP/PKA vasorelaxing effect of B2AR observed in vascular smooth muscle cells and suggesting a coupling of B3AR to G-alpha-s. Additionally, activation of the receptor in endothelial cells and the associated endothelium-dependent relaxation measured in aortic and resistance vessels from many animal species is connected to both nitric oxide synthase (NOS) dependent and independent pathways (Dessy and Balligand 2010). In human ventricular biopsies, the B3AR-induced production of NO is sensitive to pertussis toxin, suggesting a coupling to G-alpha-i coupled to NOS-dependent production of

cGMP (Gauthier et al. 1999). It is however still unclear whether B3AR can couple to G-alpha-s (and adenylyl cyclase/cAMP) in addition to G-alpha-i in cardiac myocytes, as does B2AR. This ability might well depend on the spatial confinement in specific subcellular compartments of both B3AR and effectors.

3.3 Signaling of B3AR in Cardiovascular Tissues

A major axis of the B3AR signaling pathway in the cardiovascular system is the NOS pathway. In bovine aortic endothelial cells, B3AR can promote eNOS activation through the phosphorylation of Ser^{1179} and the dephosphorylation of the inhibitory phosphoresidues Ser^{116} and Thr^{497} (Kou and Michel 2007). In a similar manner in neonatal rat cardiac myocytes, B3AR activates nNOS through phosphorylation of Ser^{1412} and dephosphorylation of Ser^{487}. Consistently with the human cardiac biopsies, these B3AR-induced posttranslational modifications have been linked to G-alpha-i in rat cardiomyocytes (Watts et al. 2013).

Further investigations in endothelial cells revealed that B3AR signaling to eNOS is dependent on the enzyme's association to Rac1, which also induces endothelial cell migration in vitro (Kou and Michel 2007). In human coronary resistance vessels, we showed that the B3AR-induced relaxation is mediated by both NO- and EDHF-dependent responses (Dessy et al. 2004, 2005; Massion et al. 2004). The definitive identity of EDHF remains unclear; one possible mediator would be extracellular K^+ driving hyperpolarization through the activation of the Na^+-K^+-ATPase (Edwards et al. 2010). The recent demonstration that B3AR protects the alpha1 subunit of the Na^+-K^+-ATPase from inactivation by oxidative S-glutathionylation, thereby maintaining the pump activity in the face of oxidant stress (Karimi Galougahi et al. 2015), as occurring, e.g., in diabetes corroborates the hypothesis of antioxidant effects of B3AR, as also observed by us in a setting of acute ischemia (Sorrentino et al. 2011). In this context, B3AR would contribute to maintain both NO and EDHF vasodilatory responses in disease as well as important paracrine protective signaling (e.g., through NO) to the underlying parenchymal cardiac tissue.

The development of the transgenic mouse model expressing the human B3AR specifically in cardiac myocytes, at levels driving a functional response similar to that observed in human biopsies, has been an important milestone in the study of B3AR signaling pathways in cardiac myocytes. Using this mouse model, we demonstrated the colocalization of B3AR with caveolin3 and eNOS and nNOS in caveolae-enriched membrane fractions, with little change of localization in hypertrophic and non-failing hearts (Belge et al. 2014). By crossbreeding our cardiac-specific B3AR transgenic mice with another mouse model expressing a cGMP-sensitive FRET sensor, we demonstrated the coupling of the B3AR to cGMP in cardiac myocytes, that was absent in B3AR KO mice (Belge et al. 2014). The inhibition of the signal by the soluble guanylyl cyclase inhibitor, ODQ, pointed to a B3AR/NOS/sGC/cGMP pathway. Downstream cGMP, we found that B3AR activates PKG-dependent signaling that results in the inhibition of hypertrophy (Belge et al. 2014). Among other targets, Titin or TnI phosphorylation would result

in decreased myofilament calcium sensitivity (Kruger et al. 2009) and improved diastolic relaxation that would promote LV filling. The coupling of B3AR to electrical currents in cardiac myocytes may vary in atrial versus ventricular cells. Most studies have examined the regulation of Ica-L, which is increased by B3AR agonists in human atrial cells ex vivo (Skeberdis et al. 2008) whereas the response is less prominent in ventricular cells (Treinys et al. 2014). Caution should be used, though in extrapolating data using high concentration of agonists in vitro with imperfect selectivity (and potential off-target effects on B1-2ARs).

4 Beta-3 Adrenoceptor in the Failing Heart

The physiological implications of B3AR in the cardiovascular system have been firstly examined on isolated cardiac muscle specimens from non-failing hearts with uncontrolled preloading conditions using high doses of agonists, such as BRL37344 (up to 1 μM) and revealed a decrease of contraction force in unloaded human cardiac biopsies (Gauthier et al. 1996); an effect reproduced in identical models from several mammalian species such as dog and rat (Gauthier et al. 1999; Imbrogno et al. 2015). Contrary to the classical positive inotropic effect observed for B1-2AR in most physiological situations, B3AR has been further confirmed by several groups (Amour et al. 2007; Cheng et al. 2001; Idigo et al. 2012) to act as a countervailing "brake" to prevent adrenergic overactivation (Gauthier et al. 1996; Moniotte et al. 2001a). Despite some variability depending on the species used and the variation in expression levels of B3AR, such negative inotropic action of B3AR has been observed in non-failing cardiac preparations from many species from mammals to teleosts and amphibians (Imbrogno et al. 2015).

β3AR is expressed in human myocardial cells and has been found in atrial and ventricular cardiac myocytes as well as in endothelial cells (Dessy et al. 2004; Gauthier et al. 1998, 1996). Ventricular expression may well be implicated in the modulation of cardiac ventricular relaxation, possibly through the activation of NOS (Angelone et al. 2008; Dessy et al. 2004) and the downstream cGMP-PKG pathway. B3AR coupling to eNOS and nNOS could mediate beneficial effects of exercise on the heart (Calvert et al. 2011) as well as myocardial protection in the setting of ischemia/reperfusion in mice (Aragon et al. 2011). Additional cellular work pointed to Akt/NO mediated prevention of mitochondrial permeability transition pore (mPTP) opening as one underlying mechanism (Garcia-Prieto et al. 2014).

The potential negative inotropic effect mediated by B3AR led the scientific community to thoroughly investigate the potential role of B3AR in the context of heart failure. Our group showed that B3AR expression is upregulated in the diseased myocardium following the development of hypertrophy or heart failure (Moniotte et al. 2001a), while being resistant to agonist-induced desensitization (Nantel et al. 1993). Studies performed in vivo in large animals or models of heart failure gave rise to divergent results. Although some conclude that there is a negative inotropic effect (mainly in heart failure models) (Cheng et al. 2001), others do not (Bundgaard et al.

2010; Donckier et al. 2001). The reasons for this discrepancy include the type and dose of agonist used (with high doses of unspecific agonists producing opposing positive inotropic effects) and control for reflex orthosympathetic reaction to intense peripheral vasodilatation (Donckier et al. 2001). One study concluded that, in goat, there is a small negative inotropic effect in healthy animals and an improvement of inotropic parameters in the context of heart failure (Bundgaard et al. 2010). This was attributed to the preservation of Na^+-K^+-ATPase function in failing cardiac myocytes that would decrease intracellular Na^+ content. Interestingly, reductions in Na^+ content and late Na^+ current were otherwise proposed to improve relaxation, which would be particularly helpful in heart failure with preserved ejection fraction (HFpEF) (Maier 2009) in addition to the direct regulation of myocyte EC coupling.

B3AR may regulate cardiac function through paracrine effects from the coronary endothelium as well. As B3AR activates NO- and EDHF-dependent vasorelaxation in human coronary resistance vessels (Dessy et al. 2004), activation of B3ARs in the coronary microvascular endothelium would also induce vasorelaxation (Dessy et al. 2004), thereby promoting myocardial perfusion together with left ventricular relaxation through paracrine NO release. Finally, expression of B3AR in the pulmonary arterial vasculature in some species (Dumas et al. 1998; Tagaya et al. 1999) could contribute to attenuate pulmonary vasoconstriction and reduce pre-capillary pulmonary hypertension. These direct and indirect mechanisms concur to modulate chronic myocardial remodeling.

The coupling of B3AR to the nitric oxide/cyclic GMP pathway results in an effect on contractility antipathetic to classical B1-2AR positive inotropic effects (Gauthier et al. 1998). As ample evidence pointed out, the sustained activation of B1-2AR has adverse effect on cardiac function, due to receptor desensitization/internalization, loss of contractile/frequency reserve, adverse remodeling, calcium overload, and myocyte loss. We reasoned that activation of the functionally antipathetic B3AR would protect against such deleterious effects of chronic adrenergic stimulation. To test this hypothesis, we used the transgenic mouse model mentioned above, with a cardiac myocyte-specific expression of the human B3AR, and submitted these mice and their littermate controls to a number of interventions leading to myocardial stress (i.e., mini-pump or intraperitoneal infusions of isoproterenol or angiotensin II, transaortic constriction (TAC)). We showed that B3AR attenuates cardiac myocyte hypertrophy in response to continuous or repetitive infusion of isoproterenol or angiotensin II (Belge et al. 2014). This was evident from a reduction of morphometric indexes (LV/TL ex vivo, echocardiography in vivo) as well as reduced myocyte transverse area in parallel with reduced re-expression of the fetal gene program typically accompanying hypertrophy. The results uniformly showed protection of the transgenic mice from the development of pathologic remodeling contrary to the WT controls (Belge et al. 2014). Importantly, this was not at the expense of LV function, which remained normal. To precisely analyze the affected signaling in cardiac myocytes, we developed a model of rat neonatal cardiac myocytes with adenoviral expression of the human B3AR (or GFP control) in which we showed a reduction of hypertrophy in response to three different agonists (phenylephrine, endothelin-1, and isoproterenol). Using NOS inhibitors, we observed that the anti-

hypertrophic effect of B3AR was NO-dependent, as NOS inhibition abrogated the protection in vitro and in vivo. Mechanistically, we showed that one of the potential targets of the B3AR was the Nuclear Factor of Activated T-cells (NFAT), the transcriptional activity of which was attenuated upon B3AR expression (Belge et al. 2014). Additionally, B3AR expression also greatly reduced myocardial interstitial fibrosis in response to isoproterenol and angiotensin II infusions, in the absence of changes in myocyte death or apoptosis. As the receptor is exclusively expressed in cardiac myocytes in our transgenic model, this suggested a paracrine signaling by B3AR to neighboring cells. Indeed, we could model this in vitro, using a system where the culture supernatant of B3AR expressing cardiac myocytes was superfused on cardiac fibroblasts cultured separately. Using this system, we observed the antifibrotic effect of B3AR mediated by its antioxidant properties.

5 Beta-3 Adrenergic in Preclinical Model of Cardiac Remodeling

On the basis of our observations in transgenic mice, one can assume that the B3AR upregulation is a protective mechanism in the face of myocardial stress. As the B3AR is typically activated by higher catecholamines concentrations (than B1-2AR), it is possible that this protective pathway is not maximally recruited even in circumstances of pathophysiological adrenergic activation. This would leave a therapeutic margin for an additional activation by a potent and specific B3AR agonist. With this aim, B3AR agonists have been tested in several preclinical models. Consistently with data obtained from transgenic mice models, protective effects of B3AR were reported with preferential B3AR agonists (BRL37344) in mice submitted to TAC, preventing myocardial remodeling with decreased hypertrophy and preserving LV function (Niu et al. 2012). The implication of NOS (i.e., specifically nNOS) in the protection was further supported from the loss of protection in NOS1-KO mice.

In a large animal model of HF such as sheep with chronic rapid pacing, B3AR agonists even produce an improvement of LV function, possibly due to cGMP-dependent preservation of Na^+-K^+-ATPase activity (Bundgaard et al. 2010). More recently, the role of B3AR was examined in the protection afforded by B1AR blockade in a dog model of heart failure due to volume overload (mitral regurgitation) (Trappanese et al. 2015). The study showed that metoprolol promoted the upregulation of B3AR expression, and in specific membrane microdomains the interaction and activation of nNOS, followed by downstream cGMP production. This was attributed to protection from oxidation of the soluble guanylyl cyclase under B1AR blockade. Therefore, some of the cardioprotective effects of B1AR blockade may well result from enhanced expression and coupling of B3AR to NO/cGMP in the remodeled heart. The effect of B3AR agonists was recently replicated in a large porcine animal model, in which pre-perfusion of the B3AR agonist, BRL37344, reduced reperfusion damage and improved long-term LV function.

Finally, the B3AR agonist properties of nebivolol conferred superior efficacy over metoprolol in protecting the infarcted mouse heart from adverse remodeling; this was associated with better preserved endothelial-dependent vasodilatation, progenitor cells mobilization, and functional recovery (Sorrentino et al. 2011). Mechanistically, nebivolol exerted a potent inhibition of NADPH oxidase activity and superoxide production that, in endothelial cells, was not sensitive to B1 (or -2) AR blockade, but abrogated by full B1-2-3 blockade, pointing to B3AR activation. Interestingly, we also showed that B3AR agonist properties of nebivolol promote neoangiogenesis (Dessy et al. 2005), which could favor myocardial revascularization.

All these protective effects at the myocardial level are probably reinforced from indirect effects in peripheral cells/tissues, i.e., through coronary vasodilatation via B3AR-induced endothelial-dependent relaxation (see above) as well as paracrine release of NO and its effects to improve LV relaxation. Moreover, the antioxidant effects of B3AR signaling may preserve the endothelium of the microvasculature from oxidative activation and the ensuing recruitment of monocytes initiating subendothelial inflammation at the core of sustained endothelial dysfunction. Whether this will prevent chronic development of vascular atherosclerosis or chronic development of diastolic dysfunction initiating HFpEF (Lim et al. 2015) will have to be tested with long-term interventions.

In summary, using preclinical models, we demonstrated that the activation of B3AR attenuates myocardial hypertrophy and fibrosis in response to neurohormonal or hemodynamic stresses, without compromising LV function (Belge et al. 2014), an effect mediated by the activation of the NOS/cGMP pathway. Furthermore, by preventing the inactivation of the Na^+-K^+-ATPase, B3AR participates to decrease intracellular Na^+ content by maintaining the pump activity in the face of oxidant stress (Bundgaard et al.). Altogether, these results suggest an antioxidant role of B3AR signaling that would contribute to maintain vasodilatory responses in disease as well as important paracrine protective signaling. Therefore, cardiac β3AR may represent a promising target in the treatment of the failing heart by the use of B3AR agonist. This strategy may be superior to the use of sGC activators/stimulators because β3AR agonists will activate the NOS/cGMP pathway in a receptor-regulated way, as opposed to indiscriminate activation in all tissues by the sGC activators/stimulators, which results in hypotension as a limiting side effect; the β3AR may then be an attractive target to prevent adverse remodeling in the face of chronic adrenergic stimulation, all the more because it is distinctively resistant to homologous desensitization and retains coupling to downstream signaling in the pathologic heart, as demonstrated by us in human diseased myocardium ex vivo (Moniotte et al. 2001a).

6 Potential Protective Effect of Beta-3 AR and Clinical Studies

Highly specific B3AR agonists are needed in order to clinically test the additional beneficial effect of activation of β3-adrenergic receptors. As for beta-blockers commonly used post-myocardial infarction or in chronic heart failure, such as metoprolol, bisoprolol, and carvedilol, their affinity and inhibitory properties at native human cardiac B3AR have not been thoroughly characterized; however, the comparison of their pharmacologic properties using recombinant human B3AR in cellular heterologous expression systems showed that metoprolol and bisoprolol have a high affinity for B1AR, as expected, with no binding to B3AR, whereas carvedilol nonselectively binds B1- and B2AR, and has 100-fold lower affinity for B3AR; none of the three molecules exhibited significant agonism or inverse agonism at B3AR (Hoffmann et al. 2004).

Recently, a new, specific agonist at human β3AR, mirabegron, was developed and approved in Europe, the USA, and Japan for the treatment of overactive bladder disease, a urological condition characterized by frequent micturition, where the drug was shown to improve bladder filling through its myorelaxant properties mediated by activation of B3AR in the detrusor muscle (Vij and Drake 2015). Compared with previous preferential agonists, it has superior specificity (for B3AR as opposed to β1-2AR) with a well-documented bioavailability and little potential for adverse effects resulting from drug interactions. Mirabegron is metabolized by CYP3A4 and is a (weak) inhibitor of CYP2D6 and P-GP; it is also eliminated by urinary excretion. Furthermore, clinical data from the mirabegron clinical studies in overactive bladder disease pointed out no cardiovascular complications in the clinical trial populations. Following mirabegron treatment, an increase of approximately 1 bpm in heart rate and an increase in systolic blood pressure of <1 mmHg were observed which was not associated with increased cardiovascular complications (European Medicines Agency mirabegron EPAR report EMA/706651/2012). Therefore, the recent availability of this new drug offers the possibility to test the potential benefit of mirabegron (vs placebo) as add-on therapy (on top of standard care) to prevent/delay myocardial remodeling in patients at high risk of developing HFpEF.

To test this postulate, we designed a phase IIb, prospective, placebo-controlled randomized trial testing the effect of mirabegron to prevent or reverse the LV hypertrophic remodeling of patients with structural heart disease (Beta3lvh trial; stage B, AHA; measured by cardiac MRI) who are at risk of developing HFpEF (a frequent complication of cardiac remodeling) (Komajda and Lam 2014). Accordingly, the progression of cardiac hypertrophy and the development of fibrosis are specifically measured in patients at the early stages of the disease, who are at risk of development or worsening of HFpEF; these parameters are quantified by state-of-the-art techniques using cardiac MRI for ventricular hypertrophy and fibrosis, and Doppler echocardiography for diastolic function. Sequential measurements of cardiac fibrosis, exercise tolerance, metabolic parameters as well as specific biomarkers reflective of myocardial remodeling and function are prospectively measured in parallel. Our study is based on a principle of "drug repurposing" for

a new cardiovascular indication. This investigator-initiated, academic trial involves 12 European partners with nine clinical recruitment sites and is funded by the European Commission under the Horizon 2020 programme. The study officially started on May 1, 2015 and is expected to end in the spring of 2020 (ISRCTN 65055502).

In addition, in a transdisciplinary endeavor, the effect of mirabegron is measured both on endothelial function (both by digital microtonometry and direct measurement of circulating NO in erythrocytes (Lobysheva et al. 2013)) and on the abundance/activity of beige/brown fat (measured by ^{18}FDG-PET combined with CT scan); this is justified from the increasingly recognized paracrine influence of the endothelium on cardiac remodeling and the recent (re)discovery of human beige/brown fat that may influence peripheral and cardiac metabolism. Indeed, although the role of brown fat on thermogenesis and energy expenditure is clearly established in rodent models, the unequivocal identification of residual brown fat in adult humans was only provided recently (Cypess et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009) and opened new possibilities for the treatment of human diseases with caloric overload, i.e., obesity and metabolic syndrome (Nedergaard and Cannon 2014). Indeed, B3AR expressed in human beige/brown adipocytes mediates adrenergic fatty acids beta-oxidation (Cypess et al. 2015). Therefore, systemic administration of B3AR agonists may confer additional cardiovascular protection by improving the metabolic status, i.e., decreased triglyceridemia, and decreased obesity, with indirect effects on insulin sensitivity and hypertension. Again, small clinical trials with nebivolol (which has ancillary B3AR agonist properties) (Dessy et al. 2005) hint at additional metabolic protective effects compared with "pure" B1AR blockers (Ayers et al. 2012).

7 Conclusion

The unique signaling by B3AR in cardiovascular (and peripheral) tissues explains its protective effects against myocardial remodeling in preclinical models. This translates into reduced myocardial hypertrophy, reduced fibrosis, and preserved coronary perfusion that ultimately should lead to preserved/improved ventricular function, especially in the context of cardiovascular risk factors with increased oxidant stress and endothelial dysfunction. The results of the ongoing Beta3lvh (and possibly other) trials with specific B3AR agonists will tell whether this prediction is verified, which should lead to the addition of this class of drugs to the therapeutic armamentarium for heart failure.

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Novel sGC Stimulators and sGC Activators for the Treatment of Heart Failure

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Abstract

The burden of heart failure (HF) increases worldwide with an aging population, and there is a high unmet medical need in both, heart failure with reduced ejection fraction (HFrEF) and with preserved ejection fraction (HFpEF). The nitric oxide (NO) pathway is a key regulator in the cardiovascular system and modulates vascular tone and myocardial performance. Disruption of the NO-cyclic guanosine monophosphate (cGMP) signaling axis and impaired cGMP formation by endothelial dysfunction could lead to vasotone dysregulation, vascular and ventricular stiffening, fibrosis, and hypertrophy resulting in a decline of heart as well as kidney function. Therefore, the NO-cGMP pathway is a treatment target in heart failure. Exogenous NO donors such as nitrates have long been used for treatment of cardiovascular diseases but turned out to be limited by increased oxidative stress and tolerance. More recently, novel classes of drugs were discovered which enhance cGMP

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_100

production by targeting the NO receptor soluble guanylate cyclase (sGC). These compounds, the so-called sGC stimulators and sGC activators, are able to increase the enzymatic activity of sGC to generate cGMP independently of NO and have been developed to target this important signaling cascade in the cardiovascular system.

This review will focus on the role of sGC in cardiovascular (CV) physiology and disease and the pharmacological potential of sGC stimulators and sGC activators therein. Preclinical data will be reviewed and summarized, and available clinical data with riociguat and vericiguat, novel direct sGC stimulators, will be presented. Vericiguat is currently being studied in a Phase III clinical program for the treatment of heart failure with reduced ejection fraction (HFrEF).

Keywords
Cardiovascular • Cinaciguat • Cyclic guanosine monophosphate (cGMP) • Heart failure (HF) • Nitric oxide (NO) • Riociguat • sGC stimulator • sGC activator • Soluble guanylate cyclase (sGC) • Vericiguat

Abbreviations

ANP	Atrial natriuretic peptide
BNP	Brain natriuretic peptide
CAD	Coronary artery disease
cGMP	Cyclic guanosine monophosphate
CTEPH	Chronic thromboembolic pulmonary hypertension
CV	Cardiovascular
DILATE	Acute Hemodynamic Effects of Riociguat in Patients With Pulmonary Hypertension Associated With Diastolic Heart Failure
eNOS	Endothelial nitric oxide synthase
GC	Guanylate cyclase
GTP	Guanosine triphosphate
HF	Heart failure
HFpEF	HF with preserved ejection fraction
HFrEF	HF with reduced ejection fraction
LEPHT	Left Ventricular Systolic Dysfunction Associated With Pulmonary Hypertension Riociguat Trial
LOCF	Last observation carried forward
LV	Left ventricle
LVEDP	Left ventricular end-diastolic pressure
LVEF	Left ventricular ejection fraction
PAPmean	Mean pulmonary arterial pressure
MI	Myocardial infarction

NO	Nitric oxide
NP	Natriuretic peptide
PAH	Pulmonary arterial hypertension
PDE	Phosphodiesterase
PDEi	Phosphodiesterase inhibitor
PDE5	Phosphodiesterase type 5
pGC	Particulate guanylate cyclase
PH	Pulmonary hypertension
PH-sLVD	Pulmonary hypertension associated with systolic left ventricular dysfunction
QoL	Quality of life
ROS	Reactive oxygen species
RV	Right ventricle
sGC	Soluble guanylate cyclase
SOCRATES	Soluble guanylate cyclase stimulator in heart failure study

1 Introduction

Current standard therapies for heart failure (HF) and associated comorbidities have improved outcomes of patients with HF with reduced ejection fraction (HFrEF) and led to prolonged life expectancy. However, event rates remain unacceptably high once HF events such as hospitalization or need for IV diuretics due to decompensation occur in patients who are receiving standard of care. In HF with preserved EF (HFpEF) currently no approved therapeutic regimen exists but treatment of etiological factors like hypertension or diabetes mellitus is recommended. Since the burden of HF increases worldwide with an aging population, there is still a tremendous medical need for new treatments. New effective treatment strategies are required that provide a mid- and long-term morbidity and mortality benefit and improvement in quality of life (QoL), in addition to short term symptom relief in both HFrEF and HFpEF.

For more than a century, nitrates have been empirically used to treat acute and chronic heart failure, and angina. Nitrates act by setting free nitric oxide (NO), the starting point of a signaling cascade with soluble guanylate cyclase (sGC) in a central role and cyclic guanosine monophosphate (cGMP) as its second messenger. This evolving knowledge was rewarded in 1998 with the Nobel Prize in physiology or medicine awarded to three dedicated researchers for the discovery of the actual mechanism of action of NO in the body (http://www.nobelprize.org/nobel_prizes/medicine/laureates/1998/illpres/illpres.html). Since then, this pathway turned out to be one of the key regulators of cardiovascular function. This emerging knowledge led to substantial efforts in research and development to target this pathway to treat cardiovascular diseases. Since upstream increase of NO availability by nitrates is limited by tolerance, the requirement of biotransformation of nitrates into active NO donors, and the aggravation of endothelial dysfunction by formation of reactive

oxygen species, downstream therapeutic targets within this pathways were explored. Thus, current treatment strategies focus on inhibition of degradation of cGMP via phosphodiesterases (PDE), mainly PDE type 5 (PDE5) in those tissues where it is expressed, and generation of cGMP by direct, NO independent stimulation of the sGC. To date, preclinical and emerging clinical evidence support that cGMP enhancing therapy and in particular the stimulation of the sGC by novel sGC stimulators may have a beneficial role in the HF treatment strategy of the future. Large outcome trials yet have to confirm this hypothesis.

This review will focus on the role of sGC and the pharmacological enhancement of cGMP generation by sGC in cardiovascular disease and discuss the differential mode of action of novel sGC activators and stimulators. Preclinical and clinical data will be reviewed. In addition, data of the most advanced compound in this indication, vericiguat, which is currently being investigated in a large Phase III clinical outcome trial for patients with HFrEF, will be summarized.

2 Physiology and Pathophysiology of NO-sGC-cGMP Signaling in the Cardiovascular System

2.1 Physiology

The NO-sGC-cGMP pathway is a highly conserved signaling pathway which is indispensable for tissue and organ homeostasis and a key regulator within the cardiovascular system. The cascade starts with the formation of endogenous NO produced by endothelial cells by endothelial nitric oxide synthase (eNOS). NOS-derived NO diffuses to the neighboring tissue such as vascular smooth muscle cells or cardiomyocytes. In these cells, NO binds to the cytosolic enzyme sGC which serves as the receptor for NO. Binding of its endogenous ligand NO to the sGC catalyzes the conversion of guanosine triphosphate (GTP) to cGMP.

The cytosolic sGC is a heterodimeric protein which is formed by an α and a β subunit. The α subunit contains the NO-binding heme group, the β subunit the catalytic domain which forms cGMP out of GTP. As a second messenger, cGMP has been shown to activate cGMP dependent protein kinase G (PKG), PDEs as well as cyclic nucleotide-gated ion channels. Overall, cGMP increase and PKG activation results in the decrease of intracellular free calcium resulting in relaxation of vascular smooth muscle cells as one main physiological effect in the cardiovascular system (Fig. 1). The highest expression of sGC, however, is actually found in the heart muscle (Wilkins et al. 2014). In line with the predominant cardiac expression pattern, mice deficient in the alpha 1 subunit of sGC develop diastolic dysfunction with impaired LV relaxation and reduced cardiac output, in addition to increased systemic afterload (Buys et al. 2008). Moreover, it turned out that cGMP has next to this lusitropic also an antiinflammatory role by inhibiting P-selectin expression and leukocyte recruitment (Ahluwalia et al. 2004) as well as antifibrotic activity. cGMP is degraded by PDEs which together with sGC activity and NO availability regulate the NO-dependent cGMP pool in the cell. In addition, cGMP pools are regulated via

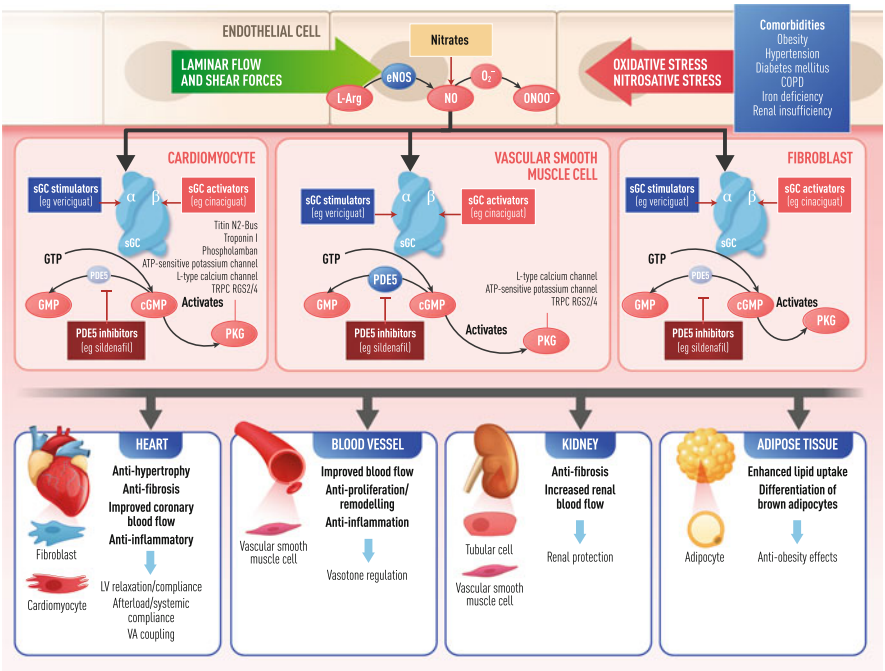

Fig. 1 sGC-derived cGMP generation in various cell types couples endothelial NO synthesis to function of organs including the heart and kidneys. cGMP is a second messenger of crucial importance to organ function. Reduced cGMP availability caused by NO deficiency due to endothelial dysfunction can be actively restored by sGC stimulation in smooth muscle cells, cardiomyocytes or fibroblasts, whereas PDE5 inhibition can only inhibit cGMP degradation. Restoration of cGMP signaling has beneficial effects in hearts, blood vessels, and kidneys and could also impact on adipose tissue. *L-Arg* L-Arginin, O_2^- peroxide, *ONOO*$^-$ peroxynitrite, *TRPC* transient receptor potential channels

natriuretic peptides (NPs) which bind to the membrane bound particulate guanylate cyclase (pGC), although individual subcellular compartmentalization of cytosolic sGC-derived as opposed to subplasmalemmal pGC-derived cGMP pools (Castro et al. 2006; Fischmeister et al. 2006; Nausch et al. 2008; Maurice et al. 2014) has been hypothesized to underlie the different responses to sGC (Su et al. 2005; Murad 1988) versus pGC stimulation (natriuresis). In addition, the NO-sGC-cGMP pathway interacts with other pathways like the prostacyclin-cAMP- or the endothelin-pathway as well as beta-adrenergic signaling (Trappanese et al. 2015; Bice et al. 2014a; Franssen et al. 2016; Belge et al. 2014; Tarone et al. 2014).

Although GCs are also expressed in the endothelium of vessels and the heart, it is important to recognize that the key role of the endothelium in this cascade is to generate NO by activity of the eNOS. The best established role of GCs as the receptor of NO signaling, however, has been characterized in vascular smooth muscle cells as well as in the myocardium, where sGC expression is highest. In vascular smooth muscle cells and cardiac myocytes sGC-derived cGMP production

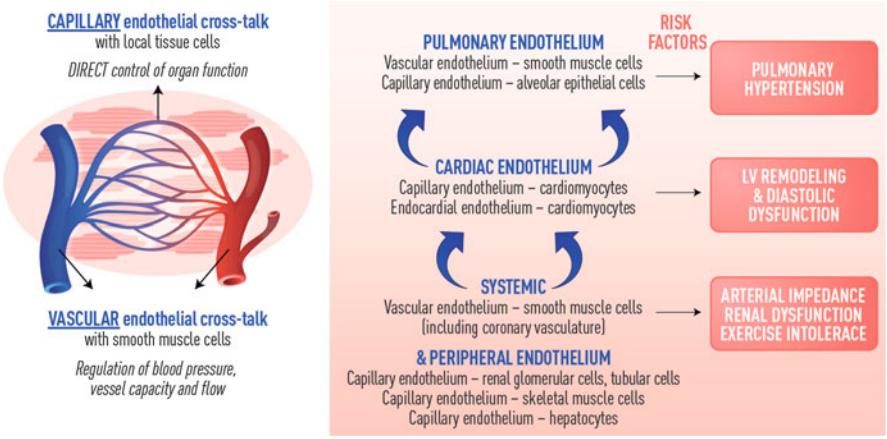

Fig. 2 Interaction of peripheral, systemic, and pulmonary vascular and capillary endothelium and endocardial and intramyocardial capillary endothelial cells with local tissue cells. Endothelial dysfunction can contribute to pulmonary and systemic arterial hypertension, cardiac and renal dysfunction as well as exercise intolerance. Adapted from Lim et al. (2015)

regulates vasotone, relaxation, and also contractility. NO generation from vascular, endocardial, and intramyocardial capillary endothelial cells stimulates sGC in coronary vessel walls as well as directly in cardiomyocytes (Fig. 2) (Lim et al. 2015; Cawley et al. 2011; Gheorghiade et al. 2013; Zhao et al. 2016; Noireaud and Andriantsitohaina 2014).

2.2 Pathophysiology

2.2.1 Role of the NO-cGMP System in CHF and Comorbidities

The NO-sGC-cGMP signaling pathway plays a major role in protection against myocardial injury, ventricular remodeling, and the cardio-renal syndrome (Gheorghiade et al. 2013). HF is associated with inflammation and oxidative stress which contributes to endothelial, skeletal muscle, and renal dysfunction (Figs. 1 and 3) (Munzel et al. 2015; Tsutsui et al. 2011; Supaporn et al. 1996; Shah et al. 2016; Boerrigter et al. 2009). NO availability as well as functionality of sGC depends on the redox status. Under conditions with oxidative stress, reactive oxygen species (ROS) disrupt the NO-sGC-cGMP signaling cascade on different levels by inactivation of eNOS, NO, and sGC. The result is reduced cGMP availability leading to endothelial dysfunction, vasoconstriction, vascular stiffness and adverse remodeling, and decreased renal and coronary blood flow with increasing impairment of respective organ function (Gheorghiade et al. 2013; Fraccarollo et al. 2014; Buys et al. 2008). In a model of sCG-alpha 1 deficient mice impaired ventricular relaxation was found to be the consequence of reduced cGMP availability (Buys et al. 2008).

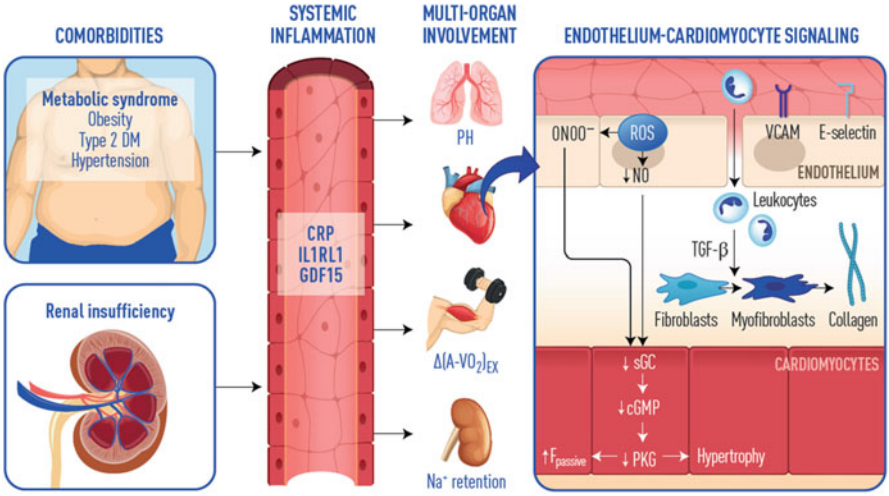

Fig. 3 Role of comorbidities resulting in inflammation and oxidative stress in HFpEF and associated organ involvement: Chronic systemic inflammation can affect the function of multiple organs. In the heart, disturbed endothelium-cardiomyocyte signaling can cause collagen deposition and reduced cGMP levels, leading to myocardial stiffening and impaired relaxation. *CRP* C-reactive protein, *IL1RL1* interleukin 1 receptor, type 1, *GDF15* growth differentiation factor 15, $\Delta(A\text{-}VO2)_{EX}$ oxygen extraction during exercise, $F_{passive}$ passive force, $ONOO^-$ peroxynitrite, *TGF-β* tumor growth factor beta, *VCAM* vascular cell adhesion molecule. Adapted from Shah et al. (2016)

Heart failure is a clinical syndrome caused by decreased CO and/or increased intracardiac pressures as a result of structural and/or functional abnormalities. Signs and symptoms like peripheral edema and shortness of breath on exertion or at rest are pathognomonic and can occur with any EF.

HFpEF is associated in many patients with the metabolic syndrome comprising of obesity, diabetes mellitus (DM), arterial hypertension, and COPD setting the stage for a proinflammatory environment. The individual components as well as any combination lead to increased morbidity and mortality in this mostly elderly population (Gerber et al. 2015). Impaired chronotropic reserve and atrial function as well as decreased vasoreactivity of systemic and pulmonary vasculature are also associated with HFpEF (Borlaug 2014).

The giant cardiomyocyte cytoskeletal protein titin modulates passive tension and stiffness, depending on phosphorylation by cGMP dependent PKG as well as phosphatase activity. When titin is phosphorylated, myocardial fibers stretch appropriately in the cardiac cycle and allow ventricular relaxation and diastolic filling as well as recoiling, major determinants of adequate cardiac output (Linke and Hamdani 2014; Stienen 2015; Hamdani et al. 2013). Hypophosphorylation of titin results in increased myocardial stiffness leading to suboptimal cardiac filling and output. In combination with enhanced afterload caused by vasoconstriction and increased vascular stiffness associated with endothelial dysfunction, cardiac failure

results and becomes symptomatic especially in situations with increased demands like exertion (Greene et al. 2013). In cardiomyocytes of HFpEF patients, an even increased stiffness compared to HFrEF patients was seen, which decreased after administration of PKG (van Heerebeek et al. 2012). However, cGMP enhancing therapy with Sildenafil did not result in improvement of clinical status and exercise capacity in a randomized placebo controlled trial in 216 HFpEF patients (Redfield et al. 2013), and also not in another study (Hoendermis et al. 2015). One potential explanation could be low generation of cGMP as substrate for PDE5 inhibition as a result of oxidative stress inactivating the sGC ligand NO. Another potential explanation is lack of PDE5 upregulation in human cardiomyocytes (Shah et al. 2016), opposed to its high expression in the corpus cavernosum. Indeed, even in HFrEF, PDE5 upregulation was reported inconsistently, and high PDE5 expression was not found in HFpEF (van Heerebeek et al. 2012). However, the failure of sildenafil to improve CV function in HFpEF has also been referred to the use of too high doses (Brutsaert and De Keulenaer 2015).

HFrEF is characterized by reduced contractility with insufficient cardiac output and associated with increased pre- and afterload. Cardiac remodeling occurs and further aggravates myocardial dysfunction. cGMP deficiency leads to impaired endothelium-dependent vasotone regulation, resulting in increased afterload and impaired myocardial microcirculation (Fig. 1) (Gheorghiade et al. 2013). cGMP dependent PKG modulates pathological Ca^{2+} signaling involved in myocardial hypertrophy (Hammond and Balligand 2012). In a rat myocardial infarction model, interstitial fibrosis, LV diastolic filling pressure (LVEDP), lung water and right ventricular (RV) hypertrophy were increased, while contractile function was markedly reduced. However, rats treated long term with the sGC activator ataciguat had reduced myocardial fibrosis and remodeling as well as improved systolic and diastolic function (Fraccarollo et al. 2014). In the dose finding SOCRATES REDUCED study, patients with HFrEF and a previous HF decompensation were treated with different doses of the sGC stimulator vericiguat or placebo. Although in the primary analysis of pooled vericiguat arms the trial did not meet the primary endpoint change in NT pro BNP, exploratory analysis showed dose-dependent lowering of NT-proBNP ($p < 0.02$), a reduction in NT-proBNP in the vericiguat 10 mg target dose arm ($p < 0.05$), and nominally improved clinical outcomes in the highest vericiguat dose groups (Gheorghiade et al. 2015).

Dysregulation of the NO-cGMP-pathway by inflammation and oxidative stress resulting in endothelial dysfunction also affects the coronary perfusion (Franssen et al. 2016; Paulus and Tschope 2013). In families with several members affected with **coronary artery disease** (CAD), next generation sequencing found mutations encoding for parts of sGC and an sGC stabilizing protein (Erdmann et al. 2013b). Importantly, when expressed in a cell line, coding variants of the sGC gene GUCY1A3 found in young MI patients are associated with compromised cGMP-generating activity. This lack of cGMP generation could be overcome by incubation of cells with the sGC stimulator BAY 41-2272. cGMP formation increased to wildtype level in all but one tested variant with low protein levels (Wobst et al. 2016). Protection against ischemic injury by sGC stimulation was seen in an MI

mouse model treated with riociguat. Treatment with riociguat at reperfusion decreased infarct size and improved LV function compared to placebo treated mice (Methner et al. 2013).

Advanced left heart dysfunction leads to increased left ventricular end-diastolic pressure (LVEDP) which is transmitted backwards into the pulmonary vasculature. In addition to passive backward failure, a vasoreactive component might rarely aggravate the resulting **pulmonary hypertension** (PH). PH in patients with left heart disease as indicator of increased LV filling pressures, however, is common and associated with a poor prognosis (Haddad et al. 2011; Damy et al. 2010). Functional status and presence of PH are correlated, and as many as 33 to 47.5% of HFrEF and up to 83% of HFpEF patients may have secondary PH (Galie et al. 2016). In a mouse model of chronic left heart failure caused by transverse aortic constriction (TAC), mice developed left ventricular hypertrophy and dysfunction and secondary pulmonary hypertension. 6 weeks after TAC, treatment with riociguat and sildenafil given over 2 weeks was investigated in comparison to placebo. Riociguat had a more pronounced effect on vascular remodeling than sildenafil. The treatment with riociguat and sildenafil maintained LV and RV function and decreased PVR and RV pressure, while in placebo treated animals LV and RV function deteriorated. Additionally, although LV hypertrophy was unaltered, collagen content in both LV and RV was reduced in actively treated mice (Pradhan et al. 2016). Despite these promising preclinical findings, the clinical results for the PDE5i came out negative. This could indicate that pulmonary vascular effects of sildenafil may not be sufficient to improve HF in the absence of cardiac effects due to reported lack of PDE5 overexpression and/or insufficient proximal sGC stimulation. In contrast, high sGC expression in the cardiac muscle and enhanced sGC-derived cGMP levels even in the absence of NO indicate promising potential of sGC stimulators. In patients with pulmonary hypertension associated with systolic left ventricular dysfunction (PH-sLVD), reduced PVR and SVR, improved stroke volume and cardiac index as well as improved quality of life (QoL) were seen under treatment with riociguat (Bonderman et al. 2013).

HF and **kidney disease** often coexist and are closely interlinked in the cardiorenal syndrome. Common pathophysiological ground is – among others – oxidative stress and endothelial dysfunction (Dubin and Shah 2016; Schinner et al. 2015; von Lueder and Krum 2015). For many reasons, heart failure and renal failure present risk factors for the progression of the respective other condition (Ronco and Di Lullo 2014). Albuminuria has been shown to be associated with LV dysfunction (Katz et al. 2014). In an eNos knockout mouse model of hypertension and diabetic nephropathy, administration of an sGC stimulator on top of an angiotensin receptor blocker resulted in reduced oxidative stress and albuminuria (Ott et al. 2012). Renoprotective effects of sGC stimulators and activators have also been shown in a number of other preclinical models (Stasch et al. 2015). Both an sGC stimulator and an sGC activator improved cardiac hypertrophy and survival in spontaneously hypertensive stroke prone rats (Costell et al. 2012). A high dose of the sGC stimulator reduced microalbuminuria and ameliorated the blood pressure rise more potently than the sGC activator. Since high salt/fat diet did not blunt vasodilation in response to both sGC stimulator and sGC

activator, the authors concluded that reduced NO bioavailability rather than sGC oxidation causes vascular dysfunction (Costell et al. 2012).

Theoretically, cGMP can be pharmacologically targeted on the level of production with exogenous NO-donors, although this approach turned out to be limited by increasing oxidative stress due to peroxynitrite formation, as already described above. In addition, cGMP can also be increased by inhibition of cGMP degradation via phosphodiesterase inhibitors (PDEi), yet this would be limited in the absence of elevated PDE5 expression in relevant target tissue opposed to the established high levels in the corpus cavernosum and pulmonary vasculature. Both pharmacological approaches have additional limitations. The recommended treatment with nitrates in African Americans with symptomatic HFrEF requires combination with hydralazine. PDEis depend on a sufficient endogenous NO-supply and cGMP production which is often impaired in cardiovascular diseases. In patients with HFpEF isosorbide mononitrate reduced physical activity and did not improve quality of life or submaximal exercise capacity (Redfield et al. 2015). Therefore, the identification of NO-independent direct stimulators of the sGC (sGC stimulators) was an important step in the pharmacological modulation of this pathway. The sGC *stimulators* bind to the heme containing form of sGC independently of NO and stimulate cGMP production. In addition, these compounds also act synergistically with NO. Besides the heme-dependent sGC stimulators, there is a second class of heme-independent sGC modulating compounds, the so-called sGC *activators*. These compounds activate the sGC in its oxidized and heme-free form which is in itself unable to produce cGMP (Munzel et al. 2015). Both compound classes have been widely profiled in preclinical animal models of cardiovascular, cardiopulmonary, or cardio-renal diseases and may have treatment potential in HF. First clinical data are available and are discussed below.

3 Clinical Data of sGC Activators and Stimulators

3.1 sGC Activators

The sGC activators target the oxidized and heme free form of the sGC, triggering cGMP production even in conditions with oxidative stress. sGC activators work independently of NO but their efficacy has been shown to be additive to endogenous NO (Fig. 1).

3.1.1 Cinaciguat

Cinaciguat is a potent and selective sGC activator which acts on sGC in its oxidized (Fe^{3+}) state and even heme free form independently of NO. The oxidation or absence of the heme moiety increases the effect of cinaciguat on the sGC (Evgenov et al. 2006) causing a significant cGMP increase. These in vitro findings have also nicely been demonstrated in vivo, in mice which express heme-free sGC (apo-sGC mice) and consequently develop hypertension. In these apo-sGC mice, the vasodilatory response to NO is blunted in comparison to WT and administration

of an NOS inhibitor only resulted in increased BP in the WT mice demonstrating the uncoupling of the NO/cGMP pathway. Administration of cinaciguat decreased the BP in both WT and aposGC mice, but significantly more pronounced in the apo-sGC mice (Thoonen et al. 2015). These findings confirm that sGC is present in both forms in vivo, in the reduced, heme containing and in the oxidized, heme free form (Evgenov et al. 2006).

In a mouse model with diabetic cardiomyopathy, diabetic mice had significantly decreased cGMP levels in plasma and myocardium as well as increased levels of PDE5 and PKG. However, PKG activity was markedly reduced. Treatment with cinaciguat lead to normalization of cGMP levels, reduced PDE 5 expression, and improved PKG activity in the DM mice. Myocardial hypertrophy was prevented and fibrosis ameliorated by treatment with cinaciguat, while clinically systolic and diastolic function improved (Matyas et al. 2015).

Treatment with cinaciguat was investigated in patients with acute heart failure. AHF, either de novo or as a result of (gradual) worsening of preexisting HF, often is a life-threatening condition requiring urgent medical care. However, despite short term stabilization and symptom control, mid- and long-term risk for HF rehospitalization and CV death remain high.

The short half-life and chemical properties resulted in an i.v. formulation for continuous application. In a first multinational, double-blind phase II b study patients with acute decompensated heart failure with an LVEF $<40\%$ and an elevated pulmonary capillary wedge (PCWP) pressure of ≥ 18 mmHg were randomized 2:1 to cinaciguat (starting dose 100 μg/h) or placebo. Patient had to present with worsening dyspnea and volume overload, and systolic blood pressure had to be at least 100 mmHg but less than 180 mmHg. Every 2 h the study drug could be uptitrated or downtitrated depending on BP, HR, and overall tolerability, to a maximum of 600 μg/h and a minimum of 50 μg/h. The primary endpoint was change in PCWP after 8 h, secondary endpoints were safety/tolerability as well as dyspnea, among others. After 158 patients in screening and with 139 in the per protocol group, the study was terminated upon recommendation of an independent data monitoring committee (DMC) because of hypotensive events without clear benefit in all dose groups except the 50/100 μg/h dose group. In the cinaciguat treated group, mean PCWP was significantly reduced from baseline (25.7 mm Hg) to 8 h/last observation carried forward (LOCF) compared with placebo (cinaciguat: −7.7 mmHg; placebo: −3.7 mmHg; difference: 4.0 mmHg). Systemic arterial mean blood pressure, right atrial pressure, mean pulmonary arterial pressure, and systemic and pulmonary vascular resistance decreased significantly while cardiac index significantly increased under active treatment from baseline to 8 h/LOCF compared with placebo. Dyspnea improved comparably in both groups. Hypotension was observed in 51% of cinaciguat and 12% of placebo treated patients, while HR increased by 5 bpm in the cinaciguat group. In total, 48 patients in the cinaciguat group had treatment-emergent hypotensive adverse events. There were more patients with ventricular tachycardia, troponin I increases, and rehospitalizations in the cinaciguat than in the placebo group, mostly at doses of 200 μg/h and higher (Erdmann et al. 2013a).

As a consequence, three further phase II b trials were conducted in the COMPOSE program (COMPOSE 1 and 2 and COMPOSE early). The goal of the COMPOSE program was to investigate the safety and efficacy of different doses of cinaciguat $\leq$200 μg/h, versus placebo starting at different time points for the treatment of patients with AHF. Patients had to present to the hospital with a history of LVEF of $\leq$40% and with worsening dyspnea and volume overload, requiring i.v. treatment. The required minimal SBP was at least 120 mmHg, a PCWP $\geq$20 mmHg and a CI $\leq$2.5 L/min/m^2. In COMPOSE 1 patients were randomized to fixed doses of 50, 100, or 150 μg/h or placebo, whereas in COMPOSE 2 patients received 10 or 25 μg/h versus placebo over 24–48 h. The primary efficacy endpoint for both studies was change in PCWP from baseline to 8 h or LOCF. In COMPOSE early, patients had to start study drug treatment (fixed doses of 50, 100, or 150 μg/h or placebo) within 12 h after admission, and the primary efficacy endpoint was change in dyspnea from baseline to 8 h or LOCF. All studies were terminated early upon recommendation of an independent DMC because of hypotensive events (COMPOSE 1 (ITT population $n = 12$) and early (ITT population $n = 62$)), and difficult patient recruitment and the risk of inconclusive results in COMPOSE 2 (ITT $n = 4$). Thus, endpoints were assessed descriptively where applicable. In COMPOSE 1, PCWP, RAP, and BP decreased but CI did not change. In COMPOSE early, dyspnea did not improve in comparison to placebo. There was a higher incidence of hypotensive events in COMPOSE 1 and early (Gheorghiade et al. 2012). It can be concluded that treatment with cinaciguat reduced pre- and afterload by potent vasodilation, very frequently at the cost of hypotension. There was no clear clinical benefit in respect of improvement in dyspnea, endorgan protection or decrease in rehospitalization rates. Although preclinical data suggest that sGC activators could be beneficial, clinical data do currently not support the use in patients with AHF. However, more research with optimized compounds seems to be worthwhile because of the potential of the unique mode of action of the sGC activators to counteract the deleterious sequelae of oxidative stress in cardiovascular disease.

Other NO- and heme-independent activators of soluble guanylate cyclase with clinical data include ataciguat (HMR1766) and the fibrate gemfibrozil (Sharina et al. 2015). A phase Ib study evaluating the safety of ataciguat in patients with moderate calcific aortic valve stenosis has been posted as completed (NCT02049203), and a phase II study evaluating the effects of ataciguat on aortic valve calcification (CAVS) is posted to be ongoing (NCT02481258).

3.2 sGC Stimulators

In contrast to sGC activators, sGC stimulators target the heme-containing non-oxidized form of sGC by binding on the regulatory domain and triggering cGMP production. The sGC stimulators work NO-independently but their efficacy is further enhanced when endogenous NO – even at very low concentrations – is present. The sGC stimulators have shown beneficial effects in a variety of

preclinical models for cardiopulmonary diseases and pulmonary hypertension (Dumitrascu et al. 2006; Weissmann et al. 2014; Lang et al. 2012). In addition, a broad database investigating the preclinical efficacy in cardiovascular diseases is available. The sGC stimulators limited hypertrophy of cardiomyocytes in vitro (Irvine et al. 2012), reduced LV and vascular fibrosis (Masuyama et al. 2006; Masuyama et al. 2009), reduced infarct size (Bice et al. 2014b), preserved ejection fraction after MI (Methner et al. 2013), protected from cardiac and renal damage and increased survival of hypertensive rats (Sharkovska et al. 2010), reduced cardiac hypertrophy and improved renal function in hypertensive rats (Follman et al. submitted). Riociguat and vericiguat are two members of this class of potent and selective sGC stimulators which are studied clinically.

3.2.1 Riociguat

Riociguat is a novel potent sGC stimulator which has been investigated and approved in the treatment of pulmonary arterial hypertension (PAH) and inoperable chronic thromboembolic pulmonary hypertension (CTEPH). In summary, PAH and CTEPH patients treated with riociguat were shown to have a better exercise capacity as measured by 6 MWD, improved hemodynamics (decreased PVR, improved CI) as well as dyspnea, WHO FC, NT-proBNP, and QoL in comparison to patients treated with placebo on top of SoC. The most common adverse events (AEs) were headache, dizziness, indigestion, peripheral edema, nausea, diarrhea, and vomiting (Ghofrani et al. 2013b; Ghofrani et al. 2013a). Treatment effects were sustained for at least 2 years in long-term studies (Rubin et al. 2015; Rubin et al. 2014; Simonneau et al. 2015; Simonneau et al. 2014).

Treatment of PH-sLVD is based on treatment of the underlying disease; however, pulmonary vasodilation in addition seems to be intuitive, especially with increased transpulmonary gradient (TPG), although parallel cardiac and systemic effects would be essential to cope with the resulting increase in pulmonary venous return. Therefore, previous studies with selective pulmonary vasodilators in HF including epoprostenol (Califf et al. 1997) and bosentan (REACH and ENABLE trials) (Mylona and Cleland 1999; Packer et al. 2005; Kalra et al. 2002) were not successful. In contrast with these selective pulmonary vasodilators, riociguat did not demonstrate pulmonary selectivity (Grimminger et al. 2009). Thus, in the randomized, placebo-controlled, double blind LEPHT study, the safety, tolerability as well as PK and PD of riociguat were investigated in patients with PH secondary to systolic LV dysfunction. Two hundred and eleven patients with PH-sLVD characterized by an LVEF $\leq$40% and mean pulmonary arterial pressure (PAPmean) $\geq$25 mmHg at rest on optimized HF therapy were enrolled. There was no minimum TPG or diastolic pulmonary gradient defined as inclusion criteria. Mean transpulmonary gradient at baseline ranged from 12.6 to 15.3 mm Hg across the treatment groups. Riociguat in doses of 0.5, 1, and 2 mg tid and placebo were administered to 201 patients in a ratio of 2:1:1:2 over 16 weeks. The primary efficacy variable was PAPmean, while secondary efficacy endpoints comprised of LVEF, exercise capacity, quality of life, and other haemodynamic and echocardiographic measurements. Under treatment with riociguat, PAPmean did not decrease

significantly, but PVR did, and stroke volume and cardiac index improved markedly without concomitant increase in heart rate. QoL improved as well and riociguat was well tolerated. The most common treatment emergent AEs in the riociguat 2 mg group were GI tract problems like nausea or diarrhea as well as dizziness, hypotension, and edema (Bonderman et al. 2013).

The hemodynamic effects, safety, and PK of riociguat were also investigated in patients with PH and HFpEF in a randomized, placebo-controlled, double blind, single dose study. Patients with an LVEF > 50%, a PAPmean of ≥25 mmHg, and a PCWP > 15 mmHg on standard HFpEF treatment received 0.5, 1, or 2 mg riociguat or placebo. The primary efficacy variable was the highest decrease in mPAP from baseline up to 6 h while secondary outcomes comprised of other hemodynamic as well as echocardiographic parameters, safety, and pharmacokinetics. In total, 36 patients were evaluable. There was no significant change in the primary efficacy variable, but 2 mg riociguat significantly increased stroke volume and CI and decreased BP and SVR without concomitant changes in HR, PVR, and PCWP. RVED and LA areas were significantly smaller after 2 mg riociguat as well. Drug-related serious AEs occurred in three patients on riociguat (2 mg group; one case of decreased CO, three cases of decreased MAP), and in two placebo treated patients (two cases of decreased CO). There were four cases in the riociguat group (one patient on 1 mg, three patients on 2 mg), and one case in the placebo group with a relevant decrease in blood pressure (SBP <80 mmHg or MAP <60 mmHg). One case of pulmonary edema occurred but was considered to be unrelated to study drug (riociguat). The author concluded that single doses of riociguat were well tolerated and that under treatment hemodynamics and echocardiographic parameters changed favorably in patients with PH and HFpEF (Bonderman et al. 2014).

3.2.2 Vericiguat

These first experiences with an sGC stimulator in patients with HF and secondary PH encouraged the continued investigation of this novel drug class in HF at low doses. For further studies in HF, the once daily compound vericiguat was chosen due to its optimized pharmacokinetic profile. Vericiguat was investigated in two phase IIb studies in HFrEF (SOCRATES reduced) and HFpEF (SOCRATES preserved) and is currently moving into phase III for HFrEF.

The phase IIb program SOCRATES comprised of two randomized, placebo-controlled, double blind studies running in parallel in patients with HFrEF (LVEF <45%) and HFpEF (LVEF ≥45%) and a previous HF decompensation. Patients were required being recently hospitalized or presenting to the ED with worsening CHF as well as elevated (NT pro) BNP levels and needing i.v. diuretics. The goal was to investigate the safety, tolerability, PK, and PD of four different doses (1.25, 2.5, 5, and 10 mg) of Vericiguat or placebo administered once daily over 12 weeks in this population at high risk for rehospitalization or death. The primary efficacy variable in SOCRATES reduced was change in NT pro BNP, and in SOCRATES preserved was change in left atrial (LA) volume after 12 weeks.

SOCRATES Reduced

In this randomized, placebo-controlled, double blind dose finding study, 456 patients with HFrEF (LVEF <45%, elevated (NT pro) BNP levels) were randomized 1:1:1:1:1 to placebo or vericiguat, 1.25, 2.5, 5, and 10 mg once daily. Patients were eligible if they had a recent hospitalization or ED visit because of worsening CHF and the need for i.v. diuretics. A systolic blood pressure of at least 110 mmHg was required, and uptitration for the 5 and 10 mg doses was guided by BP and overall tolerability. The primary efficacy end point of the study was change from baseline in log-transformed NT-proBNP level at 12 weeks. Further endpoints were safety, echocardiographic parameters as well as serum biomarkers. At baseline, patients´ NYHA class was approximately split even between I/II and III/IV, the HF etiology was ischemic in about half of the patients, and DM was present in about 50%, CKD in about 40%, and atrial fibrillation was present in about 30% of the patients. Median NT pro BNP at baseline was 3076 pg/ml. Mean LVEF was <30% in all groups, and the majority of patients received guideline recommended HF therapy as required by the protocol. Three hundred and fifty one patients completed treatment with study drug with valid 12-week NT-pro BNP levels. The primary analysis was not significantly different in the pooled vericiguat and placebo groups. The exploratory analysis suggested a dose–response relationship in which higher vericiguat doses were associated with greater reductions in NT-proBNP levels. LVEF improved in the Riociguat 10 mg group by 3.7%, and in the placebo group by 1.5%. Additional clinical exploratory endpoints were all-cause death, CV death, HF hospitalization, or the composite of CV death or HF hospitalization. There were nominally less CV deaths and HF hospitalizations in the 5 and 10 mg vericiguat arms than in all other treatment arms (Table 1 and Fig. 4).

BP and HR were unchanged in the vericiguat groups compared to placebo, and adverse event rates were the same in all treatment groups. SAE rates were highest among placebo treated patients (39.1%). Although there were more hypotensive events ($n = 14$, 10 symptomatic) and syncope ($n = 4$) in the highest vericiguat dose

Table 1 Occurrence of exploratory clinical endpoints in SOCRATES reduced

		Vericiguat			
Clinical outcome	Placebo ($n = 92$)	1.25 mg ($n = 91$)	2.5 mg ($n = 91$)	2.5 to 5 mg ($n = 91$)	2.5 to 10 mg ($n = 91$)
Up to week 12, No. (%)					
All-cause death	3(3.3)	4(4.4)	3(3.3)	3(3.3)	2(2.2)
CV death	3(3.3)	4(4.4)	2(2.2)	2(2.2)	2(2.2)
HF hospitalization	16(17.4)	16(17.6)	16(17.6)	9(9.9)	9(9.9)
CV death or HF hospitalization	18(19.6)	17(18.7)	18(19.8)	11(12.1)	10(11.0)
End of follow-up (16 wk), No. (%)					
All-cause death	6(6.5)	6(6.6)	5(5.5)	3(3.3)	4(4.4)
CV death	6(6.5)	5(5.5)	4(4.4)	2(2.2)	4(4.4)

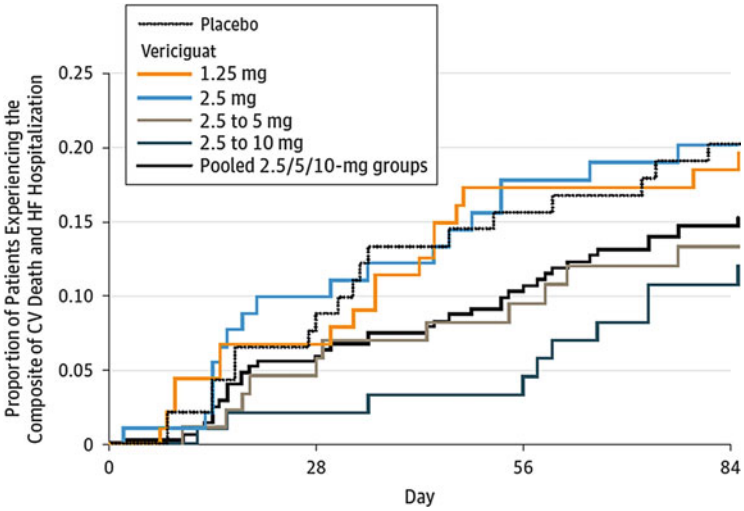

Fig. 4 Proportion of patients experiencing the composite of CV death and HF hospitalization from Gheorghiade et al. (2015)

group, 8 of 14 patients experienced hypotension between weeks 0 and 2, when the maximal vericiguat dose per the study titration schedule was 2.5 mg once daily, and the cumulative proportion of patients with treatment-emergent hypotension during the first 2 weeks in the three 2.5 mg starting dose groups was 4.4%, compared with 3.3% in the placebo group. The authors concluded that vericiguat did not change NT-proBNP levels at 12 weeks compared to placebo but was well tolerated. Further clinical trials of vericiguat based on the dose–response relationship are needed to determine the potential role of this drug in the treatment of patients with worsening chronic HF (Gheorghiade et al. 2015; Pieske et al. 2014).

A confirmatory phase III outcome trial in HFrEF patients (**VICTORIA**) has been posted in CT.gov to start in 2016 (A Study of Vericiguat in Participants With Heart Failure With Reduced Ejection Fraction (HFrEF) (MK-1242-001) (VICTORIA) NCT02861534).

The **SOCRATES preserved** phase IIb dose-finding study investigated vericiguat in patients with HFpEF (ejection fraction $\geq$45%) in a prospective, randomized, placebo-controlled double-blind design with five parallel dose arms to characterize safety, tolerability, and pharmacologic effects. Patients received vericiguat once daily at 1.25 or 2.5 mg fixed doses, or 5 or 10 mg titrated from a 2.5 mg starting dose, or placebo for 12 weeks. The two primary endpoints were change from baseline in log-transformed N-terminal pro-B-type natriuretic peptide (NT-ProBNP) and left atrial volume (LAV) at 12 weeks. As key exploratory parameters patient-reported outcomes were collected in the KCCQ and EQ-5D (Pieske et al. 2014)).

4 Conclusions

Despite therapeutic advances there is still a high unmet medical need in the treatment of HF and associated comorbidities. Endothelial dysfunction resulting in reduced NO availability has been identified as a central culprit in cardiovascular disease. The novel direct sGC stimulators stimulate the reduced and heme containing form of sGC independently of NO but also synergistically with NO. Preclinical and emerging clinical evidence support that sGC stimulators are promising candidates to be studied for a potential role in the future HF treatment strategy. Treatment with the sGC stimulator riociguat improved stroke volume and cardiac index and reduced afterload in patients with systolic HF and secondary PH. In patients with HFrEF within 1 month after a HF event, the sGC stimulator vericiguat was well tolerated. While the primary analysis was not significantly different in the pooled vericiguat and placebo groups, there was a dose-dependent decrease in NT-proBNP levels. In addition, vericiguat showed potential to improve LVEF and clinical outcomes. Based on the promising data from this dose finding, safety and PK/PD study in HFrEF a pivotal study has been posted in CT.gov to start in 2016 (A Study of Vericiguat in Participants With Heart Failure With Reduced Ejection Fraction (HFrEF) (MK-1242-001) (VICTORIA) NCT02861534).

Conflict of Interest All authors are employee of Bayer AG / Pharmaceutical Division.

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Cardiac Phosphodiesterases and Their Modulation for Treating Heart Disease

Grace E. Kim and David A. Kass

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Abstract

An important hallmark of cardiac failure is abnormal second messenger signaling due to impaired synthesis and catabolism of cyclic adenosine 3′,5′-monophosphate (cAMP) and cyclic guanosine 3′,5′- monophosphate (cGMP). Their dysregulation, altered intracellular targeting, and blunted responsiveness to stimulating pathways all contribute to pathological remodeling, muscle

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_82

dysfunction, reduced cell survival and metabolism, and other abnormalities. Therapeutic enhancement of either cyclic nucleotides can be achieved by stimulating their synthesis and/or by suppressing members of the family of cyclic nucleotide phosphodiesterases (PDEs). The heart expresses seven of the eleven major PDE subtypes – PDE1, 2, 3, 4, 5, 8, and 9. Their differential control over cAMP and cGMP signaling in various cell types, including cardiomyocytes, provides intriguing therapeutic opportunities to counter heart disease. This review examines the roles of these PDEs in the failing and hypertrophied heart and summarizes experimental and clinical data that have explored the utility of targeted PDE inhibition.

Keywords
Cyclic nucleotides • Heart failure • Myocardium • Phosphodiesterases • Protein kinase A • Protein kinase G

1 Introduction

Phosphodiesterases (PDEs) are a superfamily of enzymes that hydrolyze cyclic adenosine 3′,5′- monophosphate (cAMP) and cyclic guanosine 3′,5′-monophosphate (cGMP). Both cyclic nucleotides are synthesized in specific intracellular compartments by corresponding cyclases and are selectively catabolized by members of the PDE superfamily. The resulting localized activation elicits targeted cellular responses to multiple stimuli. Levels of both myocardial cyclic nucleotide synthesis and hydrolysis are altered by physiological and pathological stress and play an important role in diseases such as cardiac failure. Therapies to restore their signaling to improve cardiac function and suppress maladaptive organ and molecular remodeling have been and continue to be pursued. Targeting PDEs is particularly attractive as highly specific and potent small-molecule inhibitors have been developed for many of the enzymes, and their expression in particular cell types and intracellular nanodomain regulation affords targeted influences over cyclic nucleotide signaling. Inhibitors of PDE3 are in clinical use for acute heart failure, PDE4 for psoriasis and chronic obstructive lung disease, and PDE5 for erectile dysfunction and pulmonary hypertension. None are presently approved for chronic heart failure, but several are being studied for such indications. This review describes the role and potential utility of pharmacological targeting of PDEs in the diseased heart, focusing first on those for which clinical data have been generated, followed by those where only preclinical data have been obtained thus far.

2 Cyclic Nucleotides: Cardiac Second Messengers with Pleiotropic Effects

Both cAMP and cGMP modulate a wide range of myocardial properties including contraction and relaxation, diastolic stiffness, heart rate, cell growth and survival, interstitial fibrosis, vascular tone, and endothelial permeability and proliferation. Cyclic AMP is generated by adenylate cyclase (AC type 5 and type 6 in the heart), and it activates one of two cognate proteins: protein kinase A (PKA) or exchange protein directly activated by cAMP (Epac). In the cardiomyocyte, PKA phosphorylates multiple proteins controlling excitation-contraction coupling and sarcomere function. These include troponin I (TnI; Kentish et al. 2001), titin (Yamasaki et al. 2002), myosin binding protein C (Nagayama et al. 2007; Stelzer et al. 2006), phospholamban (MacLennan and Kranias 2003), ryanodine receptor (RyR2) (Reiken et al. 2003), and L-type calcium channel (Verde et al. 1999). Epac is a guanine nucleotide exchange factor (GEF) protein that can activate calcium-calmodulin activated kinase II (CamKII) signaling to alter calcium cycling and gene transcription (Gloerich and Bos 2010). In the cardiomyocyte, both PKA and Epac are engaged by cAMP synthesis coupled to β-adrenergic G-protein-coupled receptor stimulation.

Cyclic GMP is generated by either a soluble guanylate cyclase (sGC) activated by nitric oxide or a receptor-bound cyclase (GC-A or GC-B) in the intracellular domain of the natriuretic peptide receptor. The targeted kinase is cGMP-stimulated kinase (cGK1 in the heart, also called PKG-1) that phosphorylates many of the same calcium homeostasis and sarcomere proteins targeted by PKA (e.g., phospholamban, TnI, titin, myosin binding protein C). However, other cGK1 targets oppose neurohormonal stimulation pathways, such as regulator of G-protein signaling 2 and 4 (RGS2, RGS4) that counter Gq- and Gi-receptor-coupled agonism (Takimoto et al. 2009; Tokudome et al. 2008), ion channels like transient receptor potential canonical type 6 (TRPC6) that stimulate calcineurin/NFAT signaling (Kinoshita et al. 2010; Koitabashi et al. 2010; Nishida et al. 2010), and RhoA which regulates Rho-kinase signaling (Sawada et al. 2001) and myosin light chain phosphatase (Surks et al. 1999). In the myocyte, cGK1 does not stimulate L-type calcium current. Thus, cGK1 generally acts as a myocardial brake that can counter cAMP/PKA stimuli acutely and chronically suppress stress-mediated signaling.

Upon synthesis, local regulation of cyclic nucleotides is exquisitely controlled by members of the PDE superfamily. The 11 family members of PDEs are expressed by nearly 100 isoform variants (Maurice et al. 2014) that differ mainly in their N-terminus regulatory domains. By contrast, their catalytic domains are broadly conserved, with each species having subtle differences to provide cAMP and/or cGMP substrate specificity (Bender and Beavo 2006; Francis et al. 2011). PDEs pose unique opportunities for pharmacological modification of cyclic nucleotide signaling because they are selectively expressed in various cell types. PDEs 1–5, 8, and 9 are expressed in myocardium, with some species-dependent differences in isoform expression, notably in PDE1 (Table 1). PDEs 1, 2, and

Table 1 Specificity and regulation of PDEs expressed in myocardium

PDE	Substrate	Regulator
1A[a]	cAMP/cGMP	Calcium/calmodulin activated
1B		
1C[b]		
2A	cAMP/cGMP	cGMP-activated cAMP hydrolysis
3A[c]	cAMP/cGMP	cGMP inhibited
3B		
5A	cGMP	cGMP activated
9A	cGMP	None known

[a]Prominent isoform expressed in mouse and rat
[b]Prominent isoform in human, dog, rabbit
[c]Predominantly expressed myocardial isoform

3 are all dual-substrate esterases, PDE5 and PDE9 are selective for cGMP, and PDE4 and PDE8 are selective for cAMP. Preclinical studies have established a role in cardiac regulation for all of these species, while clinical data related to heart disease only exist for PDE3 and PDE5. Importantly, all of these PDEs are dysregulated in conditions of cardiac failure, infarction, and hypertrophy, often but not always displaying increased expression. This applies to disease in multiple experimental models as well as in humans (Table 2). Furthermore, studies using either selective pharmacological or genetic modulators of these PDEs have revealed their potent impact on disease pathophysiology, supporting therapeutic potential (Table 3).

3 PDE3 and Dilated Cardiomyopathy

The first exploration for a therapeutic role of PDE modulation to treat heart disease evolved with the discovery that PDE3 modulated cAMP and that its inhibition could potentially enhance PKA (and Epac)-dependent signaling in the diseased heart. PDE3 is expressed in two primary isoforms, PDE3A and PDE3B, and both are found in cardiomyocytes. PDE3A is the predominant form (Meacci et al. 1992; Sun et al. 2007), and its three splice variants differ in intracellular compartmentation (Wechsler et al. 2002) as well as regulation by PKA and PKC (Vandeput et al. 2013). These isoforms operate in microdomains. For example, PDE3A localizes to the sarcolemmal membrane, and this allows for regulation of compartmentalized PKA signaling (Leroy et al. 2008; Zaccolo 2009; Zaccolo and Pozzan 2002). While PDE3 can hydrolyze both cAMP and cGMP, it favors cAMP due to a much higher V_{max} for this substrate.

PDE3 inhibition results in increased L-type calcium current (Verde et al. 1999) which stimulates contractility (Weishaar et al. 1987), an effect principally mediated by PDE3A (Sun et al. 2007). PDE3A co-immunoprecipitates with a protein complex containing SERCA2a, phospholamban, and A-kinase anchoring protein 18 (AKAP18) in a PKA phosphorylation-dependent manner (Ahmad et al. 2015). This specifically targets a pool of cAMP to regulate calcium cycling via the

Table 2 Pathologic alterations in myocardial PDE expression (in vivo studies)

PDE family	Change	Species	Cardiomyopathy	References
1A	Increased	Rat	Hypertrophy (aortic constriction)	Yanaka et al. (2003)
		Human	Post-myocardial infarction	Miller et al. (2011)
		Mouse	Hypertrophy (ISO, AII, TAC)	Miller et al. (2009); Miller et al. (2011)
		Rat	Isoproterenol-induced HCM	
2A	Increased	Rat	Hypertrophy (aortic constriction)	Yanaka et al. (2003)
		Human	DCM, ICM, but not in AS	Mehel et al. (2013)
		Dog	Pacing-induced HF	
		Rat	Isoproterenol-induced HCM	
3A	Decreased	Human	DCM	Ding et al. (2005)
		Dog	Pacing-induced HF	Smith et al. (1997)
5A	Increased	Human	DCM, ICM	Pokreisz et al. (2009); Lu et al. (2010); Shan et al. (2012); Nakano et al. (2016)
		Human	RV hypertrophy, DCM, AS	Nagendran et al. (2007); Vandenwijngaert et al. (2013) Shan et al. (2012)
		Mouse	TAC-induced HCM	Lu et al. (2010); Vandenwijngaert et al. (2013)
9A	Increased	Human	DCM, HF, and AS	Lee et al. (2015)
		Mouse	TAC-induced HCM	

ISO isoproterenol, *AII* angiotensin II, *TAC* transaortic constriction, *HCM* hypertrophic cardiomyopathy, *DCM* dilated cardiomyopathy, *ICM* ischemic cardiomyopathy, *RV* right ventricle, *AS* aortic stenosis, *HF* heart failure

sarcoplasmic reticulum (Beca et al. 2013). Broad PDE3 inhibition can also stimulate arrhythmias by excessive calcium entry and internal release from the SR coupled to both PKA and Epac2-CamKII activation (Bobin et al. 2016). PDE3 also influences cellular apoptosis as revealed in studies of myocardial infarction. In this condition, PDE3A inhibition can damage the heart by increasing activity of ICER (inducible cAMP early repressor) to suppress Bcl2 expression and promote apoptosis (Ding et al. 2005; Yan et al. 2007). Enhancing PDE3A expression is cardioprotective against ischemia-reperfusion (Oikawa et al. 2013). However, the opposite holds for PDE3B, whose gene deletion is protective against cardiac

Table 3 In vivo myocardial effects of pharmacologic or genetic PDE interventions

PDE family	Intervention	Model/patient/ clinical trial	Outcome	References
1A	*Preclinical*			
	IC86340	Pressure overload (TAC) in mouse	Inhibition attenuated TAC-induced hypertrophy	Miller et al. (2009)
	IC86340	Myocardial infarction in mouse	Inhibition reduced collagen deposition and myofibroblast formation	Miller et al. (2011)
2A	*Preclinical*			
	–	Hypertrophy (stressor: isoprenaline) in rat	PDE2A upregulation; β-AR desensitization	Mehel et al. (2013)
	BAY 60-7550	Pressure overload (TAC) in mouse	Inhibition attenuated hypertrophy and fibrosis	Zoccarato et al. (2015)
3A	*Preclinical*			
	Genetic deletion	PDE3A$^{-/-}$ and 3B$^{-/-}$ mouse	Greater baseline heart rate in 3A$^{-/-}$ with normal contractility	Sun et al. (2007)
	Genetic deletion	Ischemia in PDE3B$^{-/-}$ mouse	Protection against infarction	Chung et al. (2015)
	Milrinone	PDE3A$^{-/-}$ and PDE3B$^{-/-}$ mice	PDE3A, but not 3B, associates with SERCA2A and phospholamban at the SR to mediate inotropic effects	Beca et al. (2013)
	Milrinone	Myocardial infarction in PDE3A1 overexpressing mouse	Hypertrophic hearts with β-AR desensitization in transgenic hearts; inhibition attenuated the beneficial protection against infarction and apoptosis in overexpressing mouse	Oikawa et al. (2013)
	Clinical			
	Milrinone	Mild to moderate HF patients in sinus rhythm	Slight improvements in exercise tolerance; increased supraventricular arrhythmias and sinus tachycardia	di Bianco et al. (1989)

(continued)

Table 3 (continued)

PDE family	Intervention	Model/patient/ clinical trial	Outcome	References
PDE3A	Milrinone	PROMISE trial	Long-term treatment did not improve cardiac function; instead increased mortality, hypotension, palpitations, and syncope	Packer et al. (1991)
	Milrinone	OPTIME-CHF	Short-term treatment did not shorten hospitalization or abate symptoms; new arrhythmias and significant hypotension	Cuffe et al. (2002)
	Enoximone	ESSENTIAL trial	Long-term treatment with lowered dose did not improve cardiac function; no increased adverse effects	Metra et al. (2009)
5A	*Preclinical*			
	Overexpression	Myocyte targeted PDE5A overexpressing mouse	Worsened cardiac hypertrophy and/or chamber dysfunction and dilation from pressure overload or myocardial infarction	Pokreisz et al. (2009); Zhang et al. (2010)
	Sildenafil	TAC-induced HCM in WT, RGS2 KO, GFPdgn TG mouse	Treatment blunted hypertrophy and protected cardiac function in each respective mouse model	Takimoto et al. (2005a); Takimoto et al. (2009); Ranek et al. (2013)
	Sildenafil	DMD mouse model	Treatment ameliorated and reversed depressed cardiac function	Adamo et al. (2010)
	Sildenafil	Hypertensive mongrel dogs	Increase in LV diastolic capacitance; B-type natriuretic peptide (BNP) stimulation amplified this effect	Bishu et al. (2011)
	Tadalafil	DMD dog model	One-month treatment slowed cardiac remodeling and dysfunction	Hammers et al. (2016)
	Sildenafil	DMD mouse model	Chronic treatment prevented DMD-associated hypertrophy and cardiac dysfunction	Seo et al. (2014)

(continued)

Table 3 (continued)

PDE family	Intervention	Model/patient/ clinical trial	Outcome	References
PDE5A	*Clinical*			
	Sildenafil	DCM patients with PH	Three-month treatment enhanced cardiac output and Vo_2, improving exercise capacity and quality of life	Lewis et al. (2007)
	Sildenafil	DCM patients	Three-month treatment improved LV function and exercise oscillatory ventilation	Murphy et al. (2011)
	Sildenafil	Patients with LV diastolic dysfunction and PH	Mean pulmonary artery pressure lowered; RV function and LV relaxation improved	Guazzi et al. (2011)
	Sildenafil	T2DM patients with diabetic cardiomyopathy	Mild improvements in cardiac strain and torsion	Giannetta et al. (2012)
	Sildenafil	RELAX trial	Long-term treatment yielded no improvements	Redfield et al. (2013)
	Udenafil	ULTIMATE trial	Exercise capacity and subjective functional capacity improved	Kim et al. (2015)
	Sildenafil	DMD/BMD patients with cardiomyopathy	Six-month treatment worsened cardiac dilation	Leung et al. (2014)
9A	*Preclinical*			
	Genetic deletion	Hypertrophy (stressor: TAC) in PDE9A$^{-/-}$ mouse	Protection against hypertrophy and fibrosis	Lee et al. (2015)
	PF-04449613	TAC-induced HCM in mouse	Inhibition attenuated cardiac hypertrophy, remodeling, and function	

TAC transaortic constriction, *KO* knockout, *HCM* hypertrophic cardiomyopathy, *RGS2* regulator of G-protein substrate 2, *GFPdgn* green fluorescent protein degron CL1 (a ubiquitin proteasome substrate), *TG* transgenic, *DMD* Duchenne muscular dystrophy, *BMD* Becker muscular dystrophy, *Vo_2* maximal oxygen uptake, *LV* left ventricle, *RV* right ventricle, *T2DM* type 2 diabetes mellitus

ischemia/reperfusion injury (Chung et al. 2015). Summary signaling is shown in Fig. 1.

Given the potential for PDE3 inhibition to improve myocardial hemodynamics by enhancing contractility while also dilating veins and arteries to reduce cardiac load, it became the first PDE targeted for inhibition to treat heart failure (HF). While acute responses appeared promising, trials with sustained PDE3 inhibition

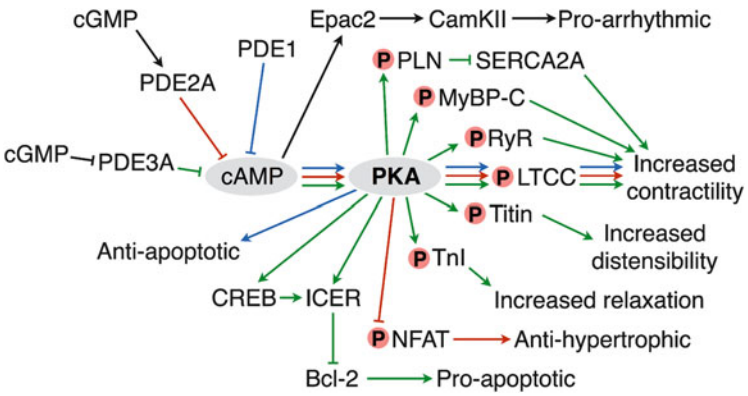

Fig. 1 Signaling pathways and their myocardial effects coupling PDE-cAMP modulation with the activation of protein kinase A, Epac, and their downstream effectors. Pathways for which published data have defined a link between a given PDE and specific downstream effects have been color coded: PDE1 in *blue*, PDE2A in *red*, and PDE3A in *green*

increased adverse events and mortality in HF patients (Cuffe et al. 2002; DiBianco et al. 1989; Metra et al. 2009; Packer et al. 1991). DiBianco et al. compared milrinone (10 mg/qid) to the cardiac glycoside digoxin (DiBianco et al. 1989) but found no added benefit over digoxin. In the pivotal 6-month PROMISE study (The Effects of Oral Milrinone on Mortality in Severe Chronic Heart Failure), Packer et al. found no improvement in heart function over placebo (Packer et al. 1991) but rather increased arrhythmia, hypotension, and greater mortality. The subsequent OPTIME-CHF (Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure) study also reported no clinical benefit over placebo (Cuffe et al. 2002). Despite this, short-term PDE3 inhibition remains in use for acute decompensated HF.

Several conclusions evolved from these studies. One was that doses yielding substantive acute inodilator responses were probably too high to avoid chronic toxicity. Another was that interventions substantively increasing intracellular calcium transients may pose arrhythmogenic risks. As these trials predated the era of broad β-adrenergic blockade use, some hypothesized that PDE3 inhibition might be safe if combined with such therapy. This was tested in the ESSENTIAL trials (Studies of Oral Enoximone Therapy in Advanced HF), examining enoximone combined with background β-blockade (Metra et al. 2009). Unlike the earlier studies, enoximone did not worsen mortality; however, it also did not improve symptoms or exercise capacity.

Despite this history, interest remains in modulating PDE3 (Movsesian et al. 2011). This has evolved in part from recent data that isoform-specific targeting might enhance beneficial effects while avoiding toxicity. For example, PDE3A rather than PDE3B is required to enhance myocyte contractility and calcium cycling (Beca et al. 2013; Sun et al. 2007). However, mice lacking PDE3B also

show protection against ischemic reperfusion, so selective targeting only of the PDE3A isoform would seem undesirable (Chung et al. 2015). However, splice variants PDE3A1 and A2 also differ, with unique phosphorylation responses to PKA and PKC (Vandeput et al. 2013) due to specific protein-protein interactions. Thus, one approach being explored is to express a disrupting peptide to interfere with a specific isoform protein complex to dislocate the PDE from its normal effectors without impacting the other isoform. If successful, this approach might avoid unwanted effects from nonselective inhibitors.

4 PDE5 and Dilated Cardiomyopathy

PDE5A was the first cGMP-selective isotype discovered. Its esterase activity is stimulated both by cGMP binding to regulatory GAF domains in the N-terminus and by cGK1 phosphorylation at S92 (Corbin et al. 2000; Francis et al. 2002; Rybalkin et al. 2003), creating a negative feedback loop. Immunocytochemical evidence has shown that PDE5A localizes to the Z-disk in the cardiomyocyte, but this can become diffuse in mammalian models of hypertrophy and heart failure (Takimoto et al. 2005a; Zhang et al. 2008) and in the absence or suppression of nitric oxide synthase III (Nagayama et al. 2008; Takimoto et al. 2005a). At the Z-disk, it predominately targets cGMP generated by the nitric oxide-soluble guanylyl cyclase (NO-sGC) pathway, having only minimal impact on natriuretic peptide (NP)-stimulated cGMP pools (Fischmeister et al. 2006; Lee et al. 2015; Takimoto et al. 2005a).

