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Delivery and tracking of therapeutic cell preparations for clinical cardiovascular applications

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Abstract

Experimental observations suggesting adult stem cell plasticity and full realization of cardiovascular regenerative medicine. This brief cross-lineage transdifferentiation have underpinned the investigation review will highlight some of these, with emphasis on the choice of cell of cell therapy for cardiovascular disease. Many challenges still face the preparation, route of cell delivery and tracking of delivered cells.

Introduction

Experimental observations suggesting adult stem cell plasticity and cross-lineage transdifferentiation have underpinned the investigation of cell therapy for cardiovascular disease [1]. A number of putative clinical indications are summarized in Table 1. Pioneering pre-clinical studies suggested the capacity of undifferentiated BM-derived progenitor cells to undergo transdifferentiation into cells with myocardial and/or endothelial phenotypes, as well as produce hemodynamic improvements in experimental models of acute myocardial infarction [2–5]. Intrinsic cardiac stem cells and BM stromal cells have been isolated that can differentiate into cells from all three germinal layers in cell culture, including cardiac myocytes and endothelial cells [6–8]. On the basis of these and other pre-clinical observations, phase I clinical trials of administering unselected autologous BM mononuclear cells to patients with established coronary artery disease have been performed in Brazil, Europe and Asia using transepicardial intramyocardial injection at the time of surgical revascularization [9–11] and percutaneous endomyocardial injection [12–15]. In Europe and Asia, phase I clinical studies of BM-derived mononuclear cells and apheresed mono-nuclear cells, administered via an intracoronary route, have also been performed in patients with recent acute myocardial infarction [16–19]. The current clinical studies of administration of autologous BM preparations have not reported a limiting adverse safety profile. Randomized placebo-controlled blinded clinical trials of intracoronary autologous BM-derived mononuclear cell delivery for acute ST segment elevation myocardial infarction have been reported in preliminary form [20] or are currently enrolling.

While the initial results are encouraging, little can be definitively concluded regarding the efficacy and long-term safety of cell therapy for clinical cardiovascular applications from the available data. Indeed, several important pre-clinical reports have failed to replicate the ability of BM-derived mononuclear cells to transdifferentiate in the setting of myocardial infarction [21,22]. In addition, the functional endpoints employed in both small and large animal pre-clinical studies should be significantly improved. This brief review will highlight some of the outstanding challenges that should be addressed before routine clinical cardiovascular cell

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therapy can be realized, with emphasis on the choice of cell preparation, route of cell delivery and tracking of delivered cells.

Cell preparations

To date, clinical studies have been performed using autologous skeletal myoblasts [23,24], autologous unselected BM mononuclear cells [12–18,25], selected BM-derived $CD133^+$ cells [10], and unselected post-mobilization apheresed mononuclear cells [19]. A variety of other cell preparations will be available for future clinical application, many of which may be amenable to genetic manipulation prior to administration. These are summarized in Table 2. Many of these cell preparations contain a multiplicity of subsets, which may be identified and isolated on the basis of specific cell-surface markers or selective culture conditions. For each preparation, the main subpopulations of cells with potential regenerative capacity are indicated, although the list is by no means exhaustive.

Functional benefits of cell therapy for cardiovascular applications may arise from induction of angiogenesis, cardiomyogenesis or mechanical interstitial support. The former two modes of benefit may result from site-specific transdifferentiation of administered cells or by secretion of paracrine factors that may stimulate endogenous repair mechanisms. On this basis, improvements in regional myocardial perfusion, systolic function, diastolic function and adverse ventricular remodeling would be predicted. The additional mechanical interstitial support provided by cell administration may itself impact beneficially on the ventricular remodeling process. Clearly these potential benefits need to be weighed against the potential toxicity from cell therapy, such as exacerbation of atherosclerosis, arrhythmogenesis, inappropriate calcification and local or ectopic tumor formation.

