Am J Cardiovasc Drugs (2017) 17:83–95
DOI 10.1007/s40256-016-0199-7

REVIEW ARTICLE

Therapeutic Targeting of Cellular Stress to Prevent Cardiovascular Disease: A Review of the Evidence

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Published online: 24 October 2016

Abstract The availability of effective drugs targeting the major risk factors of cardiovascular disease (CVD) has reduced morbidity and mortality. Cumulative relative risk of CVD events can be reduced by 75 % with a combination of aspirin, a β-adrenoceptor antagonist (β-blocker), an HMG-CoA reductase inhibitor (statin), and an angiotensin-converting enzyme inhibitor. The principal pharmacodynamics of these drugs cannot explain the entirety of their cardioprotective action, as other drugs with similar pharmacologic targets have not been associated with favorable clinical effects. This raises the possibility that the cardioprotective drugs have a unique pleiotropic activity that contributes to their clinical efficacy. Recent data suggest that reducing cellular stress such as oxidative, inflammatory, and endoplasmic reticulum stress, might be a common denominator of the drugs with proven efficacy in reducing CVD risk. In this communication, the evidence in favor of this hypothesis is discussed, and ongoing trials with therapeutic agents targeting cellular stresses are reviewed.

Key Points

Cumulative relative risk of cardiovascular events can be reduced by 75 % with a combination of aspirin, a β-adrenoceptor antagonist (β-blocker), an HMG-CoA reductase inhibitor (statin), and an angiotensin-converting enzyme inhibitor.

These cardioprotective drugs share the pleiotropic effects of reducing cellular stresses such as oxidative, inflammatory, and endoplasmic reticulum stress.

Selective targeting of oxidative stress has been shown to be ineffective in reducing cardiovascular risk.

The proof of the hypothesis that reducing cellular stress is a meaningful therapeutic target would hinge on clinical trials with pharmacologic agents that reduce cellular stress without altering the traditional risk factors such as hypertension or dyslipidemia.

1 Introduction

Over the last 5 decades, there have been remarkable advances in the prevention and treatment of cardiovascular disease (CVD) [1, 2]. Many factors account for this success, including technologic advances in interventional cardiology and coronary care units, early resuscitation and intervention protocols, and effective drugs targeting the major risk factors of CVD such as hypercholesterolemia, hypertension, platelet aggregability, and possibly

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hyperglycemia. In 2002, Yusuf [3] suggested that the cumulative relative risk of CVD events such as cardiovascular death, myocardial infarction, and stroke could be reduced by 75 % using a combination of aspirin, a β-adrenoceptor antagonist (β-blocker), an HMG-CoA reductase inhibitor (statin), and an angiotensin-converting enzyme (ACE) inhibitor. Among smokers, cessation of smoking for 2 years lowers risk of recurrent myocardial infarction by about one-half, which—along with the four drugs used—increases cumulative risk reduction to 80 %.

Although lowering of blood pressure is the principal driver of reducing cardiovascular events in most people with hypertension, some antihypertensive drug classes have been shown to confer added protection in patients with proteinuria, coronary heart disease, or heart failure [4, 5]. The 2015 American Heart Association/American College of Cardiology/American Society of Hypertension Scientific Statement on *Treatment of Hypertension in Patients with Coronary Artery Disease* [6] recommended that management should include β-blockers, ACE inhibitors, or angiotensin receptor blockers, and—in people with heart failure—aldosterone antagonists. The statement further concluded that, whereas diuretics and calcium channel blockers can be used for the management of hypertension, the evidence for improved outcomes in hypertension with coronary artery disease (CAD) is very small [6].

The drugs with proven cardioprotective properties are also effective in people with diabetes who are at high risk for CVD. In this population, in addition to statins, β-blockers, and ACE inhibitors, targeting hyperglycemia with metformin can also reduce cardiovascular events [7]. More recently, use of the sodium glucose transporter 2 (SGLT2) inhibitor empagliflozin has been associated with favorable effects [8], whereas other antihyperglycemic agents have had either modestly favorable, marginal, or detrimental effects on CVD [9–13].

The principal pharmacodynamics of these drugs targeting various risk factors of CVD cannot explain the entirety of their cardioprotective action, as many other drugs with similar pharmacologic targets have not been associated with favorable clinical effects. This raises the possibility that these latter drugs either have adverse off-target effects that limit their clinical efficacy in reducing CVD events or that the cardioprotective drugs have unique pleiotropic activity that contributes to their efficacy in reducing major cardiovascular events. Recently available data suggest that reducing cellular stress such as oxidative, inflammatory, and endoplasmic reticulum (ER) stress might be a common denominator of the pleiotropic effects of the drugs with proven efficacy in reducing CVD risk (Table 1) [14–25].

