MECHANICAL CIRCULATORY SUPPORT

Evidence of Improved Right Ventricular Structure After LVAD Support in Patients With End-stage Cardiomyopathy

Seref A. Küçüker, MD,a,e Sonny J. Stetson, BS,b,c,d,e Katy A. Becker, BS,b,c,d,e Ahmet Akgül, MD,a,e Matthias Loebbe, MD, PhD,a,e Javier A. Lafuente, MD,a,e George P. Noon, MD,a,e Michael M. Koerner, MD, PhD,b,c,d,e Mark L. Entman, MD, FACC,b,e and Guillermo Torre-Amione, MD, PhD, FACCb,c,d,e

Background: Although many reports demonstrate the hemodynamic benefits of left ventricular assist devices (LVAD) in right-sided circulation, it is not known whether the right ventricular myocardium goes through reverse remodeling after left ventricular mechanical circulatory support. Accordingly, the purposes of our studies were 1) to investigate the right ventricular changes that occur in fibrosis, in cellular hypertrophy, and in intra-myocardial tumor necrosis factor α (TNF-α) levels in patients receiving LVAD support; and 2) to determine whether the type of LVAD used influences right ventricular myocardial changes.

Methods and Results: We measured myocyte size, total collagen content, and TNF-α levels using semi-quantitative immunohistochemical analysis of myocardial samples from the right and left ventricles of control and failing myocardia, either supported by 1 of 2 distinct forms of LVADs or without support. We found that when compared with control, although myocyte size was not increased in the right ventricle of failing myocardia (p = not significant), total collagen content and myocardial TNF-α levels were decreased in the right ventricle compared with controls (p < 0.01 and p < 0.001, respectively).

Conclusion: These data demonstrate that chronic left ventricular unloading with either pulsatile or continuous-flow devices decreases right ventricular total collagen and myocardial TNF-α content. We suggest that the decreased fibrosis and normalization of cytokine milieu observed may in part contribute to the recovery of right-sided cardiac function associated with chronic mechanical circulatory support. J Heart Lung Transplant 2004;23:28–35.

From the aM. E. DeBakey Department of Surgery, Division of Transplantation and Assist Devices, bMedicine and Cardiovascular Sciences (Section of Cardiology), cGene and Judy Campbell Lab for Cardiac Transplant Research, Winters Center for Heart Failure Research, and dDeBakey Heart Center at the Methodist Hospital, Baylor College of Medicine, Houston, Texas.

Submitted August 22, 2002; revised December 20, 2002; accepted December 26, 2002.

This work was supported in part by Grant HL-42550 from the National Heart, Lung, and Blood Institute (Dr. Entman) and was funded by the Turkish Health Ministry (Drs. Küçüker and Akgül). Reprint requests: Guillermo Torre-Amione, MD, Methodist DeBakey Heart Center, Baylor College of Medicine, 6550 Fannin, Suite 1901, Houston, Texas 77030. Telephone: 713-798-1404. Fax: 713-798-8744. E-mail: gtorre@bcm.tmc.edu Copyright © 2004 by the International Society for Heart and Lung Transplantation. 1053-2498/04/$—see front matter doi:10.1016/S1053-2498(03)00057-3
It has become increasingly clear that chronic mechanical circulatory support improves left ventricular (LV) myocardial function and structure. These findings have become clinically evident in observations that some patients may improve cardiac function to the point that device removal may be possible. A critical step for improved myocardial function after left ventricular assist-device (LVAD) support is normalization of right ventricular (RV) function. Although improved RV function may be defined clinically, few or no data are available on myocardial structural changes that occur in the RV of failing myocardium after LVAD support.

The mechanisms by which LVAD support may alter RV function are 2-fold: anatomic-hemodynamic changes and normalization of the neurohormonal milieu. First, the RV and LV are interdependent structures in which changes in volume, pressure, and performance on one side may affect the other. For example, after LVAD placement, RV venous return and cardiac output increase, whereas RV after-load decreases because of decreased left atrial pressure. These anatomic and hemodynamic changes decrease the forces that stimulate hypertrophy and failure in the RV. Second, ample experimental evidence suggests that after LVAD support, the neurohormonal changes that typified the heart failure state improve. Importantly, catecholamine levels decrease as do pro-inflammatory cytokines that can directly stimulate cellular hypertrophy and promote myocardial fibrosis. Thus, normalization of these systemic effects may be important in the recovery of RV function after LVAD support.