PDE5A is expressed at very low levels in the normal myocardium but is upregulated in dilated cardiomyopathy (DCM) (Andersen et al. 2012; Shan et al. 2012) and in RV hypertrophy associated with pulmonary hypertension (Nagendran et al. 2007). There remains some controversy regarding PDE5A expression in the human heart, based primarily on differences in immunoblot data (Degen et al. 2015). As gene deletion models – even conditional ones – have not been successful, absolute proof of the role of normal PDE5A in the heart in vivo remains indirect. However, myocyte-specific overexpression models have shown that PDE5A upregulation worsens the consequences of pressure overload (Zhang et al. 2010) and myocardial infarction (Pokreisz et al. 2009) and that subsequent genetic suppression of the same gene is sufficient to reverse pathological hypertrophy/fibrosis induced by pressure overload (Zhang et al. 2010).

In experimental models, PDE5A inhibition stimulates cGK1 activity to suppress multiple cardiac signaling pathways engaged in pathological hypertrophy and HF. This includes blockade of calcineurin/NFAT signaling (Takimoto et al. 2005b), its activation of regulators of G-protein signaling (RGS2/4) to block Gq-activated cascades (e.g., from angiotensin or endothelin-1) (Takimoto et al. 2009), inhibition of transient receptor potential canonical ion channel type 6 (TRPC6) (Seo et al. 2014), improvement of proteasome degradation of misfolded proteins (Ranek et al. 2013), enhanced mitochondrial and consequent cytoprotection against ischemic injury linked to glycogen synthesis kinase 3-β

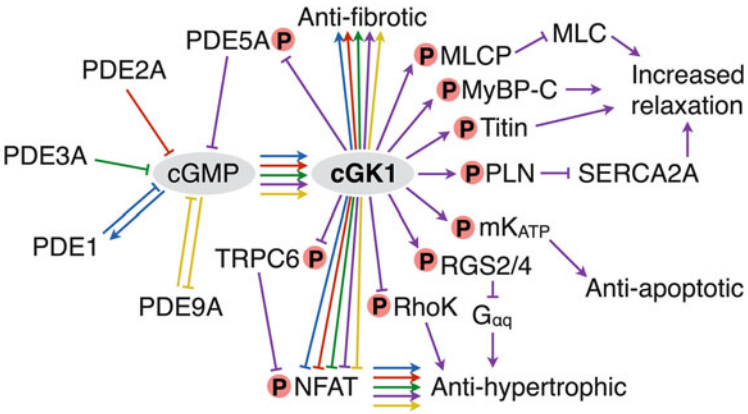

Fig. 2 Signaling pathways and their myocardial effects coupling PDE-cGMP modulation with cyclic GMP-activated protein kinase (cGK1) and their downstream effects. Pathways that regulate cardiac cGMP signaling. Pathways for which published data has defined a link between a given PDE and specific downstream effects have been color coded: PDE1 in *blue*, PDE2A in *red*, PDE5A in *purple*, and PDE9A in *yellow*

and mitogen-activated kinase ERK1/2 (Das et al. 2008), and other mechanisms. cGK1 activation can also improve diastolic function by phosphorylating titin to increase distensibility (Bishu et al. 2011). Figure 2 summarizes these PDE5A effects on cGMP/cGK1 signaling.

The clinical effects of PDE5A inhibition on dilated HF have been principally studied in patients who also had pulmonary hypertension, as the latter is an approved therapeutic target for PDE5A inhibitors. Lewis et al. (Lewis et al. 2007) tested 12 weeks of sildenafil therapy and found that peak oxygen consumption and cardiac output were enhanced, and coupled to improved exercise capacity and quality of life. Similar results were observed by others (Behling et al. 2008; Kim et al. 2015), including data showing PDE5A inhibition improves LV function and exercise oscillatory ventilation, the latter a clinical index of DCM outcome (Murphy et al. 2011). The extent to which direct myocardial effects underlie benefit from PDE5A inhibition in DCM remains speculative. Systemic arterial vasodilation is minimal, so LV unloading is unlikely, but PDE5A inhibition can dilate small arteries that may enhance skeletal muscle oxygenation (Ghofrani et al. 2004). As of now, no definitive multicenter trial of PDE5A inhibition in DCM has been conducted. A pivotal >2000 patient NIH-sponsored trial (PITCH-HF) was initiated in November 2013, but then terminated 4 months later due principally to budget constraints.

Another form of DCM for which PDE5A inhibition has been studied is Duchenne muscular dystrophy. This disease is caused by missense mutations in the dystrophin gene that results in full disruption of the dystro-sarcoglycan complex essential to normal skeletal and cardiac muscle function and survival. Duchenne patients develop near total loss of skeletal motor function by their early teens, and

heart failure generally follows. The dystro-sarcoglycan complex also anchors neuronal NOS at the plasma membrane of skeletal muscle (Brenman et al. 1995), and its absence leads to disrupted NO signaling that is thought to depress microvascular flow leading to ischemia (Kobayashi et al. 2007; Loufrani et al. 2002). The impact on NOS dyslocalization in cardiomyocytes is less clear, though cGMP signaling appears to be depressed and its activation may be therapeutic. Seo et al. (Seo et al. 2014) reported hyperactive mechano-stimulated force, intracellular calcium transients, and associated arrhythmia in myocytes from a DMD mouse model that were all potently suppressed by cGMP activation. This effect was also achieved by chronic PDE5A inhibition that further improved in vivo cardiac function and reduced hypertrophy. The latter data support studies reported from mouse and canine models of DMD (Adamo et al. 2010; Albert et al. 2012).

Human trials have shown that acute PDE5A inhibition improves microvascular flow in skeletal muscle to match contractile stress in Duchenne patients (Nelson et al. 2014). However, when tested in non-ambulatory adults with advanced Duchenne skeletal and cardiac disease, 6 months of sildenafil therapy did not improve heart function (Leung et al. 2014). Furthermore, a recently completed international multicenter trial of tadalafil in ambulatory boys with Duchenne and no cardiac disease also failed to improve motor capacity (Clinical trial NCT01359670). This could indicate that PDE5A is not the right target for improving cGMP signaling but that modulating cGMP synthesis or using other PDE inhibitors might be successful. It may also require some gene correction in addition to any modulation of cGMP signaling to achieve benefits.

5 PDE5A and Non-dilated Heart Disease

About half of all patients with heart failure symptoms have ventricles that do not dilate but rather present with an ejection fraction in the normal range. This is often termed heart failure with a preserved EF (HFpEF), a multifactor syndrome that has proven difficult to treat, with no current specific evidence-based therapy (Shah et al. 2016). While HFpEF implies a central role for heart disease, the reality is more complex as many other organ pathophysiologies contribute, including from the lung, kidney, skeletal muscle, neuroregulatory systems, and adipose tissue. Common comorbidities are type 2 diabetes, obesity, hypertension, cardiac hypertrophy, pulmonary hypertension, inflammatory disease, and renal insufficiency (Shah et al. 2016).

Since cGK1 activation can reduce hypertrophy, fibrosis, and PH, while improving metabolism and potentially renal function, it has been an attractive strategy for treating HFpEF. In patients with LV diastolic dysfunction and PH, chronic sildenafil treatment lowered mean pulmonary artery pressure and improved RV function and LV relaxation (Guazzi et al. 2011). A subsequent study, however, found no benefit on either invasive hemodynamics or exercise performance (Hoendermis et al. 2015). More convincingly, a larger 6-month multicenter trial of the same drug in HFpEF patients also reported no benefits over placebo (Redfield et al. 2013).

However, the patient cohort for this study included few subjects with PH, and a majority lacked LV hypertrophy and many did not have diastolic dysfunction. Prior animal data have shown that the influence of PDE5A inhibition to counter pressure overload-induced cardiac disease requires the presence of sufficient maladaptive remodeling so that cGK1 has something useful to suppress (Nagayama et al. 2009). Perhaps more importantly, other studies have found human HFpEF myocardium contains very little cGMP and has low cGK1 activity, neither being attributable to elevated PDE5A activation (van Heerebeek et al. 2012). This makes it less likely that PDE5A inhibition could have much impact.

HFpEF particularly affects older women, and reduced NO signaling consequent to menopause may provide another reason for little impact from PDE5A inhibition. In women, the estrogen receptor couples to NOS-dependent cGMP synthesis via a non-transcriptional signaling pathway (Haynes et al. 2000; Leung et al. 2007). As this NO-derived cGMP is the primary target of PDE5A, its decline postmenopause may limit the efficacy of PDE5A inhibition. Consistent with this hypothesis, Sasaki et al. (Sasaki et al. 2014) found that female mice with Gαq overexpression- or pressure overload-induced heart disease responded favorably to PDE5A inhibition, but this benefit was lost if the mice underwent ovariectomy. The efficacy of PDE5A inhibition was restored if exogenous estrogen was subsequently provided. This may have contributed to differences in results from the two hemodynamic studies, with negative data coming from mostly female patients (>75%) (Hoendermis et al. 2015) and positive results from mostly males (Guazzi et al. 2011).

Lastly, PDE5A inhibition has been studied in patients with type 2 diabetes and evidence of ventricular dysfunction but no heart failure symptoms. Chronic sildenafil treatment improved some indexes of LV contraction and morphology (Giannetta et al. 2012). Additional studies in this disease have not yet been reported. Further, whether this relates to myocardial, vascular, or potentially metabolic effects such as improved insulin sensitivity (Ho et al. 2014; Ramirez et al. 2015) from PDE5A inhibition remains unclear.

6 Potential Roles for PDE1, PDE2, and PDE9 in Heart Failure

6.1 PDE1

The PDE1 family is encoded by three genes: *PDE1A*, *PDE1B*, and *PDE1C*. PDE1A is selective for cGMP, whereas PDE1B and PDE1C display balanced substrate selectivity. All three isoforms are expressed in human myocardium (Table 1), with PDE1C the predominant ventricular isoform (Loughney et al. 1996; Lukowski et al. 2010; Miller et al. 2009; Vandeput et al. 2007). By contrast, PDE1A is the predominant isoform in rat and mouse heart (Miller et al. 2009). All three require activation by calcium/calmodulin making them of interest in cardiac stress conditions. In rodents, PDE1A expression is pathologically upregulated in myocytes and fibroblasts in response to Gq-coupled agonists (e.g., angiotensin II) and by myocardial stress such as infarction (Miller et al. 2011), pressure overload,

and chronic isoproterenol infarction (Miller et al. 2009). Broad PDE1 inhibition was effective in vitro in suppressing myocyte hypertrophy and fibroblast activation and in vivo against isoproterenol infusion (Fig. 1). While it is likely PDE1A is engaged in these behaviors, PDE1A knockout mice remain yet to be reported. PDE1C global knockout mice have been generated and studied for their role in the olfactory system (Cygnar and Zhao 2009). Preliminary data from these mice has revealed protection against pressure overload hypertrophy and apoptosis (Knight et al. 2014). The mechanism for the former remains unclear, while the latter appears related to cAMP rather than cGMP signaling. Broad PDE1 inhibitors have already been developed for human use and tested in patients with schizophrenia (Heckman et al. 2015). Cardiovascular studies of the hemodynamic and direct myocardial effects of nonselective PDE1 inhibitors in larger mammals are also ongoing, and clinical studies may follow.

6.2 PDE2A

PDE2A is a dual-substrate enzyme which provides important cyclic nucleotide cross talk as it is activated by cGMP binding to regulatory GAF domains to enhance hydrolysis of cAMP (Martins et al. 1982). PDE2A is encoded by a single gene giving rise to three N-terminal variants (Rosman et al. 1997). PDE2A3 is expressed in human and found in cardiomyocytes and vascular endothelial cells (Sadhu et al. 1999). Zaccolo et al. reported PDE2A modifies myocyte responses to β-adrenergic co-stimulation (Mongillo et al. 2006; Vandecasteele et al. 2001), controlling localized PKA-II at the plasma membrane in a cGMP-regulated manner (Zoccarato et al. 2015). Both NP- and NO-stimulated cGMP pathways are linked to PDE2A activation in quiescent adult and neonatal cardiac myocytes (Fischmeister et al. 2006); however, the physiological role cGMP hydrolysis in vivo remains unclear.

As with most of the other PDEs, PDE2A expression rises in the failing heart. Hypoxia increases PDE2A expression and activity through HIF1α and TNF-α signaling in cultured human umbilical vein endothelial cells (Chen et al. 2016). Myocardial expression is increased in rat hypertrophy (Mehel et al. 2013; Yanaka et al. 2003) and in canine pacing-induced HF models (Mehel et al. 2013). Similar increases are reported in human ischemic or nonischemic dilated HF, but not in hypertrophy (Mehel et al. 2013). PDE2A overexpression in isolated rat myocytes reduces L-type calcium current and corresponding calcium transients and sarcomere shortening following β-adrenergic stimulation. Chronic upregulation depresses hypertrophy induced by sustained β-adrenergic stimulation (Mehel et al. 2013), suggesting its activation is beneficial.

An alternative view was reported by Zoccarato et al. (Zoccarato et al. 2015), who found PDE2A *inhibition* suppresses norepinephrine-stimulated hypertrophy in the rat. Here, the mechanism was linked to an increase in cAMP-PKA signaling pathway that in turn increased NFAT phosphorylation. This curtailed the nuclear translocation of NFAT and downstream pro-hypertrophic signaling. Both studies

reported regulation of cAMP by PDE2A amplified by cGMP, but their differences may relate to the precise conditions and local signaling linked to the stress trigger and cyclic nucleotide cross talk. For instance, computer modeling (Zhao et al. 2016) predicts PDE2A hydrolyzes increasing amounts of cAMP as β-AR stimulation is enhanced and hydrolyzes more cGMP at low levels of NO stimulation. Differences in cyclic nucleotide regulation, such as relocalization of PDE4 and targeted stimulation of PKA isoform subtypes, are also induced by sustained adrenergic stimuli and could alter net PDE2A responses (Fields et al. 2016). Figure 2 summarizes this signaling.

Therapeutic targeting of PDE2A in the intact myocardium poses further challenges in that its expression in myocytes and vascular endothelial cells may present opposing effects. For example, Chen et al. (Chen et al. 2016) revealed that NPs released after myocardial infarction increase endothelial permeability to amplify the post-injury inflammatory response. This was related to endothelial-specific NP receptor-guanylyl cyclase activation, though not to cGMP-activated cGK1 regulation. Rather, the newly generated cGMP stimulated PDE2A activity, further potentiating the esterase's hypoxia-induced expression; this in turn lowered cAMP to promote endothelial leakiness. Thus, blockers of PDE2A might concomitantly reduce postischemic inflammation via endothelial-targeted activity, while increasing adrenergic-stimulated contractility but also suppressing hypertrophic signaling in the myocyte.

6.3 PDE9A

PDE9A is the most selective for cGMP from all the 11 species (Fisher et al. 1998; Soderling et al. 1998) and has been clinically developed and tested for its potential to treat cognitive disorders such as Alzheimer's and schizophrenia (Duinen et al. 2015). It is expressed most prominently in the brain (though still at low levels) but also in the gut, kidney, and heart. Unlike PDE1, PDE2, or PDE5, there are presently no known mechanisms regulating its hydrolytic activity. The wide range of PDE9A N-terminal splice variants are instead likely to impact subcellular targeting (Wang et al. 2003). Lee et al. reported PDE9A is also upregulated in diseased myocardium, showing protein expression increases in humans, in both dilated HF and HFpEF (Lee et al. 2015). In mice subjected to pressure overload, PDE9A deletion or its pharmacological inhibition led to improved LV function and reduced fibrosis and hypertrophy. Importantly, PDE9A regulation of cGMP is distinct from PDE5A, in that the former selectively hydrolyzes cGMP derived from NP stimulation. This was confirmed in neonatal and adult cardiomyocytes and the intact heart (Fig. 2). While inhibition of each selective PDE was linked to cGK1 activation, the protein targeting of the kinase as well as impact on transcriptional regulators as deduced by non-biased phospho-proteomics was distinct (Lee et al. 2015). These results have potentially significant therapeutic implications as NOS declines in many HF patients (Umar and van der Laarse 2010) and, as mentioned, is compromised in women by the decline in estrogen postmenopause.

Targeting PDE9A inhibition may circumvent obstacles posed by depressed NO-dependent signaling.

7 Beyond Single Small-Molecule PDE Suppression

Advances in biochemical and cloning techniques helped unveil the structural similarities as well as differences among PDE species and facilitated small-molecule design for highly potent and selective inhibitors. Still, our capacity to influence isoform subtypes remains poor, while data increasingly shows how this is central to their intracellular regulation. The concept of protein complex disruption for the cAMP signalosome may be easier to achieve given the existence of A-kinase anchoring proteins (AKAPs) (Esseltine and Scott 2013; Nygren and Scott 2015). These proteins act as coordinators of a cAMP-cyclase, targeted effector kinase, and regulatory PDEs to provide microdomain control. Equivalent proteins for cGMP-kinase signaling have not been found, and this may make it more difficult to target a specific cGK1 pool.

Another issue is whether targeting a single PDE is the optimal approach or whether strategic combinations may prove more effective. For example, both PDE5A and PDE9A regulate cGMP, but the results showing different proximal triggers and shared as well as different downstream effectors might raise the potential for combined efficacy. Synergy between PDE3 and PDE4 inhibition has been reported in myocytes (Mika et al. 2013). A particularly striking example of this synergy was reported in a study of steroid production (Shimizu-Albergine et al. 2012) in testicular Leydig cells, where inhibition of PDE4 or PDE8 alone resulted in modestly elevated testosterone production, but their combination increased this 100-fold.

8 Conclusion

Since their discovery over 50 years ago, the importance of PDEs for fine-tuning localized second messenger signaling has been increasingly recognized. While much structural chemistry and pharmacology is already established, our understanding of subcellular physiology and full therapeutic potential has lagged. Novel approaches beyond single-target small-molecule inhibitors, combining stimulation with hydrolytic suppression, using dual PDE suppression, or disrupting protein complexes to target specific isoforms, may all ultimately change the therapy landscape. New work with several PDEs previously unbeknown for relevance to heart disease has already opened up new territory and opportunities. Thus, while the PDE field has in many ways matured, its impact on human disease remains to be fully leveraged, and exciting times lie ahead.

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Steroidal and Novel Non-steroidal Mineralocorticoid Receptor Antagonists in Heart Failure and Cardiorenal Diseases: Comparison at Bench and Bedside

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_76

Abstract

Characterization of mice with cell-specific deletion or overexpression of the mineralocorticoid receptor (MR) shed a new light on its role in health and disease. Pathophysiological MR activation contributes to a plethora of deleterious molecular mechanisms in the development of cardiorenal diseases like chronic kidney disease (CKD) and heart failure (HF). Accordingly, the available steroidal MR antagonists (MRAs) spironolactone (first generation MRA) and eplerenone (second generation MRA) have been shown to be effective in reducing cardiovascular (CV) mortality and morbidity in patients with chronic HF and a reduced left ventricular ejection fraction (HFrEF). However, they remain underutilized, in large part owing to the risk inducing severe adverse events including hyperkalemia and worsening of kidney function, particularly when given on top of inhibitors of the renin angiotensin system (RAS) to patients with concomitant kidney dysfunction. Novel, potent, and selective non-steroidal MRAs (third generation) were identified in drug discovery campaigns and a few entered clinical development recently. One of these is finerenone with different physicochemical, pharmacokinetics, and pharmacological properties in comparison with the steroidal MRAs. Available data from five clinical phase II trials with finerenone in more than 2,000 patients with HF and additional CKD and/or diabetes as well as in patients with diabetic kidney disease demonstrated that neither hyperkalemia nor reductions in kidney function were limiting factors to its use. Moreover, finerenone demonstrated a nominally improved outcome compared to eplerenone in a phase IIb trial with 1,066 patients with HFrEF and concomitant type 2 diabetes mellitus (T2DM) and/or CKD.

Keywords

Chronic kidney disease • Eplerenone • Finerenone • Heart failure • Mineralocorticoid receptor antagonists • Spironolactone

Abbreviations

11β-HSD2	11β-Hydroxysteroid dehydrogenase type 2
ACEI(s)	Angiotensin converting enzyme inhibitor(s)
AGP	Alpha-1 acid glycoprotein
AngII	Angiotensin II
ARB	Angiotensin receptor blocker
BID	Bis in die (twice daily)
BNP	B-type natriuretic peptide
CCB	Calcium channel blocker
CHF	Chronic heart failure

CI	Confidence interval
CKD	Chronic kidney disease
COX-2	Cyclooxygenase-2
CTGF	Connective tissue growth factor
CV	Cardiovascular
DHP	Dihydropyridine
DKD	Diabetic kidney disease
DM	Diabetes mellitus
DN	Diabetic nephropathy
DOC	Desoxy-corticosterone
DOCA	Desoxy-corticosterone acetate
ECM	Extracellular matrix
eGFR	Estimated glomerular filtration rate
ENaC	Epithelial sodium channel
ER	Estrogen receptor
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HHF	Hospitalization for heart failure
HR	Hazard ratio
ICAM1	Intercellular cell adhesion molecule 1
Ito	Potassium transient outward current
K^+	Potassium
L-NAME	NG-nitro-L-arginine methyl ester
LV	Left ventricular
LVSD	Left ventricular systolic dysfunction
MCP-1	Macrophage chemoattractant protein-1
MR	Mineralocorticoid receptor
MRA	Mineralocorticoid receptor antagonist
Na^+	Sodium
Nedd4-2	Neural precursor cell expressed developmentally down-regulated protein 4-2
NT-proBNP	N-terminal of prohormone of BNP
OD	Once daily
PAI-1	Protein plasminogen activator inhibitor-1
PICP	Procollagen type I carboxy-terminal peptide
PIIINP	Procollagen type III amino-terminal peptide
PINP	Procollagen type I amino-terminal peptide
RAAS	Renin angiotensin aldosterone system
RAR	Retinoic acid receptor
RAS	Renin angiotensin system
ROMK	Renal outer medullary potassium channel
ROS	Reactive oxygen species
RXR	Retinoid X receptor
SERM	Selective ER modulators

Sgk1	Serum glucocorticoid kinase1
SoC	Standard of care
SRC-1	Steroid receptor co-activator-1
T2DM	Type 2 diabetes mellitus
TGFβ	Transforming growth factor beta
Tnnt2	Troponin T type 2
UACR	Urinary albumin to creatinine ratio
VCAM1	Vascular cell adhesion molecule 1
VSMC	Vascular smooth muscle cell

1 Introduction

Heart failure (HF) is a chronic, complex, and progressive syndrome characterized by a decline in cardiac output and/or elevated intracardiac filling pressures resulting in typical symptoms (e.g., breathlessness, ankle swelling, and fatigue) and signs (e.g., elevated jugular venous pressure, pulmonary crackles). Approximately 1–2% of the adult population in developed countries is suffering from HF, with the prevalence rising to $\geq$10% among persons 70 years of age or older (Mosterd and Hoes 2007). HF is the leading cause of hospitalization among adults (McCullough et al. 2002) and despite medical advances in the treatment of chronic HF over the last two decades, mortality remains high (Maggioni et al. 2013). Once hospitalized for HF exacerbation, the rate of mortality and re-admission in early post discharge within 90 days is as high as 8.6% and 29.6%, respectively (O'Connor et al. 2008). The majority of HF patients have a history of cardiovascular (CV) and non-CV comorbidities such as diabetes mellitus (DM) and chronic kidney disease (CKD) (Cleland et al. 2003) which are independent predictors for mortality and morbidity. DM and CKD are also risk factors for the development of hyperkalemia (Johnson et al. 2010) and renal failure, important adverse effects linked to the use of blockers of the renin–angiotensin–aldosterone system (RAAS) which are standard of care (SoC) among patients with HF.

Aldosterone has a well-documented role in the pathophysiology of CV diseases (Bauersachs et al. 2015; Lother et al. 2015; Jaisser and Farman 2016). HF is characterized by high plasma levels of aldosterone (Swedberg et al. 1990), which in turn are associated with reduced functional capacity (Cicoira et al. 2002) as well as CV mortality or the development of HF in postinfarction patients (Rouleau et al. 1994). Apart from its direct renal effects on sodium retention and potassium excretion, aldosterone exerts several other extra-renal effects that significantly contribute to the pathogenesis of HF. MR overactivation within the heart might cause coronary endothelial dysfunction, myocardial apoptosis, and reactive myocardial fibrosis and thus contribute to cardiac remodeling and its adverse consequences. Accordingly, blockade of MR has proven efficacy in patients with HF with reduced ejection fraction (HFrEF), arterial hypertension, and CKD (Bauersachs et al. 2015; Schwenk et al. 2015). However, current attempts to

block aldosterone's action at the MR by using the available steroidal MRAs spironolactone or eplerenone is a double-edged sword: while these drugs could be a life-saving therapy for patients with HFrEF they may also induce severe hyperkalemia and kidney dysfunction, particularly when given on top of SoC such as angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) to "real life" patients typically with variable degrees of concomitant kidney dysfunction. In fact, hyperkalemic episodes were reported in up to 36% among unselected elderly HF outpatients on spironolactone therapy with about 10% developing potential life-threatening hyperkalemia (>6 mmol/L) (Svensson et al. 2004; Dinsdale et al. 2005).

Accordingly, the demand for novel, potent, and selective, non-steroidal MRAs with a reduced risk for developing hyperkalemia initiated drug discovery programs within several pharmaceutical companies aiming for identification of novel non-steroidal MRAs with potentially different pharmacodynamics properties.

Here we review the consequences of extra-renal MR overactivation, important available pharmacological data of steroidal and novel non-steroidal MRAs and key clinical trial results with MRAs being investigated in different HF populations also highlighting differences of steroidal MRAs versus the novel non-steroidal MRA finerenone.

2 Pathophysiology of MR Overactivation

2.1 Aldosterone and MR

Simpson and Tait (1953) identified a substance of much higher potency than any previously known adrenal steroid in affecting electrolyte excretion in adrenal extracts which they designated as "electrocortin." Only a year later, the chemical structure of electrocortin was resolved (Simpson et al. 1954) and the crystalline substance was re-named aldosterone. Aldosterone is synthesized in the *Zona glomerulosa* of the adrenal cortex and its physiological role was long believed to be mainly the regulation of electrolyte homeostasis and control of blood pressure. Synthesis and release of aldosterone are stimulated by low sodium or high potassium levels in the plasma or by direct stimulation via angiotensin II. Aldosterone promotes the retention of sodium and the loss of magnesium and potassium in physiological short term feedback loops. However, chronically elevated aldosterone release participates in the generation of vascular, myocardial, and renal fibrosis, and eventually end-organ damage, dysfunction, and failure (see below).

Aldosterone levels can be inappropriately elevated in patients with HF, despite the use of ACEIs, ARBs, and beta-blockers (Albaghdadi et al. 2011). Long-term RAS blockade with ACEIs and ARBs results in incomplete suppression of serum aldosterone levels, known as the "aldosterone escape" phenomenon (Staessen et al. 1981). Exploratory clinical studies have shown that in adults with CKD exhibiting "aldosterone escape," treatment with MRAs improves kidney outcomes such as proteinuria (Fritsch Neves and Schiffrin 2003). However, even in a

randomly selected sample of the general community, elevated plasma aldosterone levels are strongly associated with the metabolic syndrome and CV diseases including CKD (Buglioni et al. 2015). Aldosterone above the normal range is strongly associated with increased incidence of all-cause mortality (Buglioni et al. 2015). However, a recent clinical study has also proven beneficial effects of MRAs in patients with low serum aldosterone levels and the absence of an aldosterone escape phenomenon (Kim et al. 2014). The authors concluded that elevated serum aldosterone levels are probably not the crucial factor for the beneficial effects of MRAs.

The before mentioned actions of aldosterone via its receptor leading to differential gene expression are summarized as "genomic" effects of aldosterone. Besides these actions, the so-called non-genomic effects of aldosterone, e.g., very rapid effects like vasoconstriction have been described. However, almost all of these "non-genomic" effects cannot be blocked directly by the available MRAs spironolactone and eplerenone (Funder 2001).

In addition to its classical renal effects, aldosterone exerts several other extra-renal effects that significantly contribute to the pathogenesis of HF, e.g., inflammation, reactive myocardial fibrosis, and cardiac remodeling processes.

An association of the renal effects of aldosterone and related mineralocorticoids with extra-renal deleterious effects was described already in the 40th of the last century. Reichstein succeeded in purification and synthesis of desoxycorticosterone (DOC), an important precursor of aldosterone and MR agonist, from an extract of beef adrenals (Reichstein and von Euw 1938). Only 2 years later Thorn et al. successfully introduced subcutaneous or intramuscular injections of desoxycorticosterone acetate (DOCA) for regaining sodium and potassium homeostasis in patients with Addison's disease (Thorn et al. 1939). Darrow and Miller (1942) described already in 1942 cardiac lesions of myocardial fibers and their replacement by fibroblasts in rats following prolonged DOCA treatment. In the same year, Selye (1942), the discoverer of the general-adaptation syndrome as the body's response to stress, discovered that DOCA injections induce nephrosclerosis accompanied by cardiac hypertrophy in animals. Later, Selye described fibrinous pericarditis as well as infiltration of basophilic, polymorphonuclear cells into myocardial tissue after prolonged DOCA application to animals in his seminal work "The general adaptation syndrome and the diseases of adaptation" in 1946 (Selye 1946). In fact, he called the mineralocorticoids "prophlogistic" corticoids already in a period when research on aldosterone was basically focussed on the interplay between adrenal glands and renal sodium retention (Selye 1955).

Aldosterone acts via a specific nuclear hormone receptor, MR, which is mainly expressed in the aldosterone target organs, most importantly the heart, kidneys, and vessels. High expression of the MR was documented also in the colon and hypothalamus. From a cellular perspective, MR is expressed besides the renal epithelial cells in vascular smooth muscle cell, endothelial cells, fibroblasts, adipocytes, and immune cells like macrophages. MR belongs to the superfamily of nuclear hormone receptors, which translocate from the cytosol to the nucleus upon ligand binding (Arriza et al. 1987).

The best understood physiological role of MR is probably the regulation of sodium transport across the epithelia of the distal nephron by initiating either a signaling cascade controlling membrane insertion or, if blocked by an antagonist, degradation of the epithelial sodium channel (ENaC) (Fuller and Young 2005). The epithelial sodium transport controlled by MR is in fact the result of a complex interplay of transcriptionally induced target gene products, most importantly the serum glucocorticoid kinase (Sgk1), which deactivates the ubiquitin ligase Nedd4-2 by phosphorylation. Nedd4-2 controls ENaC degradation by the cellular proteasome system. In the presence of aldosterone Nedd4-2 activity is blocked by the transcriptionally induced activity of Sgk1, leading to proper membrane insertion of vesicular ENaC and resulting sodium transport. In contrast, when plasma aldosterone levels are low or MR is blocked by an antagonist vesicular ENaC will ultimately be degraded. The biological half-life of membrane-inserted ENaC is about 3.5 h (Yu et al. 2008). Therefore, major rate-limiting steps in this cascade are the resynthesis of ENaC and of MR.

Besides the regulation of transepithelial sodium transport, physiological activated MR also participates in the regulation of hydrogen and potassium efflux in renal cells. This can occur either passively via an electro-neutral response to the above described MR mediated sodium flux or, with spatial separation from sodium transport actively via direct transcriptional effects on Na^+/K^+-ATPase, renal outer medullary potassium channel (ROMK) and K^+-ATPase (Rogerson and Fuller 2000).

In contrast to the relatively well understood molecular mechanisms driven by aldosterone and MR in renal epithelial cells, the precise mechanisms by which MR stimulates inflammation, fibrosis, and myocardial collagen accumulation are not fully understood yet. Meyer and Nichols (1981) discovered MR expression in cultured smooth muscle cells and fibroblasts from rat aorta. Weber and colleagues later found a pivotal role of aldosterone in the regulation of collagen synthesis within the interstitium and adventitia of intramyocardial coronary arteries (Brilla et al. 1990). Rocha et al. (2002) identified vascular inflammation as a potential mechanism of aldosterone-mediated myocardial injury in rats receiving chronically aldosterone and a sodium load. They found in histopathological analysis of rat hearts severe coronary inflammatory lesions characterized by monocyte/macrophage infiltration, elevated cyclooxygenase-2 (COX-2), macrophage chemoattractant protein-1 (MCP-1), and osteopontin mRNA expression (Rocha et al. 2002). It could also be demonstrated that aldosterone induces the transcriptional expression of inflammatory cytokines, most importantly osteopontin-1 (Sugiyama et al. 2005) as well as the prothrombotic protein plasminogen activator inhibitor-1 (PAI-1) (Chun and Pratt 2005). The latter protein is involved in a complex cascade of collagen deposition and degradation processes leading to interstitial fibrosis.

Zannad et al. (2000) examined the relationship between serological markers of collagen turnover and mortality in patients with HF. They found that elevated concentrations of procollagen type III amino-terminal peptide (PIIINP), a biochemical marker of myocardial fibrosis, were associated with an increased risk of death.

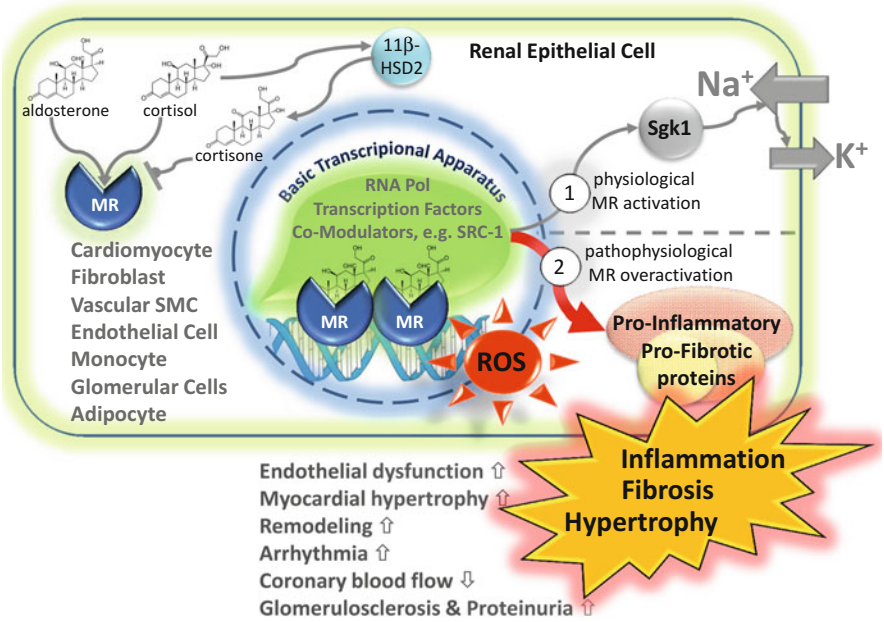


Fig. 1 Mode of action/consequences of MR overactivation. Schematic representation of the activities of intracellular MR: MR translocates into the nucleus upon binding of aldosterone or cortisol (but not of cortisone which is generated from cortisol by 11β-hydroxysteroid dehydrogenase, type 2 (11β-HSD2) in renal epithelial cells) in the cytoplasm of a target cell. An MR dimer binds to specific DNA elements in regulatory regions of target genes and interacts here with the basic transcriptional machinery including co-regulators as SRC-1. Depending on the overall situation of the intra- and extracellular milieu, MR may drive under physiological conditions transcriptional and according phosphorylation pathways via serum glucocorticoid kinase-1 (SGK1) in renal epithelial cells which enable appropriate sodium retention and potassium efflux (pathway 1). Conditions like elevated aldosterone release, high salt load, or increased generation of reactive oxygen species (ROS) may cause an MR overactivation with subsequent expression of pro-inflammatory and fibrotic proteins in the indicated cell types which ultimately lead to cardiovascular damage (pathway 2)

MR blockade significantly decreased procollagen type I amino-terminal peptide (PINP), procollagen type I carboxy-terminal peptide (PICP), and PIIINP (Zannad et al. 2000). These studies support the hypothesis that MR antagonism affects extracellular matrix turnover in humans.

Finally, it's worth mentioning also the recently discovered reciprocal cross-talk between adipose tissue and the adrenal gland which might be the basis of the epidemiological association between elevated aldosterone levels and the metabolic syndrome (for a review, see: Marzolla et al. 2012). A key cell type participating in this association seems to be the adipocyte since MR activation contributes to adipose tissue inflammation.

In summary, MR may drive under physiological conditions transcriptional pathways which enable appropriate sodium retention and potassium efflux (Fig. 1, pathway 1) while pathophysiological conditions like elevated aldosterone

release or aldosterone levels in the normal range combined with high salt load or increased generation of reactive oxygen species (ROS) may lead to an MR overactivation with subsequent expression of pro-inflammatory and fibrotic proteins in several different cell types which contribute to CV damage (Fig. 1, pathway 2). We briefly mention also glomerular cells like mesangial cells and podocytes here since they are important target cells for aldosterone which play a key role in several kidney diseases (for a review, see: Bertocchio et al. 2011).

Interestingly, cortisol has a similar affinity for the MR as aldosterone (Arriza et al. 1987). There is growing evidence that cortisol may also contribute to the progression of cardiac damage in HF via MR activation (Güder et al. 2007; Weir et al. 2011). The physiological plasma levels of cortisol are at least 100-fold higher than the respective aldosterone levels (Funder 1994). Under physiological circumstances, cortisol is prevented from binding to MR through conversion to its inactive metabolite, cortisone, via enzymatic activity of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) which is expressed in epithelial cells (Fig. 1). Because cardiomyocytes lack 11β-HSD2, cardiac MRs are probably occupied by cortisol in a tonic inhibitory fashion (Edwards et al. 1988; Funder et al. 1988; Funder 2004). However, in the context of tissue damage and generation of ROS, this inhibitory role of cortisol is transformed by the altered intracellular redox state, and cortisol may act as an MR agonist that mimics the physiological and pathophysiological effects of aldosterone (Funder 2004, 2005a; Nagata et al. 2006).

2.2 Pharmacology of MR Transgenic Animals

The MR is expressed in cardiomyocytes (Lombes et al. 1992) as well as in other cardiac cell types such as coronary endothelial and vascular smooth muscle cells (VSMC), fibroblasts and inflammatory cells like macrophages (Jaisser and Farman 2016). To assess the specific role of MR expressed in each cell type, a series of genetically modified mouse models have been generated.

2.2.1 MR in Cardiomyocytes

Overexpression of MR in cardiomyocytes induces major remodeling of cardiac ion channel expression and activity: the potassium transient outward current (Ito) is decreased while the L-type calcium current (I_{Ca}) activity is increased (Ouvrard-Pascaud et al. 2005). This prolongs the duration of the action potential and increases the frequency of early or delayed depolarization that triggers arrhythmic events (Ouvrard-Pascaud et al. 2005; Gómez et al. 2009). The activity of the ryanodine receptor is also impaired (Ouvrard-Pascaud et al. 2005; Gómez et al. 2009). This has important consequences in the control of calcium homeostasis, modulation of calcium transients, sarcoplasmic reticulum diastolic leaks and initiating cardiac rhythm disorders (Gómez et al. 2009). This major impact on cardiac electrophysiology promotes arrhythmia: conditional overexpression of the MR in cardiomyocytes is associated with ventricular extrasystoles and increased

sensitivity to triggering ventricular arrhythmias (Ouvrard-Pascaud et al. 2005). Importantly, cardiomyocyte MR overexpression also affected neighboring structure such as coronary vessels: Favre et al. (2011) demonstrated endothelial dysfunction in coronary vessels of MR overexpressing mice. This was related to increased cardiac oxidative stress generated by cardiomyocyte MR overexpression. Importantly, coronary endothelial dysfunction was prevented if mice were treated with the MRA spironolactone (Favre et al. 2011). This probably contributes to the deleterious impact of cardiac MR activation, including arrhythmia, HF, or consequences of MI.

Targeted MR gene inactivation in cardiomyocytes has been achieved by two teams using different cardiomyocyte promoters to drive the Cre recombinase (the different promoters may affect the time- and cell-specific pattern of Cre expression and therefore MR inactivation) (Rickard et al. 2012; Fraccarollo et al. 2011). Studies from Young and colleagues investigated the role of cardiomyocyte MR on the cardiac impact of the DOCA-salt mineralocorticoid challenge (Rickard et al. 2012). They showed that cardiomyocyte MR was required for the primary inflammatory response and the recruitment of inflammatory cells to the heart as well as mineralocorticoid-induced ECM remodeling (Rickard et al. 2012).

However, cardiac function was not assessed in this study. The cardioprotective effects of cardiomyocyte MR deletion upon mineralocorticoid-salt challenge may be related to a decrease of the profibrotic pathway since MR inactivation prevented the upregulation of matrix metalloprotease 2/matrix metalloprotease 9 activity and of the transforming growth factor beta (TGFβ)–connective tissue growth factor (CTGF) profibrotic pathway (Rickard et al. 2012). Bienvenu et al. (2015) analyzed the role of cardiomyocyte MR in ex vivo ischemia–reperfusion injury associated with mineralocorticoid-salt challenge. Recovery of left ventricular (LV) developed pressure and rates of contraction and relaxation post ischemia–reperfusion were greater in knockout versus wild-type hearts while the incidence of arrhythmic activity during early reperfusion was significantly higher in wild-type than in knockout hearts (Bienvenu et al. 2015). This was associated with decreased levels of phosphorylated Ca^{2+}/calmodulin protein kinase II. These data indicated that cardiomyocyte MR has significant contribution in ischemia–reperfusion-related arrhythmogenesis and systolic dysfunction (Bienvenu et al. 2015).

Fraccarollo et al. (2011) demonstrated that cardiomyocyte-specific MR deficiency improved infarct healing and prevented progressive adverse cardiac remodeling, cardiac hypertrophy of the surviving tissue, and contractile dysfunction in ischemic HF. Early tissue changes after infarct were significantly improved in cardiomyocyte MR-null mice, including increased scar thickness in the infarct zone and increased capillary density in the viable tissue (Fraccarollo et al. 2011). The first week after infarct, marked improvements in LV filling pressures, LV enddiastolic and endsystolic volumes and LV ejection fraction were observed in cardiomyocyte MR-null mice when compared with wild-type mice (Fraccarollo et al. 2011). These data underline the importance of cardiomyocyte MR for onset and development of HF post-infarction, in accordance with the benefit of MR antagonism in respective patient populations (see below). Cardiomyocyte MR

deletion was also protective in a transaortic constriction model of pressure overload (Lother et al. 2011), with a higher LV ejection fraction and decreased cardiac dilatation compared with control mice. However, loss of cardiomyocyte MR did not prevent the development of cardiac hypertrophy, fibrosis, or inflammatory response and macrophage infiltration after pressure overload (Lother et al. 2011).

2.2.2 MR in Endothelial Cells

Endothelial-specific MR overexpression induced progressive increase in BP associated with increased response to various vasoconstrictors like AngII, endothelin, or thromboxane (Nguyen Dinh Cat et al. 2010).

The impact of MR expressed in endothelial cells on cardiac structure and function upon mineralocorticoid challenge or high fat diet has been analyzed using mouse models with endothelial-specific MR gene inactivation. Mineralocorticoid challenge was achieved in unilateral nephrectomized mice treated with DOCA together with salt in the drinking water. Endothelial-specific MR inactivation (achieved with different Cre expression systems) fully blunted mineralocorticoid-induced increase in BP, cardiac hypertrophy, tissue remodeling, interstitial fibrosis, and cardiac macrophage recruitment (Rickard et al. 2014; Jia et al. 2015; Lother et al. 2016). Increased expression of the Vascular Cell Adhesion Molecule 1 (VCAM 1) (Lother et al. 2016) or Intercellular Cell Adhesion Molecule 1 (ICAM 1) (Rickard et al. 2014) by mineralocorticoid treatment was also attenuated as well as various profibrotic and pro-inflammation markers (Rickard et al. 2014; Lother et al. 2016). Interestingly, the protective effect of endothelial MR on cardiac injury upon mineralocorticoid challenge was not observed for kidney injury, suggesting that additional deleterious effects are present in the kidney (Lother et al. 2016). Endothelial MR may also be involved in cardiac comorbidities such as obesity, metabolic syndrome, and diabetes. Female animals fed with high fat diet and high carbohydrate content for 16 weeks developed diet-induced cardiac diastolic dysfunction with prolonged relaxation time, impaired diastolic septal motion, and increased LV filling pressure (Jia et al. 2015). This was associated with cardiomyocyte hypertrophy and tissue remodeling with interstitial fibrosis (Jia et al. 2015). Endothelial-specific MR knockout blunted diastolic dysfunction and improved all above parameters (Jia et al. 2015). This was associated with a decrease in cardiac oxidative stress and macrophage infiltration as well as improvements of insulin signaling (Jia et al. 2015).

2.2.3 MR in Vascular Smooth Muscle Cells (VSMCs)

Gueret and collaborators recently addressed the contribution of VSMC MR in MI (Gueret et al. 2016). VSMC-specific MR deletion has no effect in baseline condition. However, absence of MR expression in VSMC attenuated the impact of MI, improving LV elastance and compliance as well as interstitial fibrosis (Gueret et al. 2016). Importantly, this was associated with an improved coronary reserve in mice, as measured using Magnetic Resonance Imaging (MRI). Deletion of MR in VSMC reduced coronary dysfunction associated with MI: preserved coronary relaxation was associated with less oxidative stress and improved nitric oxide

availability. Of note the non-steroidal MRA finerenone demonstrated similar effects (Gueret et al. 2016).

2.2.4 MR in Macrophages

MR is expressed in macrophages and may be activated by glucocorticoid rather than aldosterone, due to the low/absent 11β-HSD2 activity in macrophages. The use of macrophage-specific MR knockout mice revealed a critical role of macrophage MR signaling in mineralocorticoid-salt induced cardiac fibrosis (Rickard et al. 2009). Macrophage recruitment in the heart was not altered but mineralocorticoid induced cardiac fibrosis was reduced. Interestingly, deletion of macrophage MR altered baseline expression of numerous pro-inflammatory genes suggesting a role of MR in normal macrophage function. Usher et al. (2010) reported that MR-deleted macrophages have an M2-type profile associated with alternative macrophage activation, typically associated with anti-inflammatory and tissue repair properties. This may explain the beneficial effect of macrophage MR deletion also in other models of cardiac injury which are not directly related to mineralocorticoid challenge (Bienvenu et al. 2012; Usher et al. 2010). Similar results were obtained in a model of aortic constriction-induced cardiac hypertrophy (Li et al. 2014).

2.2.5 MR in Fibroblasts

Human cardiac fibroblasts express a functional MR and mineralocorticoid stimulation increases expression of profibrotic and pro-inflammatory markers (Tarjus et al. 2015). Fibroblast-specific MR ablation did neither prevent development of cardiac hypertrophy nor fibrosis after pressure overload (Lother et al. 2011).

3 Pharmacology of MRAs

3.1 Steroidal MRAs and Nomenclature of MRAs

It has been recognized early that progesterone is natriuretic in dogs (Thorn and Engel 1938) and men (Landau et al. 1955). Landau and Lugibihl (1958) found that progesterone is an endogenous antagonist of aldosterone. Chemists at Searle succeeded in synthesizing the first anti-mineralocorticoid with high oral bioavailability called spironolactone in 1957 (Kagawa et al. 1957) based on the chemical structure of progesterone. Subsequently, spironolactone was launched in 1960 as a diuretic for the management of edematous conditions, primary aldosteronism, and essential hypertension. However, characteristic adverse anti-androgenic and pro-gestational symptoms such as gynecomastia, impotence, and menstrual irregularities were observed already since 1963 due to the unspecific interactions of steroidal spironolactone with the androgen and progesterone receptors (Mann 1963). Realizing the natriuretic benefits of spironolactone, on one hand, and the typical clinical side effects due to its interference with other sex hormones, the demand for a second generation of MRAs (i.e., more specific compounds) for

chronic treatment of CV diseases was raised. Ciba-Geigy succeeded with the synthesis of a 9,11α-epoxyderivative of spironolactone called eplerenone in 1987 (de Gasparo et al. 1987). In vitro, eplerenone is ~40-fold less potent than spironolactone at MR but exhibits higher selectivity with respect to the other steroid hormone receptors (Garthwaite and McMahon 2004).

Eplerenone exhibited also lower potency and efficacy in the only two published head-to-head clinical trials versus spironolactone among hypertensive patients (Weinberger et al. 2002; Parthasarathy et al. 2011). Weinberger et al. (2002) showed that 100 mg eplerenone given once daily (OD) or 50 mg twice daily (BID) have an efficacy of 50–75% compared to 50 mg spironolactone BID. These results are in line with the second head-to-head trial of Parthasarathy et al. (2011) among patients with hypertension and evidence of primary aldosteronism which demonstrated that the antihypertensive efficacy of spironolactone was significantly greater than that of eplerenone. Recently, Roush et al. (2016) determined the effects of dose doubling, relative potency, and dose equivalence of potassium-sparing diuretics including spironolactone and eplerenone on systolic BP and serum potassium in a meta-analysis. Interestingly, these authors found an eplerenone-spironolactone dose equivalency of 4.5-to-1 among hypertensive patients (Roush et al. (2016).

John Funder suggested in 2005 a nomenclature of so-called third and fourth generations of MRAs: third generation MRAs are basically non-steroidal compounds which combine the potency and efficacy of spironolactone with the selectivity of eplerenone while fourth generation MRAs should be non-steroidal, potent, and selective plus have a "renal-sparing profile" which should avoid hyperkalemia (Funder 2005b). However, as Funder also alluded later, a putative fourth generation MRA should not completely spare renal effects since this would potentially lead to the even more dangerous condition of hypokalemia and sodium retention among patients with aldosteronism (Funder 2009). Sodium release (and concomitant mild potassium retention) as a consequence of renal MRA activity is clearly beneficial and demanded. Therefore, Kolkhof and Borden (2012) suggested that a fourth generation MRA should possess a more pronounced cardiac and/or vascular activity, or in other words a balance shifted more towards a non-renal profile at least in comparison with the available steroidal MRAs.

3.2 Novel Non-steroidal MRAs

Drug discovery campaigns in several pharmaceutical companies have been launched aiming for the identification of non-steroidal MRAs with an improved therapeutic index (i.e., less risk for hyperkalemia). At least five novel non-steroidal MRAs are now in clinical development with a clear focus on the treatment of patients with CKD (Kolkhof et al. 2015). Additional novel compounds were identified in drug discovery campaigns and are in preclinical development (Collin and Jaisser 2014). We refer the interested reader to recent reviews about non-steroidal MRAs for further information on other compounds in earlier stages

(Kolkhof and Borden 2012; Kolkhof et al. 2015; Collin and Jaisser 2014; Piotrowski 2012; Tamargo et al. 2014; Yang and Young 2016) and focus here only on few selected examples.

PF-03882845 was characterized as potent and selective MRA in vitro, which demonstrated a striking reduction of BP and improved renal protection in direct comparison with eplerenone in a preclinical model of salt-induced hypertension and nephropathy (Meyers et al. 2010). Orena et al. (2013) carefully determined the respective plasma drug concentrations of eplerenone and PF-03882845 that were necessary to decrease urinary albumin and to increase serum potassium in a rat CKD model and calculated a therapeutic index, i.e., the ratio of the half maximal effective concentration (EC_{50}) for increasing serum potassium to the respective EC_{50} for urinary albumin lowering. This ratio was only 1.5-fold for eplerenone but remarkably 84-fold for PF-03882845 and therefore the first published experimental proof for an increased therapeutic index, i.e., a reduced risk for developing hyperkalemia of a non-steroidal MRA versus a steroidal MRA in a relevant preclinical model (Orena et al. 2013). The compound was advanced to clinical phase I in 2009 but discontinued in July 2012 "for strategic reasons."

In 2004, researchers from Bayer reported for the first time that compounds belonging to the chemical class of dihydropyridines (DHPs) can act as MRAs in vitro (Ergueden et al. 2005). An ultrahigh throughput screening of ca. 1,000,000 compounds yielded a single cluster of ca. 100 compounds comprised DHPs. This finding was surprising because DHPs constitute the known class of L-type calcium channel blockers (CCB) as, e.g., nifedipine, nitrendipine, or amlodipine. In the meantime several papers published in scientific journals demonstrate that DHP-based L-type calcium channel blockers do antagonize MR in vitro and in vivo (Dietz et al. 2008; Arhancet et al. 2010a; Kosaka et al. 2010; Matsui et al. 2010). Optimization programs led to potent and specific MRAs without L-type calcium channel activity (Arhancet et al. 2010b; Fagart et al. 2010; Bärfacker et al. 2012).

BR-4628 was one of the first potent and selective DHP-based MRAs (Fagart et al. 2010) with oral bioavailability in preclinical animal models (Kolkhof et al. 2006; Schupp et al. 2011). It was shown to block fibrotic remodeling in cardiac fibroblasts which suggests a potential use for the prevention of atrial fibrillation (Lavall et al. 2014). Furthermore, treatment with non-steroidal BR-4626 provided substantial suppression of mouse crescentic glomerulonephritis without causing hyperkalemia or tubular dysfunction (Ma et al. 2015) in contrast to eplerenone which exhibited signs of tubular dysfunction in the same model (Huang et al. 2014). BR-4628 prevented also from renal oxidative damage, renal dysfunction, and tubular injury induced by ischemia reperfusion in a rodent acute kidney injury model (Barrera-Chimal et al. 2016). In summary, non-steroidal BR-4628 demonstrated efficacy in preclinical models of cardiorenal diseases. Further chemical optimization led to a novel series of heterobicyclic analogues of naphthyridine derivatives which can be considered as conformationally "frozen" bioisosteres of DHP esters such as nitrendipine (Bärfacker et al. 2012). The dihydronaphthyridine finerenone (previous nomenclature BAY 94-8862) was identified as a potent MRA

(IC_{50} 18 nM) with excellent selectivity versus all other steroid hormone receptors. In contrast to BR-4628, finerenone displayed virtually no L-type Ca^{2+} channel affinity ($IC_{50} > 10$ μM) (Bärfacker et al. 2012). Thus, finerenone uniquely combines potency and extraordinary selectivity (at least 500-fold) toward MR. Table 1 summarizes the affinities of the steroidal MRAs, the CCB nitrendipine and the two DHP-based non-steroidal MRAs BR-4628 and finerenone for the steroid hormone receptor family as well for the L-type calcium channel (only DHP-based compounds).

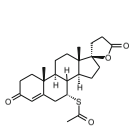
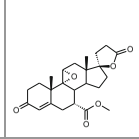
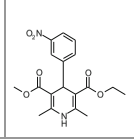
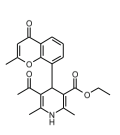
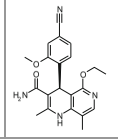
Finerenone has been investigated in different preclinical animal models of chronic hypertensive and ischemic heart and kidney diseases (Kolkhof et al. 2014; Liu et al. 2015; Gueret et al. 2016; Grune et al. 2016). Finerenone treatment prevented DOCA/salt challenged rats from functional and structural heart and kidney damage at dosages which did not reduce systemic BP. Furthermore, finerenone reduced cardiac hypertrophy, pro-B-type natriuretic peptide (BNP), and proteinuria more efficiently than eplerenone when directly comparing equi-natriuretic doses (Kolkhof et al. 2014). Gueret et al. (2016) investigated finerenone in a chronic MI model in mice induced by coronary artery ligation. Mice treated with finerenone over a period of 2 months had improved LV compliance and elastance when compared with infarcted control mice as well as reduced interstitial fibrosis. Finerenone preserved the coronary reserve assessed by MRI as well as improved the endothelial function of isolated septal coronary arteries (Gueret et al. 2016). In a mouse model of pressure-overload induced HF treatment with finerenone compared in a head-to-head manner with eplerenone resulted in a more pronounced prevention of myocardial hypertrophy (Grune et al. 2016). Very recently it was demonstrated that finerenone improves diastolic function in a preclinical model of type-2 diabetes mellitus (T2DM) (Mulder et al. 2016). Finerenone decreased proteinuria over the treatment period of 3 months. Finerenone prevented LV diastolic dysfunction and reduced LV hypertrophy as well as LV collagen density. These results would suggest that finerenone might have a benefit for patients with HF and preserved ejection fraction (HFpEF) and T2DM.

3.3 Differences Between Steroidal MRAs and Non-steroidal Finerenone

Some important differences between the steroidal MRAs and finerenone with respect to in vitro potency and selectivity, binding mode, pharmacokinetics in humans as well as physicochemical properties and related tissue distribution are summarized in Table 2.

Finerenone was found to combine spironolactone's potency with eplerenone's selectivity in vitro (Bärfacker et al. 2012; Pitt et al. 2012). Besides this superior selectivity and potency profile in comparison with the steroidal MRAs it exhibits mechanistically interesting features. Finerenone was shown not only to block wild-type MR in vitro and in vivo but also the gain-of-function S810L MR mutant

Table 1 Evolution of dihydropyridine-based non-steroidal MRAs

		Steroidal MRA		DHP-based CCB	DHP-based non-steroidal MRA	
						
		Spironolactone	Eplerenone	Nitrendipine	BR-4628	Finerenone
Steroid hormone receptor	MR IC_{50} (nM)	24	990	2,000	28	18
	GR IC_{50} (nM)	2,410	>22,000	25,760	5,470	>10,000
	AR IC_{50} (nM)	77	>21,000	10,050	4,441	>10,000
	PR IC_{50} (nM)	743[a]	>31,000	9,730	9,020	>10,000
DHP channel	L-type Ca^{2+} IC_{50} (nM)	n.d.	n.d.	0.26	1,990	>10,000

Data were taken from Fagart et al. (2010) and Pitt et al. (2012)
n.d. not determined
[a]EC_{50} because spironolactone is an agonist at PR

Table 2 Comparison of steroidal MRAs vs. finerenone

		Spironolactone	Eplerenone	Finerenone
	MRA class	Steroidal	Steroidal	Non-steroidal
Potency and selectivity	Potency	High	Low	High
	Selectivity	Low	Medium	High
Binding mode	Mode of antagonism	Passive	Passive	Bulky
	MR S810L	Partial agonist	Partial agonist	Antagonist
Pharmacokinetics	Metabolites	Multiple, active	No active metabolites	No active metabolites
	Half-life[a] (HV[b]/ patients)	Long (>12 h/ ≥24 h)	Medium/short (ca. 3 h/4–6 h)	Short (2.2–2.8 h)
Physicochemical properties and distribution	Lipophilicity (log *D*)	3.13	3.03	2.40
	Plasma protein binding	High (>90%), mostly CBG and albumin	Low (50%), mostly AGP	High (90%)
	Tissue distribution (kidney/heart)[c]	Higher in kidney (at least sixfold)	Higher in kidney (ca. threefold)	Balanced 1:1

Modified and extended from Kolkhof and Borden (2012)
[a]Half-life of active compound/metabolites
[b]*HV* healthy volunteers
[c]In rodents

(Amazit et al. 2015), which is responsible for early onset hypertension in men and gestational hypertension in women (Geller et al. 2000). Progesterone and both steroidal MRAs, spironolactone and eplerenone, paradoxically activate the S810L MR mutant (Amazit et al. 2015; Geller et al. 2000) and are therefore contraindicated among patients with this rare disease. This is a remarkable observation since it shows that structurally different MRAs can lead to an altered pharmacology.

Using an automated high-throughput microscopy of MR subcellular distribution Amazit et al. (2015) demonstrated that finerenone delays aldosterone-induced nuclear accumulation of MR more efficiently than spironolactone. Moreover, chromatin immunoprecipitation assays revealed that finerenone and spironolactone differentially affect recruitment of transcriptional cofactors on MR target promoter. While finerenone acts as an inverse agonist, i.e., reducing recruitment even in the absence of aldosterone of the crucial co-activator SRC-1 at the MR target promoter *SCNN1A* gene (which encodes the α-subunit of ENAC), spironolactone acts as a partial agonist, i.e., promoting SRC-1 recruitment but to a lesser extent as aldosterone (Amazit et al. 2015). These results indicate distinct mechanisms of action for both steroidal MRAs as compared to finerenone despite binding into the same ligand binding domain of MR. Spironolactone and eplerenone are so-called passive antagonists which quickly dissociate from the receptor and are unable to stabilize

an important helix (H12) in the C-terminal activation function 2 domain of MR. Spironolactone and eplerenone cannot prevent the H12 helix from adopting the agonist conformation, allowing co-activator binding which might explain the reported partial agonistic activity of steroidal MRAs (Cargnelli et al. 2001). In contrast, the DHP-based non-steroidal MRAs BR-4628 and finerenone are "bulky-passive" antagonists (Bärfacker et al. 2012; Amazit et al. 2015). Binding of "bulky" non-steroidal MRAs leads to a protrusion of helix 12 in the C-terminal activating function 2 domain of MR and finally to an unstable receptor-ligand complex which is unable to recruit co-regulators.

Spironolactone is rapidly metabolized into several active metabolites, most importantly 7α-thiomethyl-spironolactone (TMS) and canrenone (which is also marketed as own drug formulation in few countries, e.g., Italy) which have mean half-lives in healthy volunteers of 13.8 h and 16.5 h, respectively (Garthwaite and McMahon 2004; Karim 1978). These half-lives can be even prolonged in patients with cirrhotic ascites up to 58 h (Sungaila et al. 1992). The consequence of the complex metabolism of spironolactone is a relatively slow onset of pharmacodynamics action after the active metabolites have reached steady-state plasma levels (Sica 2005). Therefore, the long half-lives of spironolactone's active metabolites are the basis of its efficacy when applied OD among patients with essential hypertension or HF.

Eplerenone has no active metabolites and a much shorter half-life, namely 4 h in steady state after multiple dose applications of 100 mg (Cook et al. 2003). However, a study in healthy volunteers carefully investigated the pharmacokinetics and pharmacodynamics (i.e., natriuretic) effects of the immediate release formulation of different dosages of eplerenone after fludrocortisone challenge (Thosar et al. 2003). This study revealed a natriuretic action of about 10 h at 50 mg (which is the approved OD dosage in HF) despite the short half-life of only 2.9 h at this dose in healthy subjects. Weinberger et al. (2002) could demonstrate that the BP lowering efficacy of eplerenone in mild to moderate hypertensive patients is more pronounced when given BID in comparison with an OD application. However, in spite of its short half-life, eplerenone in an OD regime provides mortality reduction in HF patients (Pitt et al. 2003; Zannad et al. 2011). Therefore, one might speculate that the underlying pharmacokinetics demands for BP lowering effects (long half-life needed) and cardioprotection (i.e., anti-inflammatory, -hypertrophic and -fibrotic effects) of MRAs are different. Interestingly, the duration of natriuresis and antikaliuresis may also differ since the latter has been observed to persist for several days after discontinuation of spironolactone (Sica 2005). This characteristic is important with respect to the potential of steroidal MRAs to induce hyperkalemia when given on top of an ACEI or ARB especially on the background of reduced kidney function.

Safety, tolerability, and pharmacokinetics of finerenone were initially investigated in healthy volunteers (Lentini et al. 2016; Heinig et al. 2016). It was found that renal elimination is not a relevant route for finerenone and that renal impairment is unlikely to have a relevant effect on its pharmacokinetics. Glomerular filtration of unbound finerenone was found to be the mechanism for its renal

elimination. The plasma clearance was determined to be 0.473 L/h (7.9 mL/min) (Lentini et al. 2016). Heinig et al. (2016) carefully determined the half-life of finerenone after oral application of a 10 mg immediate release tablet which was found to be 2.2 h in individuals with normal renal function and up to 2.8 h in individuals with severe renal impairment. Renal excretion of finerenone was negligible (0.810%) in healthy individuals and was even reduced in those with renal impairment.

Besides the consequences of the DHP-based non-steroidal structure for the different binding mode within MR, the chemical structure also determines the physicochemical properties. Key physicochemical drug properties like lipophilicity and polarity have a strong impact on plasma protein binding, vascular transport, tissue penetration, and distribution. The steroidal MRAs have a 6–10-fold higher lipophilic character than finerenone while the latter exhibits greater polarity than the steroidal MRAs (Kolkhof et al. 2015). The consequence of the different lipophilicity for tissue distribution has been determined by quantitative whole-body autoradiography following the administration of [^{14}C]-labeled finerenone (Kolkhof et al. 2014). These studies revealed a balanced distribution of [^{14}C]-finerenone into heart and kidney tissues of healthy rats. This tissue distribution pattern is in clear contrast to the respective distribution pattern of spironolactone and eplerenone in rodents. Experiments using radioactively labeled eplerenone demonstrated at least a threefold higher accumulation of the drug equivalent concentration in the kidney compared with heart tissue in rats (Inspra Drug Approval Package 2003). A similar study with radioactively labeled spironolactone revealed high drug concentrations within the kidneys while radioactivity in heart tissue was below the detection limit (Platt and Pauli 1972).

Spironolactone is bound to 94% in human plasma predominantly to albumin (Chien et al. 1976). On the contrary, eplerenone's bound fraction is only 50% (Inspra Drug Approval Package 2003), thus compensating its much lower potency at MR by a higher unbound fraction of bioavailable compound. Eplerenone binds with highest affinity to alpha-1 acid glycoprotein (AGP or orosomucoid, orm-1) (Kolkhof and Borden 2012). Finerenone is bound to 89.4% to human plasma proteins in healthy individuals as well in individuals with severe renal impairment (Heinig et al. 2016).

Finally, the combination of both, a different binding mode and a different tissue distribution might ultimately lead to a differential gene expression in different tissues. Following this hypothesis, Grune et al. (2016) found a differential cardiac gene expression pattern in the hearts of mice treated either with finerenone or eplerenone. Thus, the more pronounced reduction in myocardial hypertrophy observed with finerenone might therefore result from altered myocardial gene regulation compared to eplerenone including differential expression of cardiac Tnnt2 (troponin T type 2) (Grune et al. 2016). The concept of tissue-selective modulation of nuclear co-activators and co-repressors by a steroid receptor based on the chemical structure of the agent has originally been described for the estrogen receptor (ER) on the basis of selective ER modulators (SERMs) (Shang and Brown 2002; Imai et al. 2013) as well as for retinoic acid receptor (RAR) and retinoid X

receptor (RXR) (de Lera et al. 2007). In conclusion, steroidal and non-steroidal MRAs might differentially influence the two principal pathways highlighted in Fig. 1 leading to a differential pharmacology.

4 Important Clinical Trials with MRAs

4.1 Spironolactone in HFrEF: RALES

The publication of the Randomized Aldactone Evaluation Study (RALES) trial in 1999 (Pitt et al. 1999) was the cornerstone for the role of MRAs in the treatment of HF. In this study, which was the first large, prospective, randomized mortality trial of an MRA in a HFrEF population, a total of 1,663 patients with New York Heart Association (NYHA) class III–IV and an ejection fraction $\leq$35% were randomized to either spironolactone 25 mg OD or placebo. Based on potassium levels, the dose of spironolactone could be increased to 50 mg OD after a treatment of 8 weeks. After 24 months of treatment, the mean daily dose of spironolactone was 26 mg. The overall patient population had a mean age of 65 years, was mainly male and predominantly with ischemic heart disease as major cause for HF. All patients were on loop diuretics, the majority received ACEI and digitalis with an overall low use of beta-blockers (11%). The study was discontinued early after a mean follow-up of 24 months as the primary endpoint – death from any cause – was significantly reduced in the spironolactone group (HR 0.70; 95% CI 0.60–0.82; $p < 0.001$). This impressive reduction of overall mortality was attributed to both a reduction in mortality from progressive HF and a reduction in sudden cardiac death. Hospitalization for HF (HHF) was also significantly reduced by the treatment with spironolactone (HR 0.65; CI 95% 0.54–0.77; $p < 0.001$). The incidence of serious hyperkalemia as defined of serum potassium $\geq$6 mmol/L was 2% in the spironolactone patients versus 1% in the placebo patients ($p = 0.42$), respectively.

4.2 Eplerenone in HFrEF: EPHESUS and EMPHASIS-HF

Eplerenone was investigated in patients with acute MI complicated by HF due to impaired LV systolic dysfunction (LVSD) – the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study, EPHESUS (Pitt et al. 2003). A total of 6,642 patients from 37 countries were randomized within 14 days following the acute MI to either eplerenone 25 mg OD or placebo on top of SoC. After 4 weeks of treatment, the dose was increased to 50 mg OD provided serum potassium levels were within the required range. The mean dose-equivalent of study medication was 42.6 mg in the eplerenone group. Patients had a mean of 64 years with the majority of them receiving SoC including ACEIs/ARBs, beta-blockers, diuretics, and aspirin. After a mean follow-up of 16 months, the primary endpoint, death from any cause or hospitalization for CV events, was significantly reduced by eplerenone (HR 0.87; 95% CI 0.79–0.95; $p < 0.002$). This reduction was

solely attributed to the prevention of sudden cardiac death. Serious hyperkalemia ($\geq$6 mmol/L) was observed in 5.5% of the eplerenone versus 3.9% of the placebo patients ($p = 0.002$), respectively.

The Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF), (Zannad et al. 2011) completed the investigation of steroidal MRAs in the HFrEF spectrum by investigating eplerenone in mild symptomatic HF patients. In this trial, 2,737 patients with NYHA class II, an ejection fraction $\leq$35% and a CV hospitalization within the last 6 months were randomized to eplerenone or placebo. Based on baseline estimated glomerular filtration rate (eGFR), patients were starting with eplerenone 25 mg OD or 25 mg every other day (EOD) with a potential up-titration to the next higher dose level after 4 weeks of treatment. Mean dose at 5 months in the eplerenone group was 39.1 mg. Patients had a mean age of 69 years, being mainly male and having an ischemic heart disease as the leading cause for HF. Approximately half of the patients had a previous HHF at baseline. The majority of the patients received SoC including ACEIs/ARBs as well as beta-blockers. This trial was stopped early (median follow-up 21 months) because of a significant reduction in the primary composite endpoint – death from any cause or HHF – (HR 0.63; 95% CI 0.54–0.74, $p < 0.001$) for patients being treated with eplerenone. The incidence of severe hyperkalemia (serum potassium >6 mmol/L) was 2.5% versus 1.9% ($p = 0.29$) for eplerenone versus placebo, respectively.

4.3 Spironolactone in HFpEF: TOPCAT

Around half of the patients who have symptoms and signs of HF have relatively normal or mildly impaired LV ejection fraction and are labeled as HFpEF (Yancy et al. 2006). HFpEF is the predominant form of HF in a more elderly population, more often women are affected and more commonly a history of hypertension and atrial fibrillation is present, while a history of MI is less common when comparing to HFrEF (Owan et al. 2006; Go et al. 2013). Accordingly, the prevalence of HFpEF is expected to increase with an overall aging population. Long-term mortality in HFpEF is similar to HFrEF, with less than 50% 5-year survival in community HFpEF cohorts (Owan et al. 2006; Tribouilloy et al. 2008).

On the background of the aforementioned role of MR overactivation leading to endothelial dysfunction, progressive vascular, renal, and myocardial fibrosis promoting arterial hypertension, ventricular hypertrophy and vascular stiffness contributing to the pathologic alteration in HFpEF, spironolactone was investigated in symptomatic HF patients with an ejection fraction $\geq$45% (Desai et al. 2011). The Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial was an international, prospective, randomized trial conducted in the USA, Canada, Russia, Republic of Georgia, Argentina, and Brazil. A total of 3,445 patients were randomly assigned to receive spironolactone 15 mg OD or placebo (Pitt et al. 2014). Doses of study drugs were increased to a maximum of 45 mg OD during the first 4 months. At month 8, the mean dose of spironolactone

was 25.0 mg. Mean age was 69 years with 52% being female. Approximately 90 and 35% had arterial hypertension and atrial fibrillation in their medical history, approximately 26% of patients a previous MI and 72% of the patients had been hospitalized in the previous year with management of HF as major component. The majority of the patients received diuretics, ACEIs/ARBs, and beta-blockers. After a mean follow-up of 3.3 years, spironolactone missed to show a reduction in the primary composite endpoint of death from CV causes, aborted cardiac arrest, or HHF compared to placebo (HR 0.89; 95% CI 0.77–1.04; $p = 0.14$). Of the components of the primary outcome, only HHF had a significantly lower incidence in the spironolactone group than in the placebo group (HR0.83; 95% CI 0.69–0.99; $p = 0.04$). Treatment with spironolactone was associated with a doubling of hyperkalemia rate, i.e. 18.7% vs. 9.1% in the spironolactone and placebo group, respectively, but the occurrence of hyperkalemia was not associated with any death. Interestingly, *post hoc* analyses demonstrated a ~4-fold difference in the composite event rate between the 1,678 patients randomized from Russia and Georgia compared with the 1,767 enrolled from the Americas as well as greater potassium and creatinine changes and possible clinical benefits with spironolactone in HFpEF patients from the Americas (Pfeffer et al. 2015).

4.4 Finerenone in HFrEF and Diabetic Kidney Diseases (DKD)

To date, finerenone was investigated in five clinical phase II trials in more than 2,000 patients with HF and additional CKD or diabetes as well as in patients with diabetic kidney disease (DKD) (Pitt et al. 2012, 2013, 2015; Filippatos et al. 2016; Sato et al. 2016; Ruilope et al. 2014; Bakris et al. 2015), i.e., in patient populations which are considered to reveal a high benefit from MR blockade on one side but also to be most prone for the development of hyperkalemia when receiving an MRA on top of an ACEI or ARB on the other side. All five phase II studies with finerenone were dose-finding studies investigating doses overall from 1.25 to 20 mg. Three trials (ARTS, ARTS-HF, and ARTS-HF Japan) were conducted head-to-head against with either spironolactone (ARTS) or eplerenone (ARTS-HF and ARTS-HF Japan). Overall the studies demonstrated an optimal benefit-to-risk ratio at doses of 10–20 mg finerenone OD. In September 2015, a phase III program comprising of two large outcome studies investigating 10 and 20 mg of finerenone in patients with DKD has been initiated. Therefore, these doses (10 mg [ARTS and ARTS-DN], 20 mg [ARTS-DN] and 10 → 20 mg [ARTS-HF]) were chosen for all following figures in order to enable a condensed juxtaposition of finerenone with the steroidal comparator MRAs with respect to important clinical efficacy and safety parameters.

4.4.1 ARTS

The MinerAlocorticoid-Receptor antagonist Tolerability Study (ARTS) was a Phase IIa, randomized, double-blind, placebo-controlled, parallel-group, multicenter study (Parts A and B), with an additional open-label active comparator for

Part B, that investigated the safety and tolerability of finerenone in patients with chronic HF with LVSD and mild (Part A) or moderate (Part B) CKD (Pitt et al. 2012). In Part A, 65 patients were randomized to 1 of 4 treatment groups: finerenone 2.5, 5, and 10 mg, and placebo, all given OD. In Part B, 393 subjects were randomized to 1 of 6 treatment groups: finerenone 2.5 mg OD, 5 mg OD, 10 mg OD, and 5 mg BID, placebo, and spironolactone (25 mg with up-titration to 50 mg OD after 2 weeks if serum potassium was $\leq$4.8 mmol/L; Pitt et al. 2013). All investigated doses of finerenone were safe and well tolerated. Finerenone at doses of 2.5–10 mg OD as well as 5 mg BID led to significantly smaller mean increases in serum potassium concentration (Fig. 2d), and smaller decreases in eGFR, compared to spironolactone leading to a lower number of patients with hyperkalemia, renal failure, or renal impairment when comparing finerenone at a dose of 10 mg versus spironolactone (Fig. 2f). Changes in BP in the finerenone groups were comparable with those seen in patients receiving placebo while spironolactone significantly decreased BP compared with placebo. Mean changes in serum aldosterone was most pronounced for the spironolactone treated group (Fig. 2d). Considering aldosterone synthesis being stimulated dominantly by serum potassium levels, one potential reason for this difference in the treatment groups could be directly related to the magnitude of increase in serum potassium which was more pronounced for spironolactone. Furthermore, pharmacokinetics aspects of spironolactone such as the prolonged half-life owing its active metabolites may also have contributed to this observation. Finerenone at a dose of 10 mg OD reduced N-terminal of prohormone of BNP (NT-proBNP), BNP (Fig. 2a, b), and urinary albumin to creatinine ratio (UACR) to a similar extent as spironolactone (Fig. 2c). In summary, the favorable observations in the finerenone groups over spironolactone on the undesired side effects did not occur at the expense of a lower efficacy as demonstrated here by the results on the exploratory marker NT-proBNP, BNP and UACR.

4.4.2 ARTS-HF

Despite the class IA recommendation in international guidelines to use an MRA in all patients with symptomatic HFrEF (Ponikowski et al. 2016; Yancy et al. 2013), MRAs are still being underutilized, in particular in patients hospitalized due to an acute HF decompensation in which prevalence of CKD or diabetes mellitus (DM) is high and known to further increase the risk of hyperkalemia and/or worsening renal function (Maggioni et al. 2013; Johnson et al. 2010).

The ARTS-HF Study was a randomized, double-blind, comparator-controlled, six parallel group, multicenter phase 2b dose-finding study in a high risk population – at increased risk of post-discharge mortality and morbidity, on the one hand, and, on the other hand, prone to develop hyperkalemia and/or worsening renal function (Pitt et al. 2015). Of 1,286 screened patients, 1,066 patients with HFrEF and concomitant T2DM and/or CKD were randomized within 7 days of emergency presentation to hospital for worsening chronic HF to receive finerenone or eplerenone. The 5 finerenone groups started with 2.5, 5, 7.5, 10, or 15 mg OD. The dose was increased to 5 mg, 10 mg, 15 mg, 20 mg, or 20 mg OD,

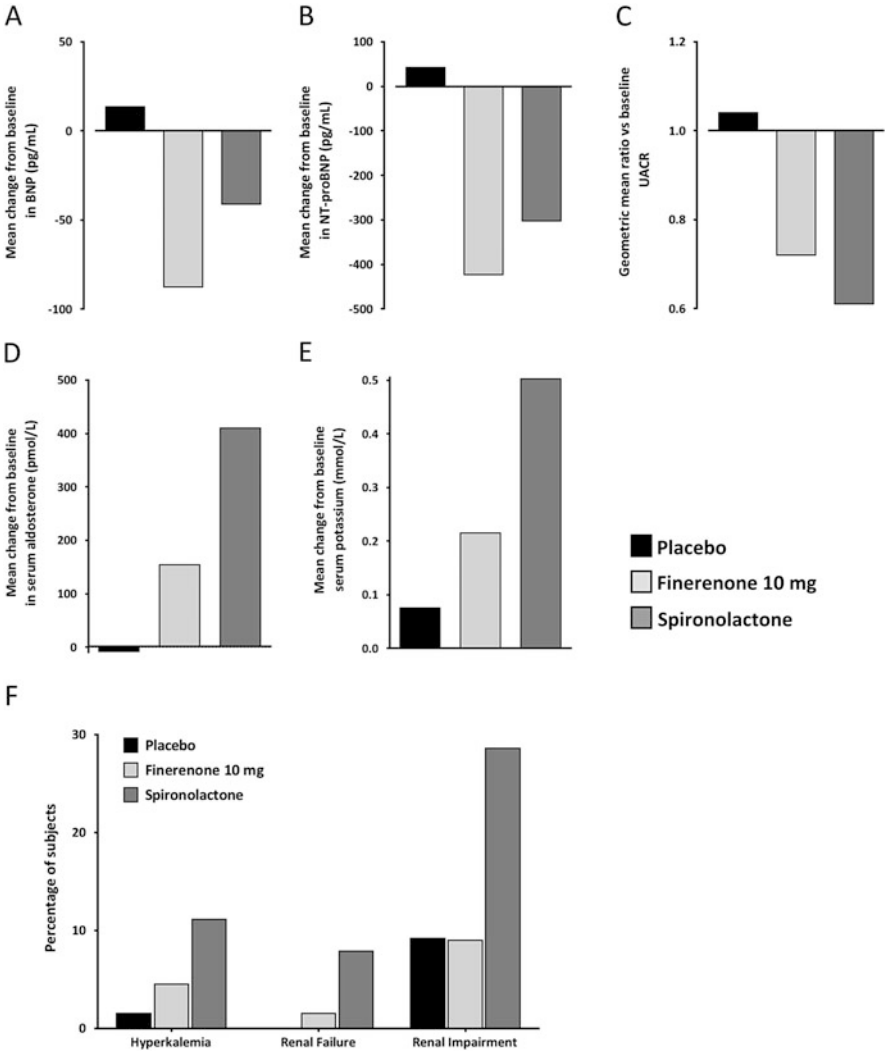


Fig. 2 Head-to-head comparison of the 10 mg finerenone dose with spironolactone in ARTS. Juxtaposition of important efficacy and safety parameters of the 10 mg finerenone dose and spironolactone in ARTS: (**a**) BNP, (**b**) NT-proBNP, (**c**) UACR, (**d**) aldosterone, (**e**) potassium, and (**f**) adverse events

respectively on Day 30, and all treatment groups were possibly sham up-titrated on Day 60. Eplerenone was started with 25 mg every other day and was up-titrated to 25 mg OD on Day 30 and to 50 mg OD on Day 60. Patients who could not be up-titrated on Day 30 were up-titrated on Day 60 to the next higher dose level. Up-titration in all treatment groups was performed only if serum potassium was $\leq$5.0 mmol/L.

The primary endpoint was the percentage of individuals with a decrease of >30% in plasma NT-proBNP from baseline to Day 90. A key exploratory endpoint was a composite clinical endpoint of death from any cause, CV hospitalizations, or emergency presentation for worsening HF until Day 90. Baseline characteristics were broadly similar across the treatment groups representing a typical HF population with ischemic heart disease (64.4%), arterial hypertension (73.5%), atrial fibrillation (40.9%) and the majority receiving recommended evidence-based therapy for chronic HF (Filippatos et al. 2016). At Day 90, all finerenone dose groups showed a similar proportion of patients who had an NT-proBNP level decrease of >30% compared to the eplerenone group with the highest proportion in the 10 → 20 mg OD group (Fig. 3a). However, the composite clinical endpoint occurred less frequently in all finerenone dose groups except for 2.5 → 5 mg OD, compared with eplerenone. The lowest incidence of events was observed in the 10 → 20 mg OD group, with a nominally improved outcome compared to eplerenone (hazard ratio, HR: 0.56 [95% CI: 0.35–0.90]); similar results were observed for the individual components of death from any cause [HR: 0.13 (95% CI: 0.02, 1.07)], CV hospitalization [HR: 0.56 (95% CI: 0.34, 0.93)], and emergency presentation to hospital for worsening chronic HF [HR: 0.58 (95% CI: 0.33, 1.02)] (Fig. 3d–f). Consistently to the observed effects on NT-proBNP as well as on the aforementioned clinical events, the most pronounced effect in decreasing UACR was seen in the 10 → 20 mg OD group (Fig. 3c).

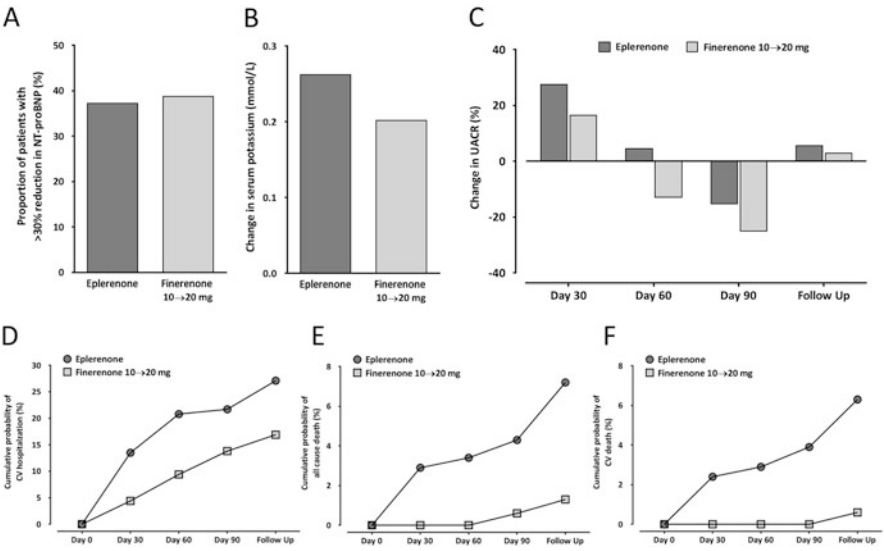


Fig. 3 Head-to-head comparison of the 10 → 20 mg finerenone dose with eplerenone in ARTS-HF. Juxtaposition of important efficacy and safety parameters of the 10 → 20 mg finerenone dose and eplerenone in ARTS-HF: (**a**) NT-proBNP, (**b**) potassium, (**c**) UACR, (**d**) CV hospitalization, (**e**) all-cause death, (**f**) CV death

All doses of finerenone had a similar safety profile to that of eplerenone. Incidences of treatment-emergent adverse events (TEAEs) were similar between the eplerenone and all finerenone dose groups. Hyperkalemia (serum potassium to ≥5.6 mmol/L) at any time post-baseline was observed in 44/1,023 subjects (4.3%) overall, with a comparable incidence amongst the treatment groups. Mean change from baseline to Day 90 in serum potassium concentration was greater in the eplerenone group (+0.262 mmol/L) than in each of the finerenone dose groups (+0.119–0.202 mmol/L, Fig. 3b).

4.4.3 ARTS-DN

Diabetes mellitus and CKD are important risk factors for the development of HF. Both risk factors are combined in patients with DKD. Hospitalization for HF (HHF) is one of the most common and prognostically important CV complications in patients with DM; the risk for HHF in patients with DKD is even amplified by the presence of albuminuria and decreased kidney function which independently increase the risk in multivariable analysis (Carr et al. 2005).

