Cell-Based Therapy in Cardiac Regeneration
An Overview

Eugene Braunwald

Although pioneering preclinical research on the use of cell therapy for cardiac regeneration was conducted in the last quarter of the 20th century,[1,2] a preponderance of advances have occurred in the 21st century, making this a relatively young field. In the first important clinical trial of cardiac cell therapy, begun in 2001, Menasché et al[3] implanted autologous skeletal myoblasts into postinfarct scar at the time of coronary artery bypass surgery. Although the transplanted cells remained viable and exhibited contraction, they formed the nidus for serious ventricular tachyarrhythmias, which led to premature discontinuation of the trial. Despite this outcome, the trial energized the field, accelerating both preclinical and clinical research, albeit not with skeletal myoblasts. The extensive progress in cardiac regeneration is reviewed in this Compendium, and as occurs frequently in science, important observations have led to more questions and challenges (Table).

Cell Types

Many cell types have been evaluated as candidates for cardiac regeneration. Among the earliest clinical trials, Zeiher's group infused autologous bone marrow-derived progenitor cells into the coronary arteries of patients with acute,[4] as well as healed myocardial infarction (MI)[5] and reported improvements in left ventricular (LV) function. However, these results have not been fully confirmed by later studies, as pointed out in the review in the Compendium by Banarjee, Bolli, and Hare.[6]

Pittenger et al[7] were among the first to direct attention to bone marrow-derived (stromal) mesenchymal stem cells (MSCs), emphasizing that these cells proliferated extensively in culture and suggesting that they could be attractive candidates for transplantation. In 2004, Chen et al[8] reported that intracoronary infusion of autologous bone marrow-derived MSCs improved cardiac function. Zimmet and Hare pointed out that MSCs lack histocompatibility type II markers and elude rejection by the host's immune system, suggesting that an allogeneic origin of these cells could be suitable.[9] MSCs may also possess antifibrotic actions.[6] In 2 randomized clinical trials, Hare et al reported similar favorable responses in LV function from autologous and allogeneic MSCs delivered by catheter-based, transendocardial injection in patients with both ischemic,[10] as well as nonischemic-dilated[11] cardiomyopathy. In a small double-blind, placebo-controlled randomized trial, Mathiesen studied patients with heart failure (HF) because of severe ischemia. Six months after randomization, MSC-treated patients showed significant reductions in LV end-systolic volume and increases in LV ejection fraction.[12] Umbilical cord MSC are simple to maintain and to expand in vitro. Preliminary studies show that they are effective in improving cardiac function in patients with HF and reduced ejection fraction.[13]

Enhancement of Cell Function

Prominent among the challenges to cell therapy referred to above is the poor potency of injected cells. The approaches taken to deal with these problems are reviewed in the Compendium by Sussman and Broughton.[14] These include preconditioning of cells by their culture in a hypoxic medium,[15] by the addition of several growth factors, such as transforming, vascular endothelial, insulin-like, and fibroblast growth factors; as well as the proto-oncogene Pim-1. An example of the clinical application of cellular preconditioning was the CHART-1 trial (Congestive Heart Failure Cardiopoietic Regenerative Therapy), a double-blind randomized trial conducted in 348 patients with advanced chronic ischemic HF and reduced LV ejection fraction[16]; the MSCs had been pretreated with a so-called cardiopoietic cocktail consisting of several growth factors.[17] Although this trial failed to achieve its primary end point, a large subgroup of patients (identified post hoc) with markedly elevated LV end-diastolic volume showed significant improvement in LV function. This hypothesis-generating observation may help to identify a patient group who will benefit from this treatment. Natsumeda et al[18] have reported the enhancement of cell function by combining allogeneic MSCs with allogeneic cardiac stem cells in a post MI porcine preparation. Clinical evidence supporting a synergistic effect of 2 cell types came from the Ixmyelocel-T trial in patients with ischemic HF in whom the transendocardial delivery of a combination of 2 bone marrow-derived mononuclear cells—MSCs and activated macrophages—resulted in a significant reduction in adverse cardiac events.[19]

Another major problem with cardiac regeneration therapy has been the poor engraftment and retention of the administered cells. Four general approaches have addressed this issue, which has been of critical importance to cell therapy. Tokita et al[20] demonstrated in a rat model of severe ischemia followed by reperfusion, that 3 separate administrations of cardiac progenitor cells improved retention and LV function compared with the usual single injection. These results were confirmed in a second species and with a second cell type, MSCs.[21] The investigators have made a compelling point in that pharmacological

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(*Circ Res*. 2018;123:132-137.
DOI: 10.1161/CIRCRESAHA.118.313484.)