Because RV function may be affected after LVAD support, we decided to further investigate RV structural changes in failing myocardia after LVAD implantation. Accordingly, the goals of our study were to compare markers of hypertrophy and myocardial cytokine expression in RV myocardial specimens after LVAD support. In addition, because of the increased use of various forms of LVAD support, we also compared the effects of continuous vs pulsatile LVAD support on RV structure.

METHODS
Source of Human Myocardium

We conducted the studies after each patient gave written, informed consent and with the approval of the Institutional Review Board of the Methodist Hospital and Baylor College of Medicine. The cohort comprised patients who underwent cardiac transplantation at the Methodist Hospital/Baylor College of Medicine Multi-Organ Transplant Center.

We obtained control myocardia (n = 5) from the left and RV free walls of donor hearts before implantation. We obtained failing myocardia from the left and RV free walls of patients (n = 5) at the time cardiac transplantation. The LVAD supported myocardia were obtained at the time of device removal for transplantation from 5 patients with DeBakey Axial Flow VAD (MicroMed Technology; Houston, TX) and from 5 patients with Novacor LVAS (Baxter Healthcare; Deerfield, IL).

Total Collagen Content

Human myocardial tissue samples were sectioned at 5 μm, embedded in paraffin, and stained for 1 hour in picro-sirius red solution (0.1% solution of Sirius Red F3B in saturated aqueous picric acid, Direct Red 80 obtained from Sigma-Aldrich Chemical; Milwaukee, WI) as described previously. The stained sections were then dehydrated rapidly in 3 changes of 95% and 100% alcohols, cleared in xylene, and mounted in xylene-based mounting medium. Total collagen content was the sum of all areas stained within the slide, including interstitial, perivascular, and microscopic scars.

Myocyte Size

We used hematoxylin and eosin staining to measure myocyte size. A point-to-point perpendicular line was drawn across the cross-sectional area of the myocytes at the level of the nucleus, and we used computer-imaging software (Image-Pro Plus, version 4.1, Media Cybernetics, Silver Spring, MD) to measure this diameter length. Transverse or oblique-sectioned myocytes were excluded. We measured 50 myocytes per slide from each tissue specimen and expressed results as mean and standard error measured.

Myocardial TNF-α Levels

We performed immunohistochemistry using a standard immunoperoxidase technique on 5-μm human tissue sections. Myocardial tissue samples were immersed immediately in 2% paraformaldehyde for 45 minutes, followed by 75% alcohol, then dehydrated in increasing concentrations of alcohols, and after that cleared, through xylene, and subsequently embedded in paraffin. For detecting TNF-α, deparaffinized sections were blocked for endogenous peroxidase activity and quenched by pre-incubating slides in 0.3% H₂O₂ in methanol for 20 minutes in a
humidity chamber. Next, the slides were flooded with \(-20^\circ\text{C}\) acetone for 3 minutes. After washing in phosphate buffer saline (PBS), the slides were incubated for 30 minutes in 1% blocking solution (1 g 99% albumin, bovine fraction V, and 10 ml PBS). The following primary and secondary antibodies were applied: a mouse monoclonal IgG_1 antibody (dilution 1:10, Santa Cruz Biotechnology; Santa Cruz, CA) incubated for 2 hours in a humidity chamber. After washing in PBS, the tissues were incubated with biotinylated anti-mouse IgG_1 (dilution 1:200, Vector Laboratories; Burlingame, CA) for 30 to 60 minutes. After washing in PBS, tissue sections were treated with streptavidin conjugated to horseradish peroxidase (Vector Laboratories) for 30 minutes. After washing in PBS, the tissues were incubated with biotinylated anti-mouse IgG_1 (dilution 1:10, Santa Cruz Biotechnology; Santa Cruz, CA) incubated for 2 hours in a humidity chamber. After washing in PBS, the tissues were incubated with biotinylated anti-mouse IgG_1 (dilution 1:200, Vector Laboratories) as the substrate at room temperature for 30 to 60 minutes. After washing in PBS, tissue sections were incubated with 3,3'-diaminobenzidine (Vector Laboratories) as the substrate at room temperature until suitable stains developed, followed by counterstaining with Harris hematoxylin for 1 minute. Sections were then dehydrated in graded alcohols and cover slipped with Cytoseal XYL (Stephens Scientific, Kalamazoo, MI). For control tissues, we substituted primary antibodies with mouse IgG_1 isotype antibody (R&D Systems; Minneapolis, MN). For preliminary experiments, we stained myocardial samples at varying concentrations of anti-TNF-\(\alpha\) antibody, ranging from a 1-to-10 to a 1-to-1,000 dilution. Peak staining always occurred at a 1-to-10 dilution, and we used this concentration of antibody for all subsequent experiments.