Oxidative and inflammatory processes have been implicated in atherogenesis and in myocardial cell injury especially after ischemia/reperfusion [26]. More recently, ER stress manifesting as abnormal accumulation of misfolded proteins has also been shown to cause cellular apoptosis and is implicated in the progression and rupture of atherosclerotic plaques [27].

In this communication, the cellular stress-reducing properties of drugs known to have reduced cardiovascular mortality in at least subgroups of patients are discussed, and ongoing trials with therapeutic agents targeting cellular stresses are reviewed.

2 Cardioprotective Drugs Targeting Cellular Stress

2.1 Statins

Although hypercholesterolemia and, more specifically, elevated low-density lipoprotein cholesterol (LDL-C) concentration, is an established risk for CVD, and partial ileal bypass-associated reductions in plasma cholesterol levels reduced myocardial infarction and subsequent morbidity [28], only statins have been shown to reduce cardiovascular and all-cause mortality [29–31]. Recently, ezetimibe, a cholesterol-absorption inhibitor, was also shown for the first time to have some cardioprotective effect commensurate with its modest lowering of LDL-C levels [32]. In this trial, patients with an acute coronary syndrome (ACS) within the previous 10 days were randomly assigned to either simvastatin 40 mg plus ezetimibe 10 mg or to simvastatin 40 mg plus placebo. At 1 year, the mean LDL-C level was 53.2 mg/dl (1.4 mmol/l) in the simvastatin-plus-ezetimibe group and 69.9 mg/dl (1.8 mmol/l) in the simvastatin-monotherapy group. At a median of 6 years, the rate of cardiovascular events was modestly but significantly lower in the simvastatin–ezetimibe group [32]. The idea that the benefit of ezetimibe in this trial is mediated solely by the lowering of LDL-C levels has been met with some skepticism because ezetimibe—like statins—has pleiotropic effects, including antioxidant and anti-inflammatory properties [33–36]. Indeed, the levels of high-sensitivity C-reactive protein (hsCRP) were significantly lower in the simvastatin–ezetimibe group than in the simvastatin monotherapy group [36]. The proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors may also prove to be clinically effective in reducing cardiovascular events [37, 38], and long-term trials could be helpful in establishing the cholesterol hypothesis. However, it is possible that the favorable effects of statins may exceed their effectiveness in lowering LDL-C. Cell cultures and studies in animals have shown the efficacy of statins in simultaneously reducing oxidative stress and ER stress [14, 15]. These properties have been associated with their ability to optimize the endothelial barrier function and prevent dextrose-induced endothelial cell death [15].

Table 1 List of drugs with established or possible cardioprotective effects demonstrated in double-blind randomized placebo-controlled clinical trials. Reducing oxidative, inflammatory, and endoplasmic reticulum stress is a common denominator of the pleiotropic effects of the drugs with established efficacy in reducing cardiovascular events

Established cardioprotective effects (strong clinical evidence)			Possible cardioprotective effects (modest clinical evidence)		
Drug class	Specific examples	Principal therapeutic target	Drug class	Specific examples	Principal therapeutic target
Statins	Simvastatin	Lowering of LDL-C	Antihyperglycemic agents	Metformin	Lowering of blood glucose
	Pravastatin			Pioglitazone	
	Atorvastatin			Empagliflozin	
	Rosuvastatin			Liraglutide	
β-Adrenoceptor antagonists	Propranolol	Lowering of BP and myocardial oxygen demand	Reproductive hormones	Estrogens	Reproductive tissues, central nervous system, endothelial cells
	Atenolol				
	Carvedilol				
ACE inhibitors	Captopril	Lowering of BP			
	Lisinopril				
	Enalapril				
ARBs	Losartan	Lowering of BP			
	Candesartan				
	Irbesartan				
Mineralocorticoid receptor antagonists	Spironolactone	Lowering of BP, mild diuretic			
	Eplernone				
Antiplatelet agents	Aspirin	Anti-thrombotic			
	Clopidogrel				

ACE angiotensin-converting enzyme, *ARB* angiotensin receptor blocker, *BP* blood pressure, *LDL-C* low-density lipoprotein cholesterol

The precise mechanism of protective effects of statins is not known. Statins have been shown to attenuate vascular smooth muscle proliferation by preventing Rho GTPase-induced down-regulation of p27Kip1 [39], and Rho GTPase is instrumental in the regulation of endothelial nitric oxide synthase (eNOS), a key enzyme in modulating vascular function [40]. In spontaneously hypertensive rats, atorvastatin treatment downregulated aortic angiotensin type 1 receptor messenger RNA (mRNA) expression, reduced mRNA expression of the essential NAD(P)H oxidase subunit p22phox, and caused an upregulation of endothelial constitutive NOS (ecNOS) mRNA expression [41]. In cell cultures, mRNA expression of superoxide dismutase isoforms and glutathione peroxidase was not modified by atorvastatin, whereas catalase expression was upregulated [42]. These changes contribute to the antioxidant effects of statins.