Circulation Research is available at http://circres.ahajournals.org
DOI: 10.1161/CIRCRESAHA.118.313484

Table. Important Challenges to Cell Therapy for Cardiac Regeneration

1	Selection of cell recipients.
2	Selection of cell donors.
3	Selection of cells.
4	Poor retention of injected cells.
5	Suboptimal cell potency.
6	Route and method of cell delivery.
7	Optimal dose, no. of doses.
8	Construction of synthetic matrices to provide cell scaffolds.
9	Standardization of preclinical experiments and clinical trials.
10	Timing of cell administration after myocardial injury.

therapy rarely uses a single administration of a drug and it would seem logical to deliver cells repeatedly in treating a persistent condition, such as chronic HF. This approach is well reviewed in this Compendium.[22] Also of importance is the administration of the cells by electro-anatomic–directed transendocardial injection, instead of coronary artery infusion.[6] With this approach the injected cells are not diverted from passing into ischemic regions by coronary obstructions. Instead, larger quantities are delivered to where they are judged to be most effective. Another approach to enhance cell engraftment is to provide structural support to the damaged myocardium with a tissue engineering technique, discussed in this Compendium by Zhang et al[23] and Vagnozzi et al.[24] Preclinical studies have utilized patches of fibrin and of synthetic scaffolds seeded with cells designed to be sewn into a necrotic, fibrotic area of the heart at the time of cardiac surgery.[25] A variety of cells have been used, including human induced pluripotent stem cells.[26] Alternatively, transcatheter injections into the myocardium of hydrogels containing stem cells have been shown to reduce infarct size, attenuate myocardial fibrosis, and accelerate functional recovery.[27] This approach is also described in this Compendium by Wysoczynski et al.[22]

As pointed out by Banerjee et al,[6] patient selection is critical. Up to now, the focus has been on patients with HF and reduced ejection fraction. Not surprisingly, the benefit of cell therapy seems to vary inversely with the severity of the HF. Autologous cells seem to be less potent in the elderly,[28] as well as in patients with diabetes mellitus and possibly other systemic illnesses as well. It would seem reasonable that young healthy individuals would be optimal cell donors.

Mechanisms of Cell Action

In the early studies of cell therapy, both preclinical and clinical, it was assumed that any observed improvement in cardiac function resulted from the differentiation of the transplanted progenitor cells into functioning cardiomyocytes, which replaced necrotic or dysfunctional cells.[29] However, further study showed, first in hematopoietic mononuclear bone marrow stem cells and subsequently in other cell types, that transplanted cells probably do not function in this manner. In 2004, Murry et al[30] reported that after cell administration, recipient hearts demonstrated few remaining transplanted cells, thought to be insufficient to exert a detectable beneficial effect on ventricular function. However, Tang et al[31] reported that although transplanted cells do not differentiate into myocytes, their paracrine mechanisms could be prolonged for cardiac progenitor cells because some do persist for 1 year.

Paracrine Hypothesis

In 2001, Fuchs et al[32] reported that bone marrow conditioned medium, that is, cell-free medium in which bone marrow cells had been cultured, acquired VEGF (vascular endothelial growth factor) and MCP-1 (macrophage chemoattractant protein) from the cultured cells. They further observed that the transendocardial injection of autologous bone marrow into pigs with induced myocardial ischemia augmented collateral perfusion and improved function in the ischemic myocardium, presumably by the above-mentioned growth factors identified in the bone marrow cells. The concept that transplanted cells can act in a paracrine manner was suggested by Kinnaird et al[33] in 2004, who showed that bone marrow-derived stromal cells released VEGF and BFGF (basic fibroblast growth factor) and other cytokines into the culture medium. When injected into a model of hindlimb ischemia, these stromal cells enhanced proliferation of endothelial and smooth muscle cells and improved collateral perfusion, presumably because of the release of arteriogenic cytokines from the injected cells.