Quantitative Analysis of Stained Areas

Six microscopic fields were photographed per specimen slide using a Diagnostic Instrument Spot II color camera (Diagnostic Instrument; Sterling Heights, MI) mounted on an AX70 fluorescence microscope (Olympus; Melville, NY). All fields were digitized to a computer database and stored for analysis. Staining was analyzed with Image-Pro Plus version 4.1 analysis software (Media Cybernetics) using color cube–based selection criteria for positive staining. We analyzed intensity level (range) and area according to the method of Matsuo et al. Results in this study are based on areas of positive staining within the color spectrum for 3,3'-diaminobenzidine (DAB for TNF-\(\alpha\)) of all intensities greater than those found in mouse IgG_1 isotype control stained sections without correction for intensity. For total collagen content, all tissue specimens were obtained, processed, and analyzed in the very same manner, and results are expressed as mean and standard error measured. An investigator masked to the origin of the samples and without knowledge of whether the slides originated from patients with LVAD support analyzed the slides. However, because variation exists between the intensity of the staining from one experiment to the other, comparisons between groups were only performed within the same experiment. Although absolute values varied from experiment to experiment, the relative number of immunopositive areas did not change.

Statistical Analysis

We performed statistical comparisons among controls, failing myocardia, and LVAD-supported groups using a 1-way analysis of variance followed by Tukey-Kramer multiple comparisons test corrections in myocyte size, total collagen, and TNF-\(\alpha\) expression. All data in the text and figures were expressed as mean \(\pm\) SEM. We considered \(p < 0.05\) as significant.

RESULTS

Demographics

Table I shows the characteristics of cohort patients studied. Five patients underwent placement of pulsatile LVAS (Novacor) and 5 patients received continuous-flow LVAD support (DeBakey Axial Flow VAD) for progressive cardiac deterioration. All of the patients supported by LVAD received maximal intravenous inotropic support. Nine of 10 patients required intra-aortic balloon pump placement before LVAD insertion. The mean duration of the LVAD support was 72.8 \(\pm\) 14.8 days for the patients treated with pulsatile pumps and 32.0 \(\pm\) 15.5 days for those treated with continuous-flow pumps.

Structural Changes in RV Failing Myocardium

Figure 1 shows myocyte size, collagen content, and myocardial TNF-\(\alpha\) content in RV (left panel) and LV (right panel) myocardial samples from control and from patients with end-stage cardiomyopathies. Figure 1A shows no difference in the myocyte size of RV failing myocardial samples compared with those of controls (22.5 \(\pm\) 1.6 \(\mu\)m vs 18.2 \(\pm\) 1.1 \(\mu\)m, \(p =\) not significant); whereas, a significant difference occurred in myocyte size of the LV failing samples compared with controls (32.3 \(\pm\) 1.8 \(\mu\)m vs 22.9 \(\pm\) 2.2 \(\mu\)m, \(p < 0.01\)). Figure 1B shows that collagen content increased in RV failing myocardia compared with controls (27.6% \(\pm\) 2.9% vs 13.2% \(\pm\) 0.7%, \(p < 0.001\)). The increase in collagen content observed in the RV was similar to the gain observed in the failing LV myocardium. Figure 1C shows that
TABLE I Patient demographics and hemodynamics before surgical interventions

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>None</th>
<th>Pulsatile</th>
<th>Continuous</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Duration of LVAD support, days</td>
<td>N/A</td>
<td>72.8 ± 14.8</td>
<td>32.0 ± 15.5</td>
</tr>
<tr>
<td>Age (mean, years)</td>
<td>54.0 ± 7.0</td>
<td>53.4 ± 6.0</td>
<td>44.4 ± 3.8</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>2/3</td>
<td>5/0</td>
<td>2/3</td>
</tr>
<tr>
<td>Cause, ICM/DCM</td>
<td>1/4</td>
<td>3/2</td>
<td>4/1</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>80.4 ± 8.9</td>
<td>74.6 ± 4.7</td>
<td>74.0 ± 2.1</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>3.3 ± 0.3</td>
<td>4.6 ± 0.5</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>10.8 ± 2.4</td>
<td>13.0 ± 1.5</td>
<td>14.0 ± 4.2</td>
</tr>
<tr>
<td>PAP (mean), mm Hg</td>
<td>38.0 ± 3.1</td>
<td>38.2 ± 3.0</td>
<td>43.6 ± 5.3</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>25.6 ± 2.2</td>
<td>25.2 ± 3.3</td>
<td>26.4 ± 3.4</td>
</tr>
<tr>
<td>TPG, mm Hg</td>
<td>12.4 ± 1.8</td>
<td>13.0 ± 4.1</td>
<td>16.8 ± 6.5</td>
</tr>
<tr>
<td>Receiving inotropic support, n (%)</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>IABP, n</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Mean ± SEM. CO, cardiac output; CVP, central venous pressure; DCM, idiopathic dilated cardiomyopathy; IABP, intra-aortic balloon pump; ICM, ischemic cardiomyopathy; LVAD, left ventricular assist device; MAP, mean arterial pressure; N/A, not applicable; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; TPG, transpulmonary gradient.

