Preconditioning Stem Cells for Cardiovascular Disease
An Important Step Forward

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The promise of stem cell therapies to regenerate new myocardium and/or to enhance intrinsic repair processes has generated enthusiasm in the scientific and clinical communities, as well as among the public and its state and national representatives. This enthusiasm is based primarily on the limited inherent cardiac regeneration capability, the increased worldwide incidence of cardiovascular disease because of the aging of the population in the developed world and adverse consequences of lifestyle changes associated with industrialization and urbanization in developing countries, the fact that left ventricular dysfunction is the final common pathway for most forms of cardiovascular disease, and the fact that despite advances in medical and device therapies, mortality, repeated hospitalization rates, and quality of life limitations in patients with left ventricular dysfunction remain at unacceptable levels.

The cell type studied most extensively in the clinical setting of chronic left ventricular dysfunction is the skeletal myoblast (SkM). Advantages of this approach include demonstrated improved cardiac performance in animal models and relatively easy accessibility to large numbers of autologous stem cells in patient populations. However, the largest and most recent placebo-controlled, randomized trial (Myo-Blast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) Trial) did not demonstrate sustained efficacy as defined by the primary end point, global ejection fraction, although secondary end points of changes in left ventricular volumes were significantly improved in the high-dose (800 million cells) group. Efficacy for any cell product is certainly dependent on delivery to the intended region, an issue largely addressed with direct injection protocols, and then successful retention at that site. Retention is one of the most significant identified issues limiting efficacy in most studied models, with some estimating a greater than 90% cell loss because of necrosis or apoptosis within 24 hours of transplantation. It may be expected that a reduction in the initial transplanted cell death would allow for transplantation of fewer cells and thus earlier administration of autologous cells after myocardial injury. There would also be decreased cellular debris which could stimulate an inflammatory response that adversely affects cell engraftment. It is also anticipated that greater numbers of surviving stem cells would translate into more paracrine stimuli with the end result being greater improvement of left ventricular function and smaller infarct size.

Several factors may play a role in early cell death, including damage during isolation and injection, hypoxia, absence of survival factors in the transplanted myocardium, disruption of cell-cell and cell-ECM contacts, ischemia because of insufficient vascular supply and elaboration of inflammatory cytokines resulting from ischemia and/or cell death. Cell death can occur by apoptosis and necrosis: apoptosis can be triggered by activation of the extrinsic or intrinsic apoptotic pathways, which ultimately result in caspase activation, cellular proteolysis and DNA degradation. Both necrosis (caspase-independent cell death) and apoptosis can be triggered by ischemia, free radicals and intracellular Ca2+ overload. Intense work is underway by several groups to identify agent(s) and interventions that improve cell survival after transplantation and include administration of growth factors (IGF-1, HGF, GCSF, VEGF), nonspecific caspase inhibitors, anti-oxidants, gene therapy to overexpress Akt (protein kinase B), Bcl-2, and HIF-1α, as well as heat shock and scaffold interventions. Linke et al demonstrated that intra-myocardial injection of IGF-1 and HGF promotes stem cell migration and myocardial regeneration in mice and dog models of myocardial infarction. IGF-1 is known to attenuate apoptosis in response to oxidative stress and to regulate cell proliferation and differentiation. Prosurvival effects of growth factors like IGF-1 are mediated by phosphoinositide 3-kinase (PI3K) and its downstream target, the serine/threonine protein kinase Akt. This pathway transduces important cell survival signals from various cell surface receptors to the mitochondria, which are the effectors of cell death (Figure), both in myocytes and transplanted stem cells. On activation of this signaling cascade by growth factor binding, Akt phosphorylates a critical serine residue on Bad, a proapoptotic protein that then dissociates from Bcl-xL, an anti-apoptotic protein, thus freeing it to promote cell survival. Bcl-2 and Bcl-xL suppress apoptosis, in part by blocking release of cytochrome c from mitochondria, a critical step in the activation of the caspase protease cascade (Figure). Significantly, nonselective caspase inhibitors like Z-VAD-FMK reduce apoptotic death both in allogeneic nigral and β-cell transplants.

The current study by Niagara et al is an important step forward in terms of identifying techniques and responsible mechanisms to improve cell retention following SkM transplantation. The authors demonstrated that "preconditioning" rat SkM by exposure to diazoxide improves...
tolerance to in vitro oxidative stress, results in increased expression of paracrine anti-apoptotic and angiogenic factors, and importantly is associated with increased cell survival at four days and improved echo-measured left ventricular function at four weeks. The authors conclude that diazoxide preconditioning improves cell survival and function and that both are related to paracrine effects and increased blood vessel formation. The inability of SkM to transdifferentiate into cardiomyocytes and to electromechanically couple with existing cardiac cells supports the notion that skeletal myoblast benefit is because of, at least in part, elaboration of homing or regeneration signals. The findings that diazoxide preconditioning improves survival in SkM is consistent with the group’s earlier report that hypoxia-induced preconditioning of bone marrow stem cells improves left ventricular function and remodeling parameters following transplantation as well. However, the authors’ findings deserve cautious interpretation and suggest additional study goals for future experiments before translation to the clinical setting. Although expressions of bFGF and HGF studied by RT PCR were upregulated by 1.44 fold and 2.26 fold respectively, use of quantitative PCR would make the findings more robust. The quantitative PCR results for the sry gene demonstrate a signal in the negative control lane and hence raise a question regarding the validity of the engraftment results using this technique; use of SkM, genetically modified with a reporter gene would strengthen the results. Engraftment was assessed at four days, but ejection fraction at 4 weeks. The interpretation that improved function can be attributed to increased paracrine signaling resulting from enhanced survival would be strengthened if future studies assess engraftment and left ventricular function at similar time points after transplantation with in vivo imaging methods like bioluminescence imaging and MRI respectively. It will also be helpful in future studies to investigate the impact of SkM transplantation on apoptosis and regeneration of cardiac myocytes. The effects of SkM transplantation on myocardial infarct size as well as myocyte apoptosis in the borderzone and un-involved myocardium are not available in the present study because of lack of detailed histologic analysis of the explanted heart. Were all Ki67 positive cells dividing SkM, or did SkM-induced myocardial neovascularization also increase cell cycling of endogenous cardiac myocytes?

In addition, studies of the effects of other factors reported to improve cell survival should be compared with those of diazoxide, as well as studies determining whether there are synergistic effects. It would also be useful, in preclinical studies, to determine whether the benefit of diazoxide “preconditioning” is age-dependent, as reported with other preconditioning protocols when functional end points following prolonged ischemic periods were examined. If so, the benefit may be limited in the typical, older patient population when autologous cells are used.

Several issues must be addressed before the preconditioning paradigm can be fully evaluated in the clinical setting. It will be important to develop methods to “track” retention and fate of administered cells so as to determine whether interventions designed to improve survival do in fact accomplish this aim. To date, it is only possible to examine retention on a short-term basis, and there is no current clinical method to assess differentiation. It will also be important to examine the effect of diazoxide and different preconditioning protocols in other cell types currently being evaluated clinically, including cardiac stem cells and mesenchymal cells, as well as newer proposed sources of progenitor cells, eg, adipose tissue and umbilical cord blood.

There is great enthusiasm for the potential of stem cell therapies in the treatment of cardiac and chronic degenerative diseases among scientists, physicians, and the patients themselves. Nevertheless, careful controlled studies are needed to explore mechanisms for any benefit and methods to enhance that benefit, as well as to identify undesirable effects and interventions to decrease their likelihood. By identifying the very significant benefits of preconditioning cells before transplantation, the authors have moved our progress on that road one very significant step forward.
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