Finerenone was investigated in patients with DKD in the ARTS-DN study. ARTS-DN was a double-blind, placebo-controlled, parallel-group, multicenter phase IIb study in patients with T2DM and albuminuria ≥30 mg/g receiving RAS blockade (Ruilope et al. 2014). Of 1,501 patients screened, 823 were randomized to 7 different doses of finerenone or placebo, all given OD. Addition of finerenone to SoC (i.e., ACEIs/ARBs) resulted in dose dependent, significant reductions in albuminuria at doses of 7.5, 10, 15, and 20 mg after 90 days of treatment. In Fig. 4a reduction of albuminuria under treatment with placebo, 10 and 20 mg of finerenone is displayed. In patients treated with 20 mg of finerenone in particular, the residual effect on albuminuria was most pronounced compared to placebo at 30 days after completion of treatment relative to baseline, which may indicate the start of a structural change rather than a purely hemodynamic induced decrease in albuminuria. Compared to placebo, there was a slight increase of serum potassium over time (Fig. 4b). Hyperkalemia leading to discontinuation was not observed in

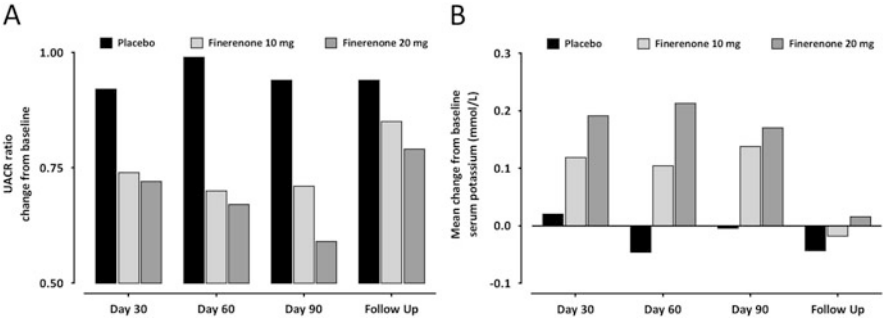


Fig. 4 10 and 20 mg finerenone compared to placebo in ARTS-DN. Important efficacy (**a**, UACR) and safety relevant (**b**, potassium) parameters of the 10 and 20 mg finerenone dose in ARTS-DN

the placebo and finerenone 10 mg groups; the incidence was 1.7% in the 20 mg group (Bakris et al. 2015).

In a recent systematic review including eight studies, the incidence of hyperkalemia increased in the combined treatment group necessitating interruption of treatment with steroidal MRAs in up to 17% of cases comparing standard treatment by an ACEI or an ARB with combined treatment by an ACEI/ARB and an MRA in patients with diabetic nephropathy. Therefore, combined treatment by an ACEI/ARB and a steroidal MRA was considered effective in decreasing albuminuria in diabetic nephropathy but it increases the risk of hyperkalemia (Mavrakanas et al. 2014).

A post-hoc analysis of the Reduction in Endpoints in non-insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) showed that a reduction of albuminuria within the first 6 months was the only predictor of long-term protection from CV outcome: 18% reduction in CV risk for every 50% reduction in albuminuria and a 27% reduction in HF risk for every 50% reduction in albuminuria (de Zeeuw et al. 2004). In ARTS-DN, 23.9% and 13.6% of patients showed a decrease in UACR from baseline to Day 90 of equal or more than 30% and 50%, respectively, in the placebo group. In the 10 mg and 20 mg finerenone group, 46% and 62.5% of patients showed a decrease in UACR of equal or more than 30%, respectively, and 62.5 and 40.2% a decrease in UACR of equal or more than 50% from baseline to Day 90 (Bakris et al. 2015).

5 Conclusions and Outlook

MRAs are considered as life-saving therapy for patients with HFrEF (Zannad et al. 2012). In three randomized controlled clinical trials, i.e., RALES (Pitt et al. 1999), EPHESUS (Pitt et al. 2003), and EMPHASIS-HF (Zannad et al. 2011) MRAs consistently reduced morbidity and mortality in the investigated HF population. Since the publication of the EMPHASIS-HF results at the latest, MRAs are considered as standard therapy in all symptomatic HFrEF patients, i.e., NYHA functional class II–IV, already receiving an ACEI and beta-blocker. This recommendation is reflected in several updates of national and international guidelines (Ponikowski et al. 2016; Yancy et al. 2013).

Despite proven benefit in HFrEF patients, the steroidal MRAs have a number of disadvantages which limit their use in clinical practice. The risk of developing hyperkalemia is the main obstacle to moving therapy with MRAs from the randomized clinical trials to everyday clinical practice (Juurlink et al. 2004). Accordingly, the demand for novel, potent, and selective, non-steroidal MRAs with a potential for a reduced risk of developing hyperkalemia initiated drug discovery programs within several pharmaceutical companies. Such novel, non-steroidal MRAs (third generation) were identified and a few entered clinical development recently. Finerenone was identified as DHP-based derivative with different physicochemical, pharmacokinetics, and pharmacological properties in comparison with the steroidal MRAs. Finerenone exhibited an improved therapeutic index in preclinical heart failure animal models when compared to eplerenone

and a nominally improved outcome compared to eplerenone in patients with HFrEF and concomitant T2DM and/or CKD.

Finerenone at daily doses of 10 and 20 mg has currently been investigated in two large outcome trials in patients with DKD (FIGARO-DKD, NCT02545049 and FIDELIO-DKD, NCT02540993). As both trials are placebo-controlled, patients with HFrEF at baseline are excluded from participating in these trials. However, in FIGARO-DKD "HHF" has been selected as component of the primary CV composite endpoint; in FIDELIO-DKD, it is part of the CV composite endpoint which has been chosen as first secondary endpoint.

Because of its unique pharmacodynamic profile based on its non-steroidal structure and the promising safety and efficacy results deduced in five phase 2 studies with more than 2,000 patients, finerenone could beneficially impact CV and renal outcomes in this population as it directly interferes with the underlying disease processes.

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Vasopressin and Vasopressin Antagonists in Heart Failure

Julie K. Vishram-Nielsen and Finn Gustafsson

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Abstract

Despite the introduction of multiple new pharmacological agents over the past three decades in the field of heart failure (HF), overall prognosis remains poor. Hyponatremia is prevalent in HF patients and has been suggested as a contributor to poor response to standard therapy. Elevated levels of arginine vasopressin (AVP), a peptide hormone produced in the hypothalamus, play a role in development of hyponatremia, and AVP and its surrogate, copeptin, are related to changes in osmolality, hemodynamics, neuro-hormones as well as in overall outcome in HF patients. Of current pharmacological interest are the selective and non-selective vasopressin receptor antagonists (VRAs), which inhibit vasoconstriction and cardiac remodeling mediated by the V_{1a} receptors in smooth blood vessels, and water retention (increased urine osmolality and decreased

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2017_28

water excretion) by increasing aquaporin-2 water channels mediated by the V_2 receptors in the renal collecting tubules. The optimal use of VRAs is yet to be determined, especially in patients with congestive HF. Although long-term effects on improvement in mortality have not been shown in the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial, the only long-term outcome trial to date, many short-term studies indicate beneficial aquaretic- and hemodynamic-effects of the VRAs. In contrast to loop diuretics, these new agents tend to increase urine flow and the excretion of electrolyte-free water (so-called aquaresis) in patients with HF, without substantial changes in sodium or potassium excretion. This chapter reviews the role of AVP and copeptin in HF, and the treatment potential of VRAs in HF.

Keywords
Arginine vasopressin • Copeptin • Heart failure • Vasopressin receptor antagonist

Abbreviations

ACTIV	Acute and chronic therapeutic impact of a vasopressin 2 antagonist
ADHF	Acute decompensated heart failure
AE	Adverse event
AVP	Arginine vasopressin
BNP	B-type natriuretic peptide
BP	Blood pressure
BUN	Blood urea nitrogen
BW	Body weight
CHF	Congestive heart failure
CI	Cardiac index
CIBIS-II	Cardiac insufficiency bisoprolol study II
CO	Cardiac output
CV	Cardiovascular
CVP	Central venous pressure
DILIPO	DILutIonal hyponatremia
ECLIPSE	EffeCt of toLvaptan on hemodynamIc Parameters in Subjects with hEart failure
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
EVEREST	Efficacy of vasopressin antagonism in heart failure outcome study with tolvaptan
HF	Heart failure
HR	Heart rate

iv	Intravenous
KCCQ	Kansas City Cardiomyopathy Questionnaire
LV	Left ventricular
MAP	Mean arterial pressure
METEOR	Multicenter evaluation of tolvaptan effect on remodeling
NE	Norepinephrine
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
OD	Once daily
PARADIGM-HF	Prospective comparison of ARNI with ACEI to determine impact on global mortality and morbidity in heart failure
PCWP	Pulmonary capillary wedge pressure
p-Na^+	Plasma sodium
PVR	Pulmonary vascular resistance
QOL	Quality of life
RA	Renin activity
RALES	Randomized aldactone evaluation study
RAP	Right atrial pressure
RAS	Renin angiotensin system
SD	Standard deviation
SIADH	Syndrome of inappropriate antidiuretic hormone
s-K^+	Serum potassium
s-Na^+	Serum sodium
SOLVD	Studies of left ventricular dysfunction
SV	Stroke volume
SVR	Systemic vascular resistance
VRA	Vasopressin receptor antagonist
WRF	Worsening renal function

1 Introduction

The prevalence of heart failure (HF) in developed countries has risen and now constitutes approximately 1–2% of adults, and even >10% amongst individuals above 70 years of age (Mosterd and Hoes 2007). At the same time, advancements in pharmacological treatments in HF, with the introduction of angiotensin-converting enzyme inhibitors (SOLVD 1991), beta-blockers (CIBIS-II 1999), mineralocorticoid receptor antagonists (Pitt et al. 1999), and the recent introduction of a neprilysin inhibitor (McMurray et al. 2014), have reduced the rates of mortality and hospitalization in patients with HF with reduced ejection fraction (EF).

Despite the multiple new therapeutic options developed over the past three decades, some patients do not respond well to standard HF therapy and the overall prognosis of patients with HF remains poor (Maggioni et al. 2013). As such, there is an on-going need for new therapeutic agents.

Hyponatremia, defined as a plasma sodium ($p\text{-}Na^+$) concentration <136 mmol/L, is a potent predictor of outcome in HF (Bettari et al. 2012) and has been suggested to contribute to the continued high mortality rates due to its high prevalence up to 20–25% among patients hospitalized with HF (Ghali and Tam 2010; Bettari et al. 2012). For instance, in outpatients with chronic HF, hyponatremia (mean $p\text{-}Na^+ = 132.4 \pm 3.2$ mmol/L) was a significant independent predictor of hospitalization and death (Balling et al. 2011). The most common type of hyponatremia seen in HF patients is hypervolemic hyponatremia, which is characterized by elevated total body Na^+ and increased extracellular fluid volume in the presence of low $p\text{-}Na^+$ (Decaux et al. 2008). The underlying pathophysiology of hyponatremia in HF is incompletely understood, but involves neurohormonal dysregulation with an interplay of increased activity of the renin-angiotensin system (RAS), the sympathetic nervous system, and of arginine vasopressin (AVP) (Lilly et al. 1984; Packer et al. 1987), the latter of which is of current pharmacological interest due to the on-going investigations of therapeutic agents targeting the AVP receptors (Goldsmith and Gheorghiade 2005).

AVP is a peptide hormone, produced in the hypothalamus and stored in vesicles at the posterior pituitary for release into the bloodstream in response to changes in serum osmolality and non-osmotic factors such as hemodynamic and neurohormonal stimuli (Goldsmith 1988). The primary functions of AVP are: (1) vasoconstriction and cardiac remodeling through activation of the V_{1a} receptors in smooth blood vessels; (2) increase in adrenocorticotropic hormone and endorphins in response to stress through activation of the V_{1b} receptors in the pituitary corticotrophs; and (3) water retention (increased urine osmolality and decreased water excretion) by increasing aquaporin-2 water channels through activation of the V_2 receptors in the renal collecting tubules (Holmes et al. 2003; Balling and Gustafsson 2016). In addition to hyponatremia, elevated AVP levels affect hemodynamics (Yamane 1968; Goldsmith et al. 1986), and seem to correlate with severity and adverse outcome in HF patients (Szatalowicz et al. 1981; Goldsmith et al. 1983), thus making antagonists of the AVP receptor attractive new therapeutic agents. However, AVP's small size and very short half-life complicate measurements in research. Instead, C-terminal pro-vasopressin, copeptin, which is secreted equimolar to AVP, can be more easily measured, and has been used as a vasopressin surrogate (Morgenthaler et al. 2006).

The aims of this chapter are to review the role of AVP and copeptin in HF, and the treatment potential of AVP receptor antagonists (VRA) in HF.

2 Vasopressin and Copeptin in Heart Failure

AVP activity has been known since 1940 (Robinson and Farr 1940). However, due to the very low AVP concentration in human plasma, research in this field has been challenged. It was not until 1968, when Yamane extended his prior method of experimental diabetes insipidus rats for a bioassay of AVP to human plasma AVP (Yamane 1968), that research in this field progressed. Yamane showed that in 33%

of patients with chronic congestive HF (CHF) and New York Heart Association (NYHA) class III–IV, plasma AVP levels were increased, but normalized after improvement of hemodynamics with digitalis or diuretics. Furthermore, in half of these patients, right HF was correlated with the hemodynamic parameters right ventricular end-diastolic pressure, right atrial pressure (RAP), but not with pulmonary capillary wedge pressure (PCWP). Since then, studies have consistently shown significant relations between elevated AVP and changes in hemodynamics (Creager et al. 1986; Goldsmith et al. 1986), neuro-hormones (Goldsmith et al. 1983), serum osmolality (Creager et al. 1986), and outcome (Szatalowicz et al. 1981; Goldsmith et al. 1983) in HF patients; many of these studies will be reviewed throughout this chapter.

In more recent years, the AVP surrogate copeptin has been proposed as a useful alternative to direct measurement of AVP concentration due to its stable nature (Morgenthaler et al. 2006), and studies have consistently shown its reliability as an outcome predictor over the entire spectrum of HF independent of the current HF markers B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) (Stoiser et al. 2006; Neuhold et al. 2008; Balling et al. 2012). For instance, in a long-term observational study Neuhold and colleagues examined the predictive value of copeptin in 786 chronic HF patients with NYHA I-IV and EF $25 \pm 10\%$ (range 5–65%). Compared to BNP and NT-proBNP, copeptin was found to be the most potent single predictor of all-cause mortality in patients with NYHA II and III (both $p < 0.0001$), whereas in NYHA IV copeptin added additional independent information, but was inferior to serum Na^+ (s-Na^+) and especially estimated glomerular filtration rate (eGFR). Of note, patients with increased levels of copeptin had a worse prognosis. Balling and colleagues further extended these previous findings by showing that the relation between copeptin and outcome is independent of Na^+ and loop diuretic dose (Balling et al. 2012). A total of 340 chronic HF outpatients with EF $< 45\%$ followed for a median of 55 months were divided into three groups according to baseline copeptin tertiles. Those patients in the highest copeptin tertile had significantly more high-risk characteristics such as older age, lower EF, systolic blood pressure (BP), eGFR, and higher NT-proBNP and loop diuretic use. Baseline p-Na^+ was the same in the three groups. In multivariate models adjusted for confounders, including p-Na^+, loop diuretic dose, and NT-proBNP, copeptin was a significant predictor of hospitalization or death (hazard ratio 1.4; 95% confidence interval 1.1–1.9; $p = 0.019$), but did not predict mortality independently of NT-proBNP. In addition, copeptin concentrations did not predict future development of hyponatremia.

Taken together, the evidence suggests that the levels of AVP and its surrogate measure copeptin are increased in HF patients and linked to excess mortality; therefore, this patient group might especially benefit from adjunct therapy with therapeutic agents targeting the AVP receptors.

3 Activators of Vasopressin in Heart Failure

In healthy individuals, vasopressin is predominantly activated by osmotic factors such as small changes in plasma osmolality, resulting in tight regulation of serum osmolality and s-Na^+ levels (Schrier et al. 1979). Moreover, in contrast to animals such as dogs where small reductions of central venous pressure (CVP) or mean arterial pressure (MAP) readily increase AVP at constant osmolality (Goldsmith 1988), severe hypotension is required in humans before AVP will be released non-osmotically (Goldsmith et al. 1989). However, in HF and left ventricular (LV) dysfunction, non-osmotic mechanisms such as baroreceptor reflexes and neuro-hormones become more prominent (Schrier et al. 1979).

Many previous studies have tried to elucidate the drivers of AVP in HF. For instance, Goldsmith et al. (1983) measured plasma AVP, norepinephrine (NE), renin activity (RA), and numerous hemodynamic parameters in 31 patients with advanced HF and 51 comparably aged normal participants. In a sub-group of 11 patients, the response of AVP to hemodynamic changes induced by nitroprusside infusion and inhibition of RAS with captopril was also studied. The mean ($\pm$ standard error of the mean) AVP levels were significantly higher in the HF patients compared to the normal participants (9.5 $\pm$ 0.89 pg/mL vs. 4.7 $\pm$ 0.66 pg/mL, respectively; $p < 0.001$). However, the AVP levels did not correlate with any hemodynamic variables, and were increased to the same degree in patients with both low and normal cardiac index (CI). Nor did acute hemodynamic changes induced by nitroprusside or captopril influence AVP levels. Furthermore, AVP correlated neither with s-Na^+ nor NE, and only modestly with RA ($r = 0.53, p < 0.02$). The authors postulate that the lack of correlation between AVP and s-Na^+ in these HF patients indicates a possible disruption of the normal osmotic regulatory mechanism, which in turn could be primary or secondary to abnormalities in the non-osmotic control pathways. Furthermore, the lack of response of AVP to acute decreases in both cardiac filling pressure and MAP may indicate that these reflexes are unimportant modulators of AVP release in patients with CHF. In addition, the fact that AVP levels under basal conditions were not confined to patients with hypotension (all the participants were normotensive) or low CI diminishes the likelihood that these factors play a role in the maintenance of increased AVP in the chronic state.

It is known that nonspecific baroreflex loading maneuvers such as head-down tilt readily suppress stimulated AVP levels in normal humans (Goldsmith 1988). Whether this holds true in patients with CHF was tested by Goldsmith (1992). They hypothesized that increased AVP levels in patients with CHF would not respond normally to baroreflex loading. A total of 12 patients with CHF had AVP levels and osmolality determined in the supine position and after 15 min of 30° head-down-tilt; 8 patients underwent further study after osmotic stimulation with mannitol. AVP levels increased from 3.5 $\pm$ 1.0 to 6.5 $\pm$ 2.0 pg/mL and did not decrease. There was no significant suppression of AVP during head-down-tilt after mannitol infusion compared with values in a time control period. These results indicate an abnormality in baroreflex suppression of AVP in chronic CHF and

suggest that such a defect may contribute to long-term high levels of AVP in this condition.

The relation between hemodynamics and AVP has also been studied by augmentation of the system (Goldsmith et al. 1986). A total of 11 patients with CHF and NYHA II-IV received exogenously administered AVP, increasing plasma AVP from mean (standard deviation, SD) 6.5 ± 2.7 to 63 ± 39 pg/mL at the highest infusion rate. There was a progressive decrease in cardiac output (CO) and stroke volume (SV), with increases in systemic vascular resistance (SVR) and PCWP, but only minimal changes in heart rate (HR) and BP. Changes in CO, SV, and SVR were evident from the first infusion rate, which increased plasma AVP from 6.5 ± 2.7 to 9.9 ± 4.6 pg/mL. Thus, it appears that small increases in AVP are related to modest but significant adverse circulatory effects in CHF patients. A fall in CO, probably as a result of increased afterload, is seen at levels of AVP within the basal range found in CHF.

Taken together, drivers of AVP in HF are not clearly defined. However, previous work indicates that AVP is correlated to severity of HF, neuro-hormonal activation, hemodynamic derangement especially CO, and is not inhibited by normal baroreceptor stimulation.

4 Vasopressin Antagonists in Heart Failure

The first VRAs were developed as peptides in the 1960s. Although the results were promising in animal models, the VRAs lost their antagonistic effect and became partial agonistic when transferred to humans (Jackson 2006). It was not until the early 1990s that non-peptide VRAs were identified and used successfully in humans (Ohnishi et al. 1993). The VRAs act by directly blocking the activation of AVP receptor subtypes – V_{1a}, V_{1b}, and V_2. To date, the most extensively studied agents are the selective V_2 RAs tolvaptan, satavaptan, lixivaptan, and mozavaptan, and the non-selective V_{1a}/V_2 RA conivaptan (Decaux et al. 2008). Whereas oral tolvaptan is the only VRA marketed in Europe with the indication euvolemic hyponatremia in patients with the syndrome of inappropriate antidiuretic hormone secretion (SIADH), both tolvaptan and intravenous (iv) conivaptan have been approved in the United States and Japan for the treatment of both euvolemic and hypervolemic hyponatremia (Peri 2013; Jovanovich and Berl 2013). However, the optimal use of VRAs has not yet been determined, especially in patients with CHF. The effects of VRAs in patients with HF have been evaluated in mainly short-term clinical studies, while there is only one long-term clinical trial, namely the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST). Although beneficial long-term effects on improvement in mortality have not been shown in the EVEREST trial, many studies indicate beneficial short-term aquaretic- and hemodynamic-effects of the VRAs. In the following, some of these clinical trials will be reviewed. The main study characteristics and results of these clinical trials are summarized in the Appendix.

4.1 Aquaretic Effects

Loop diuretics are part of the standard HF therapy. However, their use is often associated with hypotension, electrolyte abnormalities, worsening renal function (WRF), and possibly increased mortality (Cooper et al. 1999; Domanski et al. 2003). In contrast to these diuretics, the selective V_2 RAs and the non-selective V_{1a}/V_2 RAs have shown promising results in clinical trials to increase urine flow and the excretion of electrolyte-free water in patients with HF, without substantial changes in sodium or potassium excretion, leading to their designation as aquaretic agents.

Tolvaptan is an oral, once daily (OD), non-peptide V_2 RA without intrinsic agonist properties (Yamamura et al. 1998; Hirano et al. 2000). The compound binds predominantly to the V_2 receptors in the kidney, resulting in increased production of dilute urine.

The short- and intermediate-term effects of tolvaptan were evaluated in HF patients hospitalized with persistent signs and symptoms of systemic congestion despite standard therapy in the Acute and Chronic Therapeutic Impact of a Vasopressin 2 Antagonist (ACTIV) in CHF study (Gheorghiade et al. 2004). A total of 319 patients with NYHA III–IV and EF $< 40\%$ were randomized to oral tolvaptan OD of either 30, 60, 90 mg or placebo, in addition to standard therapy including diuretics. One day after randomization, there was a non-dose-dependent reduction in body weight (BW), which was significantly higher in the combined tolvaptan group (median 1.8–2.1 kg; interquartile range 0.5–3.85 kg) compared with placebo (median 0.6 kg; interquartile range 0–1.6 kg; $p = 0.008$). Consistently, urine volume was significantly higher in the tolvaptan group (mean 4,056.2–4,175.2 mL; SD 2,050.8–2,695.4 mL) compared with placebo (mean 2,296.5 mL; SD 1,134.1 mL; $p < 0.05$). Both the reduction in BW and the increase in urine volume were maintained throughout the period of hospitalization. By discharge time, significantly fewer patients in the tolvaptan group compared with placebo reported dyspnea. Furthermore, tolvaptan produced an increase in p-Na^+ in patients with hyponatremia without causing hypokalemia or WRF, and was not associated with changes in HR or BP. Although underpowered and not prospectively designed to evaluate mortality alone, a post-hoc analysis of 60-day mortality showed a trend toward lower mortality in the tolvaptan group when compared with placebo, and with this trend reaching significance in patients with high levels of blood urea nitrogen (BUN) or severe systemic congestion. There were no significant differences in worsening HF at 60 days in the tolvaptan group when compared to placebo.

The first study to examine the effect of tolvaptan monotherapy compared to furosemide and the combination of tolvaptan and furosemide in patients with HF was carried out by Udelson et al. (2011). Symptomatic patients with CHF, NYHA II–III, and EF $< 40\%$ discontinued diuretics and received a low Na^+ diet (2 g/day). After a 2-day run in period, a total of 83 patients were randomized to receive either tolvaptan 30 mg, furosemide 80 mg, the combination of tolvaptan 30 mg and furosemide 80 mg, or placebo, OD for 7 days on top of standard therapy and no fluid restriction. Compared to baseline, on day 8 patients had a decrease in BW of

1.37 ± 1.61 kg in the tolvaptan group vs. 0.54 ± 1.59 kg in the furosemide group, 1.13 ± 1.49 kg in the combination group, and an increase of 0.72 ± 2.42 kg in the placebo group. Tolvaptan was just as effective as furosemide in decreasing BW. Although the combination therapy decreased BW more on day 2 than did furosemide monotherapy, this effect did not last. Increases in urine volume when compared to baseline were similar between the tolvaptan group and the combination group, and both greater than the furosemide group and placebo. Tolvaptan monotherapy caused an increase in s-Na^+ within the normal range, and there were no changes in serum potassium (s-K^+) or BP.

Satavaptan is another orally active and highly selective non-peptide V_2 RA (Serradeil-Le Gal et al. 1996) that corrects hyponatremia in patients with SIADH (Gines et al. 2008) or cirrhosis with ascites (Verbalis 2006). The efficacy and safety of the short- and long-term treatment of satavaptan were investigated in patients with dilutional hyponatremia (s-Na^+ = 115–132 mmol/L) in the DILutIonal hyPOnatremia (DILIPO) study (Aronson et al. 2011). A total of 118 patients (90 with CHF) were randomized to oral satavaptan OD of either 25 or 50 mg for up to 4 days or placebo, followed by non-comparative open label satavaptan therapy of 25 mg OD (the investigators adjusted the dose to between 12.5 and 25 mg depending on s-Na^+ levels) for up to 343 days. The response rate (Na^+ $\geq$ 135 mmol/L or an increase in Na^+ $\geq$ 5 mmol/L above baseline) was significantly higher with satavaptan 50 mg than with placebo (61.0 vs. 26.8%; $p = 0.0035$). In patients with CHF, similar trends were seen in response rates for satavaptan. Median times to response were significantly shorter in the treatment groups compared with placebo. S-Na^+ responses were maintained during the open-label therapy after a temporary study drug discontinuation period. A significant BW reduction was only seen in the satavaptan 25 mg group compared with placebo (1.4 kg, $p = 0.032$), and this was reproducible in the subgroup with CHF. Higher rates of adverse events (AEs) occurred with satavaptan 50 mg, including rapid correction of hyponatremia. Interestingly, the planned treatment discontinuation period in the present study revealed recurrence of hyponatremia in about 50% of the patients when satavaptan was discontinued, with a prompt increase in Na^+ levels when satavaptan treatment was re-started.

Conivaptan is a non-selective VRA with strong affinity for both the V_{1a} and V_2 receptors (Tahara et al. 1997). Its pharmacokinetics has been evaluated in patients with euvolemic or hypervolemic hyponatremia with s-Na^+ $\leq$ 130 mmol/L and with or without CHF (Mao et al. 2009). The study suggests that the pharmacokinetics of conivaptan 20 or 40 mg/day do not differ by volume status or the presence or absence of CHF and that adjustment in conivaptan dosage according to volume status or CHF status is not necessary. Instead, any dosage adjustment from 20 to 40 mg/day should be considered after the first day and only if s-Na^+ does not rise at the desired rate.

Conivaptan's potential aquaretic benefits in patients with HF were examined by Goldsmith et al. (2008, 2011). In a pilot study evaluating the efficacy and safety of conivaptan in acute decompensated HF (ADHF) patients (Goldsmith et al. 2008), a total of 170 patients with NYHA III–IV and mean (SD) EF = 29.5% (15.6) were

hospitalized for worsening HF and randomly assigned to treatment with conivaptan (20 mg loading dose, followed by two successive 24 h continuous infusions of 40, 80, or 120 mg/day) vs. placebo, on top of standard therapy. The two groups did not differ significantly in patient or clinician assessment of global and respiratory status at 48 h. Conivaptan at each dose increased urine output significantly more than placebo at 24 h, with a difference of 1–1.5 L without inducing electrolyte disturbances.

In another study, the renal effects of conivaptan were compared to furosemide, and the combination of conivaptan plus furosemide in patients with stable chronic HF and on standard medical therapy (Goldsmith et al. 2011). Using a cross-over design, a total of eight male patients with NYHA II–III, EF $< 40\%$ received iv furosemide (one-half of the patient's daily outpatient oral dose given as an iv bolus, maximum 80 mg), conivaptan (as a 20 mg iv bolus followed by a continuous infusion at 1.2 mg/h iv for 4 h), or the combination on three different study days at a minimum of 1-week intervals. The study showed no significant effects of conivaptan, furosemide, or the combination on any of the renal parameters. Conivaptan and furosemide similarly increased urine volumes; the effect of the combination was significantly greater. Furosemide, but not conivaptan, increased urinary Na^+ excretion, and the combination was significantly greater than after furosemide alone.

The effectiveness of V_2 RAs on volume unloading was investigated in outpatients with HF and volume overload (Ghali et al. 2012). A total of 170 patients with NYHA II–III and EF $< 40\%$ ($n = 57\%$) and EF $\geq 40\%$ ($n = 43\%$) were randomized in a 2:1 ratio to either lixivaptan 100 mg OD ($n = 111$) or placebo ($n = 59$), on top of standard therapy for 8 weeks. BW decreased significantly with lixivaptan compared to placebo. Orthopnea and dyspnea improved in the lixivaptan group compared to placebo. Furthermore, lixivaptan was well tolerated – thirst and polyuria occurred more frequently compared to placebo.

Taken together, the evidence from these clinical trials suggests that both the selective V_2 RAs and the non-selective V_{1A}/V_2 RAs increase p-Na^+ and promote aquaresis in patients with HF, without causing adverse changes in electrolytes or renal function. The effects of some of the VRAs appear to be non-dose-dependent and just as effective as loop diuretics but without causing the electrolyte abnormalities and WRF that is seen in the treatment with loop diuretics.

4.2 Hemodynamic Effects

The relation between V1a RAs and hemodynamics was recognized early on (Creager et al. 1986). In 10 men with CHF, NYHA III–IV, and average EF $= 24 \pm 10\%$, it was found that in those with above normal baseline levels of AVP ($n = 3$) there was a systemic vasodilatory response with a decrease in SVR and an increase in CI after infusion of a V1a RA.

The acute hemodynamic effects of conivaptan (Udelson et al. 2001) and tolvaptan (Udelson et al. 2008) were evaluated in patients with symptomatic

HF. In the first study (Udelson et al. 2001), a total of 142 patients with HF, NYHA III–IV, and EF $\leq$ 40% were randomized to short-term treatment with conivaptan at a single iv dose (10, 20, or 40 mg) or placebo. Compared to placebo, conivaptan 20 or 40 mg significantly reduced PCWP and RAP 3–6 h after iv administration. Furthermore, conivaptan significantly increased urine output in a dose-dependent manner during the first 4 h after the dose. Changes in CI, SVR, pulmonary vascular resistance (PVR), BP, and HR did not differ between the groups.

In the EffeCt of toLvaptan on hemodynamIc Parameters in Subjects with hEart failure (ECLIPSE) study (Udelson et al. 2008), a total of 181 patients with HF on standard therapy were randomized to tolvaptan 15, 30, 60 mg, or placebo. Compared with placebo, all doses of tolvaptan significantly reduced PCWP and pulmonary artery pressure, and the two lower doses also reduced RAP. There were no significant changes in CI, PVR, and SVR. Furthermore, tolvaptan increased urine output by 3 h in a dose-dependent manner.

The effects of long-term administration of oral tolvaptan on LV dilation and function in patients with HF and systolic dysfunction were examined in the Multicenter Evaluation of Tolvaptan Effect on Remodeling (METEOR) trial (Udelson et al. 2007). A total of 240 stable participants with NYHA II–III and EF $\leq$ 30% were randomized to long-term treatment of tolvaptan 30 mg OD or placebo, on top of standard background therapy. After one year of tolvaptan, there was a small reduction in LV end-diastolic volume index (-1.8 ± 10.7 mL/m^2), although the between group difference was non-significant ($p = 0.21$). Thus, in a well-treated population of stable HF patients, there was no significant effect of tolvaptan therapy on LV remodeling after one year of therapy.

To differentiate the hemodynamic effects of the selective V_2 RAs from the non-selective V_{1a}/V_2 RAs, Mondritzki et al. (2011) investigated the effects of conivaptan and tolvaptan in an acute HF model produced by rapid right ventricular pacing in Mongrel dogs. After 14 days, the animals were studied under general anesthesia to evaluate their hemodynamic and urine output response to either iv bolus 0.1 mg/kg of conivaptan or tolvaptan. Prior to randomization, the animals received an iv infusion of AVP to keep the levels controlled and with increasing rates. The study showed no significant differences in effect on MAP, the rate (dP/dt_{max}) of LV pressure rise during early systole, CVP, and urine output. CO increased after conivaptan and decreased after tolvaptan. SVR increased after tolvaptan and decreased after conivaptan. At the highest AVP dose of 4 mU/kg/min, a significant rise in SVR and a significant reduction in HR and CO could be observed when compared with the baseline state. Thus, it appears that in this acute HF model, conivaptan lowered whereas tolvaptan increased afterload. Therefore, dual blockage in the acute HF setting could be beneficial compared to selective V_2 blockage.

Taken together, the evidence from these clinical trials suggests that filling pressures are reduced with V_2 RAs because of an aquaretic effect. Furthermore, an effect on SVR and CO with V_{1a} RAs – at rest – is only evident when patients or experimental animals have significantly elevated AVP levels and deranged hemodynamics. In the available studies hemodynamics were studied at rest. Whether V_{1a}

receptor activation plays an even more important role during exercise is currently being evaluated in human studies.

4.3 The EVEREST Trial

The large-scale event-driven Phase III trial – EVEREST – was designed to explore both the short- and long-term effects of the V_2 RA tolvaptan when added to standard therapy in patients hospitalized with worsening HF and with symptoms of fluid overload. Whereas the short-term Clinical Status Trials (Gheorghiade et al. 2007) investigated inpatient signs and symptoms at day 7 or discharge (if earlier), the long-term Outcome Trial (Konstam et al. 2007) investigated morbidity and mortality during a median follow-up of 9.9 months. A total of 4,133 patients – pooled together from the short-term trials A ($n = 2{,}048$) and B ($n = 2{,}085$) – and aged $\geq$18 years with NYHA III–IV and EF $< 40\%$ were randomized within 48 h of hospital admission to tolvaptan 30 mg OD or placebo on top of standard therapy for a minimum of 60 days.

In the short-term study, the primary endpoint, consisting of a composite of changes in global clinical status based on a 100-point visual analog scale and BW at day 7 or discharge, showed greater improvement in the tolvaptan group compared to placebo ($p < 0.001$). The mean (SD) reduction in BW was greater with tolvaptan compared to placebo on day 1 (trial A: 1.71 (1.80) vs. 0.99 (1.83) kg; and trial B: 1.82 (2.01) vs. 0.95 (1.85) kg) and day 7 or discharge (trial A: 3.35 (3.27) vs. 2.73 (3.34) kg; and trial B: 3.77 (3.59) vs. 2.79 (3.46) kg); all $p < 0.001$. In addition, more patients receiving tolvaptan compared to placebo reported significant improvement in dyspnea on day 1 (for trials A and B, both $p < 0.001$) and a reduction in edema on day 7 or discharge (only for trial B, $p = 0.02$), whereas improvement in general clinical status did not differ between the groups. Furthermore, at day 1 and discharge, the tolvaptan group exhibited significantly greater corrections in s-Na^+ in those patients with hyponatremia at baseline (s-Na^+ $<$ 134 mmol/L). Serious AE frequencies were similar between the groups, and without WRF or hypotension in the tolvaptan group. An overall in-hospital mortality rate of 2.4% and 2.9% was observed in the tolvaptan and placebo groups, respectively.

Despite these positive short-term outcomes, tolvaptan did not affect long-term mortality and re-hospitalization. During follow-up, 537 (25.9%) patients in the tolvaptan group and 543 (26.3%) in the placebo group died from all-causes (hazard ratio 0.98; 95% confidence interval 0.87–1.11; $p = 0.68$). The composite of cardiovascular (CV) death or hospitalization for HF occurred in 871 (42.0%) patients in the tolvaptan group and 829 (40.2%) in the placebo group (hazard ratio 1.04; 95% confidence interval 0.95–1.14; $p = 0.55$). Secondary end points of CV mortality, CV death or hospitalization, and worsening HF were not different. The Kansas City Cardiomyopathy Questionnaire (KCCQ) overall summary score was not improved at outpatient week 1, but BW and s-Na^+ effects persisted long

after discharge. Tolvaptan caused increased thirst and dry mouth, but frequencies of major adverse events were similar in the two groups.

Taken together, the evidence from these three trials does not justify continuation of tolvaptan beyond the time of improvement in fluid balance and clinical status in patients hospitalized with worsening HF.

4.3.1 Potential Factors Influencing the Long-Term Outcome in EVEREST

Consistent evidence from previous work suggests that hyponatremia is a potent predictor of outcome in HF patients, and that hyponatremia to a large extent is due to excessive secretion of AVP, since blocking the V_2 receptors raises s-Na^+ by producing aquaresis (Goldsmith 2013). In the EVEREST trial, patients were not stratified by s-Na^+ in the randomization process, and thus it did not address the potential benefits of tolvaptan among patients with baseline hyponatremia. It was not until six years later that Hauptman and colleagues investigated the short- and long-term impact of treatment with tolvaptan in patients with and without hyponatremia in a post hoc analysis of the EVEREST trial (Hauptman et al. 2013). Of note, patients with hyponatremia had signs of more advanced disease at baseline with greater volume overload, with higher jugular venous pressure, and BUN compared to patients with normonatremia. In the placebo group, patients with hyponatremia (s-Na^+ $<$ 135 mmol/L; $n = 232$), compared with those with normonatremia ($n = 1{,}785$), had less relief of dyspnea despite receiving higher doses of diuretics (59.2 vs. 69.2% improved dyspnea; $p < 0.01$) and worse long-term outcomes. In the hyponatremia subgroup from the entire trial cohort ($n = 475$), tolvaptan was associated with greater likelihood of normalization of s-Na^+ than placebo (58 vs. 20% for day 1, and 64 vs. 29% at discharge; all $p < 0.001$), greater weight reduction at day 1 (0.7 kg difference; $p < 0.001$) and at discharge (0.8 kg difference; $p = 0.008$), and greater relief of dyspnea ($p = 0.03$). Among all hyponatremic patients, there was no effect of tolvaptan on long-term outcomes compared with placebo. In patients with pronounced hyponatremia (s-Na^+ $<$ 130 mmol/L; $n = 92$), tolvaptan was associated with reduced CV morbidity and mortality after discharge ($p = 0.04$). Interestingly, these patients also had significantly higher levels of AVP ($n = 68$; mean AVP 7.26 pg/mL, range 2.8–38) compared to the normonatremic cohort ($n = 2{,}762$; mean AVP 5.65 pg/mL, range 2.3–116; $p = 0.018$). Thus, it appears that hyponatremia is associated with a more unfavorable in-hospital course and remains a potent predictor of outcomes in patients hospitalized with worsening HF. Treatment of these patients with tolvaptan improved several signs and symptoms of HF and may improve outcomes relative to standard of care in those with severe hyponatremia. This, of course, would have to be proven in a dedicated trial.

Selective V_2 antagonism did not improve long-term outcome in the EVEREST patient population. Whether AVP levels independently influence outcome in these patients is important to elucidate, as this information may determine the utility of further investigation with unselective V_{1a}/V_2 RAs in HF. A post hoc analysis of the EVEREST trial was carried out by Lanfeur et al. (2013), where they examined the

association of baseline and follow-up AVP levels with outcome and the effect of tolvaptan in 3,196 patients with valid AVP measurements. Of note, patients with elevated baseline AVP (>8 pg/mL; $n = 694$) had higher levels of natriuretic peptide and aldosterone, higher HR, and lower EF. Higher AVP was not associated with baseline s-Na^+ ($p = 0.23$). In multivariate models adjusted for baseline covariates, elevated AVP levels were independently predictive of all-cause mortality (hazard ratio 1.33; 95% confidence interval 1.13–1.55) and the composite of CV mortality or HF hospitalization (hazard ratio 1.23; 95% confidence interval 1.08–1.39). Baseline AVP level did not help identify a subset of patients in whom tolvaptan was effective ($p = 0.52$). Interestingly, tolvaptan administration significantly increased the proportion of patients with elevated AVP ($p < 0.001$), but this had no effect on mortality (hazard ratio 0.95; 95% confidence interval 0.72–1.24). Thus, it appears that AVP levels are of limited importance in predicting the long-term effectiveness of V_2 antagonism in patients hospitalized with worsening HF. In fact, previous work has shown that maximal V_2 signaling occurs at relatively low levels of plasma AVP, whereas V_{1a} effects are dose-dependent (Schrier et al. 2006). Therefore, agents with more balanced inhibition of the V_{1a} and V_2 receptors may be of benefit in a population similar to the one studied in the EVEREST trial and should be evaluated, given the potential vascular and cardiac effects of the V_{1a} receptor.

4.3.2 Considerations for Future Trials

Taken together, a combined V_{1a}/V_2 blockage might be attractive compared to selective V_2 blockage. Based on the available evidence it seems unlikely that unselected, well-treated HF patients will have significant effect on long-term outcome with AVP blockade, and it remains unclear which patients to target in future trials. Logically, patients with activated AVP system should be targeted, but with respect to the V_2 component the EVEREST data suggest that hyponatremia, as a marker of disturbances in the AVP axis, was a better marker for effect than AVP measurements as such. This may not be the case for a V_{1a} RA or combined V_{1a}/V_2 RA. Also, the poor discriminating power of AVP in EVEREST might be related to analytical difficulties with AVP as described above, and it is possible that outcome might have been different if copeptin had been measured instead. To obtain an effect of V_{1a} blockade, patients with greater activation of the AVP system should probably be targeted to achieve an effect on hemodynamics, at least at rest.

Finally, endpoints should be relevant for the population studied. In patients with ADHF, who are often elderly and fragile, symptom relief, quality of life (QOL) and hospital length of stay might be relevant, whereas in patients with CHF survival, hospitalization risk and functional capacity (measured, for instance, by a 6-minute walk test) in addition to QOL appear to be important measures.

5 Conclusions

Hyponatremia is prevalent in HF patients and is considered a contributor to the sustained high mortality rates in these patients. Elevated levels of AVP play a role in hyponatremia and AVP and its surrogate, copeptin, are related to changes in osmolality, hemodynamics, neuro-hormones, as well as in overall outcome in HF patients. Thus, therapeutic agents targeting the AVP receptors, the VRAs, seem attractive. However, the optimal use of these agents has yet to be determined, especially in patients with CHF. Although long-term effects on improvement in mortality have not been shown in the EVEREST trial, many short-term studies indicate beneficial aquaretic- and hemodynamic-effects of the VRAs. In contrast to loop diuretics, these new agents tend to increase urine flow and the excretion of electrolyte-free water (so-called aquaresis) in patients with HF, without substantial changes in sodium or potassium excretion. However, many questions remain unanswered with regard to the potential use of VRAs in HF patients. Future trials should consider the potential efficacy of longer-term treatment with non-selective VRAs, the use of more stable analytical methods such as copeptin, and the use of a relevant target population as well as endpoints.

Appendix: Main Study Characteristics and Results of the Vasopressin Receptor Antagonist Clinical Trials

Study	Design	Treatment	Target receptor	Patient characteristics	Effect on primary (important secondary) endpoints
Creager et al. (1986), $n = 10$	Controlled	iv V1a RA of 0.5 mg administered over 5 min % diuretics 24 h prior to experiment	V_{1A}	Chronic NYHA III–IV average EF = 24 ± 10% only men	↓ SVR and ↑ CI when baseline AVP was above normal
Udelson et al. (2001), $n = 142$	Multicenter trial with a baseline inpatient phase, and a randomized, double-blind treatment phase	Single dose iv conivaptan (10, 20, or 40 mg) vs. placebo in a 1:1:1:1 ratio + standard therapy	V_{1A}/V_2	Chronic NYHA III–IV EF ≤ 40%	↓ PCWP, ↓ RAP and dose-dependent ↑ in urine output in the treatment group vs. placebo no difference in CI, SVR, PVR, BP, HR between the groups
The ACTIV in CHF study (Gheorghiade et al. 2004), $n = 319$	Multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-ranging, phase 2 feasibility trial	Oral tolvaptan OD (30, 60, or 90 mg) vs. placebo in a 1:1:1:1 ratio up to 60 days + standard therapy	V_2	Acute/chronic NYHA III–IV EF ≤ 40%	Day 1+ at discharge: non-dose-dependent ↓ BW and ↑ urine volume in the treatment group vs. placebo 60-day mortality: trend toward ↓ mortality in the treatment group
The EVEREST clinical status trials (Gheorghiade et al. 2007) trial A: $n = 2{,}048$, trial B: $n = 2{,}085$	Multicenter, randomized, double-blind, placebo-controlled, phase 3	Oral tolvaptan 30 mg OD vs. placebo in a 1:1 ratio for minimum 60 days + standard therapy	V_2	Acute NYHA III–IV EF ≤ 40%	At day 7/discharge: ↓ BW, ↑ Na^+, ↓ dyspnea, and ↓ edema in the treatment group vs. placebo general clinical status did not differ between the groups
The EVEREST outcome trial (Konstam et al. 2007), trials A and B: $n = 4{,}133$	Multicenter, randomized, double-blind, placebo-controlled, phase 3	Oral tolvaptan 30 mg OD vs. placebo in a 1:1 ratio for minimum 60 days + standard therapy	V_2	Acute NYHA III–IV EF ≤ 40%	No difference in mortality or re-hospitalization between groups KCCQ summary score not improved at outpatient week 1 sustained ↓ BW and ↑ Na^+ in the treatment group

The METEOR trial (Udelson et al. 2007), $n = 240$	Multicenter, randomized, double-blind, placebo-controlled	Oral tolvaptan 30 mg OD vs. placebo in a 1:1 ratio for 1 year + standard therapy	V_2	Chronic NYHA II–III EF $\leq$ 30%	No effect on LV remodeling, although small ↓ LVEDV index in the treatment group vs. placebo (no significant between group difference) favorable effect of treatment on mortality/HF hospitalization
The ECLIPSE trial (Udelson et al. 2008), $n = 181$	International, multicenter, randomized, placebo-controlled	Oral tolvaptan 15, 30, or 60 mg OD vs. placebo in a 1:1 ratio	V_2	Acute NYHA II–III EF $\leq$ 30%	↓ PCWP, ↓ PAP (all treatment groups), ↓ RAP (tolvaptan 15 and 30 mg) vs. placebo dose-dependent ↑ in urine output no difference in CI, PVR, SVR between groups
Goldsmith et al. (2008), $n = 170$	Multicenter, double-blind, dose-ranging pilot study	iv loading dose of conivaptan 20 mg, followed by two successive 24 h continuous infusions of 40, 80, or 120 mg/day vs. placebo in a 1:1:1:1 ratio + standard therapy	V_{1A}/V_2	Acute pulmonary congestion, respiratory symptoms NYHA III–IV mean (SD) EF = 29.5% (15.6)	↑ urine output, ↓ BW in the treatment group vs. placebo no difference in worsening HF, respiratory status at 48 hours, or electrolyte disturbances between the groups
Mao et al. (2009), $n = 203$	Multicenter, 4-day open-label	iv loading dose over 30 min of conivaptan 20 mg, followed by a continuous 4-day infusion of 20 or 40 mg/day	V_{1A}/V_2	CHF ($n = 90$) s-$Na^+ \leq$ 130 mmol/L euvolemic- or hypervolemic-hyponatremia	Conivaptan concentrations: highest after 30 min loading dose, declined during day 1, and were maintained by the continuous infusion no difference with regard to status of volume or CHF
Udelson et al. (2011), $n = 83$	Multicenter, randomized, double-blind, placebo-controlled, parallel group	Oral tolvaptan 30 mg, furosemide 80 mg, or tolvaptan 30 mg + furosemide 80 mg vs. placebo in a	V_2	Acute: signs of congestion (edema, rales) NYHA II–III EF $\leq$ 40%	Day 8: ↓ BW in all treatment groups, and ↑ urine volume in the two treatment groups with tolvaptan vs. placebo ↑ Na^+ within the normal range, no

(continued)

Study	Design	Treatment	Target receptor	Patient characteristics	Effect on primary (important secondary) endpoints
		1:1:1:1 ratio + standard therapy			change in K^+ or BP in the group with tolvaptan
Goldsmith et al. (2011), $n = 8$	Randomized, cross-over study	Three separate study days separated with a 7-day washout periods: iv furosemide (<80 mg), iv loading dose of conivaptan 20 mg, followed by a continuous infusion at 1.2 mg/h for 4 h, or the combination	V_{1A}/V_2	Chronic NYHA II–III EF $\leq 23 \pm 7\%$ only men	↑ urine volume in all treatment groups and ↑ urinary Na^+ excretion with furosemide therapy (combination > monotherapy) no difference in hemodynamics, neurohormonal levels, renal blood flow, or GFR
The DILIPO study (Aronson et al. 2011), $n = 118$	Multicenter, randomized, double-blind, placebo-controlled phase; followed by a 1-year open-label non-comparative phase	Oral satavaptan OD (25 or 50 mg) vs. placebo in a 1:1:1 ratio up to 4 days, followed by satavaptan 12.5–25 mg + fluid restriction 1–1.5 L	V_2	Chronic ($n = 90$) NYHA III–IV s-Na^+ = 115–132 mmol/L	Higher ↑ in Na^+, ↓ time to response, and ↑ BW reduction in the treatment group vs. placebo similar results in the CHF subgroup the increase in Na^+ was maintained long-term
Mondritzki et al. (2011), $n = 6$	Randomized, controlled	After iv infusion of AVP to keep its levels controlled, randomization to either conivaptan or tolvaptan, both iv 0.1 mg/kg bolus	V_2 vs. V_{1A}/V_2	Mongrel dogs were paced continuously at 220 beats/min after 14 days they underwent acute testing	↑ CO and ↓ SVR with conivaptan ↓ CO and ↑ SVR with tolvaptan no difference in effect on MAP, dP/dt_{max}, CVP, and urine output between the groups

Ghali et al. (2012), $n = 170$	Multicenter, randomized, double-blind, placebo-controlled, parallel-group, phase 2	Oral lixivaptan 100 mg OD vs. placebo in a 2:1 ratio for 8 weeks + standard therapy	V_2	Chronic NYHA II–III EF $< 40\%$ ($n = 57\%$) EF $\geq 40\%$ ($n = 43\%$)	Day 1 + weeks 1, 2, 4: ↓ BW in the treatment group vs. placebo improvement in orthopnea and dyspnea in the treatment group

iv intravenous, *RA* receptor antagonist, *NYHA* New York Heart Association, *EF* ejection fraction, *SVR* systemic vascular resistance, *CI* cardiac index, *AVP* arginine vasopressin, *PCWP* pulmonary capillary wedge pressure, *PAP* pulmonary artery pressure, *RAP* right atrial pressure, *PVR* pulmonary vascular resistance, *BP* blood pressure, *HR* heart rate, Na^+ sodium, *KCCQ* Kansas City Cardiomyopathy Questionnaire, *LV* left ventricular, *LVEDV* LV end-diastolic volume, *HF* heart failure, *SD* standard deviation, *CHF* congestive HF, K^+ potassium, *CO* cardiac output, *MAP* mean arterial pressure, *CVP* central venous pressure

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Mesenchymal Stem Cell Therapy for the Treatment of Heart Failure Caused by Ischemic or Non-ischemic Cardiomyopathy: Immunosuppression and Its Implications

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Abstract

HF patients with signs and symptoms of worsening heart failure (HF), despite optimal medical therapy, have a poor prognosis. The pathways contributing to HF are multiple, probably accounting, in part, for current treatment approaches not being more effective. Stem cells, particularly mesenchymal stem cells (MSCs), have a broad range of activities, making them particularly interesting candidates for a new HF therapeutic. This review presents an overview of the studies examining the efficacy of stem cell studies administered to HF patients, focusing mainly on MSCs. It examines the issues surrounding autologous vs. allogenic stem cells, the results of different routes of administration, and implications deriving from the belief that for stem cells to be effective, they must engraft in the myocardium and exert local effects. Since intravenous administration of stem cells leads to sparse cardiac engraftment, stem cell delivery

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2017_23

strategies have uniformly involved catheter-based delivery systems. This becomes problematic in a disease that will almost certainly require delivery of the therapeutic throughout the course of the disease. Importantly, it appears that a critical contributing cause of the progressive cardiac dysfunction experienced by HF patients is the existence of a persistent inflammatory response. Since MSCs exert potent anti-inflammatory effects through paracrine mechanisms, it is possible that intravenous delivery of MSCs may be therapeutically effective. If this concept is valid, it could lead to a transformational change in stem cell delivery strategies.

Keywords
Allogeneic • Heart failure • Inflammation • Intravenous • Mesenchymal stem cells (MSCs)

1 Introduction

As our population ages, there is an increasing prevalence of heart failure (HF) (Mozaffarian et al. 2015; Gheorghiade et al. 2006). Unfortunately, despite the advances in medical and device therapies made over the past several decades, HF continues to be a major health challenge, as unacceptably high mortality rates persist. Thus, there remains an important unfulfilled therapeutic niche (Kelkar et al. 2015).

Stem cells, because of their broad range of activities, represent a unique therapeutic approach to treating patients with HF, a condition involving multiple dysfunctional pathways. Despite the rapid application of the use of stem cells to treat patients with HF, beginning in 2001 (Menasche et al. 2001), we are still at the point articulated by Sanganalmath and Bolli in 2013: "Despite this rapid progress, however, many fundamental issues remain to be resolved and, to date, no cell therapy has been conclusively shown to be effective in patients with HF" (Sanganalmath and Bolli 2013).

This review focuses mainly on the use of mesenchymal stem cells (MSCs) in patients with HF who have depressed left ventricular (LV) ejection fraction (EF) consequent to non-ischemic or ischemic cardiomyopathy (NICM or ICM).

2 Why Mesenchymal Stem Cells?

There is a vast literature assessing the efficacy of stem cells, with enormous diversity in cell type, cellular characteristics, mode of harvesting and expanding the cells, and cell culture conditions, each variation leading to differentiation or lineage specification/selection. Among some of the more frequently studied stem cells are the following:

Bone Marrow Mononuclear Cells (BMMNCs) BMMNCs were among the first "stem cells" administered to patients with either AMI or HF. It is of importance to note that the cells harvested from the bone marrow are markedly heterogeneous, with less than 2% of the harvested cells having the characteristics of stem cells (Sanganalmath and Bolli 2013).

Mesenchymal Stem Cells As specified in the position statement of the International Society for Cellular Therapy (Dominici et al. 2006), MSCs are defined as (1) being plastic-adherent when maintained in standard culture conditions, (2) must express CD105, CD 73, and CD90 surface molecules, (3) lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface molecules, and (4) must differentiate into osteoblasts, adipocytes, and chondroblasts in vitro.

Hematopoietic Stem Cells (HPCs) CD34 and CD133 are traditional markers of HPCs. These cells are purified by culture techniques and are usually obtained from mobilization of stem cells using granulocyte colony-stimulating factor (G-CSF) or similar agent. Blood is then obtained from the patient, followed by isolation and expansion of HPCs.

Cardiosphere-Derived Stem Cells (CDCs) These stem cells are harvested from the heart of patients by endomyocardial biopsy and re-administered to the patient following isolation and expansion (Makkar et al. 2012). To qualify for patient administration, more than 95% of cells have to express CD105, and fewer than 5% can express CD45 (Makkar et al. 2012). The investigators using CDCs have now transitioned to using allogenic CDCs, rather than autologous.

As noted, clinical studies have not as yet demonstrated definitive proof of efficacy of any stem cell types. It is therefore understandable that there is a paucity of data comparing their relative efficacy. One recent preclinical study using a porcine model of chronic myocardial infarction demonstrated that MSCs delivered by transendocardial injection (two injections in the infarct zone and eight injections in the border zone) perform better than BMMNCs at improving LVEF (van der Spoel et al. 2015). Interestingly, swine initially treated with BMMNCs that did not experience an improvement in LVEF received a second series of injections with MSCs; this MSC treatment did improve LVEF (van der Spoel et al. 2015). Thus, MSC injection "rescued" LV function in pigs that did not improve following BMMNC injection. However, the reliability of these results are questionable given of the small number of pigs reported in each of the subgroups ($n = 5$ or 6).

Similar questionable results were reported by Tao et al (2015). In pigs that experienced an AMI 2 weeks previously, MSCs delivered by intramyocardial injection were superior to BMMNCs in regard to improving regional wall motion and vascular density, but LVEDV, LVESV, and LVEF were not improved by either therapy. Again, this study is limited due to the small number of pigs studied in each group ($n = 7$ or 8).

Direct comparison between MSC, HPC, and BMMNC therapy is totally lacking in adequately powered clinical trials. For example, we were only able to identify

one RCT comparing MSCs, BMMNCs, and placebo. In the TAC-HFT trial (Heldman et al. 2014), Heldman and colleagues compared clinical outcomes by randomizing 19 patients to transendocardial injection of autologous MSCs, 19 patients to transendocardial injection of autologous BMMNCs, and 21 patients to control. Both BMMNCs and MSCs improved within group 6 min walk distance and quality of life scores on the Minnesota Living with Heart Failure Questionnaire (MLHFQ), while there was no improvement in the control group (Heldman et al. 2014). However, there was no significant difference for either cell therapy group compared with control in regard to changes in New York Heart Association (NYHA) HF classification and LV function. There is currently an ongoing randomized controlled trial to compare autologous BMMNCs with allogeneic human umbilical cord-derived MSCs in patients with ischemic cardiomyopathy undergoing coronary artery bypass graft surgery (Can et al. 2015). As more RCTs are performed, comparison between different stem cell strategies may become available by pooling through patient-level meta-analysis (Gyongyosi et al. 2016).

However, at present there are no large, well-conducted preclinical or randomized controlled clinical trials that allow reliable efficacy comparisons to be made between MSCs, BMMNCs, HPCs, cardiospheres, or other stem cells in either patients with AMI or in patients with ICM or NICM. Will such a study ever be conducted? Practically, it is economically prohibitive for a company with a commercial interest in a particular stem cell type to initiate the very large randomized multi-armed trial that would be necessary to generate adequately powered comparisons between two or more different stem cell types. Moreover, the risk of such a study for the funding company/companies would be enormous, particularly when considering the very real possibility that it might not be the funding company's stem cell that emerges as efficaciously the best.

It therefore appears that such a study would most appropriately be within the purview of the NIH. But even then, the wisdom of deploying the enormous resources necessary to conduct a properly powered trial is highly questionable, especially since we still don't know which, if any, stem cell will be effective, at what dose, and by what means of administration. Because of these concerns, we believe that a definitive study permitting effectiveness comparisons among the different stem cell types will never be undertaken. Therefore, when more than one stem cell type receives regulatory approval for clinical application, it will fall to each physician to evaluate the outcome data of individual trials for each approved stem cell type and decide which stem cell and associated delivery method holds the greatest potential for clinical benefit.

Because of reasons that will be detailed below, we believe that there are particular characteristics inherent in MSCs that make them especially good candidates for eventual approval for clinical use in patients with AMI, or with ICM or NICM. The following therefore summarizes existing data leading to this conclusion.

3 Mesenchymal Stem Cell Therapy for Heart Failure: Evidence from the Clinical Trials

Much of the dilemma in assessing therapeutic efficacy of stem cells for heart failure arises from the significant heterogeneity seen among the different RCTs. Fisher and colleagues conducted an excellent comprehensive systematic review and meta-analysis of the clinical studies that evaluated different cell therapies for chronic ischemic heart disease and congestive heart failure and assessed the efficacy of these therapies in regard to different clinical endpoints (Fisher et al. 2016). Though the main analysis assessed the efficacy of all cell therapies for the combination of heart failure and chronic ischemic heart disease, the authors conducted multiple sub-analyses that separated the data for better granularity for subgroup comparison. For example, the authors assessed outcomes based on whether patients received BMMNCs, HPCs, or MSCs, route of cell administration, and imaging modality utilized to assess change in LV function (Fisher et al. 2016). For the purposes of this review, we also identified papers reporting results of MSC therapy in patients with NICM.

At the time of their systematic review and meta-analysis (Fisher et al. 2016), only 15 RCTs had been performed assessing cell therapy in heart failure due to ICM. After separating out studies that assessed the role of MSCs in heart failure, we found a paucity of high-quality RCTs. When further attempting to assess the role of MSCs in either ischemic or non-ischemic cardiomyopathy (including our search), the data are even more sparse. Table 1 provides a descriptive summary of all RCTs in patients with heart failure that utilized MSCs.

The first trial assessing MSC therapy in patients with ischemic cardiomyopathy was performed in patients undergoing concomitant percutaneous coronary intervention of a chronic total or subtotal occlusion of the left anterior descending artery (Chen et al. 2006). Autologous MSCs or placebo was administered via the lumen of an over-the-wire balloon following treatment of the lesion with placement of a drug-eluting stent. Though LVEF significantly improved at 3 months following intracoronary delivery of autologous MSCs from 26 $\pm$ 6 to 37 $\pm$ 9% while there was no significant improvement in the control group, the improvement in LVEF was not sustained (Chen et al. 2006). There was, however, an improvement in NYHA HF classification and a slight increase in the level of METs achieved during exercise treadmill testing. While encouraging, it must be emphasized that the patient cohort was small (22 control patients, 23 treated).

The POSEIDON trial (Hare et al. 2012) was published 6 years later and compared transendocardial injection of autologous MSCs vs allogeneic MSCs for treatment of patients with ischemic cardiomyopathy. Much of the challenge of this study was the inclusion of three different doses of MSCs (20×10^6 vs 100×10^6 vs 200×10^6), leading to small numbers of patients in each arm. Although both groups experienced a slight non-significant improvement in LVEF and LV volumes over time, the study lacked a control arm to determine whether the beneficial trend was related to MSC therapy or was part of the natural history of patients admitted to a well-conducted clinical trial with more careful adherence to medical therapy.

Table 1 Summary of randomized controlled trials assessing MSCs in patients with heart failure

Study	Patient population	Therapeutic groups		Route of administration	Outcomes		
Chen et al. (2006)	Chronic ischemic cardiomyopathy with chronic total or subtotal occlusion of the LAD treated with PCI and stenting	Autologous MSCs (5×10^6 MSCs)	Control	Intracoronary injection through the lumen of an over-the-wire balloon		Autologous MSCs ($n = 22$)	Control ($n = 23$)
					Death	2 (SCD)	4 (2 HF, 1 SCD, 1 VF)
					METS baseline to 12 months	5 ± 2 to 7 ± 2	5 ± 3 to 5 ± 3
					NYHA class baseline to 12 months	2.7 ± 0.8 to 1.4 ± 0.7	2.9 ± 0.3 to 2.4 ± 0.4
					LVEF (%) baseline	26 ± 6	23 ± 8
					3 months	37 ± 9	25 ± 4
					12 months	30 ± 4	30 ± 5
Hare et al. (2012)	Chronic ischemic cardiomyopathy with LVEF <50%	Allogeneic MSCs ($n = 15$) 20×10^6 cells ($n = 5$) 100×10^6 cells ($n = 5$) 200×10^6 cells ($n = 5$)	Autologous MSCs ($n = 15$) 20×10^6 cells ($n = 5$) 100×10^6 cells ($n = 5$) 200×10^6 cells ($n = 5$)	Transendocardial injection into ten LV sites		Allogeneic MSCs	Autologous MSCs
					Serious adverse events at 12 months	1	3
					Heart failure exacerbation at 12 months	2	4
					Δ 6 min walk (m) at 12 months	+65.8 (27.2–104.5, $p = 0.001$)	+19.7 (−19.0 to 58.3, $p = 0.31$)
					Δ VO2 Max (mL/kg/min) at 12 months	−0.6 (−2.7 to 1.4, $p = 0.39$)	−0.6 (−2.7 to 1.4, $p = 0.39$)

					Δ MLHFQ score at 12 months	−10.2 (−31.1 to 10.7, $p = 0.34$)	−13 (−22.6 to −3.3, $p = 0.009$)
					Δ NYHA class at 12 months	28.6% improved	50% improved
						57.1% no change	42.9% no change
						14.3% worsened	7.1% worsened
					LVEF (%) baseline to 12 months	27.85 (22.3–33.4) to 29.50 (24.1–34.9)	26.23 (20.26–32.20) to 28.53 (23.4–33.66)
					EDV (mL) baseline to 12 months	260.3 (211.2–309.3) to 243.7 (200.2–287.1)	300.9 (251.3–350.5) to 291.8 (235.5–348.1)
					ESV (mL) baseline to 12 months	192 (145.8–238.1) to 176 (135.5–216.5)	225.7 (178.5–272.9) to 213.6 (164.3–262.9)
Bartunek et al. (2013)	Chronic ischemic heart failure with baseline LVEF of 15–40%. No ischemic event in the 2 months prior to enrollment	Autologous cardiopoietic cells (mean 733 × 10^6 cells, range 605–1168 × 10^6 cells) (MSCs obtained from bone marrow aspiration were treated with "cardiogenic cocktail" of 5% platelet lysate-supplemented high glucose medium	15 control patients (no BM aspiration or sham procedure)	Transendocardial injection (average 18 injections per patient) guided by electromechanical mapping using the NOGA XP system		Autologous cardiopoietic cells ($n = 21$)	Control ($n = 15$)
					SAE at 24 months	1 died at 21 months from sepsis following heart transplant	2 patients died from progressive heart failure and sudden death at 18 and 20 months, respectively
					6 min walk (m) at baseline and 6 months	394 (346–442) to 456 (391–521)	419 (382–456) to 404 (350–458)
					Δ MLHFQ score at 6 months	−7.5 (−17.5 to 2.5)	+1 (−5 to 7)

(continued)

Table 1 (continued)

Study	Patient population	Therapeutic groups		Route of administration	Outcomes			
		containing TGF-β, BMP-4, Activin A, FGF-2, cardiotrophin, a-thrombin, and diaminopyrimidine) 32 patients randomized to cell therapy. 21 patients received cell therapy			Δ NYHA class at 6 months	30% improved	25% improved	
						65% no change	60.9% no change	
						5% worsened	13% worsened	
					LVEF (%) at baseline and 6 months	27.5 (25.5–29.5) to 34.5 (32.5–36.6) ($p < 0.0001$)	27.8 (25.8–29.8) to 28.0 (26.1–30.6)	
					Δ ESV (mL) at 6 months	−24.8 (−30.7 to −18.9)	−8.8 (−16.4 to −1.2)	
					Δ EDV (mL) at 6 months	−18 (−25.5 to −10.5)	−9 (−15.0 to −3.6)	
Heldman et al. (2014)	Chronic ischemic cardiomyopathy with LVEF <50% ($n = 59$)	Autologous MSCs (randomized = 22) vs placebo ($n = 11$)	Autologous BMMNCs (randomized = 22) vs placebo ($n = 10$)	Transendocardial injection		Autologous MSCs ($n = 19$)	Autologous BMMNCs ($n = 19$)	Placebo ($n = 21$)
					Death at 12 months	1	0	1
					SAEs at 12 months	6	6	8
					6 min walk (m) at baseline and 12 months	+32.6 (−4.6 to 69.7, $p = 0.12$)	+16.9 (−14.2 to 48.0, $p = 0.30$)	+6.3 (−31.4 to 44.0, $p = 0.70$)

					Δ MLHFQ score at 5 months	−11.6 (−23.7 to 0.5, $p = 0.006$)	−15.8 (−28.6 to −3.0, $p = 0.04$)	−4.6 (−20.1 to 10.7, $p = 0.69$)
					Δ NYHA class at 12 months	35.3% improved	52.9% improved	43.8% improved
						41.2% unchanged	35.3% unchanged	50% unchanged
						23.5% worsened	11.8% worsened	6.2% worsened
					Δ LVEF (%) at 6 months	0 (−7.5 to 7.5)	Approx +2.5 (approx −8 to 13.5)	Approx +4.5 (approx −3 to 12)
Mathiasen et al. (2015)	Chronic ischemic cardiomyopathy with LVEF ≤45% and had NYHA class II–III	Autologous MSCs (randomized = 40, treatment = 39) ($77.5 \pm 67.9 \times 10^6$ MSCs)	PBS control NOGA injections (randomized = 20, treatment = 20)	Transendocardial injection (10–15/patient) with electromyocardial mapping using the NOGA-XP system		Autologous MSCs ($n = 39$)	Control ($n = 20$)	
					Death	1	1	
					HF worsening	6	2	
					NYHA Class	Significant improvement	Significant improvement	
					6 min walk test	Significant improvement	Significant improvement	
					Δ LVEF (%) at 6 months	+5.0 (3.7 to 6.2, $p < 0.0001$)	−1.3 (−3.0 to 0.5, $p = 0.14$)	
					Δ LVESV (mL) at 6 months	−7.6 (−11.8 to −3.4, $p = 0.001$)	+5.4 (−0.4 to 11.2, $p = 0.07$)	

(continued)

Table 1 (continued)

Study	Patient population	Therapeutic groups		Route of administration	Outcomes		
					Δ LVEDV (mL) at 6 months	+8.1 (1.9 to 14.3, $p = 0.012$)	+2.6 (−1.5 to 6.7, $p = 0.19$)
Perin et al. (2015)	Non-ischemic or ischemic cardiomyopathy NYHA class II or III HF with LVEF <40%	Allogeneic immunoselected (STRO-3$^+$) mesenchymal precursor cells. (25×10^6 MPCs $n = 15$) (75×10^6 MPCs $n = 15$) (150×10^6 MPCs $n = 15$)	Control with mock mapping/injection procedures ($n = 15$)	Transendocardial injections (15–20/patient)		Allogeneic MPCs (pooled $n = 45$)	Control ($n = 15$)
					Procedural events	3 (1 with hypotension, 1 with pericardial tamponade, and 1 retroperitoneal hematoma	0
					Cardiac death at 36 months	2	3
					HF exacerbation at 36 months	9	8
					6 min walk (m) at baseline and 12 months	401.6 ± 96.4 to 427 ± 115.1	319.3 ± 121.4 to 346.6 ± 121.8
					Δ NYHA class at 12 months	55.6% improved	53.4% improved
						40% unchanged	40% unchanged
						None worsened	None worsened
					Δ MLHFQ score at 12 months	−15.2 ± 19.8	−23.8 ± 14.6
					Δ LVEF (%) at 12 months	+1.2 ± 8.5	−0.4 ± 8.9

					Δ LVESV (mL) at 12 months	+2.2 ± 27.3	+3.5 ± 14.1
					Δ LVEDV (mL) at 12 months	+6.8 ± 37.4	−0.4 ± 22.9
Hare et al. (2017)	Chronic non-ischemic dilated cardiomyopathy with LVEF <40% and LVEDD >5.9 cm in men and >5.6 cm in women or LVEDV >125 mL/m^2	Allogeneic MSCs ($n = 18$) (100 × 10^6 cells)	Autologous MSCs ($n = 16$) (100 × 10^6 cells)	Transendocardial injection into ten LV sites		Allogeneic MSCs ($n = 18$)	Autologous MSCs ($n = 16$)
					SAE at 12 months	5	10
					MACE at 12 months	3	9
					Δ 6 min walk (m) at 12 months	+37 (2.0–72.0, $p = 0.04$)	+7.3 (−47.8 to 33.3, $p = 0.71$)
					MLHFQ score at 12 months	Decreased ($p = 0.002$)	Decreased ($p = 0.17$)
					Δ NYHA class at 12 months	66.7% improved	27.3% improved
						33.3% Unchanged	60.2% unchanged 12.5% worsened
					Δ LVEF (%) at 12 month	+8.0 (2.8 to 13.2, $p = 0.004$)	+5.4 (−1.4 to 12.1, $p = 0.12$)
Bartunek et al. (2016)	Chronic ischemic heart failure with LVEF ≤35% and NYHA class ≥II	Autologous cardiopoietic cells (mean 733 × 10^6 cells, range 605–1168 × 10^6 cells) (see above for details on cardiopoietic cells)	Sham control procedure (randomized = 158, procedure = 151)	Transendocardial injection (median 19 injections/ patient) guided by electromechanical mapping using the NOGA XP system		Autologous cardiopoietic cells ($n = 120$)	Sham procedure control ($n = 151$)
					Procedural complications	14 total 4 pericardial effusions (tamponade in 3) 4 with VT	2

(continued)

Table 1 (continued)

Study	Patient population	Therapeutic groups		Route of administration	Outcomes		
		(randomized = 157, injection = 120)				3 with new LBBB 1 with aortic dissection 1 TIA 1 access site thrombosis	
					CV mortality through 39 weeks	11 (9.2%)	12 (7.9%)
					Patients with 1 HF Exacerbation	11 (9.2%)	14 (9.3%)
					Patients with ≥2 HF Exacerbations	9 (7.5%)	9 (6%)
					≥10-point improvement in MLHFQ	64 (59.3%)	66 (48.5%)
					No meaningful change in MLHFQ	37 (34.3%)	60 (44.1%)
					≥10-point deterioration in MLHFQ	7 (6.5%)	10 (7.4%)
					≥40 m improvement in 6 min walk	50 (46.3%)	40 (30.5%)

					No meaningful change in 6 min walk	39 (36.1%)	69 (52.7%)
					≥40 m deterioration in 6 min walk	19 (17.6%)	22 (16.8%)
					≥4% absolute improvement in LVEF	69 (67.6%)	82 (66.1%)
					No meaningful change in LVEF	28 (27.5%)	33 (26.6%)
					≥4% absolute deterioration in LVEF	5 (4.9%)	9 (7.3%)
					≥15 mL improvement in LVESV	51 (50%)	56 (45.2%)
					No meaningful change in LVESV	33 (32.4%)	36 (29%)
					≥15 mL deterioration in LVESV	18 (17.6%)	32 (25.8%)

(continued)

Table 1 (continued)

Study	Patient population	Therapeutic groups		Route of administration	Outcomes		
Butler et al. (2017)	Non-ischemic cardiomyopathy with LVEF<40% and absent late gadolinium enhancement on CMR	Ischemia-tolerant allogeneic MSCs grown under chronic hypoxia (5% O^2) at 1.5×10^6 MSCs/kg (crossover study: 10 patients initially treated with cell therapy and then 12 placebo-treated patients received cell therapy after 90 days	Lactated Ringer's control at 1 mL/kg	Intravenous infusion		Allogeneic MSCs ($n = 22$)	Placebo ($n = 12$)
					All-cause death	0	0
					SAE	0	0
					Δ NYHA class at 90 days	37.5% improved	8.3% improved
						62.5% unchanged	91.6% unchanged
					Δ 6 min walk (m) at 90 days	+27.4 (0.3 to 54.5, $p = 0.05$)	−10.8 (−38.7 to 17.0, $p = 0.45$)
					Δ LVEF (%) at 90 days	+2.31, $p = 0.02$	+1.62, $p = 0.13$
					Δ LVESV (mL) at 90 days	−16.60, $p = 0.02$	−8.90, $p = 0.27$
					Δ LVEDV (mL) at 90 days	−17.86, $p = 0.04$	−10.56, $p = 0.22$

This same limitation is also seen in the POSEIDON-DCM trial (Hare et al. 2017) which tested the relative safety and efficacy of transendocardially injected autologous vs. allogeneic MSCs in patients with non-ischemic dilated cardiomyopathy. The total cohort tested was 37 patients. Although greater clinical benefit was reported for patients treated with allogenic vs. autologous MSCs, there was no control group in the study. Thus, we cannot be certain whether an actual improvement occurred in the allogenic vs. the autologous MSC group, or whether a deterioration in function occurred in the autologous MSC group that did not occur in the allogenic group.

The next RCT to compare autologous MSCs with control was the C-CURE trial, which utilized cardiopoietic stem cells (MSCs obtained from bone marrow aspiration treated with "cardiogenic cocktail") delivered by transendocardial injection. Patients with chronic ischemic heart failure treated with cardiopoietic cells experienced a significant increase in LVEF and decrease in LV end-systolic and end-diastolic volumes with a trend toward improved 6 min walk distance and MLHFQ scores (Bartunek et al. 2013). These encouraging findings led to the recently published CHART-1 trial, which again compared cardiopoietic stem cells delivered by transendocardial injection vs sham procedure in patients with chronic ischemic heart failure – but with very different results. This large multinational, multi-center RCT demonstrated no significant benefit from cardiopoietic stem cells compared with control in regard to functional status or LV function (Bartunek et al. 2016). However, there was a trend for benefit in patients with large end-diastolic volumes.

It is important to note that patients in the control arm also underwent a sham procedure, which was absent in many earlier studies. This allowed an assessment of adverse outcomes associated with transendocardial injection of stem cells. Fourteen patients among the 120 that received transendocardial injection of cardiopoietic stem cells experienced significant complications from the procedure, including pericardial effusion with tamponade, ventricular tachyarrhythmias, transient ischemic attack, and vascular complications (Bartunek et al. 2016).

Perin and colleagues (2015) also found no benefit from allogeneic immunoselected (STRO-3^{+}) mesenchymal precursor cells when delivered by transendocardial injection. When compared with control, the mesenchymal precursor cells failed to significantly improve functional outcomes such as 6 min walk, MLHFQ scores, and NYHA HF classification; it also failed to improve LVEF or LV volumes (Perin et al. 2015). This study, as with the CHART-1 trial, demonstrated important adverse outcomes from the transendocardial injection technique. Thus, 3 of 45 patients in the cell therapy arm experienced procedure-related complications.

The MS-HF trial of patients with ischemic cardiomyopathy utilized autologous MSCs and demonstrated MSC administration significantly improved LVEF and reduced LV end-systolic volume. Despite these beneficial effects on LVEF and LVESV, MSC injection, puzzlingly, significantly *increased* LV end-diastolic volume (Mathiasen et al. 2015). These disparate changes make it difficult to interpret the effects of MSCs on LV function. A very interesting point emerging from this study relates to "beneficial" changes that can occur just because patients participate

in a carefully conducted clinical trial. Thus, 6 min walk distance and NYHA HF class significantly improved – but improvement was seen in *both* MSC therapy and control arms (Mathiasen et al. 2015). These results emphasize how critical it is for studies to have a control arm. While LV function may improve with cell therapy, patients in randomized controlled trials are optimized on medical therapy and receive closer follow-up, changes that could in themselves lead to improved symptoms and even to improved LV function.

Butler and colleagues recently published a RCT utilizing a crossover design comparing allogeneic MSCs, which were grown under chronic hypoxic conditions (5% O_2), vs placebo control in patients with NICM (Butler et al. 2017). Importantly, the MSCs were administered intravenously. Because very few intravenously administered MSCs successfully engraft in the heart, any beneficial effects emanating from the MSCs would most likely derive from their paracrine effects. These salutary effects would probably involve, at least partly, paracrine-related anti-inflammatory actions (Kelkar et al. 2015; Eliopoulos et al. 2005) (see below). MSC-treated patients significantly improved 6 min walk distance and Kansas City Cardiomyopathy Clinical summary and functional status scores (Butler et al. 2017). There also was a trend toward improvement in LV function in the MSC-treated patients.

That paracrine-related anti-inflammatory effects contributed to the beneficial effects found is suggested by the finding that patients treated with MSCs had a significant reduction in circulating natural killer (NK) cells at 30 days, and that there was a significant inverse correlation found between NK cells and LVEF. Thus, patients with the greatest reduction in peripheral NK cells experienced the largest increase in LVEF (Butler et al. 2017). The benefits seen in this study with intravenous administration of MSCs raise the question of whether targeted delivery of cells to the heart is necessary, especially given the safety issues identified with this route of administration (Bartunek et al. 2016).

4 Autologous vs Allogeneic MSCs

There has been a major debate in the stem cell literature arising over the use of autologous vs. allogenic cells. Use of autologous cells (cells obtained from the patient being treated) means that the stem cells administered to the patient are "old" and have resided in an environment of serious disease. For example, autologous MSCs in these studies are obtained from patients who are usually in the later decades of life and have heart failure. Several studies have demonstrated that aging, risk factors for coronary artery disease, and heart failure diminish the functional capacity of stem cells (Kubo et al. 2016; Dimmeler and Leri 2008; Walter et al. 2005). Thus, allogeneic MSCs harvested from young healthy volunteers should have greater beneficial effects than autologous cells harvested from patients with heart failure, although there is still some controversy about this conclusion (Golpanian et al. 2015; Lorkeers et al. 2015).

Another consideration relating to the relative advantage of allogeneic MSCs is that use of allogeneic MSCs would eliminate the need for patients to undergo bone marrow aspiration to harvest MSCs. In addition, once a decision is made to administer stem cells to a patient, use of allogeneic MSCs avoids the long delays inherent in administering autologous cells, which entails harvesting cells from the patient, followed by the long process of isolating and expanding the autologous MSC population until a sufficient number of cells are available for injection.

5 Potential for Immune Rejection of Allogeneic Cells

An important issue that must be considered when injecting allogeneic cells into a recipient is whether an immune response to the cells develops that leads to rejection of the cells, thereby obviating any potential for beneficial effects. It has been assumed that MSCs are immunoprivileged and are capable of avoiding immune detection. However, allogeneic MSCs have been shown to induce an immune response that leads to a cytotoxic humoral response (Poncelet et al. 2007; Klyushnenkova et al. 2005), and in the randomized controlled trial by Perin and colleagues (Perin et al. 2015), 5 of 43 patients who received allogeneic mesenchymal precursor cells (STRO-3^+) developed donor specific HLA reactive antibodies. On other hand, evidence suggests that while human MSCs may initiate an alloreactive T cell response, MSCs actively inhibit T cell proliferation through suppression by production of anti-inflammatory cytokines (Klyushnenkova et al. 2005).

The answer to this conundrum has been addressed by several studies. As reviewed by Ankrum et al. (2014), allogenic MSCs administered to patients, and MSCs administered cross-species (human MSCs administered to mice), do not persist indefinitely. These authors postulated it is likely that an active immunological process is responsible for this limited persistence. Despite this lack of persistence, suggesting that MSCs are not in reality immune privileged, it appears MSCs have the capacity for *relative immune avoidance,* resulting in a delay of rejection. Ankrum and colleagues discussed the results of a study in which the relative persistence of allo-fibroblasts vs. allo-MSCs was compared. The study showed that fibroblasts died by day 10 and MSCs by day 20 – in other words, although the MSCs were not immune privileged, they had some protection against immune rejection (the authors refer to this as "immune-evasive") resulting in longer persistence than the non-MSCs. This concept was also advanced and documented by Galipeau et al. (2005, 2015). These investigators used murine MSCs transfected with the gene expressing erythropoietin as a reporter for assessing persistence of MSC functionality. MSCs were injected subcutaneously in either major histocompatibility complex (MHC)-mismatched allogeneic or matched syngeneic mice. Although expression of erythropoietin (as assayed by measuring hematocrit) lasted longer in the syngeneic mice, the mismatched MSCs still manifested an erythropoietin response for over 30 days.

More recently, we have demonstrated that human MSCs administered intravenously into mice with an AMI and in mice with ischemic cardiomyopathy persist and remain viable for at least 3 weeks (Luger et al. 2017). Most importantly, this time-period was sufficient for the MSCs to improve left ventricular function and to exert immunomodulatory effects. Thus, it appears that although MSCs are not immune privileged and eventually are eliminated from mismatched hosts through immune responses, they still can exert therapeutic actions as long as the relevant activities of the MSCs need only a limited time to produce their effects. Such a mechanism has been referred to as a "hit-and-vanish" or "hit and run" mechanism (Ankrum et al. 2014; Chinnadurai et al. 2015).

6 Best Route of Administration for MSCs: Transendocardial, Intracoronary, or Intravenous?

Until recently, the therapeutic benefit of stem cells was thought to derive from local delivery of stem cells to damaged myocardium that, once delivered, either directly transdifferentiate into functional myocardium or stimulate resident myocardial stem cells to expand and repopulate the heart with functioning healthy myocytes (Sanganalmath and Bolli 2013; Bolli et al. 2011; Malliaras et al. 2014; Yee et al. 2014; Telukuntla et al. 2013). Thus, myocardial retention of a large number of stem cells with regenerative capacity would be required. Since intravenous delivery of stem cells results in very low cellular engraftment in the damaged myocardium (Freyman et al. 2006), catheter or surgical-based intracoronary, transendocardial, or intramyocardial stem cell delivery have been, to date, the dominant delivery strategies utilized (Sanganalmath and Bolli 2013).

While there is a prior small non-randomized study assessing intracoronary vs intramyocardial injection in ICM (Chin et al. 2011), there has been only one prospective randomized controlled trial comparing intracoronary vs. transendocardial delivery of stem cells in patients with NICM (Vrtovec et al. 2013). The investigators administered CD34+ hematopoietic stem cells and compared the effects of the two delivery strategies on myocardial retention and left ventricular function. A total of 40 patients were randomized to the two therapies. Transendocardial delivery of stem cells resulted in better retention and greater improvement in LV function compared with intracoronary delivery in patients with non-ischemic dilated cardiomyopathy (Vrtovec et al. 2013). However, as demonstrated in the CHART-1 RCT, transendocardial injection of cardiopoietic stem cells to patients with ischemic cardiomyopathy is not without risk, as 11.7% of these patients receiving cell therapy had procedural complications (Bartunek et al. 2016).

As noted, and elaborated on below, recent studies suggest that direct delivery of MSCs to the heart may not be necessary, since data now indicate that the intravenous delivery of MSCs may improve outcomes (Butler et al. 2017; Luger et al. 2017). The mechanisms by which this occurs is covered in more detail in the next section. Importantly, however, because it is probable that no therapeutic, including stem cells, will "cure" the disease with a single administration, there is a need for

repeated administration of any effective therapeutic to treat HF. This need for repeated administration poses a major practical limitation to catheter or surgery-based delivery strategies for stem cell therapy in patients with HF, an issue that does not exist with intravenous delivery, if this route of administration is shown to be therapeutically effective.

7 Anti-Inflammatory Effect of MSCs

The hypotheses we have explored relating to stem cell therapeutics for cardiovascular disease are predicated on very different paradigms that have informed the design of most clinical studies to date. First, there is a growing body of data suggesting that the presence of a persistent inflammatory response is one of the mechanisms leading to progressive cardiac dysfunction both in patients experiencing AMI and those with NICM or ICM (Kelkar et al. 2015; Westman et al. 2016; Nahrendorf et al. 2015; Orn et al. 2012; Deswal et al. 2001; Wrigley et al. 2011; Briasoulis et al. 2016). Second, compelling evidence now exists demonstrating that adult stem cells neither have the capacity to transdifferentiate into functioning myocytes, nor can they stimulate transdifferentiation of sufficient numbers of resident stem cells to restore clinically important myocardial functionality (Phinney and Prockop 2007; Caplan and Dennis 2006; Peng et al. 2016). Rather, if stem cells do improve myocardial function, strong evidence suggests that any improvement would derive from the cells' capacity to stimulate reparative processes and inhibit pathways leading to progressive myocardial dysfunction via paracrine effects (Kelkar et al. 2015; Westman et al. 2016; Peng et al. 2016).

Importantly, MSCs have long been known to have potent paracrine-related anti-inflammatory activities (Kelkar et al. 2015; Phinney and Prockop 2007; Caplan and Dennis 2006; Peng et al. 2016). Consideration of these two concepts together – the causal role of inflammation contributing to progressive cardiac dysfunction, and MSCs having the capacity to secrete potent anti-inflammatory molecules–led to the hypothesis that intravenous administration of MSCs may lead to improved clinical outcomes. It is postulated that MSCs, through a complex array of paracrine-derived activities, exert anti-inflammatory effects and both a systemic and local level within the heart.

