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Bone marrow-derived mesenchymal stem cells for the treatment of heart failure

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Abstract Heart failure remains a major cause of death and disability, requiring rapid development of new therapies. Bone marrow-derived mesenchymal stem cell (MSC)-based therapy is an emerging approach for the treatment of both acute and chronic heart failure. Following successful experimental studies in a range of models, more than 40 clinical trials of MSC-based therapy for heart failure have now been registered, and the results of completed clinical trials so far have shown feasibility and safety of this approach with therapeutic potential suggested (though preliminarily). However, there appear to be several critical issues to be solved before this treatment could become a widespread standard therapy for heart failure. In this review, we comprehensively and systemically summarize a total of 73 preclinical studies and 11 clinical trial reports published to date. By analyzing the data in these reports, (1) improvement in the cell delivery method to the heart in order to enhance donor cell engraftment, (2) elucidation of mechanisms underpinning the therapeutic effects of the treatment differentiation and/or treatment secretion, and (3) validation of the utility of allogeneic MSCs which could enhance the efficacy and expand the application/indication of this therapeutic approach are highlighted as future perspectives. These important respects are further discussed in this review article with referencing latest scientific and clinical information.

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Introduction

Treatment of heart failure

Heart failure remains a major cause of death and disability, and the number of patients is predicted to sour along with the increase in the aged population [1]. Cardiac function of these patients is increasingly compromised with the progression of adverse ventricular remodeling, and many eventually develop fatal end-stage heart failure [2–4]. Current medical therapies have limited efficacy for heart failure, and heart transplantation is the only radical treatment but is problematic because of donor shortage. Therefore, the development of new therapies for the treatment of heart failure is a high priority.

Mesenchymal stem cell-based therapy

Stem cell therapy is a promising new approach for the treatment of heart failure. Many types of stem/progenitor cells have been studied as donor for this treatment, and several types of stem cells have been injected into patients with acute myocardial infarction (MI) or chronic heart failure. Based on the preclinical and clinical data available to date, bone marrow-derived mesenchymal stem (stromal) cells (MSCs) are among the most promising cell types because of following reasons. First, good medical practice–compliant protocols to isolate/expand a sufficient number of MSCs from bone marrow, which are feasible in the treatment of heart failure, have been established [5]. Second, since the first clinical trial in 1995 [6], more than

2,000 patients have been administered with allogeneic or autologous MSCs for the treatment of various diseases, including graft-versus-host disease (GVHD), hematologic malignancies, cardiovascular diseases, and neurologic diseases [7]. Results of these trials have collectively suggested safety and feasibility of MSC injection. Third, there are extensive preclinical evidence that MSC transplantation is safe and effective to treat acute MI and chronic heart failure using a variety of models (see "Preclinical studies" section) [8, 9]. Although cardiomyogenic differentiation of MSCs may be insignificant in vivo, grafted MSCs are able to secrete a range of factors, which help recovery of failing myocardium undergoing adverse remodeling ("paracrine effect"; see "Mechanism of MSC-based therapy" section). Fourth, over 40 clinical trials of MSC transplantation for treating heart failure have been registered, and results of completed clinical trials so far have suggested not only safety but also therapeutic effect of this approach (see "Clinical trials" section). Fifth, unlike other donor cell types, MSCs may be useful as allogeneic donor (see "Use of allogeneic MSCs" section) [10].

Definition of MSC

Friedenstein and colleagues originally identified MSCs as (1) adherent cells, (2) fibroblast-like cells, and (3) colony-forming unit-fibroblasts with a high replicative capacity [11–13]. Pittenger et al. [14] reported that MSCs postulate potential toward multiple mesenchymal lineages. Following the discovery of MSCs in bone marrow [13], MSCs have been isolated from a range of tissues. Among these, this review focuses on bone marrow-derived MSCs, which have been most extensively studied in preclinical and clinical research for targeting not only heart failure but also many other organ diseases, with providing promising results. Although there remains insufficiency of consensus on the definition of MSCs, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed a minimal criteria to define human MSCs as cells that (1) possess plastic adherent ability, (2) express CD105, CD73, and CD90, (3) do not express CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR, and (4) differentiate to osteoblasts, adipocytes, and chondroblasts [15].

Aim of this review article

There is convincing preclinical evidence supporting the value of MSCs as a source for stem cell therapy for the treatment of heart failure. This has encouraged initiation of many clinical studies, which has provided promising results so far. However, there appear to be several critical issues to be solved for this treatment to become a widespread standard therapy for heart disease. This review summarizes previous and latest preclinical and clinical information and discusses solution of unsolved issues for future success of MSC-based therapy for heart disease.

Preclinical studies

There are a large number of preclinical studies that investigated MSC-based therapy for heart diseases, among which we have selected those clearly describing the therapeutic effect in terms of cardiac function improvement (Table 1). There are 73 reports, of which 10, 41, 1, 17, 2, and 2 reports used heart disease models in mouse, rat, rabbit, swine, sheep, and canine, respectively. Approximately 56 % (41/73) of the reports are rat studies; 29 % (21/73) used large animal models. In these 21 large animal studies, a total of 23 treatment groups using a range of protocols were investigated for the effects (some reports studied multiple treatment groups). The majority (17 out of 23) of the study groups used acute MI models, while 6 employed chronic heart failure models. As a route for cell delivery, intramyocardial (IM) injection of MSC suspension was used in 16 treatment groups and intravenous (IV) injection of that was used in 2 groups. In addition, 6, 15, and 1 treatment groups used autologous, allogeneic, and xenogenic transplantation models, respectively (data missing in one report). In these treatment groups collectively, 17 groups showed improvement in cardiac function, while six groups showed negative results. Ratios of groups showing negative effects were nearly similar between acute MI models ($4/17 = 24$ %) and chronic heart failure models ($2/6 = 33$ %). These preclinical studies imply several important considerations for the future development of MSC-based therapy.