myocardial TNF-α levels were increased in RV failing samples compared with controls (35.4% ± 2.8% vs 1.2% ± 0.4%, p < 0.001). The increase in TNF-α observed in the RV was similar to the increase observed in the LV failing myocardium.

**Effect of LVAD Support on RV Myocardial Structure**

Figure 2A shows myocyte size of RV failing myocardia supported with either pulsatile or continuous-flow pumps. We found no difference among the 3 groups. In contrast, LV failing myocyte size was significantly smaller in patients supported with either pulsatile or continuous-flow devices when compared with LV failing myocardia with no LVAD support (23.1 ± 1.3 μm vs 23.2 ± 1.8 μm, p < 0.01 and 21.5 ± 1.5 μm vs 23.2 ± 1.8 μm, p < 0.001 for pulsatile vs no support and continuous vs no support, respectively, right panel).

Figure 2B shows decreased collagen content in patients supported with either pulsatile or continuous-flow devices when compared with RV failing myocardia with no LVAD support (20.4 ± 1.1 vs 27.6 ± 2.9, p = not significant and 14.3 ± 1.4 vs 27.6 ± 2.9, p < 0.01 for pulsatile vs no support and continuous vs no support, respectively, left panel). The decreased collagen content observed in the failing RV supported with LVAD was of similar magnitude to that observed in the failing LV supported with the same type of LVAD.

Figure 2C shows that myocardial TNF-α content in RV failing myocardia of patients supported with either pulsatile or continuous-flow LVAD was significantly less than RV failing myocardia with no LVAD support (3.0% ± 0.5% vs 35.4% ± 2.8%, p < 0.001, and 0.8% ± 0.1% vs 35.4% ± 2.8%, p < 0.001, for pulsatile vs no support and continuous-flow vs no support, respectively, left panel). The observed decrease in myocardial TNF-α content in the RV failing myocardia supported by pulsatile and continuous-flow LVAD was similar to the decrease observed in the LV failing myocardia with the same type of LVAD support.

**DISCUSSION**

Evidence of the beneficial effects of mechanical unloading on LV function and structure have been characterized previously, however, the effect of LV unloading with mechanical circulatory support on RV function and structure are not understood. Therefore, the major focus of our
investigations was to define the changes that occur in the failing RV using various markers of cellular hypertrophy that typified the failing phenotype. In this regard, the principal findings of our studies were demonstration that 1) the failing RV expresses increased levels of collagen content and myocardial TNF-α even in the absence of an increase in myocyte cell size, 2) the increased collagen content and myocardial TNF-α content were similar to the gain observed in the left failing ventricle, and 3) long-term mechanical unloading with either pulsatile or continuous-flow LVAD support leads to normalization of RV collagen and myocardial TNF-α content. The primary indication for long-term LVAD support is as bridge to transplantation, and perhaps in the near future, as a form of destination therapy; but more interestingly, the possibility of sustained myocardial improvement after LVAD support to the point that device removal is possible has led us and others to actively pursue evidence of myocardial improvement at a clinical and basic level. In this context, the demonstration of improved RV structure after mechanical unloading of the LV provides fundamental observations consistent with the possibility of sustained myocardial improvement after LVAD support.

In these studies, we investigated the effect of 2 distinct types of LVAD support on RV structure. Mechanical support can be achieved by either a device capable of generating pulsatile flow or by a continuous-flow device. Whether differences exist among these devices in terms of the cardiac response to unloading is not known. However, in a recent study from our group, we compared the effects of these 2 types of devices on cardiac hemodynamics and structure as determined by echocardiography, and we also measured the changes that occur on myocyte size and collagen content in the

**FIGURE 1** Myocardial specimens from normal controls and from end-stage myocardia obtained from the right ventricle (left panel) and the left ventricle (right panel) were used to determine myocyte size (A), collagen content (B), and myocardial tumor necrosis factor α (TNF-α) levels (C).