As previously mentioned, we have recently demonstrated that human MSCs administered intravenously into mice with ischemic cardiomyopathy significantly improved left ventricular function (Luger et al. 2017). One of the important mechanistic findings was that the intravenously administered MSCs caused potent anti-inflammatory effects. One such effect was a decrease in splenic and cardiac NK cells (Luger et al. 2017). This is of great interest because NK cells are key regulators of both the innate and adaptive immune responses in cardiovascular disease (Ong et al. 2016). That the MSC-induced decrease in NK cells played a causal role in the improved cardiac function produced by intravenous MSC infusion was strongly suggested by the finding that NK cell depletion, independent of MSC effect (achieved by administering an anti-NK cell antibody), significantly reduces

infarct size and improves LV function (Luger et al. 2017) in AMI. Interestingly, MSCs have previously been shown to significantly decrease NK cell activity and suppress inflammation (Sotiropoulou et al. 2006; Spaggiari et al. 2008; Lankester et al. 2010). The finding that MSC-mediated NK cell reduction following intravenous administration of MSCs correlates with improvement in LV function in patients with NICM (Butler et al. 2017) is very provocative and requires further investigation.

Further evidence that intravenous MSC administration exerts important anti-inflammatory effects that could lessen inflammation-induced tissue injury was provided by examining the effects of MSCs on neutrophil populations. Neutrophils have a complex role in myocardial infarction and progression to heart failure (Swirski and Nahrendorf 2013; Horckmans et al. 2016). Neutrophils are one of the first cells infiltrating the myocardium following ischemic injury, contributing to tissue damage by releasing matrix-degrading enzymes and reactive oxygen species. In addition, neutrophils appear to be involved in adverse left ventricular remodeling (Carbone et al. 2013). We found intravenously administered MSCs decrease neutrophils in the myocardium following AMI (Luger et al. 2017).

MSCs also alter the balance between peripheral and resident cardiac myeloid cell populations that orchestrate repair following MI and modulate LV remodeling (Ben-Mordechai et al. 2013; Lu et al. 2015). Ismahil and colleagues (Ismahil et al. 2014) demonstrated an increase in splenic size in a murine model of chronic ischemic heart failure accompanied by a marked depletion of splenic proinflammatory monocytes and an increased cardiac monocyte population. Interestingly, splenectomy not only decreased cardiac macrophages and dendritic cells, but also attenuated the adverse LV remodeling. This supports an integral role for the spleen in chronic heart failure. Though the importance of the cardiosplenic axis was confirmed in patients with acute MI (Emami et al. 2015), we are unaware of any published studies examining the cardiosplenic axis in patients with chronic heart failure.

The potential of intravenously administered MSCs as a treatment for NICM was examined by Butler and colleagues (Butler et al. 2017), as detailed above. The results of this study, demonstrating improved clinical outcomes associated with MSC-induced immunomodulatory effects, suggest that the paracrine-related anti-inflammatory effects of MSCs may serve as a major pathway by which MSCs improve LV function in patients with cardiomyopathy (Kelkar et al. 2015).

Interestingly, anti-inflammatory treatment with antibodies targeting tumor necrosis factor alpha did not improve outcomes in patients with heart failure (Chung et al. 2003). Other clinical trials targeting inflammation in heart failure have also failed to show clinical benefit (Chung et al. 2003; Deftereos et al. 2014). However, these trials focused on inhibition of inflammation largely through a single mechanism. MSCs secrete numerous growth factors and cytokines influencing a diverse array of pathways, such as those related to multiple inflammatory pathways, adverse LV remodeling, angiogenesis, tissue healing, apoptosis, mitochondrial dysfunction, microvascular dysfunction, and collagen deposition (Kinnaird et al. 2004a, b; Gnecchi et al. 2005, 2008; Takahashi et al. 2006; Malliaras et al. 2012).

Such multi-functional activities provide a rationale for why MSCs might provide a more effective therapeutic strategy than drugs with a more limited range of activities.

8 Conclusions

The use of stem cells to treat patients with HF began over 15 years ago. The broad range of activities stem cells possess is a particularly attractive attribute when considering new therapies for patients with HF, a condition involving multiple dysfunctional pathways. Although there are many trials in which encouraging signals have been published, definitive documentation of efficacy in clinical trials is still lacking. Virtually all previous therapeutic approaches were based on maximizing the delivery of stem cells to the heart, leading to the use of intracoronary, transendocardial, or epicardial delivery strategies. However, the recognition that progressive cardiac dysfunction in patients with HF derives, at least partly, from the existence of a persistent inflammatory response with resultant continued damage to the myocardium, and that MSCs exert potent anti-inflammatory effects through paracrine mechanisms, has stimulated what may be a transformational change in stem cell delivery strategy – intravenous delivery of MSCs. Preclinical studies and one early clinical study suggest intravenous delivery of MSCs results in potent anti-inflammatory effects and, most importantly, that such a delivery approach holds promise for efficacy. The validity of these concepts awaits the results of pivotal clinical trials.

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Platelet-Derived Growth Factor in Heart Failure

John Medamana, Richard A. Clark, and Javed Butler

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Abstract

Defective vascular and cardiomyocyte function are implicated in the development and progression of both heart failure with reduced and preserved ejection fraction. Any treatment option that augments these myocardial processes may therefore be of significant value. The platelet-derived growth factor (PDGF) family is involved in a wide range of growth processes and plays a key role in both regulating angiogenesis and mesenchymal cell development. Thus, PDGF may serve as a potent therapy for heart failure. While numerous animal studies have demonstrated beneficial cardiovascular effects of growth factor therapy, promising laboratory data has not yet translated to effective therapies. In this

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_80

review, we outline the biological role of PDGF and summarize previous studies that have focused on the cardiovascular effects of normal PDGF signaling, administration of PDGF, and the effects of PDGF on stem cell therapy.

Keywords
Angiogenesis • Animal models of human disease • Basic science research • Growth factors/cytokines • Heart failure • Journal subject code • Myocardial infarction • Stem cells

Abbreviations

Ang-2	Angiopoietin-2
CK-MB	Creatine phosphokinase-myocardial band
FGF	Basic fibroblast growth factor
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HSP	Heat shock protein
IGF-1	Insulin-like growth factor
LDH	Lactate dehydrogenase
MAP	Mitogen-activated protein
MEK	Mitogen-activated ERK-activating kinase
PDGF	Platelet-derived growth factor
PKC	Protein kinase C
PLC	Phospholipase C
VEGF	Vascular endothelial growth factor

Heart disease is currently the leading cause of death both in the United States and worldwide (Jemal et al. 2005). In 2011, the estimated cost associated with heart disease was $215.6 billion (Mozaffarian et al. 2015). Given the aging population and the increasing risk factor profile including obesity and diabetes, both the prevalence of and associated costs for the treatment of heart disease are projected to increase. While great strides have been made in the treatments of myocardial infarction, treatment options for heart failure (HF) have remained limited.

Dysfunction in vascular regulation is implicated in the development and progression of heart failure. In physiological hypertrophy, there exists an appropriate number of capillary beds to sufficiently supply the myocardium; however, in pathological hypertrophy, a decrease in capillary density is observed (Taimeh et al. 2013). It follows that this decrease in capillary density could result in myocardial hypoxia and systolic impairment. Decreased capillary density has also been implicated in heart failure with preserved ejection fraction (HFpEF). Autopsy

studies have demonstrated that HFpEF patients exhibit a reduction in coronary microvascular density and that this reduction in microvascular density is associated with an increase in myocardial fibrosis (Mohammed et al. 2015). Myocardial hypoxia is also implicated in the development of HFpEF. Cardiac ischemia can result in dysfunctional calcium removal from myocytes, and as a result, diastolic dysfunction and even HFpEF can follow (Gladden et al. 2014). Chest pain in HF patients without coronary disease has been attributed to both decreased coronary perfusion secondary to the myocardial stiffening and a decrease in coronary microvascular density (Gladden et al. 2014). In HFpEF, endothelial progenitor cells displaying an angiogenic phenotype are upregulated (Shantsila and Lip 2016). It has been shown that the potently angiogenic platelet-derived growth factor (PDGF) can induce the differentiation of endothelial progenitor cells (Miyata et al. 2005). As dysfunctional myocardial microcirculation is implicated in both systolic heart failure and HFpEF, the induction of myocardial angiogenesis, by angiogenic growth factors like PDGF, may serve to inhibit the development of both heart failure etiologies.

1 Platelet-Derived Growth Factor

The PDGF family is involved in a number of growth processes. During embryogenesis, PDGF plays a key role in vascular development by promoting proliferation and survival of vascular mural cells (Hoch and Soriano 2003). In adults, PDGF has been shown to be both a potent mitogen and survival factor for both fibroblasts and other mesenchymal cells and thus plays a key role in a wide range of processes (Shantsila and Lip 2016).

The PDGF family is made up of four different polypeptide chains: PDGF-A, PDGF-B, PDGF-C, and PDGF-D, which share a common structure. Of the four chains, PDGF-A and PDGF-B have been thoroughly studied, while PDGF-C and PDGF-D have only recently been identified. After being translated, these chains form disulfide-bonded dimers which function as biologically active growth factors. Five dimeric PDGF isoforms have been identified (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD) (Fredriksson et al. 2004). PDGFs bind two similar tyrosine kinase receptors, PDGFR-α and PDGFR-β. PDGF-A binding is restricted to PDGFR-α, while PDGF-B is able to bind both PDGFR-α and PDGFR-β. PDGF-C and PDGF-D can bind to both PDGF receptors; however, they have greater affinity for PDGFR-β (Formiga et al. 2012). Binding of PDGF to its receptor results in the activation of several signal transduction pathways which, among other things, function to promote cell survival (Fig. 1).

As PDGF exerts both anti-apoptotic and vasculogenic effects, it represents a novel treatment target for HF. Additionally, various growth factors including PDGF decrease with age, rendering study of PDGF in HF of interest as it is primarily a disease of the elderly.

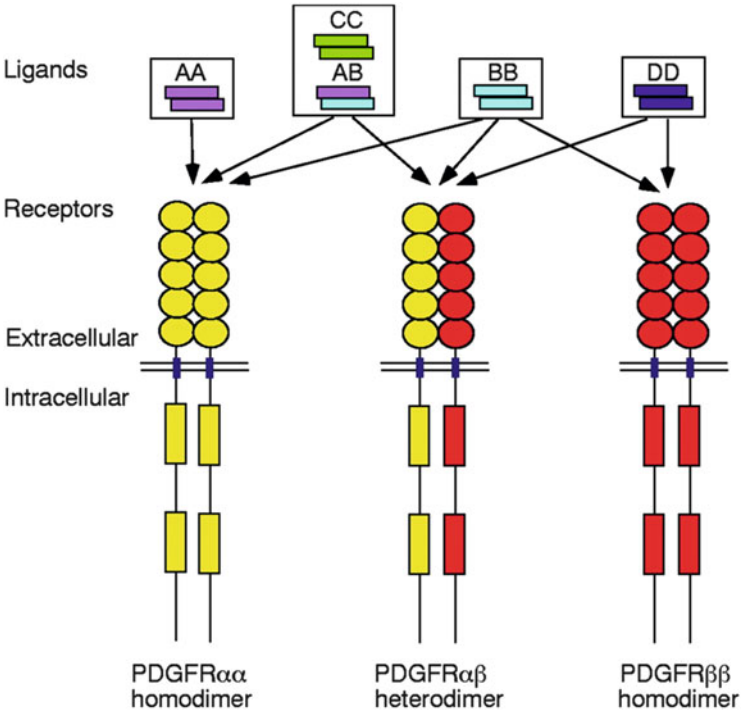

Fig. 1 The PDGF family of growth factors and receptors. The PDGF family is made up of four different polypeptide chains: PDGF-A, PDGF-B, PDGF-C, and PDGF-D. These chains form disulfide-bonded dimers which function as biologically active growth factors. Five dimeric PDGF isoforms have been identified (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD) (Fredriksson et al. 2004). PDGFs bind two similar tyrosine kinase receptors, PDGFR-α and PDGFR-β. PDGF-A binding is restricted to the PDGFR-α, while PDGF-B is able to bind both PDGFR-α and PDGFR-β. PDGF-C and PDGF-D can bind to both PDGF receptors; however, they have greater affinity for PDGFR-β (Formiga et al. 2012). Figure reproduced with permission from Roles of PDGF in animal development Renée V. Hoch, Philippe Soriano Development 2003 130:4769–4784; doi:10.1242/dev.00721

2 Cardiac Functions of PDGF in the Diseased and Healthy Heart

A few studies have investigated the role that PDGF plays either in the healthy or diseased heart. Chong et al. (2013) showed that in the fetal human heart, PDGFR-α were found in greatest numbers in the interstitial cells of the epicardium, endocardium, and myocardium of the atria and ventricles, to a lesser degree on endothelial cells and infrequently on cardiomyocytes. In addition, a subset of cells expressing PDGFR-α also expressed either c-Kit or CD146, which have been recognized as identifiers of cardiac progenitor cells. Chong et al. also investigated the expression of PDGFR-α in the diseased human heart. Expression was similar to the fetal heart;

however, a greater number of cardiomyocytes expressed PDGFR-α (Chong et al. 2013).

Shaddy et al. (1996) investigated the expression of PDGF in biopsies taken from both heart transplants and normal hearts. PDGF staining was observed in 78% of biopsies, from heart transplants, and was markedly increased with high-grade vascular rejection. All samples taken from hearts exhibiting global ischemia exhibited PDGF. In contrast, PDGF expression was only noted in 44% of hearts not exhibiting global ischemia. PDGF staining was found more in the interstitial space than in the vascular space. In contrast, biopsies from non-transplanted hearts did not stain for PDGF; however, they exhibited similar staining for basic fibroblast growth factor (FGF) (Shaddy et al. 1996). Koch et al. (2007) also investigated the expression of PDGF in posttransplant hearts. Biopsies were taken during the first 2 weeks after transplantation. Levels of PDGF increased in the first week after transplantation and then decreased to a lower, albeit elevated level. In addition, PDGF-A was significantly elevated 1 week post-surgery in patients who experienced rejection (Koch et al. 2007).

Koizumi et al. investigated serum levels of PDGF-BB in patients with ST-segment elevation myocardial infarctions, who underwent percutaneous coronary intervention, and found that serum PDGF-BB levels correlated with ischemic time and creatine phosphokinase-myocardial band (CK-MB) (Koizumi et al. 2015).

In addition to human studies, a number of studies have investigated the function of PDGF in the hearts of small animals. Zhao et al. (2011) demonstrated that all four PDGF isoforms are expressed in the normal, un-infarcted rat heart. Following infarction, PDGF-A and PDGF-D increased in the infarcted area, and PDGF-B and PDGF-C expression was decreased in the normal heart surrounding the infarction. PDGF-D expression in the un-infarcted myocardium increased 1 week following infarction and remained elevated for 5 weeks. In addition, PDGFR-α and PDGFR-β expression was increased in the infarcted sections 3 days postinfarction (Zhao et al. 2011). Zymek et al. (2006) used both anti-PDGFR-α and anti-PDGFR-β antibodies to block signaling pathways, activated by PDGF binding its receptor in the infarcted mouse heart. Inhibition of both PDGFR-α and PDGFR-β leads to a decrease in collagen deposition in the infarcted area. In addition, PDGFR-β inhibition leads to defective angiogenesis and increased permeability of the new vasculature. Seven days following infarction, mice undergoing PDGFR-β inhibition exhibited hemorrhagic areas containing erythrocytes (Zymek et al. 2006).

Together, these studies show that some baseline PDGF activity exists in the normal heart, while PDGF levels and its signaling is increased in disease states. Most of these investigators surmised that the increase in endogenously produced PDGF-BB occurs in response to injury to play an important role in cardioprotection.

3 Effects of PDGF on Cultured Cardiomyocytes

A number of studies have investigated the effects of PDGF on cardiomyocytes. Rong et al. (2015) reported that PDGF-BB expression is increased in neonatal rat cardiomyocytes cultured under hypoxic conditions. In order to investigate the effects of PDGF signaling under hypoxic conditions, a lentiviral vector was used to decrease the expression of PDGF-BB. Associated with the decreased PDGF-BB levels, an increase in lactate dehydrogenase (LDH), an increase in apoptotic cells, and a decrease in contractile function were observed (Rong et al. 2015). Taken together, these results show that PDGF signaling plays a role in promoting survival in cultured cardiomyocytes under stress.

Cheng et al. (2007) investigated the effects of growth factors on contractile property and viability of cultured engineered tissue. The engineered cardiac tissue formed three-dimensional constructs that are thought to be more representative of in vivo conditions. This study demonstrated that PDGF increased the contractile amplitude at day 4 of culture and promoted cell survival (Cheng et al. 2007). Vantler et al. (2010) found that PDGF-BB stimulation resulted in improved contractile function of engineered heart tissue and cell survival. The effect of PDGF-BB stimulation was both dose and time dependent. Vantler et al. also demonstrated that PDGF-BB signaling does not induce cardiomyocyte hypertrophy or proliferation and that the anti-apoptotic effects of PDGF-BB signaling are mediated through PI3K-AKT signaling (Vantler et al. 2010).

Edelberg et al. (1998) studied the effects of PDGF on cardiac microvascular communication. Cardiac microvascular endothelial cells were cultured both by themselves and along with cardiomyocytes. Endothelial cells constitutively made PDGF-A, resulting in the formation of PDGF-AA. When the endothelial cells and cardiomyocytes were co-cultured, PDGF-B was made along with PDGF-A, resulting in the formation of PDGF-AB. In addition, PDGF-AB resulted in production of von Willebrand factor, as well as increased endothelial levels of vascular endothelial growth factor (VEGF) and Flk-1, both known to play a role in angiogenesis (Edelberg et al. 1998). PDGF-B expression, while present in endothelial cells co-cultured with cardiomyocytes derived from young mice, is absent in endothelial cells co-cultured with cardiomyocytes derived from the hearts of aged mice. Cardiac tissue from aged mice demonstrated impaired survival as compared to cardiac tissue derived from young mice. In addition, inhibition of PDGF-AB or PDGFR-α resulted in a decreased viability of cardiac tissue derived from young mice and the addition of PDGF-AB to cardiac tissue derived from aged mice resulted in neovascularization of the tissue and improved survival (Edelberg et al. 2002).

Genneback et al. (2013) investigated the effects of growth factors on exosomes produced by cultured cardiomyocytes. Up to 47.5% of transcripts found in exosomes were attributed to PDGF-BB stimulation. The transcripts identified in the exosomes have previously been shown to exhibit signals associated with inhibiting hypertrophy and promoting the differentiation of cardiomyocytes from their mesodermal cell precursors (Backs and Olson 2006).

Hyaluronic acid is a key component of the extracellular matrix and is involved in wound healing (Price et al. 2005). Hellman et al. (2010) demonstrated that cardiomyocytes are able to produce hyaluronic acid when stimulated by PDGF-BB. When cardiomyocytes and fibroblasts were cultured together, a greater amount of hyaluronic acid was produced as compared to the amount produced by each cell type alone, suggesting that hyaluronic acid production occurs through a synergistic relationship between fibroblasts and cardiomyocytes (Hellman et al. 2010). As mentioned previously, communication in the heart between cardiomyocytes and endothelial cells induces PDGF-AB production (Edelberg et al. 1998). We would predict that PDGF stimulates even more positive feedback loops among heart cells, and their ECM then is currently recognized.

Takenaka et al. (2004) demonstrated that PDGF-BB stimulation of cardiomyocytes resulted in the phosphorylation of heat shock protein 27 (HSP 27) through the actions of the mitogen-activated protein (MAP) kinase superfamily, P38. It is known that HSP phosphorylation results in a number of wide-ranging downstream effects and that HSPs are produced in cells under stress (de Maio 1999). Liu et al. (2005) demonstrated that PDGF increased cell growth in a time- and dose-dependent manner, that PDGF-BB stimulation resulted in phosphorylation of both PDGFR-β and ERK1/2, and that long-term stimulation by PDGF increased the expression of both PDGFR and ERK1/2. A number of inhibitors were added to the cultured cells to identify participants in the signal transduction pathway of PDGF-BB. It was shown that a mitogen-activated ERK-activating kinase (MEK) inhibitor, a phospholipase C (PLC) inhibitor, and a protein kinase C (PKC) inhibitor decreased [^{3}H]-leucine in cultured cardiomyocytes stimulated by PDGF-BB (Liu et al. 2005). Thus it is clear that both MAP kinases, P38 and ERK1/2, play a critical role in the signal transduction pathways of PDGF in cardiomyocyte as has been demonstrated in fibroblasts.

From these studies, we learn that addition of PDGF to cultured cardiomyocytes promotes survival, increases contractile function, reduces apoptosis, and helps to induce signaling pathways associated with healing. Since in vitro studies are intrinsically limited, other studies have investigated the effects of PDGF administration in vivo.

4 Effect of Administration of Exogenous PDGF

Edelberg et al. (2002) showed that administration of PDGF-AB resulted in an increased vascular density in both young and old rats. In addition, pretreatment with PDGF-AB prior to induced infarction resulted in a significant decrease in infarct size (Edelberg et al. 2002). These data show the angiogenic capability of PDGF and that while PDGF-AB expression is deficient in the aged heart, the downstream transduction pathway signaling is still functional. In older rats, pretreatment with PDGF-AB provided cardioprotection with a reduction in infarct size.

PDGF-AB cardioprotection has been shown to result from a synergistic relationship between PDGF-AB and VEGF and Angiopoietin-2 (Ang-2), two downstream members of the PDGF-AB signal transduction pathway. In addition, while PDGF-AB only provided cardioprotection if administered prior to an infarction, a combination of PDGF-AB, VEGF, and Ang-2 was able to offer cardioprotection at the time of an infarct and could potentially translate as a viable therapy (Xaymardan et al. 2004a). In addition to serving a cardioprotective role in myocardial infarctions, administration of the combination of PDGF-AB, VEGF, and Ang-2 was demonstrated to restore TNF-α-associated ischemia preconditioning of ERK1/2, a cardioprotective effect, in the hearts of aged rats (Zheng et al. 2006).

These data highlight both the importance of the local environment for influencing growth factor production and the indirect angiogenic properties of PDGF. The decrease of PDGF may play a significant role in the increase of adverse cardiovascular events, in elderly patients. A patent for a potential cardioprotective therapy, combining PDGF-AB, VEGF, and Ang-2, was filed; however, a commercially available therapy has not yet followed.

Affleck et al. (2002) investigated the effects of numerous growth factors on the production of VEGF in rabbit hearts. PDGF-BB induced the greatest increase in myocardial VEGF levels, a decreased expression of $VEGF_{121}$, and an increased expression of both $VEGF_{189}$, $VEGF_{183}$, and $VEGF_{164}$. Interestingly, $VEGF_{121}$ is unable to bind heparin and is more soluble than the other VEGF isoforms (Affleck et al. 2002). As VEGF is known to have potent angiogenic capabilities (Taimeh et al. 2013), once again it is shown that PDGF has indirect angiogenic properties via its ability to induce increased expression of VEGF (see (Edelberg et al. 1998)).

Awada et al. (2015) investigated the delivery of both PDGF and VEGF, via a fibrin gel, as a treatment for infarction. VEGF is needed in the first few days of angiogenesis and is responsible for forming immature, leaky blood vessels (Lee et al. 2000). PDGF is needed later in angiogenesis and causes mural cells to cover the newly made vessels and is responsible for allowing newly formed blood vessels to mature (Betsholtz 2004). The fibrin gel synthesized was able to release VEGF followed by PDGF, and the controlled sequential release resulted in greater angiogenesis. Two weeks following infarction, controlled delivery of VEGF and PDGF resulted in a 60% improvement in cardiac function than the delivery of free growth factors. Additionally, the controlled delivery resulted in greater ventricular wall thickness and less scaring and resulted in the formation of new and stable vasculature and increased cardiomyocyte survival (Awada et al. 2015). Hao et al. (2007) investigated the dual delivery of VEGF and PDGF-BB by means of an alginate hydrogel 7 days after infarction in rats and showed that VEGF and PDGF were both able to be delivered via the hydrogels and that VEGF was released more rapidly than PDGF. Delivery of both growth factors resulted in an increased capillary density in the infarcted myocardium and an increase in the systolic velocity-time integral (Hao et al. 2007). Together, these studies demonstrate that PDGF-BB has a direct, as well as an indirect (Edelberg et al. 1998; Affleck et al. 2002), angiogenic effect.

Cui et al. (2014) investigated using a plasmid to facilitate the transfer of a combination of bFGF and PDGF to treat myocardial infarctions. Dual gene transfer was shown to establish numerous mature blood vessels, improve cardiac function, and reduce fibrosis (Cui et al. 2014). It has been shown that bFGF and PDGF work synergistically to promote the proliferation of endothelial progenitor cells and to increase VEGF release (Sufen et al. 2011); thus, the results may also reflect an induced increase in VEGF signaling. Hao et al. showed that dual transfer of PDGF-BB and bFGF in rats with a 7-day-old myocardial infarction increases both the number of capillaries and arterioles (Hao et al. 2004).

FGF-2 has been shown to stimulate angiogenesis (Schweigerer et al. 1987), while PDGF has been shown to stimulate vascular mural cells (Heldin and Westermark 1999). Thus, stable mature vasculature could be formed by administration of both growth factors. In a porcine heart model, dual delivery of PDGF-BB and FGF-2 resulted in the formation of stable vessels in the infarcted myocardium, improved regional myocardial blood flow, and increased ejection fraction. PDGF-BB signaling alone was not enough to develop mature vasculature; however, the synergistic relationship between PDGF-BB and FGF-2 proved capable of doing so (Lu et al. 2007). Thus, we learn that PDGF-BB can synergize with either FGF-2 or VEGF to elicit angiogenesis.

In another study, an adenovirus vector was delivered into the coronary arteries of rats that had received cardiac allografts and was used for overexpression of PDGF-BB that resulted in an increase in microvascular density and an increase in survival factors Bcl-2 and BMP-7. Inhibition of the PDGF receptor with imatinib mesylate resulted in an increase in ischemia reperfusion injury and chronic rejection (Tuuminen et al. 2016).

Hsieh et al. (2006a, b) demonstrated the efficacy of delivering PDGF to the myocardium via a nanofiber delivery system and that this resulted in PDGFR-β phosphorylation. The use of nanofiber delivery system resulted in sustained improvement of cardiac function. PDGF alone resulted in transient improvement in heart function; however, these effects were not sustained. This illustrates the effects of controlled release (Hsieh et al. 2006a, b).

Kim et al. (2011) administered a combination of PDGF-BB and basic fibroblast growth factor (FGF-2), via self-assembling peptides, on a rat myocardial infarction model and showed that this resulted in a decrease in both infarct size and myocyte apoptosis, an increase in angiogenesis, and increase in cardiac function. The self-assembling peptides allowed for the formation of a three-dimensional scaffold that closely mimics the extracellular matrix and is easily degradable (Kim et al. 2011) (Table 1).

These studies demonstrate that PDGF administration can serve cardioprotective, anti-apoptotic, and angiogenic functions in small animal models. However, it is known that the response to injury and inflammation in small animals does not perfectly model human inflammatory diseases (Seok et al. 2013). In addition, rat or mice heart studies are problematic to directly extrapolate to human hearts, as they often have native collaterals which limit infarction.

Table 1 Studies emphasizing the effects of in vivo administration of PDGF

Study	Growth factor(s)	Species	Results
Edelberg et al. (2002)	PDGF-AB	Rat	Increased vascular density, administration pre-induced MI resulted in ~50% reduction in infarct size
Affleck et al. (2002)	PDGF-BB	Rabbit	Statistically significant increase of VEGF expression as compared to sham ($P = 0.009$)
Awada et al. (2015)	PDGF + VEGF	Rat	60% improvement in cardiac function as compared to free GF, 68% increase in cardiac function over saline
Hao et al. (2007)	PDGF-BB and VEGF	Rat	Statistically significant increases in blood vessel density and cardiac function
Cui et al. (2014)	bFGF + PDGF-BB	Rat	14% increase in LVEF, 21% in fractional shorting as compared to control
Lu et al. (2007)	FGF-2 and PDGF-BB	Pig	Statistically significant increase in collateral index and overall myocardial function
Tuuminen et al. (2016)	PDGF-BB	Rat	Decreases IRI and causes statistically significant increase in BCL2 mRNA

Porcine hearts lack this constitutive collateral circulation, and as such, they are a better model of human heart disease (Lu et al. 2007).

5 PDGF and Stem Cells

Das et al. (2009) investigated the effects of using stem cells to overexpress VEGF and PDGF in a rat myocardial infarction model. A bicistronic vector was used to induce overexpression of VEGF and PDGF in the stem cells. This resulted in a decrease in myocardial infarction size, as well as improved angiogenesis, heart function, and exercise capacity. It was shown that the hematopoietic stem cells traveled to the site of infarction after being injected intravascularly and that stem cell therapy resulted in an increase in connexin 43, an important protein in gap junctions. Importantly, the stem cell therapy did not correspond with expression of any of a number of signaling molecules involved in oncogenesis (Das et al. 2009).

Krausgrill et al. (2009) investigated the effects of PDGF-BB on implanted bone marrow stem cells in a rat myocardial infarction model. Mice were treated with PDGF-BB prior to implantation of stem cells. Administration of PDGF-BB with bone marrow stem cells resulted in a decrease in cell loss. It was postulated that PDGF-BB results in decreased cell loss at later times but reperfusion-associated washout is mainly responsible for cell loss before PDGF-BB is able to have an effect on cell survival (Krausgrill et al. 2009). Xaymardan et al. (2004b) demonstrated that PDGF-AB resulted in a twofold increase in the speed of in vitro cardiomyocyte development from bone marrow stem cells and results in an increase in the number of cardiomyocytes formed; however, the cardiomyocytes formed as disorganized bundles, and heart function improved no more than

Table 2 Studies emphasizing the effects of PDGF on stem cells

Study	Result
Das et al. (2009)	Decrease in MI area and an increase in both heart function and angiogenesis
Xaymardan et al. (2004b)	Twofold increase in the speed of in vitro cardiomyocyte development from bone marrow-derived stem cells
Takahashi et al. (2006)	Bone marrow-derived stem cells produce VEGF, bFGF, PDGF, and IGF-1

administration of either PDGF-AB or bone marrow-derived stem cells alone (Xaymardan et al. 2004b).

Takahashi et al. (2006) investigated the mechanism by which bone marrow stem cells themselves function to improve cardiac function postinfarction and found that growth factors VEGF, bFGF, PDGF, and insulin-like growth factor (IGF-1) were produced by the bone marrow-derived stem cells. Production of these growth factors was increased under hypoxic conditions, and the bone marrow-derived stem cells were shown to improve cardiac function, induce angiogenesis, and inhibit apoptosis (Takahashi et al. 2006). Khattab et al. (2013) investigated the in vitro effects of growth factors on the transdifferentiation of umbilical cord blood-derived stem cells into cardiac myocytes. It was found that a combination of FGF-2 and VEGF promoted differentiation of the stem cells into both endothelial cells and cardiomyocytes. In addition, adding PDGF-BB to the cells committed the endothelial progenitor cells into cardiomyocytes (Khattab et al. 2013) (Table 2).

6 Conclusion

PDGF is involved in the heart's normal response to injury. Administration of PDGF both improves cardiac function and increases angiogenesis in a number of animal models. While there have been numerous promising laboratory studies involving the use of growth factors to prevent heart failure, large placebo-controlled, double-blind studies have failed to show that the use of growth factors alone is beneficial (Annex and Simons 2005). Given the results of the aforementioned large clinical trials, it seems unlikely that increasing a single growth factor will result in an effective heart failure treatment. Rather, it seems likely that successful future therapies will utilize some combination of angiogenic growth factors, like PDGF, VEGF, and/or FGF, and will be delivered by a system that allows for controlled direct release of the growth factors to the myocardium. Since PDGF has been shown to work synergistically with other growth factors, like bFGF on cardiomyocytes and endothelial cells, an enhancer of these two endogenous growth factors might be particularly advantageous (Lin et al. 2014).

Acknowledgments Funding for this manuscript was provided by a summer research grant from the Department of Dermatology at Stony Brook University Medical Center.

Conflict of Interest JM and JB report no conflict of interest. RC is the co-discoverer of a fibronectin-derived peptide that binds and enhances the activity of PDGF-BB and is the founder of NeoMatrix Therapeutics, which is developing the peptide to prevent burn injury progression, speed healing, and reduce scarring.

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Wnt Signaling in Cardiac Remodeling and Heart Failure

Vasili Stylianidis, Kevin C.M. Hermans, and W. Matthijs Blankesteijn

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_56

Abstract

Wnt signaling plays an essential role during development, but is also activated in diseases as diverse as neurodegeneration, osteoporosis, and cancer. Accumulating evidence demonstrates that Wnt signaling is also activated during cardiac remodeling and heart failure. In this chapter, we will provide a brief overview of Wnt signaling in all its complexity. Then we will discuss the evidence for its involvement in the development of cardiac hypertrophy, the wound healing after myocardial infarction (MI) and heart failure. Finally, we will provide an overview of the drugs that are available to target Wnt signaling at different levels of the signaling cascade and the results of these pharmacological interventions in cardiac disease.

Keywords

Cardiac hypertrophy • Cardiac remodeling • Frizzled • Heart failure • Myocardial infarction • Therapeutic target • Wnt Signaling

1 Introduction

1.1 Discovery of Wnt

In the last three decades, the Wnt/frizzled (Wnt/Fzd) signal transduction pathway has evolved from a study object of developmental biologists into a highly complex signaling system relevant to multiple physiological and pathological processes. After the discovery that the *Drosophila* segment polarity gene Wingless and the mouse proto-oncogene Int-1 were encoding homologous glycoproteins, which explains the name Wnt, 19 family members have been identified in the mammalian genome (Clevers 2006). In the meantime, we have learned that Wnts are glycosylated proteins of 350–400 amino acids in size with a conserved pattern of 22–24 cysteine residues. Moreover, palmitoylation of a conserved serine residue by an enzyme named Porcupine is essential for the secretion of Wnt proteins as well as for its interaction with its target (Janda and Garcia 2015).

1.2 Wnt Receptors and Coreceptors

Two decades ago, it became evident that Wnt proteins can act as ligands for proteins of the frizzled family. This family was known to control tissue patterning during *Drosophila* development and turned out to be structurally related to the large family of seven-transmembrane receptors also referred to as G protein-coupled receptors (GPCRs) (Schulte and Bryja 2007). Crystallization studies of the complex of *X*Wnt8 and the extracellular part of its receptor, Fzd8, have revealed an extraordinary interaction pattern where two extensions of the Wnt protein – referred to as thumb and index finger – can bind to two distinct binding sites on the receptor protein (Janda et al. 2012). Depending on the signaling pathway, the receptor

complex further consists of a member of the low-density lipoprotein receptor-related protein family (LRP5 or LRP6) that serves as a docking site for the intracellular signaling complex (see below) (Joiner et al. 2013).

1.3 Signal Transduction Pathways for Wnt

The Wnt/Fzd receptor complex can activate diverse signaling pathways (Anastas and Moon 2013). The best-studied pathway is the β-catenin-mediated signaling, generally referred to as the "canonical" Wnt signaling pathway (Fig. 1), in which β-catenin serves as the second messenger. Under resting conditions, β-catenin is phosphorylated by a protein complex called the "destruction complex," preparing it for degradation by the ubiquitin proteasome pathway. The destruction complex

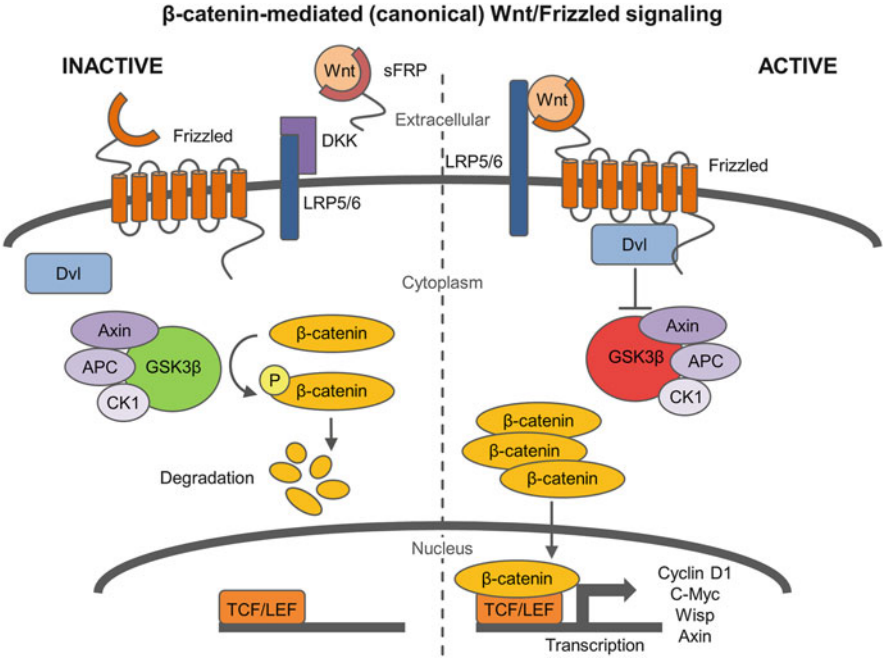

Fig. 1 Schematic representation of the β-catenin-mediated or "canonical" Wnt signaling. In the inactive state (*left*), β-catenin is continuously phosphorylated by the destruction complex consisting of glycogen synthase kinase (GSK)3β, axin, adenomatous polyposis coli protein (APC), and casein kinase 1 (CK1) which results in its degradation. When activated, a receptor complex between Wnt, frizzled, and the lipoprotein receptor-like protein (LRP) 5 or LRP 6 is formed and the disheveled (Dvl) protein is recruited to the plasma membrane. This leads to the dissociation of the destruction complex and accumulation of β-catenin which, when migrated to the nucleus, complexes with TCF/LEF transcription factors and activates gene expression. *DKK* Dickkopf, an endogenous LRP5/LRP6 inhibitor, *sFRP* soluble frizzled-related protein, a soluble Wnt-binding protein

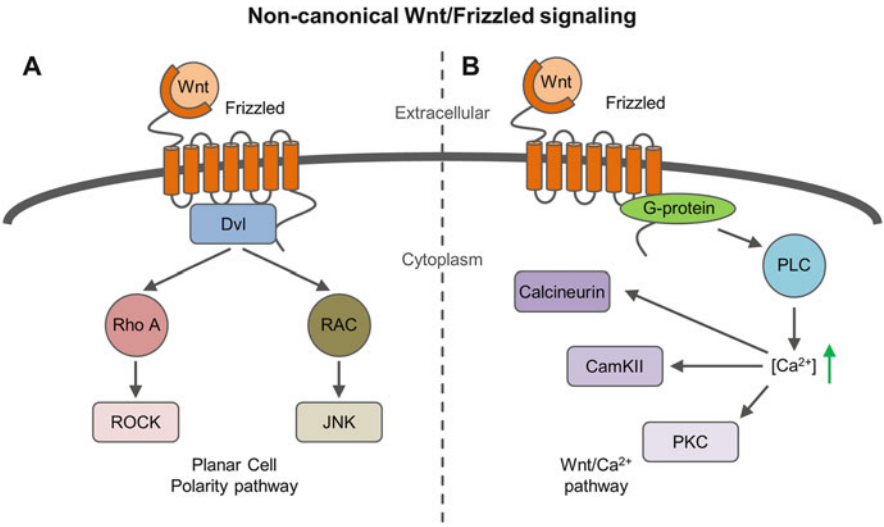

Fig. 2 Overview of the planar cell polarity pathway (**a**) and Wnt/Ca^{2+} signaling pathway (**b**). In the planar cell polarity pathway, the small G proteins RhoA and Rac are activated via disheveled (Dvl), resulting in signaling via the ROCK and Jnk signaling pathways. In the Wnt/Ca^{2+} signaling pathway, signaling is via heterotrimeric G proteins that activate phospholipase C (PLC). This induces a rise in intracellular Ca^{2+}, leading to activation of calcineurin, calcium/calmodulin-dependent kinase II (CamKII), and protein kinase C (PKC)

consists of adenomatous polyposis coli protein, axin, casein kinase 1, and glycogen synthase kinase 3β (GSK3β). Upon interaction of Wnt with Fzd, LRP5/LRP6, and the intracellular adaptor protein disheveled (Dvl), the destruction complex is recruited to the plasma membrane and disintegrates, preventing the degradation of β-catenin. The second messenger now can enter the nucleus where it can interact with TCF/LEF transcription factors (MacDonald et al. 2009).

Next to β-catenin-mediated signaling, pathways such as the planar cell polarity (PCP) pathway and Ca^{2+} pathway can be activated by Wnt proteins (Fig. 2). The PCP pathway controls cell orientation relative to neighboring cells and involves the activation of small G proteins Rac and Rho by the receptor complex, followed by the activation of Jnk and Rho-kinase pathways (Simons and Mlodzik 2008). Induction of Ca^{2+}-dependent signaling involves the activation of heterotrimeric G proteins and phospholipase C (PLC). Signaling via this branch of the Wnt pathway results in activation of Ca^{2+}-dependent enzymes with known relevance for cardiac (patho)physiology such as calcium/calmodulin-dependent kinase II (CamKII), protein kinase C (PKC), and calcineurin (Kuhl et al. 2000).

1.4 Wnt Signaling and Cardiovascular Diseases

Soon after the discovery of the Wnt proteins, it became evident that Wnt signaling can play an important role in the development of cancer. Mutations in components

of the destruction complex can give rise to defective β-catenin degradation, resulting in nuclear accumulation of this second messenger in the nucleus and uncontrolled activation of gene expression (Clevers 2006). In cardiac diseases, however, a reactivation of the fetal gene expression program is observed. Because Wnt signaling plays an important role in the development of the heart, multiple components of this pathway are reexpressed during cardiac remodeling (Hermans and Blankesteijn 2015). Therefore, an activation of the Wnt pathway through increased expression of its components, rather than through mutations, appears to be the mechanism by which Wnt signaling contributes to the control of cardiac remodeling.

In this chapter, we will briefly describe the role of Wnt signaling in cardiac development. Subsequently, we will review the experimental evidence for the activation of Wnt signaling in cardiac hypertrophy, the wound healing response following myocardial infarction (MI) and heart failure. We will finalize this review by providing an overview of the drugs that are currently available for intervention at different levels of the Wnt signaling pathway and the results that are obtained so far with the application of these drug experimental models of cardiac remodeling.

2 Wnt Signaling in Cardiac Development

During embryonic development, the heart is the first organ to be formed. During gastrulation, three germ layers (ectoderm, mesoderm, and endoderm) are formed; the mammalian heart is derived from the mesoderm. Two heart-forming fields are specified in the mesodermal layer: the first heart field which mainly contributes to the formation of the left ventricle and the second heart field from which the right ventricle and outflow tract are derived (Brade et al. 2006). Many genes that are involved in cardiac development are reexpressed during cardiac remodeling (Hermans and Blankesteijn 2015), so a better understanding of the regulation of the developmental process can help to better understand the signaling pathways activated in the adapting heart.

Both canonical and noncanonical Wnt signaling have been shown to play a role in heart development. Although conflicting results have been published about the function of the Wnt/β-catenin pathway during cardiac specification, time-dependent application of Wnt8a in zebra fish embryos provided clarity to the issue. The results of these experiments suggest a biphasic role for Wnt/β-catenin signaling during cardiogenesis, where stimulation of the pathway before gastrulation results in more cardiomyocytes, whereas stimulation after gastrulation attenuates the number of cardiomyocytes (Pahnke et al. 2016). Canonical Wnt signaling also contributes to the formation of the endocardial cushions, which give rise to internal cardiac structures such as the valves (Hurlstone et al. 2003).

Noncanonical Wnt signaling contributes to cardiac development via Wnt11. Several studies indicate that Wnt11 is required for cardiomyocyte differentiation via PKC and JNK signaling (Eisenberg et al. 1997; Pandur et al. 2002). Wnt11 is sufficient to trigger a contractile phenotype, as also observed by the Wnt/β-catenin

inhibitor DKK1 (Pandur et al. 2002). However, both DKK1 and Wnt11 are known to be canonical Wnt inhibitors (Maye et al. 2004; Weidinger and Moon 2003), suggesting that this effect is not solely due to noncanonical signaling but by suppression of β-catenin-mediated signaling as well.

In the later stages of the cardiogenesis, Wnt signaling can modulate N-cadherin which plays a vital role in cell adhesion and direct cell–cell contact, both necessary to develop a linear heart tube (Radice et al. 1997). Noncanonical Wnt signaling, in particular Wnt11 through involvement of PKC, has been shown to orchestrate N-cadherin expression. In addition, Wnt signaling regulates N-cadherin expression as well as the strength of N-cadherin-mediated cell adhesion in neonatal cardiomyocytes (Fujio et al. 2004; Toyofuku et al. 2000). Finally, there is evidence that Wnt signaling is involved in later phases of heart formation. The development of the cardiac conduction system was found to be under the control of Wnt signaling, as endothelin-1 treatment of looped chicken hearts shows an upregulation of Wnt7a and Wnt11 (Bond et al. 2003). Taken together, Wnt signaling appears to be an important regulator of cardiac development and therefore has to be under tight control, both in time and space during the different stages of heart formation.

3 Wnt Signaling in Cardiac Hypertrophy

Cardiomyocytes are terminally differentiated cells, and their proliferation rate is inadequate when the heart is confronted with an increased workload or injury (Laflamme and Murry 2011). Therefore cardiomyocytes respond to such challenges mainly by hypertrophic growth. Cardiomyocyte hypertrophy can occur in response to physiological stimuli, such as exercise or pregnancy, but also result from pathological conditions such as hypertension or a valvular defect. Generally, volume overload – resulting from, e.g., valve defects – gives rise to eccentric or outward hypertrophy, whereas pressure overload caused by aortic stenosis or hypertension initially results in concentric or inward hypertrophy. When the underlying cause of concentric hypertrophy is left untreated, this will eventually evolve into eccentric remodeling, generally referred to as dilated cardiomyopathy (Opie et al. 2006).

Physiological and pathological hypertrophy are regulated by distinct signaling pathways. Where physiological hypertrophy is mainly initiated by growth factors such as growth hormone and insulin-like growth factor, the main drivers of pathological hypertrophy are neurohumoral factors such as angiotensin II, endothelin-1, and catecholamines (Rohini et al. 2010). These insights have led to several therapeutic interventions, particularly in the renin–angiotensin system and the beta-adrenergic system, to attenuate the progression of pathological hypertrophy. Unfortunately, these interventions are not sufficient to prevent the development of dilated cardiomyopathy and the concomitant deterioration of cardiac function in a significant fraction of the patients. In an attempt to find new therapeutic targets for cardiac hypertrophy, we studied the changes in gene expression in rats exposed to aortic

banding and observed the increased expression of a rat homologue of the *Drosophila* gene Frizzled, now known as rFzd2 (Blankesteijn et al. 1996). Although by that time the function of the frizzled gene product and its position in the hypertrophic signaling cascade were obscure, it was a first indication that what the pathway now known as the Wnt/Fzd signaling cascade is activated in the pathologically stressed heart.

3.1 Modulation of Wnt Signaling at the Extracellular Level

In several studies the role of Wnt modulators, in particular sFRPs, on cardiac hypertrophy has been addressed. Deletion of the sFRP1 gene resulted in a progressive increase in heart weight/body weight ratio in mice that were left to age for 1 year. This was paralleled by attenuated cardiac function and increased cardiac fibrosis. Elevated β-catenin levels were reported as well as the induction of Wnt/β-catenin target genes, including the aging and senescence marker Wnt16. A decreased sFRP-1 expression could be confirmed in dilated cardiomyopathy patients, supporting a role for this Wnt modulator in preventing age-related cardiac remodeling (Sklepkiewicz et al. 2015). A protective role in hypertrophy development has also been suggested for sFRP2: in mice in which the plasma membrane calcium ATPase 4 (PMCA4) gene was specifically inactivated in cardiac fibroblasts, the induction of cardiac hypertrophy by aortic banding, a common intervention to induce pressure overload hypertrophy, was attenuated. The authors conclude that this effect was mediated via sFRP2, because treatment with sFRP2 neutralizing antibodies could restore the hypertrophic phenotype (Mohamed et al. 2016). Antihypertrophic effects were also reported for sFRP5, using an in vitro model of angiotensin II-induced cardiomyocyte hypertrophy (Jin et al. 2015). Although the experimental approaches in these studies were quite different, the results of these studies suggest an antihypertrophic role for sFRPs.

3.2 Modulation at the Receptor Complex

At present, no studies have been published in which a direct intervention at the level of the frizzled protein has been studied in models of cardiac hypertrophy. However, upon binding of Wnt, several proteins are recruited to the receptor complex. One of these proteins is Dvl and the role of this protein in cardiac hypertrophy has been addressed in several studies. In a study from our research group, we observed that mice lacking the Dvl1 gene showed a delayed onset of cardiac hypertrophy upon thoracic aortic constriction (TAC). This was accompanied by an increased activity of GSK3β and lower levels of β-catenin, which is indicative for attenuated canonical Wnt signaling (van de Schans et al. 2007). Malekar et al. reported the upregulated expression of Dvl1 in rats exposed to TAC. Moreover, these researchers also studied the effect of cardiomyocyte-specific overexpression of Dvl1 in mice and observed severe cardiac hypertrophy at three months of age

without any hypertrophy-inducing intervention. This phenotype evolved into overt cardiomyopathy and premature death before 6 months of age. Interestingly, the authors showed an upregulation of both canonical and noncanonical signaling in these mice, suggesting that multiple Wnt signaling pathways contribute to the phenotype (Malekar et al. 2010).

The activity of Dvl proteins can be regulated by interacting with proteins from the Dapper family. Although Dapper proteins generally are considered to inhibit Wnt signaling, Hagenmueller et al. showed that overexpression of Dapper-1 activates canonical Wnt signaling through Dvl2 rather than Dvl1 or Dvl3 (Hagenmueller et al. 2013). This resulted in increased levels of β-catenin, mediating pro-hypertrophic gene expression. On the other hand, the same authors showed that Dapper-1 also plays a role in the induction of cardiac hypertrophy via noncanonical Wnt signaling as induced by Wnt5a overexpression (Hagenmueller et al. 2014). All in all, Dvl proteins appear to be central regulators of cardiomyocyte hypertrophy by controlling both canonical and noncanonical Wnt signaling.

3.3 Modulation of Signal Transduction

A key regulator in the canonical Wnt signaling is the serine–threonine kinase GSK3β, an enzyme that phosphorylates the second messenger β-catenin, targeting it toward degradation. GSK3β can also phosphorylate other transcription factors including NFAT, thereby preventing its nuclear translocation and activation of the hypertrophic gene expression (Sugden et al. 2008). Exposure of cardiomyocytes to hypertrophic stimuli such as endothelin-1 or insulin-like growth factor 1 results in a rapid inactivation of GSK3β by phosphorylation at its serine 9 residue. A similar inhibition of GSK3β activity was observed in rats as early as 4 h after aortic banding. Overexpression of a GSK3β mutant in which Serine 9 is replaced by alanine prevented the induction of hypertrophy by endothelin-1 and phenylephrine in cultured cardiomyocytes (Haq et al. 2000). Moreover, overexpression of this mutant in mice resulted in an attenuated hypertrophic response to pressure overload and β-adrenergic stimulation (Antos et al. 2002). On the other hand, inhibition of GSK3β by administration of LiCl accelerated the development of pressure overload-induced cardiac hypertrophy in rats (Tateishi et al. 2010). However, chronic GSK3 inhibition in obesity-induced prediabetic rats using the more specific GSK3 inhibitor CHIR118637 resulted in slight pro-hypertrophic effect (Huisamen et al. 2016), a result that cannot easily be reconciled with the previously mentioned studies. Although GSK3β activation is generally thought to be antihypertrophic, it is also associated with increased apoptosis and fibrosis and decreased contractility of the heart. Therefore, it remains questionable whether interventions at the level of this enzyme can form a viable therapeutic intervention for the hypertrophic heart (Sugden et al. 2008).

3.4 Modulation of Gene Transcription

Activation of the canonical branch of Wnt signaling results in an accumulation of β-catenin, which – when translocated to the nucleus – can serve as a transcription factor for multiple hypertrophy-related genes. In cultured cardiomyocytes, overexpression of β-catenin could induce hypertrophy (Haq et al. 2003), whereas depletion of β-catenin attenuated the hypertrophic response to phenylephrine (Zhang et al. 2009). Exposure to hypertrophic stimuli such as endothelin-1 or phenylephrine also stabilized β-catenin in an in vivo model of cardiac hypertrophy, albeit that the inactivation of GSK3β in these cases is dependent on protein kinase B rather than on Wnt/frizzled signaling (Haq et al. 2003). On the other hand, TAC-induced increase in heart weight/body weight ratio was attenuated in mice harboring cardiomyocyte-specific β-catenin haploinsufficiency (Qu et al. 2007). Opposite results were reported by Baurand et al., who observed an abolished hypertrophic response to angiotensin II in mice with stabilized cardiac β-catenin but a normal hypertrophic response in β-catenin-depleted hearts (Baurand et al. 2007). The reason for this deviant result is not clear.

4 Wnt Signaling in Myocardial Infarction

Myocardial infarction (MI) is the result of ischemia induced by interrupted blood flow through a branch of the coronary arteries, depriving the cardiomyocytes that depend on this artery for their blood supply from oxygen and nutrients. Prolonged ischemia causes cardiomyocyte death, leading to a wound healing response in the affected areas of the heart. This wound healing consists of different phases: inflammation, the formation of granulation tissue, and the maturation phase in which a scar is formed. The inflammation is triggered by the release of chemokines and cytokines from the damaged cardiomyocytes and starts with the invasion of the infarct area with polymorphonuclear neutrophils (PMNs) starting at 12–16 h post-MI, followed by macrophages (starting at 3 days post-MI). During the inflammatory phase, the infarct area is cleared from necrotic debris (Cleutjens et al. 1999). Granulation tissue starts to form in the infarct area around 1 week after MI and consists of fibroblasts, newly formed blood vessels, and loosely organized extracellular matrix. Under the influence of stretch and cytokines such as transforming growth factor-β (TGFβ), the fibroblasts can differentiate into myofibroblasts, a derivative of fibroblasts expressing α-smooth muscle actin. Therefore, myofibroblasts have both contractile and matrix-secreting properties, making them very suitable to produce a compact and strong replacement for the lost cardiomyocytes (van den Borne et al. 2010). Eventually, the number of cells in the infarct area decreases, and the extracellular matrix will mature by extensive cross-linking, resulting in the formation of a stable scar (Daskalopoulos et al. 2012).

There is increasing evidence that Wnt signaling is activated in multiple disease mechanisms activated during the wound healing process following MI. Using a mouse model of MI in which Wnt signaling could be visualized by LacZ expression

under Axin-2 promoter, Oerlemans et al. showed activation of Wnt signaling in many cell types including fibroblasts, endothelial cells, and progenitor cells. The activation pattern followed the expression pattern of multiple Wnt/Fzd signaling components, with an increase in the first week after MI and a gradual decrease after the second week. Activated Wnt signaling was also observed in the cardiomyocytes in the border zone (Oerlemans et al. 2010). Similar results were obtained in mice in which the LacZ expression was under the control of a TCF/LEF promoter (Aisagbonhi et al. 2011). These studies show that Wnt signaling is activated in the inflammation, neovascularization, and fibrosis that occur during infarct healing.

Wnt signaling plays a role in inflammatory cell differentiation (Staal et al. 2008). Wnt5a has been identified as a monocyte-derived factor with autocrine and paracrine functions. Its expression is upregulated in human mononuclear cells upon microbial activation of Toll-like receptors, and, via interaction with Fzd5, Wnt5a induces the expression of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and macrophage inflammatory protein-1. Interestingly, this increased expression of pro-inflammatory cytokines is the result of activation of noncanonical Wnt signaling via CAMKII and NFAT (Pereira et al. 2008; Blumenthal et al. 2006). Because after MI the innate immune system is activated via Toll-like receptor stimulation (Timmers et al. 2008), a role for Wnt signaling, presumably mediated by Wnt5a, can be anticipated.

The restoration of the blood supply to the infarct area is an essential step in the wound healing process that follows MI. During the neovascularization of the infarct area, sprouts of existing blood vessels are formed by specialized endothelial cells. In a study from our research group, the accumulation of β-catenin in endothelial cells of newly formed blood vessels in the infarct area was observed, suggesting the activation of canonical Wnt signaling in these cells (Blankesteijn et al. 2000). In the meantime, many aspects of the role of Wnt signaling in angiogenesis and neovascularization have been studied. A detailed description would be beyond the scope of this article so we refer to review articles on this subject (Dejana 2010; Blankesteijn and Hermans 2015).

Cardiac fibrosis is a commonly observed process in most cardiac pathologies, including the remodeling process following MI (Daskalopoulos et al. 2012). Three to five days after infarction, a decline of inflammatory signals and a concomitant upregulation of pro-fibrotic signals mark the onset of the reparative phase of the wound healing after MI (Francis Stuart et al. 2016). This phase is characterized by the invasion of the infarct area with fibroblasts. Although these fibroblasts may originate from different sources, a common feature is their differentiation toward a contractile phenotype called myofibroblast during the course of the wound healing process. The cytokine TGFβ is known to play a key role in this differentiation process (Hermans et al. 2016). There is increasing evidence that Wnt signaling acts in concert with TGFβ signaling in myofibroblast differentiation. Using cultured mouse fibroblasts, Carthy et al. could demonstrate that administration of Wnt3a induced differentiation toward a myofibroblast phenotype by overexpressing TGFβ in a β-catenin-dependent manner. Recently, a more complex interaction between Wnt and TGFβ signaling was reported by Blyszczuk and colleagues using a mouse

model of autoimmune myocarditis. They showed that activation of Wnt signaling induces the release of TGFβ by a β-catenin-dependent mechanism but that TGFβ in its turn was capable to stimulate Wnt secretion. These findings suggest that the pathways are strongly intertwined, opening new options for therapeutic intervention (Blyszczuk et al. 2016).

4.1 Modulation of Wnts

The expression of members of the Wnt protein family has been determined in different studies. In all of them, overexpression of Wnt10b was detected (Aisagbonhi et al. 2011; Barandon et al. 2003; Paik et al. 2015), and in some studies, other members, like Wnt2, Wnt4, and Wnt11, were found to be upregulated, whereas the downregulation of Wnt7b was observed. Injection of recombinant Wnt3a protein into the border zone of the infarct area resulted in attenuated cardiac regeneration and an impaired cardiac function due to a decreased renewal of the cardiac side population via an insulin-like growth factor-binding protein-3-mediated mechanism (Oikonomopoulos et al. 2011). In contrast, using mice with cardiomyocyte-specific overexpression of Wnt10b, Paik et al. showed an improved cardiac repair after MI with increased arteriole formation and less fibrosis compared to wild types (Paik et al. 2015). Overexpression of Wnt11 via AAV9-mediated gene transfer suppressed the inflammatory response after infarction, reduced fibrosis, improved cardiac function, and prevented mortality (Morishita et al. 2016). These results clearly illustrate the diverse effects of the different members of the Wnt family on the wound healing process post-MI and call for a more detailed analysis of the mode of action of the Wnts.

4.2 Modulation of Wnt Signaling at the Extracellular Level

The role of sFRPs in infarct healing has been studied by different research groups. FrzA, also known as sFRP1, but also sFPR2 and SFRP4 have been reported to be upregulated after MI. The typical expression pattern for all sFPRs is that they reach a peak at 3–7 days post-MI, followed by a gradual decline in the expression to baseline at 14–28 days (Barandon et al. 2003; Kobayashi et al. 2009; He et al. 2010; Matsushima et al. 2010; Askevold et al. 2014). Increased circulating levels of sFRP3 were detected in heart failure patients, and high sFRP3 levels were associated with increased cardiovascular mortality (Askevold et al. 2014). Moreover, a genetic variant in the sFRP1 gene (rs7832767 C > T) was found to be associated with an increase in risk to develop MI (Tao et al. 2016).

In murine models of MI, genetic modulation of the expression of different sFRPs has been reported to affect the wound healing after MI. General overexpression of sFRP1 in mice reduced infarct size and improved cardiac function after MI, with reduced myocyte apoptosis and leukocyte infiltration and increased formation of capillaries and extracellular matrix (Barandon et al. 2003). The anti-inflammatory

effect could be reproduced by transplantation of bone marrow cells overexpressing sFRP1 into wild-type mice that subsequently were subjected to MI (Barandon et al. 2011). Protective effects of sFRP2 and sFRP4 were described by Mirotsou et al. and Matsushima et al., who injected this protein into the infarct area of rats and observed reduced ventricular fibrosis and attenuated adverse remodeling of the left ventricle at 4 weeks post-MI (Matsushima et al. 2010; Mirotsou et al. 2007). Similar beneficial effects on infarct healing were reported in sFRP2 knockout mice, although this effect was attributed to the procollagen C proteinase-activating effect of this sFRP rather than its interference with Wnt signaling (Kobayashi et al. 2009). Recently, the role of sFRP5 in response to cardiac ischemia/reperfusion was studied using sFRP5 knockout mice. These mice showed larger infarcts with stronger inflammatory infiltrates, more cardiomyocyte apoptosis, and reduced cardiac function (Nakamura et al. 2016). Therefore, the general picture that emerges is that most of the sFRPs have a protective effect in the wound healing process following MI, suggesting that an inhibition of Wnt signaling is beneficial for this process.

4.3 Modulation of the Receptor Complex

The first indication of the activation of Wnt/Fzd signaling in the wound healing process after MI came from a study from our research group, where the expression of Fzd2 was studied in infarcted rat hearts. The Fzd2 expression gradually increased till day 7 and then declined to baseline levels at day 28 post-MI. Fzd2 expression co-localized with myofibroblasts migrating into the infarct area, suggestive for a role in migration and alignment (Blankesteijn et al. 1997). A similar expression pattern has been observed for the Dvl1 expression in the infarcted heart (Chen et al. 2004). In the meantime, the overexpression of multiple Fzds, including Fzd1, Fzd2, Fzd5, and Fzd10, and the downregulation of Fzd8 expression have been confirmed by others (Barandon et al. 2003). At present, no studies have been published in which the effect of interventions in these genes on infarct healing was assessed. Injection of DKK2, an antagonist of Wnt signaling that binds to the coreceptor LRP5/LRP6, was shown to be beneficial for infarct healing in a rat model of cardiac ischemia/reperfusion, as it reduced infarct size at 1 week post-MI and improved function at 3 weeks post-MI (Min et al. 2011).

4.4 Modulation of Signal Transduction

No interventions have been reported that specifically target the Wnt signaling complex in non-cardiomyocytes in the infarct area. However, cardiomyocyte-specific deletion of GSK3β resulted in an attenuated ventricular dilation and an improved cardiac function at 8 weeks post-MI. This effect could mainly be attributed to an increased hypertrophic response of the cardiomyocytes remote from the infarct, rather than an effect on the wound healing in the infarct area (Woulfe et al. 2010).

4.5 Modulation of β-Catenin

In the canonical Wnt signaling pathway, β-catenin acts as a second messenger molecule that – when migrated into the nucleus – can activate the transcription of genes relevant for the cardiac remodeling process. In several studies, the effect of modulation of β-catenin on the wound healing after MI was addressed. Overexpression of a constitutively active form of β-catenin by adenoviral transduction resulted in increased myofibroblast differentiation and proliferation in the infarct area, combined with antiapoptotic and pro-hypertrophic responses in cardiomyocytes in the border zone (Hahn et al. 2006). Knocking out β-catenin in epicardial cells resulted in attenuated fibroblast generation due to a blockade of epithelial-to-mesenchymal transition, which is dependent on Wnt1/β-catenin signaling. This indicates that canonical Wnt signaling is necessary for the fibrotic response following MI (Duan et al. 2012). However, Zelarayan et al. reported opposite results in a model of cardiomyocyte-specific β-catenin depletion. In this model, beneficial effects on infarct healing were observed, with improved function, less dilatation, and the presence of cardiac progenitor cells (Zelarayan et al. 2008). The reason behind these contradictory findings still has to be resolved, but it raises doubt about the therapeutic potential of targeting Wnt signaling at the level of β-catenin.

5 Wnt Signaling and Heart Failure

Many cardiovascular conditions, including cardiac hypertrophy and MI, can lead to the development of heart failure. In several studies addressing heart failure in humans, a link with increased levels of sFRPs was observed. Increased mRNA levels of sFRP3 and sFRP4, but not sFRP1 and sFRP2, were observed in failing human hearts and were related to decreased β-catenin levels (Schumann et al. 2000). The increased expression of sFRP3 was recently confirmed by Askevold and colleagues, who also reported that the increased level of sFRP3 in serum of heart failure patients was associated with cardiovascular mortality (Askevold et al. 2014). These observations suggest that Wnt signaling is inhibited in heart failure by the expression of the endogenous Wnt antagonists. It has to be noted, however, that the correlation between sFRP3 levels and the occurrence of a cardiovascular event just failed to reach statistical significance in a 1-year follow-up of a group of 142 heart failure patients (Motiwala et al. 2014). In contrast, evidence for an activation of canonical Wnt signaling in human as well as murine failing hearts was recently published by Hou et al. In this study, nuclear accumulation of β-catenin and activation of the transcription factor TCF7L2 resulted in the increased expression of c-Myc, a Wnt target gene expressed during cardiac stress (Hou et al. 2016).

6 Therapeutic Interventions in the Wnt Signaling Pathway

Aberrant Wnt signaling has not only been observed in cardiovascular diseases but also in many other conditions such as osteoporosis (Joiner et al. 2013), neurodegenerative diseases (Libro et al. 2016), and cancer (Anastas and Moon 2013). This has generated significant interest in the development of therapeutic agents, preferably small molecules that allow interventions in this pathway in order to modulate the disease process (Daskalopoulos et al. 2013). Due to the complexity of the pathway, interventions can be developed that target the signal transduction cascade at different levels, as depicted in Fig. 3. Here, we will describe the different groups of therapeutic agents that have been developed and subsequently discuss their potential application in the therapy of cardiac remodeling and heart failure.

6.1 Agents Affecting the Secretion of Wnt Proteins

In order to be biologically active, Wnt proteins are extensively modified by palmitoylation and glycosylation. A member of the O-acyltransferases called porcupine is responsible for the palmitoylation of Wnt in the endoplasmic reticulum; this modification is essential for the excretion of Wnt from the cell but also for its interaction with the cysteine-rich domain of Fzds (Langton et al. 2016). Two compounds have been described that can inhibit porcupine, thereby reducing the

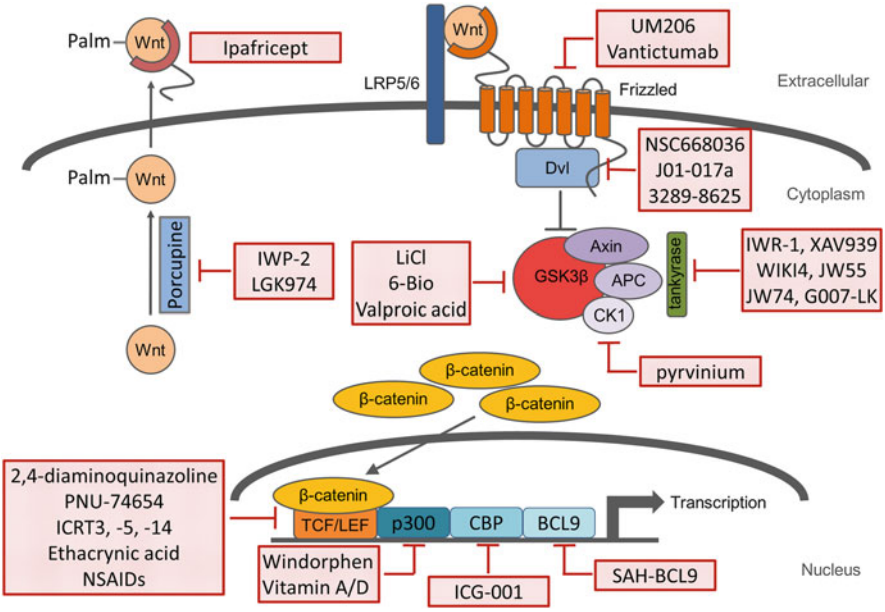

Fig. 3 Sites of pharmacological modulation of Wnt/frizzled signaling. The molecular targets of the drugs mentioned in the chapter are schematically represented in this figure

amount of biologically active Wnt that is available. The first one, IWP-2, has been shown to attenuate the improvement of cardiac conduction induced by transplantation of mesenchymal stem cells in cardiac tissue, suggesting a Wnt-dependent paracrine induction of connexin 43 expression (Mureli et al. 2013). For a second porcupine inhibitor, LGK974, no studies have been published in which the compound was tested in cardiac remodeling.

6.2 Agents Interfering with Wnt Signaling at the Extracellular Level

Wnt signaling activity can be regulated by endogenous modulators such as sFRPs. Choosing a similar approach, a Wnt scavenger (OMP-54 F28/ipafricept) was developed consisting of the cysteine-rich domain of Fzd8 fused to the human immunoglobulin Fc domain. Moreover, vantictumab (OMP-18R5), an antibody directed against Fzd7 but having cross-reactivity with Fzd1, Fzd2, Fzd5, and Fzd8, and a selective Fzd10 monoclonal antibody (OTSA101) were developed (Blagodatski et al. 2014). These compounds are currently in clinical trials for cancer therapy but, to our knowledge, have not been tested in cardiovascular diseases.

An alternative approach to modulate Wnt signaling at the level of the receptor complex is by using peptide fragments of Wnt proteins. The research team led by Andersson was the first to demonstrate that this approach can yield both a mimetic [Foxy-5 (Safholm et al. 2006)] and an inhibitor [Box5 (Jenei et al. 2009)] of Wnt5a. These compounds were shown to be effective in models of cancer cell migration but to date no studies are published on applications in the cardiovascular field. Following similar lines, our research group tested several Wnt5a-derived peptides for interference with the Wnt3a-mediated activation of canonical Wnt signaling. This resulted in the identification of UM206, an 11-amino acid peptide containing two cysteine residues. Administration of UM206 to mice in which MI was induced by permanent ligation of the left ascending coronary artery resulted in reduced infarct expansion, increased infarct thickness, and higher myofibroblast numbers in the infarct area. This was accompanied by a marked reduction in mortality due to heart failure, decreased lung weight, and improved cardiac function (Laeremans et al. 2011). Similar improvements in infarct healing were observed when UM206 was administered to a swine model of MI in which myocardial injury was induced by a temporary occlusion of the left circumflex coronary artery followed by reperfusion. Continuous infusion of UM206 for 5 weeks resulted in a significant decrease in infarct mass and halting of the adverse remodeling compared to saline-infused swine. However, this was accompanied by a reduction rather than an increase in myofibroblasts in the infarct area (Uitterdijk et al. 2016).

6.3 Agents Interfering with the β-Catenin Destruction Complex

As discussed before, the cellular β-catenin content is tightly regulated by a protein complex generally referred to as the β-catenin destruction complex. Inhibitors for several components of the destruction complex have been described, including the Dvl inhibitors NSC668036, J01-017a, and 3298-8625, but none of these have been tested in the context of cardiac remodeling. Tankyrase-1 and tankyrase-2 are members of the poly(ADP-ribose) polymerase (PARP) family and can stimulate the degradation of Axin (Riffell et al. 2012). Multiple tankyrase inhibitors have been described, including IWR-1 and XAV939. These two compounds can inhibit β-catenin-mediated Wnt signaling in stem cells, thereby inducing differentiation toward a cardiomyocyte phenotype in embryonic (Wang et al. 2011) or pluripotent (Weng et al. 2014) stem cells.

Pyrvinium, an FDA-registered antihelminthic compound which – next to other mechanisms – interferes with the β-catenin destruction complex by targeting casein kinase 1α, has been tested in infarct healing in mice in two independent studies. Saraswati and colleagues reported that a single intramyocardial injection of pyrvinium after induction of MI by coronary artery ligation reduced adverse cardiac remodeling and induced myocyte proliferation in the peri-infarct and remote areas, but did not improve cardiac function (Saraswati et al. 2010). Murakoshi et al. reported that administration of pyrvinium via oral gavage for 14 days post-MI reduced the size of the scar and improved cardiac contractility in a mouse model of MI (Murakoshi et al. 2013). These results underscore that inhibition of canonical Wnt signaling after MI has beneficial effects on the adverse cardiac remodeling by interfering with fibroblast function and stem cell differentiation.

6.4 GSK3β Inhibition

Several drugs that are frequently used in the clinic have been shown to act as inhibitors of GSK3β. These drugs include LiCl and valproic acid which are commonly used to treat bipolar disorders. However, as these drugs are likely to have multiple targets besides GSK3β, they are not suitable to study the role of this particular enzyme in cardiac remodeling. In the meantime, more specific inhibitors of GSK3β have been introduced, including the compound 6-BIO. This compound was shown to induce proliferation of adult rat cardiomyocytes by activation of canonical Wnt signaling (Tseng et al. 2006). However, as this compound lacks selectivity between GSK3α and GSK3β, two GSK3 isoforms with distinct effects on cardiomyocytes, further research with either isoform-specific antibodies or RNAi-interventions is needed to confirm the role of GSK3β in this context (Lal et al. 2015).

6.5 Agents Interfering with the Transcriptional Activity of β-Catenin

In the nucleus, β-catenin forms a complex with transcription factors from the TCF/LEF family and coactivators such as P300, CBP, and Bcl-9. In many forms of cancer, a mutation in the β-catenin destruction complex induces uncontrolled activation of the signaling transduction pathway, leading to high levels of β-catenin in the nucleus. Therefore, much effort has been made in the field of cancer therapeutics to develop agents that interfere with the interaction of β-catenin and its nuclear partners, in order to inhibit the transcription of Wnt target genes. These target genes play a stimulating role in cell proliferation and migration, thereby contributing to tumor development (Blagodatski et al. 2014).

Because the interaction of β-catenin with TCF/LEF is critical for activation of the transcription of target genes, several compounds (e.g., PNU-74654; iCRT3, iCRT5, and iCRT14; and ethacrynic acid) have been identified that block this interaction (Zhang and Hao 2015). However, to our knowledge, none of these compounds have been tested in a context of adverse cardiac remodeling or heart failure. Inhibition of the β-catenin-mediated gene transcription can also be achieved by targeting the cofactors that form the transcription complex. The small molecule ICG-001, also named PRI-724, inhibits Wnt-induced gene transcription by blocking the interaction between β-catenin and the coactivator CREB-binding protein (CBP). ICG-001 was tested in a rat model of MI, where it was administered during the first 10 days after induction of the infarct. This treatment resulted in a significant improvement of cardiac function at 4 weeks post-MI. This was accompanied by a trend toward reduced infarct expansion and increased expression of genes involved in cardiac regeneration, such as Gdfl5, kit, and Sdf1, in epicardial cells. The authors conclude that inhibition of β-catenin-mediated Wnt signaling may hold promise to improve infarct healing by stimulating epicardial stem cell differentiation (Sasaki et al. 2013). Interestingly, nonsteroidal anti-inflammatory drugs such as sulindac and celecoxib can attenuate β-catenin-mediated gene transcription too. Although the precise mode of action is unclear, it appears to be independent from cyclooxygenase inhibition which is the main mode of action of this class of drugs (Gurpinar et al. 2014), and it is unclear how this pleiotropic effect can affect cardiac remodeling.

7 Conclusion

Over the last years, evidence has accumulated that Wnt signaling plays an important role in the control of cardiac development and in the adaptive response of the heart to different challenges. Many studies have been published in which the effects of interventions in Wnt signaling on cardiac remodeling were addressed. However, it has to be noted that in the vast majority of these studies, genetic interventions (either overexpression or inactivation of genes) have been used. Only in a minority of the studies, pharmacological interventions have been used, although from a

translational perspective this approach would be favorable. Because of the importance of Wnt signaling in other disease areas, including cancer and bone metabolism disorders, many drugs have been developed that allow interventions at different levels of the signal transduction cascade. Because in cardiac diseases dysregulations rather than mutations in the Wnt signaling pathway are thought to contribute to the maladaptive response, these drugs can facilitate the next step in the exploration of Wnt signaling as a novel therapeutic target for cardiac diseases and help to identify the levels in the signaling cascade that are most suitable for intervention.

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Gene Therapy in Heart Failure

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Abstract

Heart failure is a significant burden to the global healthcare system and represents an underserved market for new pharmacologic strategies, especially therapies which can address root cause myocyte dysfunction. Modern drugs, surgeries, and state-of-the-art interventions are costly and do not improve

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_81

survival outcome measures. Gene therapy is an attractive strategy, whereby selected gene targets and their associated regulatory mechanisms can be permanently managed therapeutically in a single treatment. This in theory could be sustainable for the patient's life. Despite the promise, however, gene therapy has numerous challenges that must be addressed together as a treatment plan comprising these key elements: myocyte physiologic target validation, gene target manipulation strategy, vector selection for the correct level of manipulation, and carefully utilizing an efficient delivery route that can be implemented in the clinic to efficiently transfer the therapy within safety limits. This chapter summarizes the key developments in cardiac gene therapy from the perspective of understanding each of these components of the treatment plan. The latest pharmacologic gene targets, gene therapy vectors, delivery routes, and strategies are reviewed.

Keywords

Gene therapy • Gene therapy vectors • Heart failure • Molecular targets • Routes of gene delivery

Abbreviations

AAV	Adeno-associated virus
AC6	Adenyl-cyclase type 6
Akt	Serine-threonine protein kinase
Bcl-2	B-cell lymphoma 2 gene
CCN family	Extracellular matrix-associated proteins
cDNA	Complementary DNA
CHF	Congestive heart failure
CVD	Cardiovascular diseases
dATP	Deoxy adenosine triphosphate
DNA	Deoxyribonucleic acid
GRK2 G	Protein-coupled receptor kinase 2
LacZ	Intracellular enzyme encoded beta-galactosidase
LVEF	Left ventricular ejection fraction
MCARD	Molecular cardiac surgery with recirculating delivery
microRNA	Small non-coding RNA
modRNA	Modified RNA
ODN	Antisense oligodeoxynucleotides
P-13	Chromosomally encoded integral outer membrane protein
PLN	Phospholamban
RNA	Ribonucleic acid
S100A1	Calcium-binding protein A1
SERCA2a	Sarcoplasmic reticulum calcium ATPase
siRNA	Short interfering RNA

SR	Sarcoplasmic reticulum
TGF beta	Transforming growth factor beta
VEGF	Vascular endothelial growth factor
βARKct	Carboxyl terminus of beta-adrenergic receptor kinase

1 Introduction

Approximately 85 million Americans suffer from acute and chronic cardiovascular diseases (CVD). The associated mortality rates are stark, accounting for at least 33% of all deaths post 2010. This rate is 4.4 times more than cancer related morbidity and growing inline with record levels of obesity and diabetes. The total direct and indirect costs of CVD in the USA are estimated to be $315.4 billion, which is unsustainable (Go et al. 2013). These statistics clearly demonstrate that CVD are still the leading cause of mortality and morbidity in developed countries despite an increased awareness and focus on preventative measures.

Coronary artery disease is the prime risk factor and direct cause of CVD, prevalent in at least 65–75% of cases (St. John-Sutton et al. 1997). Coronary vessel disease progression typically results in single or successive myocardial infarction events, which permanently alter global function. Despite numerous interventions available to limit the initial postinfarction damage in the acute phase, the downward progression toward the congestive heart failure (CHF) condition is largely inevitable. Through the remodeling disease process strained myocytes are either lost, transition, or remain in a dysfunctional state typically out of sync with viable myocardium.

The CHF condition is the most expensive burden on the global healthcare system featuring accelerating annual costs alone exceeding $34 billion. The majority of these patients are destined for heart failure status despite significant efforts in accumulated, yet ineffective pharmacological interventions. All clinically available heart failure drugs, devices, and corrective interventional procedures only address secondary symptoms. Consequently, an additional 22 million CHF patients are diagnosed per year with 50% mortality within 5 years, despite cumulative failed therapeutic regimens.

Cardiac transplantation is the best option for end-stage CHF for long-term outcomes; however, more than 35% of patients unfortunately die waiting for a matched donor heart. Even more disturbing, as many as 60,000 patients per year could benefit from a transplant (Frazier and Myers 1998). Thus, given the shortcomings of the best available pharmacologic based therapies and donor hearts, there is a significant unmet need for newer, more efficacious, and more cost-effective treatments for severely afflicted patients.

There are two alternative treatment paradigms to improve long-term outcomes in both the early and late stage of CVD. These two paradigms are either a replacement (i.e., cell therapy) approach or a restorative genetic reprogramming of cells with gene therapy via vector transfer of nucleic acids. Although much more attention has

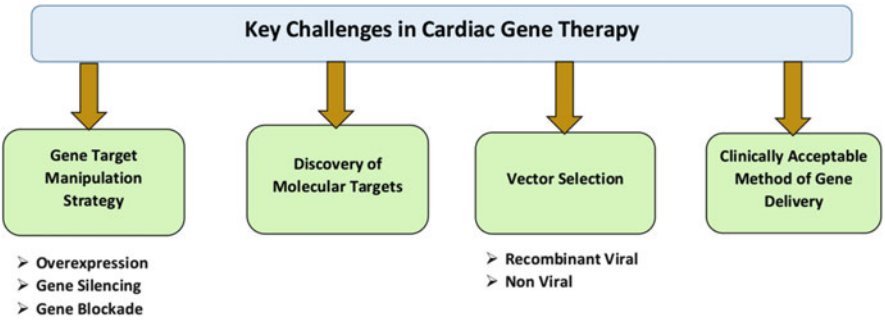

Fig. 1 Key challenges in cardiac gene therapy

been placed in cellular replacement strategies, these approaches do not address improving contractility function which is essential for increasing survival outcomes in CHF. The focus in late stage management is improving contractility, whereby the opportunity to arrest infarct expansion and global LV remodeling has past. Therefore, the transfer of therapeutic genes for the treatment has emerged as the more attractive strategy given its therapeutic impact on directly enhancing contractility.

The field of cardiovascular gene therapy has generated significant interest in recent times having completed a Phase II placebo controlled clinical trial in a robust heart failure population. A large base of extensive preclinical studies have provided solid proof of concept data indicating gene therapy's clinical potential, whereby the expression of selected transgenes in the myocardium enhances contractility, restores global function, and in some cases completely reverses CHF. Although promising, a number of challenges must be addressed in order to surpass regulatory clearance and ultimately reach the clinic.

The key translational challenges that must be addressed with this new therapeutic class are the following (Fig. 1), which will be extensively covered in the following text:

1. The validation of the specific target's genetic manipulation in the heart failure condition including overexpression and knockdown approaches
2. Selection of the best vector to manipulate the target that is both safe and results in efficacious expression
3. A clinically feasible method of delivery to safely achieve sufficient transfer in the myocardium
4. The discovery of novel targets that address root causes of myocyte function at the protein, RNA, and in some cases microRNA level
5. Synthesizing the said elements above such that the treatment is safe and effective as well as cost efficient for the healthcare provider.

2 Cardiac Gene Therapy Manipulation Strategies

A number of key gene therapeutic strategies have been investigated for improving outcomes in heart failure patients. The most commonly applied strategy features overexpression of a target gene, which involves either the replacement of a missing or restoring a gene's resultant protein levels. Dysfunctional genomic regulation in the heart can either be due to inherited genetic and or acquired with progressive cardiac disease. A common example of a purely inherited genetic is the X-linked recessive Becker's cardiomyopathy, which is an autosomal recessive gene defect such as those associated with alpha sarcoglycan deficiency. It is important to note that less research has been dedicated to this cause since a very low number of patients suffer from these defects. More commonly, however, ischemic cardiomyopathy induced CHF is characterized by certain genes that are consistently downregulated (e.g., SERCA2a). With the advent of biotechnology via rapid DNA/RNA profiling, these perturbations are readily identified against healthy controls. Moreover, viable mechanisms and interactions are in place but perturbed due to the degradation process secondary to the original infarction events. In the case of heart failure as covered later in this text, there are several key targets that have influential roles in driving contractility, energetics, survival, and structural integrity.

An alternative strategy to manipulating cardiac gene regulation is with gene silencing or blockade, which relates to the inactivation of dominant negative gene function involved in disease etiology or progression (Quarck and Holvoet 2004; Melo et al. 2005). By comparison with overexpression whose sole aim is to achieve a factor fold higher expression level, the degree of necessary efficacious interactions with this class is inherently much more complex since the expressed target must then interact further with subcomponents. Effective silencing often requires overexpression of the silencing element plus sufficient interaction with associated mechanisms of the disease mechanism, which may not be well established. Thus, due to the degree of conflicting interactions, these are much more difficult to execute in large animal model and human cardiovascular systems despite proof of concept in basic science experiments.

2.1 Target Overexpression Approaches

A gene's physiological function may be impaired or downregulated as a result of a mutation or a pathological process. Therefore, the restoration of function through exogenous DNA delivery to replace the deficient gene's action seems quite logical, but challenging to execute in vivo. In this case, full-length or partial cDNA encoding the deficient gene is delivered to the heart using a vector system capable of expressing the therapeutic protein (Quarck and Holvoet 2004). Several steps in the gene overexpression process may be modulated including the transcription, RNA splicing, translation, and posttranslational modification of a protein.

2.2 Silencing and Blockade Approaches

2.2.1 Antisense Oligodeoxynucleotides

Antisense oligodeoxynucleotides (ODN) are used as inhibitors of specific gene expression without any change in function of other genes. Single stranded ODN may be delivered either by direct administration (as a pharmacological agent) or by transfection with a vector encoding the ODN. The ODN binds to the target mRNA transcript and prevents translation. This mechanism of action is based on the presence of two forms of ODN: the RNase H-dependent ODN, which induces the degradation of mRNA, and the steric-blocker ODH, which physically blocks the progression of mRNA translation.

In CVD applications, the antisense ODH approach has been evaluated to prevent restenosis after balloon angioplasty (Quarck et al. 2001). In one study, treatment with antisense ODN directed against VEGF receptors could prevent VEGF-mediated arteriogenesis (Marchand et al. 2002). In another example, the systemic delivery of an antisense ODN induces silencing of miR-208a in the myocytes, thus improving cardiac function and survival in hypertensive-induced heart failure in rats (Montgomery et al. 2011).

