Brave New World
Ventricular remodeling and ultimately heart failure are the inexorable consequences of substantial myocardial infarction. In recent years, the understanding that regenerative processes exist at the level of the myocardium has placed stem cell research at center stage in cardiology. Through cellular therapies, the concept of “growing” heart muscle and vascular tissue and manipulating the myocardial cellular environment has revolutionized the approach to treating heart disease. Unfortunately, however, the vast field of possibilities opened by stem cell therapy has frequently given rise to more questions than answers. A few of these questions include: Which patients with cardiovascular diseases should be considered for stem cell therapy? Which type of stem cell(s) should be used? What quantity and concentration of cells should be administered? By what mechanisms do stem cells engraft, survive, and differentiate? Is the functional and morphological cardiac improvement achieved actively (ie, by increasing contractility) or passively (ie, by limiting infarct expansion and remodeling)? What is the lifespan of transplanted stem cells in the heart? How safe is this therapy, and is there potential tumorigenesis of stem cells? What might be the potential benefits of cell transplantation in nonischemic heart failure?

In this report, we wish to review available information about cardiovascular stem cell therapy, share our early experience in this new field, and speculate about future directions. Although embryonic stem cells have been shown to have greater potency for proliferation and differentiation than adult stem cells, their lack of availability and ethical issues hamper clinical applications. This report will, therefore, focus on the therapeutic applications of adult stem cells.

Therapeutic Use of Stem Cells
The diverse literature on stem cell research comprises the work of basic and clinical scientists from many different subspecialties. This may account for the heterogeneous mixture of models, methods, types, quantity, and nature of the cells employed and the timing of experiments. Certain landmark findings and concepts should be highlighted, however, as they have shaped our understanding of what may be accomplished and what potential mechanisms may be explored to achieve clinically successful results in the future.

Paradigm Shift
The pivotal finding by Ashahara and colleagues [1] that postnatal vasculogenesis exists (ie, that stem cells contribute directly to the formation of new blood vessels in adults) provided new insights into mechanisms of cardiac repair. In the adult, neovascularization does not rely exclusively on angiogenesis (sprouting from preexisting blood vessels). Furthermore, endothelial progenitor cells (EPCs) that originate in the bone marrow play a role in vasculogenesis (physiological and pathological) and circulate in adult peripheral blood. [1] The intriguing observation in heart transplant patients that putative stem cells and progenitor cells from a recipient were present in the transplanted heart further supports the notion of ongoing regenerative and reparative mechanisms mediated by circulating stem cells from the bone marrow. [2]

Finding a Home
The microenvironment plays a fundamental role in the transdifferentiation of stem cells. Human mesenchymal stem cells (from adult bone marrow), when engrafted into murine...
hearts, seem to differentiate into cardiomyocytes that are indistinguishable from the host’s cardiomyocytes.3

Although its mechanism is incompletely understood, the “homing” of stem cells to the injured myocardium is essential, as it concentrates the implanted cells in an environment favorable to their growth and function. Ischemia or hypoxia may increase vascular permeability, enhance the release of chemoattractive factors, and promote the expression of adhesion proteins, which may facilitate the homing process.

**Putting Stem Cells to Work**

Because the normal regenerative mechanisms seem to be overwhelmed when clinically significant myocardial injury occurs, a logical next step would be to artificially amplify one part of this response by locally applying stem cells in the setting of ischemia or infarction when a large amount of heart muscle has been injured. Experimentally, circulating EPCs have been shown to be mobilized endogenously in response to tissue ischemia (or exogenously by cytokine therapy), after which they augmented the neovascularization of ischemic tissues.4 Implantation of bone marrow mononuclear cells into ischemic myocardium in swine enhanced collateral perfusion and regional myocardial function.5 This therapeutic angiogenesis may have been due to the natural ability of the bone marrow cells to secrete potent angiogenic ligands and cytokines, as well as to be incorporated into foci of neovascularization.6 Other observations have shown that EPCs prevented cardiomyocyte apoptosis, reducing remodeling and improving cardiac function in areas of neovascularized ischemic myocardium in rats.7