Firstly, it is likely that MSC-based therapy using either syngeneic, allogeneic, or xenogeneic MSCs is safe. There are no reports providing evidence of tumor formation in the heart or other organs, or arrhythmia occurrence following MSC transplantation in large animal or rat models. Although there are a few reports suggesting tumor or bone formation after MSC transplantation in mouse [16, 17], these adverse events might have occurred due to chromosomal instability specific to mice [18, 19]. Anyway, these possible complications should continue to be carefully monitored in further preclinical research and clinical trials.

Secondly, these preclinical studies suggest that it is important to improve the cell delivery route. The majority of previous studies used IM, intracoronary (IC), or IV injection (Table 1). However, analyses of donor cell survival in the heart showed that all of these cell delivery methods are associated with disappointingly poor retention and survival of donor cells [20]. Refinement of these

Table 1 Preclinical studies of bone marrow-derived MSC-based therapy for heart diseases

Species	Cell delivery route	MSC type	Cell numbers	Model type	Follow-up period (weeks)	Functional outcome MSC group versus control group	References
Mouse	IV	Syngeneic	5×10^5	Acute	2	Improve, max d*P*/d*t* 8,738 versus 5,234 mmHg/s	Boomsma et al. [92]
Mouse	IV	Human	1×10^6	Acute	1	Improve, max d*P*/d*t* 5,300 versus 3,700 mmHg/s	Van Linthout et al. [93]
Mouse	IM	Syngeneic	5×10^5	Acute	4	Improve, LVFS 80 versus 69 %	Noiseux et al. [24]
Mouse	IM	Syngeneic	5×10^5	Acute	6	Not improve, LVFS 32 versus 30 %	van der Bogt et al. [94]
Mouse	IM	Syngeneic	5×10^5	Acute	6	Not improve, LVEF 33 versus 32 %	van der Bogt et al. [95]
Mouse	IM	Syngeneic	1.5×10^5	Acute	12	Improve, LVFS 18 versus 15 %	Cho et al. [96]
Mouse	IM	Allogeneic	2×10^5	Acute	4	Improve, LVEF 47 versus 26 %	Buccini et al. [97]
Mouse	IM	Human	2×10^5	Acute	2	Improve, LVEF 24 versus 16 %	Grauss et al. [98]
Mouse	IM	Human	1×10^5	Acute	3	Not improve, LVEF 33 versus 20 %	Li et al. [99]
Mouse	Patch	Syngeneic	5×10^5	Chronic	2	Improve, max d*P*/d*t* 7,655 versus 6,115 mmHg/s	Derval [100]
Rat	IV	N/A	2×10^6	Acute	4	Improve, LVFS 18 versus 10 %	Mills et al. [101]
Rat	IV	Syngeneic	3×10^6	Acute	2	Improve, LVEF 71 versus 57 %	Ohnishi et al. [102]
Rat	IV	Syngeneic	5×10^6	Acute	4	Improve, LVEF 53 versus 39 %	Nagaya et al. [103]
Rat	IV	Allogeneic	1×10^6	Acute	1	Not improve, LVEF 37 versus 37 %	Li et al. [104]
	IM	Allogeneic	1×10^6	Acute	1	Improve, LVEF 42 versus 37 %	
Rat	IV	Allogeneic	$3–10 \times 10^6$	Acute	32	Improve, LVEF 48 versus 20 %	Lopez et al. [105]
Rat	IV	Allogeneic	5×10^6	Chronic	4	Improve, LVEF 65 versus 46 %	Wang et al. [106]
	IM	Allogeneic	5×10^6	Chronic	4	Improve, LVEF 65 versus 53 %	
Rat	IM	Syngeneic	5×10^5	Acute	2	Improve, LVEF 67 versus 57 %	Khan et al. [107]
Rat	IM	Syngeneic	3×10^6	Acute	4	Improve, LVEF 56 versus 35 %	Jiang et al. [108]
Rat	IM	Syngeneic	1×10^7	Acute	4	Improve, max d*P*/d*t* 5,200 versus 3,800 mmHg/s	Tang [109]
Rat	IM	Syngeneic	5×10^6	Acute	4	Improve, LVFS 23 versus 17 %	Imanishi et al. [8]
	IM	Allogeneic	5×10^6	Acute	4	Improve, LVFS 21 versus 17 %	
Rat	IM	Syngeneic	5×10^6	Acute	4	Improve, LVEF 62 versus 44 %	Tsubokawa et al. [73]
Rat	IM	Syngeneic	2×10^6	Acute	4	Improve, LVEF 57 versus 37 % (young MSC)	Jiang et al. [110]
	IM	Syngeneic	2×10^6	Acute	4	Improve, LVEF 47 versus 37 % (old MSC)	
Rat	IM	Syngeneic	4×10^6	Acute	4	Improve, LVEF 48 versus 36 %	Narita et al. [48]
	Cell sheet	Syngeneic	4×10^6	Acute	4	Improve, LVEF 54 versus 36 %	
Rat	IM	Syngeneic	1×10^6	Acute	4	Improve, LVEF 42 versus 27 %	Davani et al. [111]
Rat	IM	Syngeneic	6×10^6	Acute	6	Improve, LVEF 36 versus 28 %	Furlani et al. [112]
Rat	IM	Syngeneic	1×10^6	Acute	6	Improve, LVEF 42 versus 37.3 %	Afzal et al. [71]
Rat	IM	Syngeneic	1×10^6	Acute	3	Improve, LVFS 29 versus 20 %	Hahn et al. [62]
					7	Not improve, LVFS 28 versus 22 %	
Rat	IM	Syngeneic	5×10^5	Acute	7	Not improve, LVFS 13 versus 13 %	Al Kindi et al. [113]
	IM	Syngeneic	$1.5–5.0 \times 10^6$	Acute	7	Improve, LVFS 22 versus 13 %	
Rat	IM	Syngeneic	1×10^7	Acute	8	Improve, LVFS 49 versus 19 %	Tang et al. [28]
Rat	IM	Syngeneic	5×10^6	Chronic	4	Improve, LVFS 34 versus 20 %	Nagaya et al. [9]
Rat	IM	Syngeneic	3×10^6	Chronic	4	Improve, LVEF 52 versus 42 %	Wang et al. [114]
Rat	IM	Syngeneic	3×10^6	Chronic	24	Improve, LVFS 29 versus 18 %	Huang et al. [76]
	IM	Allogeneic	3×10^6	Chronic	12	Improve, LVFS 32 versus 16 %	