2.2 β-Adrenoceptor Antagonists

A host of clinical trials with several β-blockers have shown the capacity of this class of agents to reduce major cardiovascular events in high-risk people with CAD and previous myocardial infarction as well as in those with congestive heart failure (CHF) and low ejection fraction [43, 44]. The efficacy of β-blockers in patients with stable coronary heart disease has been questioned as there are no recent randomized controlled trials demonstrating that β-blockers are cardioprotective in patients who are offered reperfusion interventions and intensive medical therapy with aspirin, statin, ACE inhibitors, and angiotensin receptor blockers [44]. Although β-blockers are effective antihypertensive agents, their enhanced beneficial effects are attributed to their anti-arrhythmogenic activity as well as their effectiveness in reducing myocardial oxygen demand through reducing the heart rate and myocardial contractility. However, many known anti-arrhythmogenic drugs do not reduce cardiovascular mortality [45]. Furthermore, calcium channel blockers, which are effective antihypertensive agents and can reduce the heart rate and myocardial contractility, are not associated with survival advantage, although they can be effective in controlling angina [46, 47]. More recently, ivabradine, an agent that selectively reduces the heart rate without affecting blood pressure or left ventricular systolic function, has been shown to improve outcomes in patients with systolic heart failure [48]. Ivabradine also reduces ischemia in patients with stable angina pectoris but has failed to reduce the incidence of fatal and nonfatal myocardial infarction in a trial in over 19,000 patients with stable CAD [49]. In the latter trial, a disconcerting finding was that ivabradine was associated with a significant increase in the incidence of the

primary endpoint among patients with class II or higher angina (7.6 vs. 6.5 % with placebo; $p = 0.02$) [49, 50].

Overall, clinical trials suggest that the known cardioprotective properties of β-blockers cannot solely be explained by their ability to reduce blood pressure, heart rate, or myocardial contractility. Given this uncertainty, it is possible that the cardioprotective effects of β-blockers could be the results of hitherto underestimated pleiotropic effects as reducers of cellular stresses such as oxidative stress and ER stress [16–19]. The underlying mechanisms of the antioxidant activity of β-blockers are not known. Experiments in vivo and in cell cultures have shown that some β-blockers act as effective scavengers of reactive oxygen species [51, 52].

2.3 Renin Angiotensin Aldosterone System Inhibitors

The renin angiotensin aldosterone system (RAAS) is a hormonal cascade with a pivotal role in maintaining blood pressure and serum electrolyte homeostasis. In addition to these systemic effects, the discovery that the myocardial tissue and endothelial cells have the full complement of the components of RAAS has expanded its physiologic and pathophysiologic effects within these tissues [53]. Activation of this system in people with diabetes has been suspected to play a key pathophysiologic role in premature atherosclerosis, nephropathy, and retinopathy [54–59].

Interrupting RAAS with either ACE inhibitors or angiotensin II (A-II) receptor antagonists (A-II receptor blockers [ARBs]) has been shown to have favorable effects in cardiovascular events and has been the cornerstone of therapy aimed at preventing diabetic nephropathy [58]. Blocking aldosterone receptors with spironolactone or eplerenone has also been effective in reducing major cardiovascular events in patients with CHF [60–62]. The renin inhibitor aliskiren may also be an effective agent in reducing cardiac events in patients with CHF. However, a recent study found that the addition of aliskiren to enalapril led to more adverse events without an increase in benefit, and aliskiren non-inferiority compared with enalapril could not be shown [63].

The vascular-protective effects of RAAS inhibition cannot be explained solely by the changes in blood pressure, as other equally effective antihypertensive agents do not impart the same degree of clinical benefits. A recent study in 253 patients with end-stage renal disease receiving non-heart failure dialysis found that treatment with spironolactone 25 mg once daily for 2 years significantly reduced the primary outcome of a composite of death from cardiocerebrovascular (CCV) events, aborted cardiac arrest, and sudden cardiac death (7.2 vs. 18.0 %; adjusted hazard ratio [HR] 0.42; 95 % confidence interval [CI] 0.26–0.78) [64]. Death from CCV events occurred in 4.0 % of patients in the spironolactone group and in 11.7 % of patients in the control group. The secondary outcome of death from all causes occurred less frequently in the spironolactone group than in the control group (9.6 vs. 19.5 %; adjusted HR 0.52; 95 % CI 0.29–0.94) [64]. The beneficial effect was independent of blood pressure changes or changes in salt or potassium handling in the kidney [64].