Two papers published in 2005 extended the paracrine actions to the heart. Gnecchi et al[34] demonstrated that the intracardiac injection of cell-free MSC culture medium modified with the survival gene *AKT1* reduced infarct size in rats with coronary occlusion. These investigators concluded: "Our data support a 'paracrine hypothesis' of stem cell action in tissue protection and repair. These findings would suggest that the isolation of the secreted factor(s) may have important therapeutic application for the prevention of ischemic tissue damage." Yoshioka et al[35] extended these observations to cynomolgus monkeys whose cultured $CD34^+$ stem cells had secreted VEGF. The myocardium treated with these cells contained high concentrations of VEGF. These investigators suggested that this and other angiogenic cytokines secreted by the transplanted cells acted in a paracrine manner to enhance the angiogenic activity of the host's endogenous cells and thereby reduced infarct size.

Based on these and other studies, the paracrine mechanism is now widely accepted as being primarily responsible for the improvement in cardiac function observed with the administration of most cell types.[22] Embryonic and induced pluripotent stem cells may be exceptions because they seem to retain their ability to differentiate into cardiomyocytes.[36]

Extracellular Vesicles-Exosomes

Not surprisingly, there has been intense interest in identifying the mediators of paracrine action of transplanted cells. As mentioned initially, these mediators were thought to be a variety of cytokines, chemokines, and growth factors. Increased attention has focused on extracellular vesicles, structures which seem to play a critically important role in intercellular communication.[37,38] This important topic is reviewed in this Compendium by Garikipati et al.[39] The smallest extracellular vesicles are exosomes, which are of endosomal origin within a lipid bilayer, are between 30 and 120 nm in diameter, and have been studied most intensively. They contain many

mRNAs, miRNAs, transmembrane proteins and lipids, derived in varying proportions from their parental cells[40,41] and which act on the recipient (target) cells.

A variety of cell types have been demonstrated to produce exosomes.[40] Sahoo et al[41] reported that human CD34+ CPCs secrete exosomes with potent, independent angiogenic activity. Arslan et al observed that MSC-derived exosomes administered to mice whose hearts had been subjected to severe ischemia reduced infarct size and prevented adverse ventricular remodeling.[42] Gallet et al found that exosomes obtained from cardiospheres reduce scarring and attenuated adverse remodeling in a porcine model of MI,[43] whereas exosomes obtained from human umbilical cord MSCs have also been shows to relieve acute myocardial ischemia in rats after coronary artery ligation.[44] Induced pluripotent stem cells might also be able to deliver cardioprotective exosomes to autologous dysfunctional myocardium.[39,40]

In an effort to identify the constituent(s) of the exosomes that are responsible for their salutary paracrine effects, attention has been directed to their miRNAs. Mathiyalagan et al found that the cardioprotection derived from naked miRNA obtained from human CPCs was inferior to the same miRNA delivered within the exosome, suggesting that the naked miRNA might have undergone enzymatic digestion before it reached its target, but was protected when it was carried within an exosome.[45]

Exosomes and larger extracellular vesicles seem to exhibit the immunosuppressive activity of their parent cells.[46] They might be used as drug delivery vehicles, which can be produced in robust quantities in vitro by immortalized stem cells.[47] It may be speculated that the generations of exosomes by a variety of progenitor cells might allow the selection of exosomes that treat specific cardiac disorders. As suggested by Silvestri and Menasché: "The natural evolution of the stem cell theory for cardiac regeneration may end with the development of cell-free strategies with multiple cellular targets, including cardiomyocytes."[48]