The optimal cell preparation for each of the variety of potential cardiovascular applications remains to be determined. It cannot be assumed that one cell preparation will be equally efficacious for all clinical applications, and different cell preparations may have varying toxicity profiles. Indeed, it is unclear if administration of a highly selected cell population is preferable to a heterogeneous unselected or combination cell product. Other unresolved issues include determination of the optimal number of cells to be delivered, timing of cell administration, importance of growth factor preconditioning of cellular products prior to administration, effects of *ex vivo* cell expansion and prolonged cell culture prior to administration, and the use of allogeneic rather than autologous cell populations (Table 3).

Method of delivery

For therapeutic purposes, cells should be delivered to the target tissue of interest in sufficient number to confer functional benefit with minimal toxicity to the patient.

Systemic delivery

For cardiovascular applications, modes of cell delivery may be broadly categorized as systemic or local (Table 4). Systemic delivery may consist of intravenous infusion of cells or cytokine mobilization of cells into the circulation. The homing of systemically administered cells to the target tissue of interest requires appropriate chemokine signaling from the target tissue. The intensity of these homing signals appears to decline with time following an acute injury and may determine the time window during which systemic cell administration may confer functional benefit. Studies in rodents have demonstrated the ability of human progenitor cells from G-CSF-mobilized leukapheresis products [4] and rat allogeneic mesenchymal stromal cells [26] to home to regions of acute myocardial infarction following intravenous administration. In the former study, improvements in ejection fraction and attenuation of

adverse cardiac remodeling were noted on the basis of echocardiography. Other rodent studies have demonstrated that cytokine mobilization may confer hemodynamic benefits in a murine model of acute coronary artery ligation [3]. Recent clinical data of G-CSF mobilization in patients with severe symptomatic coronary disease and previous myocardial infarction have not demonstrated improvements in regional or global left ventricular dimensions, contractile function or perfusion [19]. These preliminary data suggest that systemic cell delivery may not be optimal in the setting of acute or chronic coronary syndromes, although further studies are required.

Local delivery

Local delivery is theoretically more attractive than systemic in that larger numbers of cells may potentially be administered to specific regions of interest within the target organ. Local delivery systems should be biocompatible with the cell preparation to be administered, such that there is minimal loss of cell number and cell viability as a consequence of passage through the delivery system. Clinically, local cell delivery can be achieved by direct injection at the time of open heart surgery or by percutaneous catheter-guided intracoronary infusion or intramyocardial injection.

Surgical delivery

Surgical exposure is excellent for performing intramyocardial cell injection targeted to infarct borders under direct vision. A number of phase I trials of intramyocardial injection of autologous BM mononuclear cells [9,11], $CD133^+$ cells [10] and autologous skeletal myoblasts [23,24] at the time of coronary artery bypass grafting have been performed. All these studies report little procedural toxicity, although skeletal myoblast therapy has been associated with increased risk of malignant ventricular arrhythmia. Phase II double-blind randomized controlled clinical trials of autologous skeletal myoblast therapy are currently enrolling patients, with all eligible patients requiring an implantable cardiac defibrillator prior to randomization. No firm conclusion about the efficacy of cell therapy can be made due to the uncontrolled and unblinded nature of the studies currently reported in the literature and the confounding beneficial effects of revascularization. The surgical approach for local delivery of cytotherapy is attractive as an adjunct to surgical coronary revascularization, but is unlikely to find widespread clinical application for the sole purpose of targeted cell delivery.

