Editorial

Cortical Bone Stem Cells Administered at Reperfusion Attenuate Remote Zone Myocyte Remodeling

John M. Canty Jr, Brian R. Weil

Heart failure arises from intrinsic diseases of cardiac muscle or idiopathic dilated cardiomyopathy as well as consequences of coronary artery disease after acute myocardial infarction and chronic ischemia. In patients with ST-segment–elevation myocardial infarction (MI) from a coronary thrombus, immediate reperfusion is the most effective therapy to prevent the loss of myocytes. The routine use of β-blockers, angiotensin inhibition therapy, and spironolactone blocks neurohormonal activation and prevents subsequent left ventricular (LV) remodeling. Stem cell therapy has emerged as an adjunctive approach to further attenuate postinfarction remodeling by stimulating myocyte proliferation and mitigating myocyte loss arising from within and outside of the infarct region. Despite favorable preclinical and early clinical studies in humans using bone marrow mononuclear cells, meta analyses of completed trials have failed to demonstrate a significant impact on clinical outcomes and LV function.[1] Because substantial myocyte loss occurs during the first few days after infarction, the lack of efficacy may reflect delays in administering therapy that arise from synthesizing autologous cell products after the event. This and the increasing recognition of the role that paracrine effects play led to interest in allogeneic cells, such as cardiosphere-derived cells (CDCs) and mesenchymal stem cells (MSCs). These are relatively immune privileged and can be administered at the time of percutaneous coronary intervention and reperfusion. Cortical bone stem cells (CBSCs) are a particularly proliferative mesenchymal cell subtype[2] that, in vitro, have characteristics similar to murine cKit+/Sca1+ cells and can to some extent transdifferentiate into cardiac myocytes.[3] Enriching the cKit+ CBSC population could potentially provide an allogeneic cell therapy platform similar to cardiac stem cells (CSCs) without the need for a myocardial biopsy. Completed studies in nonreperfused murine infarct models have demonstrated that they attenuate postinfarction remodeling.[3]

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In this issue of the journal, Sharp et al[4] translate prior beneficial effects of CBSCs in murine models from this group to a large animal model of reperfused MI. Closed-chest swine subjected to a 90-minute occlusion received allogeneic CBSCs via percutaneous intramyocardial injection into the infarct border zones immediately after reperfusion without immunosuppression. Animals studied after 72 hours demonstrated no effect of CBSCs on infarct size or ejection fraction, but residual CBSCs were identified within the injection sites. Chronic studies evaluating serial echocardiographic function demonstrated a progressive decline in LV ejection fraction in both groups with relative preservation of systolic function in the CBSC-treated animals developing between 2 and 3 months after treatment. Although the ischemic area-at-risk was not assessed, postmortem analysis demonstrated a significant reduction in infarct size. Of equal importance, CBSCs exerted a prominent effect on myocyte size and myocyte nuclear density throughout the viable remote region, as well as the narrow peri-infarct border zone. In saline controls, there was substantial myocyte loss and myocyte cellular hypertrophy in conjunction with a progressive decline in global function. In contrast, myocyte nuclear density and area were only slightly different from normal hearts after treatment with CBSCs. Although ejection fraction improved versus saline-treated animals, it remained significantly depressed compared with noninfarcted controls. Like other cell types, the authors conclude that intramyocardial CBSC injection retards the development of postinfarction remodeling by preventing the time-dependent decline in LV function.

There are many strengths to this study's conduct and design. First is the blinded, randomized nature of treatment allocation, data acquisition, and data analysis. Few large animal studies have accomplished this for parameters other than cardiac imaging, and it reinforces the notion that this standard can be accomplished in a single-center study design.[5,6] Second, the extensive functional and hemodynamic assessments reinforce the consistency of the physiological observations using multiple blinded independent methods. Third, sex-mismatched donor–recipient pairs and EGFP (enhanced green fluorescent protein) labeling allowed the fate of the injected CBSCs to be assessed. Although cell retention was not quantified, fluorescence microscopy demonstrated persistent localization of CBSCs confined to the injection sites at the 72-hour time point. No CBSCs were identified after 3 months. Weaknesses of the study include the inability to assess the ischemic area-at-risk or serial infarct size to ensure that, like the 72-hour study, the initial infarct was the same in the chronic treatment groups. A chance difference in the initial infarct size could be particularly problematic with such small sample sizes (n=5 per group) and also contribute to some of the findings.