Table 1 continued

Species	Cell delivery route	MSC type	Cell numbers	Model type	Follow-up period (weeks)	Functional outcome MSC group versus control group	References
				Chronic	24	Not improve, LVFS 18 versus 18 %	
Rat	IM	Allogeneic	1×10^6	Acute	1	Improve, LVEF 59 versus 41 %	Zeng et al. [115]
Rat	IM	Allogeneic	2×10^6	Acute	4	Improve, LVEF 44 versus 39 %	Dai et al. [116]
					24	Not improve, LVEF 42 versus 42 %	
Rat	IM	Allogeneic	2.5×10^6	Acute	4	Not improve, LVEF 24 versus 21 %	Guarita-Souza et al. [117]
Rat	IM	Allogeneic	2×10^6	Acute	4	Improve, LVEF 23 versus 14 %	Amsalem et al. [118]
Rat	IM	Allogeneic	8×10^6	Acute	4	Improve, LVEF 58 versus 46 %	Gao et al. [119]
Rat	IM	Allogeneic	4×10^6	Acute	4	Improve, max d*P*/d*t* 5,054 versus 3,945 mmHg/s	Wang et al. [120]
Rat	IM	Allogeneic	5×10^6	Acute	4	Not improve, 55 versus 40 %	Flynn et al. [121]
Rat	IM	Allogeneic	5×10^6	Acute	6	Improve, LVEF 68 versus 53 %	Wang et al. [122]
Rat	IM	Allogeneic	1×10^7	Chronic	4	Not improve, LVFS 18 versus 16 %	Enoki et al. [123]
Rat	IM	Human	2×10^6	Acute	4	Improve, change in LVEF +5 versus −6 %	Hou et al. [124]
Rat	IM	Mouse	1×10^7	Acute	4	Improve, LVEF 57 versus 39 %	Wang et al. [125]
Rat	IM	Human	1×10^6	Acute	4	Not improve, LVEF 66 versus 64 %	Bayes-Genis et al. [126]
Rat	IM	Human	1.2×10^6	Acute	4	Improve, LVFS, 32 versus 26 %	Arminan et al. [127]
Rat	IM	Mouse	1×10^6	Acute	4	Improve, LVEF 61 versus 46 %	Herrmann et al. [128]
Rat	IM	Human	1×10^6	Acute	4	Not improve, LVEF 57 versus 58 %	Rasmussen et al. [129]
Rat	IM	Mouse	1×10^6	Acute	6	Improve, max d*P*/d*t* 3,000 versus 2,100 mmHg/s	Hu et al. [90]
Rat	IC	Allogeneic	1×10^6	Chronic	4	Improve, Change in LVFS +0.2 versus −1.7 %	Molina et al. [130]
Rat	Collagen sheet	Syngeneic	1×10^6	Acute	6	Not improve, LVEF 62 versus 64 %	Mokashi et al. [131]
Rat	Collagen sheet	Human	1×10^6	Acute	4	Improve, LVEF 61 versus 46 % (young MSC)	Kang et al. [132]
	Collagen sheet	Human	1×10^6	Acute	4	Not improve, LVEF 46 versus 46 % (old MSC)	
Rabbit	IV	Allogeneic	1×10^8	Acute	4	Improve, LVEF 42 versus 35 %	Xu et al. [133]
Swine	IV	Allogeneic	3.2×10^8	Acute	12	Improve, LVEF 49 versus 44 %	Price et al. [134]
Swine	IV	Allogeneic	$1–10 \times 10^6$/kg	Acute	12	Improve, LVEF 52 versus 45 %	Halkos et al. [135]
Swine	IM	N/A	2×10^8	Chronic	12	Improve, LVEF 40 versus 29 %	Schuleri et al. [136]
Swine	IM	Autologous	3×10^7	Acute	6	Not improve, LVEF 41 versus 42 %	Yang et al. [137]
Swine	IM	Autologous	6×10^8	Chronic	4	Not improve, LVEF 52 versus 40 %	Huang et al. [138]
Swine	IM	Autologous	6.2×10^9	Chronic	12	Improve, LVEF 55 versus 35 %	Schuleri et al. [139]
	IM	Autologous	3.2×10^8	Chronic	12	Not improve, LVEF 44 versus 35 %	
Swine	IM	Allogeneic	5×10^7	Acute	4	Improve, LVEF 46 versus 35 %	Wang et al. [140]
Swine	IM	Allogeneic	2×10^8	Acute	8	Improve, LVEF 25 % (pre; 25 %)	Amado et al. [85]
Swine	IM	Allogeneic	2×10^8	Acute	8	Improve, LVEF 39 versus 29 %	Amado et al. [141]
Swine	IM	Allogeneic	2×10^8	Acute	8	Improve, LVEF 39 versus 27 %	Schuleri et al. [142]
Swine	IM	Allogeneic	1×10^8	Acute	8	Improve, LVEF 36 versus 30 %	Hatzistergos et al. [29]
Swine	IM	Allogeneic	$2.4–44 \times 10^7$	Acute	12	Not improve, LVEF 33–35 versus 32 %	Hashemi et al. [87]