Cell Therapy in Infants

There has been growing interest in extending the study of stem cell therapy from adults to newborns and children, as reviewed by Bittle et al in the Compendium.[49] Indeed, there is evidence for an inverse relationship between age and responsiveness to cell therapy. Utilizing right atrial tissue obtained at operation, Mishra et al reported that human CPCs are more abundant in neonates than in older infants, and then rapidly decline in concentration during postnatal maturation.[50] Also, there is evidence that CPCs obtained from neonates are superior to those from adults in the recovery of cardiac function.[51] The greater expression of heat shock factor-1 in neonatal CPCs may account, in part, for this superiority.[51] Similarly, Agarwal et al found that when studied in rats with MI the exosomes obtained from CPCs of human neonates were superior to those from older children.[52]

Because the right ventricle (RV) is primarily at risk in infants and children with congenital heart disease, the effects of cell therapy on this chamber have been examined. Studies on animal models of RV overload have shown improvement of function with stem cell therapy.[49,53] The epicardial injection of stem cells obtained from human umbilical cord blood improved RV function in a 1-week-old ovine model in which RV pressure overload was produced by pulmonary artery banding.[54] Similarly, in a neonatal swine model of RV pressure overload, the intramyocardial injection of human MSCs resulted in new vessel formation, which attenuated ventricular remodeling and hypertrophy.[55] Also, in lambs with chronic RV volume overload, the implantation of autologous mononuclear cells improved diastolic function.[56]

Hypoplastic left heart syndrome (or other causes of single ventricle physiology) is the most common congenital cardiac malformation on which cell therapy has focused. In this anomaly, the RV is subjected to both pressure and volume overload.[49,53] PERSEUS (Cardiac Progenitor Cell Infusion to Treat Univentricular Heart Disease) is the largest randomized trial of cell therapy in children with congenital heart disease that has been reported thus far.[57] In this phase II randomized trial conducted in 41 patients, the intracoronary infusion of CDCs resulted in improved ventricular function, reduced HF, and myocardial fibrosis and reversed ventricular remodeling.

Meta-Analysis

Although a considerable number of randomized, placebo-controlled, phase I and II clinical trials of cell therapy have been reported, by definition they have not been of sufficient size to provide definitive results of clinical outcomes. Many meta-analyses of these trials have been conducted to aid in planning larger, definitive phase III clinical outcome trials and to do so, in part, by identifying the phenotypes of patients most likely to exhibit clinical benefits. These are ably summarized by Gyongyosi et al[58] in the Compendium. However, the interpretation of these meta-analyses have been limited by the heterogeneities of the individual trials. These differences have involved the patient populations, the types of cells administered, their preparation, doses, methods of delivery, and the duration of follow-up. An additional difficulty is that most meta-analyses reported many of the same trials repetitively,[59] which can be avoided by conducting trial sequential analysis.[60]

Despite these issues, there are some lessons that can be learned from the meta-analyses. They indicate that in patients with chronic ischemic heart disease and reduced ejection fraction, the intramyocardial delivery of cells has resulted in modest improvements in LV function and a trend to clinical benefit.[61] Phase III trials using currently available technology may require upwards of 7000 to 10 000 patients to provide unambiguous evidence of clinical benefit. These numbers will decline with further advances in the technology and patient selection used. Meta-analyses of trials in patients with acute MI have not demonstrated improvements in hemodynamics or clinical outcome, using trial sequential analysis[60,62] or using individual patient data.[63] However, there are hints that the timing of cell delivery after the MI is important, with better results achieved when delivery is performed 3 to 7 days after the acute event.[64]

Although careful analyses of previous trials are certainly useful, in a field that is moving forward as rapidly as cardiac regeneration therapy, it is something akin to looking in the rear-view mirror. Importantly, however, the meta-analyses have demonstrated that with careful conduct, cardiac cell therapy appears to be safe.