Despite challenges in identifying de novo myocyte formation in a large animal model, Sharp et al used the thymidine

analogue EdU (5-ethynyl-2′-deoxyuridine) to identify newly formed myocytes. Myocyte EdU incorporation was restricted to the infarct and lateral border zone with no EdU-positive myocytes identified in the remote zone. Nevertheless, this only amounted to ≈1% to 1.5% of the total myocytes present in the peri-infarct region because EdU administration was limited to the first week post-MI. Unlike the lack of myocyte EdU in remote myocardium, there was a prominent reduction in myocyte apoptosis throughout all viable regions of the heart. The net effect of antiapoptotic and proliferative effects resulted in normalization of myocyte cross-sectional area in CBSC-treated pigs. Because myocardial mass was not different in either group, the reduced myocyte size and increased myocyte nuclear density are consonant with the notion that postinfarction myocyte loss and cellular hypertrophy were prevented by the early administration of CBSC therapy. This arose from tissue immediately surrounding the scar but perhaps more prominently from the viable remote regions distant from the injection site.

The remote zone remodeling in the present study is quantitatively similar to several previous studies using other cardiac cell therapies in swine with acute infarction and viable, chronically dysfunctional myocardium, which are both substrates encountered in patients with ischemic cardiomyopathy. The Table summarizes selected studies quantifying myocyte nuclear density and myocyte size (cross-sectional area) in remote myocardium after cell therapy in swine. Suzuki et al[7] demonstrated prominent myocyte cellular remodeling after global intracoronary infusion of autologous MSCs in hibernating myocardium. These changes were devoid of confounding effects of cell therapy on infarct size because infarct scar is absent in this model. Subsequent studies in the same model demonstrated similar effects of intracoronary CDCs.[8] A blinded, head-to-head comparison of intracoronary allogeneic CDCs and MSCs found similar effects with no difference between the 2 cell types.[5] When allogeneic cells were administered immediately after reperfusion in porcine infarct models, favorable remote zone remodeling was seen after mesenchymal precursor cells[6] and CBSCs[4] from the present study. Directionally similar results in myocyte area were found after allogeneic CDCs administered after reperfusion although myocyte nuclear density was not reported.[9] In contrast, several studies using regional cell administration several weeks after MI have demonstrated reductions in infarct size but failed to show major effects on remote zone myocyte hypertrophy.[10–12] This suggests that the greatest impact of cell therapy on myocyte remodeling may be achieved by preventing myocyte apoptosis arising in the hours or days after reperfusion. Regardless of cell source, all allogeneic cell therapies administered at the time of reperfusion reduced infarct volume over time.

There are 2 somewhat perplexing findings in the present study. The first is the fact that intramyocardial CBSC injection confined to the infarct border region exhibited such pronounced effects on myocyte nuclear density and size in remote myocardium. The authors clearly demonstrate no evidence of CBSCs migrating to or being retained in the remote regions. While confirming a paracrine mechanism of action, it is difficult to conceive how growth factors, exosomes, or other paracrine mediators would diffuse such a great distance from the border zone and remain in the myocardial tissue without escaping into the vascular compartment. Thus, it seems plausible that the mechanism of action of CBSCs may actually reflect acute modulation of the host inflammatory response to myocardial injury in the ischemic and remote region of the heart. Further studies will be required to investigate this, but similar immune modulatory actions have already been identified for CDCs[9] and MSCs.[13] The second relates to the time

Table. Effects of Cell Therapy on Remote Zone Myocyte Cellular Hypertrophy, Myocyte Number, and Infarct Size in Large Animals