Table 1 continued

Species	Cell delivery route	MSC type	Cell numbers	Model type	Follow-up period (weeks)	Functional outcome MSC group versus control group	References
Swine	IM	Allogeneic	2×10^8	Chronic	12	Improve, LVEF 41 versus 32 %	Quevedo et al. [86]
Swine	IM	Human	2×10^8	Acute	4	Improve, LVEF 37 versus 30 %	Williams et al. [143]
Swine	IC	Autologous	2×10^6	Acute	4	Improve, change in LVEF +9.6 versus +2.0 %	Valina et al. [144]
Swine	IC	Autologous	$3–6 \times 10^6$/kg	Acute	8	Improve, LVEF 79 versus 69 %	Peng et al. [145]
Swine	IC	Allogeneic	1.1×10^9	Acute	6	Not improve, LVEF 45 versus 48 %	Dubois et al. [146]
Sheep	IM	Allogeneic	$2.5–45 \times 10^7$	Acute	8	Improve, LVEF 22 versus 14 %	Dixon et al. [147]
Sheep	IC	Allogeneic	$1.3–3.8 \times 10^8$	Acute	8	Improve, LVEF 50 versus 39 %	Houtgraaf et al. [148]
Canine	IM	Allogeneic	1×10^8	Acute	2	Improve, LVEF 47 versus 33 %	Perin et al. [149]
	IC	Allogeneic	1×10^8	Acute	2	Not improve, LVEF 33 versus 33 %	
Canine	IM	Allogeneic	1×10^8	Chronic	4	Improve, LVEF 48 versus 25 %	Silva et al. [65]

Some data are from graphs in original papers. Data are shown as MSC group versus control group

IC intracoronary injection, *IM* intramyocardial injection, *IV* intravenous injection, *LVEF* left ventricle ejection fraction, *LVFS* left ventricle fractioning shortening

methods, or development of new technologies such as the cell sheet technique [21, 22], is needed to enhance donor cell engraftment and obtain the maximum benefits from MSC-based therapy. This issue will be further discussed in "Cell delivery route to the heart" section.

The third issue raised is the mechanism by which MSCs improve cardiac function. Originally, cardiomyogenic differentiation was expected to be the major mechanism for MSCs to recover damaged myocardium based on in vitro findings indicating cardiomyogenic potency of MSCs [23]. However, accumulating preclinical evidence indicates that differentiation of MSCs to cardiomyocytes does not occur to a significant extent in vivo [24]. Instead, the "paracrine effect" derived from secretion of MSCs is now believed to be the dominant mechanism. MSCs can secrete a group of cytokines, growth factors, and chemokines, which would beneficially modulate failing myocardium by reducing pathological fibrosis, increasing neovascular formation, attenuating cardiomyocyte apoptosis and hypertrophy, reducing inflammation, and stimulating endogenous stem/progenitor cells for myocardial regeneration [25–30]. This aspect will be further discussed in "Mechanism of MSC-based therapy" section.

Fourth, MSCs have a great advantage over other cell types as donor for cell therapy in being able to be used as allogeneic donor. MSCs have relatively low-immunogenic phenotypes and possess a powerful immunosuppressive secretion [10], allowing transplanted allogeneic or xenogeneic MSCs to survive and function to improve cardiac function without causing significant immunorejection. In previous large animal studies, 13 out of 16 (81 %) treatment groups using allogeneic and xenogeneic transplantation models showed positive therapeutic effects without showing serious complications, indicating great potential of this strategy. This issue will be further discussed in "Use of allogeneic MSCs" section.

Finally, among 23 treatment groups studied in the previous preclinical reports in large animals we listed, only seven treatment groups were investigated for the effects for more than 12 weeks. While five groups showed positive cardiac function improvement, while remaining 2 groups showed contradicting results. Further preclinical studies and clinical trials to investigate the long-term effect of MSC-based therapy are warranted.

Clinical trials

Promising results of preclinical studies have encouraged clinicians to undertake clinical trials of MSC-based therapy for heart diseases (Table 1). Currently, more than 40 clinical trials have been registered to evaluate the safety and/or efficacy of MSCs for the treatment of several types of heart diseases, including acute MI, ischemic heart failure, dilated cardiomyopathy, and Duchenne muscular dystrophy [31]. The results of 11 clinical trials have been reported to date (Table 2). Target diseases were either acute/subacute MI or ischemic heart failure, which were treated with MSC transplantation as a sole therapy [32–36] or in conjunction with percutaneous catheter intervention (PCI) [37–42] or coronary artery bypass surgery [37]. The results from these trials collectively showed that transplantation of MSCs was feasible and safe for the treatment

of heart diseases. Although the patient numbers and observation periods may not be sufficient, there has been no report of serious cardiovascular adverse events, including tumor formation and arrhythmia occurrence, after MSC transplantation. This is generally consistent to the results from clinical trials of MSC-based therapy in other organs diseases. To date, more than 2,000 people have been injected with MSCs [43], and systemic analysis of these trials has confirmed the safety of MSC injection [7]. Efficacy of MSC-based therapies observed in these clinical trials is inconsistent, although many of them were not designed to evaluate the effect of the therapy. Improvement in LVEF was seen in 7 out of 11 trials (64 %) compared with the control groups or baseline, and even four "negative" reports suggested some benefits from MSC-based therapy including MI size reduction, wall motion score, and improvement in NYHA score. It is important to understand that LVEF is not the perfect indicator of the cell therapy efficacy, although it has been most frequently used as an endpoint. It is a load-dependent factor [32, 33], and the technique for measurement may not be fully consistent among centers and examiners (Table 2). Development of more objective and reproducible indicators, i.e., infarct size, is warranted.