Systemic and local growth factors almost certainly influence stem cells homing and biology. EPCs and their precursors are mobilized during an acute myocardial infarction. This mobilization may be due to the increased levels of vascular endothelial growth factor (VEGF) associated with acute myocardial infarctions, as VEGF has been shown to augment the mobilization of EPCs from bone marrow.8 Increased levels of transcriptional activators for VEGF have been found in response to early ischemia and infarction.9

**A Call to Arms**

There is intense, ongoing investigation into the identification of the ideal cell for use in therapeutic applications. On the basis of experimental evidence, several types of stem cells might be administered for therapeutic angiogenesis. These include filtered bone marrow cells, mononuclear bone marrow cells, or even a subtraction of bone marrow stem cells, including endothelial precursor cells, as well as stromal or mesenchymal cells and hemopoietic stem cells. Selection of a specific cell type may require highly sophisticated facilities and technology. A sufficient quantity of cells may be obtained from direct bone marrow aspiration (as with mononuclear bone marrow cells), but expansion of a selected cell will likely necessitate cell culture. Cells may also be obtained from peripheral blood after cytokine or growth factor stimulation. Skeletal myoblasts are easily harvested from a small sample of skeletal muscle, which is subsequently processed, and further expansion of myoblasts is achieved through culture.10 Stem cells may also be harvested and cultured from other autologous sources such as adipose tissue. Umbilical cord blood harboring a large number of autologous embryonic stem cells may be saved for future use.

Fresh administration of cells after brief manipulation (for a matter of hours) offers a simpler approach than culturing cells, which involves more extensive manipulation (for 2 weeks) and a greater concern about infection.

**Administration of Stem Cells**

**General Considerations**

Although the ideal route for administering stem cells has still yet to be determined, it may be important to take certain factors into consideration. The strength of homing signals may vary in different clinical scenarios. In more acutely ischemic scenarios, the stem cells may be administered either peripherally or locally through the circulatory system. When the homing signals may be less intense, as may be the case for chronic ischemia or nonischemic cardiomyopathies, injection of the cells directly into the cardiac muscle may produce a more favorable outcome. Certain stem cells, such as skeletal myoblasts, are best administered by means of direct tissue injection because of the potential for embolization when large numbers of these cells are administered.

**Surgical Intramyocardial Injection**

Although the most invasive approach, this method is suited to patients who already have a surgical procedure scheduled. The injection process is simple and can be performed under direct visualization, allowing evaluation by direct inspection of the potential target zones. Not all areas, however, can be readily accessed with this approach.

**Transendocardial Injection**

This method primarily involves the NOGA system (Cordis), for which previous experimental11 and clinical12 experience is available. An injection catheter incorporates the mapping capabilities of the system. This provides a means by which tissues with different degrees of viability13 and ischemia can be mapped in detail, allowing therapy to be precisely targeted (eg, at the border zone of an infarct). NOGA application in humans has a long learning curve but has been used very safely in our experience.

Ultimately, noninvasive imaging, possibly with MRI, echocardiography, or computed tomography, needs to develop for the purpose of monitoring stem cell concentrations in cardiac and vascular tissue and their contributions to tissue function and angiogenesis.

**Intracoronary/Transvascular Injections**

Performed successfully in a clinical trial,14 intracoronary injection is especially well suited for the delivery of cells to a specific coronary territory. It is less complex than transen-
docardial delivery, and because of the segmental nature of coronary artery disease, may prove very practical. Retention of cells in the target area remains a central issue that suggests that this technique will be particularly suited for treating relatively intense ischemia. The quantity of cells and time of infusion should be carefully considered to avoid coronary flow impairment and myocardial cell necrosis. This technique may not be suitable for certain types of larger stem cells, such as skeletal myoblasts, which may be prone to embolization.