2.2.2 Short Interfering

Gene silencing via siRNA technology is a promising strategy with great therapeutic potential despite continued problems with translation into the clinic due to delivery issues. siRNA is a short dsRNA molecule that induces sequence specific posttranscriptional gene modification. This mechanism is called RNA interference (RNAi). Recently, this strategy was used for the treatment of HF and the results showed that the restoration of cardiac function was most likely through the reduction of hypertrophy (Suckau et al. 2009). Once transferred into the cytoplasm, the siRNA incorporates into the nuclease complex, where they then disrupt the translation of the targeted genes. Successful left ventricular intracavitary delivery of DNA/siRNA complexes by means of sonoporation was demonstrated in murine hearts (Tsunoda et al. 2005). The incorporation of siRNA into terminally differentiated adult rat cardiac myocytes using adenovirus has also been reported (Rinne et al. 2006).

Due to sustainability challenges with blockage and silencing approaches, single gene target overexpression continues to dominate in the field since the posttranslational interactions and immune barriers are more lucid. Safe long-term overexpression provides the best possible chance to impact cardiac dysfunction provided sufficient levels of cDNA are present to drive therapeutic protein. siRNA, ODN, and other blockade strategies often have difficulty due to either insufficient delivery or other unknown mechanisms that counteract the intervention at the mRNA level. These strategies, however, are more attractive in angiogenesis applications where it is more desirable for the treatment duration to be short-term for repair. This is in direct contrast with the CHF patient's need in driving contractility.

3 The Molecular Basis of Congestive Heart Failure: Established and Novel Targets

Generally, the accepted clinical definition of CHF is the pathophysiologic state of impaired heart function that can result from any structural or functional cardiac disorder. These individually or compounded impair the ability of the ventricles to work as pump for maintaining metabolic requirements of the body's tissue and organs (Go et al. 2013). CHF is a progressive disease and irrespective of the cause, is accompanied by deterioration of cardiac function. From the pathophysiological molecular perspective, CHF includes alterations in the myocytes gene expression, qualitative changes in cardiac cell types, and composition of extracellular matrix. These alterations compounded eventually lead to structural changes of left ventricle geometry.

CHF can be categorized as systolic or diastolic. Systolic CHF is characterized by a reduction in left ventricular ejection fraction (LVEF), enlargement of the left ventricle, a reduction in contractility, and pulmonary congestion. In post-myocardial infarction (post-MI), the systolic function is primarily impaired. On the other hand, diastolic CHF with normal LVEF is currently diagnosed in approximately 40–45% of heart failure patients. This type of CHF can mainly be attributed to LV diastolic dysfunction. The contributing factors including impaired LV relaxation, decreased LV distension, and increased LV end-diastolic stiffness.

In the last decade there has been tremendous increase in knowledge concerning the molecular mechanisms underlying both types of heart failure. The most recent developments indicate that the human heart in CHF is subjected to numerous important gene, cell, and organ level molecular changes which include these primary alterations: (1) in excitation–contraction coupling leading to changes in the contractile properties of the myocyte; (2) cytoskeletal proteins such as sarcomeric, membrane-associated, and proteins of the intercalated disc; (3) myosin heavy chain expression; (4) the maladaptive progression of β-adrenergic desensitization; (5) occurrence of hypertrophy, and myocytolysis with myofibrillar degeneration and progressive loss of myofilaments; and (6) abnormal myocardial energetics secondary to mitochondrial dysfunction (Shah and Mann 2011).

These changes in turn cause myocyte loss due to necrosis, apoptosis and autophagy, and alterations in composition of the extracellular matrix including enhanced matrix degradation and myocardial interstitial and perivascular fibrosis (Mann et al. 2012). Pharmacological management of CHF, such as angiotensin converting enzyme inhibitors, β-adrenergic receptor blockade, calcium-channel blockers, diuretics, and inhibition of renin-angiotensin-aldosterone system. These have provided benefit to decrease morbidity and mortality. However, this treatment is symptom-oriented and cannot stop the disease progression and reversal CHF to healthy state, thus increasing the value of a gene therapy solution which directly focuses on permanently treating the mechanism. Here we summarize the current state of main existing molecular targets in cardiac gene therapy (Fig. 2).

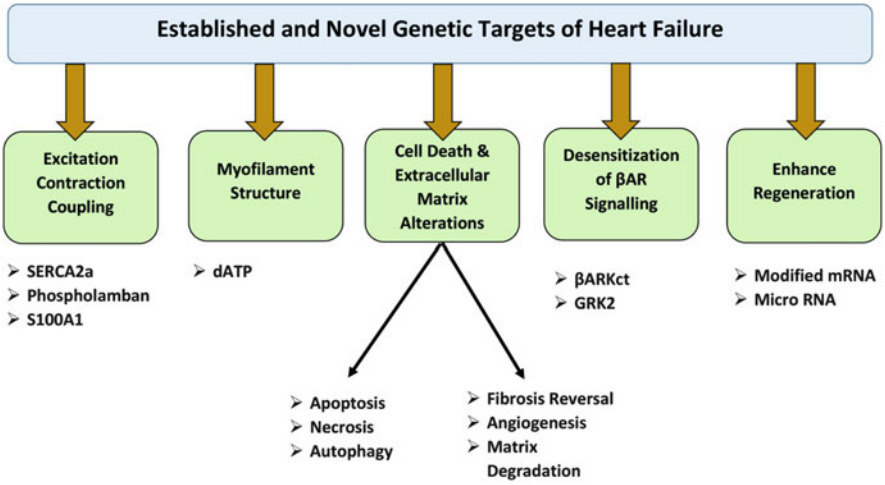

Fig. 2 Established and novel genetic targets in heart failure. *SERCA2a* sarcoplasmic reticulum calcium ATPase, *S100A1* calcium-binding protein A1, *dATP* deoxy adenosine triphosphate, *βARKct* carboxyl terminus of beta-adrenergic receptor kinase, *GRK2* G-protein-coupled receptor kinase 2

3.1 Excitation–Contraction Coupling

To understand the molecular defects in heart failure, we need to briefly describe the processes occurring in cardiac excitation–contraction coupling. During the cardiac action potential, Ca^{2+} enters the cell through depolarization-activated Ca^{2+} channels as an inward Ca^{2+} current, which contributes to the action potential plateau. This action lead to triggers Ca^{2+} release from the sarcoplasmic reticulum (SR) via ryanodine receptors located at the SR membrane. This, in turn, allows Ca^{2+} to bind to the myofilament protein troponin C, resulting in sarcomere shortening and muscle contraction. For relaxation to occur, it is necessary to have a decrease in intracellular Ca^{2+} concentration, thus allowing Ca^{2+} to dissociate from troponin. This requires Ca^{2+} transport out of the cytosol by pathways involving SR Ca^{2+} ATPase, sarcolemmal Na+/Ca^{2+} exchange, sarcolemmal Ca^{2+} ATPase, or mitochondrial Ca^{2+}. (Hajjar et al. 2000).

3.1.1 SERCA2a

Deficient SR Ca^{2+} uptake during myocyte relaxation has been identified in failing hearts from both humans and animals and is associated with a decrease in the expression and activity of the sarcoplasmic reticulum calcium ATPase (*SERCA2a*). This protein is a Ca^{2+} ATP-dependent pump of the sarcoplasmic reticulum that has a critical role in Ca^{2+} regulation. The importance of *SERCA2a* in HF has been studies extensively in rodents and large animal models and it was tested in clinical trials. Gene transfer of *SERCA2a* restored the calcium transit and significantly improved contraction and relaxation velocity in all experimental models of heart

failure. The mechanism of improved heart function induced by *SERCA2a* overexpression includes an enhanced SR Ca^{2+} uptake during diastole, increased SR Ca^{2+} content, and more efficient Ca^{2+} efflux during systole. Moreover, *SERCA2a* gene delivery with normalization of intracellular Ca^{2+} prevents mitochondrial dysfunction and decreases energy cost for regulation. This results in the restoration of cardiac energetics by improving mechano-energetic efficiency and by enhancing energy supply (Hajjar et al. 1998). On basis of these promising results, the CUPID 2 clinical trial was designed to assess whether gene transfer with SERCA2a improves outcome in patients with moderate to severe heart failure. CUPID 2 was the largest gene transfer study done in patients with HF so far. However, the results of AAV1/SERCA2a at the dose and delivery mode tested did not support the positive findings seen in a pilot trial (Greenberg et al. 2016).

3.1.2 Phospholamban Inhibition

Phospholamban (PLN) regulates the homeostasis of SR Ca^{2+}, mediating slower cytosolic Ca^{2+} decay in cardiomyocytes, which translates into diastolic relaxation. It is hypothesized that the suppression of the inhibitory effect of PLN is a promising approach to improve cardiac function. Indeed, AAV-mediated overexpression of a mutant form of PLN improved LV function and mitigated adverse remodeling (Tsuji et al. 2009). Silencing of PLN expression after tachycardia-induced CHF in sheep increased ejection fraction and decreased LV end-diastolic area (Kaye et al. 2007).

3.1.3 S100A1

In cardiomyocytes, S100A1 primarily plays a role in increasing SERCA2a activity. This is achieved via diminishing diastolic SR Ca^{2+} leak, reinforcing function of the ryanodine receptors during systole, leading to an overall gain in SR Ca^{2+} cycling. S100A1 regulates SERCA2A/Phospholamban function, resulting in an amplification of SR Ca^{2+} release and uptake as well. The molecular level of S100A1 is reduced at the development of CHF (Most et al. 2007). This confirms that S100A1 may be a promising factor in the treatment of CHF. A study in a postinfarction pig model with CHF revealed improvement in contractility and also restoration of high-energy phosphate homeostasis (Pleger et al. 2011).

3.2 Targeting the Myofilaments

The use of 2-deoxy adenosine triphosphate (dATP) instead of adenosine triphosphate (ATP) as the energy source improves muscle contractility by enhancing crossbridge binding and cycling kinetics (Regnier et al 2004; Nowakowski et al. 2013). It was developed as an approach to elevate dATP in vivo by increasing the expression of the enzyme ribonucleotide reductase. This results in increased levels of 2-deoxy ATP (dATP). It was shown that increasing dATP in intact cardiomyocytes via adenovirus-mediated transfection increased contractile magnitude and kinetics (Moussavi-Harami et al. 2015). dATP can increase contraction

and the rate of crossbridge cycling in cardiac muscle from patients with end-stage heart failure, without retarding relaxation. It was also found that raising cultured rat cardiomyocyte dATP levels to only ~1% by adenoviral vector-mediated overexpression of ribonucleotide reductase under control of the constitutive cytomegalovirus promoter, significantly enhances the magnitude and rate of contraction, as well as the rate of relaxation, without altering intracellular Ca^{2+} transient amplitude (Kolwicz et al. 2016). Based on these results, overexpression of RNR and increased dATP content appear to have significant potential as a therapeutic strategy with the myofilament approach for treating heart failure.

3.3 Inhibiting Apoptosis

3.3.1 Bcl-2

Apoptosis regulates the balance between pro-death and pro-survival cell signals. The Bcl-2 family of proteins has emerged as a key component of the cell death process. Bcl-2 can prevent the opening of mitochondrial transition pores and the resultant release of cytochrome *c* that directly triggers apoptosis. Also, Bcl-2 can block cytokine-mediated apoptosis via the nuclear factor-kB signaling pathway. In an animal model of HF, group treated with Bcl-2 had superior preservation of LV geometry with less ventricular dilatation and wall thinning. There was also reduced apoptosis compared with the controls (Chatterjee et al. 2002).

3.3.2 Akt, P-I3

The Akt proto-oncogene, a serine/threonine protein kinase, controls multiple responses in the cardiac cells including the ability to inactivate pro-apoptotic proteins like Bad and caspase 9. Act is activated by the products of PI-3 kinase reaction and eventually led to inhibition of cardiac myocytes apoptotic death. Intracoronary delivery of adenoviral construct encoding Akt in small animal models revealed reduced infarct size and protected cardiac myocytes from apoptosis (Miao et al. 2000).

3.4 Desensitization of βAR-Signaling

3.4.1 βARKct

The βAR system is a hallmark signaling pathway for the regulation of cellular communication in cardiac contractility and is therefore an attractive molecular target (Koch et al. 1995). Long-term neuro-hormonal activation in CHF induces the desensitization of β-adrenergic signaling transduction including β-adrenergic receptor (βAR) down regulation, upregulation of GRK2 (βAR kinase), and increased inhibitory G-protein alpha-subunit function (Raake et al. 2008). Extensive research studies revealed that the βAR system includes two main components: β-receptors and the GRK2. GRK2 is a cytosolic enzyme that, upon receptor activation, binds the released Gβγ subunit of activated heterotrimeric G-proteins

at the plasma membrane allowing for phosphorylation of βARs, which subsequently bind inhibitory proteins called β-arrestins (Brinks et al. 2010). βARs that are bound to this complex undergo receptor desensitization. Abnormal βAR responsiveness leads to upregulation of sympathetic drive with chronic catecholamine release that contributes to further cardiac deterioration.

The clinical use of β-blockers in pharmacological management of HF has shown great success by blocking chronic βAR activation. However, β-blockers do not act on the molecular pathways of the CHF. Therefore, gene treatment is much more promising for long-term management. A GRK inhibitor was developed to effectively reverse βAR desensitization permanently via overexpression. It consists of the carboxyl terminus of βARK, known as βARKct which directly competes with GRK2. Many studies in preclinical models of CHF have demonstrated that myocardial overexpression with βARKct reversed ventricular remodeling and lowered sympathetic outflow of catecholamines. This implies that the βARKct overexpression and targeting GRK2 inhibition are very fruitful therapeutic targets in CHF (Raake et al. 2013).

3.5 Activation of Cardiac Adenyl-Cyclase Expression

Adenyl-cyclase (AC6) regulates the transfer of adenosine triphosphate to cyclic adenosine monophosphate. This sequence initiates many cardiac intracellular and extracellular signaling pathways. CHF is associated with decreased expression and activity of AC6, and a desensitization of β-adrenergic receptors. Cardiac-directed expression of AC6 in genetic animal models of CHF increases impaired LV function, enhances cAMP capacity in response to βAR stimulation, normalizes PKA activity, increases phospholamban phosphorylation, and increases sarcoplasmic reticulum (SR) Ca2+ uptake (Tang et al. 2011).

3.6 Enhance Regeneration

CHF results in comparatively large-scale loss of myocardium. As opposed to the theory of the postmitotic organ differentiation, studies in animal models have shown that myocytes can divide and express growth-related genes after myocardial infarction (Bergmann et al. 2009). The evaluation of cardiac cell cycle events, as well as genetic and metabolic fate mapping, have provided new data that CMs are not terminally differentiated cells. Studies performed in the mice and zebrafish suggest that cell turnover during normal conditions and after injury leads to appearance new mononucleated and polynucleated CMs with their proliferative capacity. Also, molecular markers associated with mitosis were described in the presence of human myocardium (Anversa et al. 2002).

Furthermore, it has been proven that the majority of new CMs are derived from preexisting CMs through cell division rather than activation of undifferentiated stem or progenitor cells (Kikuchi et al. 2010). These data altogether demonstrate

that mammalian hearts maintain a regenerative capacity throughout life, providing a rationale for the development of a new direction to restore function after significant myocardial damage. Postnatal mammalian CMs are being studied in two major ways that may be utilized independently or together: exogenous cell transplantation and stimulation of endogenous regenerative processes (Katz et al. 2016a).

3.6.1 Modified RNA

Recently, a new approach to manipulate the gene program of the adult cardiomyocyte has been reported via the generation of chemically modified mRNA (modRNA). The use of modified RNA technology in delivering paracrine factors into a damaged region in the heart has important implications for cardiogenesis, and the pathways that might trigger heart regeneration. It was demonstrated that modRNA is an efficient approach for transient, high level, and localized gene transfer into the heart. Moreover, intramyocardial injection of modRNA encoding human vascular endothelial growth factor-A resulted in the expansion and directed differentiation of endogenous heart progenitors in a murine HF model. VEGF-A/modRNA markedly improved heart function and enhanced long-term survival of recipients. This improvement was in part due to mobilization of epicardial progenitor cells and redirection of their differentiation toward cardiovascular cell types (Zangi et al. 2013).

3.7 Activation of Cytoprotective Mechanisms

Reactive oxygen species and oxidative stress have been implicated in a number of pathological processes that contribute to HF, including vasoconstriction, cardiac hypertrophy, apoptosis, fibrosis, inflammation, and myocardial stunning (Zablocki and Sadoshima 2013). Angiotensin II and norepinephrine, two mediators whose production/release is abnormally upregulated in failing hearts, promote oxidative stress by activating NAD(P)H oxidase and feeding the hydrogen peroxide-generating enzyme mono amino oxidase. The selective VEGFR-1 ligands VEGF-B and PlGF prevent mitochondrial superoxide and cytosolic hydrogen peroxide overproduction in cultured neonatal cardiomyocytes exposed to angiotensin II. Moreover, VEGF-B could mitigate hydrogen peroxide overproduction in cultured cardiomyocytes exposed to norepinephrine. These results suggest that a mechanism underlying the therapeutic action of VEGF-B, in vivo, might consist of antagonizing the pro-oxidant effects of angiotensin II and norepinephrine (Woitek et al. 2015).

3.8 Reverse Established Cardiac Fibrosis

One of the most important contributors to the development of cardiac fibrosis is the transforming growth factor beta (TGFβ1)-SMAD signaling cascade, which stimulates collagen expression and other downstream profibrotic targets and is

markedly upregulated in HF (Dobaczewski et al. 2011). A growing body of evidence from studies conducted in the heart indicates that CHF-activated TGFβ1-SMAD signaling system is phosphorylated and subsequently translocated to the nucleus to regulate target gene transcription (Rosenkranz 2004).

3.8.1 CCN Family

A group of matricellular proteins known as the CCN family (CCN1 to CCN6) are participated with many cellular functions including TGFβ1-SMAD system (Holbourn et al. 2008). CCN5 was significantly decreased in the myocardium of patients with severe CHF. A study evaluated the effects of adeno-associated virus (AAV)-mediated overexpression of CCN5 on established fibrosis with concomitant cardiac dysfunction. CCN5 was found to reverse fibrosis, as shown by its effects on collagen contents and the cardiac myofibroblasts. CCN5 inhibited endothelial to mesenchymal transition mediated by TGF-beta signaling and transdifferentiation of fibroblasts into myofibroblasts and thus CCN5 can reverse established fibrosis (Jeong et al. 2016).

3.8.2 SERCA2a

In another study it was demonstrated that SERCA2a gene delivery disrupts activation of the TGFβ1/SMAD signaling cascade, inhibiting de novo collagen synthesis and downregulating angiotensin II and its receptor. Moreover, it was found that TGFβ1 signaling genes are dramatically upregulated in CHF in all myocardial regions (Katz et al. 2016b).

4 Cardiac Gene Therapy Vectors: The Evolution

Gene therapy continues researching improvements with delivery, specifically in addressing the rate limiting translational gaps from animal models to clinical applications. Here, a key focus is often placed on host considerations such as anatomical, physiologic, disease, and immune barriers. Mammalian cells were not designed to uptake foreign genetic material, thus the delivery challenge is recognized as the greatest impediment once the target is validated. Choosing the right vector for the intended cardiovascular application is the most important decision, since safe and efficacious therapeutic genetic manipulation is absolutely demanded for successful gene therapy.

The availability of vectors for gene transfer has improved dramatically attributable to both increased available basic science research and the emerging clinical trial data. The ideal vector should have the following characteristics for the intended CVD application: (1) Cardiotrophic, since the myocyte microenvironment contains a disproportional population of endothelial cells, (2) Result in long-term expression (i.e., a must for contractility genes and enough to induce repair in the case of regeneration), (3) Minimize the risk of innate and adaptive immune responses, and (4) Possess a large coding capacity to incorporate the gene and enhancing promoters (Gaffney et al. 2007).

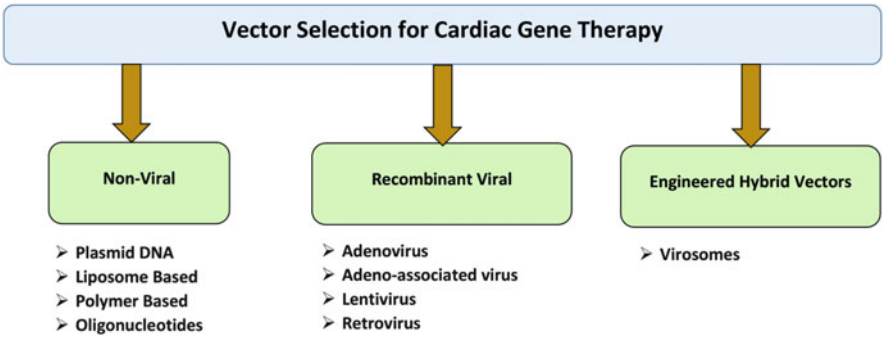

Fig. 3 Vector selection for cardiac gene therapy

The key challenges for a cardiac gene therapy vector are as follows: (1) Escaping the neutralizing effects of specific antibodies and non-specific adsorption to other blood and off target cellular components, (2) Overcoming the endothelial barrier and penetrating the vascular wall for diffusion through the extracellular matrix, and (3) Uptake into the cell at the level of the plasma membrane and efficient trafficking to the nucleus, and (4) Synthesis by the host of the complimentary DNA strand for single stranded delivery vectors followed by transcription and translation of the transgene (Müller et al. 2008). Various vectors have been used to achieve myocardial gene transfer, modified or selected to enhance the probability of overcoming each of these challenges. All vectors can be classified into two main categories, either non-viral, recombinant viral, or engineered hybrid (Fig. 3).

4.1 Non-Viral Vectors

Non-viral vectors are grouped as plasmid DNA, liposome-DNA complexes (lipoplexes), and polymer-DNA complexes (polyplexes). Oligonucleotides are also considered non-viral vectors (Felgner et al. 1997). In 1990, Lin and associates injected plasmid DNA into the left ventricle and demonstrated that the lacZ gene could be introduced and expressed in cardiac myocytes (Lin et al. 1990). Although non-viral vectors have the major advantage of production in large quantities at low cost while at the same time possess fewer toxic or immunological problems, their transfer efficiency is generally poor independent of delivery route (Nabel 1995). Nevertheless, a large number of human cardiac clinical trials are based on plasmid-mediated gene transfer investigating angiogenesis in myocardial ischemia (Losordo et al. 1998; Vale et al. 2001; Kastrup et al. 2005). A major advantage of this approach is that it avoids many of the biosafety concerns associated with viral vectors. However, the level of transgene expression and the efficiency of gene transfer (percent of target cells expressing the transgene) are low and expression is restricted to the zone of the injection site. DNA complexes are relatively more efficient, but not at the level of viral vector driven expression (Qin et al. 1998).

Despite significant reformulation efforts with a variety of chemical and biomimetic polymeric strategies, the key problem is that the nucleic acid content typically remains unstable and is often cleared or biologically inactivated following delivery. This is especially the case for transvascular delivery systems which impose significant contact with blood. An additional disadvantage of these vectors is their short biological half-life due to intracellular degradation and non-specific binding (Kizana and Alexander 2003).

The demonstration of plasmid gene transfer opened a new era of cardiovascular therapy. Despite numerous efforts to enhance efficiency through modification, direct myocardial plasmid injection basically remains a proof of concept tool, although there are a growing number of clinical trials employing plasmid gene transfer (Wasala et al. 2011). Despite their attractiveness in terms of cost and better risk/reward profile, the delivery problem becomes clear when comparing the data obtained between in vivo and in vitro efficacy. In most cases the scale and model system data require very large, unrealistic levels of DNA to achieve measurable benefit that cannot be administered clinically.

4.2 Viral Vectors

Since successful cardiac gene therapy for chronic patients demands efficient myocardial transduction long-term, viral vectors are the prime choice due to their unmatched performance. Since 2000, the performance of viral vectors in terms of efficiency has improved significantly due to key investments in basic and preclinical research evaluations. These key investigations have resulted in innovations that have addressed several rate limiting problems including cell specific targeting and at the same time reduced immunogenicity concerns via recombinant tropism selection. Moreover, the growing body of long-term clinical trial safety data has bolstered the case that only viral vectors appear to meet the demands of cardiovascular performance that could be executed in the clinic (Vinge et al. 2008; Hinkel et al. 2011).

Compared to non-viral vectors, viruses simply have an evolutionary advantage in their interactions with the cellular surface receptors, directly leading to more efficient intracellular trafficking of packaged DNA to the nucleus. This is important to note since many constructs can successfully enter the target cell but fail to achieve any expression due to the complex trafficking interactions. Furthermore, their protein capsid protects the message from degradation in lysosomes (Rapti et al. 2011; Ding et al. 2005). Some viral vectors are able to integrate into the host genome, whereas others remain episomal. Integrating viruses result in persistent transgene expression while viruses in episomal form lead to long-term expression in predominantly non-dividing tissues (e.g., adult myocardium) but only transient expression in rapidly dividing tissues (e.g., the hematopoietic system). It should be noted that for some disorders, short-term expression in a relatively small proportion of cells would be sufficient or even desirable (e.g., angiogenesis

post-myocardial infarction) whereas other pathologies might require long-term expression (e.g., autosomal recessive cardiomyopathy).

4.2.1 Lentiviruses

These vectors were initially developed for HIV therapy. Lentiviral vectors can infect non-dividing cells, cause long-term expression, and do not typically induce an inflammatory or immune response. The major limitation is the risk for mutagenesis and oncogenesis, thus limiting their desirability for cardiovascular applications (Wasala et al. 2011). The new generation of lentiviruses, containing an mRNA and a nuclear import sequence, have been used for successful myocardial transduction, although expression is usually short-term (Zhao et al. 2002; Bonci et al. 2003). Fleury et al. in a study with rat cardiomyocytes in vivo succeeded in obtaining persistent GFP transfer for up to 10 weeks (Fleury et al. 2003). In another study the transduction efficiency of lentiviral vector-mediated SERCA2 gene transfer was about 40% and the positive physiological effect persisted 6 months later (Niwano et al. 2008).

4.2.2 Adenoviruses

Adenoviral vectors have historically been the most frequently used transfer system in experimental studies. This is attributed to the vector's advantages of the ability to transduce non-dividing cells, ease of manufacture in very high titers, rapid peak onset of transgene expression, and a large transgene cloning capacity. However, their use is limited clinically due to the resultant transient gene expression. In addition, adenoviral vector particles are highly immunogenic and cause inflammatory and toxic reactions in the host. This is due to the fact that the adenovirus stimulates both the innate and adaptive immune systems. Using a rat model, it was confirmed that adenovirus was several orders of magnitude more efficient in transducing myocytes than plasmid DNA expressing the same construct (Guzman et al. 1993). Another study featuring direct intramyocardial injection of replication-deficient adenovirus demonstrated gene expression in a large animal model. However, the authors noted a robust T-cell-mediated immune response against the vector and limited distribution of the reporter gene (French et al. 1994).

Simultaneously, several groups confirmed the possibility to achieve significant cardiac gene expression after catheter-mediated delivery of adenovirus encoding phospholamban and the β2-adrenergic receptor (Hajjar et al. 1998; Maurice et al. 1999). Despite sophisticated modifications in an attempt to attenuate the host immune response to the adenovirus, the risk is too high to further advocate the use of this delivery vector for clinical cardiovascular applications of chronic nature.

4.2.3 Adeno-Associated Viruses

The adeno-associated virus (AAV) is a small (20 nm), non-enveloped virus that belongs to the dependovirus genus of the parvovirus family. AAVs have a single stranded DNA genome. The viral genome is approximately 4.7 kb in length, and is composed of two major open-reading frames which encode a Rep (replication) and

Cap (capsid) proteins (Berns and Giraud 1996). For an infection to occur, wildtype AAV requires co-infection with a helper virus such as adenovirus. This allows the viral genome to replicate episomally, and leads to synthesis of the AAV proteins. AAV is one of the smallest viruses, with a capsid mean diameter of 22 nm.

The first AAV2 infectious clone was created in 1982 by Samulski and colleagues (1992). One of the major advantages of AAV vectors is that in multiple animal models and humans, it has been demonstrated that after reaching a steady state level, AAV expression may last for years with an absence of a significant immune response to the transgene (Rivera et al. 2005). Moreover, AAV vectors can be engineered to provide a wide range of cell type tropism with the ability to transduce both dividing and non-dividing cells. Due to their biological properties and advantages over other viral vector systems, AAV has gained great popularity in the last decade in many clinical trials. Seventy-five clinical trials using AAV have been initiated over the past 15 years although only approximately 10% indicated for CVD (Coura Rdos and Nardi 2007).

The process of AAV endocytosis and intracellular trafficking is complex and cannot be underscored in understanding problems with clinical outcomes. Despite the availability and diversity of AAV vectors, several biological barriers appear to limit the effectiveness of AAV mediated gene therapy (Ziello et al. 2010; Coura Rdos and Nardi 2008). Understanding the fundamental basis of these barriers has led to the establishment of methods to improve the efficiency of rAAV-mediated gene delivery. Clarification of the processes by which a virus first enters and traffics through a cell helps to understand the life cycle of the virus and its ability to transduce cardiac muscle. The transport activity of AAV is mainly determined by selective receptor-mediated vesicle transcytosis (Di Pasquale and Chiorini 2006). This intracellular route does not appear to alter the properties of the AAV.

AAV transport to the myocyte's microenvironment can be abrupted by neutralizing antibodies, temperature, and physical and chemical inhibitors through a time and dose-dependent process. In vivo studies have noted that several serotypes of AAV are able to cross vascular endothelium with different efficiencies (Wang et al. 2005). It is known that AAV2 has a relatively poor tropism for vascular cells, although reasonable levels of transduction have been achieved in cardiac myocytes (Melo et al. 2002). Local delivery of AAV2 led to transduction of underlying vascular smooth muscle cells and sequestration of AAV in the extracellular matrix around endothelial cells thus preventing cell binding and entry. The potential of the AAV6 vector for cardiac gene therapy was achieved through the use of VEGF to increase vascular permeability. To date, AAV1, 6, and 9 are considered the best choices for CVD (Gregorovic et al. 2004).

5 Route of Administration Methods for Cardiac Gene Delivery

An important prerequisite for introducing cardiac gene therapy (i.e., following vector/gene construct) into clinical practice is the development of safe and efficient gene delivery techniques. During the last two decades, the field has witnessed the development of several experimental gene delivery strategies with potential therapeutic value for the transition from the preclinical phase to clinical trials. Yet, efforts will need to resolve several problems that exist with delivering sufficient quantities of therapeutic vector in order to establish the efficacious expression profile within safety limits (Katz et al. 2010).

The key challenges with delivery are as follows: sufficient delivery to the myocytes and not collaterals organ systems, improved efficacy in the heart, prevention of injury from the procedure itself, the creation of devices to accommodate the techniques, and avoiding deleterious immune responses. Although cardiac tissue-specific promoters may mitigate collateral organ gene expression, only a true cardiac specific gene delivery method can diminish the biodistribution of vector capsids to extra cardiac organs.

Systemic exposure of vector in the blood can result in unsafe levels of exposure to antigen presenting cells. Antigen presenting cells provide another mechanism to increase the potential for a T-cell-mediated immune response to the vector capsid and or packaged transgene. Ideally, the most optimal gene delivery system should be combined with an appropriate vector, whereby the selections transduce cardiomyocytes but avoid all collateral and immune cells.

Cardiac gene delivery methods can be classified into two main categories: direct or transvascular system approaches. Each of these categories has subsets of particular approaches that range from minimally invasive to very invasive access points (Fig. 4). Direct delivery methods administer vector directly into the cardiac muscle. Typically, this is achieved via intramuscular needle injection or other physical methods that penetrate the heart while depositing vector. On the other hand, the more commonly employed transvascular system approach seeks to leverage the heart's vast network of arterial, venous, and capillary transport system for broad distribution to adjacent cardiac tissue from the site of administration. Here, vector is infused through a designed access point in the coronary anatomy with a specific time interval, pressure, and flow rate. The end goal is to diffuse the vector through the endothelial barriers, where they become bioavailable in the cardiomyocyte compartments.

5.1 Direct Myocardial Delivery

Direct gene delivery methods have been utilized for more than two decades and many authors continue to reference and utilize them in their preclinical cardiac gene therapy studies. Numerous methods have also successfully been translated for use in clinical trials, which range in the degree of invasiveness. The most relevant of

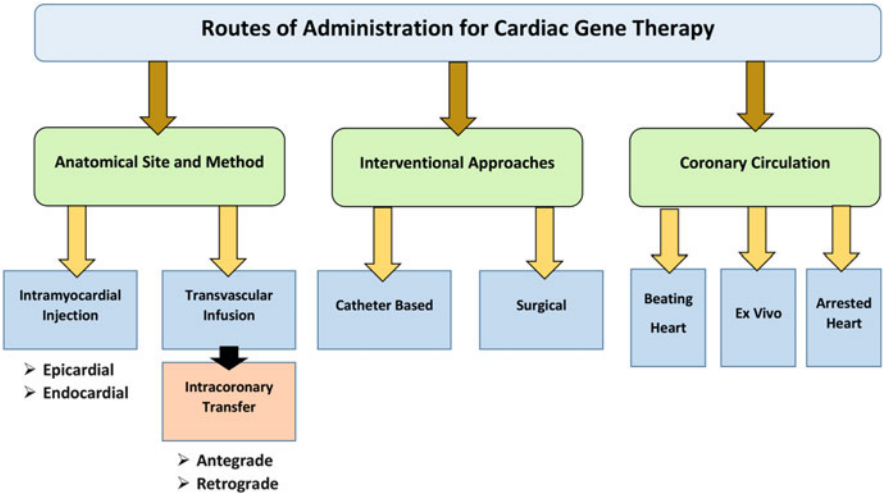

Fig. 4 Routes of administration for cardiac gene delivery

them are usually classified as either an open technique, that is to include the surgical opening of the chest or a closed technique, i.e., transcutaneous or minimally invasive.

5.1.1 Intramyocardial Injection

The majority of successful preclinical studies have involved direct administration of vector via standard needle. This technique allows for the application of a high concentration of vector directly at the target site with excellent specificity and control. Several groups have demonstrated the feasibility of delivering transgenes to the heart via direct intramyocardial injection of plasmid DNA (Acsadi et al. 2002; Buttrick et al. 1992; von Harsdorf et al. 1993). Although these studies have been encouraging because plasmid DNA may be expressed for up to 6 months by cardiomyocytes adjacent to the area of injection, estimates of the number of myocytes that can be transfected in vivo consistently have been as low as 60–100 cells per injection (Acsadi et al. 2002). This low efficiency has made it difficult to measure the physiological effects of gene expression in myocytes, making it unlikely that clinically significant effects will result since there is an ultimate limit to the number of injections that can be performed without permanent injury (von Harsdorf et al. 1993).

The low transduction efficiency of plasmid DNA vectors leads to the search for improved gene transfer efficiency with direct injection of an adenovirus vector. Hearts transfected with an adenovirus vector containing the β-galactosidase gene showed significantly increased β-galactosidase enzymatic activity compared with hearts injected with β-galactosidase plasmid. Unfortunately, the gene expression persisted for only 1 week after injection and it included acute inflammatory response, which the authors considered to be related to the injury produced by

direct injection as well as a cellular immune response against the adenovirus itself (Guzman et al. 1993).

French et al. first demonstrated in a porcine model these important points relevant to needle injection: (1) Direct intramyocardial injection of replication-deficient adenovirus is 140,000 times more efficient than injection of an equal number of genome copies of recombinant plasmid DNA, (2) The impact of this procedure on cardiac function appears to be negligible provided the number of injections is reasonable and across myocardial surface area, (3) The amount of recombinant protein produced increases with the amount of virus, but plateaus, (4) The expression of recombinant genes following intramyocardial injection is similar in the left and right ventricles; and (5) The percentage of cardiomyocytes expressing β-galactosidase in the needle track adjacent to the injection, but rarely are lacZ positive cells detected farther than 5 mm from any given injection site (French et al. 1994).

Large animal model studies generally report robust expression within weeks 1–4 with a decline in follow-up timepoints. For example, in a canine study using adenovirus encoding chloramphenicol acetyl transferase, peak gene expression was noted at 2 days and decreased by an order of magnitude 14 days after direct single myocardial administration. In this study, there was not significant transduction of distant organs and no documented changes in global or regional LV function (Magovern et al. 1996). However, the feasibility of adenovirus-mediated gene transfer has been limited by the cellular immune response which causes myocardial inflammation and results in transient recombinant gene expression (Barr et al. 1994).

In summary, the direct gene delivery approach was the first among others that helped establish the therapeutic efficacy of cardiac gene therapy. Furthermore, the use of this method in some experimental models resulted in successful therapeutic myocardial angiogenesis, and focal treatment of cardiac arrhythmias through effects on cellular electrophysiology; thus, making this platform widely used. Finally, this approach has been successfully utilized in Phase I/II clinical trials demonstrating its potential therapeutic relevance. This branch of delivery continues to evolve with some new concepts such as liquid jet injection, sonoporation, and electroporation applications being tested in preclinical evaluations.

5.2 Transvascular Gene Delivery

Transvascular delivery approaches are more widely practised due to the high frequency, reliability, and safety of interventional catheter based procedures. Effective therapy demands a gene delivery method capable of globally transducing the myocardium while minimizing the systemic exposure to collateral organs (Hajjar et al. 2000; Donahue et al. 1997). This addresses the key rate limiting problem with direct delivery, where only a high concentration of expression is limited to the injection site. Transvascular applications can result in a more homogenous profile with a greater area of myocardium transduced from the original

infusion site. This paradigm is particularly valid in heart failure gene therapy where most authors agree that gene transfer should be as diffuse and homogeneous as possible to access maximum failing myocardium. Furthermore, a diverse number of configurations without sacrificing the degree of invasiveness are available with modification of the catheter including antegrade (arterial infusion via left/right main branches), retrograde (greater cardiac vein, coronary sinus), and both via concomitant blockage and delivery to increase local perfusion delivery gradients.

Additional transvascular delivery procedures that achieve more robust delivery profiles at the cost of invasiveness include surgical approaches that use cardiopulmonary bypass. Bridges group have pioneered the use of molecular cardiac surgery with recirculating delivery (MCARD) which features complete cardiac isolation of the heart with retrograde delivery to maximize vector transfer to the heart with minimal collateral circulation in the context of open heart surgery (Fargnoli et al. 2013; Katz et al. 2014).

5.2.1 Antegrade Intracoronary Delivery

The most preferred gene delivery route at present involves percutaneous catheter based vector infusion into the coronary arteries. The benefits of this technique include its minimal invasiveness, the possibility of transgene delivery to all four myocardial chambers, and the delivery of vector genomes using endovascular coronary catheterization. Early reports using simple antegrade intracoronary delivery achieved very limited myocardial transduction efficiency (Magovern et al. 1996; Hayase et al. 2005; Kaplitt et al. 1996). The low degree and variability in transduction was due to a number of factors including animal species, biocompatibility of catheter and vector, different pharmacological agents used to permeabilize the vasculature, and vector-related variables such as vector serotype and dose (Ding et al. 2004).

The critical parameters influencing the efficiency of intracoronary perfusion included exposure time, high coronary flow rate, perfusion pressure, the use of crystalloid solution as opposed to whole blood, virus concentration, and temperature (Donahue et al. 1997). Further investigations supported that the most critical variable for intracoronary gene transfer is the short residence time of vector within the coronary circulation of a beating heart (Boekstegers and Kupatt 2004). Increasing perfusion pressure and flow augments myocardial expression perhaps by increasing the fenestration width between capillary endothelial cells, permitting better viral transendothelial transfer and enhancing virus–myocyte interaction (Wright et al. 2001).

5.2.2 Advanced Selective Retrograde Intracoronary and Surgical Approaches

The feasibility of percutaneous retrograde gene delivery by selective pressure-regulated retroinfusion of the coronary veins has been demonstrated by Boekstegers et al. This was achieved with a custom device consisting of a pump unit, extracorporeal circuit, and retroinfusion catheter coupled with a suction device. The authors demonstrated advantages of retrograde delivery compared to antegrade and

confirmed the results from several groups that blocking the venous outflow and coronary ischemia can significantly increase viral transfection of the myocardium (Boekstegers and Kupatt 2004).

Unlike previous studies that utilized a single-pass perfusion technique, Bridges group was the first to create an isolated "closed loop" recirculating model of vector-mediated cardiac gene delivery in the large animal heart using cardiopulmonary bypass with an antegrade delivery approach, allowing for vector recirculation for 20 min. Later, they used CPB with high-pressure retrograde coronary sinus infusion with multiple-pass recirculation of vector through the heart and washed out of the cardiac circuit prior to weaning from CPB, which limited extracardiac gene expression. They were able to show an increase of several orders of magnitude in cardiac marker gene activities compared with controls. Furthermore, there was minimal gene expression in the liver and other collateral organs (Fargnoli et al. 2013; Katz et al. 2014). These results validate this surgical technique as a potentially clinically translatable approach for cardiac gene therapy in carefully selected cardiac surgical patients.

6 Future Perspectives

The outlook for cardiac gene therapy is very bright since robust safety data is now available from numerous Phase I and II trials. A large pool of patients has completed trials without any major adverse events, specifically with the adeno-associated virus and plasmid DNA vectors. The ideal cardiac gene treatment plan, depending on target manipulation strategy, is to safely administer the least amount of product that would result in improved survival outcome for chronic heart failure patients. Currently, efficient delivery remains a key issue as doses are high. Improvements in vector design, medical device based delivery technologies, and genetic discovery are necessary to advance the field toward its goal for first FDA approved treatment.

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Noncoding RNAs in Heart Failure

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Abstract

Heart failure is a major contributor to the healthcare burden and mortality worldwide. Current treatment strategies are able to slow down the transition of healthy heart into the failing one; nevertheless better understanding of the complex genetic regulation of maladaptive remodeling in the failing heart is essential for new drug discovery.

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_99

Noncoding RNAs are key epigenetic regulators of cardiac gene expression and thus significantly influence cardiac homeostasis and functions.

In this chapter we will discuss characteristics of noncoding RNAs, especially miRNAs, long noncoding RNAs, and circular RNAs, and review recent evidences proving their profound involvement during different stages of heart failure progression. Several open questions still prevent the extensive use of noncoding RNA-modulating therapies in clinics; yet they are becoming an attractive target to define novel regulatory mechanisms in the heart. In-depth study of their interaction with gene networks will refine our current view of heart failure and revolutionize the drug development in coming years.

Keywords
Circular RNA • Heart failure • Long noncoding RNAs • miRNAs • ncRNA therapy

1 Introduction

The first decade of post-genomic era showcased enormous discoveries to highlight key regulatory roles of novel epigenetic modifiers exploiting the high-throughput multi-omic tools. Among these modulators, the noncoding RNAs (ncRNAs) represent a diverse class of RNA transcripts with significant association to human pathophysiology (Dangwal and Thum 2014; Thum 2014). Being incapable of encoding proteins, these RNA transcripts were referred to as the dark matter of the genome until recent studies demonstrated their ability to fine-tune the genomic interactions with various environmental factors to maintain hemostasis in a biological organ system. Dysregulation of such regulatory RNA transcripts can therefore disturb the physiological balance of functional protein expression leading to complex pathologies including heart failure, diabetes, and cancer (Beermann et al. 2016; Dangwal and Thum 2014).

Heart failure is one of the largest contributors to human morbidity and mortality. Despite the wide range of available therapeutic options, the prevalence and healthcare costs associated with cardiovascular disorders continue to increase worldwide (Gaziano 2005; Go et al. 2013). Recent advances of heart failure treatment are able to slow down the disease progression to some extent; yet in-depth understanding of novel pathological mechanisms is essential to develop better treatments and novel drugs. In this chapter we overview characteristics, working mechanisms of ncRNAs, and their biological functions with special emphasis on prevalence and progression of heart failure or associated pathologies.

1.1 ncRNAs: Classification and Characteristics

Based on the transcript length ncRNAs can be broadly classified as short or long ncRNAs. Short ncRNAs (<200 nucleotides) mainly comprise much studied, highly conserved microRNAs (miRs/miRNAs). To date, approximately 2000 miRNAs have

been mapped to the human genome and more are expected to be discovered (Beermann et al. 2016; Dangwal and Thum 2014). On the other hand, long ncRNAs (lncRNAs, >200 nucleotides) lack >100 amino acid classical open reading frame and their discovery is still at a preliminary stage (Beermann et al. 2016). LncRNA transcripts account for the major part of the noncoding transcriptome and encompass nearly 30,000 different transcripts in humans. Another classification is based on the linearity of transcripts and can be classified as linear, e.g. miRNAs and lncRNAs, or circular, e.g. circular RNAs (circRNAs). The 3′- and 5′-ends of linear RNA transcripts join together to make a loop during the biogenesis of circRNAs which also confers high stability to these transcripts compared to linear forms (Beermann et al. 2016; Jeck et al. 2013). Due to their high abundance paired with a high phylogenetic conservation, circRNAs possess very attractive characteristics that could potentially be exploited for ncRNA-based therapies. However, their functional importance in the heart represents a widely unexplored territory.

ncRNAs are detectable in most tissues and the body fluids. High-throughput screenings and expression profiling have revealed the stimuli or stress responsiveness and spatiotemporal variations in ncRNA expression. Compared to protein-coding genes, ncRNAs are more tissue specific and show greater variation between tissues (Derrien et al. 2012). When mapped on human genome, some ncRNAs are located in the intergenic or intronic regions while lncRNAs also overlap pseudogenes and as natural antisense transcripts to coding genes (Milligan and Lipovich 2015). Based on the position and direction of transcription in relation to other coding genes, lncRNAs can be classified into several different subtypes, e.g., sense or antisense overlapping, intergenic or intronic, bidirectional, and processed transcripts (Mattick and Rinn 2015; Peschansky and Wahlestedt 2014). Location wise, lncRNAs may exclusively reside in the nucleus but some are cytoplasmic or both nucleic and cytoplasmic. On the other hand, mature miRNAs carrying protein complexes and circRNAs are located in cytoplasm (Beermann et al. 2016).

1.2 Mechanism of Action

MiRNAs carry nucleotide seed sequences that are complementary to binding sites on the 3′-untranslated regions (3′-UTRs) of coding or messenger RNA (mRNA) transcripts (Bartel 2004). This miRNA base-pairing represses gene translation upon binding to mRNAs and thereby regulates vital cellular functions mediated by them. In mammals, such repression of functional protein-coding genes remains modest (Guo et al. 2010); however, roles of specific miRNAs or their families often become prominent under stress indicating their importance as suitable therapeutic targets in human disorders (van Rooij and Olson 2012; van Rooij et al. 2012). Changes in miRNA expression profiles observed during progression of heart disease can affect gene expression in different cellular compartments of heart in a direct or paracrine manner (Bang et al. 2014; Care et al. 2007; Kumarswamy and Thum 2013; Thum et al. 2008; Thum 2012). Compared to miRNAs, lncRNAs act through versatile mechanisms and seem to have a wide range of functions in cellular and developmental processes. Despite the RNA polymerase

II-dependent transcription common to mRNAs, lncRNAs also show distinct characteristics, for instance facilitating their biological functions by forming secondary and tertiary conformations of RNA structures. Recent studies suggest their exceptional ability to regulate bidirectional gene expression at any level by controlling chromatin methylation, histone modifications, mRNA transcription, stability or translation, etc. (Boon et al. 2013; Quiat and Olson 2013; Thum 2012). Moreover, this regulatory impact on gene regulation can be very prominent as the antisense transcription overlaps more than one-fourth of coding genes. The mechanism of circRNAs is mostly unclear but a few are known to have miRNA sponge function as they can carry repetitive complementary miRNA sequences. For instance, CDR1AS/CirS-7 and Hrcr are able to sponge miR-7 and miR-223, respectively (Hansen et al. 2013). However, very few lncRNAs and circRNAs have been characterized in detail.

1.3 ncRNA Therapeutics

Functional modulation of ncRNAs in cardiovascular disease animal models or genetically modified mice has demonstrated their importance and catalyzed research interest towards developing novel ncRNA-based therapeutic strategies (Dangwal and Thum 2014; Quiat and Olson 2013). Unlike conventional drug molecules, miRNAs may target multiple mRNAs simultaneously to influence the translation of a group of genes which often results in a strong impact on common cellular and biological end-point functions (van Rooij and Olson 2012). This is indeed advantageous over conventional drugs due to the least target desensitization. However, the possibility of their off-target effects due to unspecific uptake by various tissues upon systemic delivery may pose limitation to these strategies. Most of these limitations could be minimized using advanced drug delivery techniques, e.g., site-directed drug delivery.

ncRNAs can be delivered in vivo using either nonviral methods or viral infections. Several commercially available oligonucleotide chemistries allow high-affinity interactions with miRNAs activated in pathological states using miRNA interference, i.e., antisense oligonucleotides that bind and block target miRNAs in vivo. Unlike low-molecular-weight small molecules, miRNA inhibitors show rapid and high plasma clearance rates within hours of administration due to high volume of distribution (Geary et al. 2001; Levin 1999). Therefore, most of the miRNA inhibitors are chemically modified to lower the drug clearance by increasing plasma protein binding, conferring stability against enzymatic hydrolysis by nucleases, and enhancing cellular uptake as seen in phosphorothioate backbone modification or cholesterol linkage. A miRNA inhibitor (e.g. antagomir and anti-miRNA) is a small synthetic RNA oligomer that is complementary either to the seed sequence or to the full-length sequence of the specific miRNA. An antagomir contains a cholesterol construct conjugated via a 2′-O-methyl (2′-OMe) group linkage and several phosphorothioate moieties to improve cellular uptake, in vivo stability, and hepatic or other organ uptake (Krutzfeldt et al. 2005). Newly developed antisense oligonucleotides use unconjugated phosphorothioate with different 2′ sugar modification via either a 2′-OMe group or a

2′-*O*-methoxyethyl (2′-MOE) group to increase stability and efficiency (Broderick et al. 2011).

Unlike miRNAs, lncRNAs show fairly low evolutionary conservation along the phylogenic tree and it is therefore challenging to use cross-species experimental disease models to study most of the lncRNA candidates which have been screened from human clinical samples. Nonetheless, lncRNA modification can be carried out using commercially available single-stranded DNA-LNA chimeric antisense oligonucleotides "GapmeRs." These oligonucleotides catalyze degradation of target lncRNAs by recruiting RNase H present in the nucleus and cytoplasm and degrade RNA–DNA heteroduplexes (Beermann et al. 2016).

2 Bioinformatics: A Valuable Tool in ncRNA Research

A growing number of noncoding transcripts such as miRNAs, lncRNAs, and circRNAs are suggested to be involved as potential therapeutic targets in cardiac vascular disease. However, due to some intrinsic difficulties such as less conservation, secondary structure effects, and cell type-specific expression profiles, the exact biological and molecular functions of most disease-associated noncoding transcripts remain largely unknown. Bioinformatics approaches including specialized database development, functional prediction, and interactive network construction, as well as utilization of the substantial data generated from high-throughput technologies, are tremendously necessary before and during the wet-lab investigations.

2.1 Microarray and RNA-Sequencing

Microarray has been widely used over decades in medical research to discover human disease-correlated molecules. The basic technical principle behind microarray is the hybridization between nucleotides with complementary base pairs, which also leads to the limitation of this approach that novel transcripts or unannotated transcripts cannot be detected. With the tremendous development of deep sequencing technology and the drop of price in recent years, RNA-sequencing (RNA-seq) has become a new prominent trend improving the genome-wide identification and expression profiling of noncoding RNAs, which has less annotation resources compared to the coding genes. In one of the earliest studies of hypertrophic regulating lncRNA *chrf* (Wang et al. 2014b), microarrays of miRNAs and lncRNAs were performed in parallel to identify the deregulated transcripts in response to angiotensin II treatment. Another cardiovascular associated lncRNA *Bvht* (Klattenhoff et al. 2013) was identified by genome-wide screening of expression profiling dataset from RNA-seq. Although there are still lot of disputes about the profiling accuracy and sensitivity of the two most popular platforms, RNA-seq has been suggested to be more efficient detecting low-abundance transcripts in samples from a big range of complexity (Wang et al. 2014a).

2.2 Expression Profiling

The aim of expression profiling is to identify the deregulated genes or transcripts involved in pathological or physiological situations by comparing the output value from high-throughput platforms, such as normalized mapped read count from RNA-seq or signal intensities from microarray. As a well-developed approach, microarray raw data can currently be easily transformed and analyzed by researchers without strong statistical or bioinformatical techniques. Commercial packages such as GeneSpring GX provide pipelines for raw data analysis from Agilent or Affymetrix platform. Free tools with GUI such as BRB-ArrayTools (Simon et al. 2007) and Expander (Shamir et al. 2005) have also been designed for scientists to further explore their own data.

On the other hand, intensive bioinformatics supports or standard pipelines are necessary for precisely processing and analyzing the raw data from various next-generation sequencing platforms. As a standard procedure, after quality control the raw reads in FASTQ format are either mapped to a reference genome assembly or ncRNA transcript models using Bowtie2 (Langmead and Salzberg 2012) or BWA (Li and Durbin 2009). The mapping files can then be imported to ncRNA prediction or expression-level quantification tools such as DARIO (Fasold et al. 2011) to make further analysis. However, most tools or pipelines currently available for specific scientific questions such as detection of the alternative splicing events for lncRNAs or circRNAs were written in scripts or only executable from command line (Sun et al. 2015) (Memczak et al. 2013).

2.3 Functional Annotation of Noncoding RNAs

After the determination of noncoding transcripts which are differentially expressed in a case-control study, functional annotation of those candidates is routinely followed to interpret the profiling results; relevant information such as genomic location, neighboring coding genes, and full transcript sequences can be retrieved from general or specific databases. As one of the earliest and most comprehensive databases for miRNAs, Mirbase (Kozomara and Griffiths-Jones 2014) provides integrated information from primary sequences to expression levels as well as links to target prediction tools. In 2016 NONCODE (Zhao et al. 2016) has updated their knowledge-based sequences and functional databases for noncoding transcripts in 16 species except tRNA and rRNA. Although circRNAs represent a comparably new field in the noncoding RNA world, scientists can still find useful information such as splice sites and their genomic background in circBase (Glazar et al. 2014). All the mentioned databases provide annotation tracks integrated to the UCSC genome browser (Speir et al. 2016).

2.4 Coding Potential and Conservation

Unlike miRNAs, that generally only contain 18–22 nucleotides, most lncRNAs are much longer in length and some of them have the potential to code for a protein (Cabili et al. 2011). Till now the Coding Potential Calculator (Kong et al. 2007) and PhyloCSF (Lin et al. 2011) are two of the most cited but distinct algorithms to assess the coding potential for putative noncoding transcripts. The CPC discriminates coding and noncoding sequences by scoring the open reading frame as well as the sequence homology to known proteins across multiple species while PhyloCSF is a comparative genomics method based on statistical phylogenetic model.

In the research field of translational medicine, a highly conserved homologous molecule in the human genome is always desired for any pathological state-associated candidate. As a therapeutic target miRNA is by no means one of the best choices because the mature sequences in one family can be fully conserved or identical in the seed region (Mathelier and Carbone 2013). Exonic circRNAs have also been suggested to be highly conserved between humans and rodents (Jeck et al. 2013). LNCipedia (Volders et al. 2015), a knowledge-based human lncRNA resource, evaluates the genomic locus conservation between human, mouse, and zebra fish. For characterized lncRNAs, another database lncRNAdb (Quek et al. 2015) covering multiple species can be used as a reference for scientists looking for confident homologs of their candidates. It is still a big challenge to find homologs of tissue-specific lncRNA in primary sequence level especially the antisense or intergenic transcripts due to the low expression level and lack of annotations.

Although the primary sequence of *Xist* between human and rodents gives poor overall conservation, the secondary structure was suggested to have more impacts on its molecular functions (Maenner et al. 2010). RNA folding algorithm such as RNAfold and RNAalifold (Gruber et al. 2015) can be used to predict the structure either from a single transcript or multiple sequence alignment.

2.5 Interaction Between Noncoding Transcripts and Other Molecules

Computational prediction of the interactions between noncoding transcripts and other molecules such as mRNA, transcription factors, RNA-binding proteins, or even DNA duplex is efficiently applicable in order to theoretically elucidate the functional potencies for single and multiple candidates. For example, an energy model-based algorithm RNAhybrid (Rehmsmeier et al. 2004) was used to assess the interaction between miR-489 and UTR from putative noncoding candidates in the study of *Chrf*. Triplexator (Buske et al. 2012) was used to predict the triplex target sites and triplex-forming oligonucleotides for MEG3 before the wet-lab triplex assay (Mondal et al. 2015).

2.6 lncRNA-Protein Interactions

Inspired by the CeRNA hypothesis (Salmena et al. 2011) as well as the fast-growing high-throughput sequencing data many ncRNA databases constructed network models to elucidate the interactions between the different RNA classes. Due to the large proportion of uncharacterized noncoding transcripts, functional enrichment analysis of miRNAs or lncRNAs is either based on gene lists from target prediction approaches or from co-expression patterns of coding genes.

3 ncRNAs: Key Regulators of Heart Failure Pathophysiology

Compensatory cardiac remodeling in response to stress is characterized by progressive cellular hypertrophy, which ultimately leads to declined cardiac function clinically known as heart failure. Reversing or slowing the transition to the decompensated state is a major goal of current treatment. A growing body of evidences shed light on the complex molecular mechanisms by which ncRNAs affect the transition to the failing heart (Table 1). In this section, we will discuss the role of ncRNAs as modulators of genetic networks responsible for heart failure progression through various pathological processes such as myocardial ischemia, hypertrophy, and fibrosis.

3.1 Heart Failure Due to Acute Myocardial Infarction

Myocardial infarction is known to be one of the leading causes of heart failure worldwide, and although there are less incidences of premature coronary artery disease in Western countries, acute myocardial infarction remains an important cause of morbidity and mortality. Around two million people die of acute myocardial ischemia per year, of which 500,000 episodes occur in the USA, representing a great socioeconomic burden (Wang et al. 2015; Weir et al. 2006).

Myocardial infarction occurs when atherosclerotic plaques start forming in the major arteries until they block the blood flow, therefore causing acute coronary and other ischemic syndromes. Recent studies suggest that the spleen, the nervous system, the bone marrow, and multiple leukocyte subclasses are interconnected to play key role in the plaque formation (Libby et al. 2016). This hypoxic condition causes cardiomyocyte necrosis and apoptosis and the loss of heart contractility. Consequently, a cascade of immune-inflammatory responses triggers wound healing and repair. At first, these processes occur as an adaptive response to stress but eventually develop into tissue remodeling which causes cardiomyocyte hypertrophy, fibrosis in the remote area, and ventricular dilatation. Cardiac tissue remodeling ultimately leads to heart failure and therefore death of the patient (Li and Maegdefessel 2016; Frantz et al. 2009; Dobaczewski et al. 2010). All of these processes are the outcome of a complex cross talk among different players. During the last decade many noncoding RNAs have been shown to play an important role in the initiation and development of ischemic diseases.

3.1.1 Arteriosclerosis

Arteriosclerosis, and particularly one of its specific forms—atherosclerosis—represents a maladaptive and chronic inflammatory process of the wall of the arteries. It starts with an endothelial injury and subsequent subendothelial lipoprotein retention and flow-mediated inflammatory changes in endothelial cells. These processes are followed by plaque rupture and thrombus formation (Hansson et al. 2015).

Cholesterol homeostasis is a crucial step in atherosclerosis. The imbalance of the ratio between low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol is critical to promote atherosclerosis, and many miRNAs, like miR-223, are associated with the metabolism of this molecule. This miRNA inhibits cholesterol biosynthesis by repressing sterol enzymes 3-hydroxy-3-methylglutaryl-CoA synthase 1 and methylsterol monooxygenase 1, and controls the uptake of HDL cholesterol by repressing scavenger receptor BI (Vickers et al. 2014). Another miR that has been shown to play role in the cholesterol levels in blood is miR-122. The inhibition of this miRNA in mice results in reduced cholesterol levels in plasma, increased hepatic fatty-acid oxidation, and a decrease in hepatic fatty-acid and cholesterol synthesis rates (Esau et al. 2006). MiR-33 has also been widely studied in the atherosclerosis process. It regulates HDL biogenesis in the liver and modulates the expression of genes involved in cellular cholesterol transport. It inhibits the expression of the adenosine triphosphate-binding cassette (ABC) transporter, thus attenuating cholesterol efflux to apolipoprotein A1 (Rayner et al. 2010).

On the other hand, many miRNAs have been involved in macrophage foam cell formation, which involves the inflammatory response of macrophages and the stimulation of lipid uptake by oxidized LDL (oxLDL). This is the case for miR-125a, which mediates lipid uptake and decreases the secretion of some inflammatory cytokines in oxLDL-stimulated monocyte-derived macrophages. This same study also showed that miR-146a/b and miR-155 were aberrantly expressed after oxLDL treatment of primary monocytes (Chen et al. 2009). However, for this to happen, arterial endothelial cells (which generally resist attachment of the white blood cells) express adhesion molecules like vascular adhesion molecule-1, intracellular adhesion molecule-1, and E-selectin that capture leukocytes on their surfaces when subjected to stress stimuli such as hyperlipidemia (Libby et al. 2011). One of the major pathways controlling the activation of these adhesive molecules is NF-κB, and miR-181b has been shown to regulate this pathway, thus playing a key role in atherosclerosis. This miR inhibits proinflammatory genes, influx of macrophages and CD4+ T cells in the vessel wall, and activation of NF-κB in the vascular endothelium (Sun et al. 2014).

3.1.2 Ischemia

Following the arteriosclerosis and thrombus formation, cardiomyocyte apoptosis is caused by oxidative stress, ischemia, and hypoxic injury and reperfusion (Huang et al. 2016). It has been found that miRNAs play a role in myocytes' adaptation and survival during hypoxia/ischemia (Huang et al. 2016; Cheng et al. 2010; Rane et al. 2009). MiR-199a was reported to be downregulated in cardiomyocytes under hypoxia. This reduction upregulates its target: hypoxia-inducible factor (Hif)-1alpha. Overexpression of this miRNA under hypoxic conditions inhibits Hif-1alpha and its

stabilization of p53 and thus reduces apoptosis (Rane et al. 2009). Another cardioprotective miRNA is miR-451. The miR-451 upregulation attenuates the loss of cardiomyocyte viability and decreases the apoptosis of cardiomyocytes during anoxia/reoxygenation injury by inhibiting high-mobility-group box 1 expression (Xie et al. 2016).

Recent studies have also shown that not only microRNAs but also lncRNAs play a role in myocardial infarction. For example, one of the first lncRNAs to be identified that confers risk of myocardial infarction was myocardial infarction-associated transcript (MIAT) (Ishii et al. 2006). Another cardiac specific lncRNA myosin heavy chain-associated RNA transcript (MHRT) was significantly elevated in the blood from AMI patients compared with the healthy control. This lncRNA was shown to be upregulated in apoptotic cardiomyocytes after treatment with hydrogen peroxide (H_2O_2) (Zhang et al. 2016). These results altogether show that ncRNAs are key players in the origin and development of acute myocardial infarction.

3.2 Heart Failure Due to Hypertension and Metabolic Disorders

Challenged by deteriorating cardiac stress stimuli such as hypertension, ageing, or metabolic disorders, the heart usually compensates by enlargement of both cardiomyocytes (hypertrophy) and extracellular matrix, which organizes the structure of the heart. Excessive accumulation of extracellular matrix, termed fibrosis, results from an increase in both proliferation rates and collagen deposition of resident cardiac fibroblasts. This triggers myocardial stiffness with concomitant alterations in left ventricular filling and relaxation of the heart muscle, a condition known as diastolic heart failure (HF) or heart failure with preserved ejection fraction (HFpEF). Weakened by the exhausting attempt to compensate, the ventricles eventually dilate, resulting in end-stage systolic HF or heart failure with reduced ejection fraction (HFrEF) (Braunwald 2013).

3.2.1 Cardiac Hypertrophy

Prolonged hypertrophic growth of cardiomyocytes favors progression to end-stage HFrEF, a life-threatening condition, and therefore constitutes a promising therapeutic target (Bisping et al. 2014).

In 2006, miRNAs were found to be deregulated in both rodent and human failing hearts (van Rooij et al. 2006). Due to the small size of miRNAs, deep sequencing currently is the method of choice for such studies, as it allows enhanced specificity by nucleotide-to-nucleotide synthesis. Although a miRNA array was used in the above-mentioned study, this observation laid the foundation of intense research on miRNA-based therapies and diagnostics in cardiovascular diseases in the following years.

Hence, only 1 year later, the first pro-hypertrophic miRNA was discovered (van Rooij et al. 2007). In this study, the first ever miRNA knockout animal in the field of heart failure research was generated. In-detail phenotyping of these miR-208$^{-/-}$ mice revealed diminished adverse hypertrophy, fibrosis, and pathological myosin switching

in response to pressure overload or expression of activated calcineurin, stimuli that both induce cardiac remodeling. Importantly, this miRNA is both cardiac specific and conserved in humans, highlighting its potential translational relevance for heart failure treatment. In line with this, preclinical studies showed that transgenic overexpression of miR-208a in the mouse heart is sufficient to induce pathological hypertrophy as well as arrhythmias (Callis et al. 2009). Subsequently, miR-208a inhibition via a systemic delivery of an antisense oligonucleotide was reported to improve cardiac function and survival in a rat model of hypertension-induced heart failure (Montgomery et al. 2011). Of note, inhibition of cardiac specific miR-208a also leads to resistance to high-fat diet-induced obesity as well as to improved insulin sensitivity and glucose tolerance in mice (Grueter et al. 2012), implying a role of the heart in modulation of whole-body metabolism, and providing first evidence for miR-208a to be a potential therapeutic target also for metabolic disorders.

In parallel to the above-mentioned pioneered study by Eva van Rooij and colleagues, miR-1 levels were reported to be decreased in response to aortic constriction-induced cardiac hypertrophy (Sayed et al. 2007). The same observation was made both in transgenic mice with cardiac overexpression of a constitutively active form of the Akt kinase and in exercised rats, thereby mirroring both pathological and physiological hypertrophy (Care et al. 2007). Of note, muscle-enriched miR-1 belongs to the most abundant miRNAs in the heart (Lagos-Quintana et al. 2002; Rao et al. 2009). Whether miR-1 plays a detrimental or protective role in the heart still remains a matter of debate: Identified growth-related targets suggest an induction of hypertrophy through loss of the repressive influence ("derepression") of miR-1 (Elia et al. 2009; Sayed et al. 2007), and even more importantly, cardiac targeted delivery of miR-1 reverses pressure overload-induced cardiac hypertrophy in the rat (Karakikes et al. 2013). Conversely, miR-1 also exacerbates arrhythmogenesis in a rat model of myocardial infarction (Yang et al. 2007). However, in the latter study, in vivo gene transfer was achieved by direct injection of synthetic miR-1 through a needle into the myocardium, which causes additional injury to the heart. Thus, methodological issues as well as different heart failure models used might account for the divergent results on this miRNA.

At the same time as miR-1 was discovered, the anti-hypertrophic and muscle-enriched miR-133a was studied, for the first time using antagomiR chemistry for miRNA inhibition in vivo (Care et al. 2007). MiR-133a levels were found to be decreased not only in mouse and rat models of pathological as well as exercise-induced hypertrophy, but also in human hearts of patients displaying hypertrophic cardiomyopathy or atrial dilatation. The latter observation highlights the potential clinical implication of this miRNA. Preclinical studies show that inhibition of this miRNA is sufficient to induce hypertrophy not only in vitro but also in vivo. Interestingly, subsequent studies of miR-133a revealed also an anti-fibrotic nature of this miRNA (see the sect. 3.2.2. Cardiac Fibrosis). A miRNA-based therapeutic strategy using miR-133a would therefore be a replacement therapy to regain its protective effects.

Considering the plethora of common drawbacks of miRNA-replacement therapies, the identification of detrimental miRNAs that can be blocked in the clinical setting would be favorable. Hence, the discovery of the pro-hypertrophic, evolutionarily

conserved miR-212/132 family was of great importance (Ucar et al. 2012). Expression levels of this miRNA family are elevated specifically in cardiomyocytes in a murine model of pressure overload and in vitro upon treatment with various hypertrophic stimuli. Moreover, this miR-212/132 family is both sufficient and required for the hypertrophic response in vitro and in vivo. Of note, the pro-autophagic transcription factor FOXO3 was determined to be a direct target of both miR-212 and miR-132. Thus, 212/132 family blocks autophagy in cardiomyocytes, a recycling process that was described as an adaptive response that protects the failing heart from hemodynamic stress (Nakai et al. 2007).

The very first lncRNA with implications in heart failure entered the stage in 2014 with an article published in Circulation Research (Wang et al. 2014b). This study pioneered the field describing *Chrf* (cardiac hypertrophy-related factor), an lncRNA that is induced in vitro in cardiomyocytes after treatment with angiotensin II, in pressure overload-induced cardiac hypertrophy in a murine TAC model as well as in human heart failure samples, underscoring translational relevance. Overexpression of *Chrf* induces hypertrophic responses in vitro and increases cardiomyocyte apoptosis in vivo, whereas knockdown attenuates cardiac hypertrophy in the angiotensin II-induced mouse model of heart failure. Mechanistically, *Chrf* sponges miRNA-489, a miRNA that prevents hypertrophic responses both in vitro and in vivo in response to angiotensin II infusion. However, there is no data available using state-of-the-art knockdown of *Chrf* by GapmeRs chemistry. Moreover, toxicological evaluation would be urgently needed to prevent possible side effects due to targeting this lncRNA.

A research letter published in Nature (Han et al. 2014) uncovered a cluster of antisense lncRNA transcripts from the murine myosin heavy-chain 7 (*Myh7*) locus designated as myosin heavy-chain-associated RNA transcripts (*Myheart*, or *Mhrt*). Cardio-protective role of this cardio-specific lncRNA could be demonstrated in a murine model of pressure overload induced by transverse aortic constriction (TAC). Restoring *Mhrt* to pre-stress levels by transgenic overexpression leads to inhibition of cardiac hypertrophy and failure. In the healthy heart, *Mhrt* sequesters Brg1, a chromatin-remodeling factor in the nucleus, preventing it from repression of its target gene Myh6. In contrast, in the hypertrophied heart, stress-induced *Mhrt* downregulation allows Brg1 to repress Myh6 and activate Myh7, leading to a pathological isoform switch from Myh6 to Myh7. Of note, human *MHRT* is repressed in hearts of patients with hypertrophic, ischemic, or idiopathic cardiomyopathy, pointing towards a translational relevance of this cardio-protective lncRNA. As for miR-133a, an *Mhrt*-replacement therapy would be the strategy of choice to translate these findings into clinics. However, there are a plethora of obstacles to be overcome before this route can be taken.

To date, in the most recent publication demonstrating strong therapeutic potential of lncRNAs, *Chast* (cardiac hypertrophy-associated transcript) was identified as a pro-hypertrophic lncRNA in a murine model of pressure overload (TAC) (Viereck et al. 2016). Importantly, inhibition of *Chast*, which was found to be induced after TAC operation specifically in the cardiomyocyte fraction of the heart, prevents or attenuates cardiac remodeling before and after induction of hypertrophy,

respectively. Conversely, in vivo overexpression of *Chast* is sufficient to induce hypertrophy in the mouse heart. Mechanistically, *Chast* promotes hypertrophy through its anti-autophagic function. Similar to murine *Chast*, also human *CHAST* drives hypertrophy in vitro and is activated in hypertrophic hearts from patients with aortic stenosis highlighting the clinical relevance of *CHAST* as a potential new therapeutic target for hypertrophy. Careful toxicological evaluation is needed to stratify the risk of an oligonucleotide-based therapy targeting *CHAST*. However, GapmeR-mediated inhibition of *Chast* in hypertrophic mouse hearts did not provoke any significant changes in plasma marker of kidney and liver damage.

The only observation showing therapeutic implication of a circRNA in heart failure stems from a preclinical study published in 2016 in the European Heart Journal (Wang et al. 2016). Here, overexpression of heart-related circRNA (*Hrcr*) ameliorates hypertrophic responses in vitro as well as in vivo in both TAC-operated and isoproterenol-infused mice. Mechanistic insights suggested a sponging function of *Hrcr* of pro-hypertrophic miR-223 resulting in the increase of apoptosis repressor with CARD (ARC) domain in cardiomyocytes.

3.2.2 Cardiac Fibrosis

Since myocardial fibrosis correlates with a higher long-term mortality and persists in patients when medicated following the official guidelines for HF treatment, anti-fibrotic therapeutic strategies are indispensable (Heymans et al. 2015).

The very first miRNA to be identified to control cardiac fibrosis, miR-21, at the same time constitutes the most intensively studied pro-fibrotic miRNA, in regard to fibrosis development not only in the heart but also in the lung and kidney (Bao et al. 2014; Liu et al. 2010; Thum et al. 2008). In a groundbreaking study, miR-21 levels were found to be increased in failing mouse and human hearts (Thum et al. 2008). Importantly, miR-21 is selectively upregulated in the fibroblast fraction of failing hearts of β1-adrenergic receptor transgenic mice. Inhibition of miR-21 by a specific cholesterol-modified antisense oligonucleotide (antagomir) in a pressure overload-induced heart failure mouse model ameliorates cardiac function and fibrosis both in a preventive and in a therapeutic study. Mechanistically, miR-21 targets sprouty homolog 1 (Spry1), thereby augmenting fibroblast survival and growth factor secretion. As expected for systemic delivery of oligonucleotide-based therapies, the miR-21 antagomirs were also taken up into other organs. However, liver morphology and function both proved to be normal, thereby showing promising results in a first early toxicological evaluation. Further studies revealed a miR-21-mediated endothelial- and epithelial-to-mesenchymal transition, thereby adding more layers of complexity to the contribution of this miRNA to fibrosis development (Bronnum et al. 2013; Ghosh et al. 2012; Kumarswamy et al. 2012). Strikingly, miR-21 knockout mice develop fibrosis comparable to wild-type littermates following a plethora of cardiac stresses (Patrick et al. 2010). However, as for the deletion or mutation in any gene, there are also limitations of miR knockout animal models: like genes, also many miRNAs may have redundant roles and biological processes are generally complex, opening up a Pandora's box. Therefore, it does not come as a surprise that many researchers reported different outcomes in genetic deletion and pharmacologic inhibition of the

same target. As mentioned above, pharmacologic inhibition of miR-21 proved to be a successful therapeutic strategy to prevent fibrosis in the heart, the lung, and the kidney (Bao et al. 2014; Liu et al. 2010; Thum et al. 2008). Of note, passenger strand of miR-21, miR-21*, is highly enriched in cardiac fibroblast-derived exosomes and induces cardiomyocyte hypertrophy, thus serving as a paracrine signaling mediator (Bang et al. 2014). This finding underscores the functional importance of miRNA passenger strands. For a long time, miRNA passenger strands were thought to be degraded in the cells, as only the guide strand is incorporated into the RNA-induced silencing complex, which guides the miRNA to the 3′-UTR of the target mRNA.

In contrast to miR-21, miR-133a was identified as a cardio-protective miRNA, as described in the sect. 3.2.1. Cardiac Hypertrophy. Although this miRNA is muscle enriched, transgenic overexpression of miR-133a in the heart ameliorates cardiac fibrosis and function but not the hypertrophic response in a pressure overload-induced (Matkovich et al. 2010) as well as in a diabetic mouse model of heart failure (Chen et al. 2014). A direct link between miR-133a and fibrosis could be established by the identification of its targets connective tissue growth factor (CTGF) (Duisters et al. 2009) and collagen α1 (I) chain (Castoldi et al. 2012). Whereas collagen 1 fibers are highly cross-linked and predominate over thinner collagen 3 fibers in adverse fibrosis, CTGF is a regulatory key player in the fibrotic response (Heymans et al. 2015). Importantly, inhibition of CTGF can reverse fibrosis in the heart, the liver, and the lung (Lipson et al. 2012). Therefore it comes as no surprise that a pharmacologic CTGF inhibitor currently is being tested in a phase II clinical trial (http://www.fibrogen.com/clinical_pipeline).

MiR-29, another cardio-protective and well-studied miRNA, was found to be downregulated in response to cardiac stress (van Rooij et al. 2008). Subsequent studies uncovered a plethora of targets involved in fibrosis, such as collagens, matrix metalloproteinases, and leukemia inhibitory factor, insulin-like growth factor 1, and pentraxin 3, thereby explaining the anti-fibrotic nature of this miRNA (Abonnenc et al. 2013). Importantly, restoration of miR-29 to pre-stress levels prevents angiotensin II-mediated cardiac fibrosis and dysfunction (Zhang et al. 2014). Remarkably, a noninvasive ultrasound-mediated gene transfer of an inducible miR-29b vector was used in these studies. Conversely, LNA-based inhibition of miR-29 induced the expression of extracellular matrix, preventing angiotensin II-induced dilation of the aorta and rupture of aortic walls in mice (Boon et al. 2011).

Apart from single study showing a decrease of long noncoding RNA-NR024118 upon treatment of cardiac fibroblasts with angiotensin II (Jiang et al. 2015), evidence of fibrosis-associated lncRNAs seems to be sparse. As a matter of fact, the existence of fibroblast-specific lncRNAs with mechanistic function remains to be explored.

Table 1 Preclinical studies on noncoding RNA-based therapeutic strategies for cardiac diseases

ncRNA	Animal model	Manipulation/ intervention	Phenotypic outcome	Reference
miR-223	Humans	Overexpression	Inhibits cholesterol biosynthesis	Vickers et al. (2014)
miR-122	Murine model	Inhibition	Reduces cholesterol levels in plasma	Esau et al. (2006)
miR-33	Murine model	Overexpression	Reduces circulating HDL levels	Rayner et al. (2010)
miR-125-a	Human primary monocytes	Overexpression	Mediates lipid uptake	Chen et al. (2009)
miR-146a	Murine model	Inhibition	Decreases cardiomyocyte apoptosis under ischemia/hypoxic conditions	Huang et al. (2016)
miR-181b	Murine model	Overexpression	Inhibits activation of NF-κB in the vascular endothelium	Sun et al. (2014)
miR-208	Murine pressure overload model/ calcineurin activation	miR-208$^{-/-}$ KO	Diminished hypertrophy and fibrosis	van Rooij et al. (2007)
miR-208a	Murine model	CM-specific transgenic overexpression	Pathological hypertrophy and arrhythmias	Callis et al. (2009)
miR-208a	Rat model of hypertension	Pharmacologic inhibition	Improved cardiac function and survival	Montgomery et al. (2011)
miR-1	Rat model of pressure overload	Cardiac-targeted delivery	Reversal of hypertrophy	Karakikes et al. (2013)
miR-21	Murine model of pressure overload	Pharmacologic inhibition	Prevention and amelioration of cardiac function and fibrosis	Thum et al. (2008)
miR-21	Murine models of cardiac stress	Global KO	No obvious phenotype	Patrick et al. (2010)
miR-133a	Murine model of pressure overload and diabetes	Cardiac-specific transgenic overexpression	Amelioration of cardiac function and fibrosis	Matkovich et al. (2010), Chen et al. (2014)
miR-133a	Murine model	Pharmacologic inhibition	Pathological hypertrophy	Care et al. (2007)
miR-29	Murine model of hypertension	Ultrasound-mediated gene transfer of an inducible miR-29b vector	Prevention of cardiac fibrosis and dysfunction	Zhang et al. (2014)
miR-29	Murine model of hypertension	Pharmacologic inhibition	Prevention of dilation of the aorta and rupture of aortic walls	Boon et al. (2011)

(continued)

Table 1 (continued)

ncRNA	Animal model	Manipulation/ intervention	Phenotypic outcome	Reference
miR-132/212	Murine model	CM-specific transgenic overexpression	Pathological hypertrophy	Ucar et al. (2012)
miR-132/212	Murine model of pressure overload	Global KO	Protection from cardiac hypertrophy	Ucar et al. (2012)
MIAT	Human samples	Overexpression	Confers risk of myocardial infarction	Ishii et al. (2006)
Chrf	Murine model	Overexpression	Increases CM-apoptosis	Wang et al. (2014b)
Chrf	Murine model of hypertension	Knockdown	Attenuation of hypertrophy	Wang et al. (2014b)
Mhrt	Murine model of pressure overload	Transgenic overexpression	Inhibition of hypertrophy and HF	Han et al. (2014)
Chast	Murine model of pressure overload	Pharmacologic inhibition	Prevention and attenuation of cardiac remodelling	Viereck et al. (2016)
Hrcr	Murine model of pressure overload and isoproterenol-infusion	Overexpression	Amelioration of hypertrophy	Wang et al. (2016)

4 Conclusion

Progress in ncRNA research has widened our knowledge of eukaryotic genome diversity and inspired overwhelming enthusiasm to identify novel mechanisms linked to human pathologies. As described in this chapter ncRNAs may serve as potential targets for novel therapeutics; yet their role as diagnostic or prognostic disease biomarkers cannot be overlooked. Early diagnosis is essential for effective patient management and blood biomarkers may provide a quick, effective, and reliable informative tool in clinical settings. Circulating ncRNAs show high level of sensitivity, stability, and specificity due to disease-specific release into the bloodstream. Alterations in plasma or serum concentrations of ncRNAs during disease progression and their correlations deliver an advantage of minimal invasive procedures over tissue biopsies (Vausort et al. 2014). For instance, biomarker potential of several miRNAs, e.g. miR-1, miR-133a, mir-208b, miR-499, and miR-21, is well established in relation to the heart failure pathologies and recent meta-analysis studies have attempted to find the best combination of miRNA biomarkers (Cheng et al. 2014; Yang et al. 2015). Likewise, circulating mitochondrial lncRNA LIPCAR is reported to be a predictor of adverse cardiac remodeling and is associated with survival in heart failure patients (Kumarswamy et al. 2014). Similarly, ANRIL and KCNQ1OT1 concentrations in blood can predict left ventricular dysfunction (Vausort et al. 2014). However, the

clinical use of circulating ncRNAs as biomarkers still needs further validation to set their basal cutoff levels, measurement reproducibility and method uniformity.

Understanding overall biological significance of a vast variety of newly discovered RNA transcripts is a major challenge to researchers and ncRNAs represent an unexplored mine of potential disease biomarkers and novel drug targets. While miRNA therapy could successfully enter phase II human clinical trials for HIV treatment (Janssen et al. 2013), different therapeutic approaches to modulate lncRNAs or circRNAs in vivo are still in infancy. Several open questions still prevent the use of ncRNA-based therapies in clinics; yet this attractive research area is refining our current view of the human pathologies which will revolutionize the new drug discoveries in coming years.

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Cardiac Myosin Activation with Gene Therapy Produces Sustained Inotropic Effects and May Treat Heart Failure with Reduced Ejection Fraction

Sam L. Teichman, Kassandra S. Thomson, and Michael Regnier

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Abstract

Chronic inotropic therapy is effective for the treatment of heart failure with reduced ejection fraction, but has been limited by adverse long-term safety profiles, development of tolerance, and the need for chronic parenteral administration. A safe and convenient therapeutic agent that produces sustained inotropic effects could improve symptoms, functional capacity, and quality of life. Small amounts of 2-deoxy-adenosine triphosphate (dATP) activate cardiac myosin leading to enhanced contractility in normal and failing heart muscle. Cardiac myosin activation triggers faster myosin crossbridge cycling with

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_31

greater force generation during each contraction. This paper describes the rationale and results of a translational medicine effort to increase dATP levels using a gene therapy strategy to deliver and upregulate ribonucleotide reductase (R1R2), the enzyme responsible for dATP synthesis, selectively in cardiomyocytes. In small and large animal models of heart failure, a single dose of this gene therapy has led to sustained inotropic effects with a benign safety profile. Further animal studies are appropriate with the goal of testing this agent in patients with heart failure.

Keywords
Cardiac myosin activation • dATP • Gene therapy • Heart failure • Inotropic therapy • Ribonucleotide reductase • Translational medicine

Non-Standard Abbreviations and Acronyms

AAV6-R1	Single AAV6 vector expressing R1 subunit
AAV6-R1.2	Single AAV6 vector expressing R1 & R2 subunits, linked by P2a
AAV6-R2	Single AAV6 vector expressing R2 subunit
AV-R1	Single AV vector expressing R1 subunit
AV-R2	Single AV vector expressing R2 subunit
BB-R12	AAV6 viral vector with a cardiac-specific promoter cTnT455 to overexpress R1R2 in the heart
dATP	2-Deoxy-adenosine triphosphate
HF	Heart failure
R1	Rrm1 subunit of ribonucleotide reductase
R1R2	Ribonucleotide reductase
R2	Rrm2 subunit of ribonucleotide reductase
TgRR	Transgenic mouse model overexpressing R1R2

1 Introduction

Chronic heart failure (HF) remains a significant, growing cause of morbidity, hospitalizations, medical costs, and mortality and the condition has been characterized repeatedly as an epidemic (Redfield 2002; Lüscher 2014; Yancy et al. 2013). In patients with moderate-to-severe HF with reduced ejection fraction, decreased cardiac systolic function and compensatory mechanisms are the underlying causes of the clinical manifestations of HF, including the development and worsening of symptoms, declining functional capacity, episodes of decompensation requiring hospitalization, progressive dysfunction, and premature death. Chronic use of inotropic agents acting through the adrenergic system or cyclic AMP has improved symptoms and exercise capacity through an increase in ejection fraction,

but the benefit of these drugs has been limited by problematic long-term safety and tolerance, and by the need for parenteral administration (Cohn et al. 1998; Lowes et al. 2000; Francis et al. 2014). Data from the use of cardiac resynchronization and left ventricular (LV) assist devices suggest that chronic improvement of cardiac function may prevent or reverse the progression of HF (Solomon et al. 2010; Ambardekar and Buttrick 2011).

Recently, gene therapy has been tested for the treatment of HF and offers the promise of durable results after a single exposure through sustained expression of a therapeutic gene. These therapies primarily target improvement of dysfunctional calcium cycling that triggers myosin contractions, either directly or through the adrenergic system and cyclic AMP (Tilemann et al. 2012; Pleger et al. 2013). The three most advanced programs have completed clinical trials, including gene therapy targeting sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a) which regulates calcium movement between the cytoplasm and the sarcoplasmic reticulum, stromal cell-derived factor 1 (SDF-1) which is involved in endogenous myocardial repair, and adenylyl-cyclase type 6 (AC6) which catalyzes cyclic AMP formation (Pleger et al. 2013).