The first randomized trial was reported by Chen and colleagues in 2004 [38]. PCI was performed within 12 h following the onset of acute MI, and then, patients were randomly divided into two arms: additional treatment of autologous MSCs injection via IC injection and normal saline. The average period from PCI to MSC or saline injection was 18.4 and 18.2 days, respectively. MSC injection group displayed significant improvement in cardiac function at 6 months after the treatment without any complications including arrhythmia occurrence. A recent C-CURE trial reported a corresponding result. IM injection of MSCs improved cardiac function for 2 years, without occurrence of adverse events, in chronic ischemic heart failure patients [34]. More recently, the TAC-HFT trial has reported that IM injection of autologous MSCs was similarly safe to IM injection of autologous bone marrow mononuclear cells during the 1-year follow-up period, but that MSCs appeared to achieve superior therapeutic effects [32]. Although LVEF was not changed, improvement in quality of life and reduction in infarct size were found after MSC injection. Not only autologous MSCs but also allogeneic MSCs have been injected to patients with heart disease, resulting in preliminary but promising outcome. The POSEIDON trial compared the safety and effects in IM injection of autologous MSCs and allogeneic MSCs for 13 months in patients with chronic ischemic heart failure [33]. There was no severe immunological response after allogeneic MSC injection with some therapeutic benefits, which appeared to be similar to those by autologous MSC injection.

These previous clinical trials raised several issues for consideration. Firstly, MSC doses are largely different between clinical trials, requiring further comparison (dose–effect) studies. Secondly, many cell delivery routes, including IM, IC, and IV injection, were used. Given preclinical information ("Preclinical studies" section), we will need to compare and improve these current methods and also develop novel cell delivery technologies. Thirdly, the methods of MSCs isolation and expansion have not been standardized. Culture materials (flask and culture medium and supplement), seeding density, passaging methods, and cryostorage protocols widely differ among trials, though these could significantly affect the quality of MSCs [39, 40]. In addition, longer-term safety and effect need to be investigated. Global consensus of the MSC preparation protocols and appropriately designed further clinical trials are warranted. Finally, although LVEF has been commonly used as the primary endpoint of the clinical trials, this may not be a perfect indicator, and development of a more precise indicator of therapeutic effects is warranted.

Future perspectives

From the discussion in preclinical studies and clinical trials, it is clear that further research and development is essential for MSC-based therapy to become a widely adopted standard therapy. The issues to be solved include the improvement in cell delivery route, elucidation of the mechanism of therapeutic effects, and validation of the use of allogeneic MSCs.

Cell delivery route to the heart

Current routes for MSC delivery for the treatment of heart disease include IV injection, IC injection, and IM injection. These have their own advantages and disadvantages, but importantly all these remain suboptimal. Particularly, engraftment of donor cells by any of these methods was disappointingly poor. Freyman et al. [20] reported that donor cell presence at 14 days after MSC administration was 6, 3, and 0 % after IC, IM, and IV injection, respectively, in a swine MI model. This indicates requirement of improvement in these methods and/or development of new cell delivery technologies.

IV injection

Systemic IV injection is an easier, less invasive, and more economical approach than other methods. It has been suggested that MSCs have a unique ability to recruit ("home") into the injured heart [44], encouraging the use

Table 2 Clinical trials of MSC-based therapy for heart diseases

References	Disease	Study design	Cell number Cell type	MSC type	Cell delivery route	Follow-up period (months)	Concurrent Procedure	LVEF Pre (%)	LVEF Post (%)	Patient Number
Chen et al. [38]	AMI		$4.8–6.0 \times 10^{10}$ MSC	Auto	IC	6	PCI	MSC 49	67*,†	34
								Cont 48	54	35
Katritsis et al. [40]	AMI		$2.0–4.0 \times 10^{6}$ MSC + EPC	Auto	IC	4	PCI	MSC 41.5	43.45	11
								Cont 46.1	47.72	11
Bonab [37]	ICM		5.55×10^{6} MSC	Auto	IM or IC	18	CABG or PCI	MSC 38.75	48.75*	8
								Cont 41.88	42.50	8
Hare et al. [41]	AMI	Phase 1	$0.5, 1.6, 5 \times 10^{6}$/kg MSC	Allo	IV	12	PCI	MSC 50.4	56.9*	39
								Cont 48.7	56.1	21
Yang et al. [42]	AMI		Group 1: 1.22×10^{7} MSC	Auto	Group 1: IC (culprit coronary)	6	PCI	Group 1 42.1	52.0*	8
			Group 2: 1.32×10^{7} MSC	Auto	Group 2: IC (noninfarct-relative artery)	6	PCI	Group 2 41.6	50.5*	8
Lasala et al. [35]	ICM	Phase 1	1.5×10^{7} BMMNC + MSC	Auto	IC	6	None	MSC 37.9	43.1*	10
Williams et al. [36]	ICM	Phase 1/2	$1.0–2.0 \times 10^{8}$ BMMNC or MSC	Auto	IM	12	None	MSC 32.5a	36.0[a]	8
Hare et al. [33]	ICM	Phase 1/2	$0.2–2.0 \times 10^{6}$ MSC	Auto	IM	13	None	Allo	29.50	15
				Allo	IM	13	None	Auto 26.23	28.53	15
Bartunek et al. [34]	ICM	Phase 2	7.33×10^{8} MSC-derived CSC	Auto	IM	24	None	MSC 27.5	34.5*,†	21
								Cont 27.8	28.0	24
Gao et al. [39]	AMI		3.08×10^{6} MSC	Auto	IC	24	PCI	MSC 50.8	55.1*	21
								Cont 51.4	54.9	22
Heldman et al. [34]	ICM	Phase 1/2	2.0×10^{8} MSC	Auto	IM	12	None	MSC 35.7	+0	16
								Cont 28.1	+5	17

