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. In addition to early reperfusion, blockade of the neurohormonal axis with pharmacological therapies including angiotensin inhibition, β-blockers, aldosterone antagonists, and now nephrilysin 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 well as recurrent myocardial infarction. This has motivated approaches to regenerate the myocytes and myocardium lost using cardiac cell therapies, which has become the focus of considerable preclinical and clinical investigation during the last 15 years. Although much research has focused on directly injecting cells into the myocardium, intra-arterially infused cardiopoietic stem cells delivered at the time of myocardial reperfusion are emerging as promising candidates to improve LV dysfunction.

The feasibility of global intracoronary cell infusion without interrupting coronary flow was initially demonstrated over 10 years ago by Dawn et al. They showed that 1 million intra-arterially administered cKit+/lin− cardiac stem cells (CSCs) delivered to the entire heart could attenuate LV remodeling in rats after ischemia and reperfusion. Not only was systolic function improved but EGFP labeled CSCs produced increased adult appearing myocytes in as little as 35 days in the infarct as well as remote regions of the heart. At a cellular level, there were measurable increases in global myocyte number with reductions in myocyte cell volume. A subsequent study using cKit+/CD45− cardiac progenitor cells (CPCs) confirmed these relatively rapid beneficial physiological actions at a similar time point. There was dose dependency of functional effects after intra-arterially infused CPCs, which plateaued >0.75 million CPCs with higher doses of CPCs (≈6 million CSCs) causing increased mortality. Global intra-arterial CPC infusion elicited a prominent reduction in apoptosis within the infarct. Interestingly, reductions in apoptosis were equally as prominent in the normally perfused remote region of the heart. There were increases in myocyte proliferation leading to a net increase in BrdU+/α-sarcomeric actin–positive (α-SA+) cells, reflecting small immature myocyte precursors. The long-term fate of the CPCs whether myocyte precursors developed into mature myocytes and the durability 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 in the cardiac stem cells in patients with ischemic cardiomyopathy (SCIPIO) trial.

In this issue of the Journal, Tang et al 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<sup>pos</sup> cells continued to reflect small myocyte precursors with few attaining a size and morphometry consistent with a mature cardiomyocyte. The possibility that CPCs stimulated endogenous myocyte formation as the mechanism for functional improvement was explored using BrdU pulse studies administered for 1-month intervals at selected time points. Proliferating α-SA<sup>pos</sup> cells were increased throughout the heart after infarction but were ≈2-fold greater in CPC-treated hearts versus controls and much greater than BrdU<sup>+</sup> cells arising from sex-mismatched CPC donors. Nevertheless, like CPC-derived α-SA<sup>pos</sup> cells from donors, few endogenous BrdU<sup>+</sup> α-SA<sup>pos</sup> 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<sup>+</sup> 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<sup>+</sup> cardiopoietic progenitor cells in other model systems.8,9 The small BrdU<sup>+</sup> α-SA<sup>pos</sup> cells that the authors interpret as immature myocytes do not result in mature myocytes, decline in frequency between 3 and 12 months and are not associated with functional improvement during this time period. Thus, rather than reflecting a delayed progression toward a mature myocyte phenotype, it seems that these immature myocytes may be in a futile cycle where they begin proliferating but then exit the cell cycle before ever reaching maturity. Alternatively, they may be undergoing rapid senescence.

If there are no mature BrdU<sup>+</sup> myocytes present after 1 year, where does the increase in myocardium demonstrated in the risk region of CPC-treated animals come from? The measurements of myocyte nuclear density and myocyte numbers demonstrate significant increases after CPCs but the size of the remaining myocytes (as well as the size cutoff used to exclude immature myocytes) was not provided. Lacking evidence of significant new mature myocyte formation from BrdU<sup>+</sup>, there seems to be 2 possible mechanisms to explain increased myocardium in the risk region. The first is that most of the proliferation of CPCs into mature myocytes could have occurred in the first 2 months after therapy. Myocyte proliferation with increases in myocyte number and reductions in myocyte size have been demonstrated within 2 to 4 weeks after intra-arterial infusion after CSCs in rats<sup>4,5</sup> as well as cardiosphere-derived cells (CDCs) and mesenchymal stem cells in swine by others.<sup>10,11</sup> Because BrdU<sup>+</sup> cells in this study were not started until 3 months after CPC administration, the absence of mature de novo BrdU<sup>+</sup> cardiomyocytes would not be surprising. The second possibility is that the increased myocardium in the risk region actually reflects an antiapoptotic effect of CPCs administered shortedly after reperfusion. Here, the authors’ recent study in the same model provides some insight.<sup>3</sup> Although the effect of CPCs on remodeling was dose dependent, a comparable dose range as used in this study (0.75–1.5 million CPCs) resulted in an increased infarct wall thickness, a reduction in infarct size and improved systolic function at 35 days. Although BrdU<sup>+</sup> α-SA<sup>pos</sup> cells were increased at this time, they were 100-fold lower than in this study (≈3/10000 at 35 days versus ≈1000 in 100000 nuclei at 3 months). At the same time, apoptosis at 35 days was increased and substantially reduced by CPCs (although the frequency of myocyte apoptosis was not reported). Thus, it seems likely that the increased myocardium in the risk region along with the preservation of function after CPCs is to some extent related to paracrine factors inhibiting apoptosis in the infarct as well as remote myocardium.

The complexity of studying myocardial repair in vivo rests on determining the relative importance of cell therapies in preventing death of existing myocytes versus promoting new myocyte formation. New myocytes can arise from the injected cells or by stimulating endogenous myocyte proliferation. It is straightforward to quantify the contribution of injected cells to new myocyte formation when sex-mismatched donors are a feasible approach as in the study of Tang et al.<sup>7</sup> In contrast, quantifying 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<sup>+</sup> myocytes is attractive to identify de novo myocytes yet potentially problematic to interpret in vivo.<sup>12</sup> 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 |
|---------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Average myocyte size            | ↑                               | ↑                               | ↑                               |
| Myocyte number per gram or myocyte nuclear density | ↑                               | ↓                               | ↓                               |
| Left ventricular mass            | ↑ or ↓                          | ↑↑                              | ↑                               |
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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