A new and promising method of improving cardiac function in HF patients is to target cardiac myosin directly instead of altering calcium regulation within the cardiomyocyte (Teerlink 2009). Cardiac myosin activation triggers faster myosin crossbridge cycling and greater force generation during each contraction. This approach bypasses the adrenergic and cyclic AMP systems, as well as the complex calcium regulation mechanisms. Omecamtiv mecarbil is the only cardiac myosin activating drug currently in development and encouraging clinical results have been reported preliminarily (Malik et al. 2011; Cleland et al. 2011; Greenberg et al. 2015; Teerlink et al. 2016). Omecamtiv is a myocardial ATPase activator that increases LV function independent of calcium levels by prolonging systole without an increase in +dP/dt. However, as a small organic molecule, omecamtiv mecarbil requires chronic repeat administration via injections or oral dosing.

A gene therapy strategy to cardiac myosin activation could represent the intersection between the promise of a single-exposure therapy leading to a durable improvement in cardiac function. Here we review the published work on the development of BB-R12, a novel gene therapy for HF that targets cardiac myosin contraction directly by increasing production of 2-deoxy-adenosine triphosphate (dATP) in cardiomyocytes.

2 Overview

Original observations showed that dATP is a more efficient contractile substrate for myosin than ATP, and we hypothesized that increasing intracellular dATP levels in the myocardium could lead to improved cardiac function. dATP exerts positive inotropic effects on cardiomyocytes as a complementary energy source to ATP via a direct interaction with myosin. dATP increases +dP/dt (with a corresponding increase in –dP/dt) and does not prolong systole. Cellular production of low levels

of dATP occurs normally in mammalian cells by action of ribonucleotide reductase (R1R2), the enzyme that catalyzes the removal of a hydroxyl group from the 2-position on the ribose ring of ADP to produce dADP, which is then rapidly converted to dATP via phosphorylation by creatine kinase. Small amounts of dATP (and other deoxy-NTPs) are normally produced by R1R2 as a substrate for DNA synthesis and repair in replicating cells.

R1R2 has been extensively characterized and consists of two subunits: the larger R1 subunit contains the catalytic site and two allosteric sites that can bind dATP, while the smaller R2 subunit contains the free radical generator. R1R2 is tightly allosterically regulated, with no more than approximately 5% of the ATP pool present as dATP (Caras and Martin 1988; Ahmad and Dealwis 2013). However, since cardiomyocytes are non-replicating cells, the expression of R1R2 is normally markedly downregulated and dATP levels in cardiomyocytes are less than 10% of that in other cells (Korte et al. 2011). dATP concentration can be increased by the overexpression of R1R2, the rate-limiting enzyme in its production, using gene therapy.

BB-R12 is a designed, multi-component gene therapy consisting of a recombinant serotype-6 adeno-associated viral vector (AAV6) that carries a genome containing a human cardiac troponin-specific promoter (cTnT455) linked to a transgene that codes for the human sequences of both the large (R1) and small (R2) subunits of R1R2 to overexpress the enzyme selectively in myocardium. BB-R12 increases dATP levels in cardiomyocytes for the treatment of chronic HF with reduced ejection fraction. Though BB-R12 is nominally a gene therapy, the therapeutic algorithm employed is novel. Rather than correct a genetic or proteomic defect, increased levels of dATP act as a locally produced inotropic small molecule drug treatment for the failing heart. In essence, BB-R12 creates a drug production and delivery system within the heart using gene therapy. Local, intracellular production of dATP may also lower the risk of off-target cardiac or systemic side effects. Based on the initial insight that dATP is a more efficient energy source for muscle contraction, the gene therapy approach of upregulating myocardial R1R2 has progressed from testing in cardiomyocytes and myofibrils to small animal HF models, and most recently to a pilot large animal HF model. The results of these published studies are summarized below. Further testing is underway with the goal of testing this therapy in patients with HF.

3 dATP Is a More Potent Source of Energy for Myosin Contraction

The original pharmacologic insight that 2-dATP is a more potent source of energy for myosin contraction occurred almost 20 years ago. Studies initiated by Dr. Michael Regnier evaluating ATP and ATP analogs (NTPs) for their effects on skeletal muscle contractility provided a detailed analysis of the chemo-mechanical processes of myosin–actin crossbridge cycling that is normally fueled by ATP. These studies showed increased crossbridge cycling rates with dATP compared to

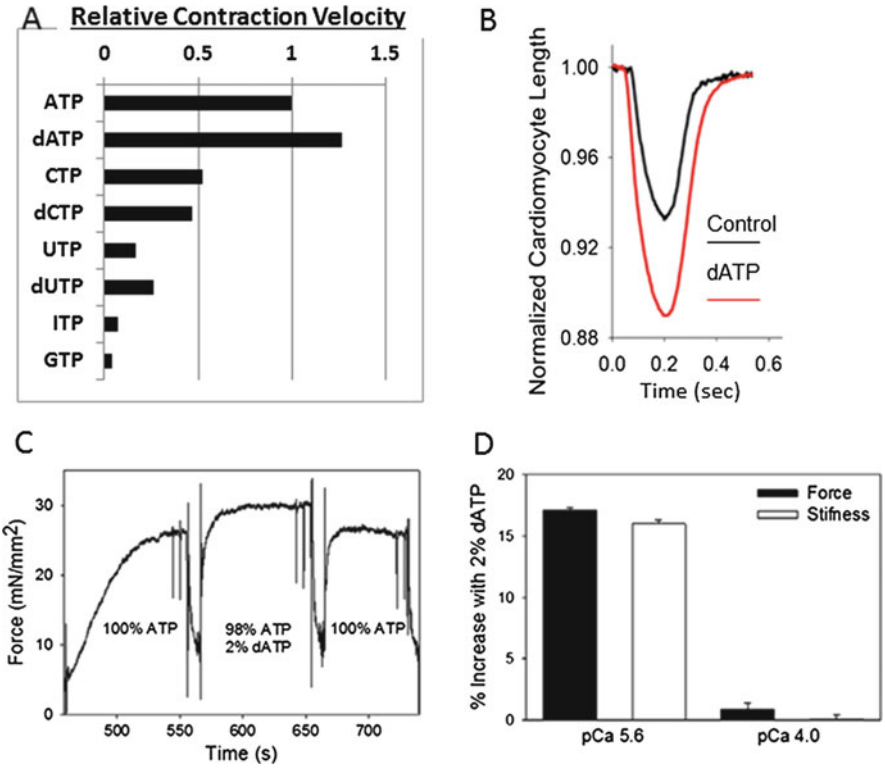

Fig. 1 (**a**) Relative contraction velocity with different NTP and dNTP substrates (ATP = 1). (**b**) The effect of 2-deoxy-adenosine triphosphate (dATP) on cardiomyocyte contraction compared to control. (**a**) and (**b**) adapted from Regnier et al. (1998a). (**b**) Effects on force production and stiffness with [2% dATP/98% ATP] compared to 100% ATP (5 mM total) in rat cardiac muscle. Example force trace (pCa 5.6) showing increased force production with transfer from 100% ATP to [2% dATP/98% ATP], and reduction of force after transfer back to 100% ATP solution. (**c**) and (**d**) % increases in force production and stiffness of cardiac trabeculae with [2% dATP/98% ATP] at submaximal (pCa 5.6) and maximal (pCa 4.0) calcium levels. (**c**) and (**d**) adapted from Korte et al. (2011)

ATP, indicating dATP improved myosin binding with faster detachment of the myosin head from actin, thus making dATP a more effective contractile substrate than ATP (Regnier et al. 1998a; Regnier et al. 1998b; Regnier and Homsher 1998). Only dATP increased force generation compared to ATP, while all other NTPs and dNTPs produced weaker contractions (Fig. 1). Furthermore, dATP increased force generation through an increase in +dP/dt (with a corresponding increase in –dP/dt) and no prolongation of systole (Fig. 1).

Later studies investigating how the chemo-mechanical transduction pathway differs in cardiac muscle showed dATP substitution for ATP in demembranated cardiac trabeculae isolated from adult rats resulted in increased isometric force at all levels of calcium activation. Muscle "stiffness" (an experimental measurement

of crossbridge binding) and force were increased similarly with dATP in cardiac muscle, suggesting increased isometric force resulted from increased strong crossbridge binding. In contrast, dATP caused elevated stiffness and force generation in skeletal muscle only at submaximal levels of calcium, demonstrating a myosin-mediated enhancement of contraction that was more potent in cardiac muscle. The substitution of dATP for ATP reversibly increased force production, stiffness, crossbridge cycling, and calcium sensitivity of force in cardiac muscle. In both cardiac and skeletal muscle, contractile kinetics were enhanced with dATP; however, increased maximal force production was only seen in cardiac muscle (Regnier et al. 2000; Regnier et al. 2004).

An experiment in rat cardiac muscle demonstrated that addition of low levels of dATP to the ATP pool at physiological calcium levels resulted in significant increases in force development. Briefly, demembranated rat cardiac trabeculae were exposed to solutions containing 5 mM NTP (either 100% ATP or [2% dATP/98% ATP]) at submaximal (pCa 5.6) and maximal (pCa 4.0) calcium concentrations. Force production at pCa 5.6 was increased by 17% with a solution of [2% dATP/98% ATP] compared to 100% ATP solution. Furthermore, the resultant increase in force was reversible upon transfer back to 100% ATP solution. Stiffness was increased with [2% dATP/98% ATP] compared to 100% ATP at pCa 5.6, indicating that increased force production with dATP is due to increased crossbridge binding. At pCa 4.0, these increases in force production and stiffness were not seen (Fig. 1). This study demonstrated that even small amounts of cellular dATP added to ATP are sufficient to significantly increase contractile force in cardiac tissue by increasing the number of strong crossbridges (Korte et al. 2011). These data, confirmed by the work of others, suggest that the analogy to a "fuel additive" is more appropriate than "higher-octane fuel" (Schoffstall et al. 2006; Schoffstall and Chase 2008; Baker 2011).

Dilated cardiomyopathy (DCM), a prevalent form of HF, is characterized by ventricular dilatation, as well as a loss of systolic function. A widely accepted canine model of naturally occurring DCM was used to evaluate whether dATP could improve contractility in DCM cardiac tissues. When myofibrils isolated from DCM canine hearts were treated with dATP, contractility was significantly increased and function was restored to the control (non-failing) cardiac tissue levels, without affecting relaxation (Cheng et al. 2016).

In order to evaluate the potential for a human therapy, cardiac tissue obtained from patients with end-stage HF was treated ex vivo with dATP to determine whether the same effects would be seen. Adult LV wall tissue was obtained from 16 end-stage HF patients undergoing LV assist device placement or cardiac transplantation. Demembranated tissues were mounted on a custom biomechanical assay platform where stiffness and steady-state isometric force were measured at varying calcium concentrations (pCa) in the presence of varying ratios of ATP:dATP (5 mM total). Cardiac myofibrils isolated from the tissue samples were used to assess the activation and relaxation kinetics with ATP or dATP (2 mM). Contractility of the failing human cardiac muscle was significantly enhanced in the presence of dATP without affecting relaxation kinetics. Isometric force at saturating

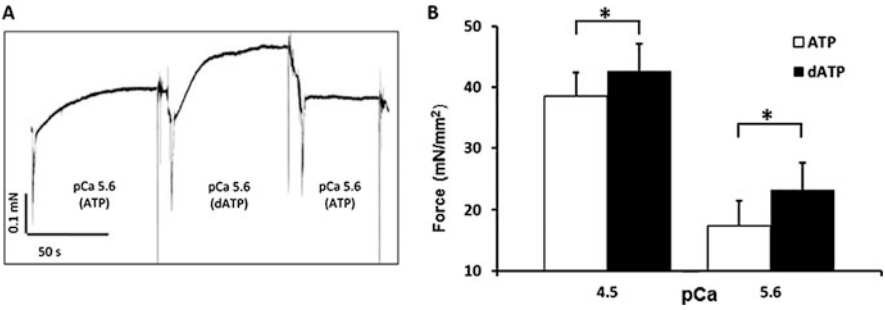

Fig. 2 Effects of ATP vs. dATP in ex vivo human cardiac tissue from end-stage HF patients demonstrating dATP increases force produced. (**a**) Representative isometric force trace of demembranated tissue at pCa 5.6 with ATP or dATP. (**b**) Force measured at maximal (pCa 4.5) and submaximal calcium concentrations (pCa 5.6) with ATP or dATP. Measurements are mean ± SEM. $*P < 0.05$ by paired student's *t*-test. Adapted from Moussavi-Harami et al. (2015)

calcium levels (pCa 4.5) was increased by 11% with dATP compared to ATP, and increased by 35% at submaximal calcium levels in the range where the heart normally operates. Isometric force was also shown to increase linearly ($R^2 = 0.985$) with increasing dATP content at pCa 5.6. Myofibrils showed a 13% increase in force production and a 44% increase in activation rate with dATP compared to ATP, with no effect on relaxation rates and no prolongation of systole. In agreement with previous studies in rat cardiac tissues, increased force production, calcium sensitivity, crossbridge cycling, and rate of activation at physiological calcium levels were seen with low levels of dATP, resulting in increased force production and more robust contractile kinetics (Fig. 2) (Moussavi-Harami et al. 2015).

The studies of rat, canine, and human tissues ex vivo characterized the increased magnitude and rate of force production with dATP in cardiac muscle. However, the structural basis for improved myosin binding and detachment with dATP in muscle was not yet understood. In order to investigate the underlying chemo-mechanical mechanisms for improved contractility seen with dATP, atom-level differences in pre-powerstroke myosin structure and dynamics with dADP binding were evaluated with molecular dynamics (MD) simulations. Effects of dADP on myosin affinity for actin were measured by an in vitro motility (IVM) assay to validate MD simulation results. A well-characterized myosin structure in the pre-powerstroke state was used as a starting structure for MD simulations. Simulation results showed that binding of dADP in the myosin cleft alters the intramolecular contacts within the nucleotide binding pocket (starting with Phe129) compared to ADP, resulting in a change in both the structure of the binding pocket and the position of the nucleotide within the pocket. This initiates a cascade of altered contacts in the protein structure, causing myosin to undergo global conformational changes towards a conformation seen in strong actin binding states. dADP binding stabilizes a myosin conformation that is more energetically favorable for actin binding (closed cleft conformation) resulting in more exposed polar residues on the actin

binding surface of myosin, thus increasing the probability of electrostatic interactions between actin and myosin. MD simulation results were supported by IVM analysis, which indicated that dATP enhances weak binding electrostatic interactions between actin and myosin. These studies suggest that the missing hydroxyl group in dATP modifies the chemo-mechanical interaction of the nucleotide with the binding site on myosin, inducing global conformational changes in the myosin head structure. These changes result in an increased rate and affinity of actin binding. The increased frequency and strength of crossbridge cycling ultimately increase force production in cardiac muscle (Nowakowski et al., 2014, unpublished data) (Nowakowski et al. 2013a).

To evaluate the effects of constitutive upregulation of dATP in vivo, a transgenic mouse model that overexpresses R1R2 (TgRR) was studied. R1R2 levels are typically higher in replicating than in quiescent cells or in post-mitotic differentiated cells such as cardiomyocytes, since deoxynucleotides are needed for DNA synthesis and repair. The development of this animal model therefore initially addressed the challenge of providing heart cells in an intact animal with an adequate supply of dATP. Both in vivo and ex vivo cardiac assessments were conducted, including cardiac function, energetics, tissue morphology, gene expression, and cardiomyocyte contractility. TgRR mice overexpress both the R1 and R2 subunits of R1R2 and wild-type (WT) control transgenic mice were bred similarly to TgRR mice. Results showed that adult TgRR mice have enhanced basal ventricular function in vivo (fractional shortening and ejection fraction), and produce increased contractile force and hemodynamic parameters ex vivo (LV developed pressure, positive and negative maximum LV pressure waves), without exhibiting increased heart rate, cardiac hypertrophy, LV dilation, or adverse cardiac remodeling. Hearts were able to respond to high workload β-adrenergic challenge (dobutamine infusion) and performed similarly to normal hearts during the high workload for 20 min. High-energy phosphocreatine reserves were mildly reduced at baseline (though the levels remained high), but were similar to normal hearts after high workload challenge without affecting cellular ATP levels under normal conditions. No differences in body weight, heart weight, cardiomyocyte size, organization, or fibrosis were seen in hearts from TgRR and WT mice at 3 and 12 months, suggesting no hypertrophy or cardiac remodeling resulted from chronically elevated dATP. This transgenic animal model demonstrated that elevated cardiac function can be maintained over the long-term with R1R2 overexpression without noticeable side effects or structural adaptation of the heart, indicating that long-term upregulation of dATP may be a viable therapeutic mechanism (Nowakowski et al. 2013b).

4 Translational Medicine Challenge: Developing a Gene-Delivery System to Increase dATP Specifically in Cardiac Tissue

The body of work characterizing the effects of dATP on cardiac muscle contractility demonstrates how this ATP analog increases the magnitude and rate of force development. However, chronic administration of dATP directly to the heart is not a viable strategy to achieve long-term therapeutic cardiac effects. The transgenic mouse model demonstrated that sustained systemic overexpression of R1R2 increases basal cardiac function. In order to translate these findings into a viable therapy for cardiac diseases, a method of increasing intracellular dATP specifically in cardiomyocytes was needed and viral vector systems were designed to overexpress R1R2.

The initial in vitro evaluation of viral-mediated overexpression of R1R2 to increase cellular dATP concentration used a delivery system consisting of two separate recombinant adenoviral (AV) vectors that expressed the R1 or R2 subunits separately with a GFP reporter under the cytomegalovirus (CMV) promoter. These vectors were administered simultaneously in equal doses (this treatment is referred to as AV-R1 + AV-R2, Table 1). When cultured rat cardiomyocytes (both adult and neonatal) were transduced with AV-R1 + AV-R2, the treatment resulted in increased intracellular dATP content and an increased magnitude of contraction and rate of contraction and relaxation, without affecting calcium transient properties. These results suggest that the increased contractility seen with R1R2 overexpression is due to increased myofilament responsiveness to calcium. Cells treated with AV-R1 + AV-R2 maintained the relative increase in relaxation kinetics at all stimulation frequencies, indicating no impairment of relaxation. Contractile response (defined as fractional shortening/maximal Fura-2 fluorescence) was increased in cells treated with AV-R1 + AV-R2 compared to controls at all stimulation frequencies (Fig. 3a). Measurement of cellular R1, R2, and dATP levels indicated a significant increase in R1 and R2 protein content, and an approximate tenfold increase in dATP over that seen in control cells, equating to approximately

Table 1 Description of viral vector systems

Sequence species	Viral vector	Gene(s) expressed	Vector system design	Treatment name
Rat	AV	R1 + GFP	Two separate vectors	AV-R1 + AV-R2
Rat	AV	R2 + GFP		
Rat	AAV6	R1	Two separate vectors	Rat AAV6-R1 + AAV6-R2
Rat	AAV6	R2		
Human	AAV6	R1	Two separate vectors	Human AAV6-R1 + AAV6-R2
Human	AAV6	R2		
Human	AAV6	R1-P2A-R2[a]	Single vector	Human AAV6-R1.2 (BB-R12)

[a]P2A is a 20 amino acid peptide linker that enables co-translation of two proteins from a single strand of mRNA and subsequent cleavage of the two proteins

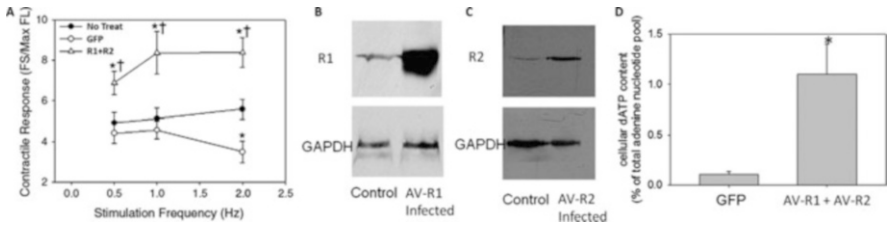

Fig. 3 Effects of AV-R1 + AV-R2 treatment on adult rat cardiomyocytes (ARCs). (**a**) Contractile response of ARCs at different stimulation frequencies. AV-R1 + AV-R2 treated cells (*open triangles*) showed significantly greater response to calcium at all frequencies. AV-GFP treated cells (*open circles*); non-transduced cells (*closed circles*). $*P < 0.05$ compared to non-transduced; $\dagger P < 0.05$ compared to AV-GFP treated. (**b–d**) R1R2 protein expression in ARCs after AV-R1 + AV-R2 treatment. Increased (**b**) R1 and (**c**) R2 protein expression in AV-R1 + AV-R2 treated neonatal rat ventricular myocytes (NRVMs). (**d**) Increased intracellular dATP in AV-R1 + AV-R2 treated NRVMs. $*P < 0.05$ compared to AV-GFP treated NRVMs. Adapted from Korte et al. (2011)

1% of the total adenine nucleotide pool (Fig. 3b–d). This study provided the first proof of principle that dATP levels could be increased within cardiomyocytes, and that small elevations of dATP levels enhanced contractility (Korte et al. 2011).

When the AV-R1 + AV-R2 system was used analogously to transduce human cells (human embryonic stem cell-derived cardiomyocytes, hESC-CMs), contractility was again significantly increased (Lundy et al. 2014). To evaluate the therapeutic potential of this delivery system, AV-R1 + AV-R2 was used to transduce cardiomyocytes isolated from infarcted adult rat hearts. Contractility of the treated infarcted cardiomyocytes was significantly improved compared to untreated infarct cells with the magnitude and rate of contraction restored to levels comparable to healthy (uninfarcted) cardiomyocytes (Feest et al. 2014).

The transfer of small molecules such as ATP and dATP between cells is facilitated by gap junctions. It was hypothesized that dATP could diffuse through gap junctions between physically coupled cardiomyocytes to enhance the contractility of neighboring cells that were not themselves overexpressing R1R2. To test this hypothesis, the transfer of fluorescein-labeled dATP via gap junctions between AV-R1 + AV-R2 transduced and non-transduced (WT) cardiac cells (both rat and human) and the resulting effects on contractility were measured. Rapid transfer of dATP between coupled cells (Fig. 4a, b) was demonstrated, and this effect was blocked when a gap junction inhibitor was introduced. R1R2-overexpressing human cardiomyocytes (hESC-CMs) had significantly larger contraction magnitudes than WT cells (Fig. 4c, d). WT hESC-CMs coupled to AV-R1 + AV-R2 treated hESC-CMs also had increased contraction magnitudes and contraction velocity, but showed no significant differences in time to peak contraction or time to 90% relaxation (Fig. 4d). When these R1R2-overexpressing human cardiomyocytes were transplanted into adult rat hearts in vivo, they delivered dATP to the host heart muscle and significantly increased global cardiac performance within 5 days (Fig. 4e) (Lundy et al. 2014).

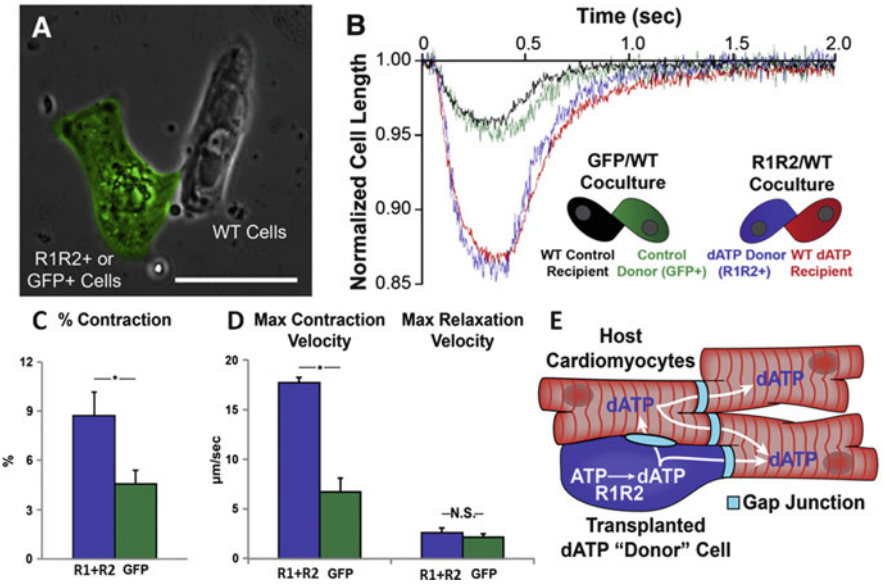

Fig. 4 R1R2 overexpression increases human embryonic stem cell-derived cardiomyocyte (hESC-CM) contractility and enhances contractility of coupled cardiomyocytes. (**a**) Example of coupled GFP-expressing cell and WT cell. Contractile measurements were made individually on each cell in a doublet, as well as on uncoupled WT cells in culture. (**b**) Representative traces showing increased contractility of AV-R1 + AV-R2 treated cells and coupled WT cells compared to control cells with no prolongation of systole. AV-R1 + AV-R2 treated hESC-CMs showed (**c**) significantly increased magnitude of contraction compared to AV-GFP treated cells, and (**d**) significantly increased maximum contraction velocity without affecting relaxation velocity. $^*P < 0.05$; *NS* not significant. (**e**) Schematic showing dATP generation in transplanted R1R2-overexpressing cells (dATP "Donor" cells) and the gap junction-mediated transfer of dATP through coupled host cardiomyocytes. Adapted from Lundy et al. (2014)

In order to reduce immune responses to the delivery system relative to AV and achieve long-term expression of R1R2 in the myocardium, adeno-associated virus vector (AAV6) systems were developed that targeted cardiac tissue (Table 1). The next translational therapy was to develop a dual vector system consisting of two separate recombinant AAV6 vectors that express rat sequences of R1 or R2 under the cardiac-specific promoter cTnT455 (a regulatory cassette composed of enhancer and promoter portions of the human cardiac troponin gene (TNNT2)), which would be delivered simultaneously at equal doses (treatment referred to as Rat AAV6-R1 + AAV6-R2). When delivered systemically via intravenous infusion to healthy mice, the resulting R1R2 overexpression increased global cardiac systolic function at all doses (1.5×10^{13}, 4.5×10^{13}, or 1.35×10^{14} total vector genomes (vg)/kg), and the effects persisted for at least 13 weeks post-injection. A larger effect in the high dose group was seen at 1–3 weeks and persisted, suggesting an early dose–response, but by 6 weeks the effect in the lower dose groups had increased to reach the level seen in the high dose group. These results indicate that

the improvements in heart function seen with higher doses of vector-mediated R1R2 overexpression can be achieved with lower doses of the vector over time (Kolwicz et al. 2016). This pattern is consistent with the hypothesis that transduction of fewer cardiomyocytes in the lower dose groups required more time to establish a sufficient concentration gradient for dATP to diffuse from these cells to distal non-transduced cells.

Further translational research led to the development of a single vector system using an AAV6 vector containing a transgene cassette expressing optimized human sequences of both R1 and R2 subunits separated by a protein cleavage site under a single cardiac-specific promoter (cTnT455) (Table 1). This vector system, called BB-R12 (AAV6-R1.2 in this publication, Fig. 5a), was designed to restrict the increase of intracellular R1R2 to cardiac muscle cells. To evaluate this single vector delivery system and determine if the increased cardiac function seen with the dual vector systems used previously could be replicated, BB-R12 was delivered systemically via tail vein injection (7×10^{13} vg/kg) to healthy adult mice and cardiac function was monitored for 4 weeks and compared to other vector systems as described in Table 1. While significant viral genome uptake was seen in liver and ventricular tissues of treated mice compared to controls (with negligible uptake in the gastrocnemius tissue, Fig. 5b), increased levels of R1 and R2 proteins were only found in ventricular tissue (Fig. 5c), confirming the specificity of the cTnT455 promoter. BB-R12 significantly increased basal cardiac function by ~20–30%.

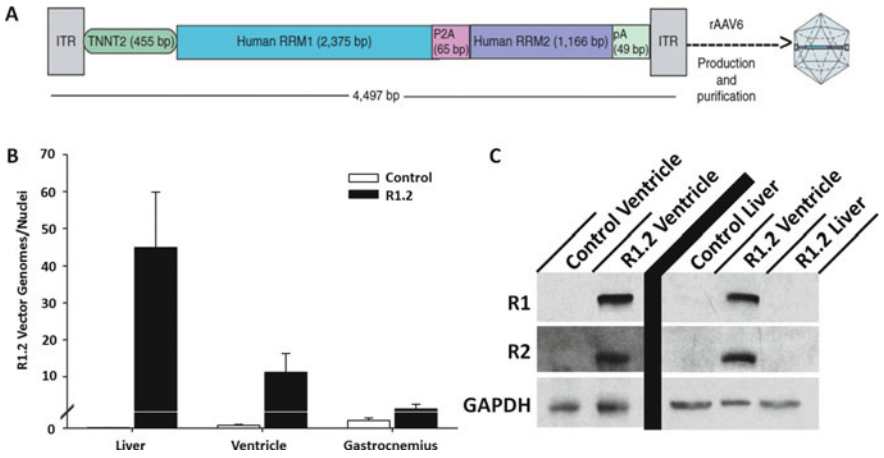

Fig. 5 BB-R12 vector in healthy mouse tissues. (**a**) Schematic diagram detailing the BB-R12 (AAV6-R1.2) vector construct containing a cardiac-specific promoter (cTnT455) and human sequences of the R1 (RRM1) and R2 (RRM2) subunits separated by a self-cleaving P2A peptide linker. (**b**) BB-R12 vector genome biodistribution in healthy mice tissues. qPCR results quantifying the BB-R12 vector genomes/nuclei of liver, heart (ventricle), and gastrocnemius tissues. *Bars* represent the mean ± SD. (**c**) R1R2 is selectively overexpressed in cardiac tissue of healthy mice treated with BB-R12. Western blot analysis of control and BB-R12-treated mouse heart (ventricle) and liver tissue, probing for R1, R2, and a loading control (GAPDH). Adapted from Kolwicz et al. (2016)

Responsiveness to high workload challenge (dobutamine infusion) indicated that β-adrenergic signaling remained intact with AAV6 treatment, and responses were similar to those seen in transgenic mice (Kolwicz et al. 2016).

Finally, to determine whether the BB-R12 system was capable of improving in vivo cardiac function in a disease model, BB-R12 (2.5×10^{13} vg/kg) was injected directly into the adjacent non-infarcted myocardium of adult rats 5 days after myocardial infarction (MI) induced by permanent ligature of the left anterior descending coronary artery. Effects on cardiac function were monitored with echocardiography for 8 weeks post-MI. At 2 weeks post-treatment, fractional shortening remained significantly depressed in the MI group compared to sham controls (uninfarcted). However, by 4 and 8 weeks, cardiac function had recovered in BB-R12 treated rats to levels comparable to the sham control hearts (Kolwicz et al. 2016).

5 Large Animal Model of HF

After characterization in vitro, ex vivo, and in vivo in rodent systems and models, the definitive translational evaluation of BB-R12 was to determine whether viral-mediated overexpression of R1R2 could increase dATP levels and improve cardiac function in a standard large animal (swine) MI/HF model (Dixon and Spinale 2009; Ishikawa et al. 2012). Yucatan minipigs were screened for AAV6 neutralizing antibodies. Seronegative animals had MI induced by balloon occlusion of the left anterior descending coronary artery. At 2 weeks post-MI, echocardiographic and hemodynamic data confirmed that all groups showed the effects of the induced MI and ensuing HF. Animals were not immunosuppressed and were administered BB-R12 via free-flowing, antegrade intracoronary infusion after onset of HF 2 weeks post-MI (day 0). Doses were 1×10^{13} (high), 5×10^{12} (medium), or 1×10^{12} (low) doses of vector genomes. The cardiovascular responses over the 2 months post-treatment were compared to sham controls (MI + saline). As expected, during the 2-month observation, the sham group showed progression of HF with continued deterioration in LV ejection fraction (LVEF), increased LV end diastolic pressure (LVEDP), and decreased maximum rate of pressure rise (+dP/dt), a parameter of systolic function.

In contrast, animals treated with the high dose of BB-R12 had improved LVEF by 1 month post-treatment, with further improvement after 2 months. Interestingly, the medium dose group showed no response at 1 month, but had significantly increased LVEF by the 2-month time point compared to sham (Figs. 6 and 7a). This is consistent with the hypothesis that fewer transduced cells in the medium dose group required more time for dATP to diffuse from these cells to non-transduced cells and produce a response. Hemodynamic data show that LVEDP and +dP/dt were significantly improved in the high dose groups by 2 months post-treatment, with similar results in –dP/dt, a parameter of early diastolic function (Fig. 7b–d). There was no effect on LV end diastolic diameter, and no differences in blood pressure or heart rate were seen. No humoral or cellular

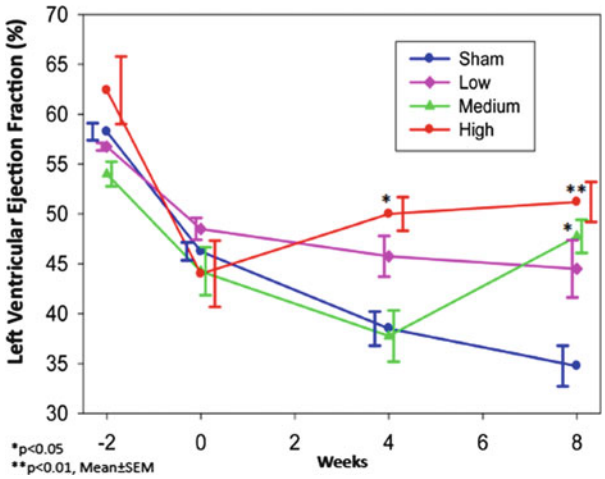

Fig. 6 BB-R12 effect on LV ejection fraction (LVEF) in swine model of MI/HF. LVEF at each time point in the 2-month study of swine MI/HF for sham (MI + saline) vs. BB-R12 therapy at low, medium, and high doses ($n = 4$–5 per group). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to sham, mixed effects regression model; data represented as mean ± SEM. Adapted from Kadota et al. (2015)

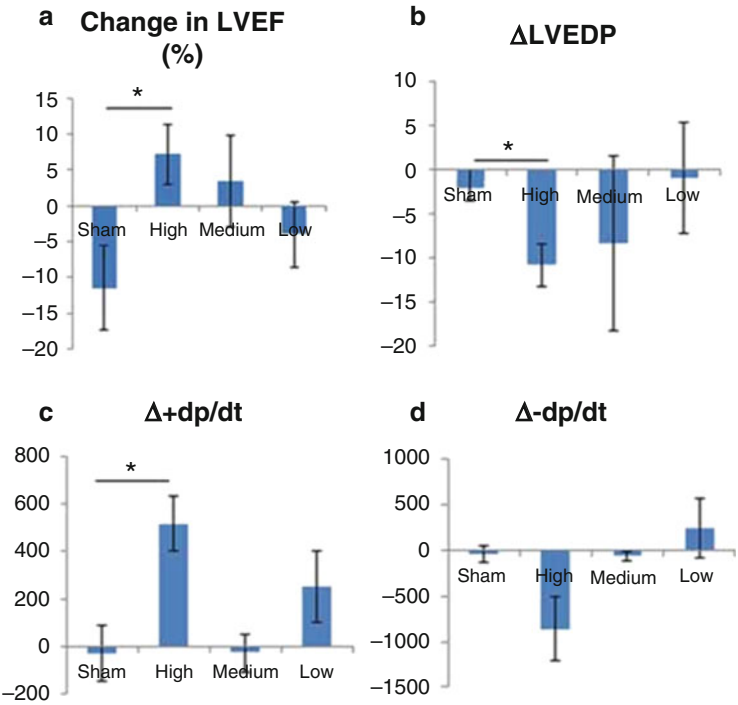

Fig. 7 BB-R12 effect on hemodynamic parameters in swine model of MI/HF. Day 0 (pre-treatment) to Day 56 changes in (**a**) LVEF, (**b**) LV end diastolic pressure (LVEDP), (**c**) +dP/dt, and (**d**) –dP/dt. Data are mean change ± SEM. $^{*}P < 0.05$. Adapted from Kadota et al. (2015)

immune responses to BB-R12 occurred, and no adverse effects on blood counts, chemistries, or liver enzymes were seen at any time point. At the end of the study, there were no gross or microscopic pathological findings in any organ nor any histopathologic differences in the infarct area. The results of this pilot large animal cardiac pharmacological study of the effects of BB-R12 in an MI/HF swine model extended the findings from rodent pharmacology studies, with safe and sustained increases in global cardiac function observed following a single administration and in a dose-responsive manner (Kadota et al. 2015).

6 Limitations and Future Directions

Data from the studies described above are consistent with the interpretation that elevated dATP levels increase cardiac contractility due to a direct interaction with myosin. dATP increases myosin–actin crossbridge cycling rates causing increased force generation. dATP levels can be increased with a single dose of gene therapy that selectively upregulates R1R2 in transduced cardiomyocytes. dATP can diffuse through gap junctions from a small number of transduced cells into a larger number of neighboring, non-transduced cells amplifying the effect.

However, dATP may exert additional effects via enhancing the functions of other excitation–contraction coupling components that bind ATP, or enhancement of metabolic or mitochondrial energy pathways. Since R1R2 also converts other NTPs into deoxy-NTPs, elevated levels of one or more of these could affect cardiac contractility. A more complete understanding of the mechanisms underlying this approach is desirable, but not essential to evaluating further its therapeutic potential.

Additional studies are warranted to further our understanding of important translational aspects of this technology. These include replication of the pilot swine study findings in a larger study, direct measurements of dATP and R1R2 activity in treated animals, and confirmation of the response in a second large animal experimental model of HF. Further work will assess the durability of the observed response, evaluate the effects on oxygen consumption (both at the sarcomere and, more importantly, the whole-heart level), and further assess the acute and longer-term cardiac and non-cardiac safety profile, in particular the arrhythmic potential.

From a practical perspective, gene therapy may have a valid target and fail due to insufficient transduction efficiency, i.e., too few cells are transduced to produce the desired effects. BB-R12 contains an AAV6 viral vector, which has greater affinity for the heart compared to most other myogenic AAV serotypes (Palomeque et al. 2007; Arnett et al. 2013; Gao et al. 2011). The effect of dATP appears potent, since small increases in dATP can increase cardiac contractility, and the diffusion of dATP from transduced (“factory”) cells through gap junctions to neighboring and distal non-transduced cells amplifies the effect. Even though the potency and amplification effects may make BB-R12 less critically dependent upon transduction efficiency, optimization of dose and delivery techniques will increase the likelihood

of observing a beneficial therapeutic response. Antegrade intracoronary infusions produced a favorable effect in pigs, and systemic injections and direct myocardial injections have also been evaluated preliminarily. Before human testing, further animal studies should evaluate antegrade intracoronary infusions with balloon occlusions, as well as retrograde coronary sinus infusions that may increase transduction efficiency.

Lastly, because adeno-associated viruses are endemic, there is antiviral immunity in the human population. The animals in the swine MI–HF study were from a closed herd and were all seronegative. Circulating neutralizing antibodies to AAV6 are present in a large proportion of the human population and the clinical utility of this approach would be limited. However, if the therapeutic utility can be demonstrated in an initial, carefully selected population, then alternative approaches to delivery can be considered. These may include alternative AAV vectors, cell-based therapies, or direct myocardial injections.

7 Conclusion

The observation that dATP activates cardiac myosin in healthy and failing cardiomyocytes has been translated into BB-R12, a gene therapy agent capable of upregulating R1R2 selectively in the heart leading to the local synthesis of dATP. Testing to date suggests that a durable increase in cardiac performance can be produced safely after a single exposure. The approach represents the intersection of the promise of both cardiac myosin activation and gene therapy. The extensive in vitro, ex vivo, and in vivo research into experimental HF described here support further animal studies, and may eventually lead to clinical testing of BB-R12 as a therapy for the many patients with HF due to systolic dysfunction.

Disclosures MR is a scientific founder and equity holder in BEAT Biotherapeutics Corp. SLT is an employee of BEAT Biotherapeutics Corp.

Sources of Funding
Work in MR's laboratory was supported by NIH grants R01 HL061683, R01 HL065497, R01 HL111197, and R21 HL091368.

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Direct Myosin Activation by Omecamtiv Mecarbil for Heart Failure with Reduced Ejection Fraction

Mitchell A. Psotka and John R. Teerlink

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2017_13

Abstract

Myosin is the indispensable molecular motor that utilizes chemical energy to produce force for contraction within the cardiac myocyte. Myosin activity is gated by intracellular calcium levels which are regulated by multiple upstream signaling cascades that can be altered for clinical utility using inotropic medications. In contrast to clinically available cardiac inotropes, omecamtiv mecarbil is a novel direct myosin activator developed to augment left ventricular systolic function without the undesirable secondary effects of altered calcium homeostasis. Its identification and synthesis followed high-throughput screening of a reconstituted sarcomere, deliberate optimization, exquisite biochemical evaluation, and subsequently promising effects in animal models were demonstrated. Physiologically, it prolonged the duration of left ventricular systole in animal models, healthy adults, and patients with heart failure with reduced ejection fraction (HFrEF) without changing the velocity of pressure development, as assessed in animal models. It has been formulated for both intravenous and oral administration, and in both acute and chronic settings produced similar alterations in the duration of systole associated with beneficial increases in cardiac output, improvements in left ventricular volumes, and reductions in heart rate and often of natriuretic peptides. Small, asymptomatic increases in troponin were also observed in the absence of clinically evident ischemia. Clinically, the question remains as to whether the possible harm of this minimal troponin release is outweighed by the potential benefits of reduced neurohormonal activation, increased stroke volume and cardiac output, and improved ventricular remodeling in patients treated with omecamtiv mecarbil. The resolution of this question is being addressed by a phase III outcomes trial of this potential novel therapy for heart failure.

Keywords

Cardiac output • Heart failure • Myosin activator • Omecamtiv mecarbil • Systolic ejection time

Abbreviations

ACE	Angiotensin-converting enzyme
ARB	Angiotensin receptor blocker
ARNI	Angiotensin receptor neprilysin inhibitors
ATP	Adenosine triphosphate
BNP	Brain-type natriuretic peptide
cAMP	Cyclic adenosine monophosphate
CO	Cardiac output
dP/dt	Maximal rate of pressure rise during systole
FS	Fractional shortening

HF	Heart failure
HFrEF	Heart failure with reduced ejection fraction
LV	Left ventricle
LVEDP	Left ventricular end-diastolic pressure
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end-systolic volume
MRA	Mineralocorticoid receptor antagonists
MVO_2	Myocardial oxygen consumption
NCX	Sodium–calcium exchanger
OM	Omecamtiv mecarbil
PDE	Phosphodiesterase
P_i	Hydrolyzed phosphate
SERCA2a	Sarcoplasmic reticulum calcium ATPase 2a
SET	Systolic ejection time
SR	Sarcoplasmic reticulum
SV	Stroke volume

1 Introduction

Decreased cardiac contractility, principally quantified by reduced left ventricular ejection fraction (LVEF), is the underlying pathologic change responsible for heart failure (HF) with reduced ejection fraction (HFrEF) and its substantial associated worldwide morbidity and mortality (Yancy et al. 2013; Ponikowski et al. 2016; Mozaffarian et al. 2016; McMurray 2010). Current treatment modalities combat the negative consequences of hemodynamic and neurohormonal compensatory responses to maintain adequate cardiac output (CO) in HFrEF. Evidence-based therapies that improve mortality include β-adrenergic receptor blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB), angiotensin receptor neprilysin inhibitors (ARNI), mineralocorticoid receptor antagonists (MRA), and hydralazine with nitrates, while additional agents including ivabradine and digoxin offer benefits for morbidity without substantial improvements in mortality (Yancy et al. 2016; Psotka and Teerlink 2016). Those agents that improve mortality also typically secondarily increase LVEF, decrease left ventricular end-diastolic volume (LVEDV), and decrease left ventricular end-systolic volume (LVESV), while most agents unable to positively influence mortality typically fail to augment LVEF or improve ventricular dimensions (Kramer et al. 2010). Therapeutics to improve ventricular contractility, addressing an underlying cause of HFrEF, remain conceptually attractive for both the acute and chronic management of HFrEF. Unfortunately, currently available inotropic medications lack evidence of benefit at best and increase mortality at worst (Hasenfuss and Teerlink 2011). This chapter will review the pharmacokinetics, preclinical and clinical efficacy, and safety of the novel small-molecule direct myosin activator omecamtiv mecarbil (CK-1827452; AMG-423).

Omecamtiv mecarbil (OM) has the potential to fill the widely applicable role of a clinical therapeutic that improves LVEF with concomitant benefits on clinical outcomes, ideally in both acute and chronic HFrEF.

2 Cardiac Contractility

2.1 Myocardial Force Production

Since cardiac myocytes are connected by tight junctions, they form an electrical syncytium over which a wave of depolarization sweeps to locally activate them in unison and produce contractile force (Solaro 2011). The principal unit of cardiac contraction and the critical site of its regulation is the sarcomere. Within the sarcomere, the force-producing unit is cardiac myosin, the molecular motor that utilizes cellular energy from the hydrolysis of adenosine triphosphate (ATP) to pull on actin filaments, resulting in myocardial contraction. Myosin functions as a dimeric protein consisting of two light chains and two heavy chains. The latter possesses an ATPase domain responsible for accessing the energy stored as intracellular ATP as well as a binding site for actin for the purpose of translocation (Spudich 1994; Malik et al. 2011). The myocardial sarcomere is composed of interdigitating myosin thick filaments and actin thin filaments with associated troponin and tropomyosin regulatory complexes (Fig. 1). At basal intracellular calcium levels, prior to the influx of calcium through the cell membrane or release from an intracellular membranous system called the sarcoplasmic reticulum (SR), tropomyosin complexed with troponins C, I, and T blocks actin–myosin cross-bridge formation. Following myocyte depolarization, increased intracellular calcium binds to troponin C and causes a conformational change in the tropomyosin complex allowing subsequent myosin binding and procession through the mechanochemical cycle.

2.2 Mechanochemical Actin–Myosin Cycle

The myosin mechanochemical cycle couples ATP hydrolysis to mechanical movement of the actin myofilament by approximately 10 nm, generating 3–6 pN of force per myosin head (Spudich 1994, 2014; Liu et al. 2015). It is conceptually useful to start discussion of the myosin cycle at the termination of the previous cycle, when the myosin head has completed mechanical movement yet remains strongly bound to the actin myofilament (Fig. 2). ATP binding to the myosin head rapidly releases post-powerstroke myosin from actin. Myosin hydrolysis of ATP to adenosine diphosphate (ADP) and phosphate (P_i) resets the myosin lever arm to a cocked position prepared for mechanical transduction and allows a weak interaction between the myosin head and actin filament. Release of the hydrolyzed P_i is rate-limiting and occurs concomitantly with transition to a strong interaction between myosin and actin, followed by the force-producing mechanical transduction of the myosin lever arm. The approximately 100-ms cycle completes with the release of ADP whereupon

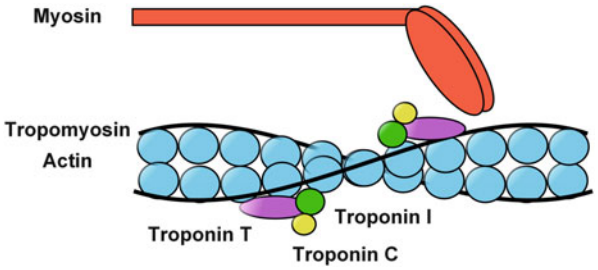

Fig. 1 Actin and myosin regulatory complex. Increased intracellular calcium binds to troponin C resulting in a conformational change of the tropomyosin–troponin I–troponin T complex on the actin myofilament, exposing the actin so that the myosin head may attach and enter the myosin cross-bridge cycle

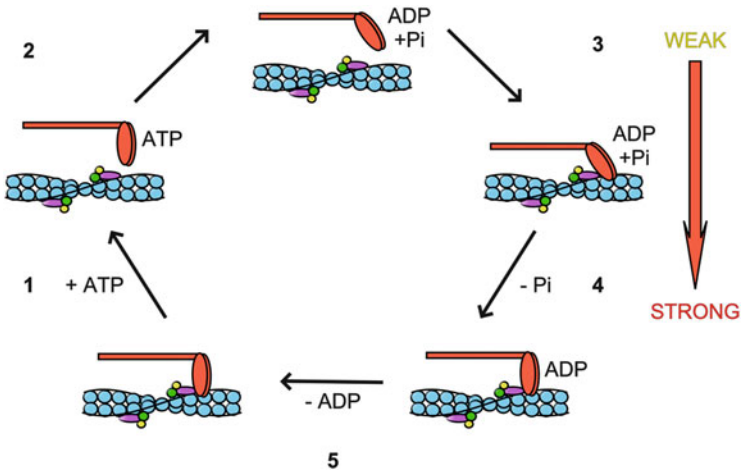

Fig. 2 The mechanochemical cycle of actin and myosin, modified from Malik et al. (2011) and Spudich (1994). The mechanochemical cycle begins with release of myosin bound to the actin myofilament by interaction with cytoplasmic adenosine triphosphate (ATP) (1). Hydrolysis of ATP into adenosine diphosphate (ADP) and inorganic phosphate (P_i) cocks the myosin lever arm into the loaded position (2). The myosin–ADP–P_i complex weakly binds to the actin myofilament and scans for an appropriate binding site (3). Concomitant with transition to strong binding of actin, myosin releases the P_i and the myosin lever arm shifts to translocate the actin filament (4). Following movement of the lever arm, ADP is released and the myosin–actin complex awaits the arrival of another ATP molecule

a new ATP rapidly binds to myosin releasing it from the actin myofilament. In cardiac tissue, the physiologic correlates to the myosin cycle are ventricular systole (active cardiac contraction) during which myosin translocates actin, and ventricular diastole (cardiac relaxation and filling) during which myosin is unbound or weakly affiliated with actin awaiting the following cardiac cycle.

2.3 Endogenous Regulation of Myosin Activity

The final common myosin regulatory pathway for a diverse group of endogenous stimuli is the myocyte intracellular calcium concentration during myocardial activation and the calcium sensitivity of the myofibril regulatory apparatus (Solaro 2011; Hasenfuss and Teerlink 2011). When an action potential reaches a myocyte, it causes opening of sarcolemmal calcium channels that transport a small amount of calcium into the cytoplasm. Through calcium-induced calcium release, the small initial calcium current activates additional calcium release through the ryanodine receptors from the internal SR network. Depolarization-induced calcium influx contributes approximately 20% of the intracellular calcium rise while the rest principally comes from the SR. As previously described, as concentrations of intracellular calcium increase, calcium occupies the exchangeable calcium binding sites on troponin C, causing the troponin complex to move tropomyosin, thus exposing actin and facilitating the myosin cycle. Following cell repolarization, calcium is returned to the SR by SERCA2a, a calcium ATPase, and extruded by the sodium–calcium exchange protein NCX and a sarcolemmal calcium ATPase. In the basal state, prior to depolarization, there is not sufficient cytoplasmic calcium to saturate myofilament troponin C, and thus the myosin cycle does not occur. In the event of rapid heart rate, delayed inactivation of the cell membrane calcium channels occurs leading to increased intracellular calcium accumulation to facilitate more rapid sarcomere activation. In this way, there is heart rate- or frequency-dependent increased myocardial contractility.

Extracellular stimuli modify myocyte intracellular calcium fluxes (Teerlink 2009; Solaro 2011). Autonomic nervous system signaling controls the levels of catecholamines and acetylcholine that, respectively, activate or inhibit myocyte membrane-bound G-protein coupled receptors. Activation of adrenergic G-protein coupled receptor signaling stimulates adenylyl cyclase to transform ATP into cyclic adenosine monophosphate (cAMP) that activates protein kinase A (PKA) to phosphorylate a host of downstream molecules. Phosphodiesterase breaks down cAMP and inhibits this activation of PKA. PKA phosphorylation of phospholamban leads to increased calcium uptake into the SR by SERCA2a, increasing both intracellular calcium and the rate of reuptake following contraction in preparation for another cycle. PKA also phosphorylates SR membrane calcium channels which facilitates increased calcium release with depolarization, as well as troponin I which eases calcium-induced troponin C release. These actions in concert augment calcium-mediated activation of the myosin cycle and increase cardiac contractility as well as rapidity of contraction.

Apart from intercellular and intracellular signaling mechanisms previously discussed, length-dependent activation of cross-bridges also increases myocardial contractility (Holubarsch et al. 1996). Increased ventricular preload with concomitant increased precontraction sarcomere length increases contractile strength via the calcium-independent Frank–Starling mechanism. These changes are mediated by a combination of effects on sarcomere regulatory proteins responsible for determining calcium sensitivity. Unfortunately, this mechanism becomes overwhelmed as the heart continues to dilate in severe HFrEF.

3 Myocardial Contractility

3.1 Inotropy Defined

An inotrope is an agent that increases myocardial contractile force and speed. There are several determinants of myocardial contractility that are capable of modulation. The first component is the number of myosin–actin cross-bridges participating during each cycle of systole. Because the force produced by each myosin head is constant, the total force production during contraction is dependent on the number of myosin–actin cross-bridges; more cross-bridges result in a greater force of contraction. The number of cross-bridges depends on the amount of calcium available to bind troponin C, the calcium affinity of the troponin complex, the number of myosin heads ready to undergo actin binding once their binding sites become available, and finally the duration of thin filament activation, which if prolonged can allow additional myosin heads to participate during the same contraction. At the conclusion of systole, the non-force-producing period begins, during which the myosin cross-bridges detach, representing the diastolic period during which ventricular relaxation and coronary artery filling occur.

All currently known inotropic agents alter calcium homeostasis and thus indirectly effect an increase in generated force by myosin by changing the amount of calcium available to bind troponin C (Hasenfuss and Teerlink 2011). Although not critical for augmented contractility, interventions that heighten calcium concentrations or enhance calcium sensitivity also result in increased velocity of force development, quantified by the maximal rate of pressure rise during systole (dP/dt), and also increase lusitropy, or the maximal rate of pressure decline during diastolic relaxation.

3.2 Myocardial Energetics of Inotropy

In the detached state, myosin binds and hydrolyzes ATP but is very slow to release the P_i or ADP. If it does so before binding to the actin filament, then the energy of ATP hydrolysis is wasted. However, if the myosin is able to bind the actin filament during systole, ATP is productively used in a 1:1 ratio by each participating myosin head during a mechanochemical cycle (Hasenfuss and Teerlink 2011). Since only a fraction of available myosin heads normally forms productive cross-bridges during each cycle, the actin-independent ATP turnover by myosin essentially wastes chemical energy without force production. The total energy use by myosin is thus the summation of the two processes; the efficiency of the system depends on the amount of ATP hydrolyzed as part of force production versus independent of actin compared to the quantity of generated force–time integral. In the case of cAMP-augmented contractility, the increased rate of calcium fluxes reduces the attachment time of each cross-bridge cycle. Thus increases in dP/dt and lusitropy occur at the expense of worsened myocardial energetic efficiency. In addition, cAMP-augmented contractility also requires increased energy of ATP-dependent ion pumps in the context of these increased calcium fluxes.

3.3 Mechanisms of Available Inotropic Agents

Currently available inotropic agents for clinical use include adrenergic receptor agonists (dobutamine and dopamine) and phosphodiesterase 3 (PDE) inhibitors (milrinone and enoximone), both of which signal through cAMP and PKA, calcium sensitizers (levosimendan), and sodium–potassium ATPase inhibitors (digitalis) that impair establishment of the sodium gradient utilized by NCX to extrude calcium (Hasenfuss and Teerlink 2011). All of these agents intensify cardiac contractility through their common mediator: intracellular myocardial calcium. Most of these agents inflate energy utilization because calcium extrusion and importation into the SR are an ATP-dependent process. They also increase heart rate by aggravating the compensatory mechanisms known to be harmful in HFrEF (Hasenfuss and Teerlink 2011; Haikala et al. 1995; Teerlink 2009). Levosimendan mediates potent vasodilation by activating ATP-dependent potassium channels and may function as a calcium sensitizer at the level of the troponin complex (Haikala et al. 1995; Hasenfuss et al. 1998; Yokoshiki and Sperelakis 2003), though others have suggested that it also has significant PDE-3 inhibitor activity at clinically relevant concentrations (Endoh 2014; Orstavik et al. 2014).

3.4 Clinical Effects of Available Inotropic Agents

The underlying premise of increased contractility for the treatment of HFrEF is intuitive: the central pathophysiologic change thought to be responsible for the syndrome is decreased systolic function, thus improved systolic function should prevent or reverse maladaptive neurohormonal activation and ventricular remodeling, reduce symptoms, and increase survival. In spite of this logic, the fulfillment of this potential benefit has been elusive. Inotropic agents have repeatedly failed to improve clinical outcomes. In the most recent guidelines from the USA and Europe, the use of available non-glycoside inotropic agents may be considered only for the treatment of cardiogenic shock or hypoperfusion associated with acute HF but they are not recommended because their usefulness and efficacy is poorly established. Additionally, they are contraindicated in the absence of cardiogenic shock except in palliative settings due to their negative impact on patient outcomes including increased mortality (Ponikowski et al. 2016; Yancy et al. 2013). Digoxin is the exception; it may be used for patients in sinus rhythm who remain symptomatic despite multiple preferable therapies (ACEi/ARB/ ARNI, beta-blockers, diuretics, MRA, hydralazine with nitrates, and ivabradine) to decrease hospitalization during or after other agents have been employed, and it may be indicated as second-line therapy for rate control of atrial fibrillation in the setting of HF.

The lack of benefit with these available inotropes may be due to the mechanism by which they augment contractility. The adverse clinical effects of cAMP mediated inotropy have been demonstrated in multiple clinical trials. In the ESCAPE trial, the use of inotropes (primarily dobutamine, milrinone, and dopamine) was associated with increased 6-month all-cause mortality (Elkayam et al. 2007). In OPTIME-

CHF, 951 patients with acute HF not in cardiogenic shock (systolic blood pressure > 90 mmHg) were randomized to a 48-h infusion of the PDE inhibitor milrinone or placebo and followed for 60 days (Cuffe et al. 2002). Milrinone was not superior to placebo for the primary endpoint of the proportion of time spent in the hospital, was not associated with substantially improved patient well-being, and produced statistically significantly more adverse events including sustained hypotension and new atrial arrhythmias, with numerically more ventricular arrhythmias, myocardial infarctions, and deaths. In the subset of patients with known coronary artery disease, milrinone therapy increased 60-day mortality (11.6% versus 7.5% for placebo, $p = 0.03$) (Felker et al. 2003). Use of oral milrinone compared to placebo was investigated in the PROMISE trial, where 1,088 chronic HFrEF stable outpatients were randomized (Packer et al. 1991). Milrinone increased all-cause mortality by 28% as well as increased hospitalizations. Similar results were reported from a meta-analysis of randomized trials of intravenous dobutamine, in which there was a trend towards increased mortality with dobutamine use with odds ratio 1.47 [95% confidence interval (CI) 0.98–2.21, $p = 0.06$] (Tacon et al. 2012). Finally, the PDE inhibitor vesnarinone demonstrated clear dose-dependent increased mortality in the Vesnarinone trial, which terminated its development as a therapeutic (Cohn et al. 1998).

Clinical results with the calcium sensitizer levosimendan have been similarly disappointing. In the SURVIVE trial, 1,327 patients with acute HF in need of intravenous inotropic support were randomized to levosimendan or dobutamine for at least 24 h (Mebazaa et al. 2007). There was no difference between the groups in the primary outcome of 180-day all-cause mortality, and although levosimendan was associated with a greater decrease in the marker of vascular congestion, brain-type natriuretic peptide (BNP), there was no benefit in terms of patient global assessment. Levosimendan did however increase atrial fibrillation and hypokalemia. In the 700-patient REVIVE trials, intravenous levosimendan for acute HF provided improved symptomatic relief, decreased BNP, and less clinical worsening, however was associated with a numerical elevation in 90-day mortality and statistically significant increases in hypotension, heart rate, episodes of ventricular tachycardia, and atrial fibrillation (Packer et al. 2013).

Although now with a diminished role in the treatment of HFrEF, digoxin was once commonly administered to increase cardiac contractility. It exerts its cardiac function by inhibiting the sodium–potassium ATPase, limiting calcium extrusion by NCX, and thus elevating intramyocyte calcium. The DIG trial randomized 6,800 patients with chronic HFrEF to oral digoxin or placebo and followed them for mean 37 months (The Digitalis Investigation Group 1997). There was no difference in the primary outcome of mortality between the treatment arms, although patients treated with digoxin were hospitalized less for cardiovascular causes or worsening HF than in the placebo arm (risk ratio 0.72, 95% CI 0.66–0.79, $p < 0.001$). Additional analyses suggested that patients with serum digoxin concentrations of 1.2 ng/mL and higher had a 12% greater absolute mortality rate (Rathore et al. 2003). Digoxin's use in the outpatient arena has been largely replaced by therapeutics with demonstrated mortality benefit, although some have suggested that its role should be expanded to improve symptoms and reduce worsening heart failure (Ambrosy et al. 2014).

4 Omecamtiv Mecarbil (OM)

Omecamtiv mecarbil (OM) represents the first drug candidate in a novel therapeutic class whose mechanism of action is to directly alter the myosin cross-bridge cycle. It specifically acts by increasing the rate of transition of myosin from a weakly bound to a strongly bound state as indicated by an increase in the rate of actin-dependent phosphate release, thus allowing more myosin heads to enter the strongly bound force-producing state during systole. The increased number of myosin cross-bridges augments contractility without altering calcium homeostasis or changing the velocity of pressure development (dP/dt); however, it does increase the duration of systole manifest as an increase in systolic ejection time (SET). In contrast to available inotropes, OM improves cardiac contraction independent of changes in cAMP and calcium transients. Because not all myosin heads participate in each cycle of systole (generally $<10\%$ of the available cross-bridges), the addition of more myosin heads enhances myosin-generated force and myocardial contraction (Spudich 2014).

4.1 Screening and Optimization

It was hypothesized that direct activation of the human sarcomere could augment cardiac performance without the adverse effects of known inotropic agents or calcium sensitizers. OM was identified by high-throughput screening of small-molecule compounds in a biochemically reconstituted sarcomere and followed by optimization of candidate molecules for pharmacologic and clinically desirable properties (Malik et al. 2011; Morgan et al. 2010; Malik and Morgan 2011). The reconstituted calcium-responsive sarcomere was composed of polymerized purified bovine actin, with subsequent addition of stoichiometrically appropriate troponin–tropomyosin complexes and the S1 subfragment of type II β-cardiac myosin with ATP and calcium. Increases in measured myosin ATPase signaled small molecules capable of sarcomere activation among approximately 400,000 screened agents. Specificity for cardiac myosin was demonstrated by contrasting these results with a similarly constructed system using skeletal muscle myosin. The identified compounds were highly selective for the cardiac sarcomere, and they were subsequently shown to increase contractility in isolated rat cardiac myocytes with no effect on myocyte calcium fluxes, confirming the original hypothesis (Morgan et al. 2010).

The compounds discovered in the original screen were subsequently modified to allow for intravenous and oral administration by improving stability and aqueous solubility, decreasing protein binding, removing undesirable chemical moieties, increasing potency, and avoiding off-target effects including vasodilation via potassium channel alteration and hepatic enzyme inhibition that would lead to unfavorable drug interactions (Morgan et al. 2010). Systematic iterative optimization of the parent compound addressed these issues while maintaining the targeted effect of myosin activation in the reconstituted sarcomere, isolated cardiomyocytes, and on cardiac function as observed using echocardiography in normal Sprague-Dawley rats. After synthesizing more than 1,700 compounds, the terminal chemical structure of OM (Fig. 3),

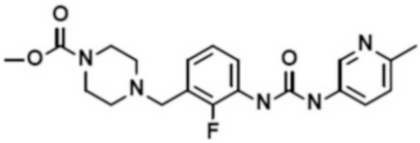

Fig. 3 Chemical structure of omecamtiv mecarbil

also called CK-1827452 and AMG-423, possessed a molecular weight of 401.43 g/mol, and demonstrated in vitro and in vivo pharmacokinetic and pharmacodynamic properties suitable for further evaluation (Malik and Morgan 2011).

4.2 Omecamtiv Mecarbil Mechanisms of Action

OM selectively activates cardiac isoforms of myosin and does so by directly binding to the myosin head and allosterically modulating its progression through the mechanochemical cycle (Malik et al. 2011). In the reconstituted bovine sarcomere, OM accelerated the transition of myosin from the weakly actin-bound to strongly actin-bound state measured by release of P_i (Fig. 2, step 4), without changing the rate of subsequent ADP release that determines the amount of time in the strongly actin-bound state (Fig. 2, step 5), nor the rate of ATP binding and release of actin (Fig. 2, step 1). These in vitro biochemical findings were corroborated and expanded with porcine myosin by a separate research group (Liu et al. 2015). In this system, OM appeared to shift the equilibrium towards myosin ATP hydrolysis without affecting the rate of hydrolysis (Fig. 2, step 2), in addition to accelerating the rate of P_i release. Because the transition from weak to strong interaction between myosin and actin together with release of P_i is the rate-limiting step in the mechanochemical cycle, OM expedites the entry into the force-production state, expanding the number of myosin heads participating in force production during each cross-bridge cycle. This increases the total contractile impulse generated. Notably, OM decreased the rate of P_i release when actin was removed from the reconstituted bovine system and this was recapitulated in the in vitro porcine system (Liu et al. 2015; Malik et al. 2011). This decrease in actin-independent ATP hydrolysis potentially increases the overall energetic efficiency of the system by diminishing ATP use not associated with mechanical work.

OM exerted its effects independent of known secondary messenger systems including β-adrenergic signaling and calcium homeostasis (Malik et al. 2011). OM demonstrated an additive effect with isoproterenol on fractional shortening (FS) of freshly isolated adult rat cardiac myocytes in the absence of change in the calcium transient, and the OM-augmented contractility was not blocked by treating with the adrenergic antagonist carvedilol. OM can mediate its effect in the presence of these typical inotropic mechanisms because it binds directly to myosin. In the bovine reconstituted in vitro sarcomere, OM bound at a stoichiometry of one molecule of OM for each myosin head. An OM analog with UV-crosslinking identified the binding cleft in close proximity to both the actin-binding and ATP-binding regions of myosin, allowing allosteric modulation of these areas (Malik et al. 2011). On the cellular level, OM increased the contraction duration of freshly isolated adult rat cardiomyocytes without

altering the speed of contraction or relaxation compared to untreated cells. OM is thus postulated to mediate its physiologic effect in intact organisms by increasing the number of myosin heads participating during a contractile cycle, thus increasing the time of thin filament activation, resulting in an increase in SET. The overall result is an increase in the overall extent of contractile force produced.

4.3 Omecamtiv Mecarbil in Healthy Animal Models

The OM-induced augmentation of the myosin mechanochemical cycle was demonstrated to have the anticipated consequences in a variety of animal models. OM infusions for 30–60 min to generate plasma concentrations of 100–1,000 ng/mL produced dose-dependent elevations in echocardiographically measured fractional shortening of 10–20% in both Sprague-Dawley rats and beagle dogs (Malik et al. 2011). In normal mongrel dogs with implanted hemodynamic and ultrasonographic sensors, OM as an intravenous bolus of 0.5 mg/kg followed by 0.5 mg/kg/h for 15 min increased fractional shortening, myocardial wall thickening, SET, and stroke volume. OM decreased heart rate without statistically significant changes in CO, dP/dt, mean arterial pressure, left ventricular end-diastolic pressure (LVEDP), or total vascular resistance.

4.4 Omecamtiv Mecarbil in Animal Models of Heart Failure

The conscious mongrel dog with implanted hemodynamic and ultrasonographic sensors was used to investigate the effects of OM in a model of HFrEF. HF was created by ligation of the mid-anterior descending coronary artery followed by 1–2 weeks of recovery, and then 3–4 weeks of rapid ventricular pacing at 240 bpm (Shen et al. 2010). These cardiac insults increased resting heart rate, left atrial pressure, LVEDP, and decreased dP/dt (Shen et al. 2010). Like similarly monitored healthy dogs, OM as an intravenous bolus of 0.5 mg/kg followed by 0.5 mg/kg/h for 15 min increased ventricular wall thickening, FS, and SET, and decreased heart rate, without changes in dP/dt in dogs with HF. However, OM also significantly raised CO (29% versus 1%) and SV (61% versus 10%) substantially more in the HF dogs than in healthy dogs, despite a 17% decrease in heart rate (Malik et al. 2011). When compared with a 10 μg/kg/min dobutamine infusion, a 1 mg/kg bolus of OM demonstrated separate effects on time-dependent elastance during pressure–volume loop monitoring, again clarifying the effects of OM as distinct from those of traditional inotropes.

When OM was administered to the dog model as an intravenous bolus of 0.25 mg/kg followed by 0.25 mg/kg/h for 24 h, similar changes were maintained as with short-term dosing; this included increases of 26% in SET, 22% in cardiac output, and 44% in stroke volume, with coincident decreases of 15% in heart rate, 12% in left atrial pressure, and 16% in LVEDP (Shen et al. 2010). These hemodynamic changes are summarized in Fig. 4. There was no evidence of desensitization with sustained OM infusion, there remained no effect on dP/dt, and in some animals the infusions were continued for 72 h without abatement of physiologic improvements. Myocardial energetics in the live

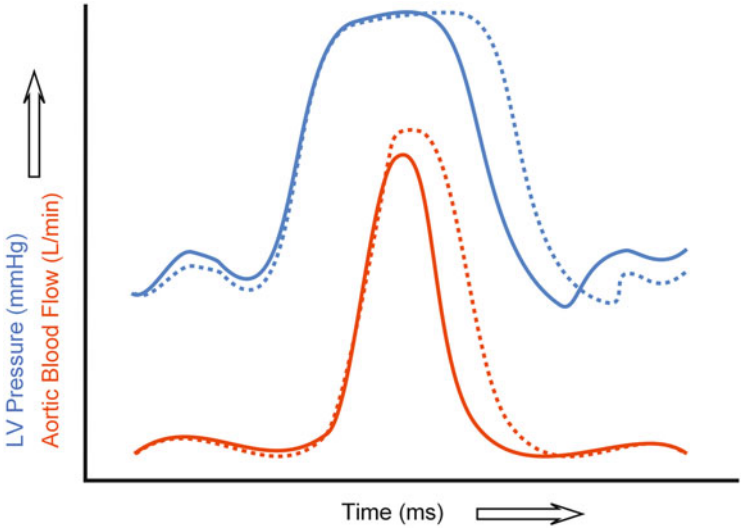

Fig. 4 Schematic hemodynamic effects of omecamtiv mecarbil on the cardiac left ventricle, adapted from Shen et al. (2010). Omecamtiv mecarbil (*dashed lines*) prolongs the systolic ejection time (SET) compared to untreated ventricles (*solid lines*), thus increasing the duration of elevated left ventricular pressure without changing the velocity of pressure development (dP/dt) or relaxation. Prolonged SET increases aortic blood flow (cardiac output) proportionally

animals were ascertained by measuring myocardial oxygen consumption (MVO_2). OM was associated with no significant change in myocardial MVO_2, confirming the hypothesis that cardiac activity could be enhanced without negatively affecting energetics.

A second model of nonischemic hypertrophied HF in conscious dogs can be induced by placing a Teflon cuff around the ascending aorta to create a 50%-reduced aortic diameter for 1 year (Shen et al. 2010). This model may better approximate the hypertrophied cardiac pathology of many patients with HFrEF. The resultant hypertrophied dog heart is then subjected to 3–4 weeks of rapid ventricular pacing at 240 bpm as in the infarction model above. Like the infarction model, these dogs develop elevated resting heart rate, left atrial pressure, LVEDP, and depressed dP/dt. OM similarly boosted SET and systolic wall thickening, and diminished heart rate and LVEDP in this model, demonstrating the potential for OM to improve cardiac physiology due to multiple etiologies.

An alternative hemodynamically and ultrasonographically monitored postischemic pig model with ischemia induced by approximately 20 min of 11 repetitive left coronary artery occlusions reported unfavorable effects for OM on myocardial energetics (Bakkehaug et al. 2015). This ischemic stunning without infarction produces transiently impaired left ventricular function with mildly decreased dP/dt and SV, although only 90 min of hemodynamic stability has been published (Korvald et al. 2001). Following administration of 0.75 mg/kg OM as a bolus over 10 min then 0.5 mg/kg/h in the 20 min prior to MVO_2 measurement (equivalent to 0.92 mg/kg in

20 min compared to 0.33 mg/kg in 20 min in the previously reported dog studies), there was a linear increase in SET, CO, and LVEF as reported in other model systems. Although there was also a reported decrease in cardiac efficiency with OM compared to baseline in this model with increased MVO_2, the changes in MVO_2 were not compared to untreated ischemic controls at similar time points following ischemia, making this conclusion difficult to support. Furthermore, not only was OM given at three times higher a dose than used in all other systems, using doses without clinical relevance, OM infusion was associated with increases in dP/dt in both healthy and ischemic animals, a finding not corroborated by data from any other animal or human system. These factors and methodological issues, among others not discussed here (Teerlink et al. 2015), limit the ability of this work to currently undermine the extensive data supporting improved myocardial energetics with OM treatment.

5 Omecamtiv Mecarbil Clinical Development Program

Following the extensive in vitro and in vivo preclinical demonstration of beneficial physiologic effects with OM administration, initial clinical studies were performed to pursue its use as a therapeutic. Table 1 contains a summary of the principal results from the main clinical studies examining OM efficacy, tolerability, and safety reported thus far. Phase I trials in healthy participants primarily focused on determining the pharmacokinetics, pharmacodynamics, and metabolism of OM when administered orally and intravenously, while phase II investigations have determined optimal dosing as well as provided more insight into its pharmacodynamics and clinical effects.

5.1 Phase I Clinical Studies

The first-in-human study of OM enrolled 34 healthy young men and administered escalating doses in 6-h intravenous infusions with the primary aim to establish the highest tolerated dose, and secondary goals to determine its safety, pharmacokinetics, and pharmacodynamics (Teerlink et al. 2011) (Table 1). OM was formulated as a colorless and clear solution with 50 mmol/L citrate in sterile water, with pH adjusted to 5.0 (Cleland et al. 2011). In this predominantly white population with mean age 27 years, OM infusion was tolerated at a protocol-defined maximal dose of 0.5 mg/kg/h, however many tolerated 0.625 mg/kg/h as well (Teerlink et al. 2011). The mean elimination half-life was 17.1–21.0 h, with clearance 132–207 mL/h/kg, and mean volume of distribution of 3.7–5.2 L/kg. OM caused dose-dependent increases in SET (85 ms increase at the highest dose), FS (8%), SV (15 mL), and LVEF (7%), starting at plasma levels of 100 ng/mL for SET and 200 ng/mL for SV, all with $p < 0.0001$. The linear correlation between SET and OM plasma concentration was excellent, and plasma levels closely corresponded with FS and LVEF. OM also decreased ventricular dimensions, with changes in echocardiographic diastolic function parameters consistent with increased left atrial contractile function. The dose-limiting effect of symptomatic

Table 1 Reported phase I and phase II clinical studies of omecamtiv mecarbil

Trial (phase), year(s)	Subjects (n)	Dose (route)	Design	Endpoints	Major outcomes	Reference
NCT01380223 (I), 2005–2006	Young, healthy, 85% white, males in four successive cohorts of eight ($n = 34$)	0.005–1.0 mg/kg/h for 6 h (IV)	Double-blind, placebo-controlled, dose-escalating, crossover, single-center trial	(1) Maximum infusion dose tolerated in $\geq$8 subjects for 6 h (2) Safety, tolerability, pharmacokinetics, and pharmacodynamics	(1) Maximum tolerated infusion 0.5 mg/kg/h (2) Dose-dependent linear increase in SET ($r^2 = 0.99$), with increased LVEF, FS, and SV. $t_{1/2} = 17.1$–21.0 h. Cardiac ischemia at plasma concentrations >1,200 ng/mL	Teerlink et al. (2011)
NCT00624442 (II), 2007–2009	Stable, 87% male, 64% ischemic, HFrEF, LVEF $\leq$ 40% or LVEF $\leq$ 30% in cohort 4 ($n = 45$)	Loading 0.125–1.0 mg/kg/h; maintenance 0.0625–0.5 mg/kg/h (IV)	Double-blind, placebo-controlled, dose-escalating, crossover, multicenter international trial	(1) Safety and tolerability for 2, 24, and 72 h (2) Establish tolerated range of pharmacologically active plasma concentrations	(1) Cardiac ischemia at plasma concentrations >1,300 ng/mL (2) Dose-dependent increases in SET, FS, LVEF, SV, and CO; decreased HR, QTc, LVESV, LVEDV, and standing SBP and DBP	Cleland et al. (2011)
NCT00682565 (II), 2008	Stable, 80% male, ischemic HFrEF with LVEF $\leq$ 35% and angina ($n = 94$)	Loading 24–48 mg/h for 2 h, then maintenance 6–11 mg/h for 18 h (IV)	Double-blind, placebo-controlled, randomized, multicenter study	(1) Safety and tolerability exercise during infusion; proportion stopping ETT due to angina at earlier stage than baseline (2) Duration of exercise, angina, and ECG changes	(1) Only one patient (receiving placebo) stopped ETT early due to angina (2) No statistical difference in time to angina, exercise duration, or ischemic ECG changes	Greenberg et al. (2015)
NCT01786512 **ATOMIC-AHF** (IIb), 2013–2015	Acute HFrEF, 77% male, 88% white, 62% ischemic, with LVEF $\leq$ 40%, dyspnea, and elevated	Loading 7.5–20 mg/h for 4 h, then maintenance 1.5–4 mg/h for	Double-blind, placebo-controlled, dose-escalating, randomized	(1) Dyspnea relief by 7-point Likert scale at 6, 24, and 48 h without worsening HF or death (2) Safety and	(1) No benefit for dyspnea relief at 6, 24, or 48 h vs. pooled placebo; improved dyspnea in third cohort	Teerlink et al. (2016a)

(continued)

Table 1 (continued)

Trial (phase), year(s)	Subjects (*n*)	Dose (route)	Design	Endpoints	Major outcomes	Reference
	BNP ($n = 613$)	44 h (IV)	international multicenter trial	tolerability, biomarkers, hospital length of stay, 30-day morbidity, and mortality	vs. paired placebo (2) No benefit for 30-day death or worsening HF, length of stay, or NT-proBNP; increased SET, SBP, decreased HR, and LVESD	
NCT01300013 **COSMIC-HF** (IIb), 2011–2015	Expansion phase: stable, 83% male, >90% white, HFrEF with LVEF $\leq$ 40%, and elevated NT-proBNP ($n = 448$)	25 mg twice-daily or PK-guided titration to 50 mg twice daily (oral)	Double-blind, placebo-controlled, dose-escalating, randomized, multicenter, international trial	(1) Pharmacokinetics of modified release dosing over 20 weeks (2) Safety and tolerability, echocardiographic and biomarker changes over 20 weeks	(1) Modified release allowed titration to 50 mg twice daily in 60% of patients (2) Increased SET, SV, and decreased HR, LVESV, LVEDV, and NT-proBNP; asymptomatic small increased troponin at 20 weeks: 0.001 ng/mL with 25 mg and 0.006 ng/mL with 25–50 mg twice daily	Teerlink et al. (2016b)

BNP B-type natriuretic peptide, *CO* cardiac output, *DBP* diastolic blood pressure, *ECG* electrocardiogram, *ETT* exercise tolerance test, *HF* heart failure, *HFrEF* heart failure with reduced ejection fraction, *HR* heart rate, *IV* intravenous, *LVEDD* left ventricular end-diastolic dimension, *LVEDV* left ventricular end-diastolic volume, *LVEF* left ventricular ejection fraction, *LVESD* left ventricular end-systolic dimension, *LVESV* left ventricular end-systolic volume, *NT-proBNP* N-terminal fragment of prohormone B-type natriuretic peptide, *PK* pharmacokinetic, *QTc* corrected electrocardiographic Q–T interval, *SBP* systolic blood pressure, *SET* systolic ejection time, *SV* stroke volume, $t_{1/2}$ plasma half-life

myocardial ischemia was established to occur at plasma concentrations over 1,200 ng/mL obtained with infusions of 0.75–1.0 mg/kg/h, associated with increases in SET over 110 ms, identifying the likely upper limit of the therapeutic range. In concordance with the extensive attention during development of OM, no off-target adverse effects of the drug were observed.

Additional phase I studies determined the pharmacokinetics of OM as an oral formulation as well as potential drug–drug interactions. A single-center study observed proportional dose responses at lower and higher doses in men and women with single doses and at steady state (Cytokinetics 2008). A single-center, open-label study in healthy males investigated the interaction of a single oral dose of OM with the cytochrome P450 3A4 inhibitors ketoconazole and diltiazem with respect to their genotype of another cytochrome P450 enzyme, 2D6 (Cytokinetics 2008). Based on 2D6 status, subjects were divided by whether they were extensive or poor metabolizers. Ketoconazole lengthened the elimination half-life in the extensive metabolizer group from 22 to 27 h ($p < 0.01$) by mildly reducing clearance of OM, associated with a 50% increase in the total exposure of OM without a change in the maximal plasma concentration. Diltiazem co-treatment had no effect on the total OM exposure nor the maximal plasma concentration, and only minimally increased the OM half-life from 18 to 20 h. As the maximal plasma dose best correlated with intolerable side effects in the other phase I trials, these data suggest low potential for clinically significant drug–drug interactions with OM.

Bioavailability of immediate-release and modified-release formulations, as well as the effects of coincident food consumption, was ascertained in 65 healthy, 85% male, 72% white, young adults as part of a randomized, open-label, crossover study (Palaparthy et al. 2016). Compared to the immediate-release formulation for which 25 mg of OM produced a maximal concentration of 262 ng/mL after 30 min, the various delayed-release forms produced maximal plasma concentrations of 34–78 ng/mL occurring 2–10 h after administration. The plasma half-life was similar for all the formulations, ranging from 18 to 21 h. The relative bioavailability of most of the oral formulations was over 75%, and the presence or absence of recent food consumption caused minimal differences in OM pharmacokinetics without altering the total exposure to the drug, changes unlikely to be clinically meaningful. OM was also found to be approximately 82% bound to plasma proteins, and primarily metabolized by decarbamylation with less substantial metabolism by hepatic cytochrome P450 enzymes 3A4 and 2D6 (Palaparthy et al. 2016). Following a single administration of OM, 8% of the unmodified compound was excreted in the urine up to 14 days afterwards suggesting extensive metabolism; however, multiple preclinical and clinical studies have demonstrated returns to normal physiology 24 h following cessation of administration (Teerlink et al. 2011; Palaparthy et al. 2016; Cleland et al. 2011).

5.2 Phase II Clinical Studies

The purpose of phase II clinical studies is to establish the dosing and concentration-response relationships for the novel agent as well as safety and tolerability in the intended patient population as both an oral and intravenous agent.

The first intravenous study of OM in patients with HFrEF was a double-blind, placebo-controlled, dose-ranging crossover trial of 45 stably treated patients with LVEF $\leq$40%, 64% of whom had ischemic cardiomyopathy (Cleland et al. 2011) (Table 1). These patients were well-treated with over 98% on neurohormonal therapy with ACE inhibitor or ARB and β-adrenergic blockade, 51% were prescribed an MRA, and 18% were on digoxin. They were hemodynamically stable with mean heart rate 69 bpm, systolic blood pressure 121 mmHg, and mean CO 4.4 L/min; however, 51% had ongoing stable angina. In this population as with healthy individuals, OM loaded at 0.125–1.0 mg/kg/h and maintained at infusions of 0.0625–0.5 mg/kg/h for 2, 24, or 72 h, with the goal to keep plasma concentrations under 1,200 ng/mL, produced dose-dependent increases in SET, SV, CO, FS, and LVEF, with decreases in systolic and diastolic ventricular volumes. Shortening of diastolic filling was partially attenuated by the slowed heart rate that occurred with each increased dose of OM. Minimal decreases in blood pressure without orthostatic changes or symptomatic hypotension were noted as well as a decreased heart rate corrected QT interval. The pharmacokinetics of OM administration in these HFrEF patients were similarly linear as healthy patients across these plasma concentrations. SV increased 5–10 mL at plasma concentrations over 200 ng/mL and plateaued at 400 ng/mL, LVEF increased above 300 ng/mL, and improvement in ventricular volumes occurred at 500 ng/mL, but there was no change in NT-proBNP at 24 or 72 h of infusion. The beneficial effects were all apparent for the duration of infusion from 2 to 72 h and there was again no evidence of desensitization to OM.

Adverse events associated with plasma OM concentrations above the targeted limit were noted. One patient experienced an unintended overdose of OM, receiving four times the intended high dose (2.2 mg/kg/h) and developed symptoms of cardiac ischemia after 45 min with electrocardiographic changes, and an increase in troponin to 2.3 ng/mL when his plasma OM level was >1,400 ng/mL. Symptoms resolved rapidly with cessation of the infusion. The other patient with symptoms of ischemia had very low clearance of OM and developed cardiac ischemic symptoms at a plasma OM concentration of >1,300 ng/mL. Otherwise, one additional patient with a baseline BP of 182/116 mmHg had an asymptomatic increase in troponin lasting the duration of the infusion. Overall, these results supported the relative safety and potential impact of OM, as well as its continued development.

Because the dose-limiting adverse effect of OM was myocardial ischemia due to decreased diastolic-filling time with increased SET, its safety and tolerability were assessed in patients with ischemic cardiomyopathy and angina (Greenberg et al. 2015) (Table 1). Ninety-five predominantly white male patients with chronic stable ischemic HFrEF, LVEF $\leq$35%, and angina were randomized to placebo or intravenous OM bolus 24–48 mg/h for 2 h followed by 6–11 mg/h for 18 h (targeting plasma concentrations of 295 ng/mL or 550 ng/mL). Patients were all able to perform at

least 4 min of an exercise tolerance test (ETT) on both of two attempts prior to the OM infusion, and the safety endpoint was defined by the proportion of patients who stopped the ETT during infusion at a stage earlier than baseline due to angina. There was no difference in the primary endpoint between the groups, as only one patient (in the placebo group) stopped their on-treatment ETT a stage early due to angina. Exercise time increased for all groups, and there were no statistically significant differences in ETT duration or change from baseline ETT duration with OM infusion, though the study was underpowered to detect a difference in these results. Pharmacokinetics of OM were again linear and predictable from data gathered previously in healthy individuals.