Allo allogeneic, *AMI* acute myocardial infarction, *Auto* autologous, *BMMSC* bone marrow mononuclear cells, *CABG* coronary artery bypass grafting, *Cont* control group, *CSC* cardiopoietic stem cells, *EPC* endothelial progenitor cells, *IC* intracoronary injection, *ICM* ischemic cardiomyopathy, *IM* intramyocardial injection, *IV* intravenous injection, *LVEF* left ventricular ejection fraction, *PCI* percutaneous coronary intervention

* $p < 0.05$ between baseline

† $p < 0.05$ compared to the control group

[a] Data from graphs

of the IV route. Potential homing signals include stromal cell-derived factor (SDF)-1α, interleukins (ILs), matrix metalloproteases (MMPs), and adhesion molecules at the myocardial side and integrins, selectins, and chemokine receptors at the MSC side. It is known that MSCs express CXCR4 (for SDF-1α), IL6RA and IL6ST (for IL-6), and CCR2 (for CCL7), E-selectin ligand, VLA-4 (integrin α4/β1), integrin α6/β1, integrin α8/β1, and α9/β1 [45]. However, it is likely that such homing systems are not sufficient even when the heart is suffering acute MI. Barbash and colleagues demonstrated that very few MSCs were accumulated in the heart after IV injection of ^{99m}Tc-labeled MSCs in rats with acute MI, and the most cells were localized in the lung [46]. Development of strategies to enhance such homing signals is needed for the success of this cell delivery route.

IM injection

This method has been frequently used in animal studies and clinical trials. By the IM injection, MSC suspensions are directly delivered into the myocardium via a needle inserted. Three approaches for IM injection have been reported: epicardial, endocardial, and transvascular approaches [47]. The epicardial IM injection allows to deliver cells into the aimed area under the direct vision or under the endoscopic observation. In contrast, endocardial IM and transvascular IM injection are performed using transcatheter techniques. For the endocardial approach, the percutaneously inserted catheter is advanced to the left ventricular cavity, and MSCs are injected into left ventricle walls, while for the transvascular approach, the catheter is placed in the coronary artery or the cardiac vein, and cells are injected into the myocardium via a needle penetrating the vascular wall.

The IM injection method is advantageous in delivering a large number of cells into a targeted area selectively. This method is free from the risk of coronary embolism unlike IC injection. In addition, the epicardial method is quite easy for surgeons to add routine cardiac surgery such as coronary artery bypass grafting. However, in addition to the poor efficiency of retention and survival of donor MSCs [48], formation of islet-like donor cell clusters is a problem for this method. Such isolated clusters are consisted of injected donor cells as well as host inflammatory cells, generating physical, biological, and electrical heterogeneity in the host myocardium, potentially resulting in arrhythmia occurrence [49].

Several reasons for the poor donor cell engraftment after IM injection have been reported. Donor cells prepared using trypsinization lose the cell surface proteins, reducing the cell–cell affinity and thereby being easily flushed out [50]. Mechanical injury induced by direct needle injection may cause donor cell damage and death directly (mechanical injury) or secondarily via inducing inflammation [51, 52]. It will be important to optimize injection pressure, volume of cell suspensions, and the needle type to improve donor cell engraftment and subsequent therapeutic effect from MSC-based therapy by IM injection.

IC injection

This is infusion of MSC suspensions into the coronary circulation. Two different approaches are available to undertake IC injection of MSCs, antegrade and retrograde IC injection [53]. These techniques are usually performed by the percutaneous catheter technique. Unlike the IM injection, the IC injection method can achieve more homogenous cell distribution in the target areas without producing cell clusters and with less inflammatory response [54].

However, donor cell engraftment after IC injection is similarly poor to that after IM injection. Ly and colleagues demonstrated the poor initial retention of injected cells, with 15 % of injected MSCs engrafted at 2 min and only 5 % detected at 1 h [55]. The injected cells by IC injection have to adhere to coronary endothelial cells via adhesion molecules or to be entrapped passively in the vessel lumen. Subsequently, these cells need to undergo transendothelial migration into the myocardial interstitium or integration into the vascular walls [56]. It is likely that these processes, which are usual in myocardial accumulation of inflammatory cells, do not appropriately occur after IC injection of MSCs, resulting in poor donor cell engraftment [20, 55].

Another important issue associated with IC injection of MSCs, which are relatively larger cells in size, is the risk of coronary embolization. Vullite and colleagues reported ST-T changes in ECG, increased plasma concentrations of cardiac troponin I, and histological findings of scattered regions of dense fibroplasias, suggesting occurrence of coronary embolisms leading to MI, after IC injection of MSCs in a dog model [57]. This risk will be more critical when the cells are injected into diffusely diseased and narrowed coronary arteries.