Catheter delivery

Percutaneous catheter-based approaches are available for delivery of cells to the myocardium via intracoronary infusion, endomyocardial injection, transcoronary vein intramyocardial injection, transcoronary sinus retrograde infusion and intrapericardial injection. Phase I clinical trials of intracoronary and endomyocardial injection of autologous BM-derived mononuclear cells have been performed [12–18,25]. In addition, catheter-based delivery of skeletal myoblasts has been performed [27]. These studies also report little procedural toxicity, and the available data suggest improvement in ventricular dimensions, systolic function and myocardial perfusion. However, extreme caution needs to be taken when interpreting these initial results, which are derived from non-randomized unblinded studies without inclusion of placebo groups, particularly as significant improvements in left ventricular function can be observed as a result of routine clinical management of acute myocardial infarction [28]. Randomized, blinded placebo-controlled trials of intracoronary and endomyocardial autologous BM mononuclear cell administration for acute myocardial infarction [20] and ischemic cardiomyopathy, respectively, are currently enrolling patients.

A number of methods are available for targeting cell administration using catheter-based techniques. Clinical procedures are currently performed under X-ray fluoroscopic guidance.

Intracoronary infusions can be performed down the infarct-related artery [17,18] and endomyocardial injections can be localized to peri-infarct regions using an electromechanical mapping system [12,13]. The former approach is safely and easily performed using conventional over-the-wire angioplasty balloons, with cells being delivered to the distribution territory of the infarct-related artery. The appeal of the latter system is that it creates an interpolated color-coded map that can register measures of electrical and mechanical function in a 3-dimensional surface rendering. In addition, a patent conduit vessel supplying the target myocardium is not required. The disadvantage of the system is that, apart from electrocardiographic gating, a retrospective mechanical 'roadmap' is used to relate current geometric catheter position to anatomy, despite complex non-periodic cardiac and pulmonary motion. Therefore, these electromechanical maps, which can take up to 2 h or more to acquire, may not accurately represent the true position of the catheter device in relation to the target delivery site at the precise time of cell injection. Targeted cell delivery may also be achieved using real-time magnetic resonance imaging (MRI) [29,30] techniques and cells labeled with particles (Figure 1) [31], which appear as signal voids on the magnetic resonance (MR) image (Figure 1). Using this sophisticated technology, cell delivery can be targeted in real time precisely to infarct borders (Figure 1). In the future, this may become an increasingly attractive imaging environment in which to perform these types of procedure.

There are several major unresolved issues in cell delivery. The optimal delivery route has not been established. There are few quantitative data addressing cell distribution and cell retention as a function of the mode of cell delivery. Biodistribution studies using technetium-99m labeled BM-derived mesenchymal stromal cells in recently infarcted rats suggest that, following intravenous infusion, the vast majority of infused cells are entrapped in the lungs with little distribution to the heart [32]. The number of cells in the heart was increased by infusion of cells directly into the left ventricular cavity [32]. Retention of cells following direct intramyocardial injection was not assessed in this study. Other pre-clinical data suggest that at best only 30–40% of particulate material is retained within the myocardium following a successful endomyocardial injection [33]. Clearly, significant improvements in cell retention and its quantification are required. Furthermore, the biocompatibility of interventional devices with therapeutic cell preparations needs further assessment. Variability in cell number, viability, migratory, proliferative and differentiation capacity as a function of cell handling and interaction with the delivery system may be important determinants of the efficacy of cell therapy. Finally, the importance of targeting cell delivery has yet to be established, although preliminary data from skeletal myoblast administration suggest that the risk of arrhythmia is greater when myoblasts are injected into necrotic myocardium rather than peri-infarct zones.

Cell labeling and tracking

There are few methods available for performing cell labeling and tracking in humans *in vivo*. The use of cells labeled with iron particles that produce susceptibility artifacts when imaged with MRI is one potential approach. Inert polymer microspheres on a micron [34] or nanometer [35] scale containing iron oxide have been used successfully to label mesenchymal stromal cells, $CD34^+$ and $CD133^+$ cells. The latter approach is particularly attractive as it employs clinically approved reagents.