A-II receptor signaling has been identified as the mediator of oxidative, inflammatory, and ER stresses in various tissues [20–24]. Interrupting RAAS with ACE or A-II receptor inhibitors or the aldosterone receptor blocker spironolactone has been shown to reduce oxidative stress [20–24]. Spironolactone and ARBs are also effective in reducing ER stress [24]. These pleiotropic effects of diverse inhibitors of RAAS may contribute to the salutary effects of these agents in reducing vascular disease. It is noteworthy that it can be argued that the antioxidative and ER stress-reducing effects of RAAS inhibitors should not be considered pleiotropic effects as the A-II type 1 receptor has a pivotal role in oxidative stress and ER stress [24].

2.4 Antiplatelet Therapy

It is generally accepted that intracoronary artery clot formation is a common terminal event in a large number of people with myocardial infarction. Thus, the effectiveness of antiplatelet agents in reducing major cardiovascular events is supported by strong pharmacologic plausibility and by empiric data from large clinical trials [65–67]. It is intriguing that the two most commonly used antiplatelet agents, namely aspirin and clopidogrel, have significant pleiotropic effects that ameliorate cellular stresses, notably oxidative and inflammatory stresses [68–70]. In one study, the total antioxidant capacity of plasma from people receiving aspirin or clopidogrel was significantly increased [69]. Clopidogrel improves endothelial nitric oxide bioavailability and diminishes biomarkers of oxidant stress and inflammation in patients with symptomatic CAD [70]. It is noteworthy that aspirin at clinically achievable plasma concentrations activates AMP-activated protein kinase (AMPK), a central regulator of cell metabolism [71]. Clopidogrel has also been shown to activate AMPK and the phosphoinositide 3 kinase (PI3 K)-Akt pathway, thereby enhancing vascular protection [72, 73].

2.5 Antihyperglycemic Agents

The role of increased blood glucose concentrations in the pathogenesis of accelerated atherogenesis in diabetes has been challenged because clinical trials have not been able to consistently demonstrate reductions in cardiovascular

events associated with improved blood glucose control [10–13]. This failure to demonstrate efficacy has been attributed to a variety of factors, including the peculiarities of patient populations studied, relatively small differentials in blood glucose levels between the intensively treated and standard care groups, off-target effects of the antiglycemic agents used, or lack of sufficient time to observe clinical benefits [13]. However there have been two remarkable exceptions so far. One is the demonstration of cardioprotective effects of metformin in a study of overweight people with diabetes [7] and the other is the recent clinical trial using empagliflozin as an add-on therapy [8].

The main mechanism of action of metformin is believed to be through activation of AMPK. However, metformin has a number of pleiotropic effects that would theoretically contribute to its cardioprotective role [74–76]. These effects include reducing cellular oxidative stress and possibly interfering with advanced glycation end-product accumulation [74–76]. Hyperglycemia and hypertension impair endothelial function in part through oxidative stress-activated poly (adenosine diphosphate [ADP]-ribose) polymerase 1 (PARP1). Metformin and temisartan (an ARB) but not metoprolol reduced PARP1 activity via AMPK activation [77]. In addition, metformin activates the synthesis of Nrf2, which controls the expression of many antioxidant enzymes and blocks the synthesis of the transcription factor nuclear factor (NF)-κB, which controls several molecules involved in the generation of inflammatory responses [74]. Thus, metformin can reduce both oxidative and inflammatory stresses and as such can be designated an antioxidant inflammation modulator (AIM). It is noteworthy that another known AIM molecule is bardoxolone, which in clinical trials failed to show significant reductions in cardiovascular events [78]. It is likely that metformin, through pleiotropic effects, has additional cardioprotective activity.

Pioglitazone is another antiglycemic agent that possesses antioxidative and anti-inflammatory activity that may well contribute to some of the cardioprotective effects observed in a few clinical trials [79–82]. Although peroxisome proliferator-activated receptor (PPAR)-γ activation provides cardioprotection from oxidative stress caused by hydrogen peroxide [79], only pioglitazone but not rosiglitazone has been found to be associated with cardioprotection. However, the US FDA issued a 'black box warning' for pioglitazone because of the high risk of developing CHF while using it.

More recently, two additional antiglycemic agents were found to have some cardioprotective effects. In a large randomized study, treatment with empagliflozin, an SGLT2 inhibitor, resulted in a significant reduction in the primary outcome (death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke: 10.5 % in the pooled empagliflozin group vs. 12.1 % in the placebo group; HR in the empagliflozin group, 0.86; $p = 0.04$ for superiority [8]. In addition, empagliflozin was associated with a slower progression of kidney disease in patients at high cardiovascular risk [83].

