JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
© 2017 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER

VOL. 70, NO. 3, 2017
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2017.05.040

Cell-Specific Pathways Supporting Persistent Fibrosis in Heart Failure

Stephen D. Farris, MD,[a] Creighton Don, MD, PhD,[a] Deri Helterline, MS,[a] Christopher Costa, BS,[a] Tabitha Plummer, BS,[a] Susanne Steffes, DNP, RN,[c] Claudius Mahr, DO,[a] Nahush A. Mokadam, MD,[b] April Stempien-Otero, MD[a]

ABSTRACT

BACKGROUND Only limited data exist describing the histologic and noncardiomyocyte function of human myocardium in end-stage heart failure (HF).

OBJECTIVES The authors sought to determine changes in noncardiomyocyte cellular activity in patients with end-stage HF after left ventricular assist device (LVAD)-induced remodeling to identify mechanisms impeding recovery.

METHODS Myocardium was obtained from subjects undergoing LVAD placement and/or heart transplantation. Detailed histological analyses were performed, and, when feasible, mononuclear cells were isolated from fresh, dissociated myocardium for quantitative reverse transcription polymerase chain reaction studies. Echocardiographic and catheterization data were obtained during routine care.

RESULTS Sixty-six subjects were enrolled; 54 underwent 8.0 ± 1.2 months of LVAD unloading. Despite effective hemodynamic unloading and remodeling, there were no differences after LVAD use in capillary density (0.78 ± 0.1% vs. 0.9 ± 0.1% capillary area; n = 42 and 28, respectively; p = 0.40), cardiac fibrosis (25.7 ± 2.4% vs. 27.9 ± 2.4% fibrosis area; n = 44 and 31, respectively; p = 0.50), or macrophage density (80.7 ± 10.4 macrophages/mm^2 vs. 108.6 ± 15 macrophages/mm^2; n = 33 and 28, respectively; p = 0.1). Despite no change in fibrosis or myofibroblast density (p = 0.40), there was a 16.7-fold decrease (p < 0.01) in fibroblast-specific collagen expression. Furthermore, there was a shift away from pro-fibrotic/alternative pro-fibrotic macrophage signaling after LVAD use.

CONCLUSIONS Despite robust cardiac unloading, capillary density and fibrosis are unchanged compared with loaded hearts. Fibroblast-specific collagen expression was decreased and might be due to decreased stretch and/or altered macrophage polarization. Dysfunctional myocardium may persist, in part, from ongoing inflammation and poor extracellular matrix remodeling. Understanding these changes could lead to improved therapies for HF.
(J Am Coll Cardiol 2017;70:344–54) © 2017 by the American College of Cardiology Foundation.

Heart failure (HF) affects almost 8 million Americans and carries >50% 5-year mortality from time of diagnosis (1). Although HF etiology and clinical trajectories are variable, myocyte-myofibril dysfunction, excess fibrosis, arrhythmias, and chronic volume overload uniformly contribute to disease progression. Detailed analysis of myocardium and in vivo biological mechanisms of dysfunction and remodeling were limited in human subjects before the advent of the left ventricular assist device (LVAD) as a bridge to transplant (BTT). Initial studies sought to evaluate histological changes after mechanical unloading to understand potential mechanisms of disease and recovery; however, subjects in these early studies primarily had now obsolete pulsatile LVADs, limited histological parameters

Listen to this manuscript's audio summary by *JACC* Editor-in-Chief Dr. Valentin Fuster.

From the [a]University of Washington, Department of Medicine, Division of Cardiology, Seattle, Washington; [b]University of Washington, Department of Cardiothoracic Surgery, Seattle, Washington; and the [c]University of Washington, School of Nursing, Seattle, Washington. This work was supported by grants R01 HL094384 and Tall Family Foundation (Dr. Stempien-Otero), UL1TR000423 ITHS (Dr. Don), Locke Family Foundation (Drs. Stempien-Otero and Farris), and an American College of Cardiology Foundation Fellowship (Dr. Farris). Dr. Modadam has consulted for Heartware and St. Jude. Dr. Mahr has consulted for HeartWare, St. Jude (Thoratec), and Abiomed. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received December 6, 2016; revised manuscript received May 11, 2017, accepted May 16, 2017.

were evaluated, and results were inconsistent (2–7). Newer-generation, continuous-flow LVADs have less pulsatility, greatly improved mechanical durability, and are an essential clinical tool in contemporary management of medical therapy refractory advanced systolic HF (8). Nevertheless, recovery after device implantation remains uncommon and histological studies continue to provide unclear answers (9).

