Widespread Intracoronary Cardiopoietic Cell Infusion
Treating at the Time of Myocardial Reperfusion to Prevent Rather Than Reverse Established Left Ventricular Dysfunction Moves Us Closer to Practical Clinical Translation

John M. Canty Jr, Brian R. Weil, Gen Suzuki

The acute management of ST-segment–elevation myocardial infarction is now primarily focused on rapid restoration of myocardial perfusion to reduce myocardial infarct size, improve systolic function, and attenuate long-term postinfarction left ventricular (LV) remodeling. Indeed, in the contemporary reperfusion era, LV systolic function is now relatively preserved with postinfarction ejection fractions averaging 50%. As a result, postinfarction heart failure after primary coronary intervention now develops in fewer than ≈5% of patients and most have only New York Heart Association functional class I or II symptoms.1 In addition to early reperfusion, blockade of the neurohormonal axis with pharmacological therapies including angiotensin inhibition, β-blockers, aldosterone antagonists, and now neprilysin inhibitors have significantly improved prognosis. These therapies further limit postinfarction remodeling and variably increase LV systolic function, resulting in improved functional capacity and increased survival. Yet, despite these significant therapeutic successes, a minority of patients go on to develop progressive LV dysfunction as a result of the short-term functional improvement were unknown. Nevertheless, the administration of CSCs to patients using the stop-flow technique has demonstrated durable increases in myocardial function in patients with ischemic cardiomyopathy (SCIPiO) trial.6

In this issue of the Journal, Tang et al7 provide important new insight into the long-term effects of intra-arterial CPC infusion at the time of reperfusion. Using echocardiography and state-of-the-art hemodynamic assessment, they show that the beneficial physiological effects previously demonstrated at 35 days after infarction were maintained at 3 months. Nevertheless, although favorable differences in function in CPC-treated versus control groups persisted during the subsequent 9 months, there was no further absolute increase in ejection fraction or fractional shortening demonstrated with CPC treatment. From an anatomic perspective, the preservation of function was accompanied by reductions in infarct size along with increases in viable LV mass within the risk region. The use of sex mismatched but otherwise syngeneic rats as CPC donors allowed the long-term CPC viability and differentiation into myocytes at 1 year to be clearly quantified. The results demonstrate persistence of CPC-derived cells for as long as 1 year and the absence of any tumorigenic effect (a critically important contribution for safety and clinical translation).
Surprisingly, although immunohistochemical staining demonstrated that the transplanted CPCs expressed contractile proteins, most α-SA^{POS} cells continued to reflect small myocyte precursors with few attaining a size and morphology consistent with a mature cardiomyocyte. The possibility that CPCs stimulated endogenous myocyte formation as the mechanism for functional improvement was explored using BrdC pulse studies administered for 1-month intervals at selected time points. Proliferating α-SA^{POS} cells were increased throughout the heart after infarction but were ≈2-fold greater in CPC-treated hearts versus controls and much greater than BrdU+ cells arising from sex-mismatched CPC donors. Nevertheless, like CPC-derived α-SA^{POS} cells from donors, few endogenous BrdU+ α-SA^{POS} cells had a size and morphological appearance of a mature myocyte. Thus, although this study provides further evidence that CPCs demonstrate a durable effect on preventing the progression of postinfarction LV dysfunction, CPCs are not a source of new mature myocytes. Although the role of cKit+ cells in cardiac regeneration continues to be controversial, this study adds support to an increasing number of preclinical studies assessing cell fate that have failed to identify significant numbers of de novo myocytes derived from cKit+ cardiopoietic progenitor cells in other model systems.

The small BrdU+ α-SA^{POS} cells from donors demonstrate significant increases after CPCs but the size and morphometric approaches can quantify whether the new myocardium generated from endogenous cells reflects inhibiting cell death, myocyte cellular hypertrophy, or myocyte proliferation is challenging. The Table summarizes the complimentary approaches that can be used in this assessment. At the most fundamental histological level, morphometric approaches can quantify myocyte size, nuclear density, and the number of myocytes per gram of tissue. A reduction in myocyte size coupled with an increase in nuclear density (where the average number of nuclei per myocyte remains the same) indicates new myocyte formation because the number of myocytes per gram of tissue would have to increase. In contrast, a reduction in myocyte nuclear density along with an increase in myocyte size is compatible with myocyte cellular hypertrophy. Cellular hypertrophy can arise physiologically from normal growth in the young animals used in virtually all preclinical studies, myocyte loss from apoptosis or LV hypertrophy (regional or global). Because of this, attributing changes in viable LV mass to proliferation from cell therapy can be complex. For example, favorable myocyte regeneration could cause a resolution of postinfarction remodeling such that total viable LV mass and LV mass/body weight ratio is actually lower in treated versus untreated animals as in this study. Similar effects could also occur by inhibiting myocyte apoptosis. Assessing endogenous myocyte formation by quantifying BrdU+ myocytes is attractive to identify de novo myocytes yet potentially problematic to interpret in vivo. One concern relates to sensitivity of the technique because it is unknown how much BrdU is required to visualize