Another antiglycemic agent shown to have cardioprotective properties is liraglutide. In a randomized study of patients with type 2 diabetes mellitus and established CVD, liraglutide also demonstrated a significant reduction of the composite endpoint of cardiovascular death, non-fatal myocardial infarction, or non-fatal stroke [84]. The precise mechanisms of the cardioprotective effects of these agents are not known. Multiple factors could have contributed to the cardioprotective effects of empagliflozin, such as changes in arterial stiffness and cardiac oxygen demand, as well as reductions in albuminuria and plasma uric acid concentrations [8].

It is noteworthy that empagliflozin has also been shown to have anti-inflammatory and antioxidative properties [85, 86]. Similarly, the pleotropic effects of liraglutide include reductions in cellular stresses such as inflammatory, oxidative, and ER stresses [87–92]. The glucagon-like peptide (GLP)-1 agonist lixisenatide and three dipeptidyl peptidase (DPP)-4 inhibitors (alogliptin, saxagliptin, and sitagliptin) studied were not associated with a lower rate of cardiovascular events compared with the control of diabetes with other drug classes [93–96]. A number of other trials are in progress [97].

2.6 Hormone-Replacement Therapy

There is considerable debate in the literature as to the cardioprotective effects of estrogen and possibly testosterone. Estrogens reduce ischemic damage during cerebrovascular and coronary artery occlusion [98, 99], and low plasma testosterone levels in men are a predictor of CVD [100, 101]. However, findings from the Women's Health Initiative suggest that the risk for coronary heart disease was not significantly changed in the conjugated equine estrogens plus medroxyprogesterone acetate trial (HR 1.18; 95 % CI 0.95–1.45) or in the conjugated equine estrogens-alone trial (HR 0.94; 95 % CI 0.78–1.14) [102]. It has been postulated that estrogen may slow early stages of atherosclerosis but may have adverse effects on advanced atherosclerotic lesions [103, 104] This is consistent with the observation that, in women aged <50 years, combination estrogen–progestogen was associated with a reduction in a composite endpoint that included CVD [103]. In addition, preliminary findings from ELITE (Early versus Late Intervention Trial with Estradiol) show that estrogen slows atherosclerosis progression among women in early but not late menopause [104]. However, in ELITE, the primary outcome was surrogate endpoint of carotid artery

intima-media thickness (CIMT) and the study population was not large enough to assess cardiovascular events [104].

Estrogens have diverse effects on a host of variables that may change cardiovascular risk. One of the pleiotropic effects of estrogens is their antioxidative and ER stress-reducing effect [105–107]. In endothelial cell cultures, physiologic concentrations of estradiol and testosterone suppress oxidative stress and inhibit dextrose-induced ER stress [107]. The effect of testosterone was attributed to its aromatization to estradiol since non-metabolizable analogs of testosterone such as dehydrotestosterone and 5-methyl testosterone were not effective, and pharmacological inhibition of aromatization prevented testosterone-related reductions in ER stress or superoxide production [108]. Indeed, in a cell-free system, testosterone did not have antioxidant capacity when aromatization was not possible [108].

3 Ongoing Trials Targeting Cellular Stress

Oxidative stress and inflammation play a critical role in the pathogenesis of atherosclerosis [25]. Several drugs with antioxidative and anti-inflammatory properties are being developed for the treatment of atherosclerosis (Table 2) [109–113].

The clinical development of AIMs such as bardoxolone or a synthetic triterpenoid derivative of oleanolic acid, RTA 408, is now being pursued as treatment for premature vascular disease [78, 114]. These novel compounds attenuate the degradation of Nrf2, which promotes the production of important antioxidant enzymes and inhibits pro-inflammatory transcription factors including, NF-κB and STAT3 [109, 110]. However, it is noteworthy that in the BEACON (Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes Mellitus: the Occurrence of Renal Events) trial, bardoxolone methyl increased risk of heart failure and cardiovascular events [78]. It is possible that this unfavorable effect may have been secondary to drug interactions with ARBs or ACE inhibitors, thereby decreasing their bioavailability and reducing their effectiveness as cardioprotective agents [115].