These labeling protocols appear not to affect the demonstrable proliferative or differentiation capacity of these cells *in vitro*. Using this general approach, cells may be imaged serially at multiple time points, allowing assessment of the migratory capacity of these cells following local or systemic delivery. Unfortunately, there is no clear quantitative relationship between the size and/ or intensity of the signal voids on the MRI image and cell number, thus making quantification of cell retention or accumulation difficult to achieve. Preliminary pre-clinical

data suggest that homing of mesenchymal stromal cells and $CD133^+$ cells to infarcted regions after intravenous administration can be demonstrated in rat models of myocardial infarction by MRI. In addition, serial imaging studies suggest that iron-labeled mesenchymal stromal cells do not appear to migrate towards infarcted regions following direct endomyocardial injection.

This technique is attractive as it has the potential to allow tracking of cells over time *in vivo* and, furthermore, may enable confirmation of cell administration following either X-ray or MRI-guided delivery. While the *in vitro* assays are encouraging, the impact of the cell label on *in vivo* biological activity of administered cells has yet to be determined. In addition, it is known that following direct myocardial injection, the majority of retained cells undergo cell death [36], resulting in the potential deposition of iron that may not be contained within the originally injected cells. This may hinder image interpretation and may be potentially toxic to myocardium.

Conclusion

Many challenges still face the full realization of cardiovascular regenerative medicine. This is a complex therapy, the success of which will require an integrated multi-disciplinary collaboration between cardiologists, hematologists, cell-processing experts, basic scientists and industry.

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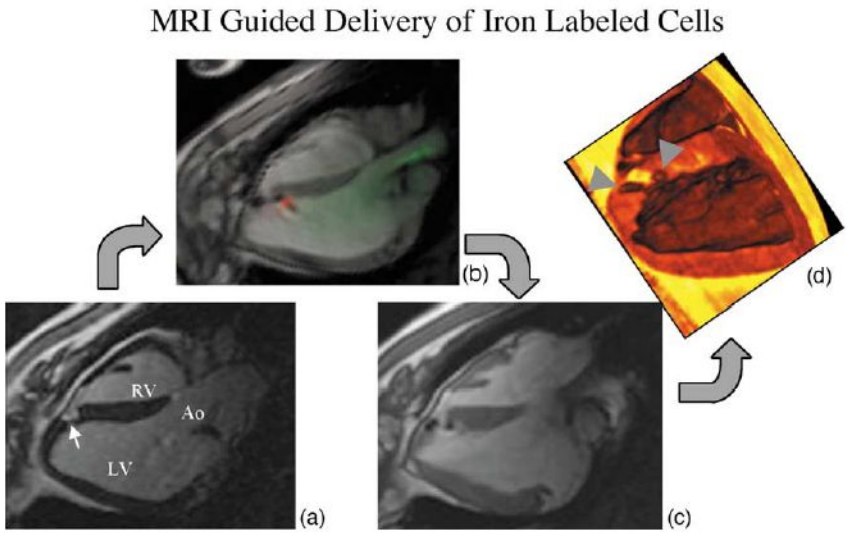

Figure 1.

(a) A long axis cardiac MR image demonstrating a small localized area of myocardial infarction (arrow) in the apical septum following gadolinium injection. (b) An endomyocardial injection catheter is used to deliver iron-labeled mesenchymal stromal cells precisely to the infarct border. The catheter shaft is green and the catheter tip is red. (c) The iron-labeled cells appear as signal voids (dark spots) on the MR image and can be seen on either side of the area of infarction (white). (d) This shows an ex vivo *high-resolution volume-rendered MR image demonstrating the area of infarction and the location of the iron-labeled cells, which appear as signal voids. LV, left ventricle; RV, right ventricle; Ao, aorta.*

Table 1

Clinical cardiovascular indications for cell therapy

	Acute	Chronic
Cardiac	Acute myocardial infarction	Ischemic cardiomyopathy Idiopathic-dilated cardiomyopathy
Peripheral artery disease		Intermittent claudication Critical lower limb ischemia

