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Mechanisms of Bone Marrow–Derived Cell Therapy in Ischemic Cardiomyopathy With Left Ventricular Assist Device Bridge to Transplant

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ABSTRACT

BACKGROUND Clinical trials report improvements in function and perfusion with direct injection of bone marrow cells into the hearts of patients with ischemic cardiomyopathy. Preclinical data suggest these cells improve vascular density, which would be expected to decrease fibrosis and inflammation.

OBJECTIVES The goal of this study was to test the hypothesis that bone marrow stem cells (CD34+) will improve histological measurements of vascularity, fibrosis, and inflammation in human subjects undergoing left ventricular assist device (LVAD) placement as a bridge to cardiac transplantation.

METHODS Subjects with ischemic cardiomyopathy who were scheduled for placement of an LVAD as a bridge to transplantation underwent bone marrow aspiration the day before surgery; the bone marrow was processed into cell fractions (bone marrow mononuclear cells, CD34+, and CD34–). At LVAD implantation, all fractions and a saline control were injected epicardially into predetermined areas and each injection site marked. At the time of transplantation, injected areas were collected. Data were analyzed by paired Student *t* test comparing the effect of cell fractions injected within each subject.

RESULTS Six subjects completed the study. There were no statistically significant differences in complications with the procedure versus control subjects. Histological analysis indicated that myocardium injected with CD34+ cells had decreased density of endothelial cells compared to saline-injected myocardium. There were no significant differences in fibrosis or inflammation between groups; however, density of activated fibroblasts was decreased in both CD34+ and CD34– injected areas.

CONCLUSIONS Tissue analysis does not support the hypothesis that bone marrow–derived CD34+ cells promote increased vascular tissue in humans with ischemic cardiomyopathy via direct injection. (J Am Coll Cardiol 2015;65:1424–34) © 2015 by the American College of Cardiology Foundation.

Ischemicheart disease is characterized by a loss of myocardial cells and vasculature leading to end-stage heart failure. Cell therapy offers an appealing solution to replace these components and restore normal structure and function. Bone marrow cells are an easily accessible source of cells that have been shown to differentiate into cardiac components that potentially provide paracrine factors to improve healing of injured myocardium.

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More than a decade ago, studies in small animal models showed that injection of bone marrow–derived stem cells improved cardiac remodeling after myocardial infarction (MI) (1). Specifically, injection of either bone marrow stem cells (CD34+) or bone marrow mononuclear cells (BMMC) improved vascularity, decreased scar burden, and resulted in improved cardiac function compared to noninjected animals (2,3). Follow-up studies using large animal models of bone marrow cell injection after MI showed similar results (4,5).

SEE PAGE 1435

In vitro studies of human bone marrow cells produced tantalizing data that these findings could be easily translated. Stem cells isolated from bone marrow or circulated blood carrying the markers CD34 and/or CD133 can differentiate into endothelial cells and form vascular structures (6,7). Furthermore, in vitro studies have shown that other bone marrow cell components, including CD34+ cells and mesenchymal stem cells, can provide paracrine factors to promote angiogenesis and cardiomyocyte cell survival; the same is observed when these cells are transplanted into immune-deficient rodents (8–11).

Clinical trials of bone marrow cell therapy in humans with ischemic heart disease have not yielded as dramatic results as in animals. Direct injection of bone marrow cells into ischemic myocardium has shown the most benefit in a review of these trials (12). Some subjects with chronic ischemic cardiomyopathy undergoing intramyocardial injection with BMMCs have improved function and perfusion on noninvasive imaging studies compared to untreated subjects (13–18). However, other trials in similar populations have failed to show a significant benefit to this therapy (19). Furthermore, a trial in subjects with nonischemic cardiomyopathy showed similar benefit (20), implying that the in vivo mechanisms responsible for improvements in animal models remain to be validated in humans.

We sought to analyze the potential mechanisms by which bone marrow cells could improve cardiac function in ischemic cardiomyopathy. We developed a novel model in which tissue from humans with severe ischemic heart disease could be analyzed for structural changes after injection of bone marrow–derived cell fractions. Our studies revealed that animal studies do not necessarily predict the effects of cell therapy in humans, but do indicate potential therapeutic beneficial effects. Furthermore, these studies shed light on the dynamic nature of repair even in the presence of severe ischemic cardiomyopathy.

METHODS

Twenty-six subjects with ischemic cardiomyopathy scheduled for placement of a left ventricular assist device (LVAD) as a bridge to transplantation (BTT) were screened for the trial (**Figure 1**). Inclusion criteria included age >18 years, ischemic cardiomyopathy, and need for LVAD implantations as a BTT. Exclusion criteria included presence of an intra-aortic balloon pump, emergent LVAD, and inability of the subject to consent. Eight subjects consented to the study. One subject withdrew and a second was too unstable for injection. Of the 18 subjects who refused consent or met exclusion criteria, 13 consented to safety data collection as controls.

The day before LVAD implantation, 15 to 20 ml of marrow was collected from the iliac crest and transported to the Fred Hutchinson Cell Therapeutics facility. Marrow was processed into 3 fractions for injection: 0.5×10^6 CD34+ cells, 1.0×10^6 CD34- cells, and 1.0×10^6 BMMC cells, and placed in numbered vials. An additional vial was loaded with 1 ml of Plasmalyte (Baxter International Inc., Deerfield, Illinois) as a control. The contents of all vials were blinded to the investigators (Online Figure 1). The doses of cells correspond to the average cell number per injection site reported in earlier clinical trials (Online Table 1).

Preoperatively, technetium single-photon emission computed tomography rest scans were reviewed and areas of 50% to 75% perfusion identified for injection. In the operating room, the contents of each vial were drawn up into a sterile syringe fitted with a sterile 25-gauge needle. Pilot sheering studies indicated minimal cell death (viability >98% by trypan blue stain) with this diameter. Areas to be injected were identified on the heart's surface and a 1-cm diameter marker sewn on. The cells for each fraction and for saline were injected separately into the area's 4 quadrants. Thus, each area received 4 250-μl injections of a single cell fraction or saline. After injection, the LVAD implantation proceeded per routine.

Case report forms were completed 24 h and 7 days after the LVAD implantation. Usage of blood products (packed red blood cells [PRBCs]), fresh frozen plasma (FFP), and platelets (PLT)] was recorded at each time point. Chart notes, 12-lead electrocardiograms, and telemetry were reviewed to define episodes of ventricular tachycardia in the postoperative period. Safety data were reviewed by the principal investigator and the safety monitor after each subject enrollment to assess for safety endpoints that would preclude enrollment of additional subjects.

ABBREVIATIONS AND ACRONYMS

BMMC = bone marrow mononuclear cells

FFC = fresh frozen plasma

LVAD = left ventricular assist device

MI = myocardial infarction

PRBC = packed red blood cells