Another agent with antioxidant and anti-inflammatory properties tested in phase II trials is succinobucol. This compound is a monosuccinic acid ester of probucol, a lipid-lowering agent that has been abandoned in clinical

Table 2 A select list of drugs in development that target oxidative and inflammatory stress to reduce coronary artery disease risk

Drug or drug class	Principal mechanism of action
AIMs, e.g., bardoxolone; RTA 408	Attenuate the degradation of Nrf2, which promotes the production of antioxidant enzymes and inhibits pro-inflammatory transcription factors including NF-κB and STAT3
HDL-related agents, e.g., D-4F, an apolipoprotein A-I mimetic peptide	Anti-inflammatory and antioxidant activity. Facilitates reverse cholesterol transport
Probucol analogs, e.g., succinobucol	Potent antioxidant. Clinical trials have been discontinued because of adverse effects
Colchicine	Anti-inflammatory agent that reduces myocardial tissue production of IL-1β, IL-18, and IL-6
Low-dose methotrexate	Reductions in IL-6 and C-reactive protein
IL-1β inhibitors, e.g., gevokizumab and canakinumab	Monoclonal antibodies that neutralize IL-1β and attenuate inflammation associated with myocardial infarction
Tumor necrosis factor inhibitors	Anti-inflammatory agents through inhibition of pro-inflammatory transcription NF-κB
Lipoxygenase pathway inhibitors	Anti-inflammatory effects through inhibition of leukotrienes
Cyclopentenone prostaglandins	Cyclopentenone structure have protective effects in inflammation and tissue injury. Targets identified include glutathione transferase and aldo-keto reductases that have key roles in redox signaling
Phospholipase A(2) inhibitors, e.g., darapladib and varespladib	Phospholipase A_2 hydrolyses phospholipid molecules thereby increasing oxidative stress and production of atherogenic lipid moieties capable of activating multiple inflammatory pathways
LPA antagonists; selective LPA receptor inhibitors	LPA are lipid-signaling molecules that regulate diverse cellular events, such as motility, chemotaxis, and wound healing
P-selectin blockers	P-selectin is an adhesion molecule involved in recruitment of leukocytes into the atherosclerotic lesions. Inhibition of P-selectin signaling may attenuate inflammatory responses
Serpin (viral-derived)	Serp-1 is an inhibitor of tissue and urokinase-type plasminogen activators and has anti-inflammatory effects
PAI-1 activity inhibition by small organic molecules	Ameliorate atherosclerosis and thromboembolic diseases

AIMs antioxidant inflammation modulators, *HDL* high-density lipoprotein, *IL* interleukin, *LPA* lysophosphatidic acid, *PAI-1* plasminogen activator inhibitor 1

practice because of its adverse effects on cardiac arrhythmias [116, 117]. In the ARISE (Aggressive Reduction in Inflammation Stops Events) trial, 6144 patients with recent ischemia were randomized to receive either succinobucol or placebo and followed for the primary composite endpoint of cardiovascular death, resuscitated cardiac arrest, myocardial infarction, stroke, unstable angina, or revascularization [116]. There were no benefits on this primary endpoint, and serious adverse events were significantly increased in the active treatment arm of the study. Similarly, in a second trial among 232 patients undergoing elective percutaneous coronary interventions (PCIs), succinobucol had no effect on plaque volume or atherosclerotic regression [117]. Given the results of these trials, succinobucol is no longer considered a potential therapeutic agent.

Colchicine is an anti-inflammatory agent commonly used in clinical practice to treat gout or periodic polyserositis. It has also been tested in small clinical trials as a potential cardioprotective agent. In one study, 21 patients with ACS were randomized to receive either oral colchicine or no treatment and compared with nine untreated healthy controls. Colchicine treatment reduced intracellular and secreted levels of interleukin (IL)-1β [118]. In another study of 40 patients with ACS (33 with stable CAD) and ten controls, oral colchicine treatment significantly reduced trans-coronary gradients for IL-1β, IL-18, and IL-6 [119]. The clinical implications of this observation are not yet known.

The anti-IL-1β antibody gevokizumab is in early clinical trials to investigate its ability to reduce the risk of diabetic kidney disease and CVD [120, 121]. Canakinumab is a human monoclonal antibody that selectively neutralizes IL-1β and is currently used for the treatment of a rare group of IL-1β-mediated inflammatory diseases [122, 123]. The outcome of long-term IL-1β inhibition will be evaluated in CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study). In this study, the rates of major acute cardiovascular events, such as recurrent myocardial infarction, stroke, and cardiovascular death, will be evaluated among 17,000 stable patients with persistent hsCRP elevation after myocardial infarction [123].