Overall adverse events were similar between OM and placebo-treated patients; however, one patient in the OM infusion arm had intolerable angina during his baseline and then on-treatment ETTs and after stabilization with nitroglycerin required percutaneous coronary revascularization of the left anterior descending coronary artery (Greenberg et al. 2015). The maximum plasma concentration in this patient was 651 ng/mL and after PCI the patient developed a troponin elevation to 2.45 ng/mL as well as electrocardiographic changes consistent with myocardial infarction. Two other patients who received OM developed elevated troponin to 0.13 and 1.1 ng/mL without any clinical signs of symptoms of cardiac ischemia. Although relatively few patients were studied, given the rarity of any adverse events during the study, the overall safety of OM in a population prone to inducible ischemia was supported.

The most recently published investigation of OM as an intravenous agent was the study of Acute Treatment with Omecamtiv Mecarbil to Increase Contractility in Acute Heart Failure (ATOMIC-AHF; Table 1) (Teerlink et al. 2016a). This randomized, placebo-controlled, double-blind, sequential-cohort, international multicenter dose-escalation trial enrolled 613 and treated 606 mostly white male patients hospitalized for acute HF with LVEF $\leq$40%, dyspnea, and elevated natriuretic peptides, but not requiring other inotropic agents, in three cohorts. In the three groups, placebo or OM at 7.5, 15, or 20 mg/h over 4 h were infused as a bolus followed by a 44-h infusion of 1.5, 3, or 4 mg/h, respectively. Therapy was started on average 15 h after hospital presentation. The volume infused was 30 mL/h for loading and 6 mL/h for maintenance, which patients received for a total of 48 h. Patients were followed daily during their hospitalization, and then at 1 and 6 months.

Patients achieved mean 48-h plasma concentrations of 148, 311, and 425 ng/mL in the three respective cohorts with concentration-dependent increases in SET and systolic blood pressure, and with decreases in heart rate and left ventricular end-systolic dimensions. ATOMIC-AHF did not demonstrate a difference in its primary outcome of 48-h improvement in dyspnea on a 7-point Likert scale without clinically worsening HF or death in the OM treatment groups compared to the pooled placebo groups. However, there were also significant differences between the cohorts enrolled earlier and later in the trial, with decreasing proportions of nonwhite patients and patients hospitalized for HF within the previous year, as well as increasing enrollment from Eastern Europe and greater baseline troponin concentrations in later cohorts (Teerlink et al. 2016a). Accounting for these differences, a prespecified secondary analysis of the primary endpoint between placebo and OM

treatment within each cohort demonstrated a 41% relative increase in dyspnea relief at 48 h with the highest OM dose (95% CI 2–93%, 14% absolute improvement, $p = 0.034$), a finding supported by other sensitivity analyses. There were no differences between OM and placebo for secondary endpoints including patient global assessment response, length of hospital stay, total intravenous loop diuretic usage, health resource use, or change in NT-proBNP from baseline. Adverse events were similar, with numerically fewer OM than placebo patients developing renal failure (11.9% versus 17.2%) or supraventricular tachycardias (8.0% versus 1.0%) during the infusions, or requiring all-cause rehospitalization within 30 days afterwards (12.9% versus 15.5%).

Due to the potential adverse effect of OM at plasma concentrations above the targeted therapeutic range, troponin levels were followed closely by a blinded core laboratory (Teerlink et al. 2016a). Although the median 48-h difference from baseline was unchanged at 0.000 ng/mL for the pooled OM groups, it decreased 0.004 ng/mL for the pooled placebo groups, differences that were not statistically significantly different. Importantly, neither the maximum OM plasma concentration nor the induced change in SET predicted the change in troponin from baseline. Overall ATOMIC-AHF provided additional evidence for the tolerability of OM, suggested clinical benefit of improved dyspnea in the high dose group, and supported its potential to demonstrate therapeutic efficacy in an appropriately dosed and powered trial in acute HF.

The Chronic Oral Study of Myosin Activation to Increase Contractility in Heart Failure (COSMIC-HF) investigated the role for chronic oral dosing of OM in the treatment of stable HF in a placebo-controlled, randomized, double-blind, international multicenter, two-part study (Teerlink et al. 2016b). The COSMIC-HF initial dose-escalation phase randomized 96 mostly male patients with stable outpatient HFrEF and LVEF $\leq$40% 1:1:1:1 to placebo or three modified-release oral formulations of OM in two ascending dose cohorts, using either 25 or 50 mg twice daily. Plasma levels were sampled for pharmacokinetics over 35 days and the primary endpoint of evaluation and selection of a single modified-release formulation for further testing in a larger expansion cohort was achieved.

The trial expansion phase utilized 448 mostly white male outpatients with chronic HFrEF, LVEF $\leq$40%, with elevated natriuretic peptides and HF symptoms (NYHA II or III). These patients were well-treated with guideline directed medical therapy including ACE inhibitors, ARBs, β-blockers, and MRAs. Patients were randomized 1:1:1 to placebo or the selected formulation of 25 mg of oral OM twice daily, or an oral OM pharmacokinetic-guided dose titration group where 25 mg twice daily could be increased to 50 mg twice daily depending on plasma concentrations after 2 weeks of the lower dosing. Patients received study drug for 20 weeks and were followed for an additional 4 weeks after study drug discontinuation. Of those in the dose titration group, 60% were increased to 50 mg twice daily. This expansion phase documented stable pharmacokinetics of the selected formulation over the extended time period and the secondary endpoints of the trial demonstrated the expected physiologic changes with OM; however, they additionally suggested beneficial morphologic and functional effects of this OM administration scheme.

Compared to placebo, OM increased SET 25 ± 3 ms, SV 4 ± 2 mL, and decreased heart rate approximately 3 bpm by 20 weeks, as anticipated from prior data. OM also caused a statistically significant decrease in NT-proBNP by 970 ± 357 pg/mL that persisted for 4 weeks after discontinuation of study drug. Significant improvements in left ventricular end-diastolic and end-systolic dimensions and volumes were also evident. These changes were progressive from 12 to 20 weeks, with further improvements in LVESV, LVEDV, and NT-proBNP between the 12- and 20-week time points. Patients in the OM pharmacokinetic-guided titration group had a significant improvement in the total symptom score of the Kansas City Cardiomyopathy Questionnaire at 20 weeks with trends in improvements in both frequency and burden of symptoms (Teerlink et al. 2016c). Adverse events were comparable to placebo. Although underpowered to detect a statistically significant difference in outcomes, 2.7% of placebo-treated patients versus 1.4% of those receiving OM died during the study. Troponin levels were closely monitored, and there were no independently adjudicated events deemed to be myocardial ischemia or infarction. However, treatment with OM for 20 weeks caused asymptomatic increases in plasma troponin of 0.001 ng/mL with the 25 mg dose, and 0.006 ng/mL in the pharmacokinetic dose titration group. The clinical significance of these increased troponin levels in contrast to the improvements in parameters of cardiac function seen with chronic OM therapy remains unclear.

Throughout these clinical studies, OM has had tolerability and adverse events similar to placebo and no off-target adverse effects. In contrast to currently available inotropes and consistent with its mechanism of action, OM has demonstrated no increase in atrial or ventricular arrhythmias, myocardial ischemia, or hypotension. However, two issues have emerged: the small increase in troponins and the potential to decrease diastolic-filling times. The increased troponin appears to be a small amount and of the magnitude that is evident after vigorous endurance exercise (Shave et al. 2010) and during diurnal variation (Klinkenberg et al. 2014). There is no correlation of the troponin increases with peak OM plasma concentrations or change in SET, and no apparent clinical events. In addition, rather than increasing, as would be consistent with myocardial damage, OM patients had progressively decreasing NT-proBNP, as well as heart rate, and favorable ventricular remodeling, making it unlikely that this troponin is due to myocardial damage. While it is possible that other, noninjury, mechanisms (Waldenstrom and Ronquist 2014) may be responsible, they remain undefined. Another issue is the potential of the increased SET to impinge upon diastolic filling resulting in myocardial ischemia. Although it is no longer widely known, prior research has shown that patients with HFrEF have decreased SET by 10–70 ms, correlating with the degree of reduced systolic function (Weissler et al. 1968), such that OM could be viewed as increasing SET towards normal. In the COSMIC-HF study, the pharmacokinetic-guided titration group had a mean increase of 25 ms with 95% confidence intervals of 18–32 ms in SET. For the baseline heart rate of 70 bpm (860 ms cycle length) with an SET of 300 ms, diastole was approximately 560 ms. At 20 weeks, the mean heart rate was approximately 67 bpm (895 ms cycle length) and the SET was on average 325 ms, resulting in a diastolic duration of 570 ms. The exercise treadmill study in patients with HFrEF and angina tested whether these increases in SET would be clinically relevant at higher heart rates and did not find

any evidence of increased ischemia. Both of these issues will need to be addressed by a large outcomes trial to fully assess the benefit-to-risk of OM.

The clinical efficacy of OM can only be established in a large phase III outcomes trial. However, the improved dyspnea in the high dose group of ATOMIC-AHF and the improved total symptom score in COSMIC-HF suggest that OM may provide some symptom benefit. The favorable effects on NT-proBNP and ventricular remodeling in COSMIC-HF are also supportive of a possible role in reducing long-term mortality in patients with chronic HFrEF (Kramer et al. 2010; Cleland et al. 2009).

5.3 Phase III Clinical Trials

There have been no completed phase III clinical trials with OM to date. However, the Global Approach to Lowering Adverse Cardiac outcomes Through Improving Contractility in Heart Failure (GALACTIC-HF; NCT02929329) trial is currently enrolling patients. GALACTIC-HF will enroll approximately 8,000 male or female patients, aged 18–85 years with symptomatic (NYHA II–IV) chronic HF on standard of care therapy, LVEF $\leq$35%, current or within 1 year HF-related urgent care visit, and elevated natriuretic peptides. Patients will be randomized 1:1 to placebo or to a titrated dose of omecamtiv mecarbil twice daily. The primary endpoint of the trial is the time to cardiovascular death or first heart failure event, and multiple other secondary endpoints will be evaluated.

6 Summary

The novel direct cardiac myosin activator omecamtiv mecarbil (OM) has been formulated with predictable pharmacokinetics and pharmacodynamics as both an intravenous and oral agent. The OM development program has provided the essential data to determine dosing regimens for treatment of both acute and chronic heart failure. Pharmacologic efficacy has been demonstrated in multiple domains, including a consistent and predictable pharmacodynamic effect (increases in SET), augmented systolic function (increased stroke volume, cardiac output, and ejection fraction; reduced LVESV), reduced LV wall stress (decreased NT-pro-BNP), attenuation of neurohormonal activation (lower heart rate and NT-proBNP), beneficial LV remodeling (reduced LV end-diastolic and end-systolic dimensions and volumes), and symptom improvement at clinically relevant doses (improved dyspnea and KCCQ symptom scores). However, small asymptomatic increases in troponin have been found with acute and chronic OM dosing, and the significance of this finding is currently unknown. In the phase II clinical studies, OM has tolerability and adverse events similar to placebo with no evidence of increased heart rate, supraventricular or ventricular arrhythmias, or hypotension. It has shown no adverse effect on renal function or serum potassium, suggesting that it will have no interference with initiation or titration of current therapies. Given the sum total of the beneficial associations

demonstrated with OM, the final determination of the utility of this novel therapeutic for treatment of chronic HFrEF will await the results of the GALACTIC-HF trial.

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Mitochondrial Therapies in Heart Failure

Albrecht von Hardenberg and Christoph Maack

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Abstract

The current therapy for patients with stable systolic heart failure is largely limited to treatments that interfere with neurohormonal activation. Critical pathophysiological hallmarks of heart failure are an energetic deficit and oxidative stress, and both may be the result of mitochondrial dysfunction. This dysfunction is not (only) the result of defect within mitochondria per se, but is in particular traced to defects in intermediary metabolism and of the regulatory interplay between excitation-contraction coupling and mitochondrial energetics, where defects of cytosolic calcium and sodium handling in failing hearts may play important roles. In the past years, several therapies targeting mitochondria have emerged with promising results in preclinical models. Here, we discuss the mechanisms and results of these mitochondria-targeted therapies, but also of

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_123

interventions that were not primarily thought to target mitochondria but may have important impact on mitochondrial biology as well, such as iron and exercise. Future research should be directed at further delineating the details of mitochondrial dysfunction in patients with heart failure to further optimize these treatments.

Keywords
Calcium • Energetics • Heart failure • Mitochondria • Reactive oxygen species

1 Introduction

The current evidence-based (pharmacological) therapy for heart failure acts by modulation of neurohormonal systems, such as the renin–angiotensin–aldosterone and the sympathetic nervous systems (Ponikowski et al. 2016). Since these therapies are limited by hemodynamic side effects like not only hypotension and bradycardia, but also electrolyte disturbances or renal dysfunction, there is a need for novel drugs without these disadvantages (Gheorghiade et al. 2016). Mitochondrial therapies are one promising option for such hemodynamically neutral drugs. However, the development of such drugs is challenging, and the most prominent impediments are the lack of specific mitochondrial targets and the difficulties of delivering the respective agent to the mitochondrial compartment (Szeto and Schiller 2011).

Similar as diabetes and neurodegenerative diseases, heart failure is a secondary form of mitochondrial dysfunction. In contrast to primary mitochondrial diseases, secondary dysfunction is acquired and not caused by a primary genetic defect of the mitochondrial synthesis machinery or respiratory chain complexes (Smith et al. 2012). Mitochondria are ubiquitous in mammalian cells, but targeted mitochondrial therapies preferentially act on cells with high mitochondrial content such as cardiac or skeletal myocytes, which comprise ~30–40% of mitochondria. Nevertheless, due to mitochondria's ubiquity, mitochondrial therapies are feasible in many different indications. In general, mitochondrial therapies aim to improve disease burden rather than to achieve complete recovery, since they have an impact on common damaging disease pathways (Smith et al. 2012). As pointed out by Wallace et al. (2010) and McKnight (2010), however, our limited understanding of bioenergetics underlies the so-far disappointing progress in the development of treatments targeting metabolism and mitochondria. Therefore, we should place mitochondrial diseases and their therapeutics into a broader context of organismal and cellular bioenergetics (McKnight 2010; Wallace et al. 2010). Accordingly, a better understanding of intermediate metabolism and redox biology in the cardiovascular system and in particular, of mitochondrial bioenergetics may avoid previous failures such as vitamins, whose lack of benefit in cardiovascular diseases may be related to non-specific targeting and potentially paradoxical effects on redox biology at higher doses (Münzel et al. 2015).

In the following, we will provide an overview of the development and progression of mitochondrial dysfunction in heart failure and introduce different mitochondrial therapy strategies.

2 Mitochondrial Biology and Regulation

The heart consumes about 6 kg of ATP per day for pumping 10 tons of blood through the vascular circulation of the body (Marín-García 2012). Excitation-contraction coupling is the main consumer of ATP in the heart: about 2% of the cellular ATP is consumed per heart beat (Balaban 2002). Such efficient energy utilization was facilitated by the endosymbiosis of an alphaproteobacterium to an eukaryotic progenitor cell 4 billion years ago. The proteobacterium survived until today in form of mitochondria and enables the highly effective way to use oxygen (O_2) to produce ATP from food molecules, increasing the efficiency of glycolysis from 2 to 30 molecules of ATP (Lane and Martin 2010). However, the cost of this increase in efficacy by aerobic bioenergetics is the generation of reactive oxygen species (ROS), against which a whole battery of anti-oxidative enzymes are installed to prevent oxidative stress. If under pathological conditions, production of ROS overwhelms the anti-oxidative capacity, oxidative stress occurs that may contribute to the development and progression of heart failure (Dai et al. 2011b; Nickel et al. 2015).

Mitochondria have an outer (OMM) and an inner membrane (IMM) (Zick et al. 2009). Invaginations of the IMM form the cristae where the complexes of the respiratory chain assemble to respiratory "supercomplexes" or "respirasomes" (Cogliati et al. 2016; Gu et al. 2016). The cristae formation increases membrane surface and thereby enhances the capacity of oxidative phosphorylation (OXPHOS). Therefore, dense cristae formation is typical for mitochondria in highly energy-demanding tissues. Central to the cristae and respirasome formation is cardiolipin (CL), a phospholipid that is uniquely expressed in mitochondria, and in particular, on the IMM (Paradies et al. 2010).

OXPHOS regenerates ATP at the electron transport chain (ETC) (Mitchell and Moyle 1967). NADH and $FADH_2$, the main products of the Krebs Cycle, deliver electrons to the ETC, inducing sequential redox reactions which induce proton translocation across the IMM, establishing a proton gradient (ΔpH) which together with the electrical gradient ($\Delta\psi_m$) constitutes the proton motive force ($\Delta\mu_H$) (Fig. 1). This $\Delta\mu_H$ is the driving force for ATP production at the F_1F_o-ATP synthase (Balaban 2009). The Krebs cycle is fueled by energetic intermediates primarily from fatty acids as substrates (70%), and to a lesser extent glucose (30%), lactate and amino acids. In principle, however, the metabolic pathways of these different fuels are interwoven to a net of redundancy, variability, and effectiveness (Taegtmeyer 2007).

Cardiac workload and thus ATP consumption changes constantly and requires rapid and efficient adaptation of energy supply to demand. Two major regulators of oxidative phosphorylation are Ca^{2+} and ADP (Maack et al. 2007; Cortassa et al.

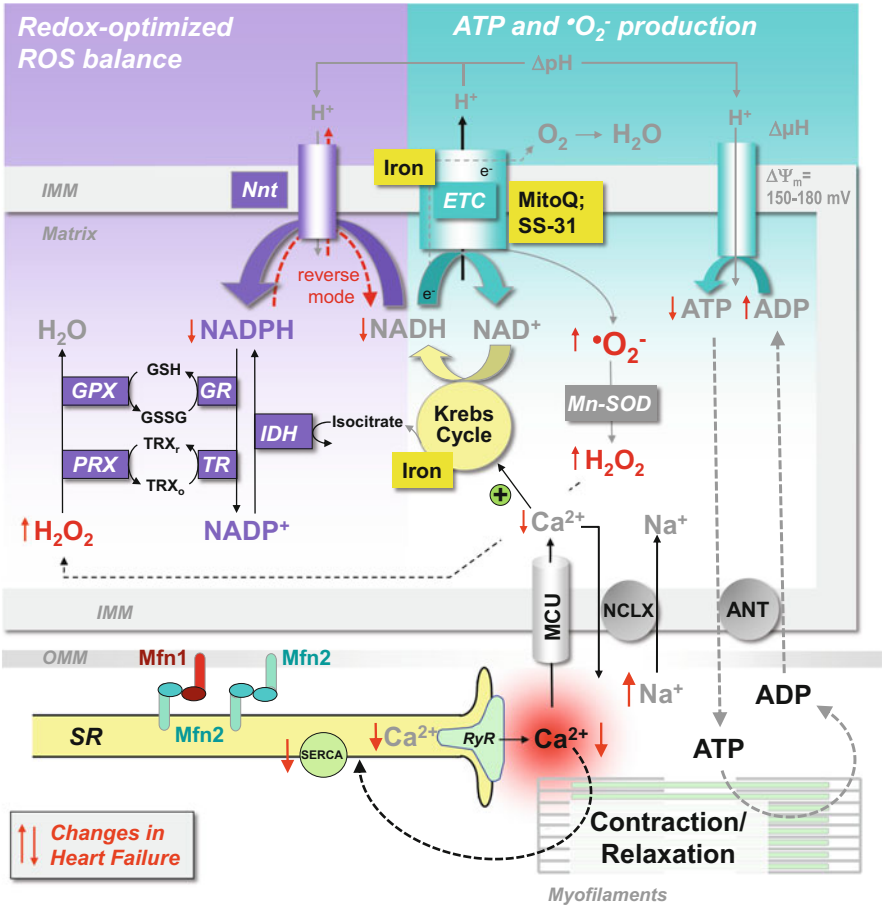

Fig. 1 Mechanisms of mitochondrial energetics and their regulation through ADP and Ca^{2+}. The points of intervention of mitochondria-targeted therapies (MitoQ, SS-31, iron) are highlighted in *yellow*. *Nnt* nicotinamide nucleotide transhydrogenase, *Mn-SOD* Mn^{2+}-dependent superoxide dismutase, *PRX* peroxiredoxin, *GPX* glutathione peroxidase, $TRX_{r/o}$ reduced/oxidized thioredoxin, *GSH/GSSG* reduced/oxidized glutathione, *TR* thioredoxin reductase, *GR* glutathione reductase, *MCU* mitochondrial Ca^{2+} uniporter, *NCLX* mitochondrial Na^+/Ca^{2+} (and Li^+) exchanger, *ANT* adenine nucleotide translocator, *RyR* ryanodine receptor, *SR* sarcoplasmic reticulum, *SERCA*, SR Ca^{2+} ATPase, *Mfn* mitofusin, *IMM* inner mitochondrial membrane, *OMM* outer mitochondrial membrane, $\Delta\mu_H$ proton motive force

2009). When ATP consumption increases, such as during β-adrenergic stimulation, ADP stimulates ATP production at the F_1F_o-ATP synthase, which slightly dissipates $\Delta\mu_H$ and accelerates electron flux along the ETC (Brand and Murphy 1987). This increased electron flux oxidizes NADH and $FADH_2$ (Fig. 1). At the same time, β-adrenergic stimulation increases the amplitude and frequency of cytosolic Ca^{2+} transients, facilitating the accumulation of Ca^{2+} in the mitochondrial matrix, where

Ca^{2+} stimulates Pyruvate dehydrogenase (PDH) and rate-limiting dehydrogenases of the Krebs cycle (isocitrate- and α-ketoglutarate dehydrogenases) to increase the rate of NADH and $FADH_2$ regeneration (Fig. 1). Thus, the balance of ADP-induced acceleration of respiration and Ca^{2+}-induced stimulation of the Krebs cycle maintains a constant redox state of NADH/NAD^+ and $FADH_2$/FAD and thus, a sufficient electron reserve to generate ATP (Nickel et al. 2013).

Already under physiological conditions, superoxide (O_2^-) is produced at complexes I and III of the ETC, which is rapidly dismutated to H_2O_2 by the Mn^{2+}-dependent superoxide dismutase (Mn-SOD) (Fig. 1). H_2O_2 is then eliminated by several enzymes (such as glutathione peroxidase and the thioredoxin/peroxiredoxin system) that require NADPH, which in turn is regenerated by three enzymes that derive their substrates from the Krebs cycle, such as isocitrate dehydrogenase, malic enzyme and the nicotinamide nucleotide transhydrogenase (Nnt) (Ying 2008) (Fig. 1). Therefore, mitochondrial Ca^{2+} uptake is not only required to adapt energy supply to demand, but also to regenerate the NADPH-coupled anti-oxidative capacity to prevent excessive emission of H_2O_2 from mitochondria (Kohlhaas et al. 2010).

3 Pathophysiology of Heart Failure: Focus on Mitochondria

In patients with heart failure, an energetic deficit can be detected in vivo, leading to the well-known concept of the heart as an "engine out of fuel" (Neubauer 2007). A reduction of the myocardial phosphocreatine (PCr) to ATP ratio, measured non-invasively by ^{31}P-MR spectroscopy, is an indicator of energy shortage predicting an adverse outcome of heart failure patients (Neubauer et al. 1997). Starling et al. (1998) observed an inverse correlation between ATP content and pulmonary wedge pressure. The decline of myocardial ATP was primarily associated with diastolic rather than systolic dysfunction (Starling et al. 1998). Increased energetic demand and/or energetic mismatch (Gorski et al. 2015) are supported by different experimental heart failure models, such as hypertension (Eirin et al. 2014a), pacing (Marín-García et al. 2001), and transaortic constriction (Patten and Hall-Porter 2009).

Two important aspects of the energy starvation concept, however, are presently incompletely resolved. First, the underlying reasons for the energetic deficit are unclear. Several studies ranging from 50 years ago at the National Institutes of Health (NIH) (Chidsey et al. 1966; Sobel et al. 1967) to more recent studies (Cordero-Reyes et al. 2014; Holzem et al. 2016) have suggested that the electron transport chain function per se is not impaired in failing versus nonfailing hearts, although conflicting data exist (Sharov et al. 2000). Instead, substrate metabolism, i.e., the capacities of glycolysis and fatty acid oxidation to provide acetyl-coenzyme A to the Krebs cycle, and also Krebs cycle activity per se – responsible for the production of NADH and $FADH_2$ from acetyl-coenzyme A, appear to be impaired in failing hearts (Nickel et al. 2013; Cordero-Reyes et al. 2014). Second, it is unclear as to how far the energetic deficit – mostly monitored by decreased PCr levels – actually contributes to the contractile deficit per se and the progression of

heart failure (through induction of maladaptive remodeling), or whether the energetic deficit may rather impair only cardiac function under maximal exertion, as discussed in more detail previously (Nickel et al. 2013). In fact, in mice that are completely deficient of PCr, no impairment of cardiac function at baseline or after myocardial infarction could be observed, while maximal cardiac output in response to β-adrenergic stimulation or the recovery of cardiac function from ischemia was slightly reduced (Lygate et al. 2013). These data argue against a maladaptive role of a deficit of the final currency of energy, i.e., ATP, for cardiac dysfunction. In contrast, the mentioned defects of substrate metabolism may rather result in the accumulation of metabolic intermediates that can become toxic and/or induce maladaptive signaling in their own right (Chatham and Young 2012; Nickel et al. 2013).

One important consequence of the changes in intermediary metabolism is oxidative stress. In patients with heart failure, oxidative stress occurs in the plasma and LV myocardium and correlates with LV dysfunction (Belch et al. 1991; Maack et al. 2003). Increased levels of ROS can deteriorate Ca^{2+} handling (Xu et al. 1997; Zweier and Talukder 2006; Maack et al. 2009), cause arrhythmias (Akar et al. 2005; Wagner et al. 2013), and induce hypertrophic signaling (Ago et al. 2008; Erickson et al. 2008), apoptosis, and necrosis (Halestrap 2005; Biasutto et al. 2016). Ca^{2+} calmodulin kinase II (CaMKII) activation through a ROS-dependent pathway led to increased Ca^{2+} leak of the sarcoplasmic reticulum (Viatchenko-Karpinski et al. 2014). But oxidative stress in cardiac myocytes can be either adaptive (Zhang et al. 2010) or maladaptive (Dai et al. 2011b), depending on its source, timing, and quantity.

Besides NADPH oxidases and other enzymes, one major source of ROS is mitochondria. In a dog model of heart failure, excessive production of O_2^- at complex I is transformed to H_2O_2 and (via the Fenton reaction) hydroxyl radicals (Ide et al. 2000). Besides an increase in ROS *production*, decreased ROS *elimination* is another key contributor to mitochondrial oxidative stress (Nickel et al. 2014). In the failing heart, defects in Ca^{2+} homeostasis result in smaller amplitudes and slower velocities of cytosolic Ca^{2+} transients (Bers 2006), which deteriorate mitochondrial Ca^{2+} uptake to stimulate key enzymes of the Krebs cycle (Kohlhaas and Maack 2013). Furthermore, increased cytosolic Na^+ concentrations, as observed in heart failure, accelerate mitochondrial Ca^{2+} export via the mitochondrial Na^+/Ca^{2+} exchanger (Maack et al. 2006; Liu and O'Rourke 2008) (Fig. 1). Under physiological conditions, microdomains between the sarcoplasmic reticulum (SR) and mitochondria mediate efficient mitochondrial Ca^{2+} uptake (Kohlhaas and Maack 2013). During systole, very high Ca^{2+} concentrations in the immediate vicinity of the ryanodine receptors (RyRs) of the SR in close juxtaposition to mitochondria and the mitochondrial Ca^{2+} uniporter (MCU) allow uptake of Ca^{2+} into mitochondria despite the relatively low affinity of the MCU for Ca^{2+} (Kohlhaas and Maack 2013). These microdomains are controlled by tethering proteins between the SR and mitochondria, among which mitofusin (Mfn) 1, located on the OMM, and Mfn2, located on both the OMM and the SR, play important roles (de Brito and Scorrano 2008; Chen et al. 2012). In animal models of heart failure, decreased

expression of Mfn1 and Mfn2 may deteriorate the well-organized spatial pattern of mitochondria within cardiac myocytes, but potentially also the SR-mitochondrial Ca^{2+} microdomain (Goh et al. 2016; Maack 2016). Finally, the open probability of the MCU is reduced in mitochondria from human failing hearts (Michels et al. 2009).

Together, these data indicate that in heart failure, decreased mitochondrial Ca^{2+} uptake during cardiac workload transitions impairs the Ca^{2+}-induced stimulation of the Krebs cycle and thereby, the regeneration of NADH and NADPH, required for energy production and the anti-oxidative capacity (Nickel et al. 2014). One important consequence of this energy supply-and-demand mismatch is oxidation of NADH, which favors the reverse mode of the mitochondrial nicotinamide nucleotide transhydrogenase (Nnt) during elevated cardiac workload, which oxidizes NADPH and therefore dissipates the anti-oxidative capacity (Nickel et al. 2015). The depleted NADPH-coupled anti-oxidative capacity is then overwhelmed by ROS production by NADH-coupled respiration at the ETC. This imbalance appears to be a core mechanism of oxidative stress during pressure-overload induced heart failure, since in animals that lack a functional Nnt, no oxidative stress and less systolic dysfunction or premature death occurred (Nickel et al. 2015).

According to the concept of "redox-optimized ROS balance" (R-ORB), Aon et al. (2010) proposed that "mitochondria have been evolutionarily optimized to maximize energy output while keeping ROS overflow to a minimum by operating in an intermediate redox state" (Aon et al. 2010). This implies that the optimal condition for cardiac mitochondria is when extreme oxidation, as outlined above, or reduction of the mitochondrial redox state (such as during ischemia) is avoided. The R-ORB concept proposes that under highly reduced conditions, high ROS production at the ETC overwhelms the anti-oxidative capacity. However, considering that the working heart constantly produces ADP, which physiologically accelerates respiration and thereby oxidizes the respiratory chain, increased oxidative stress in heart failure is unlikely due to a pure net increase of ROS production, but rather due to diminished ROS scavenging capacity (Nickel et al. 2015).

Oxidative stress leads to a vicious circle by exacerbating the energy supply and demand mismatch (Kohlhaas and Maack 2011). Mitochondria are in the center of the scene, since they contain typical targets of oxidative damage like iron sulfur clusters, unsaturated fatty acids, and densely packed proteins and mitochondrial DNA (mtDNA) that are all essential to mitochondrial function (Murphy 2009). The proximity of ROS production to the components of the ETC including cardiolipin makes them most vulnerable to oxidative damage (Lesnefsky and Hoppel 2008). Therefore, oxidative stress directly affects enzyme function of ETC complexes and leads to peroxidation of cardiolipin due to the high content of unsaturated fatty acids (Paradies et al. 2010). Peroxidation of cardiolipin impairs cristae formation and disrupts the respirasome and the detachment of cytochrome *c*, a mobile electron carrier in the IMM (Szeto 2014). The net result of these changes is a reduced ATP synthesis and a further increase in electron slippage to oxygen, therefore setting up a feed-forward cycle of ROS-induced ROS production (Zorov et al. 2006). Furthermore, mtDNA is associated with the IMM and vulnerable to oxidative damage by

missing protective histones. Damage of mtDNA further leads to reduced ETC activity and exacerbating the feed-forward cycle of ROS production (Ide et al. 2001; Hebert et al. 2010).

4 Strategies for Mitochondrial Therapies

Initial attempts to improve the outcome of patients with cardiovascular risk and/or disease were performed with the supplementation of vitamins C and E, however, these attempts were not successful (Yusuf et al. 2000). One reason for this failure is that these anti-oxidants do not achieve sufficiently high concentrations within mitochondria (Münzel et al. 2015; Murphy 2016). Furthermore, depending on its concentration, duration, and sources, ROS can also serve physiological signaling roles (Jones and Sies 2015). In this context, the concept of "mitohormesis" promotes ROS-induced health benefits depending on exposure time and concentration (Ristow 2014). For instance, the application of vitamins C and E in healthy young men prevented the health benefits of exercise, in particular on insulin signaling (Ristow et al. 2009). Furthermore, antioxidants prevent myocardial protection provided by "preconditioning" episodes of brief ischemia/reperfusion (Baines et al. 1997; Kaeffer et al. 1997; Vanden Hoek et al. 1998). To some extent, these data conflict with the classical free radical theory of aging (Liochev 2013).

Therefore, other strategies were employed to target anti-oxidants more specifically to those compartments where ROS are thought to produce most damage, i.e., to mitochondria. The principles of such mitochondrial therapies have been reviewed in more detail previously (Smith et al. 2012). Here, we will briefly review the concepts and (if available) clinical results of different mitochondrial therapies – and therapies that affect mitochondria – in the context of heart failure. One approach is to target drugs specifically to mitochondria by coupling a pharmacon to the cation triphenylphosphonium (TPP^+), whose lipophilicity allows the passage across the cell and mitochondrial membranes, and its negative charge facilitates the Nernst's distribution law to accumulate in the negatively charged mitochondrial matrix. The most prominent example of this strategy is MitoQ, where a ubiquinone derivative is coupled to TPP^+ (Kelso et al. 2001).

Ubiquinone is synonymous to coenzyme Q, which is a physiological component of the ETC and functions as an electron carrier. In patients with heart failure, decreased levels of coenzyme Q correlated with the severity of the disease and could be slightly increased by conventional oral coenzyme Q supplementation (Folkers et al. 1985). In fact, a recent clinical trial observed that oral coenzyme Q supplementation was associated with improved morbidity and mortality in patients with heart failure (Mortensen et al. 2014). As an electron acceptor, coenzyme Q can also function as an anti-oxidant, and since it is unclear to what extent non-conjugated coenzyme Q is really taken up by mitochondria, the rationale of the development of MitoQ was therefore the coupling of coenzyme Q (ubiquinone) to TPP^+, forming MitoQ (Smith et al. 2012).

Initially considered a similar class of drugs as MitoQ (Smith et al. 2012), the Szeto-Schiller peptides comprise of alternating aromatic and basic amino acid residues, where the aromatic residues were thought to allow the passage across membranes, and the positive charge attracting the molecule to the mitochondrial matrix and finally, the dimethyltyrosine residue providing anti-oxidative properties (Zhao et al. 2004). However, more recent data suggest that SS-31, the most promising candidate of this family, does not have direct anti-oxidative effects (Brown et al. 2014), but binds to cardiolipin, an essential phospholipid of the IMM (Szeto 2014). This interaction with SS-31 protects cardiolipin from oxidation and dysfunction, preventing disassembly of the ETC supercomplexes and thereby, energetic deficit and mitochondrial ROS production (Szeto et al. 2001; Szeto 2014).

Another important catalytic factor in mitochondria is iron, which participates in numerous iron-sulfur (Fe/S) clusters of the ETC and the Krebs cycle. In patients with heart failure, iron deficiency predicts adverse outcome (Jankowska et al. 2010), and in human failing myocardium, decreased iron levels are associated with decreased respiratory capacity (Melenovsky et al. 2016). Intravenous iron application improves functional status and exercise capacity in heart failure patients (Jankowska et al. 2016), and it may be assumed that this is primarily the result of improved mitochondrial function, although this is not entirely proven and also it is unclear whether this is an effect on cardiac and/or skeletal muscles (Stugiewicz et al. 2016).

Finally, physical exercise improves symptoms and quality of life in patients with heart failure, and this effect may be (at least to some extent) related to an improvement of mitochondrial biogenesis. We discuss these aspects in more detail in the following passages.

5 Coenzyme Q_{10} Supplementation: Myth or Reality?

Coenzyme Q_{10}, also known as ubiquinone, coenzyme Q and ubiquinol (reduced form), is a crucial component of the electron transport chain by transporting electrons from complex I, II and the electron transfer flavoproteins to complex IV (Schwarz et al. 2014). Therefore, Q_{10} undergoes cyclic oxidation-reduction. The molecular structure is related to vitamin k, where "Q" connotates the quinone-, and "10" the 10-isoprene group as the molecular structure found in humans. Ubiquinones are ubiquitous in most mammalian cells, and particularly in organs with the highest energy demand, such as the heart (Crane 2007). The cyclic oxidation-reduction rate is slower than the rate of cytochrome c, but this is compensated by 10 times higher coenzyme Q_{10} concentrations than that of other carriers (Klingenberg 2010). Accordingly, depleting coenzyme Q_{10} concentrations in heart mitochondria can slow mitochondrial respiration, while replenishing Q_{10} accelerates respiration (Redfearn and Burgos 1966). Preclinical data suggest that Q_{10} has a critical role in ATP production, is a potent anti-inflammatory agent, and may improve endothelial function (Sharma et al. 2016). In fact, besides mitochondria, coenzyme Q_{10} is also found in other cellular membranes where it controls

the function of endothelial nitric oxide synthase (eNOS), whose uncoupling upon coenzyme Q_{10} depletion can make it an additional source for ROS and thereby, shifts the nitroso-redox balance towards oxidation (Mugoni et al. 2013).

Under physiological conditions, ~50% of coenzyme Q_{10} is ingested, while the other half is synthesized endogenously through the mevalonate pathway, which is blocked by statins (Bentinger et al. 2007; Crane 2007). Therefore, treatment of patients with cardiovascular risk and/or disease with statins is associated with decreased coenzyme Q_{10} levels (Banach et al. 2015). In patients with heart failure, decreased levels of coenzyme Q_{10} correlated with the severity of disease (Mortensen et al. 1990; Mortensen 2015). As a mechanism of decrease in coenzyme Q_{10} concentrations, lipid oxidation was suggested as a consequence of oxidative stress (Forsmark-Andrée et al. 1997). Coenzyme Q_{10} was initially described as an independent predictor of mortality in 236 patients with heart failure (Molyneux et al. 2008). A pre-specified analysis of the much larger CORONA study, however, could not confirm this (McMurray et al. 2010). In CORONA, rosuvastatin did not reduce the primary endpoint of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke in patients with ischemic cardiomyopathy (Kjekshus et al. 2007). In these patients, rosuvastatin reduced coenzyme Q_{10}, but even in patients with a low baseline coenzyme Q_{10}, rosuvastatin treatment was not associated with a worse outcome (McMurray et al. 2010). Accordingly, reduced coenzyme Q_{10} was not an independent prognostic variable in heart failure (McMurray et al. 2010). Together, these data call into question a causal role of the coenzyme Q_{10} deficit in patients with heart failure, with or without statin treatment.

As an established mitochondrial therapy, coenzyme Q_{10} cures a defect in Q_{10} biosynthesis by a permanent dietary Q_{10} supplementation. This is an exception to the mostly (therapeutically) orphaned group of primary mitochondrial diseases (Rötig et al. 2000; Montini et al. 2008). In cardiovascular diseases, however, coenzyme Q_{10} supplementation has been pursued without firm evidence of benefit for several decades. While a meta-analysis of several rather small trials indicated that coenzyme Q_{10} may improve LV ejection fraction, additional well-designed studies were required to assess the effect of coenzyme Q on the outcome of these patients (Fotino et al. 2013). In the recent Q-SYMBIO trial, coenzyme Q_{10} was tested against placebo in 420 patients with systolic heart failure, and indeed, coenzyme Q_{10} improved symptoms and substantially reduced major adverse cardiovascular events (Mortensen et al. 2014). However, since the study was underpowered to prove a benefit in this population, the issue whether coenzyme Q_{10} supplementation really improves outcome and symptoms in heart failure is not fully settled (Ezekowitz 2014), and coenzyme Q10 is not even mentioned in the most recent guidelines on the treatment of heart failure (Ponikowski et al. 2016).

6 MitoQ

Mito Q is a mitochondrial targeted antioxidant, where a ubiquinone moiety is linked by a 10-carbon alkyl chain to the cation triphenylphosphonium (TPP^+), which takes advantage of the electrochemical gradient (150–180 mV) across the IMM. Therefore, compounds coupled to TTP^+ are several hundred-fold higher concentrated in mitochondria (Murphy 2016). The ubiquinol moiety of MitoQ is an antioxidant running through a redox cycle via oxidation by ROS to an ubiquinone and reduction by complex II back to ubiquinol (James et al. 2007).

MitoQ can be administered orally, which is safe in rodents (Rodriguez-Cuenca et al. 2010). Preclinical studies show protection against oxidative damage. Feeding MitoQ to rats reduced cell death and mitochondrial damage and thereby improved cardiac function after ischemia-reperfusion of Langendorff-perfused hearts (Adlam et al. 2005). In a spontaneously hypertensive rat model of heart failure, administration of the mitochondria-targeted antioxidant MitoQ protects against the development of hypertension, improves endothelial function, and reduces cardiac hypertrophy (Graham et al. 2009). The favorable outcome may also be attributed to the reduction in blood pressure and improvement in endothelial function observed in the MitoQ group (Bayeva et al. 2013). Moreover, MitoQ protected against anthracycline- (Chandran et al. 2009), endotoxin- (Supinski et al. 2009), and cocaine-induced cardiotoxicities (Vergeade et al. 2010).

These positive preclinical data led to the assessment of MitoQ in a human phase II trial in Parkinson's disease (PD), the PROTECT trial (NCT00329056) which showed safety, but no difference between MitoQ and placebo on any measure of PD progression (Carlisle et al. 2015). A second small trial with MitoQ was performed on patients with chronic hepatitis C virus (the CLEAR trial; NCT00433108), in which MitoQ significantly decreased liver damage in patients with chronic HCV infection (Gane et al. 2010). Despite these favorable clinical data that also confirmed safety of MitoQ, the drug has not been tested yet in clinical trials on patients with cardiovascular diseases.

7 Szeto-Schiller Peptides (Lead Compound SS-31, Also Known as Elamipretide or Bendavia)

The discovery of the Szeto-Schiller (SS) peptides was serendipity while Hazel Szeto and Peter Schiller were working on a family of dermorphins. SS peptides are a new class of compounds that selectively accumulate 1,000- to 5,000-fold in the inner mitochondrial membrane (IMM) compared to the cytosolic compartment. The mitochondrial uptake does not depend on the mitochondrial membrane potential ($\Delta\Psi_m$), which may be of advantage in conditions in which $\Delta\Psi_m$ is (partly) dissipated, such as ischemia or heart failure.

The central mechanism of action of SS-31 is binding selectively to cardiolipin via electrostatic and hydrophobic interactions. SS-31 protects cardiolipin from damage by oxidative stress (Zhao et al. 2004; Szeto 2014) and thereby maintains

proper function of the respiratory chain, avoiding aberrant slippage of electrons to O_2 to produce superoxide anion radical (O_2^-). Furthermore, SS-31 prevents cardiolipin from converting cytochrome c into a peroxidase while protecting its electron carrying function (Szeto and Schiller 2011; Szeto 2014). Usually, peptides are unsuitable drugs mainly due to rapid degradation through peptidases and their inability to cross cellular membranes. However, SS peptides are water soluble due to their cationic character. After administration, SS-31 is rapidly distributed to highly perfused organs, including the heart, kidney, lung, and brain (Szeto 2008). The enzymatic degradation is low and stable even after 2 h of incubation in whole blood (Szeto et al. 2001).

In mice, a 4-week infusion of angiotensin II induces mitochondrial oxidative stress, myocardial hypertrophy, fibrosis and diastolic heart failure, and SS-31, but not the non-specific antioxidant *N*-acetyl cysteine ameliorated these parameters (Dai et al. 2011a). In mice with trans-aortic constriction, the afterload-induced increase in oxidative stress is caused by the reversal of the mitochondrial transhydrogenase, provoking elevated mitochondrial ROS emission which then causes necrosis, LV dysfunction, and premature death (Nickel et al. 2015). In this model, application of SS-31 prevented the afterload-induced increase in necrosis after 3 days and premature death over 6 weeks (Nickel et al. 2015). In a porcine renovascular hypertension model, SS-31 improved diastolic function and oxygenation and reversed myocardial tissue damage after renal angioplasty and stenting (Eirin et al. 2014b). In a dog model of systolic heart failure, SS-31 not only improved systolic function in the long term (i.e., after 3 months of treatment), but also in the short-term: A 48-h treatment with SS-31 increased LVEF and stroke volume and decreased end-systolic volume, with no changes of heart rate or systemic vascular resistance (Sabbah et al. 2016).

The promising preclinical data and its pharmacokinetic profile warranted further clinical testing in patients with cardiovascular diseases. Since April 2016, the international nonproprietary name of SS-31 is Elamipretide (ELA-, no meaning; MI-, mitochondrial; PR-, protection; TIDE, stem of every peptide and glycopeptide; www.stealthbth.com). Elamipretide entered into clinical development with a for-profit commercial sponsor (Stealth Biotherapeutials Inc., Newton, MA, USA) in 2010. The first clinical phase II trial with Elamipretide to reduce the ischemia-reperfusion injury among subjects with first-time anterior STEMI who underwent successful PCI, intracoronary administration of Elamipretide was safe and well tolerated. However, in this single-dose study, the treatment with Elamipretide was not associated with a decrease in myocardial infarct size as assessed by AUC0 – 72 of CK-MB, but some hypothesis-generating positive signals on LV function were noted (Gibson et al. 2015).

This spurred the launch of a clinical programme comprising three phase II trials on patients with heart failure. The first trial evaluates the effects of 4 weeks' treatment with subcutaneous Elamipretide on LV function in subjects with stable heart failure with preserved ejection fraction (HFpEF) by comparing the delta in E/e' at rest and during submaximal exercise between the Elamipretide and placebo groups (NCT02814097). A second phase II trial evaluates the cardiac and renal effects

of short-term treatment with Elamipretide in patients hospitalized with congestion due to heart failure. The primary outcome measures the change in NT-proBNP between baseline and day 8/early discharge (NCT02914665). A third phase II trial examines the effect of multiple subcutaneous injections of Elamipretide on various measures of heart function in patients with chronic heart failure with a reduced ejection fraction (HFrEF). The primary outcome measures are the change from baseline in left ventricular end systolic volume (LV ESV) assessed by cardiac MRI (NCT02788747). The first results of these heart failure trails are expected for February 2017.

8 Iron Supplementation

Iron is not only required for oxygen transport via hemoglobin and oxygen storage by myoglobin, but is also essential in cellular bioenergetics. Iron is either embedded into a heme molecule or an iron-sulfur (Fe/S) cluster. The biogenesis of Fe/S clusters is a highly complex and coordinated process in living cells (Hentze et al. 2010). Their main purpose is electron transfer by switching between oxidative states as part of the complexes of the mitochondrial ETC (Lill 2009). Furthermore, Fe/S clusters play an important role for the function of various Krebs cycle enzymes, such as aconitase.

Both iron deficiency and iron overload can negatively affect human health (Abbaspour et al. 2014). Due to the importance of iron in tissues with high metabolic demand like the heart, the balance between iron deficiency and overload requires precise regulatory control. As a reactive metal, free iron catalyzes production of highly toxic hydroxyl radicals via the Fenton reaction (Eaton and Qian 2002). In the context of chronic iron overload during hemochromatosis, iron-catalyzed ROS can induce heart failure in addition to liver failure and type II diabetes (Gao et al. 2010; Dixon and Stockwell 2013). Also in patients with β-thalassemia, myocardial iron overload resulting from chronic and excess hemolysis can induce heart failure that is ameliorated by iron chelators (Tanner et al. 2007; Kremastinos et al. 2010). Deficiency of frataxin, a regulator of mitochondrial iron homeostasis, has similar effects in Friedreich's ataxia: Patients develop a mitochondrial iron overload combined with mitochondrial dysfunction and oxidative damage (Whitnall et al. 2008; Payne 2011). Furthermore, doxorubicin induces mitochondrial iron accumulation and thereby contributes to ROS-mediated cardiotoxicity (Ichikawa et al. 2014). Finally, during ischemia/reperfusion injury, upregulation of the mitochondrial iron exporter decreased mitochondrial iron content and protected against ischemia/reperfusion damage in mice (Chang et al. 2016).

On the other hand, in patients with chronic heart failure, serum iron deficiency (ID) is quite prevalent, affecting roughly one third of all heart failure patients, and is associated with decreased exercise capacity and adverse outcome independent of ID-associated anemia (Jankowska et al. 2010; von Haehling et al. 2015). These clinical observations and pathophysiological considerations fostered the development of efficient therapies to refill the depleted iron stores in patients with

heart failure. Ferric carboxymaltose is an intravenous drug in which a ferric hydroxide core is stabilized by a carbohydrate shell, allowing for controlled delivery of high amounts of iron to target tissues (Lyseng-Williamson and Keating 2009). In fact, intravenous iron supplementation over 24 weeks improved the 6-min walk distance, functional status and well-being and reduced hospitalizations of patients with systolic heart failure (Anker et al. 2009; Ponikowski et al. 2015; Jankowska et al. 2016). In contrast, no effects on total or cardiovascular mortality were noticed so far (Jankowska et al. 2016). Therefore, the application of intravenous iron has received a class IIa, A recommendation in the recent ESC Guidelines on the treatment of heart failure (Ponikowski et al. 2015).

It is presently unresolved, however, whether the beneficial effects of iron are mediated by improvement of cardiac or skeletal muscle function, or both (Stugiewicz et al. 2016). In fact, the impact of serum ID on mitochondrial iron content in failing hearts is presently unclear. In a rat model of experimental iron deficiency, the activities of various ETC complexes and mitochondrial respiration were reduced (Blayney et al. 1976). In patients with heart failure, the *total* myocardial iron content is reduced (Maeder et al. 2011; Leszek et al. 2012; Haddad et al. 2016; Melenovsky et al. 2016). However, myocardial iron content did not correlate with serum iron, serum transferrin saturation or the severity of the disease in a cohort of 33 patients with systolic heart failure and a mean of LVEF 22% (Leszek et al. 2012, 2015). In those failing hearts in which myocardial iron content was decreased, the activity of respiratory chain complexes was preserved, while the activity or protein expression of aconitase, citrate synthase, and anti-oxidative enzymes was reduced (Melenovsky et al. 2016). Importantly, in a study that differentiated between *cytosolic* and *mitochondrial* iron contents, mitochondrial iron was actually *increased*, while cytosolic iron was *reduced* (Khechaduri et al. 2013). In mitochondria, iron is integrated in heme molecules which in turn are integrated into the ETC complexes or Krebs cycle enzymes. The mitochondrial heme content was also increased in human failing hearts, and in cell systems, increased heme expression was associated with higher production of ROS (Khechaduri et al. 2013). Together, although most studies show that in human failing hearts, the *total* iron content is reduced, this decrease may not occur in mitochondria (Khechaduri et al. 2013) and appears unrelated to serum iron status (Leszek et al. 2012), severity of the disease (Leszek et al. 2015) or the activity of respiratory chain complexes (Melenovsky et al. 2016).

Cells take up iron bound to transferrin via transferrin receptor 1 (Trf1), which in turn is under the control of iron-regulatory proteins (Irp) 1 and 2 (Hentze et al. 2010). In human failing hearts, Irp activity and Trf1 expression are downregulated (Maeder et al. 2011; Haddad et al. 2016). In mice with cardiomyocyte-specific deletion of both Irp1 and Irp2, myocardial iron stores and Trf1 expression were reduced, and this decreased PCr/ATP ratios and decreased the maximal inotropic response to β-adrenergic stimulation in vivo (Haddad et al. 2016). These energetic defects were related to decreased complex I activities in the LV myocardium and decreased maximal respiration in isolated cardiac myocytes of Irp1/2-deficient mice. Furthermore, after myocardial infarction, the development of LV hypertrophy

and systolic dysfunction was aggravated in mice with cardiomyocyte-specific ID (Haddad et al. 2016). These defects could be restored by systemic application of ferric carboxymaltose, suggesting that in patients with heart failure, iron supplementation may improve cardiac function. However, before translating these experimental results to the human situation, one needs to consider that – as mentioned above – in human heart failure, iron content was not reduced in cardiac mitochondria (Khechaduri et al. 2013), and decreased total myocardial iron content was not associated with serum ID (Leszek et al. 2012) or decreased myocardial ETC activity (Melenovsky et al. 2016). Nevertheless, the study by Haddad et al. (2016) sheds some new light onto cardiac myocyte iron metabolism and supports the notion that therapeutic iron may improve cardiac function of patients with heart failure.

Together, while it is clear that serum ID predicts an adverse outcome and ferric carboxymaltose improves symptoms and quality of life in patients with heart failure, these effects are presumably related to improving skeletal muscle function (Stugiewicz et al. 2016) and potentially also of cardiac function (Haddad et al. 2016). Nevertheless, more research is needed to further elucidate the impact and regulation of iron in cardiac mitochondria.

9 Exercise

Physical exercise appears to be a systemic and genuinely mitochondrial therapeutic strategy. In fact, our growing understanding of the regulation of mitochondrial biogenesis and function also helps to understand how exercise may have positive impacts on health through improving mitochondrial function (Safdar et al. 2011; Picard et al. 2016). In fact, even small improvements in physical fitness are associated with a significantly lowered risk of death (Erikssen et al. 1998). One central molecular hub for several exercise-associated signaling pathways, and in particular, for mitochondrial biogenesis in skeletal and myocardial muscle is the peroxisome proliferator-activated receptor gamma co-activator (PGC-1α) (Handschin and Spiegelman 2008; Safdar et al. 2011). PGC-1α regulates the coordinated expression of key mitochondrial proteins of the respiratory chain and the Krebs cycle (Scarpulla 2008; Ventura-Clapier et al. 2008). Furthermore, the levels of PGC-1α expression correlate with OXPHOS capacity, and in patients with heart failure, myocardial PGC-1α expression is decreased (Garnier et al. 2003; Sebastiani et al. 2007). During physical exercise, ROS activate PGC-1α (and several other signaling pathways) which in turn increases the expression of anti-oxidative enzymes (St-Pierre et al. 2006). In fact, the application of non-selective antioxidants such as vitamins C and E can attenuate endurance training-induced and ROS-mediated enhancements in antioxidant capacity, mitochondrial biogenesis, cellular defence mechanisms, and insulin sensitivity (Ristow et al. 2009). This may further explain the observation that vitamin supplementation (as a *non-targeted* anti-oxidative intervention) did not have any positive impact on the cardiovascular outcome of patients at risk (Yusuf et al. 2000; Münzel et al. 2015).

In the HF Action trial on patients with systolic heart failure, exercise improved quality of life and self-reported health status (Flynn et al. 2009). After adjustment for highly prognostic predictors of the primary end point, exercise training was associated with modest significant reductions for both all-cause mortality or hospitalization and cardiovascular mortality or heart failure hospitalization (O'Connor et al. 2009). Also in patients with heart failure with preserved ejection fraction (HFpEF), exercise training improved exercise capacity (peak oxygen consumption), quality of life and diastolic function (Edelmann et al. 2011; Fukuta et al. 2014; Nolte et al. 2015). The comparison of high intensity interval training versus moderate intensity continuous training showed equal effect in improving quality of life and functional capacity in HF patients (Benda et al. 2015; Ulbrich et al. 2016). Therefore, the current ESC Guidelines recommend exercise training for the treatment of heart failure to improve quality of life and reduce hospitalizations (Ponikowski et al. 2016).

10 Conclusions

A precise understanding of pathophysiological mechanisms affecting mitochondrial function and metabolism in heart failure is key to design efficient drugs that may improve mitochondrial function. While iron therapy and exercise are already recommended in the current ESC Guidelines for the treatment of heart failure, novel drugs such as MitoQ and SS-31 are still under preclinical and clinical investigation and may be promising additions to our current armament of neurohormonal interventions.

Acknowledgments The research of C. Maack was and is supported by the Deutsche Forschungsgemeinschaft (DFG; Heisenberg Programm; SFB-894; Ma 2528/7-1), Deutsche Herzstiftung (Margret Elisabeth Strauß-Projektförderung) and Corona-Stiftung.

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Anticoagulation Therapy and NOACs in Heart Failure

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Abstract

Current evidence indicates that heart failure (HF) confers a hyper-coagulable state that is associated with adverse events including stroke, systemic embolism, and mortality. This may be due to the elevated levels of pro-thrombotic and pro-inflammatory cytokines that are seen in patients with acute and chronic HF. Left ventricular wall motion abnormalities in patients with systolic dysfunction predispose to local thrombosis due to blood stasis as does atrial fibrillation (AF) which leads to blood stasis in regions of the atria. The high risk of thromboemboli in HF patients with AF has resulted in the use anticoagulation therapy to prevent the occurrence of catastrophic events. There is evidence, however, that the pro-inflammatory, pro-thrombotic state that exists in HF puts patients who are in sinus rhythm at risk. The novel oral anticoagulants (NOACs)

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2016_126

have been shown in RCT to have at least equivalent efficacy in reducing stroke as warfarin while exposing patients to a lower risk of bleeding. The fact that the NOACs don't require routine monitoring to assure that patients remain within the therapeutic range and have relatively simple dosing requirements and a safer risk profile makes them attractive substitutes to warfarin in HF patients with atrial fibrillation and other conditions (e.g. deep venous thrombosis). Post hoc analyses from a subset of HF patients from the RCTs in AF patients have demonstrated similar findings as were reported in the entire populations that were included in the trials. As a result, NOACS are commonly used now in HF patients with AF. For HF patients with reduced ejection fraction in sinus rhythm, the use of warfarin in randomized clinical trials (RCT) to reduce stroke has been disappointing and associated with increase bleeding risk when compared to aspirin. The advantages of the NOACs over warfarin, however, raise the question of whether they might improve outcomes in HF patients who are in sinus rhythm. The currently ongoing COMMANDER-HF trial has been designed to address this issue. In this chapter we review evidence of existence of a prothombotic state in HF, the pharmacodynamics and clinical trials of the NOACs and the outcomes from NOAC substudies in the HF subgroup. We also discuss the rationale for using anticoagulation in HF independent of arrhythmia burden.

Keywords
Atrial fibrillation • Bleeding • Heart failure • Novel oral anticoagulant • Randomized clinical trials • Stroke • Thrombosis

1 Introduction

Heart failure (HF) is an important and growing cause of morbidity and mortality in both the United States and around the world. The overall costs of caring for HF patients are enormous, related in large part to the frequency of hospitalization in this population (Rosamond et al. 2007). Although currently recommended therapies have improved outcomes, there is a pressing need for novel treatment strategies. As the worldwide prevalence of HF increases, this need will become even more apparent (De Lorenzo et al. 2003). There is evidence suggesting that HF confers a hypercoagulable state (De Lorenzo et al. 2003; Lip and Gibbs 1999) and many HF complications may be the result of a pro-thrombotic state. Thus, antithrombotic therapies may be of potential benefit in the HF population.

In considering the use of antithrombotic therapy in HF patients, the potential benefits must be weighed against associated bleeding risks. In the past, such discussions focused on the relative risk benefit ratio of warfarin and anti-platelet agents. With the advent of novel oral anticoagulants (NOACs) – among them direct thrombin and factor Xa inhibitors (Fig. 1) – several agents are now available that have demonstrated equivalent, and, in some cases, superior stroke and bleeding outcomes when compared to warfarin in treating patients with non-valvular atrial fibrillation (AF) (Connolly and Ezekowitz 2009; Patel et al. 2011; Granger et al. 2011; Giugliano et al. 2013). In patients with venous thromboembolism (VTE),

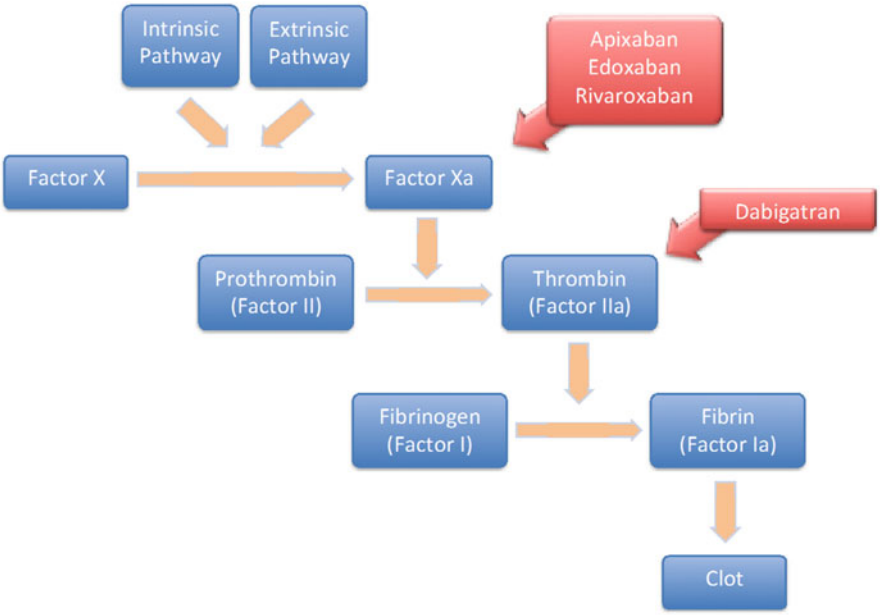

Fig. 1 Simplified coagulation cascade with site of novel oral anticoagulant activity

NOACs have been shown to have equivalent efficacy and lower risks of bleeding compared to warfarin (Schulman et al. 2014; Agnelli et al. 2013; Hokusai et al. 2013; Prins et al. 2013). An appealing aspect of NOACs is that they do not require frequent laboratory monitoring and dose adjustment as is common with the use of warfarin.

Information about the impact of thrombosis on HF outcomes (Zannad et al. 2013) and evidence of the safety and efficacy of NOACs has resulted in renewed interest in utilizing antithrombotic therapies in the HF population. In fact, NOACs are already frequently utilized in HF patients owing to the high prevalence of AF and VTE (Beemath et al. 2006; De Ferrari et al. 2007). The focus of this chapter is to review the rationale for NOACs in HF patients, their current use in this population, and potential new directions for antithrombotic therapy.

2 Thrombosis and Embolism in HF

Several lines of evidence link HF with a hypercoaguable state (Zannad et al. 2013). High levels of circulating markers of fibrin generation, fibrinolysis, and platelet activation have been described in HF patients (Yamamoto et al. 1995; O'Connor et al. 1999; Jafri et al. 1993).

HF has been associated with increases in pro-inflammatory cytokines that may contribute to a prothrombotic mileu (Levine et al. 1990; Marcucci et al. 2006;

Rauchhaus et al. 2000). Markers of endothelial dysfunction (von Willebrand factor and thrombomodulin) and activation (e-selectin) are elevated in HF, and these may predispose to leukocyte adhesion, inflammation, and thrombosis (Chong et al. 2006).

Stagnation of blood flow in dyskinetic regions of the heart accentuated by abnormalities of the endocardial surface may also predispose to local thrombosis (Fukuchi et al. 2001; Schoner et al. 2015). For instance, patients with HF with reduced ejection fraction are at risk for the development of left ventricular thrombi owing to stasis of blood in hypokinetic or akinetic regions of the left ventricle (LV). When these LV thrombi break loose into the systemic circulation, they can have catastrophic results. For this reason, systemic anticoagulation is recommended for patients with LV thrombus, and is often prescribed to those at high risk of developing LV thrombi, such as following anterior MI with apical akinesis or dyskinesis (Vandvik et al. 2012; O'Gara et al. 2013).

HF patients are known to be at increased risk for additional forms of thrombosis and embolism including atrial thrombosis and embolic stroke in the setting of atrial fibrillation, deep venous thrombosis (DVT) and pulmonary embolus (PE), and ischemic stroke, all of which contribute to poor outcomes. In addition, coronary thrombosis can result in the onset and progression of HF. Although relatively low rates (2–4%) of fatal and nonfatal myocardial infarction (MI) are reported in clinical trials of HF, the true rate of MI is likely underestimated (Zannad et al. 2013). In patients with sudden cardiac death, acute coronary events, including acute coronary syndrome (ACS) and coronary embolism, are frequent but often go unrecognized (Uretsky et al. 2000). Furthermore, troponin (Tn) elevations in the blood occur in HF patients and they have been associated with poor outcomes (Horwich et al. 2003; Peacock et al. 2008). Although increases in levels of this biomarker of myocardial injury could be due to a variety of causes (e.g. myocardial oxygen supply-demand mismatch, effects of inflammatory mediators, reactive oxygen species) coronary micro-thrombosis is also a potential contributor to Tn release.

Atrial fibrillation is highly prevalent in patients with HF, and may be more common in those patients with preserved rather than reduced ejection fraction (Lubitz et al. 2010). The diagnosis of AF confers a substantial impact on morbidity and mortality in HF patients secondary to a variety of clinical sequelae, including systemic thromboembolism (Benjamin et al. 1998; Chugh et al. 2001). The presence of AF has traditionally been linked to stroke through the stasis of blood leading to thrombus formation in a dilated left atrium and hypocontractile left atrial appendage, with additional evidence suggesting AF confers an independent pro-thrombotic effect (Chugh et al. 2001; Hart and Halperin 2001). As a result, risk-based antithrombotic therapy is the standard recommendation for stroke prophylaxis, when tolerated (January et al. 2014).

Venous thromboembolism in the form of DVT is common in HF patients, particularly those hospitalized for an episode of decompensation (Beemath et al. 2006), and can lead to PE which further complicate the clinical course. Heart failure is an independent risk factor for VTE, and its presence conveys a two- to threefold

increased risk (Samama 2000). The occurrence of PE has been associated with an increased risk of death or re-hospitalization in HF patients (Darze et al. 2007), and there is evidence that the frequency of PE in HF patients may be underestimated as one series of patients with idiopathic dilated cardiomyopathy noted that 39% had PEs at autopsy (Roberts et al. 1987).

Ischemic stroke is also common in HF patients. In one cohort study, HF was associated with a 17-fold increase in the risk of stroke compared to the general population (Witt et al. 2006). This is likely due in part to shared risk factors of stroke and HF, such as diabetes and hypertension, as well as the associations of HF with AF, LV thrombus, and hypercoagulability (Haeusler et al. 2011).

3 Prior Clinical Trials of Conventional Antithrombotic Therapy in HF

Despite evidence of a hypercoaguable state in HF patients, randomized controlled trials of antithrombotic therapy in this population have thus far failed to demonstrate an overall clinical benefit. The Heart Failure Long-Term Antithrombotic Study (HELAS) and Warfarin Aspirin Study in Heart Failure (WASH) studies included patients in normal sinus rhythm with HF and an LV ejection fraction (LVEF) less than 35% (Cleland et al. 2004; Cokkinos et al. 2006). In HELAS, patients with a history of MI were randomized to warfarin or aspirin, and patients with non-ischemic cardiomyopathy were randomized to warfarin or placebo. In WASH, patients were randomized to aspirin, warfarin, or no therapy. Antithrombotic therapy was not associated with improved outcomes compared to controls in either study. However, both trials were small (197 patients in HELAS and 279 patients in WASH) and underpowered. The Warfarin and Antiplatelet Therapy in Chronic Heart Failure (WATCH) trial randomized HF patients with an LV ejection fraction of less than 35% to aspirin, clopidogrel, or warfarin therapy (Massie et al. 2009). Trial enrolment was terminated at 1,587 out of a planned 4,500 patients due to slow enrollment. There were no differences in the primary endpoint of the composite outcome of all-cause mortality, nonfatal MI, and non-fatal stroke among the treatment arms. However, warfarin was associated with fewer strokes than aspirin and clopidogrel, and fewer hospitalizations for worsening heart failure than aspirin. These benefits were offset by significantly higher risk of major hemorrhages in patients in the warfarin arm compared to those in the aspirin arm.

The largest trial of antithrombotic therapy in HF to date is the Warfarin vs. Aspirin in patients with Reduced Cardiac Ejection Fraction (WARCEF) trial (Homma et al. 2012). In WARCEF, 2,305 patients with LVEF less than 35% were randomized to warfarin plus placebo or aspirin plus placebo. The primary outcome was time to first event in a composite endpoint of ischemic stroke, intracerebral hemorrhage, or death from any cause. The secondary outcome was time to first event in a composite endpoint of the primary outcome plus MI or hospitalization for HF. There were no significant differences in the primary and secondary outcomes between the two arms. However, warfarin was associated with

a significantly decreased risk of stroke compared to aspirin (hazard ratio 0.52; 95% confidence interval 0.33–0.82), and no significant difference in the risk of intracranial and intracerebral hemorrhage. The rate of major hemorrhage was higher with warfarin compared to aspirin (1.78 events per 100 patient-years vs. 0.87 per 100 - patient-years), largely driven by more frequent occurrence of major gastrointestinal bleeding.

Overall, the benefits of warfarin in these trials were offset by bleeding complications associated with this drug. The findings of the clinical trials using warfarin, however, raise the possibility that NOACs, with favorable safety profiles and relative simplicity of use, could provide a more favorable risk to benefit ratio in HF patients than warfarin therapy.

4 NOACs for AF

In randomized controlled trials and meta-analyses, warfarin has demonstrated efficacy in preventing stroke in patients with AF, particularly those with clinical features correlating with a higher stroke risk (Philip et al. 1990; McBride et al. 1994; Hart et al. 2007). Warfarin has also demonstrated superiority over aspirin in preventing stroke (van Walraven et al. 2002). However, the risk of bleeding complications, including intracranial and gastrointestinal hemorrhage, limits its application in those with an increased risk of clinically significant bleeding. Moreover, even with the frequent laboratory monitoring that is required to achieve and maintain a therapeutic dose of warfarin, patients receiving warfarin may have sub-therapeutic international normalized ratios (INRs) for substantial periods of time, with out of therapeutic range values increasing their risk for thromboembolism (when levels are too low) or bleeding (when they are too high) (Nelson et al. 2015a, b; Schein et al. 2016).

Until recently, warfarin has been the standard anticoagulant therapy for AF patients at a high risk of stroke. Several NOACs – among them dabigatran, rivaroxaban, apixaban, and edoxaban – have been developed to overcome the limitations of warfarin and other vitamin K antagonists. Key characteristics of these agents are summarized in Table 1. Their rapid onset of action and lack of necessity for routine coagulation monitoring has allowed them to have a wider therapeutic application. Evidence supporting their equivalent and/or superior efficacy to warfarin in the prevention of stroke and systemic embolism (SSE) has been demonstrated in a series of clinical trials comparing the NOACs to warfarin in patients with non-valvular AF. A discussion of these agents in patients with non-valvular AF follows below.

Dabigatran The Randomized Evaluation of Long Term Anticoagulant Therapy (RE-LY) study included 18,113 patients with AF randomized to warfarin vs. double blinded treatment with either dabigatran 150 or 110 mg BID. The study was designed to assess the non-inferiority of the two doses of dabigitran to warfarin for the primary outcome of stroke or systemic embolism (SSE). The primary

Table 1 Novel oral anticoagulants characteristics (Hull and Gersh 2015; Gomez-Outes et al. 2015)

Mechanism of action	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
	Direct thrombin inhibitor	Factor Xa inhibitor	Factor Xa inhibitor	Factor Xa inhibitor
Bioavailability	3–7%	80–100% (10 mg dose) 76% (20 mg, decreased without food)	50%	62%
T_{max} (h)	1–2 h	2–4 h	3–4 h	1–2 h
Protein binding	35%	92–95%	87%	55%
Volume of distribution (V_d)	50–70 l	50 l	21 l	107 l
$T½$ (half-life)	12–17 h (27 h in severe renal impairment)	5–9 h (13 h, elderly)	12 h	10–14 h
Excretion	Renal (80% unchanged)	Renal (36% unchanged)	Renal (27% unchanged)	Renal (35% unchanged)
Metabolism	Conjugation	Oxidation (CYP3A4/5, CYP2J2) and hydrolysis	Oxidation (CYP3A4 > CYP1A2, CYP2C8, CYP 2C9, CYP2C19, CYP2J2)	Minimal (hydrolysis, conjugation and oxidation by CYP3A4)
Dosing (non-valvular atrial fibrillation)	150 mg BID (75 mg BID in CrCl 15–30 ml/min)	20 mg daily (15 mg daily in CrCl 15–50 ml/min)	5 mg BID (2.5 mg BID if meets 2 of 3 criteria: age ≥ 80, Scr ≥1.5 and/or weight ≤ 60 kg)	60 mg daily if CrCl 51–95 ml/min (30 mg daily if CrCl 15–50 ml/min)
Dosing (venous thromboembolism)	150 mg BID in CrCl >30 ml/min	15 mg BID × 21 days, then 20 mg daily ≥30 ml/min	10 mg BID × 7 days, then 5 mg BID	60 mg daily (30 mg daily if CrCl 15–50 ml/min or weight ≤ 60 kg)
Reversal agent	Idarucizumab (Praxbind) Dose: 5 gm IV × 1	None approved	None approved	None approved
Major drug interactions	p-GP inhibitors[a] and inducers[b]	Dual p-GP inhibitors and strong CYP3A inhibitors[c]; dual p-GP inducers and strong 3A4 inducers[d]	Dual p-GP inhibitors and strong CYP3A inhibitors[c]; dual p-GP inducers and strong 3A4 inducers[d]	p-GP inhibitors[a] and inducers[b]

[a]E.g., verapamil, quinidine, azithromycin, clarithromycin, erythromycin, oral itraconazole, or oral ketoconazole
[b]E.g., rifampin
[c]E.g., ketoconazole, itraconazole, ritonavir, indinavir, conivaptan, clarithromycin
[d]E.g., carbamezapine, phenytoin, rifampin, St. John's wort

outcome results demonstrated non-inferiority for both doses of dabigitran and superiority with the 150 mg dose (34% relative risk reduction vs. warfarin and 27% relative risk reduction vs. 110 mg dose). Rates of hemorrhagic stroke were lower for both dabigatran doses compared to warfarin (0.12% per year-110 mg; 0.10% per year-150 mg; 0.38% per year-warfarin), as well as a decrease in intracranial hemorrhage (ICH) events (0.23% per year-110 mg; 0.30% per year-150 mg; 0.74% per year-warfarin, $p < 0.001$) (Connolly and Ezekowitz 2009). Rates of life threatening bleeding, major or minor bleeding were higher with warfarin compared to both doses; however a higher rate of major gastrointestinal bleeding was noted with the 150 mg dose than with warfarin. Dosing of dabigitran is summarized in the table. Of note is the fact that more than 80% of dabigatran is renally eliminated and those with severely impaired kidney function (creatinine clearance [CrCl] <30 ml/min) were excluded from the RE-LY study. However, the FDA has approved a dose of 75 mg twice daily for AF patients with CrCl of 15–30 ml/min (Lehr et al. 2012).

Rivaroxaban The Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared With Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET-AF) included 14,264 patients with a higher stroke risk (CHADS2 score $\geq$ 2). The primary outcome was non-inferiority of rivaroxaban to warfarin for the primary endpoint of SSE. The results confirmed that rivaroxaban was non-inferior to warfarin (1.7% per year vs. 2.2% per year; HR 0.79 95%, CI 0.66–0.96; $p < 0.001$ for non-inferiority). The safety outcome for major and non-major clinically relevant bleeding events was not different (14.9% vs. 14.5% per year; HR 1.03, 95% CI 0.96–1.11; $p = 0.44$) but there was less fatal bleeding (0.2% vs. 0.5%, $p = 0.003$) and ICH (0.5% vs. 0.7%, $p = 0.02$) with rivaroxaban (Patel et al. 2011). Dosing for rivaroxaban is outlined in the table. As with other NOACS, rivaroxaban clearance decreases as renal dysfunction becomes more severe, thus dose adjustment is recommended as rivaroxaban exposure can increase by approximately 52–64% in moderate (CrCl 30–49 ml/min) and severe renal impairment (<30 ml/min) (Kubitza et al. 2010).

Apixaban The Apixaban for the Prevention of Stroke in Subjects With Atrial Fibrillation (ARISTOTLE) double-blind non-inferiority trial enrolled 18,201 patients with AF and at least one additional risk factor for stroke. The primary outcome was ischemic or hemorrhagic stroke or systemic embolism. Patients were treated with apixaban (5 mg twice daily) or warfarin (target INR 2.0–3.0). Apixaban was superior to warfarin regarding the primary outcome of SSE (HR 0.79, 95% CI 0.66–0.95; $p = 0.01$ for superiority) after a median 1.8-year follow-up. Apixaban was also associated with lower rate of major bleeding including intracranial bleeding (HR 0.69, 95% CI 0.60–0.80; $p < 0.001$) and lower rate of death from any cause (HR 0.89, 95% CI 0.80–0.99; $p = 0.047$). As with other NOACs, caution should be taken when using apixaban especially in high-risk populations such as elderly patients with several comorbidities as decline in renal function overtime may increase bleeding events. As summarized in the table, dose

adjustment is recommended in those with renal function <50 ml/min due to high risk of major bleeding (Hijazi et al. 2016).

Edoxaban This selective inhibitor of factor Xa was assessed in the Effective aNticoaGulation with factor xA next GEneration in Atrial Fibrillation (ENGAGE AF-TIMI 48) study, a multicenter double-blind trial comparing edoxaban 30 and 60 mg to warfarin in 21,105 AF patients who were at moderate-to-high risk for thromboembolism. The results of ENGAGE AF-TIMI 48 showed edoxaban to be non-inferior compared to warfarin with respect to the primary efficacy end point of SSE, in both high and low doses [1.5% events per year for warfarin, 1.18% events per year for high-dose edoxaban (HR 0.79, 97.5% CI 0.63–0.99; $p < 0.001$ for on-inferiority) and 1.61% events per year for low-dose edoxaban (HR 1.07, 97.5% CI, 0.87–1.31; $p = 0.005$ for non-inferiority)]. Annualized hemorrhagic stroke rate was 0.47%, 0.26%, and 0.16% for warfarin, high dose and low dose edoxaban, respectively. Major bleeding occurred less frequently with both edoxaban doses compared to warfarin (2.75% patients per year – high dose, 1.61% patients per year – low dose vs. 3.43% patients per year – warfarin) with hazard ratios favoring edoxaban use (high dose HR 0.8; 95% CI, 0.71–0.91; $p < 0.001$ and low dose HR 0.47; 95% CI 0.41–0.55; $p < 0.001$). Overall major bleeding, intracranial bleeding, and gastrointestinal bleeding occurred less frequently with the lower dose use compared to the other groups. Gastrointestinal bleeding was seen more frequently with higher dose use compared to warfarin. The primary net clinical outcomes of death from any cause, SSE, or major bleeding were significant less with both edoxaban regimens compared to warfarin (6.79% low dose, 7.26% high dose and 8.11% warfarin). Dosing recommendations are provided in the table.

NOAC Reversal A primary concern regarding the use of NOACs is the lack of availability of an agent to rapidly reverse their anti-coagulant effect when emergent situations arise. Recently, a new monoclonal antibody fragment reversing agent, idarucizumab, has been developed to reverse the effects of dagibitran. The use of idarucizumab was studied in the prospective multicenter RE-VERSE AD (A Study of the RE-VERSal Effects of Idarucizumab on Active Dabigatran) trial. This study included 90 patients who developed serious bleeding or required urgent surgery while being treated with dagibitran. The median percentage reversal of the anticoagulation effect was 100%, showing efficacy for complete reversal of anti-coagulation. A total of 21 patients suffered an adverse event, which included 5 thrombotic events and 18 deaths. The latter were proposed to be the result of the severity of illness of the -population enrolled in this study, which had a mean age of 76.7 years, and a substantial burden of comorbidities, including 35% with intracranial bleeding on study entry. The FDA approved idarucizumab in October 2015 for the treatment of patients treated with dabigatran when reversal of the anticoagulant effects is needed for emergency surgery/urgent procedures, or in life-threatening or uncontrolled bleeding (Pollack et al. 2015).

A novel antidote to factor Xa inhibitors that acts as a human factor Xa decoy protein has been developed and studied in an open label prospective multicenter

trial. The ANNEXA-4 trial of Andexanet Alfa (andexanet) included 67 patients with a mean age of 77 years who had acute major bleeding within 18 h of receiving a factor Xa inhibitor (included apixaban, rivaroxaban, edoxaban, or enoxaparin only). Patients received a low or high dose depending on the type of Xa inhibitor received. The 30-day outcomes assessed were anti-Xa factor activity and hemostasis achieved 12 h after andexanet infusion. A relative decrease of anti-Xa factor activity was more pronounced with apixaban than other Xa inhibitors. Clinical hemostatic efficacy was achieved in 79% of the patients. A total 12 patients suffered a thrombotic event (1 with acute MI, 5 with stroke, 7 with DVT, and 1 with PE) and rate of death was 6% at 45 days. Of note was the fact that 34% of patients in this cohort had a history of HF (Connolly et al. 2016). Andexanet Alfa is currently under FDA review and if approved could be used as an antidote for factor Xa inhibitors.

5 NOACs for VTE

Additional randomized clinical trials have explored the use of NOACs in other thrombotic states (Schellong 2015). The RE-COVER and RE-COVER II trials were randomized non-inferiority clinical trials evaluating the efficacy and safety of dabigatran 150 mg twice daily vs. warfarin on recurrent symptomatic, confirmed VTE and related deaths at 6 months. Secondary end points included symptomatic DVT, symptomatic non-fatal PE, VTE-related death, and all cause death (Schulman et al. 2009, 2014). For both studies the results showed that dabigatran was comparable to warfarin for the primary and secondary outcomes (2.4% vs. 2.2% based on pool analysis). The pooled analysis data of both trials showed a statistically significant reduction in major bleeding events with dabigitran and comparable clinically relevant non-major bleeding events.

Two randomized open-label clinical trials in more than 8,281 patients assessed the efficacy and safety of rivaroxaban for the treatment of DVT, PE, and extended risk reduction for DVT and PE. The results of these studies showed that rivaroxaban is non-inferior to warfarin (EINSTEIN-DVT trial, 2.1% vs. 3%; HR 0.68, 95% CI 0.44–1.04; $p < 0.001$ for non-inferiority; EINSTEIN-PE trial, 2.1% vs. 1.8%; HR 1.12, 95% CI 0.75–1.68; $p = 0.003$). Similar to other NOAC trials, rivaroxaban showed rates comparable to warfarin in the safety endpoints of major bleeding or non-major clinically relevant bleeding (EINSTEIN-DVT 8.1% for both groups; HR 0.97, 95% CI 0.76–1.22; $p = 0.77$. EINSTEIN-PE 10.3% vs. 11.4%; HR 0.90, 95% CI 0.76–1.07; $p = 0.23$) (EINSTEIN Investigators et al. 2010; EINSTEIN–PE Investigators et al. 2012).

Apixaban has been studied in acute VTE including symptomatic proximal DVT or PE (with or without DVT). In a randomized, double-blind study of apixaban 10 mg twice daily for 7 days followed by 5 mg daily for 6 months compared to conventional therapy (enoxaparin followed by warfarin), the primary outcome for recurrent VTE or death was non-inferior compared to warfarin (2.3% vs. 2.7%; RR 0.84, 95% CI 0.60–1.18; $p < 0.001$ for non-inferiority). The safety outcome of major bleeding and clinically relevant non-major bleeding occurred less frequently

in the apixaban group compared to the warfarin group (4.3% vs. 9.7%; RR 0.44, 95% CI 0.36–0.55; $p < 0.001$) (Agnelli et al. 2013).

Edoxaban was studied as a treatment for symptomatic VTE. In a randomized non-inferiority study of 4,921 patients with DVT and 3,319 with PE, 60 mg (or 30 mg in patients with a CrCl of 30–50 ml/min or a body weight of 60 kg or less) of daily edoxaban was non-inferior to warfarin for the primary outcome of recurrent VTE and 3.2% vs. 3.5%; HR 0.89, 95% CI 0.7–1.13; $p < 0.001$ for non-inferiority. Major bleeding or clinically relevant non-major bleeding was less in edoxaban than in warfarin treated patients (8.5% vs. 10.3%; HR 0.81, 95% CI 0.71–0.94; $p = 0.004$ for superiority) (Hokusai et al. 2013).

6 Impact of Heart Failure in NOAC Trials in AF

Heart failure is a strong predictor for AF development with increasing prevalence as worsening NYHA class ensues (AF is present in almost 30% of NYHA III-IV and up to 50% of NYHA IV) (Maisel and Stevenson 2003).

Furthermore, HF patients are at increased risk for stroke, thromboembolism, and death especially in those with a higher CHA_2DS_2-VAS_c Score (Melgaard et al. 2015). Several secondary analyses of the NOAC trials in AF evaluating the subset of patients with HF have provided insights into the efficacy of NOACs in this higher risk population. These secondary analyses defined HF as the presence of symptoms of HF, an LVEF of <40%, or both. Although the original trials were not powered for any definitive conclusion regarding NOAC vs. warfarin in HF, the overall effects of NOACS in HF patients with AF showed similar treatment efficacy and safety for the primary outcomes of SSE and major bleeding. The benefits of these agents were comparable between AF patients with HF and without HF regardless of the dosing regimen. The clinical characteristics of the patients evaluated and the major efficacy and safety outcomes are summarized in Tables 2 and 3.

In RE-LY, 27% of the overall study cohort was comprised of HF patients, defined as those patients having NYHA class $\geq$ II symptoms and LVEF $\leq$40% in the last 6 months before randomization. All HF patients experienced a lower annual SSE rate with either dose of dabigatran administered in the trial in comparison to warfarin [1.92% per year for warfarin, 1.90% per year for dabigatran 110 mg (HR 0.99, 95% CI 0.69–1.42) and 1.44% per year for dabigatran 150 mg (HR 0.75, 95% CI 0.51–1.10)]. Importantly, HF patients on either dose of dabigatran had lower major bleeding, ICH, or total bleeding episodes compared to warfarin. No interaction between treatment effect of either dose of dabigatran and the presence or absence of HF was noted.

In ROCKET-AF, the prevalence of heart failure in the study population was 63.7%. Patients with HF had similar number of SSE events than non-HF patients. After adjustment of baseline variables, rivaroxaban had similar efficacy outcomes for SSE compared to warfarin regardless of the presence or absence of HF (HF: HR 0.91, 95% CI 0.74–1.13 vs. Non-HF: HR 0.84, 95% CI 0.65–1.09; $p = 0.62$). The risk of major or non-major bleeding was similar to warfarin (HF: HR 1.06, 95% CI

Table 2 NOAC heart failure substudy baseline characteristics

Trial	RE-LY (dabigatran)		ROCKET-AF (rivaroxaban)	ARISTOTLE (apixaban)
Dose	110 mg	150 mg	20 mg	5 mg BID
HF definition entry criteria	Left ventricular ejection fraction of less than 40%, New York Heart Association class II or higher heart-failure symptoms within 6 months before screening		Clinical HF or left ventricular EF <40%	Symptomatic HF within 3 months; LVSD with EF ≤40%
Number	1,641	1,640	4,530	2,736
EF < 40%	427 (44%)	429 (44%)	1,043 (33.3)	2,736 (100%)
CHADS2	2.6 ± 1.1	2.7 ± 1.2	3.7 (0.9)	2.22 (1.20)
ICD/CRT	NR	NR	148 (3.6)	304 (5.1)
SBP (mmHg)	127.3 (109.9–144.7)	127.3 (109.9–144.7)	130 (120–140)	126 (114–138)
HR BPM	76.1 (61–91.2)	76.1 (61–91.2)	77 (68–86)	NR
ASA	NR	NR	1,373 (30.3)	938 (34)
ARB/ACEI	1,296 (79)	1,314 (80)	2,792 (61.6)	2,205 (81)
Beta blocker	1,118 (68.1)	1,155 (70.4)	3,113 (68.7)	2,043 (75)
Digitalis	NR	NR	2,023 (44.7)	1,299 (47)
Diuretic	1,182 (72.0)	1,189 (72.5)	3,235 (71.4)	1,992 (73)
Amiodarone	NR	NR	NR	415 (15)
Statin	NR	NR	NR	1,184 (43)
MRA	NR	NR	NR	62 (2)
NT-proBNP (pg/mLe)	1,450 ± 1,399	1,640 ± 2,417	NR	NR
Warfarin	NR	NR	2,658 (58.7)	1,656 (61)

NR not reported in substudy

0.96–1.16 vs. Non-HF: HR 1.05, 95% CI 0.93–1.18; $p = 0.92$). A trend towards risk reduction of hemorrhagic stroke in HF patients with rivaroxaban was noted (van Diepen et al. 2013).

