The Mechanisms of Cell Therapy for Clinical Investigations:

An Urgent Need for Large-Animal Models

Running title: Zhang et al.; The mechanisms of cell therapy

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Evidence from experiments performed in animal models and from early-stage clinical trials demonstrate that the contractile performance of hearts with severe ischemic injury can be improved by transplanting a variety of cell types into the injured heart.1-5 As a result of these investigations progenitor cells have emerged as a promising therapeutic agent both for limiting postinfarction left-ventricular (LV) remodeling and for improving cardiac performance in hearts with severe LV dysfunction secondary to other diseases, such as dilated cardiomyopathy and concentric LV hypertrophy.6,7 Some of the most encouraging clinical results were obtained in the SCIPIO trial4, which investigated the use of autologous cardiac progenitor cells (CPCs) in patients with severe LV dysfunction undergoing coronary artery bypass graft surgery. Although the small patient population and open-label design preclude definitive mechanistic conclusions, CPCs infusion was associated with significant improvements in LV ejection fraction from before treatment to 4 months afterward, and even greater improvement was observed at 12 months. These and other promising results could soon lead to the initiation of more definitive, large-scale, phase 2 clinical trials.

Novel therapies have often been adopted into clinical practice before their mechanisms of action have been completely deciphered, provided their safety and beneficial effects are reproducible. For the emerging cellular therapy, however, the optimal cell type, dose, timing, and route of administration need to be identified to ensure that cell therapies produce the best possible benefit for each patient while avoiding overtreatment. Many of these variables are likely to depend both on the specific disease state and on complex interactions among numerous mechanisms that function seamlessly in an in-vivo system. The availability of clinically relevant large animal models of LV dysfunction are critical for identifying, characterizing and quantifying responses to cell therapy.
This issue of Circulation includes a report by Bolli, et al., that is among the first investigations of cell therapy to be performed in a large-animal model of ischemic cardiomyopathy with a mature LV scar that mimics the ongoing clinical trial. The authors induced infarction in the hearts of swine by ligating a coronary artery for 90 minutes followed by reperfusion, and then allowed the animals to recover for three months before infusing the reopened infarct related coronary artery with autologous CPCs that expressed c-Kit and had been expanded from samples of atrial tissue collected at the time of infarction. The treatment led to significant improvements in LV-chamber and regional contractile function that was accompanied by improvements in myocardial structure and evidence of cardiomyocyte and vascular-cell regeneration. Importantly, both the treatment administered and the experimental results closely mimic those reported for the SCIPIO trial, which suggests that this animal model can provide a useful platform for addressing many unmet needs and unanswered mechanistic questions in the field of cardiovascular cell therapy.

In analogy with novel pharmacological treatments, cell therapy has been adopted clinically prior to the recognition and characterization of their mechanisms of action proven that it is safe and feasible. Dr. Bolli’s current work points to growth and differentiation of the administered CPCs as the prevailing modality of recovery of the infarcted heart. The accumulated results provide critical insights concerning the fundamental issues currently faced by clinical cardiology. The major questions include the implementation of the most effective cell type, its dose, timing of delivery, and route of administration. These variables depend on the disease state, the functional competence of the progenitor cell category, and the complex interactions between cells and the microenvironment of the injured myocardium. Some of these problems are briefly discussed below.
Myocardial regeneration secondary to progenitor cell therapy

The impressive results achieved in recent clinical trials as well as in the preclinical trials likely involve a seamless interplay of numerous processes that can be loosely grouped into two categories: myocardial protection and myocardial regeneration (Figure 1). In addition to myocardial regeneration from the differentiation of the engrafted CPCs, protective effects of cell therapy are induced through the release of trophic factors9,10, such as VEGF, IGF, and FGF etc, which subsequently reduce apoptosis and adverse cardiac remodeling by promoting neovascularization and improved perfusion9-12. Because the experiments presented in Dr. Bolli’s current report were performed in animals with mature infarcts, the observed response to treatment is most likely attributable to regenerative, rather than protective mechanisms. The study also provides convincing evidence that the administered CPC differentiated into functional cardiac cells, but did not include experiments to determine whether the rate of endogenous myocyte turnover also changed in response to cell therapy, which is a limitation of the report.8

Myocyte turnover

The concept of cardiomyocyte turnover in the adult heart is relatively new. The first significant report from direct observations in humans that challenged the dogma that the adult heart is a post-mitotic organ was by Beltrami et al 13 who carefully examined the histology of the border zone myocardium in patients dying of acute myocardial infarction. After these original reports, the existence of myocyte turnover in the normal adult heart has been demonstrated by numerous studies such as the recent one by Bergmann et al using carbon-14 pulse-chase analyses that was made possible by above-ground nuclear testing in the 1950s and 1960s.14 Myocyte turnover in adult mammalian hearts can occur through the proliferation of pre-existing cardiomyocytes or the activity of endogenous CPCs. The relative importance of these two mechanisms, as well as
the myocyte turnover rate in the adult heart, remain matters of intense debate. Once the signaling pathways of specific CPCs population are deciphered, the methods used for cell delivery can be refined to promote cardiomyocyte turnover, leading to more comprehensive activation of mechanisms for cardiac repair.