Another therapeutic target is arachidonate 5-lipoxygenase (5-LO), a key enzyme in the synthesis of leukotrienes, such as the potent chemoattractant leukotriene B_4 (LTB4) and the vascular permeability-enhancing leukotriene E_4 (LTE4) [124]. The leukotrienes participate in host defense reactions and pathophysiological conditions such as immediate hypersensitivity and inflammation. Inhibitors of the 5-LO pathway have been tested in small clinical trials [125, 126]. In one of these, 191 patients with ACS were randomly assigned to receive VIA-2291 at doses of 25, 50, or 100 mg or placebo daily for 12 weeks. Levels of whole blood-stimulated leukotriene LTB4 and urine leukotriene LTE4 were reduced; in a subset of 60 compliant patients, coronary computed tomography (CT) examination showed that new coronary plaques were less frequent in the active treatment group [126]. However, another study found that inhibition of 5-LO with VIA-2291 was not associated with significant reductions in vascular inflammation (via fluorodeoxyglucose positron emission tomography [FDG-PET]) or in blood inflammatory markers [126].

Phospholipase A2 inhibitors have been tested for the treatment of atherosclerosis [127]. Darapladib (GlaxoSmithKline) is an inhibitor of lipoprotein-associated phospholipase A2, and varespladib (Anthera) inhibits several forms of the secreted phospholipase A2s [127, 128]. Unexpectedly, in the VISTA-16 trial, treatment with varespladib was associated with an increased incidence of myocardial infarction, cardiovascular mortality, and stroke in patients with ACS despite lower levels of LDL-C and CRP than those receiving placebo [129]. The adverse outcome in the latter study could be the result of lack of specificity of the approach, as prostaglandins are not the only products of arachidonic acid metabolism. Similarly, darapladib did not reduce cardiovascular events in the STABILITY (Stabilization of Atherosclerotic plaque by Initiation of darapladib therapy) and SOLID-TIMI 52 (Stabilization of Plaques Using Darapladib-Thrombolysis in Myocardial Infarction) trials [130, 131]. The precise mechanism behind the increase in the incidence of myocardial infarction has not been elucidated. However, other drugs modulating the prostaglandin metabolites have shown a detrimental effect on the incidence of myocardial infarction [130, 131]. These negative results highlight the complexity of the pathogenesis of the atherosclerotic process. Nevertheless, several anti-inflammatory drugs are still in clinical development, including the p38 mitogen-activated protein kinase (MAPK) inhibitor losmapimod and low-dose methotrexate.

P-selectin belongs to a family of adhesion molecules that are involved in recruitment of leukocytes into atherosclerotic lesions, and inhibition of P-selectin signaling may attenuate inflammatory responses [132, 133]. Two ongoing randomized trials are testing the efficacy and safety of RO4905417 (inclacumab), a novel recombinant monoclonal antibody, against P-selectin. The SELECT-ACS trial is evaluating the efficacy of the drug in reducing procedural damage during PCI, assessed with troponin I levels, and the SELECT-CABG (SELECT-Coronary Artery Bypass Graft) trial tested the efficacy of this compound in preventing saphenous vein graft disease in patients undergoing CABG surgery [132, 133]. The SELECT-ACS trial demonstrated the efficacy of inclacumab in reducing myocardial damage following PCI, whereas the SELECT-CABG trial found that inclacumab

Table 3 Large randomized double-blind placebo-controlled trials of antioxidant vitamins in preventing cardiovascular disease-related events

Source	Population	Antioxidant	Outcome
Nutrition Intervention Trials [136]	29,584 Chinese adults in Linxian, China. 5.4-year follow-up	Vit E 30/carotene 15 mg, selenium 50 μg daily	9 % decrease in total mortality and decreased incidence of stomach cancer
ATBC [137]	29,133 Finnish male smokers. 5–8 years follow-up	Vit E 50 mg, β-carotene 20 mg daily	Vit E: No effect on CHD mortality; increased lung cancer and CVD with carotene
CHAOS [138]	2002 UK patients with CHD. 1.4-year follow-up	Vit E = 400–800 IU daily	77 % decrease in non-fatal MI. Randomization was not very successful
Physician Health Study I [139]	22,071 male physicians aged 40–84 years	β-Carotene 50 mg on alternate days	No adverse effect beneficial effect even after 12 years of supplementation
CARET [140]	18,314 men and women at high risk for lung cancer	β-Carotene 30 mg and retinal palmitate 25,000 IU daily or placebo	No cardiovascular protection. Increased lung cancer
GISSI-P [141]	11,324 Italian patients with MI. 3.5-year follow-up	Vit E 300 mg ± ω-3 fatty acids 1 g daily	Vit E: no benefit; ω-3 fatty acids decreased risk
HOPE Trial [142]	9541 high-risk patients	Vit E 400 IU daily	No change in cardiovascular events
Primary Prevention Project [143]	4495 Italian patients with one or more risk. Follow-up of 3.6 years	Vit E 300 mg ± aspirin 100 mg daily	Vit E: no benefit. Aspirin: 29 % reduction in risk
HPS [144]	20,536 high-risk men and women receiving simvastatin or placebo	Vit E 600 mg, vit C 250 mg, carotene 20 mg daily	Antioxidants do not decrease risk of CHD
Women's Health Study [145]	39,876 healthy women. The vitamin E arm was followed up for an average of 10.1 years	β-Carotene 50 mg, vit E 600 IU, aspirin 100 mg on alternate days	Vit E did not decrease risk of CHD or stroke. No harm or benefit demonstrated
Women's Antioxidant and CV Study (WACS) [146]	8171 women with CHD or 3 CHD risk factors. 9.4 years follow-up	Vit E 600 mg, vit C 500 mg, carotene 50 mg, folate/vit B_6/vit B_{12} on alternate days	No effects on cardiovascular events
Physicians' Health Study II [147]	14,641 healthy male physicians. Mean duration of follow-up of 8 years	Multivitamin + vit E 400 IU, vit C 500 mg, carotene 50 mg daily	No effect on cardiac events but vit E increased risk of hemorrhagic stroke