In ARISTOTLE, there was information about HF status and left ventricular systolic dysfunction (LVSD) in 14,671 patients. In a subgroup analysis, these patients were divided into three groups: (1) No HF and no LVSD; (2) HF preserved LV systolic function (HFPEF), and (3) LVSD with/without symptomatic HF. Only 22% of preserved EF group and 13% of the LVSD group had reported symptomatic HF. The adjusted rates of SSE, although not statistically significant across the three groups, were higher for LVSD and HFPEF patients compared to no HF/no LVSD groups (1.39, 1.52, and 1.37 per 100 patient-year follow up, respectively). The rates for major or clinically relevant non-major bleeding were also not statistically different across the three groups (5.53, 5.37, and 4.88 per 100 patient-years for LVSD, HFPEF, and no HF/LVSD,

Table 3 Rates of major efficacy and safety outcomes in patients with and without heart failure (HF)

	RE-LY (dabigatran)		ROCKET-AF (rivaroxaban)		ARISTOTLE (apixaban)			ENGAGE AF-TIMI 48 (edoxaban)	
Agent	Dabigatran		Rivaroxaban		Apixaban				
Study year	2013		2013		2013			2016	
(*n*)	HF (4,904)	No HF (13,209)	HF (9,033)	No HF (5,138)	LVSD (2,736)	HFPEF (3,207)	No LVSD/No HF (8,728)	HF (1,801) NYHA III-IV	No HF (5,926)
Follow up (years)	2		1.94		2			2.8	
Event rate (*n*)	% per year		100 patient-year		100 patient-years			% per year	
Efficacy outcome									
Stroke or systemic embolism	1.75 (164)	1.35 (355)	1.99 (343)	2.32 (232)	1.39 (67)	1.52 (89)	1.37 (224)	2 (92)	1.66 (267)
HR (95% CI), *p* value	1.08 (0.89–1.31), 0.46		0.94 (0.78–1.13), 0.51		1.01 (0.77–1.33), 0.71	1.11 (0.87–1.42), 0.71		1.45 (1.12–1.88), 0.004	
Safety outcome									
Major bleeding	320 (3.42)	842 (3.19)	14.12 (1,766)	15.73 (1,158)	3.09 (135)	2.55 (134)	2.50 (372)	2.83 (104)	3.31 (430)
HR (95% CI), *p* value	0.96 (0.83–1.10), 0.53		1.00 (0.92–1.08), 0.99		1.23 (1.01–1.50), 0.11	1.02 (0.84–1.24), 0.11		1.31 (1.06–1.65), 0.002	
Intracranial bleeding	35 (0.37)	120 (0.46)	0.53 (74)	0.77 (65)	0.45 (20)	0.45 (24)	0.60 (90)	0.61 (23)	0.68 (90)
HR (95% CI), *p* value	0.72 (0.49–1.06), 0.10		0.84 (0.58–1.22), 0.36		0.75 (0.46–1.22), 0.30	0.75 (0.48–1.18), 0.30		1.48 (0.93–2.37), 0.07	

(continued)

Table 3 (continued)

	RE-LY (dabigatran)		ROCKET-AF (rivaroxaban)		ARISTOTLE (apixaban)			ENGAGE AF-TIMI 48 (edoxaban)	
Agent	Dabigatran		Rivaroxaban		Apixaban				
Study year	2013		2013		2013			2016	
Cardiovascular outcome[a]									
Vascular death	4.69 (439)	1.67 (441)	3.53 (600)	1.75 (176)	NR			5.43 (257)	2 (332)
HR (95% CI), *p* value	2.26 (1.96–2.61), <0.0001		1.65 (1.37–1.98), <0.0001					3.18 (265–3.8), <0.001	
All cause death	NR		5.26 (879)	3.37 (335)	7.07 (348)	4.32 (258)	2.40 (400)	6.45 (305)	3.20 (532)
HR (95% CI), *p* value			1.34 (1.17–1.55) <0.0001		2.96 (2.57–3.42) <0.0001	1.81 (1.55–2.11) <0.0001		2.43 (2.08–2.83) <0.001	
Hospitalization	2,098 (22.41)	5,102 (19.35)	NR		5.99 (274)	3.24 (185)	1.12 (189)	15.47 (591)	9.77 (1,397)
HR (95% CI), *p* value	1.13 (1.07–1.20) <0.0001				5.07 (4.21–6.11) <0.0001	2.77 (2.26–3.40) <0.0001		1.83 (1.64–2.04) <0.001	

CI confidence interval, *HF* heart failure, *HR* hazard ratio (HF vs. no HF)
[a]All outcomes adjusted for baseline variables

respectively). There was no evidence of treatment heterogeneity according to LV function or HF status, with equivalent efficacy across all groups (McMurray et al. 2013).

In an ENGAGE-AF TIMI 48 substudy, 58% of 14,071 patients studied were found to have a history of HF, with 13% of them being classified as having severe HF, defined as NYHA III-IV. Among patients with severe HF, the rate per year of SSE was higher compared to non-HF patients (2.00% vs. 1.66%, $p = 0.012$). After adjustment for baseline characteristics, SSE was still higher in those with mild and severe HF (HR 1.19 and HF 1.45 vs. Non-HF). HF patients also had higher risk of major bleeding (HR 1.31, 95% CI 1.05–1.65; $p = 0.02$) including fatal bleeding and gastrointestinal bleeding. The overall outcomes for efficacy of edoxaban in preventing an SSE compared with warfarin were similar among patients with and without HF (edoxaban vs. warfarin: No-HF: HR 0.87, 95% CI 0.69–1.11; NYHA class I–II: HR 0.88, 95% CI 0.69–1.12; NYHA class III–IV: HR 0.83, 95% CI 0.55–1.25; p-interaction =0.97) (Magnani et al. 2016).

A meta-analysis of these four main trials demonstrated that in all patients with HF and AF on a single or high dose NOAC regimen reduced the risk of SSE by 14% compared to warfarin with an OR 0.86 (95% CI, 0.76–0.98). Additionally single and/or high dose NOAC regimen had a 24% reduction in major bleeding with an OR 0.76 (95% CI, 0.67–0.86). HF patients with AF on NOACs had a 41% lower risk of ICH compared with those without HF (OR 0.59, 95% CI 0.40–0.87) (Xiong et al. 2015).

7 NOACs for HF with Sinus Rhythm: Potential for Benefit?

Given the evidence linking HF to a prothrombotic state, it is not surprising that HF patients in sinus rhythm are at increased risk for thromboembolic events. In clinical trials of HF patients, the annual incidence of ischemic strokes was observed to be 0.6–2.3% and total thromboembolic events to be 1.5–2.3% with greater risk as EF declines (de Peuter et al. 2009). Two recent HF trials, the Controlled Rosuvastatin in Multinational Trial Heart Failure (CORONA) and the Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza cardiaca-Heart Failure trial (GISSI-HF), have analyzed risk factors for stroke in 9,585 HF patients (3,531 had AF and 6,054 had no AF). The stroke rate per 100 HF patient-years was 4.7% for AF vs. 3.4% for no AF. The investigators identified age, NYHA class (III and IV), insulin-dependent diabetes, body mass index, NT pro BNP, and previous stroke as predictors for stroke in those without AF. Using a risk-score based on the predictors, HF patients without AF in the third tertile had a rate of stroke similar to AF patients with no anticoagulation (2.0% per year vs. 2.2% per year, respectively) (Abdul-Rahim et al. 2015).

While clinical trials of conventional antithrombotic therapies for HF patients failed to demonstrate a net clinical benefit, the advent of NOACs and their demonstrated safety and efficacy in AF and VTE trials have led to a renewed interest in utilizing anticoagulants to improve outcomes in HF patients in sinus rhythm. In the ATLAS ACS 2-TIMI 51 trial, 15,526 patients within 7 days of a hospital admission for ACS were randomized to twice daily 2.5 or 5 mg doses of

rivaroxaban or placebo for a maximum follow-up of 31 months. Rivaroxaban was associated with a reduction for the composite primary outcome of cardiovascular death, myocardial infarction, and stroke (HR compared to placebo, 0.84; 95% confidence interval [CI], 0.74–0.96; $p = 0.008$) (Mega et al. 2012). A subgroup analysis showed that patients having HF at the time of their ACS, rivaroxaban 2.5 mg twice daily reduced the primary outcome of cardiovascular death, MI or stroke (18.6% placebo vs. 11.6% rivaroxaban, $p < 0.001$) and reduced all cause death (11.1% placebo vs. 5.3%, rivaroxaban $p < 0.001$). The findings from this study support the notion that anticoagulation in patients with acute HF may have an impact on outcomes.

The findings from the ATLAS ACS 2-TIMI 51 trial provided rationale for a randomized clinical trial assessing HF patients in sinus rhythm for the risk of stroke and/or other cardiovascular events in HF patients. The COMMANDER HF trial is a randomized double-blinded placebo-controlled, event-driven trial of ~5,000 patients that will assess rivaroxaban 2.5 mg twice daily compared to placebo in patients with HF with reduced EF (EF $\leq$ 40%) with significant CAD and in sinus rhythm. Patients are enrolled into the study after an episode of acute HF exacerbation within the last 30 days (Zannad et al. 2015). The primary efficacy outcome is a composite of all cause mortality, myocardial infarction, or stroke. The safety outcome is a composite of fatal bleeding or bleeding into a critical space with potential permanent disability, bleeding events requiring hospitalization, or major bleeding events according to International Society on Thrombosis and Hemostasis bleeding criteria (Schulman and Kearon 2005).

8 Conclusion

Heart failure patients with AF are a high-risk group for stroke and systemic embolism. This population requires effective anticoagulation approaches to minimize morbidity. Results of clinical trials carried out over the past decade indicate that NOACs are an efficient and safe alternative to warfarin to treat patients with AF and HF. The results of secondary analyses of NOAC trials in AF demonstrate, on balance, similar efficacy and safety in HF patients. Given evidence that HF is associated with a prothrombotic state, the use of NOACs in HF patients in sinus rhythm may be of potential benefit. Although there is some data suggesting that anticoagulation can improve outcomes in HF patients in sinus rhythm, well-designed randomized clinical trials are needed to determine if this is the case. The ongoing COMMANDER-HF trial is designed to determine the effects of low dose rivaroxiban on morbidity and mortality in this population, and the results of this study are eagerly awaited as they well open a door for future applications of NOACs.

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Role of Hyperkalemia in Heart Failure and the Therapeutic Use of Potassium Binders

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2017_25

Abstract

Hyperkalemia can be a life-threatening disorder, especially for at-risk patients with heart failure, chronic kidney disease, with diabetes, and patients on certain drugs like renin-angiotensin-aldosterone system antagonists and mineralocorticoid receptor antagonists. There are limited therapeutic options available for hyperkalemia, and they have narrow effectiveness because of their unfavorable side effects profile in long-term and high cost utilization requiring inpatient care. Patiromersorbitex calcium and sodium zirconium cyclosilicate are novel potassium-lowering compounds for the treatment and prevention of hyperkalemia in at-risk population. These therapeutic agents have shown encouraging results in early phase II and phase III clinical trials. However, there is need to further study their efficacy and safety in heart failure population in order to establish their clinical use. The focus of this chapter will be to promote better understanding of potassium homeostasis in heart failure patients and the mechanistic overview of novel drugs, with emphasis on heart failure population.

Keywords

Angiotensin converting enzyme inhibitors • Angiotensin receptor blockers • Chronic kidney disease • Heart failure • Hyperkalemia • Mineralocorticoid receptor antagonist • Patiromer • Renin-angiotensin-aldosterone system inhibitors and sodium polystyrene sulfonate • Sodium zirconium cyclosilicate

Approximately 26 million people globally have heart failure (HF) (Ponikowski et al. 2014). By virtue of their disease, comorbidities, and medical therapy, these patients are at risk for hyperkalemia, which is a frequent disorder in these patients. Its incidence is up to 10% in hospitalized patients while 75% of these cases are due to medications. Hyperkalemia can potentiate the already elevated risk of arrhythmias in heart failure patients. Heart failure patients have a high prevalence of chronic kidney disease, which further heightens the risk of hyperkalemia, especially when using renin-angiotensin-aldosterone system inhibitors (RASSi). The current treatment for acute hyperkalemia may not be tolerated for chronic or preventive purposes. Recent randomized clinical studies on two novel drugs for hyperkalemia, namely patiromer and sodium zirconium cyclosilicate, have shown favorable data to treat and prevent high serum potassium levels on a more chronic basis. This has caused increased interest in the treatment of hyperkalemia as well as the potential use of RAASi in intolerant patients.

1 Introduction of Hyperkalemia

1.1 Definition and Diagnosis

Serum potassium level higher than 5.0 mmol/L is defined as hyperkalemia (An et al. 2012). Hyperkalemia signs or symptoms include weakness, fatigue, nausea, chest pain, shortness of breath, and paralysis. However, most patients with hyperkalemia

are asymptomatic. Hyperkalemia is usually discovered through routine blood screening and once diagnosed, additional investigations such as electrocardiograms are performed to determine the severity.

1.2 Epidemiology and Risk

Among hospitalized patients for any cause, the prevalence of hyperkalemia has been estimated at 1–10% (Acker et al. 1998). Patients with chronic medical conditions such as chronic kidney disease (CKD), heart failure (HF), diabetes mellitus, and those using renin-angiotensin-aldosterone system inhibitors (RAASi) are at 2–3 times higher risk for developing hyperkalemia (Jain et al. 2012; McMurray et al. 2012; Yancy et al. 2013a, b). With the rise in the aging population, there is an increase in the number of patients with CKD and HF. Therefore, there is an increase in the number of patients who are utilizing RAASi and mineralocorticoid receptor antagonists (MRA). Hyperkalemia can be a life-threatening condition due to an increased risk for arrhythmias (An et al. 2012; Khanagavi et al. 2014). Angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARBs), and mineralocorticoid receptor antagonists (MRAs) all have proven benefit in patients who are also at high risk for hyperkalemia (Juurlink et al. 2004). Hyperkalemia secondary to these conditions and therapies leads to intolerance or suboptimal dosages of RAASi and MRA. The discontinuation of RAASi due to hyperkalemia represents an unfavorable clinical compromise. Importantly, many clinical trials have excluded patients with advanced CKD and hyperkalemia or at risk for hyperkalemia, leaving behind a critical evidence gap in pharmacotherapy for these high-risk patients.

2 Potassium Homeostasis Under Physiologic Conditions

Potassium is freely filtered by the renal glomeruli and is predominantly regulated by the kidneys, which under normal circumstances have a substantial capacity to excrete potassium without clinically significant hyperkalemia (Fig. 1). Under physiologic conditions, the kidneys are responsible for excreting 90% of the potassium that is consumed daily (Rabelink et al. 1990). Most of the potassium is absorbed in the proximal tubule and loop of Henle, while only 10% reaches the distal tubule (Gumz et al. 2015; Palmer 2004). If potassium is in excess in the serum, it is secreted into the tubular lumen by the principal cells present in the renal collecting duct. Potassium excretion is also mediated by the luminal membrane potassium channels that respond to the electrochemical gradient generated by the basolateral membrane Na-K-ATPase pump and a luminal membrane sodium channel.

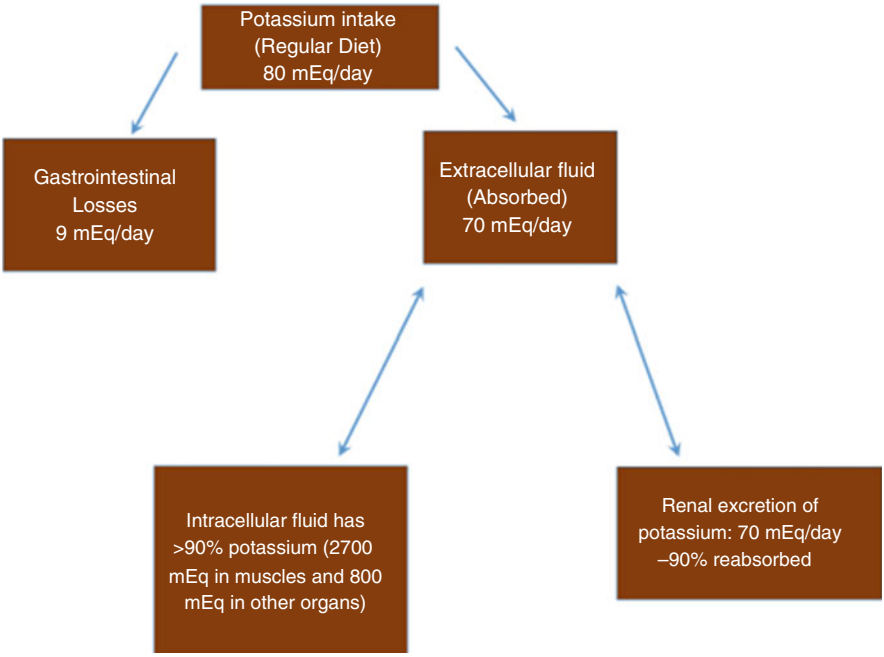

Fig. 1 Potassium homeostasis. Kidneys tend to maintain the potassium levels and regulate its excretion and reabsorption

2.1 Regulation of Potassium by RAAS

Serum potassium concentration and RAAS regulate the secretion of aldosterone. Renin is secreted by the juxtaglomerular cells present in the afferent arterioles in response to low renal perfusion pressures and stimulates the conversion of angiotensin I, derived from angiotensinogen in the liver, to angiotensin II by angiotensin-converting enzyme (ACE) (Fig. 2). Angiotensin II stimulates the zona glomerulosa in the adrenal glands to release aldosterone. Aldosterone regulates the secretion of potassium in the collecting duct and sodium concentration in the distal tubule (Palmer 2004). In addition, high serum potassium directly stimulates aldosterone to excrete potassium from the distal tubules and leads to decreased serum potassium levels (Young et al. 1984; Pratt et al. 1989).

3 Mechanism of Hyperkalemia in Heart Failure

Under physiologic conditions, serum aldosterone concentration varies inversely with the delivery of sodium to the distal nephron so that potassium excretion remains independent of changes in the extracellular fluid volume (Rabelink et al. 1990; Palmer 2004; Gumz et al. 2015). However, in HF, increased aldosterone causes increased absorption of sodium in the proximal tubules resulting in its

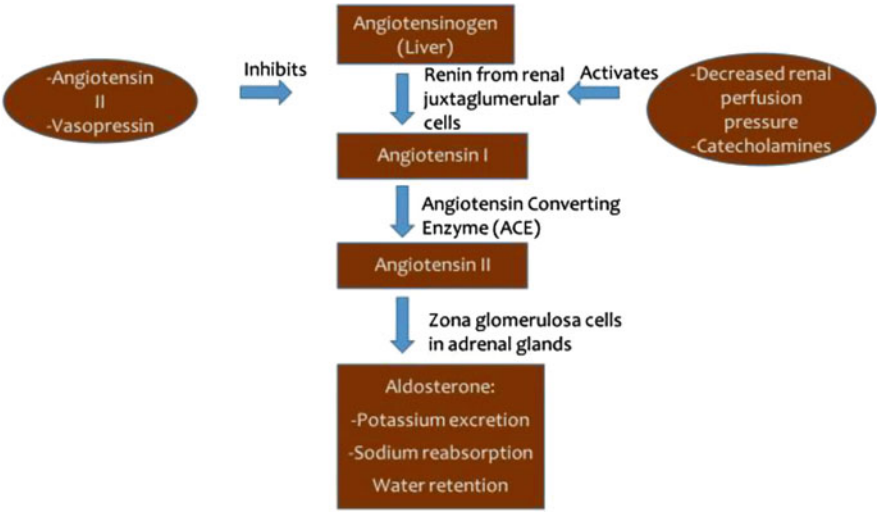

Fig. 2 Activation of renin angiotensin aldosterone system (RAAS)

decreased delivery to the distal nephrons and thus decreased potassium excretion. The use of RAASi in the presence of tubulointerstitial renal disease increases the risk of hyperkalemia by developing resistance to already reduced aldosterone levels (Young et al. 1984; Palmer 2004). Many of the diseases that affect tubular function also impair the release of renin and result in hyporeninemic hypoaldosteronism and impaired tubular function (Palmer 2004).

Renal hypoperfusion in HF leads to renin elevation and stimulates the synthesis of aldosterone, causing the excretion of potassium while high potassium concentrations can directly inhibit RAAS (Dargie 1990). ACEis block the stimulatory effect of angiotensin II on aldosterone secretion, while ARBs prevent angiotensin II from binding to its adrenal receptors (Dargie 1990). In addition, both of these drugs may interfere with angiotensin I production locally within the adrenal glands (Shier et al. 1989). RAASi such as ACEis, ARBs, MRAs, and direct renin inhibitors are associated with an increased risk of hyperkalemia, particularly when administered in combination (Takaichi et al. 2007).

Hyperkalemia can also develop secondary to decreased delivery of sodium to the distal nephron, aldosterone deficiency, and abnormal functioning of the cortical collecting tubule. Hence, these abnormalities can result from the effects of other drugs, underlying disease, or commonly combination of both. These mechanisms are explained below:

1. *Decreased delivery of sodium to the distal nephron tubule*: In normal circumstances, serum aldosterone concentration varies inversely with the delivery of sodium to the distal nephron so that potassium excretion remains independent of changes in extracellular fluid volume. However, in HF, the increased aldosterone causes increased absorption of sodium in the proximal tubules resulting in its decreased delivery to the distal nephrons. Despite the increased concentration of

aldosterone, the decreased sodium availability results in decreased potassium excretion (Na^{+}/K^{+} pump). In this setting, these drugs may also cause the serum creatinine concentration to rise due to the reduction in intraglomerular pressure that is no longer offset by an increase in glomerular perfusion.
2. *Decreased aldosterone levels*: The use of RAASi in heart failure reduces the aldosterone concentration, but RAASi are often not sufficient to impair the excretion of potassium in most cases. The development of hyperkalemia due to decreased aldosterone levels is usually seen before the administration of RAASi.
3. *Adrenal cortical dysfunction*: The use of RAASi in the presence of tubulointerstitial renal disease increases the risk of hyperkalemia by developing resistance to already reduced aldosterone levels. Many of the diseases that affect tubular function also impair the release of renin. As a result, hyporeninemic hypoaldosteronism and impaired tubular function may coexist (Palmer 2002, 2004).

4 Magnesium Homeostasis

The role of magnesium and its pathophysiology in heart failure remains less well studied when compared to other electrolyte abnormalities. However, it is established that early and effective correction of magnesium disturbances is favorable in HF subjects (Douban 1996, p. 398).

In decompensated HF, an excess catecholamine release can significantly influence the trans-cellular magnesium shift. Furthermore, hypomagnesaemia in HF stems from reduced dietary intake, impaired absorption of micronutrients due to intestinal edema, altered distribution of the ion, and renal losses. Respiratory alkalosis may produce a decrease in serum magnesium due to the shift of magnesium into the intracellular compartment. In animal models, magnesium deficiency leads to the development of mitochondrial alterations with calcium accumulation, cell death, and multifocal myocardial necrosis. Hypomagnesaemia seems to have vasoconstrictor properties secondary to the inhibition of prostaglandin-induced relaxation and to the enhancement of the activity of the vasoconstrictor neurohormones through alterations in calcium uptake. In addition, magnesium depletion is a feature of diuretic therapy but there is a paucity of published data concerning the relationship between magnesium and the activity of the renin-angiotensin system in HF (Dargie 1990, p. 399).

5 Causes of Hyperkalemia

Some etiologies of hyperkalemia include the following (see Figs. 3 and 4):

1. Hormonal disorders, for example: Addison's disease, hyporeninemic hypoaldosteronism.
2. Chronic conditions, for example: Diabetes mellitus, CKD, and diseases with cell membrane instability that can cause intra- and extracellular potassium shifts (Bramlage et al. 2016; Packham and Kosiborod 2016).

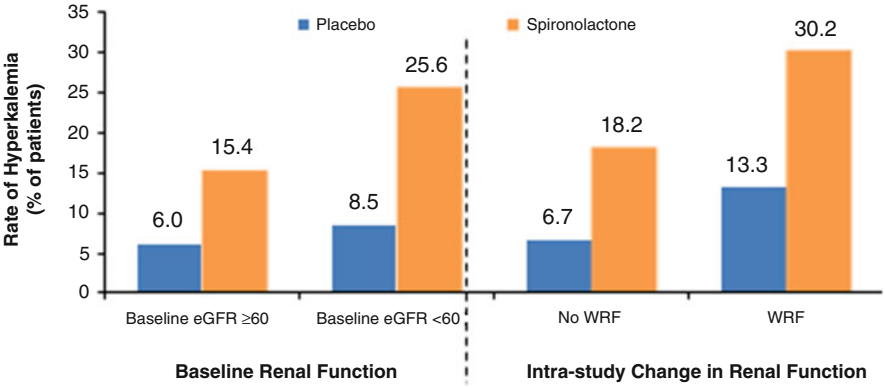

Fig. 3 Relationship of hyperkalemia in heart failure patients as renal function declines. Impaired renal function increases the risk of hyperkalemia in both placebo and MRA-treated patients. *WRF* worsening renal function, *eGFR* estimated glomerular filtration rate. Adapted and modified from: Go AS, et al. N Engl J Med. 2004;351:1296–1130

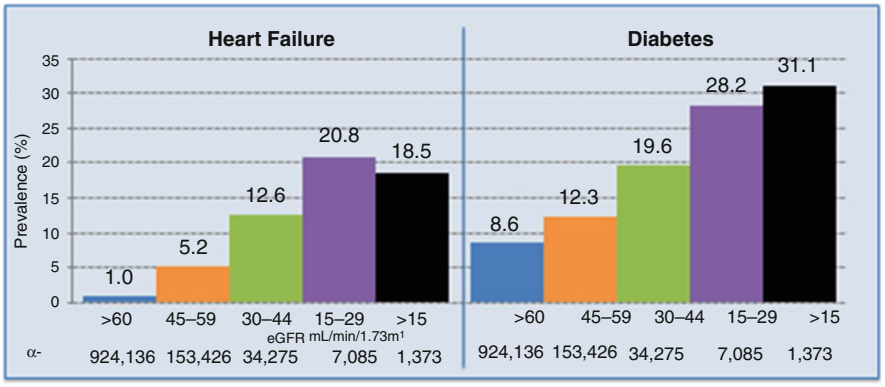

Fig. 4 Relation of chronic kidney disease with the prevalence of heart failure and diabetes mellitus. Prevalence of HF and diabetes mellitus may increase the risk of hyperkalemia based on disease and concomitant therapies. *eGFR* estimated glomerular filtration rate, *N* sample size. Adapted and modified from: Go AS, et al. N Engl J Med. 2004;351:1296–1130

3. Medications, for example: RAASi, MRA, nonsteroidal anti-inflammatory drugs, diuretics, and heparin. MRAs or RAASi increase the risk of hyperkalemia (Yancy et al. 2013a, b). The use of ACEi is attributed to the development of hyperkalemia in 10–38% of hospitalized patients (Acker et al. 1998; Ahuja et al. 2000) while hyperkalemia develops in up to 10% of the outpatient population within 1 year of prescribing RAASi (Reardon and Macpherson 1998). Patients with renal insufficiency or diabetes are at higher risk of hyperkalemia (Palmer 2004).
4. Excess dietary intake of foods high in potassium or sodium supplements that contain high potassium content can also cause hyperkalemia (Aaron and Sanders 2013; Go et al. 2004; Vardeny et al. 2012).

6 Hyperkalemia Affects Optimization of RAASi Therapy

RAASi therapy (ACEi, ARBs, and MRAs) improves survival and decreases hospitalization in high-risk heart failure patients. However, in clinical practice, these drugs are often discontinued or dosages are decreased to suboptimal levels due to hyperkalemia. RAAS is implicated in cardio-renal syndrome (both types 1 and 2). These patients develop diuretic resistance due to sodium retention, which has led to the increased use of continuous ambulatory peritoneal dialysis. However, this form of dialysis often leads to hyperkalemia, which in turn leads to the discontinuation or suboptimal dosing of RAASi therapy.

In the PARADIGM-HF trial, despite renal function, potassium-based eligibility criteria, and a run-in period, about 15% of patients in both the LCZ696 and enalapril arms developed hyperkalemia (McMurray et al. 2014). In the Randomized Aldactone Evaluation Study (RALES) trial, the beneficial effect of spironolactone continued in the treatment group versus placebo group despite higher potassium levels in the treatment group (4.54 ± 0.49 versus 4.28 ± 0.50 mmol/L; $P < 0.001$), but there was higher mortality risk observed when potassium levels increased above 5.5 mmol/L in the spironolactone group (Vardeny et al. 2014). This beneficial effect of spironolactone compared to placebo was maintained at all levels of hyper- or hypokalemia showing a U-shaped relationship between potassium levels and mortality. The use of spironolactone increased post RALES trial, but hospitalizations attributed to hyperkalemia also increased from 2.4/1,000 in 1994 to 11/1,000 patients in 2001 ($P < 0.001$) (Juurlink et al. 2004). Additionally, the rate of in-hospital hyperkalemia-associated death in HF patients also increased from 0.10/1,000 patients in 1994 to 0.39/1,000 in 2001 ($P < 0.001$) (Juurlink et al. 2004). This data reflects the importance of hyperkalemia while optimizing the RAASi dose and the need to carefully monitor the potassium levels (Sarwar et al. 2016).

7 Management of Hyperkalemia

In general, the management of hyperkalemia depends on the severity and etiology as summarized below and in Table 1;

1. *Emergent*: In the presence of electrocardiographic changes (for example: peaked T-waves, short QT interval, wide QRS complex) and/or potassium levels above 7.0 mmol/L regardless of electrocardiographic changes, intravenous calcium is administered to patients to prevent arrhythmias (Weisberg 2008). In addition, insulin and beta-2 adrenoceptor agonists are used to rapidly lower serum potassium levels by redistributing potassium from the extracellular to intracellular space. However, this is a temporizing measure (Palmer 2004).
2. *Intermediate*: In patients with poor kidney function or unresponsive to other treatments, dialysis can be used. Sodium bicarbonate is an effective strategy to minimize increases in the potassium concentration in patients with CKD and metabolic acidosis (Wilmer et al. 2003). Loop diuretics also lower potassium by

Table 1 Treatment options for hyperkalemia

Severity	Treatment	Goal
Emergent	Calcium gluconate Insulin Beta-adrenoreceptor agonists	Cellular membrane stabilization Intracellular potassium shift Intracellular potassium shift
Intermediate	Dialysis Loop diuretics Thiazide diuretics Sodium bicarbonate in patient with metabolic acidosis	Potassium removal from body Potassium removal from body Potassium removal from body Intracellular potassium shift
Maintenance	Review of medication list and dietary supplements Low potassium diet Reduce renin angiotensin-converting enzyme inhibitors dose Stop offending medications Potassium binding resins	Target potassium balance between intake/production and excretion

increasing the delivery of sodium to the collecting duct and increasing the excretion of potassium (Palmer 2004).

3. *Maintenance*: Several measures can be taken including dietary potassium intake restriction, dose reduction, administration on an every-other-day basis, or complete discontinuation of the drugs that impair potassium excretion (Palmer 2004). The decision is made after reviewing all dietary and herbal supplements in addition to salt substitutes. Potassium binding resins can also be used. However, until recently, the only approved ion-exchange resin was sodium polystyrene sulfonate, which was not well tolerated and may have caused colonic necrosis and intestinal injury (Sterns et al. 2010). Patiromer has recently been approved for use, expanding the few options of therapies available for the management of these patients. However, chronic therapy targeting prevention of hyperkalemia and subsequent optimization of RAASi needs further studies.

8 Clinical Perspective

The risk of hyperkalemia in HF patients is increased in the presence of CKD. In the Acute Decompensated Heart Failure National Registry (ADHERE), over 60% ($n = 105{,}388$) of patients had kidney disease (Adams et al. 2005). The prevalence of hyperkalemia in CKD patients can be up to 20% and is associated with increased mortality, major adverse cardiovascular events, and discontinuation of RAASi therapy (Luo et al. 2016). The use of RAASi in high-risk patients with cardiovascular disease, myocardial infarction, HF, diabetes, and CKD can improve outcomes. However, most trials excluded patients with moderate or severe CKD, which is common in HF patients – especially at an advanced stage (Butler and Givertz 2014). Recent development of effective and safe potassium binders may allow the assessment of RAASi in this high-risk patient population.

8.1 Use of RAASi in CKD

Since the use of novel potassium binders is still in the early clinical stages, the following considerations are important in RAASi therapy in patients with CKD:

1. Potassium supplements or salt substitutes containing potassium need to be discontinued or used with close monitoring, if needed.
2. Using low doses of both RAASi and MRA may be more beneficial over using a high dose of one drug and not using the second class of drugs altogether. Although optimal doses of ACEi and ARB are associated with better outcomes in heart failure with reduced ejection fraction (HFrEF) (Packer et al. 1999; Konstam et al. 2009), low doses of RAASi are better than not using these drugs altogether.
3. With declining renal function, the risk of hyperkalemia and the rate of worsening renal function need to be monitored. At present, there are no specific guidelines but it is suggested that ACEi or ARB doses may be reduced or stopped temporarily or even permanently with eGFR less than 15–30 mL/min, while they can be used in dialysis patients with careful monitoring. Currently, MRAs are contraindicated in patients with eGRF <30 mL/min. However, it is advised that these decisions be individualized, as there is no consensus and limited evidence is available in such situations.

9 Use of Potassium Binding Resins in Treatment of Hyperkalemia

9.1 Sodium Polystyrene Sulfonate (SPS)

Sodium polystyrene sulfonate (SPS) was the first potassium binding resin approved by the Food and Drug Administration (FDA) in 1958. It is an organic compound that exchanges electrolytes, including potassium for sodium, in the gastrointestinal tract. It is often combined with sorbitol to enhance its delivery to the GI tract and avoid constipation. Due to its adverse side effect profile, sodium polystyrene sulfonate has significant limitations for chronic use and has not been evaluated in large randomized trials (Kessler et al. 2011; Harel et al. 2013; Yuan et al. 2013). In addition to gastrointestinal side effects that include severe and even fatal gastrointestinal complications, its use in HF is limited as it may worsen edema by exchanging sodium for potassium ions (Nepal et al. 2010).

9.2 Novel Therapies for Hyperkalemia

Patiromer (patiromersorbitex calcium/RLY5016) and sodium zirconium cyclosilicate (ZS-9) are two new promising potassium-lowering compounds (Table 2).

Table 2 Comparison of sodium polystyrene sulfonate (SPS), patiromersorbitex calcium (RLY5016), and sodium zirconium cyclosilicate (ZS-9)

	Sodium polystyrene sulfonate	Patiromersorbitex calcium	Sodium zirconium cyclosilicate
FDA approval	Approved as Kayexalate	Approved as Veltassa	Under review
Structure	Benzene, diethenyl-polymer, with ethenylbenzene, sulfonated, sodium salt, organic polymer	100 μm bead, organic polymer	Octahedral, micropore ring 3 Å diameter, inorganic crystal
Mechanism of action	Binds Na^+, K^+, Ca^{2+}, or Mg^2 High selectivity for Ca^{+2} (Stavros et al. 2014) Works mostly in colon (Stavros et al. 2014)	Binds Na^+, K^+, Ca^{2+}, or Mg^2 Works mostly in colon (Stavros et al. 2014)	Selectivity for K^+ Works in entire GI tract (Stavros et al. 2014)
Administration	15–60 g, up to 4 times daily (Bashour et al. 1975)	21–35 g, twice daily, oral (Weir et al. 2015b)	5–15 g, once daily, oral (Packham et al. 2015)
Storage temperature	Room temperature (Sterns et al. 2010)	2–8°C (Weir et al. 2015b)	Room temperature (Packham et al. 2015)
Efficacy			
Normalize serum K^+	Variable and not known	1 week (mean) (Weir et al. 2015b)	2.2 h (mean) (Kosiborod et al. 2014)
Normokalemia maintained	Variable and not known	52 weeks (so far known) (Bakris et al. 2015)	52 weeks (so far known) (FDA 2014)
Safety			
Edema	Not known	None	1.3% (14 days) (Packham et al. 2015), 7.9% (28 days) (Kosiborod et al. 2014)
Worsening of CKD	Not known	6.3% (over 52 weeks) (Bakris et al. 2015)	Not known
Mild-to-moderate GI AE	Variable (Lepage et al. 2015)	15% (52 weeks) (Bakris et al. 2015)	5.3% (open-label phase) (Kosiborod et al. 2014) 1.8% (maintenance phase) (Kosiborod et al. 2014)
AE			
Severe GI AE	Colonic necrosis: case reports (Sterns et al. 2010; Watson et al. 2010)	None	None
Hypomagnesemia	Reported (Bashour et al. 1975)	7.2–24% (Pitt et al. 2011; Bakris et al. 2015; Weir et al. 2015b)	None

(continued)

Table 2 (continued)

	Sodium polystyrene sulfonate	Patiromersorbitex calcium	Sodium zirconium cyclosilicate
Hypokalemia/ increased QTc	Reported (Einbinder et al. 2015)	3–5.6% (Bakris et al. 2015; Pitt et al. 2015)	0–11% (Kosiborod et al. 2014), dose dependent
Calcium	Reported hypercalcemia (Bashour et al. 1975)	Possible hypocalcemia (Allon and Shanklin 1996), rare	None
Phosphosphate	Not known	None to minimal (Pitt et al. 2011, 2015)	None

FDA US Food and Drug Administration, Na^+ sodium ion, Ca^{2+} calcium ion, Mg^2 magnesium ion, K^+ potassium ion, *Å* angstrom unit, *GI* gastrointestinal, *AE* adverse events, *CKD* chronic kidney disease, *QTc* QT interval

9.2.1 Patiromer

Patiromer is a non-absorbed polymer that was recently approved for clinical use by the FDA that is designed to bind potassium in the gastrointestinal tract and reduce serum potassium levels. It was developed to have greater potassium binding ability than SPS by using calcium instead of sodium as the exchange cation. It is fully ionized for ion exchange at the physiological pH in the colon, where the potassium ion concentration is highest. Its properties are further described below:

Structure

Patiromer is comprised of a polymer anion and calcium-sorbitol counterion (RLY5016S) (Fig. 5). However, unlike SPS, the sorbitol amount in patiromer is 5–10 times less and does not induce diarrhea. Patiromer is a synthetic carboxylic acid compound made as a 100 μm bead (lower molecular weight than SPS) with optimized flow and viscosity properties. It is made in a high-yield 2-step process with polymerization followed by hydrolysis (Li et al. 2016). This process forms an emulsion; the organic component forms spheres containing the monomer, which forms cross-links to make a polymer. Due to its lower pKa, patiromer is acidic and thus allows it to sustain its potassium binding capacity at the physiologic pH of the small and large intestines (Li et al. 2016, p. 397).

Mechanism of Action

Patiromer is administered twice daily and promotes ionization of the polymeric potassium-binding moiety under pH conditions present along the full length of the gastrointestinal tract, but predominantly in the colon (Sterns et al. 2010). As a result of this structure, patiromer exchanges monovalent (sodium ion-Na^+) and divalent (calcium ion-Ca^{2+}, magnesium ion-Mg^{2+}) cations throughout the length of the gastrointestinal tract, preferentially binding potassium in the colon where its concentration is substantially higher than that of Na^+, Ca^{2+}, or Mg^{2+} (Fordtran and Locklear 1966). Increased potassium secretion in the colon through big-potassium channels represents an adaptive response to hyperkalemia, leading to the net effect

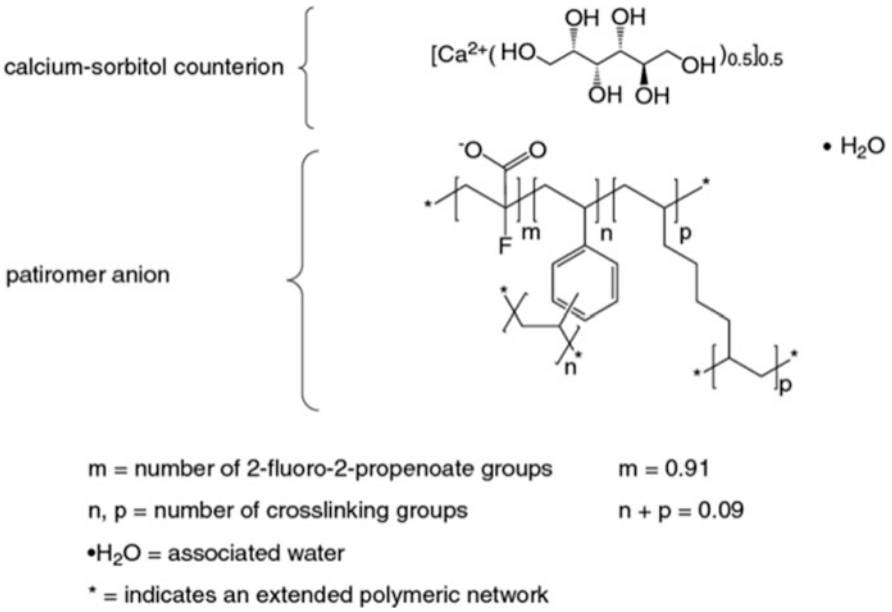

Fig. 5 Chemical structure of patiromer. http://www.relypsa.com/veltassa/prescribing-information/

of a reduction in serum potassium (Sorensen et al. 2010). Patiromer particles are too large to be absorbed in the gastrointestinal tract. Studies using quantitative whole-body autoradiography on rats treated with ^{14}C-RLY5016 did not detect any radiolabeled patiromer-polymer outside the GI tract.

Efficacy

The Study Evaluating the Efficacy and Safety of Patiromer for the Treatment of Hyperkalemia (OPAL-HK) included 237 CKD patients with potassium levels ranging from 5.1 to 6.4 mmol/L who were on RAASi (Weir et al. 2015b). The study consisted of two phases: a 4-week phase in which patients received patiromer 4.2 or 8.4 g twice a day followed by an 8-week withdrawal phase in which patients were randomized to continue the initial dose or switched to placebo. Seventy-six percent of patients achieved normal potassium levels in 4 weeks. During the withdrawal phase, the incidence of hyperkalemia was 15% in the treatment arm and 60% in the placebo group. The Patiromer in the Treatment of Hyperkalemia in Patients With Hypertension and Diabetic Nephropathy (AMETHYST-DN) (Bakris et al. 2015) was a multicenter, open-label, dose-ranging, randomized trial with a total of 306 patients with diabetes mellitus, CKD (estimated glomerular filtration rate, 15 to <60 mL/min/1.73 m^2) and serum potassium levels above 5.0 mmol/L. All patients received RAASi prior to and during study treatment. Treatment with patiromer in both the mild and moderate hyperkalemia groups resulted in reduction in potassium levels at 4 weeks and was maintained at 52 weeks. It is important to

note that patients in both the OPAL-HK and AMETHYST-DN trials were able to maintain RAASi therapy.

Efficacy Trials in Heart Failure

A subgroup analysis in the OPAL-HK trial studied the effects of patiromer in HF patients (Pitt et al. 2015). Of the 102 patients in the first 4 weeks, 76% patients achieved serum potassium levels ranging from 3.8 to 5.0 mmol/L. In the second withdrawal phase, hyperkalemia occurred in 52% ($n = 22$) of the patients on placebo and 8% ($n = 27$) of patients on patiromer ($P < 0.001$). The Evaluation of Patiromer in Heart Failure Patients (PEARL-HF) trial studied patiromer in patients with chronic HF (Pitt et al. 2011). Patients either had estimated glomerular filtration rate of <60 mL/min or had a history of hyperkalemia resulting in the discontinuation of a RAASi. A total of 155 patients were started on 25 mg/day of spironolactone and were randomized to double-blinded treatment with 30 g/day of patiromer or placebo for 4 weeks. At 4 weeks, the patiromer treatment group had significantly lower potassium levels (7.3% vs. 24.5%, $P = 0.015$) and was more likely to have spironolactone increased to 50 mg/day (91% vs. 74%, $P = 0.019$).

Safety and Tolerability

Patiromer is not absorbed by the gastrointestinal tract and therefore has a safer side effect profile. In addition, the uniform spherical shape, bead size, and low swelling ratio may contribute to the improved tolerability of patiromer. In the OPAL-HK trial the most common adverse side effect was constipation (11%) followed by diarrhea (8%), hypomagnesemia (8%), and hypokalemia (3%). Magnesium replacement therapy was initiated in 4% of patients during the initial phase. In the withdrawal phase, constipation, diarrhea, and nausea (4% each) were the most commonly reported gastrointestinal events with patiromer while none of these events occurred in the placebo group (Pitt et al. 2015). Hypomagnesemia is reported in clinical trials studying patiromer, but there are no significant neuromuscular or cardiac abnormalities noted with treatment (Pitt et al. 2011; Bakris et al. 2015; Weir et al. 2015b; Fulton n.d.).

In the PEARL-HF trial, the most common adverse events were gastrointestinal (12%, $n = 21$: flatulence, diarrhea, constipation, or vomiting) (Pitt et al. 2011). When patiromer is used as preventive therapy, hypokalemia is an important concern in HF patients. In the PEARL-HF trial, hypokalemia (<3.5 mmol/L) was present in 6% of patients on patiromer versus 0% of placebo patients ($P = 0.094$) (Pitt et al. 2011). In addition, hypomagnesemia (<1.8 mg/dL) was observed in 24% of patients in the treatment group versus 2.1% of patients in the placebo group. Adverse events resulting in patient withdrawal were similar in both groups (Pitt et al. 2011).

Interaction with Other Drugs

In vitro studies showed patiromer binding greater than 30% in 14 of the 28 drugs tested. The following is a list of drugs and the extent of interaction with patiromer:

- 30–50% binding to: clopidogrel, furosemide, metformin, warfarin, metoprolol, verapamil, and lithium.
- >50% binding to: amlodipine, cinacalcet, ciprofloxacin, levothyroxine, quinidine, thiamine, and trimethoprim (Department of Health and Human Services 2015).
- 30% reduction in availability of valsartan and rosiglitazone in preclinical coadministration studies in rats (Pitt et al. 2011).

However, the Phase 1 open label three-way crossover randomized trial conducted per FDA recommendations on 12 of 14 drugs tested in healthy patients showed (1) no clinically meaningful reduction in absorption and no impact on peak concentration (C_{max}) of lithium, trimethoprim, verapamil, and warfarin; (2) no clinically meaningful reduction in absorption with some reduction in C_{max} of amlodipine, cinacalcet, clopidogrel, furosemide, and metoprolol; and (3) reduced absorption and C_{max} of ciprofloxacin, levothyroxine, and metformin. Quinidine and thiamine were not tested (http://www.investor.relypsa.com/releasedetail.cfm?releaseid=951581).

9.2.2 Sodium Zirconium Cyclosilicate (ZS-9)

ZS-9 is an inorganic, orally administered, potassium-binding compound that has recently been investigated in Phase II and III clinical trials. It is under review by the FDA for approval for clinical use.

Structure

The structure of ZS-9 is composed of octahedrally and tetrahedrally oxygen linked zirconium (Zr) and silicon atoms (Fig. 6). Zirconium has very low toxicity and has been known to selectively extract ammonium from cation mixtures. ZS-9 is administered once daily and was developed after the modification of the Zr molecule so that it would resemble the physiologic potassium ion channel. It selectively captures potassium by filtering ions based on diameter. This is accomplished by the use of a selectivity filter (Fulton n.d.). To pass through the channel, the potassium ion must shed its hydration sphere to allow interaction with the carbonyl oxygen in the channel. Sodium and potassium would both fit in the ZS-9 structure ring but after shedding their hydration shells, only potassium fits in the ZS-9 pore (Stavros et al. 2014) (Fig. 7). The average size of the pore is approximately 3 Å.

Mechanism of Action

ZS-9 is not absorbed in the gastrointestinal tract due to its insoluble properties and is available as free-floating, odorless, tasteless, white crystalline powder. Unlike sodium polystyrene sulfonate, ZS-9 is an inorganic compound that specifically traps monovalent cations (potassium and ammonium) rather than divalent cations (for example: Ca^{2+} and Mg^{2+}). Since it is not systemically absorbed, the risk of systemic toxicity is low (Kosiborod et al. 2014; Ash et al. 2015; Packham et al. 2015). The potassium exchange capacity of ZS-9 was studied using a simulated gastrointestinal

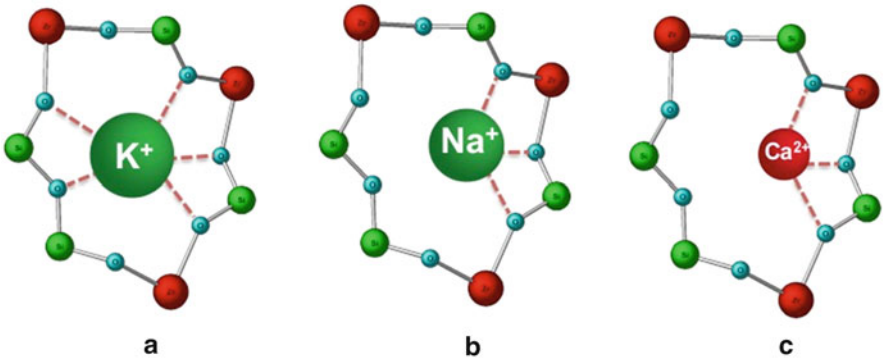

Fig. 6 ZS-9 pore detail with a potassium ion (**a**), a sodium ion (**b**), and a calcium ion (**c**). The specificity for the potassium is likely due to the size and binding sites of the pores. Adapted from: Stavros S, et al. PloS one. 2014;9:e114686

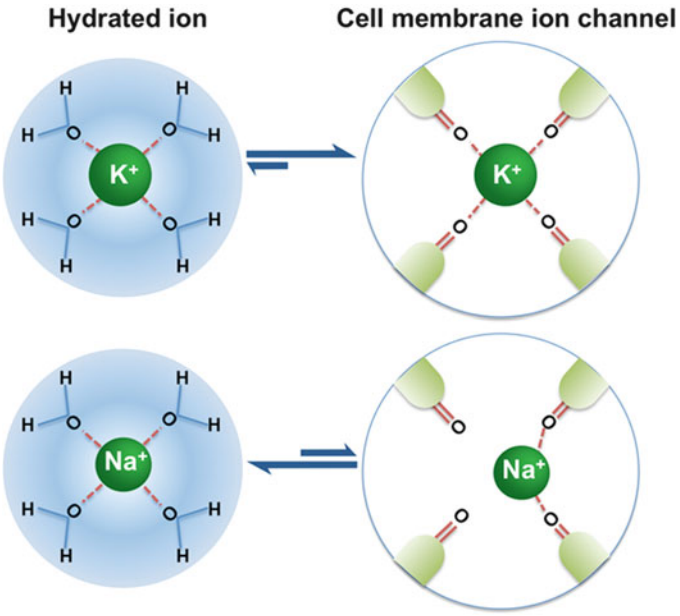

Fig. 7 Physiologic potassium channel and its selectivity to K^+. After shedding the water coat, the K^+ is able to pass through the channel while Na^+ is not, by the virtue of its small size and inability to interact with the oxygen atoms. Adapted from: http://dx.doi.org/10.1371/journal.pone.0114686.g002

tract medium with varying concentrations of ZS-9 and medium pH (Stavros et al. 2014). In simulated gastric fluid (pH ~1.2), there is an observed initial drop in potassium concentration that reverses soon afterwards. In the small intestine (pH ~4.5), there is an immediate uptake of potassium followed by a small release

with equilibrium reached in about 20 min. In the large intestine (pH 6.8), there is a rapid uptake of potassium during the first 10 min followed by slower but continuous uptake over the next hour with no further increase thereafter. It was also observed that the binding capacity of ZS-9 increased proportionally with increasing concentrations of the drug, ranging between 0.5 and 50 mg/ml. These results concluded that in environments mimicking the human gastrointestinal tract, ZS-9 led to potassium equilibrium relatively quickly (less than 20 min) (Stavros et al. 2014). After shedding its water coat, magnesium has a diameter of 1.44 which is not sufficient to capture the oxygen bonds of ZS-9 (Paula et al. 1996; Stavros et al. 2014).

Efficacy

ZS-9 led to a dose-dependent reduction in serum potassium levels over 14 days, including at 48 h in the ZS-003 trial (Packham et al. 2015). In another short-term phase II study by Ash et al., even lower doses of ZS-9 showed favorable results with significant reductions in potassium levels (Ash et al. 2015).

The Hyperkalemia Randomized Intervention Multi-Dose ZS-9 Maintenance Clinical Trial (HARMONIZE) (Kosiborod et al. 2014) enrolled ambulatory patients with hyperkalemia (>5.1 mmol/L). In the first 48 h, patients ($n = 258$) received 10 g of ZS-9 3 times a day, which resulted in the reduction of potassium levels from 5.6 to 4.5 mmol/L. The median time to normalization was 2.2 h. Normokalemia was achieved in 84% of patients within 24 h and 98% of patients within 48 h. This was followed by the maintenance phase, where patients who achieved normokalemia were randomized to receive ZS-9 5 g ($n = 45$), ZS-9 10 g ($n = 51$), ZS-9 15 g ($n = 56$), or placebo ($n = 85$) daily for 28 days. Maintenance of normokalemia was observed with once daily doses of ZS-9 at 5, 10, and 15 g with serum potassium levels of 4.8, 4.5, and 4.4 mmol/L, respectively versus placebo (5.1 mmol/L). The trial also illustrated that patients with higher baseline potassium levels experienced greater absolute reduction after ZS-9 treatment.

Efficacy in Heart Failure

A subgroup population analysis of heart failure patients ($n = 94$) from the HARMONIZE trial showed that all three doses of ZS-9 (5, 10, and 15 g) were effective in lowering and maintaining normal potassium levels with similar safety profile, including patients on RAASi therapy (Anker et al. 2015).

Safety and Tolerability

In the ZS-003 trial, the rate of adverse events was similar in the ZS-9 group (12.9%) and placebo group (10.8%), with diarrhea being the most common side effect in both groups (1.7% in ZS-9 vs. 2.2% in placebo). In the HARMONIZE trial, adverse events occurred in 53%, 29%, and 44% of patients receiving ZS-9 doses of 5 g, 10 g, and 15 g, respectively, versus a 32% adverse events occurrence in patients receiving the placebo. Edema was more common in the Zs-9 15 g group. Gastrointestinal side effects in the Zs-9 group occurred at similar rates in the placebo group

(7%, 2%, 9% for 5 g, 10 g, and 15 g groups, respectively, vs 14% placebo) (Ash et al. 2015).

10 Effect of Patiromer and ZS-9 on Aldosterone and Blood Pressure

10.1 Aldosterone

In HF pathophysiology, renal hypoperfusion activates the RAAS system which increases norepinephrine (NE) and angiotensin II, causing vasoconstriction and release of aldosterone through α-adrenergic and angiotensin II type 1 receptors (Holtz 1993). Salt and water retention is stimulated along with renal potassium excretion and activation of the sympathetic nervous system (Sobotka et al. 2012). RAASi inhibit aldosterone resulting in decreased potassium excretion (Palmer 2004; Urso et al. 2015). Weir et al. (2015a) analyzed the effect of patiromer on serum aldosterone levels in patients with CKD in the OPAL-HK trial. Patiromer utilization for 4 weeks and 8 weeks resulted in a reduction in plasma aldosterone levels and urine aldosterone to creatinine ratio. Similarly, a reduction in systolic and diastolic blood pressures and urinary albumin to creatinine ratio was also observed. Data from the HARMONIZE trial showed a 30% reduction in serum aldosterone with ZS9 after 28 days of treatment. This improvement in laboratory data needs to be studied further in a clinical setting and its importance needs to be established in regards to clinical outcomes.

10.2 Blood Pressure

A subgroup analysis on a cohort of 79 out of 306 patients with diabetic kidney disease and resistant hypertension (defined as systolic blood pressure [SBP] $>$140 mmHg on four or more classes of antihypertensive drugs) who were treated with patiromer for hyperkalemia and continued on RAASi therapy for 52 weeks showed a decrease in systolic and diastolic blood pressures of -18 ± 17 mmHg/-9.0 ± 13 mmHg, respectively (Epstein et al. 2016). The interim analysis of 711 patients enrolled in an ongoing ZS-9 study (ZS005) to evaluate the long-term (52 weeks) efficacy and safety of ZS-9 showed hypertension in 7% (48 out of 684) of patients (Tumlin 2015). Hence, treating hyperkalemia with patiromer in addition to maintenance of RAASi therapy may improve blood pressure control. In a post hoc analysis of OPAL-HK data in patients with CKD, hypertension and on diuretics, it was determined that the efficacy of patiromer was not compromised in this population (Weir 2017, p. 396). Further prospective data on patiromer is needed to further evaluate its clinical benefit on blood pressure in addition to hyperkalemia, especially when used chronically and preventively.

11 Chronic Use for Prevention of Hyperkalemia

The treatment of acute hyperkalemia is well recognized. It is equally important to develop chronic preventive treatments that can be used both by previously hyperkalemic patients and in those who are at risk of developing hyperkalemia. The utility of this approach will require long-term data on the safety of these compounds as well as the efficacy of such an approach to optimize RAASi utilization. This will require both clinical outcome and quality of life data that compares patients' willingness to continue therapy versus using other approaches, such as dietary potassium restriction in patients already on low carbohydrate and low sodium diets. In addition, the risk of rebound hyperkalemia in HF patients optimized on RAASi who are noncompliant with preventive hyperkalemic agents or abruptly discontinue therapy needs to be studied.

12 Hyperkalemia: State of the New Treatments

Further data on the safety of these new drugs needs to be considered. Additionally, this will provide an opportunity to study those groups of patients who were excluded from clinical trials due to hyperkalemia. Though subgroup analysis of HF patients supported the ability of these agents to continue RAASi, further trials to evaluate the up-titration of RAASi to optimal dosing regimens in HF are needed (Anker et al. 2015). Based on these evidence gaps, the following areas for future research are proposed:

(a) Prevention of hyperkalemia and the ability to optimize RAASi. It is unknown if this is possible even if hyperkalemia is prevented since patients may still be intolerant of optimal doses of RAASi due to hypotension or worsening renal function.
(b) Broadening the use of RAASi to patients with eGFR <30 mL/min, who have generally been excluded from previous trials. These patients may benefit more from RAASi due to higher risk but it is not known. Additionally, in this high-risk group the efficacy of novel binders may be different.
(c) Assessing higher doses of RAASi than previously used, e.g., high dose in the setting of acute HF.
(d) Cost-effectiveness data that may include impact of novel agents on length of stay in the hospital, admission to the intensive care unit, and need for rescue emergent treatment (such as death, arrhythmias, dialysis, and other emergent measures).
(e) Patient demographics, including genetics and ethnicity. Outcome data is required through trials and more importantly through easy to conduct observational studies in the inpatient and outpatient setting.
(f) These evidence gaps would likely require large-scale morbidity and mortality trials in the long term. However, short-term safety and tolerability trials would be more practical if the newer potassium lowering agents reduced hyperkalemia, rendering

the use of RAASi to improve outcomes in high-risk populations where it has not yet been tested.

13 Conclusion

The current HF guidelines recommend that MRAs should not be used in patients with eGFR less than 30 mL/min or serum potassium levels greater than 5.0 mmol/L (McMurray et al. 2012; Yancy et al. 2013a, b). This leads to the exclusion of a significant group of patients (18–40%) with HFrEF who are not prescribed MRAs (Maggioni et al. 2013; Tebbe et al. 2014). Similarly, the use of ACEi or ARB is only seen in 55–63% of patients with CKD in primary care patients. However, there is a lack of literature quantifying the role of hyperkalemia that results in decreased utilization of RAASi in this population (Mold et al. 2014). The promising initial results shown by patiromer and ZS-9 may impact this paradigm favorably. Although both compounds have demonstrated positive short-term and longer-term efficacy and tolerability, the long-term optimization of RAASi with the use of potassium binders needs to be evaluated, especially when compared to the standard of care treatment.

It is evident that there is need for treatment for both acute and chronic hyperkalemia, especially in the high-risk population with renal impairment and heart failure. If the development of newer agents like patiromer and ZS-9 is established by long-term safety and efficacy data, it may bring a positive paradigm shift to the management of hyperkalemia.

Funding Source

None.

Conflict of Interest, Disclosures, and Relationship with Industry

CS and AAB report no disclosures. JB reports receiving research support from the National Institutes of Health, and European Union, and serve as a consultant to Amgen, Astra Zeneca, Bayer, Boehringer Ingelheim, Cardiocell, CVRx, Luitpold, Novartis, Relypsa, and ZS Pharma. S.D.A. has received honoraria from, and has been a consultant to ThermoFisher Scientific, Cardiorentis, Bayer HealthCare AG, ZS Pharma, Relypsa, Novartis Pharma AG, and Vifor Pharma.

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Iron Deficiency Treatment in Patients with Heart Failure

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Abstract

Iron deficiency (ID) is one of the major risk factors for disability and mortality worldwide, and it was identified as a common and ominous comorbidity in patients with heart failure (HF), both with and without anaemia. Based on two clinical trials (FAIR-HF and CONFIRM-HF) and other epidemiological evidence, ID has been recognized as an important therapeutic target in symptomatic patients with HF and LVEF $\leq$45%.

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J. Bauersachs et al. (eds.), *Heart Failure*,
Handbook of Experimental Pharmacology 243, DOI 10.1007/164_2017_30

Intravenous iron supplementation has been demonstrated to be safe and effective for iron repletion and related with an improvement in clinical status, exercise capacity, and quality of life. Ongoing trials are testing the hypothesis that such a therapy may also reduce the risk of HF hospitalizations and cardiovascular death.

Keywords
Ferric carboxymaltose • Ferritin • Heart failure • Hepcidin • Intravenous iron • Iron deficiency • Iron therapy • Transferrin saturation soluble transferrin receptor

1 Introduction

Iron deficiency (ID) is one of the leading risk factors for disability and mortality worldwide, affecting an estimated 2 billion people (Zimmermann et al. 2007). The prevalence of ID in different populations varies according to host factors including age, gender, as well as some physiological, pathological, environmental factors, and socioeconomic conditions (Zimmermann et al. 2007; Andrews 1999; Clark 2009; McLean et al. 2009; Lundström 1994).

ID reflected as iron deficiency anaemia accompanies many chronic diseases most often associated with chronic inflammation, autoimmune disease (particularly in rheumatoid arthritis), kidney disease, or neoplastic process.

It needs to emphasize that ID can exist with or without anaemia. Although untreated ID can result in anaemia as a consequence of more advanced and longer lasting ID, ID itself reveals several clinical unfavourable effects. In recent decades, heart failure (HF) has been identified as a disease which is commonly accompanied by ID, both with and without anaemia (Anker et al. 2009a; Ponikowski et al. 2015). Moreover, ID occurring in patients with HF has been identified as an important therapeutic target (Ponikowski et al. 2016; Drozd et al. 2016).

2 Significance of Iron for Human Physiology

Iron is a biologically critical element for almost all living entities, ranging from organella, through cells, tissues, up to whole organisms, as it participates in a wide variety of metabolic processes, including oxygen transport (as haemoglobin component), oxygen storage (as myoglobin component), ribonucleic acid synthesis, and electron transport (Andrews 1999; Kell 2008; Fairbanks and Beutler 2001; Hower et al. 2009; Rouault and Tong 2005; Beard 2001; Carrondo 2003; Cairo et al. 2006; Dunn et al. 2007).

Iron exists in two oxidative states as bivalent ferrous (Fe^{2+}) and trivalent ferric (Fe^{3+}) iron, and these states can be changed in metabolic reactions. Iron is necessary for the formation of haem enzymes and other iron-containing enzymes like flavin-iron enzymes, transferrin, and ferritin involved in electron transfer and oxidation–reductions (Hurrell 1997; McDowell 2003). However, as iron can form free radicals, its concentration in body tissues must be tightly regulated because in excessive amounts, it

can lead to tissue damage. Human beings are unable to excrete iron actively, so its concentration in the body must be regulated predominantly at the site of iron absorption (Zimmermann et al. 2007).

Almost two-thirds of the body iron is found in the haemoglobin present in circulating erythrocytes, 25% is contained in a readily mobilizable iron store, and the remaining 15% is bound to myoglobin in muscle tissue and in a variety of enzymes involved in the oxidative metabolism and many other cell functions (Institute of Medicine 2001; Stugiewicz et al. 2016). Iron is important not only for haematopoietic system (mainly erythron, but not only), but having in mind that ID leads to mitochondrial dysfunction, there is growing evidence that preserved intracellular iron status is needed for optimal functioning of tissues of high energy demand, such as skeletal muscle tissue (Stugiewicz et al. 2016; Haddad et al. 2016).

3 Pathogenesis of Iron Deficiency in Heart Failure

In general, the origin of ID is related with reduced iron intake, increased iron losses, and/or the abnormal iron distribution when it is functionally not available for body (Fairbanks and Beutler 2001).

There is scarce evidence that ID in HF partially results from inadequate diet iron intake (Hughes et al. 2012; Lourenço et al. 2009), low bioavailability of iron, or handicapped gastrointestinal absorption (also as a consequence of intestinal interstitial oedema, the use of drugs lowering gastric pH such as omeprazole or other H2 receptor antagonists, and the use of drugs reducing iron absorption such as calcium, tannins, oxalates, phytate, and phosphates) (Hallberg and Hulthén 2000; González-Costello and Comín-Colet 2010). Patients with HF demonstrate reduced iron intake in their diet (Hughes et al. 2012; Lourenço et al. 2009), but its significance in the development of overt ID seen in circulating biomarkers has not been shown yet. Iron loss may be increased in the course of gastrointestinal disorders (peptic ulcer, esophagitis, gastritis, and duodenitis), menstrual blood loss, excessive blood sampling, to name but a few (Fairbanks and Beutler 2001). Moreover, there is no direct relationship between the prevalence of ID and the use of anticoagulants or/and antiplatelet drugs in patients with HF (Klip et al. 2013; Jankowska et al. 2010). Importantly, although anticipated, all aforementioned pathomechanisms have not been confirmed to be present in patients with HF, hence still remain hypothetical.

It has been established that inflammatory state characterizing several chronic diseases (including HF) is considered to be responsible for impaired iron absorption, recycling, and release from body stores (Kell 2008; Wessling-Resnick 2010; Balla et al. 2007). It has been anticipated that the pathogenesis of ID in the course of HF resembles the pathomechanisms (related directly with inflammatory status) seen in the course of chronic kidney disease (CKD). Patients with CKD demonstrate mainly the so-called functional ID, namely due to inflammation and related high circulating hepcidin iron is present in the body but trapped in reticuloendothelial cells and therefore not available for metabolic needs (Weber et al. 2013; Jankowska et al. 2013a; Nemeth and Ganz 2006; Nicolas et al. 2002; Silvestri 2013). Importantly, in

some observational prospective studies, one recruiting patients with stable HF, the second one performed among acute HF patients, there were no direct relationships between parameters reflecting iron status and circulating inflammatory biomarkers (Jankowska et al. 2013b, 2014). Moreover, patients with HF both in chronic (Jankowska et al. 2013b) and acute settings (Jankowska et al. 2014) demonstrate extremely low (but not high) circulating hepcidin, which suggests that ID seen in the course HF is the consequence of depleted iron stores in the body (the so-called absolute ID).

4 Assessment of Iron Deficiency Based on Circulating Biomarkers

Bone marrow aspiration is the most accurate method to assess iron status (Moreno Chulilla et al. 2009; Goddard et al. 2011; Gale et al. 1963; Pasricha et al. 2010; Goodnough et al. 2010), but this examination is invasive, not widely available, and unsuitable to assess ID in the clinical practice. Actually, laboratory blood tests based on circulating iron biomarkers are indirect but acceptable methods to diagnose and monitor ID (Fairbanks and Beutler 2001).

Ferritin is one of the most useful laboratory measures of iron status (Mei et al. 2005). It reflects the body iron stores. Ferritin level <100 μg/L is considered to reflect an absolute ID in HF. Ferritin values are proportionally lowered when iron stores are more depleted in the body. In a general population, the cut-off of serum ferritin to diagnose absolute ID is usually 30 μg/L, (Goodnough et al. 2010; Koulaouzidis et al. 2009), although lower values (i.e. 12–15 μg/L) have also been applied (Ali et al. 1978; WHO 2011).

Ferritin is also an acute-phase protein. Therefore, in healthy individuals, ferritin is directly proportionally related to the amount of body iron stores; however, its values increase in case of concomitant acute or chronic inflammation (Umbreit 2005). Also, its values are unreliable in patients with malignancy, hyperthyroidism, liver disease, or heavy alcohol intake (Umbreit 2005). Therefore, in such clinical scenarios, the interpretation of circulating ferritin needs to be very critical.

Hepcidin is considered as the major regulator of iron metabolism and a part of an innate immune response (Kemna et al. 2008; Handelman and Levin 2008; Nemeth and Ganz 2009; Franchini et al. 2010; Viatte and Vaulont 2009; Babitt and Lin 2010). It is a very conservative polypeptide produced by hepatocytes. Hepcidin synthesis by hepatocytes is precisely regulated in order to optimize and synchronize iron metabolism and/or antimicrobial response (Kemna et al. 2008; Handelman and Levin 2008; Nemeth and Ganz 2009; Franchini et al. 2010; Viatte and Vaulont 2009; Babitt and Lin 2010). Major and independent stimuli decreasing hepcidin expression in the liver and its release into the circulation are: depleted iron stores, hypoxia, and ineffective erythropoiesis, whereas inflammation/infection produces the opposite effect.

Hepcidin correlates with iron stores more precisely than ferritin, particularly low circulating hepcidin reflects depleted iron stores, even in the presence of concomitant inflammation. Circulating hepcidin causes the reduced expression of proteins

involved in transmembrane iron import to enterocytes and also an internalization of ferroportin, the only protein able to export intracellular iron (Kemna et al. 2008; Handelman and Levin 2008; Nemeth and Ganz 2009; Franchini et al. 2010; Viatte and Vaulont 2009; Babitt and Lin 2010; Hentze et al. 2010). Hence, hepcidin blocks intestinal absorption of iron (contributing to absolute ID) and diverts iron from the circulation into the reticuloendothelial system, resulting in iron depletion in target tissues (leading to functional ID) (Nemeth and Ganz 2009; Franchini et al. 2010; Viatte and Vaulont 2009; Babitt and Lin 2010; Hentze et al. 2010).

The measurement of circulating hepcidin is challenging and not standardized yet, hence until now its assessment is used only for research purposes. Another parameter used for clinical assessment of iron status is transferrin saturation (Tsat) (Pasricha et al. 2010; Goodnough et al. 2010; Wish 2006; Briggs et al. 2009). It is calculated as a ratio of serum iron and circulating iron bound to transferrin (TIBC, total iron binding capacity) expressed in percentage (%). This inexpensive and easily available index reflects roughly the amount of iron available for cellular metabolism (Jankowska et al. 2013a). Tsat <20% is used for the diagnosis of depleted iron available for target cells, and this cut-off is applied in both a general population and patients with chronic diseases accompanied by low-grade inflammation (e.g. HF).

Soluble transferrin receptor (sTfR) is a relatively novel iron biomarker already used by haematologists for diagnosis of ID-related anaemia; however, it should be emphasized that regardless of haemoglobin level high sTfR reflects insufficient intracellular iron availability for metabolic needs (red). TfR is the major transmembrane molecule which facilitates an iron influx to the cell. In case of deficient iron within cells, its membrane expression as an adaptive response is increased, and excessive particles are shed to the circulation. What is important, level of circulating sTfR is not substantially affected by acute-phase response and/or inflammation (Cook 2005).

The commonly accepted criteria for detecting ID in patients with HF are serum ferritin <100 μg/l (identifying absolute ID), or serum ferritin 100–299 μg/l in combination with Tsat <20% (identifying functional ID) (Anker et al. 2009a, b; Jankowska et al. 2013a). It should be emphasized that neither serum iron nor serum transferrin (or TIBC) alone is reliable and sufficient for the assessment of iron status in patients with HF. The aforementioned definition of ID has already been used in two major clinical trials in patients with symptomatic HF, where ID was supplemented with intravenous ferric carboxymaltose (FCM) (Anker et al. 2009a; Ponikowski et al. 2015).

There are also attempts to apply novel iron biomarkers in order to improve the accuracy of the definition of ID in the clinical setting of HF. Recently, we have proposed a new pathophysiological definition of ID based on the combined assessment of low serum hepcidin (reflecting depleted iron stores) and sTfR (unmet metabolic needs regarding intracellular iron amount) (Jankowska et al. 2013b, 2014).

The 2016 ESC/HFA guidelines on HF management, as following 2012 ESC/HFA guidelines, emphasize the need to screen all patients with HF for the presence of ID based on serum ferritin and Tsat, regardless of haemoglobin level in order to detect reversible/treatable causes of HF and comorbidities interfering with HF (Ponikowski et al. 2016; McMurray et al. 2012).

5 Prevalence of Iron Deficiency in Heart Failure

ID constitutes a frequent comorbidity in patients with HF. So far, only Nanas et al. investigated the prevalence of ID based on the direct assessment of iron stores in bone marrow and reported ID in anaemic, decompensated HF patients to be approximately 73% (Nanas et al. 2006). Severely depleted iron stores assessed directly in bone marrow tissue have also been demonstrated in half of patients with multivessel coronary artery disease undergoing elective CABG (approx. 50% of them had LV systolic dysfunction as well) (Jankowska et al. 2015).

The prevalence of ID in patients with HF using standard definition ranges from 33 to 74% (Klip et al. 2013; Jankowska et al. 2010, 2014; Parikh et al. 2011; Comín-Colet et al. 2013; Kasner et al. 2013; Rangel et al. 2014; Yeo et al. 2014; Schou et al. 2015; Ebner et al. 2016; Núñez et al. 2016a; Enjuanes et al. 2016; Okonko et al. 2011). ID is more prevalent in anaemic (43–78%) vs. non-anaemic subjects (15–65%) (Klip et al. 2013; Jankowska et al. 2010, 2014; Parikh et al. 2011; Rangel et al. 2014; Yeo et al. 2014; Okonko et al. 2011), and those with decompensated (65–74%) (Jankowska et al. 2014; Nanas et al. 2006; Núñez et al. 2016a; Cohen-Solal et al. 2014) vs. stable HF (34–65%) (Klip et al. 2013; Jankowska et al. 2010; Parikh et al. 2011; Comín-Colet et al. 2013; Kasner et al. 2013; Rangel et al. 2014; Yeo et al. 2014; Schou et al. 2015; Ebner et al. 2016; Enjuanes et al. 2016; Okonko et al. 2011; de Silva et al. 2006; Belmar Vega et al. 2016; Przybylowski et al. 2016).

The majority of data on the prevalence of ID in HF comes from European HF with reduced ejection fraction (HFrEF) cohorts (Jankowska et al. 2010; Rangel et al. 2014; Schou et al. 2015; Okonko et al. 2011; de Silva et al. 2006); however, few data on patients with both reduced and preserved LVEF have shown similar percentages of iron-deficient enrolees (Klip et al. 2013; Comín-Colet et al. 2013). Importantly, in one study regarding multi-ethnic Southeast Asian population of patients with HF, the prevalence of ID was higher than in the European cohorts (Yeo et al. 2014). Besides anaemia (Klip et al. 2013; Jankowska et al. 2010), the following characteristics can be considered as factors associated with the higher prevalence of ID in patients with HF: female sex (Klip et al. 2013; Jankowska et al. 2010; Cohen-Solal et al. 2014), advanced NYHA class (Klip et al. 2013; Jankowska et al. 2010), higher N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) (Klip et al. 2013; Jankowska et al. 2010), and higher high-sensitivity C-reactive protein (hsCRP) (Jankowska et al. 2010).

6 Clinical and Prognostic Consequences of Iron Deficiency in Heart Failure

In patients with HFrEF, the presence of ID is accompanied by impaired aerobic capacity as reflected by lower peak oxygen consumption (peak VO_2), higher ventilatory response to exercise (VE-VCO_2 slope) (Ebner et al. 2016; Okonko et al. 2011; Jankowska et al. 2011), and shorter 6-min walking test (6-MWT) distance (Ebner et al. 2016). Importantly, in patients with HFrEF, the impact of ID on both peak VO_2

and VE-VCO_2 slope is independent of and much stronger than the effect of anaemia alone (Jankowska et al. 2011).

Similar relationships have been reported in patients with HFpEF (Klip et al. 2013; Comín-Colet et al. 2013; Yeo et al. 2014; Núñez et al. 2016a; Enjuanes et al. 2016). Nunez et al. have shown that in these patients low Tsat and ferritin correlate with impaired functional capacity as assessed in cardiopulmonary exercise test (CPX) (Núñez et al. 2016a). Importantly, in one study including both HFrEF and HFpEF patients, the 6-MWT distance was also decreased in subjects with ID vs. without ID (Enjuanes et al. 2016).

The presence of ID is also associated with decreased health-related quality of life (HRQoL) as assessed using Minnesota Living with Heart Failure Questionnaire (MLHFQ) (Comín-Colet et al. 2013; Enjuanes et al. 2014).

In patients with both HFrEF and HFpEF, concomitant ID predicts increased long-term all-cause mortality independently of the presence of anaemia (Klip et al. 2013; Jankowska et al. 2010, 2014; Okonko et al. 2011) or ethnicity (Yeo et al. 2014).

In patients hospitalized for acute HF, ID increases the risk for re-hospitalization within 30 days after discharge (Núñez et al. 2016b), and the risk for combined all-cause death or non-fatal cardiovascular event (hospitalization for congestive HF, acute coronary syndrome, severe arrhythmia, or stroke) (Rangel et al. 2014), as well as combined death or heart transplantation (Jankowska et al. 2010).

7 Benefits of Intravenous Iron Supplementation in Heart Failure

Although it might be expected that iron-deficient patients with HF may benefit from oral iron supplementation, it should be emphasized that there is no evidence that such a therapeutic approach efficiently replenishes body iron stores. In the recently presented prospective, randomized clinical trial (IRON-OUT), polysaccharide iron complex (150 mg twice daily for 16 weeks) as compared to placebo had a little (if any) effect on circulating ferritin and Tsat in replacing iron stores, and improved neither peak VO2, a 6-MWT distance, oxygen kinetics, ventilatory efficiency, or quality of life in symptomatic patients with HFrEF (Lewis et al. 2016a, b). There is no available evidence to recommend oral iron supplementation in symptomatic patients with HF.

It should be emphasized that previously used parenteral iron preparations were toxic, generated a lot of non-transferrin bound iron, induced oxidative stress, and led to frequent adverse events, such as: hypotension, nausea, vomiting, abdominal and lower back pain, peripheral oedema, and a metallic taste (Silvestri 2013; Heath et al. 1932; Goetsch et al. 1946; Evans et al. 2007). These side-effects have been circumvented due to the introduction of compounds containing iron in a core surrounded by a carbohydrate shell (Macdougall 2009). Until now, only five new parental iron formulations have been studied in patients with HF. Iron sucrose was used in seven studies (136 treated patients in total) (Bolger et al. 2006; Toblli et al. 2007, 2015; Okonko et al. 2008; Usmanov et al. 2008; Terrovitis et al. 2012; Beck-Da-Silva et al. 2013). Iron dextran, iron isomaltose, and ferric gluconate were used

only in small single-centre non-comparative studies with only 40, 20, and 13 treated patients, respectively (Gaber et al. 2012; Kaminsky et al. 2016; Hildebrandt et al. 2010; Reed et al. 2015). FCM was used in two multi-centre, randomized, placebo-controlled, double-blind trials (454 treated patients in total) (Ponikowski et al. 2015).

In the FAIR-HF trial, 304 ambulatory patients with symptomatic HF,LVEF $\leq$40% (NYHA II) or $\leq$45% (NYHA III) and ID (defined as serum ferritin $<$100 ng/mL or ferritin 100–300 ng/mL with Tsat $<$20%) with haemoglobin between 9.5 and 13.5 g/dL were randomized in a 2:1 ratio to receive i.v. FCM or i.v. saline. In this trial, the Ganzoni formula (Ganzoni 1970) was used to calculate the required cumulative FCM dose, namely the cumulative iron deficit [mg] = bodyweight [kg] $\times$ (target haemoglobin − actual haemoglobin) [g/dL] $\times$ 2.4 + iron storage depot [mg]. In patients weighing $<$35 and $\pm$35 kg, the target haemoglobin should be 13 and 15 g/dL, respectively, and the iron storage depot should be 15 mg/kg and 500 mg, respectively (Anker et al. 2009a, b). The dosing frequency was 200 mg of FCM weekly until iron repletion was achieved (the correction phase) and then every 4 weeks during the maintenance phase, which started at week 8 or week 12, depending on the required iron-repletion dose. Primary endpoints were self-reported PGA at week 24 and NYHA class at week 24, adjusted for baseline NYHA class (Anker et al. 2009b), both of which improved in patients treated with FCM as compared to those receiving saline. The improvement in these parameters was found in both anaemic and non-anaemic patients, even though the clinical improvement in non-anaemic patients was not associated with an increase in haemoglobin level (Filippatos et al. 2013). Iron supplementation with FCM resulted also in an increase in the 6-MWT distance and quality-of-life scores (Anker et al. 2009a).

In the CONFIRM-HF trial, ambulatory patients with HF in NYHA class II–III with LVEF $\leq$45%, BNP $>$100 pg/mL, or NT-proBNP $>$400 pg/mL with ID (defined as in the FAIR-HF study) and haemoglobin level $<$15 g/dL were randomized 1:1 to treatment with i.v. FCM or i.v. saline. FCM was administered in a single dose as an undiluted bolus injection of up to 1,000 mg according to a fixed scheme based on the subject's weight and haemoglobin value at screening and administered at weeks 0 and 6. Further FCM doses were administered at weeks 12, 24, and 36 if ID was still present (Ponikowski et al. 2014), but importantly more than 75% of treated patients required a maximum of two doses (Ponikowski et al. 2015). This new dosage pattern appeared to be convergent with a total iron dose administered in patients recruited in the FAIR-HF trial (Filippatos et al. 2013). Treatment with FCM increased the 6-MWT distance at week 24 (the primary endpoint). The treatment effect of FCM was consistent in all clinical subgroups and was sustained to week 52. Throughout the study, an improvement in NYHA class, PGA, quality of life, and fatigue score in patients treated with FCM was demonstrated with a statistical significance confirmed from week 24 onwards. Treatment with FCM was associated with a reduction in the risk of hospitalizations for worsening HF at week 52 (Ponikowski et al. 2015).

In other small studies with other intravenous iron preparations in patients with HFrEF, the following advantageous effects of such a therapy were found: an increase in LVEF, a reduction in LVSD, LVDD, LVPW, IVS thickness, left ventricular mass index, left ventricular end systolic volume, an improvement in S′, E′, a

decline in E/E′, a reduction in peak systolic strain rate (Bolger et al. 2006; Toblli et al. 2007; Okonko et al. 2008; Usmanov et al. 2008; Gaber et al. 2012) as well as a reduction in plasma NT-proBNP and CRP (Toblli et al. 2007).

Several meta-analyses regarding iron therapy in patients with HF and ID have been published (Kapoor et al. 2013; Avni et al. 2012; Desai et al. 2010; Qian et al. 2016; Jankowska et al. 2016). The most recent one compiled the results of five trials performed among HF patients with LVEF ≤45% (509 patients received i.v. iron preparations as compared with 342 controls), with at least a single-blind randomization, and without a concomitant therapy with any erythropoiesis-stimulating agent [139]. The performed meta-analysis has demonstrated that i.v. iron supplementation reduces the risk of urgent HF hospitalization, the risk of combined endpoint of all-cause death or cardiovascular hospitalization, and the risk of combined cardiovascular death or hospitalization for HF worsening, but without the impact on either all-cause or cardiovascular mortality (which might be due to a limited number of reported events and short follow-up) [139]. Additionally, it has been shown that i.v. iron supplementation improves exercise capacity, alleviates HF symptoms, and improves quality of life assessed using questionnaires either specific for HF or those reflecting patients' general medical condition [139].

8 Conclusions and Clinical Perspectives

The recent 2016 ESC/HFA guidelines on HF management (Ponikowski et al. 2016) emphasize the need to screen all patients with HF for the presence of ID based on serum ferritin and Tsat (using the aforementioned definition), regardless of haemoglobin level in order to detect reversible/treatable causes of HF and comorbidities interfering with HF (Ponikowski et al. 2016; McMurray et al. 2012). Intravenous iron supplementation should be started in symptomatic patients with ID and HFrEF in order to alleviate HF symptoms and improve exercise capacity and quality of life (Anker et al. 2009a; Ponikowski et al. 2015). Only i.v. route of iron supplementation has been demonstrated to be safe and effective for iron repletion in these patients. Oral iron supplementation is not effective in this group of patients and cannot be recommended.

Additionally, in order to verify if iron repletion improves outcomes, morbidity, and mortality, trials have already been launched in a population of patients with acute HF (www.clinicaltrial.gov: AFFIRM-AHF, NCT02937454) (www.clinicaltrial.gov: IRONMEN, NCT02642562) and with chronic HF (www.clinicaltrial.gov: FAIR-HF2, NCT03036462).

Funding

This research was financially supported by the National Science Centre (Krakow, Poland) grant allocated on the basis of the decision number DEC-2012/05/E/NZ5/00590.

Conflict of Interest

Wroclaw Medical University received an unrestricted grant from Vifor Pharma. M.D. reports personal fees from Fresenius. E.A.J. reports personal fees from Vifor Pharma and Fresenius. P.P. reports personal fees from Vifor Pharma and AMGEN.

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