Conclusions

At present, there is considerable skepticism, especially in the clinical community, about the potential role of cell therapy in the treatment of HF and acute MI. For example, a Task Force of the European Society of Cardiology stated: "Since 2006 advances in the field have been small … Although there have been advances in our understanding of the basic biology regarding the effector mechanisms involved in cell therapy these have not as yet led to significant improvements in the results of clinical trials."[65] A position paper of the European Society Working Group on Cell Biology and the Heart began its conclusions with a simple statement: "The early promise of cell therapy has not yet been fulfilled."[66] No regulatory authority has yet approved any cells for the treatment of HF or acute MI, nor have any practice guidelines recommended their use. Indeed, at the time of this writing, no phase III trial showing clinical benefit in patients with these disorders has been reported. Nevertheless, the cardiac journals are filled with interesting and encouraging preclinical and some phase II clinical results, as summarized in this Compendium.

What accounts for this disconnect? Looking back, during the first decade of this century, many encouraging results of preclinical studies demonstrating cardiac regeneration were reported at major scientific meetings and published in well regarded journals; many of these papers were accompanied by upbeat editorials. These reports reached the attention of the popular press, which, sometimes abetted by overenthusiastic investigators, led to excessive hype. This, in turn, resulted in unrealistic clinical expectations. In the transition from experiments in rodents to human trials, the immense differences between healthy young mice and seriously ill elderly humans with their multiorgan diseases, in both the vigor of their stem cells[28] and in the clinical responses, were rarely discussed. As a consequence, the human trials provided unimpressive results, which are responsible for the aforementioned skepticism. Although many phase III trials in patients with HF or acute MI with clinical end points are ongoing, some have hypothesized unrealistically favorable benefits and may therefore be underpowered, which may lead to further discouragement when their results become available.

However, despite these challenges, there have been some encouraging findings about the clinical potential of stem cell therapy. For example, autologous $CD34^+$ cells delivered intramyocardially have been shown to improve exercise capacity and reduce mortality and angina frequency in patients with refractory angina.[67] Also, MSCs and cardiosphere-derived cells have been found to be hypoimmunogenic, making possible allogeneic off the shelf products obtained from young, healthy human donors. The potency of these healthy cells can be stimulated further by a variety of additional interventions.[6] Another important piece of good news is, as already mentioned, when properly conducted, cardiac cell therapy seems to be safe.

Appreciation of the importance of the paracrine mode of action of transplanted cells represents an enormous step forward. The apparent key role of exosomes as paracrine mediators suggests that the production and harvesting of such nanovesicles from cultured cells, and perhaps in the future, the isolation of their protective constituents could be of immense importance. This could bring the pharmaceutical industry into this field and thereby provide increases both of scientific strength and financial resources for both preclinical and clinical research.

In assessing the overall development of cardiac regeneration, it may be useful to compare it to what are now the established cornerstones of cardiovascular therapy. The successful management of hypertension, the dyslipidemias, acute MI, as well as valvular and congenital heart disease did not occur overnight, but required decades of research. Indeed, although work on these conditions was begun almost a century ago, efforts for achieving further improvements are still underway. In contrast, intensive research on cardiac regeneration began <2 decades ago. Going forward, it seems advisable to support the recommendation of a recent global position paper on cardiovascular regenerative medicine, which stated: "The opportunity to… make real progress in the regeneration of human cardiovascular tissue is through worldwide multidisciplinary cooperation. By pooling the efforts of leading expert groups, we will collectively be able to develop effective treatments that will improve the prognosis of patients with a wide range of heart and vascular diseases."[68]

Disclosures

For this paper, Dr Braunwald reports no disclosures. For outside the submitted work, Dr Braunwald reports grant support to his institution from Daiichi Sankyo, AstraZeneca, GlaxoSmithKline, Merck, and Novartis; uncompensated consultancies and lectures with Merck and Novartis; Consultancies with The Medicines Company, Cardurion and Theravance; and personal fees for lectures from Medscape.

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KEY WORDS: Editorials ■ bone marrow ■ heart failure ■ myocardial infarction ■ regeneration ■ umbilical cord

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Cell-Based Therapy in Cardiac Regeneration: An Overview

Eugene Braunwald

Circ Res. 2018;123:132-137
doi: 10.1161/CIRCRESAHA.118.313484
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571