Table 2

Sources of autologous cells for clinical cardiovascular applications

Source	Cell subsets with potential regenerative capacity
BM	
Unselected mononuclear cells	Stromal cells, CD117/c-kit$^+$ cells, CD34$^+$ cells, CD133$^+$ cells, endothelial progenitor cells
Stromal cells	Mesenchymal stem/stromal cells, multipotent adult progenitor cells, side population cells
CD34$^+$ cells	CD133$^+$ cells, VEGFR-2$^+$ cells
CD133$^+$ cells	VEGFR-2$^+$ cells
Endothelial progenitor cells	CD34$^+$ late outgrowth cells, CD133$^+$ cells, VEGFR-2$^+$ cells, monocyte/macrophages
Post-mobilization leukapheresis product	
Unselected mononuclear cells	CD117/c-kit$^+$ cells, CD34$^+$ cells, CD133$^+$ cells, endothelial progenitor cells, monocytes
CD34$^+$ cells	CD133$^+$ cells
CD133$^+$ cells	VEGFR-2$^+$ cells
Endothelial progenitor cells	CD34$^+$ late outgrowth cells, CD133$^+$ cells, VEGFR-2$^+$ cells, monocyte/macrophages
Pheripheral blood	
Endothelial progenitor cells	Circulating bone-marrow derived endothelial progenitor cells, CD34$^+$ cells, CD133$^+$ cells, VEGFR-2$^+$ cells, CD14$^+$ myeloid cells, side population cells, circulating endothelial cells
Umbilical cord blood	
CD34$^+$ cells	CD133$^+$ cells, VEGFR-2$^+$ cells
CD133$^+$ cells	VEGFR-2$^+$ cells
Multipotent adult progenitor cells	
Unrestricted somatic stem cells	
Adipose tissue	
Stromal cells	
CD34$^+$ cells	CD133$^+$ cells, VEGFR-2$^+$ cells
Skeletal muscle	
Skeletal myoblasts	
Skeletal muscle stem cells	‘Satellite’ cells, hematopoietic progenitor cells, multipoptential muscle cells
Cardiac muscle	
Endogenous cardiac stem cells	CD117/c-kit$^+$ cells, sca-1$^+$ cells
Embryonic stem cells	

Abbreviations: VEGFR, Vascular Endothelial Growth Factor Receptor

Table 3

Comparison of allogeneic and autologous cell preparations

	Autologous	Allogeneic
Advantages	• No immune rejection of cells • Minimal risk of anaphylaxis, transfusion reaction and alloimmunization • Minimal risk from transmissible infectious agents • Rapid access to large numbers of cells, e.g. post-mobilization leukapheresis product	• ‘‘Off the shelf ’’ cell product, immediate access useful for acute interventions • Immediate access to large numbers of cells • Immediate access to genetically modified cells • BM aspirate, cytokine mobilization ± leukapheresis or skeletal muscle biopsy not required • Cells from young donors may overcome issues of age-related decline in regenerative capacity
Disadvantages	• BM aspirate, cytokine mobilization ± leukapheresis or skeletal muscle biopsy required • Time-consuming *ex vivo* expansion may be required (e.g. skeletal myoblasts, stromal cells), cell administration for acute conditions delayed • Potential age-related loss of regenerative capacity • Extra expense of patient-specific cell processing	• Anaphylaxis, acute transfusion reaction, alloimmunization • Potential immune rejection • Risk of transmissible infectious agents • Decline in regenerative capacity and accumulation of cytogenetic abnormalities as a consequence of prolonged *ex vivo* expansion and culture

Table 4

Routes of cell delivery

Systemic	Local
• Intravenous infusion + cell homing	• Cardiac: percutaneous catheter **based**
• Cytokine mobilization + cell homing	• Selective intracoronary infusion
	• Transcoronary sinus retrograde infusion
	• Endomyocardial needle injection
	• Transcoronary vein
	• intramyocardial injection
	• Intrapericardial
	• Cardiac: surgical
	• **Open** chest transepicardial intramyocardial injection
	• Lower limb
	• **Intra-**arterial infusion
	• Direct intramuscular injection