In addition, both the coronary sinus and the great cardiac vein provide low-pressure venous conduit access to the interior and anterolateral left ventricular myocardium by which ultrasound-directed (Transvascular Inc) intramyocardial injections of stem cells may be performed.

**Intravenous Injections**

This is the simplest method of cell administration, but a greater degree of dependence on homing of the stem cells is required in order for them to reach the myocardium. Intravenously injected cells may become trapped in other organs (eg, liver, spleen, lung, etc) so that only a small portion enters the coronary circulation and migrates into ischemic myocardium. Homing signals are also present at other sites in the body, particularly in lymphoid tissues. Dosing will likely play an important role in the viability of this method.

**How Much Is Enough?**

An important issue concerning the therapeutic use of stem cells is the quantity of cells necessary to achieve an optimal effect. In current human studies of autologous mononuclear bone marrow cells, empirical doses of 10 to $40 \times 10^6$ are being used with encouraging results. However, further studies are needed to explore the efficacy of different doses. Recently, in a study designed to treat peripheral vascular disease with autologous bone marrow, much larger doses were administered to the gastrocnemius muscle ($2.7 \times 10^9$ cells), with minimal inflammation and positive results.

When the primary focus is not on angiogenesis, but rather on transplantation of contractile muscular tissue, a much higher quantity of cells may be needed to obtain a clinical effect.

**Initial Experience in Humans**

The currently available knowledge and the scope of the clinical problem are compelling and have prompted the initiation of clinical trials. They are currently being performed as single-center studies using mononuclear bone marrow cells or skeletal myoblasts. Safety and feasibility have initially been favorable in trials utilizing human adult bone marrow stem cells, but unresolved issues remain regarding the initial arrhythmogenic potential of myoblasts.

In the published reports of human stem cell trials thus far, the use of mononuclear bone marrow cells, delivered via the intracoronary route in patients with previous myocardial infarcts but a preserved ventricular ejection fraction, was shown to improve function and perfusion in a small number of patients at 3- and 4-month follow-up.

Several other groups, including our own (in collaboration with Procardiaco Hospital in Rio de Janeiro, Brazil) are actively pursuing clinical studies. We have delivered mononuclear bone marrow cells using electromechanical mapping in patients with end-stage heart failure. Viable/ischemic zones were targeted for delivery. According to our as yet unpublished data, these patients have shown symptomatic improvement associated with increased contractility (Figure) and perfusion at 4- and 6-month follow-up examinations.

**Future Perspectives**

Combining stem cell therapy with other treatments may increase therapeutic options in the future. Gene therapy has shown encouraging results in promoting angiogenesis, but the safety of gene delivery by means of viral vectors has become of increasing public concern. Stem cells may serve as new vehicles for carrying genes into tissues. For instance, through genetically manipulated stem cells, a VEGF gene that is critical for angiogenesis can be transferred into stem cells that, in turn, may have a more potent action than VEGF-negative cells. Treatment achieved with adenoviral vector-aided delivery of genes encoding VEGF has shown positive results in a hind-limb experimental ischemia model.

Combined pharmacological, surgical, and interventional treatments with stem cell therapy (eg, injection of stem cells...
during placement of a ventricular assist device) may also provide added benefit, but it is too early to speculate further.

The Fountain of Youth

Recently, a study suggested that cellular restoration of the platelet-derived growth factor pathway by young bone marrow-derived EPCs could reverse the aging-associated decline in cardiac angiogenic activity in mice.18 The very notion that within each one of us lies a treasure-trove of renewable life that can be directed toward healing and revitalizing cardiac function should be reason enough to propel us on a journey to answer the myriad questions involved in understanding the application of stem cells and their associated therapies. As the song suggests, “the future’s so bright, [we’ve] gotta wear shades”!19

References

Adult Stem Cell Therapy in Perspective
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