Table. Morphometric and Anatomic Alterations Distinguishing Myocyte Regeneration, Ventricular Hypertrophy, and Physiological Growth

<table>
<thead>
<tr>
<th>Measure</th>
<th>Myocyte Regeneration</th>
<th>Ventricular Hypertrophy</th>
<th>Physiological Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average myocyte size</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Myocyte number per gram or myocyte nuclear density</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Left ventricular mass</td>
<td>↑ or ↓</td>
<td>↑</td>
<td>↑</td>
</tr>
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</table>
a positive nucleus. The second issue is that continuous BrdU administration after treatment and appropriate costaining to identify myocytes is required to quantify the total number of myocytes formed. A final concern relates to the fact that cells can become positive because of DNA repair. In light of potential limitations of all approaches, the most definitive conclusions about myocyte cell proliferation can be made in a circumstance where apoptosis is absent and include measurements of regional and global LV mass, morphometric measurements (myocyte nuclear density, average myocyte size, and distribution) and administration of BrdU. If the latter is not possible, point estimates of proliferation using Ki67 staining and phospho-histone-H3 staining for mitosis can serve as surrogates with the caveat that they reflect instantaneous rates and likely change over time.

The study by Tang et al\textsuperscript{7} adds to a growing body of preclinical literature demonstrating the efficacy of intra-arterially infused cardiopoeitic cells in the absence of techniques, which require stop flow. Although this requires transient aortic occlusion and is technically challenging in the rodent heart, it is straightforward to accomplish in humans and large animals by direct intracoronary infusion. Suzuki et al\textsuperscript{10,11} and Weil et al\textsuperscript{13} demonstrated that continuous intracoronary infusion was efficacious without cessation of coronary flow and stimulated myocyte proliferation throughout the diseased but not normal heart after widespread intracoronary mesenchymal stem cell and CDC administration to swine with hibernating myocardium. Others have shown that transient occlusion or stop flow is not required for CSC or CDC uptake and demonstrated functional efficacy of CDCs administered without cessation of flow after acute myocardial infarction.\textsuperscript{14,15} Importantly, previous studies have identified substantial myocyte apoptosis, leading to myocyte loss in remote myocardium immediately after infarction in patients.\textsuperscript{16} The ability of widespread intracoronary cell therapies to prevent apoptosis and promote cell proliferation in ischemic as well as remote myocardium may provide a 2-pronged approach to prevent postinfarction remodeling and preserve LV systolic function. Global intracoronary stem cell infusion may also provide a relatively simple administration technique to treat patients with chronic ischemic as well as nonischemic cardiomyopathy. The latter possibility is being examined in an ongoing clinical trial using allogeneic intracoronary CDCs in humans.\textsuperscript{14}

Finally, although chronic cardiomyopathy can be treated electively using autologous cell platforms which are expanded ex vivo and then readministered to the donor, allogeneic cardiopoeitic cell therapies are required to treat patients presenting with an acute myocardial infarction because cells need to be immediately available. Although allogeneic mesenchymal stem cells and allogeneic cardiopoeis-derived cells have emerged as potential off-the-shelf products, it is unclear as to whether allogeneic CPCs will be a viable alternative to treat patients with an acute myocardial infarction like the syngeneic CPCs have been demonstrated to by Tang et al.\textsuperscript{7} Although this is worthy of future study, the long-term presence of CPCs and CPC-derived cells in the heart may actually be a disadvantage because these mismatched survivors would provide a stimulus for tissue immune rejection. Regardless of the specific cell therapy platform, it seems time to begin evaluating allogeneic cell therapy strategies that use global intracoronary cell infusion at the time of reperfusion as a practical approach to prevent rather than reverse LV dysfunction.

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**Disclosures**

None.

**References**


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