**Myocardial Protection**

The severity of LV dysfunction and remodeling after myocardial infarction (MI) is linearly related to the size of the initial infarct. 

Thus, one of the primary goals for the treatment of postinfarction LV remodeling is to protect the peri infarct border zone (BZ) myocardium and to reduce the infarct size. Many reports indicate that apoptosis declines when cell transplantation is performed shortly after an infarct event, and cell transplantation is frequently associated with improved myocardial perfusion and increased BZ myocardial resistance vessel density, which may preserve functional myocardial tissue by relieving BZ subendocardial ischemia. Bolli’s study found small regions of viable myocardial tissue within the scar that appeared to be derived from the transplanted cells. However, whether these observations were accompanied by significant declines in infarct size was not determined. At present a fair evaluation of the reduction in LV infarct size in response to a therapy remains controversial. The existing technology for quantification of the extent to which damaged myocytes cannot recover from injury remains controversial. The gold standard for evaluating cardiomyocyte irreversible damage is Triphenyltetrazolium staining (TTC), which monitors the leakage of dehydrogenase enzymes and cofactors from myocytes, but the technique is performed on explanted hearts and, consequently, cannot be used to determine the proportion of damaged cells that may recover or are destined to die. Thus, cardiovascular science will benefit from new technologies that can noninvasively track the fate of injured cardiomyocytes, particularly at the BZ, where apoptosis
and programmed necrosis are active\textsuperscript{12,16}. Measurements of infarct size reduction can be confounded by infarct shrinkage and LV dilation that occur during acute phase post infarction LV remodeling. Currently, the appropriate quantification of infarct size is obtained by cardiac MRI and the assessment of the ratio of LV scar surface area to the LV surface area.\textsuperscript{12,16} 

Only a small fraction of transplanted cells are retained in the infarcted myocardium, and the survival of engrafted cells is poor; however, cell engraftment and survival can be improved by incorporating the cells into a cardiac patch before administration.\textsuperscript{12,16} One week after transplantation, the engraftment rate was 4-fold greater when the cells were administered in a fibrin patch than when they were injected directly into the injured myocardium.\textsuperscript{16} In a swine MI model, patches containing human embryonic stem cell derived vascular cells (hESC-VC) led to reduced cardiomyocyte apoptosis, and to improvements in the recruitment and activation of endogenous CPCs, and in border-zone perfusion and contractile function.\textsuperscript{16} Similar benefits have been observed after treatment with patches containing VCs derived from human induced-pluripotent stem cells (hiPSCs).\textsuperscript{12} The patches can also be modified to contain cytokines that promote the activity of the transplanted cells or induce cytoprotective and regenerative mechanisms in resident cardiac cells\textsuperscript{18}. However, clinical application of this patch-based approach will require the development of a practical and minimally invasive delivery method.

In conclusion, the preclinical study reported by Bolli, et al. demonstrates that the benefits associated with intracoronary CPC infusion in patients with severe LV dysfunction can be successfully reproduced in swine and, consequently, that this model may be an appropriate platform for the development of new analytical techniques for comparing the effectiveness of different cell types, doses and delivery methods, and for mechanistic experimental investigations. Their report is also among the first large-animal studies to investigate the response to cell
therapy administered late after the initial infarct event when the injured tissue has been replaced by a thin fibrotic scar. Thus, the findings are particularly important for guiding the clinical trials needed to develop and optimize the emerging cellular therapy for cardiac repair.

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Figure Legend:

**Figure 1.** Mechanisms of cell therapy for myocardial repair. The therapeutic mechanisms induced by cell transplantation can be grouped into two categories: cardiac regeneration and myocardial protection. Regenerative mechanisms include the transdifferentiation of engrafted CPCs into cardiomyocytes and vascular cells, as well as the release of trophic factors that subsequently activate resident CPCs or stimulate cell-cycle progression and proliferation in native cardiac cells. Trophic factors can also induce cytoprotective mechanisms that impede apoptosis immediately after the infarct event and promote neovascularization, which continues to increase cardiac performance at later time points through the beneficial feedback from improvements in perfusion, metabolism, and LV chamber function.
Cell therapy for myocardial repair

- CPCs
  - Transdifferentiation
  - Activation of Cardiac Progenitor Cells
  - Cardiomyocyte Proliferation
  - Anti-apoptosis
  - Neovascularization
  - Improved perfusion and metabolism; reduction of infarct size
  - Myocardial Regeneration
  - Myocardial Protection
  - Functional Recovery

Paracrine Effects

Trophic factors
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