		Infarct Size (Percentage of Left Ventricle)		Remote Zone Myocyte Remodeling					
				Myocyte Area, µm²			Myocyte Number or Nuclear Density (per mm²)		
	Cell Type	Vehicle Treated	Cell Treated	Vehicle Treated	Cell Treated	Change, %	Vehicle Treated	Cell Treated	Change, %
Suzuki et al[7]	Autologous MSCs	NA*		235±17	119±15	−49	1027±55	1219±131	+19
Suzuki et al[8]	Allogeneic CDCs	NA*		120±7	71±6	−41	981±22	1574±145	+60
Weil et al[5]	Allogeneic MSCs	NA*		308±30	229±30	−26	1005±51	1387±155	+38
	Allogeneic CDCs	NA*		308±30	225±12	−27	1005±51	1545±124	+54
Houtgraaf et al[6]	Allogeneic MPCs	18.4±1.5	12.0±0.7	378±32	252±30	−33	1763±122	2645±242	+50
Sharp et al[4]	Allogeneic CBSCs	16.2±1.9	8.5±2.2	401±2	277±1	−31	623±48	934±85	+50

Values are mean±SEM. CBSCs indicates cortical bone stem cells; CDCs, cardiosphere-derived cells; LAD, left anterior descending; MPCs, bone marrow-derived mesenchymal precursor cells; MSCs, bone marrow-derived mesenchymal stem cells; and NA, not applicable.

*Swine with hibernating myocardium do not exhibit a myocardial infarct. Instead, a proximal LAD artery stenosis results in chronic, repetitive myocardial ischemia in the territory supplied by the LAD. The viable dysfunctional region encompasses ≈35% to 45% of the left ventricle. Myocyte area in these studies was calculated based on published measurements of myocyte diameter.[5,7,8]

course of the functional improvement in CBSC-treated animals. Most studies show rapid divergence of ejection fraction with treatment within the first 4 weeks after infarction, which persists over time. Although this pattern was also seen in non-reperfused murine infarcts treated with CBSCs,[3] functional effects in swine were delayed with ejection fraction only becoming significantly different 3 months after CBSC treatment when there were no residual CBSCs. It is plausible that this lack of early effect may reflect the statistical power of the study because sample sizes were small.

Although intramyocardial injection of allogeneic CBSCs reduces LV remodeling and does not require immunosuppression, there are several unanswered questions of importance. First, are the in vivo effects of CBSCs that are selected based on cKit positivity any different from the other allogeneic cell types (Table) that have been shown to reduce scar volume and preserve myocyte nuclear density in remote myocardium when administered at the time of reperfusion? Like CDCs and MSCs, CBSCs have many similarities in terms of mesenchymal cell markers (eg, CD90 and CD105), and few, if any, of the CBSCs differentiate into myocytes in vivo.[3,4] Second, is it necessary (or clinically feasible) to perform intramyocardial injection of CBSCs at the time of reperfusion after a ST-segment–elevation MI? Clearly, an intracoronary infusion could be more widely implemented, and small cell types like CSCs are effective when given in this manner.[14] Further studies evaluating intracoronary CBSCs would be informative.

Appropriately powered, blinded, and randomized preclinical trials in large animals comparing CBSCs to other cell types are needed to identify whether there are measurable differences in physiological end points among cell platforms that may have clinical impact. In this regard, there continues to be a note of caution on the translational path. Small initial preclinical studies are typically underpowered, yet positive results are rapidly translated to early-phase clinical trials. While positive preclinical results may be replicated in small phase I trials, the literature is replete with disappointment from an increasing collection of larger phase II trials failing to translate the favorable effects. Although confirmatory large animal studies are costly, they are considerably faster and less expensive than conducting a clinical trial on human subjects. Perhaps it is time to begin thinking about developing an approach to test promising cell-based platforms like CBSCs in a fashion similar to the way that we conduct larger phase II trials to avoid the consistent lack of translation that we have faced thus far.[15]

Disclosures

None.

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