Coincident with advances in LVAD technology, numerous human cell therapy trials have shown nonsignificant increases in systolic function with cell therapy; however, cellular and tissue characterization and potential mechanisms of action are absent (10–13). We sought to determine if recovery could be augmented by combining cell therapy and LVAD unloading. In a phase 1 study, with the purpose of defining safety and directly analyzing the effect of cell therapy on myocardial tissue in patients with end-stage HF, we injected purified, patient-derived bone marrow cell fractions into ischemic, viable myocardium (defined by single-photon emission computed tomography imaging) during LVAD placement (14). Upon cardiac explantation, we found that epicardial injection of either CD34+ or CD34-depleted cell fractions reduced activated fibroblast density compared with injected saline control without changes in fibrosis or microvessel density (14). These data provided evidence for a unique paracrine effect of cell injection independent of cell type in unloaded myocardium. Before initiating a larger clinical study to determine if larger scale inhibition of fibroblast activation would translate into significant reductions in fibrosis and augment recovery, we sought to better understand the effects of LVAD unloading on components of cardiac fibrosis.

SEE PAGE 355

Cardiac fibrosis is affected by diverse cell-specific pathways. Fibroblasts, the major collagen-expressing cell, can become activated by mechanical stretch to migratory, proliferative, and secretory phenotypes. Macrophages can adopt a pro-fibrotic (M2) phenotype that stimulates fibroblasts to express matrix or a pro-inflammatory (M1) phenotype to proteinases that degrade matrix. Hypoxemia from capillary rarefaction in advanced heart disease can also stimulate fibrosis. Because complete recovery on LVAD support remains a rare phenomenon, identifying histologic changes and which pathways are most active in response to unloading is critical to developing cell-based therapies to modulate fibrosis and potentiate reverse remodeling (15–20). Ultimately, such therapies may lead to improved myocardial recovery and survival.

To this end, we performed a prospective, observational study collecting clinical data and tissue from human subjects with end-stage HF undergoing LVAD implantation and/or cardiac transplantation. This study included histological analysis and gene expression studies of isolated cardiac fibroblasts and macrophages to test the hypothesis that unloading generates improvements in interstitial remodeling as manifest by: 1) increased capillary density; 2) decreased fibrosis; and 3) decreased expression of pro-fibrotic genes in isolated cardiac fibroblasts and macrophages.

METHODS

Subjects gave informed consent and were prospectively enrolled at the University of Washington Medical Center before LVAD implantation or transplantation. Study groups include subjects with LVAD use (as either BTT or destination therapy [DT]), primary transplant, or total artificial heart implantation. Given the inherent, clinically driven crossover of DT and BTT indications, not all subjects enrolled underwent cardiac transplantation. All study protocols were approved by the University of Washington internal review board and adhered to the Helsinki Principles for Human Subjects Research.

STUDIES AND ANALYSIS. Subjects underwent routine clinical care and established protocols at the University of Washington. Before LVAD implantation and/or heart transplantation, information was obtained via neurohormonal medication use, cardiac catheterization, echocardiography, and measurements of B-type natriuretic peptide (BNP). Left ventricular chamber measurements were made using 2-dimensional ultrasound techniques in the parasternal long axis.

Cell isolation was performed as previously described (21,22). In brief, ventricular myocardium was placed into cold phosphate-buffered solution without calcium or magnesium immediately in the operating room. Left ventricular myocardial tissue underwent immediate dissociation and cell isolation for RNA or formalin fixation and paraffin embedment for histology.

Fresh tissue was minced into 1-mm pieces and digested with thermolysin and deoxyribonuclease I. Sequentially, 100- and 70-μM filters were used. Ammonium-chloride-potassium lysing buffer was used to lyse red blood cells. Mononuclear cells were then incubated with anti-human CD14 antibody conjugated to magnetic beads and passed over a

ABBREVIATIONS AND ACRONYMS

ACE = angiotensin-converting enzyme

ANOVA = analysis of variance

αSMA = alpha smooth muscle actin

BNP = B-type natriuretic peptide

BTT = bridge to transplant

DT = destination therapy

GAPDH = glyceraldehyde 3-phosphate dehydrogenase

HF = heart failure

LVAD = left ventricular assist device

MMP = matrix metalloproteinase

PECAM = mouse anti-human platelet endothelial cell adhesion molecule