**FIGURE 2** Failing myocardial specimens from the right ventricle (left panel) and from the left ventricle (right panel) were obtained from patients with end-stage cardiomyopathies who were treated with either pulsatile or continuous-flow pump support. Myocardial specimens from these groups of patients were used to determine myocyte size (A), collagen content (B), and myocardial tumor necrosis factor α (TNF-α) levels (C).
Right ventricular myocardial samples from controls and from patients with end-stage heart failure without LVAD support or with support from pulsatile or continuous-flow pumps were stained for myocyte size, collagen content, and myocardial tumor necrosis factor α (TNF-α) content. The picture depicts representative immunostainings (original magnification ×40).
LV after LVAD support. We found that both types of devices were capable of inducing regression in hypertrophy. The current study expands those observations by demonstrating that both pulsatile and non-pulsatile devices induce beneficial changes that translate into normalization of RV structure. The evidence presented in this article is consistent with the ability of pulsatile or continuous-flow support to improve myocardial structure; however, the implication of these changes in their translation to functional improvements is not known.

The mechanisms by which RV structure are improved may be 2-fold: 1) the direct hemodynamic effect caused by decreased pulmonary pressure leads to decreased RV load, and 2) the effect of LV support decreases the expression of a paracrine growth factor that may stimulate RV hypertrophy, for example, TNF-α capable of producing hypertrophy. Whether any of these mechanisms is the primary one is not known; but currently we are pursuing this question as part of a larger multi-institutional effort directed at defining the cellular and functional changes induced by LVAD support in failing myocardia.

In summary, the current study provides fundamental observations that demonstrate improved RV myocardial structure after mechanical support and, furthermore, suggests that the beneficial effects of mechanical unloading may be achieved with the use of either pulsatile or continuous-flow pumps. These observations may be important in the future application of these devices for long-term use or for myocardial recovery.

Study Limitations

The number of patients in this study is small; however, the observations presented are clear. The myocardial samples studied were not paired samples because we had not systematically obtained RV myocardial samples of failing myocardia at the time of LVAD support.

The authors thank Alida J. Evans and Stephanie Butcher for excellent technical assistance.

REFERENCES


**TABLE II** Effects of LVAD support on myocardial structures

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>None</th>
<th>Pulsatile</th>
<th>Continuous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Ventricle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocyte size (microns)</td>
<td>18.2 ± 1.1</td>
<td>22.5 ± 1.6</td>
<td>21.7 ± 1.3 ‡</td>
<td>21.0 ± 0.9 ‡</td>
</tr>
<tr>
<td>Collagen content (% tissue area stained)</td>
<td>13.2 ± 0.7</td>
<td>27.6 ± 2.9</td>
<td>20.4 ± 1.1 ‡</td>
<td>14.3 ± 1.4 *</td>
</tr>
<tr>
<td>TNF-α (% tissue area stained)</td>
<td>1.2 ± 0.4</td>
<td>35.4 ± 2.8</td>
<td>3.0 ± 0.5 †</td>
<td>0.8 ± 0.1 *</td>
</tr>
<tr>
<td>Left Ventricle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocyte size (microns)</td>
<td>22.9 ± 2.2</td>
<td>32.3 ± 1.8</td>
<td>23.1 ± 1.3 *</td>
<td>21.5 ± 1.5 *</td>
</tr>
<tr>
<td>Collagen content (% tissue area stained)</td>
<td>10.8 ± 0.8</td>
<td>29.9 ± 2.3</td>
<td>20.2 ± 1.8 ‡</td>
<td>13.6 ± 1.8 †</td>
</tr>
<tr>
<td>TNF-α (% tissue area stained)</td>
<td>1.3 ± 0.3</td>
<td>37.8 ± 3.8</td>
<td>4.2 ± 0.6 †</td>
<td>1.2 ± 0.3 †</td>
</tr>
</tbody>
</table>

Mean ± SEM.

Statistical analysis was performed among LVAD = supported cohort (pulsatile and continuous) vs no support.

†p < 0.01; ‡p < 0.001; §p < 0.05; #p = not significant. LVAD, left ventricular assist device; TNF-α, tumor necrosis factor α.
11. Taegtmeyer H, Dietze GJ. Perspective from increased energy metabolism to cardiac hypertrophy and failure: mediators and molecular mechanisms. Am J Cardiol 1999;83(12A):1H–2H.