CHD coronary heart disease, *CVD* cardiovascular disease, *MI* myocardial infarction, *vit* vitamin

had no favorable effects on saphenous vein graft disease progression.

Serp-1 is a secreted glycoprotein of the serpin superfamily, is an inhibitor of tissue and urokinase-type plasminogen activators, and has anti-inflammatory effects [134, 135]. A phase II randomized trial of 48 patients with ACS undergoing a coronary stent procedure and receiving 6 months of treatment with the viral-derived Serp-1 indicated that patients treated with higher doses of Serp-1 experienced fewer major adverse cardiac events than those receiving the lower dose or placebo [134]. Plasminogen activator inhibitor 1 (PAI-1) is the main inhibitor of tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA), and inhibition of PAI-1 activity by small organic molecules is a potential therapeutic strategy to ameliorate atherosclerosis [135].

To date, evidence supporting the use of anti-inflammatory therapies for prevention of CAD is limited to observational studies or small trials with surrogate endpoints.

4 Failure of Selective Targeting of Oxidative Stress

The antioxidative and anti-inflammatory properties of the various cardioprotective drugs cannot fully account for their effectiveness, as large clinical trials with potent antioxidants have failed to show any clinical benefit and some trials have found evidence for increased harm (Table 3) [136–147]. It can be argued that antioxidants used in the clinical trials were not selective enough to target oxidative stress in key cellular compartments. Many factors could explain this paradoxical outcome with antioxidant therapy, including the possibility that antioxidants may aggravate ER stress while reducing oxidative stress [148–150]. It is entirely possible that, given the multiplicity of cellular compartments where stress can occur, an effective strategy is to target the multiple stresses simultaneously rather than selectively. In this regard, the evolution of AIMs seems to be in the right direction.

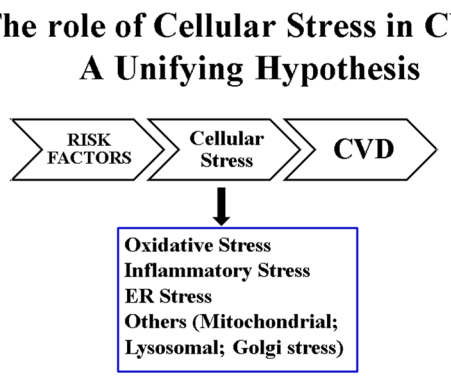

Fig. 1 A diagrammatic representation of the hypothesis that risk factors of cardiovascular disease (CVD) promote atherosclerosis through a common pathogenetic mechanism of increased cellular stresses. *ER* endoplasmic reticulum

5 Conclusions

Despite the diversity of pharmacologic profiles of the drugs with proven efficacy in reducing CVD, reducing cellular stress is a shared pleiotropic effect. These drugs have potent antioxidative and anti-inflammatory activity and are often effective in reducing ER stress that is increased in atherosclerotic plaques associated with ACS [27]. It is tempting to speculate that all the known risk factors of CAD promote atherosclerosis through a common pathogenetic mechanism of increased cellular stress (Fig. 1). However, this pathogenesis is extraordinarily complex and much is yet to be learned about the precise underlying mechanisms of cellular stresses. Understanding the nature of the cross talk among these multiple cellular stresses can increase the possibilities of developing new therapeutic agents that can reduce CVD risk. The ultimate proof of the hypothesis that reducing cellular stress is a meaningful therapeutic target would hinge on clinical trials with pharmacologic agents that reduce cellular stress without altering traditional risk factors such as hypertension or dyslipidemia.

Compliance with Ethical Standards

Funding No sources of funding were used to conduct this study or prepare this review.

Conflict of interest Arshag D. Mooradian has no conflicts of interests that are directly relevant to the content of this review.

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