New technologies for MSC delivery

As discussed above, IV, IM, and IC injection all result in poor donor cell engraftment. This has encouraged developing new, more effective cell delivery techniques to the heart, including tissue engineering technologies. One of the most promising technologies for MSC delivery will be the "cell sheet" technique, developed by Okano et al. [21, 22]. They have developed a culture dish, the bottom of which is coated with temperature-responsive polymer (poly-*N*-isopropylacrylamide). At 37 °C, the polymer is hydrophobic, and cells can adhere to and grow on the dish to become

confluent. While once the temperature is dropped to below 25 °C, the polymer turns to hydrophilic and swollen, letting the cells spontaneously to detach from the dish as a scaffold-free "cell sheet." Cells in the cell sheet are supposed to have better-preserved cell surface proteins, cell–cell communications, and the underlying extracellular matrix, compared to cells dissociated by trypsinization. We have recently reported the utility of this technology for MSC-based therapy for heart disease [48]. Epicardial placement of an MSC sheet largely increased initial retention and subsequent presence; 94.8 and 61.4 % of grafted MSCs retained on the myocardial surface at 1 h and 3 days after MSCs sheet placement, respectively. These rates were 6.4-fold and 6.1-fold increased compared to IM injection, respectively (14.9 and 10.1 % at 1 h and day 3). More importantly, this effect by MSC sheet technique was correlated with significantly improved cardiac function recovery, in association with amplified paracrine effects, compared to IM injection. The cell sheet technology has entered a clinical trial with skeletal myoblasts [58], and clinical development of MSC sheet is warranted.

Mechanism of MSC-based therapy

Mechanisms by which transplanted MSCs improve cardiac function remain uncertain. Possible major mechanisms reported include transdifferentiation of MSCs toward cardiomyocytes and/or vascular cells and "paracrine effect" due to secretion of a range of cytokines and growth factors.

Differentiation to cardiomyocytes

It is a major aim of stem cell therapy to generate new cardiomyocytes derived from donor cells compensating cardiomyocyte loss by myocardial injury. There are reports showing cardiomyogenic differentiation potency of MSCs in vitro and in vivo. Makino and colleagues demonstrated that cultured MSCs can differentiate to cardiomyocyte-like cells in response to demethylating agent, 5-azacytidine in vitro [23]. Toma and colleagues reported that human MSCs intramyocardially injected into the mice heart obtained cardiomyocyte-specific features in vivo [59]. It was reported that bone morphogenetic protein (BMP)-2, fibroblast growth factor (FGF)-4, hepatocyte growth factor (HGF), insulin-like growth factor (IGF)-1, and transforming growth factor (TGF)-1β would play a role in the mechanism of cardiomyogenic differentiation of MSCs [60–62]. In addition, importance of direct cell–cell contact in differentiation of MSCs to cardiomyocytes has been suggested [63]. However, more recently, there have been accumulating in vivo reports showing that cardiomyogenic differentiation of transplanted MSCs does not occur to such a degree that the new cardiomyocytes influence global cardiac function [24]. Taken poor donor cell survival together, it is unlikely that cardiomyogenic differentiation of MSCs is the central mechanism of cardiac function improvement by MSC-based therapy [24].

Differentiation to vascular cells

Differentiation of MSCs to endothelial cells and vascular smooth muscle cells has also been reported. Oswald and colleagues demonstrated that MSCs differentiate toward the endothelial lineage and form capillary-like structures in vitro [64]. They also identified the role of vascular endothelial growth factor (VEGF) to enhance endothelial differentiation of MSCs. In vivo, Silva et al. [65] observed differentiation of IM-injected MSCs into smooth muscle cells and endothelial cells in a dog model of chronic myocardial ischemia. We also observed MSC-derived endothelial cells after IM injection and epicardial placement of MSC sheets in rat [48]. Thus, the frequency of in vivo transdifferentiation of MSCs to endothelial or smooth muscle cells appears to be more significant compared to differentiation to cardiomyocytes. However, the functional role of the vascular differentiation in cardiac function improvement after MSC-based therapy remains unclear, requiring further investigations.

Paracrine effect

Instead of differentiation, the "paracrine" mechanism is now focused as the key mechanism of the therapeutic effects induced by MSC-based therapy. Transplanted MSCs can secrete a range of growth factors, cytokines, and chemokines, which could help repairing failing myocardium undergoing adverse remodeling in association with persistent ischemia and inflammation in heart failure. Such factors could also be secreted from recipient cardiac cells such as cardiomyocytes, fibroblasts, and endothelial cells, in response to the stimuli occurred by MSC transplantation. Targets of the paracrine effects include many cell types including cardiomyocytes, fibroblasts, endothelial cells, accumulated inflammatory cells, and endogenous stem/progenitor cells [25–30].

Inflammation underpins the pathophysiology in both acute MI and chronic heart failure [2]. MSCs may be able to attenuate such inflammation via production of anti-inflammatory cytokines including IL-10, resulting in protection of the viable myocardium [66, 67]. Neovascularization, encompassing angiogenesis and vasculogenesis, is an important process to save and repair damaged tissues. There has been extensive experimental evidence that MSCs improve neovascular formation in the heart in association with upregulation of pro-angiogenic factors including

VEGF, FGF, TGF-β, tumor necrosis factor (TNF)-α, and placenta growth factor [26]. Pathological fibrosis is a typical event in chronic heart failure [68]. Transplanted MSCs modulate expression of MMPs and tissue inhibitor of metalloproteinases (TIMPs) [67, 69], leading to favorable modulation of extracellular matrix (ECM) accumulation, increased ECM stability, and consequently prevention from the left ventricle dilatation and structural disorganization. Apoptosis of cardiomyocytes plays a role in progression of heart failure, with which two kinds of pathways including caspases and Bcl-2 are involved [70]. MSCs can modulate both apoptotic mechanisms through secreted factors such as HGF, IGF, FGF-2, Ang-2, VEGF, and Sfrp2, which lead to modulation of mitogen-activated protein kinase (MAPK) and PI3K-AKT signaling pathways [71–73]. In addition, there are increasing evidence that MSCs enhance proliferation and differentiation of endogenous cardiac stem cells in a paracrine manner for myocardial regeneration [29, 30].

