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Effect of chronic left ventricular unloading on myocardial remodeling: Multimodal assessment of two heterotopic heart transplantation techniques

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heterotopic heart transplantation;
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BACKGROUND: Cardiac recovery is possible by means of mechanical unloading yet remains rare. Excessive unloading-associated myocardial atrophy and fibrosis may adversely affect the process of reverse remodeling. In this study, we sought to evaluate the effect of different intensities of chronic left ventricular (LV) unloading on myocardial remodeling.

METHODS: Twenty-five isogenic Lewis rats underwent complete LV unloading (CU, $n = 15$) induced by heterotopic heart transplantation or partial LV unloading (PU, $n = 10$) by heterotopic heart-lung transplantation. Information obtained from serial echocardiography, 2-deoxy-2[^{18}F]fluoro-D-glucose (^{18}F-FDG)-positron emission tomography, and an LV pressure-volume catheter were used to evaluate the morphology, glucose metabolism, and hemodynamic performance of the orthotopic hearts and heterotopic transplants over 4 weeks. Cell size, collagen content, tissue cytokines (interleukin [IL]-1α, IL-2, IL-6, IL-10, tumor necrosis factor-α, and vascular endothelial growth factor), and matrix metalloproteinase-2 and -9 were also determined. The recorded parameters included LV end-systolic dimension, LV end-diastolic dimension, posterior wall thickness, diastolic interventricular septum thickness, LV fractional shortening, and LV ejection fraction.

RESULTS: We demonstrated an LV load-dependent relationship using echo-based structural (left posterior wall thickness, diastolic interventricular septum thickness, and left ventricular end-diastolic dimension) and functional (LV fractional shortening and LV ejection fraction) parameters, as well as an ^{18}F-FDG uptake (all $p < 0.05$). This load-dependent relationship was also evidenced in measurements from the pressure-volume conductance catheter (stroke volume, stroke work, cardiac output, dP/dTmax, and –dP/dTmin; all $p < 0.05$). Significant myocardial atrophy and fibrosis were observed in unloaded hearts, whereas concentrations of cytokines and matrix metalloproteinases were comparable in both unloading conditions.

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CONCLUSIONS: Partial and complete unloading affected the remodeling of non-failing hearts in a rodent model to different extents on myocardial atrophy, fibrosis, glucose metabolism, and mechanical work. Cardiac atrophy is the prominent change after mechanical unloading, which exaggerates the proportion of total collagen that is responsible for diastolic dysfunction.
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Heart failure is characterized by an alteration in ventricular geometry and impaired cardiac function (ventricular remodeling) secondary to myocardial injurious events of various etiologies, including acute and chronic ischemia, exposure to toxins, genetic abnormalities, infectious agents, and cardiac pressure/volume overload.[1] Over the past few years, there has been increased interest in the therapeutic strategy involving reducing left ventricle (LV) overload while preserving ventricular contractility.[2,3] Promising clinical outcomes have been reported with regard to cardiac recovery under ventricular assist devices (VADs).[2,3] Although the mechanistic details are still being refined, the benefit of mechanical unloading can to some extent be ascribed to the regression of myocytes hypertrophy, upregulation of β-adrenergic receptors and sarcomeric proteins, improved calcium handling, and normalization of the neurohormonal environment.[4,5]

However, sustained myocardial recovery that allows for the removal of the LVAD without recurrence is, however, a rare phenomenon,[6] with clinical and experimental studies having indicated that long-term LV unloading might trigger myocardial atrophy that is detrimental to cardiac recovery.[7,8] These observations have brought into question whether there is a limit in the duration and intensity of ventricular unloading before a detrimental effect occurs on efficient reverse remodeling. Oriyanhan et al[8] conducted a study that suggested the optimal duration of mechanical unloading for improving myocardial recovery was 4 weeks. The effect of different unloading levels on myocardial contractility and extracellular matrix turnover, however, remains a matter of some debate.[9–14]

In this study, we used 2 murine heterotopic heart transplantation procedures, namely, heterotopic heart transplantation[15] to simulate LV complete unloading (CU) models and heterotopic heart-lung transplantation[16] to simulate partial unloading (PU) models. By applying several non-invasive (transthoracic echocardiography and 2-deoxy-2[^{18}F]fluoro-D-glucose [^{18}F-FDG]-positron emission tomography (PET) examinations and invasive (conductance catheter) functional tests in 2 animal models, we investigated the relative contribution of different LV unloading conditions to the evolution of graft morphology, contractile function, and glucose metabolism. Histomorphometric evaluation and biochemical tissue analysis were also performed to assess the ventricular unloading effects on cellular and molecular levels of inflammatory cytokines (interleukin [Il]-1α, IL-2, IL-6, IL-10, and tumor necrosis factor [TNF]-α) the angiogenesis cytokine vascular endothelial growth factor (VEGF), and matrix metalloproteinase (MMP)-2 and -9, and also to investigate the effect of inflammation, tissue ischemia, and collagen degradation on unloading-induced cardiac remodeling.

Methods

All animals used in this study received care in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Animals and animal models

The study used adult male Lewis rats (Genest, St Isle, France) weighing 300 to 400 g and aged 8 to 10 weeks. Animal anesthesia was obtained by inhalation of isoflurane (2-3 vol%) and oxygen (2 liters/min) through a nosecone or by tracheal intubation connected to mechanical ventilation (Minerve, Esternay, France).

Heterotopic heart transplantation (CU group, $n = 15$) and heterotopic heart-lung transplantation (PU group, $n = 10$) were performed as previously described.[7] Briefly, for heart procurement, heparin (500 U/kg) was injected into donor rats through the penile vein, and 10 mL Celsior cardioplegic solution (Genzyme Corp, Boston, MA) was administrated through a catheter inserted into the ascending aorta after cross clamping. The heart or heart-lung grafts were thereafter harvested. For transplantation in the CU group (Figure 1A and C), the donors' ascending aorta and pulmonary artery were anastomosed to the recipients' abdominal aorta and inferior vena cava (IVC), respectively; and the donors' superior vena cava (SVC), IVC, and pulmonary veins (PVs) were ligated. In the PU group (Figure 1B and D), the donors' ascending aorta was anastomosed to the recipients' abdominal aorta, the donors' SVC and IVC were ligated, and the pulmonary artery and PVs were intact. The anastomosis was sutured with 8-0 Prolene (Ethicon, Somerville, NJ).

The surgical success rate was in 80% (12 of 15) in the CU group and 70% (7 of 10) in the PU group. The graft lost was due to hemorrhage (2 in CU group and 1 in PU group) and primary graft dysfunction (1 in CU group and 2 in PU group). No secondary graft loss was observed during follow-up.

Echocardiography

Echocardiography was performed serially at Postoperative Day (POD) 0, 14, and 28 on the heterotopic and orthotopic hearts in the recipients. Data acquisition and analysis were obtained with a commercially available echocardiograph system (Philips Medical Systems, Best, The Netherlands) equipped with a 14-MHz frequency transducer. Two-dimensional, color Doppler and Doppler flow models (Figure 2) were performed for qualitative measurements. M-mode tracings were recorded with a 2-dimensional short-axis view at the level of mitral papillary