Use of allogeneic MSCs

As donor for cell-based therapy, autologous cells are more convenient because these cells do not require immunosuppression and do not include ethical issues. However, the use of autologous cells is associated with several concerns and limitations. Stem cells collected from aged, diseased patients may have deteriorated cellular function including the ability of in vitro expansion and the capability to repair the damaged myocardium [74]. In addition, in the case of MSCs, it usually takes 4–8 weeks to collect/expand a sufficient number of autologous MSCs for heart failure treatment [5]. This prolonged culture period will delay the treatment and is associated with a risk of contamination by bacterial, fungus, and virus [75]. The use of allogeneic MSCs will solve many of these concerns. Ultimately, it will be possible to develop a bank of allogeneic MSCs, which can supply off-the-shelf, highly competent, quality controlled/assured MSCs upon requirement for treatment without delay. Therefore, if allogeneic MSCs can survive after transplantation to a similar degree to autologous MSCs without immunosuppression, it will be a great advantage, enhancing the therapeutic effects and expanding the application of MSC-based therapy.

Immunomodulation by MSCs

Research has shown that MSCs have relatively low expression of major histocompatibility complex (MHC) class antigens and also have the ability to secrete immunosuppressive factors. The expression level of MHC class Ia, which is a component recognized by T cells and a mediated lysis target of natural killer (NK) cells, is relatively low in MSCs [76]. In addition, expression of MHC class II, which plays a role in presenting antigens for T-cell activation, is absent or extremely low in MSCs [76]. Also, co-stimulators for T-cell activation (CD40, CD80, and CD86) are not expressed in MSCs [77]. In addition, MSCs are able to secret immunosuppressive soluble factors such as TGF-β, prostaglandin E2, HGF, soluble HLA-G, nitric oxide, galectin-1, and indoleamine 2,3-dioxygenase [78]. These result in attenuation of immunological response against allogeneic cells by inhibiting T-cell proliferation [79], B-cell proliferation, and immunoglobulin [80], response of NK cells [81], and dendritic cells activity [82].

Allogeneic MSC transplantation

The specific features of MSCs in low MHC antigen expression and immunosuppressive ability may allow allogeneic MSCs to survive and work for myocardial repair in the host heart without causing significant immunorejection. In fact, allogeneic MSCs have been injected into patients for the treatment of GVHD [83], autoimmune diseases [84], heart failure [33], and so on. Although a small number of reports suggested that administrated allogeneic MSCs were associated with some graft rejection, injection of allogeneic MSCs has been proven safe in general [25].

Among 15 preclinical studies of allogeneic MSC transplantation to the heart in large animals, 12 (80 %) reports demonstrated positive therapeutic effects, either in the short term, mid-term, or long term, in terms of cardiac function recovery (Table 1). Amado and colleagues reported that IM injection of allogeneic MSCs improved cardiac function without rejection by 8 weeks in a swine acute MI model [85]. Quevedo et al. [86] also demonstrated long-term MSC survival and engraftment by 12 weeks in a swine chronic MI model, contributing to improvement in LVEF and reduction in infarct size. Hashemi et al. [87], however, showed that, in swine post-MI acute heart failure model, IM injection of allogeneic MSCs reduced the infarct size by 12 weeks but did not improve LVEF. Allogeneic MSCs have been injected into patients with heart diseases [33]. This did not appear to induce severe immunological response, while obtaining some therapeutic benefits, which appeared to be similar to those by autologous MSC injection. Further investigations into preclinical and clinical trials are needed to elucidate whether allogeneic MSCs are really useful as donor for cell therapy for heart failure.

Conclusions

MSCs have great potential to become an established standard therapy for heart failure. However, for this ultimate

goal, there remain several issues to be solved. In terms of safety, there are considerable amounts of data supporting the safety of MSCs in preclinical and clinical research; however, it is also true that there are reports warning about a risk of complications after MSC injection. Jeong and colleagues have shown that MSCs injected into the mouse heart with acute MI resulted in tumor formation with features of malignant sarcoma [17]. It was also reported by Breitbach et al. [16] that the MSCs injected in the myocardium formed bone-like structures in mice. However, these observations in mice should be interpreted with caution, as it is known that small rodents have genetic/chromosomal instability in MSCs, compared to human and large animals [18, 19, 88]. There was no such a finding in large animal studies so far. Nonetheless, one clinical trial reported a case, which developed eccrine poroma, a benign tumor after MSC injection for the treatment of stroke [89]. It is of importance to keep our extreme caution on the safety of MSCs, in particular long-term tumorigenicity and unwanted differentiation, in future preclinical and clinical research.

Most of the previous clinical trials reported preliminary but encouraging results in the therapeutic efficacy of MSC injection. However, it is obvious that the effect is limited by poor donor cell retention and survival. It is important to continue our effort to improve the current injection methods or development of new, more effective, cell delivery technologies. Comparisons of the effects among different cell delivery methods in homogenous patient populations using similar competent MSCs will be needed to decide the optimal protocol for MSC-based therapy. Continuation of challenges to enhance the therapeutic efficacy of MSC-based therapy by improving the capability of MSCs by physical and genetic pre-treatment may also be useful for the future success of MSC-based therapy [90, 91].

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