Myocardial Alterations in Adrenoreceptors After Ventricular Unloading with a Pulsatile *vs* a Nonpulsatile Device

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Abstract: We have recently shown that ventricular unloading with an implantable left ventricular assist device (LVAD) leads to improved calcium handling and membrane integrity and redistribution of alpha adrenoreceptors (AARs) and beta-adrenoreceptors (BARs). Here, we used fluorescence deconvolution microscopy to examine the effect of LVAD type (pulsatile *vs* non-pulsatile) on upregulation and redistribution of adrenoreceptors in core biopsy samples of the myocardium before and after the removal LVAD. We noted no major differences between the pulsatile and non-pulsatile groups; however, an individual patient's 'recovery' in adrenoreceptor numbers depended on the pre-LVAD number of receptors. These findings suggest that ventricular unloading is beneficial regardless of LVAD type; however, the degree of repair and recovery may correlate with the patient's level of ventricular dysfunction at implant and the pre-LVAD number of adrenoreceptors (130).

Keywords: Heart failure, adrenoreceptors, microscopy, ventricular unloading.

INTRODUCTION

Alpha-adrenoreceptors (AARs), beta-adrenoreceptors (BARs), and their subtypes are intimately involved in cell signaling [1] and in modulating heart failure [2]. We have recently shown that ventricular unloading with an implantable left ventricular assist device (LVAD) may lead to improvements in calcium handling and membrane integrity, and to redistribution of both AARs and BARs from perivascular to intramyocytic areas [3,4]. Improvements in cardiac function after ventricular unloading with all types of LVADs have been documented [5-10] even in patients who require continued LVAD support [5,9,10]. Increased myocardial adrenoreceptor content and sensitivity is a major benefit of ventricular unloading [11], resulting in restoration of optimum myocardial contractility. The use of adrenoreceptors as therapeutic targets has not been well studied. Targeting specific receptor subtypes for treatment regimens may be beneficial and may depend on the type of LVAD used. Thus, we investigated the upregulation and redistribution of adrenoreceptors by comparing tissue samples from patients who were supported by either a pulsatile or a non-pulsatile LVAD.

MATERIALS AND METHODOLOGY

Myocardial AAR and BAR densities and localizations were compared at the time of LVAD insertion and at either LVAD removal or organ transplantation. This investigation was approved by our center's institutional review board, and the patients provided written informed consent. All procedures conformed to guidelines dictated by the University of Texas Medical School at Houston and the Texas Heart Institute. A left ventricular myocardial core removed from the apex (1-1.5–cm wide) for LVAD placement served as the initial biopsy specimen. A second biopsy sample taken at LVAD explant after heart transplantation was obtained near the apex of the left ventricle but away from the suture line used for LVAD implant. Patient characteristics and preoperative hemodynamic measures were similar in the 2 patient groups before LVAD implantation (Table **1**). All patients accepted for heart transplantation were in New York Heart Association class-IV heart failure. The nonpulsatile LVAD patients had an average pulmonary capillary wedge pressure (PCWP) of 25±4.6 mmHg, a cardiac index (CI) of 1.67±0.22 L/min/m2, and a central venous pressure (CVP) of 12.2±2.8 mmHg. The pulsatile LVAD patients had an average PCWP of 20±3.4 mmHg, a CI of 2.08±0.28 L/min/m2, and a CVP of 8±3.1 mmHg.

Tissue Preparation and Staining

Core samples of fresh cardiac tissue were embedded in OCT compound (Tissue-Tek, Torrance, California.) and frozen on dry ice. We used a Reichert HistoSTAT cryotome at 4°C to obtain sections (10 ± 3 μm–thick), which were placed on poly-L-lysine-coated (Sigma-Aldrich Corp., St. Louis, Missouri) coverslips and soaked in 3.7% paraformaldehyde (5 minutes at room temperature). Sections were stained with fluorescent-receptor probes (5 nmol/L for 30 minutes at 37°C) and placed on a glass slide under 1 drop of Elvanol (DuPont Antifade, Wilmington, Delaware). To localize AARs, we used the Bodipy 558/568-tagged prazosin probe (Invitrogen/Molecular Probes Inc., Carlsbad, California) at a *K*d of 0.13 nmol/L for prazosin. We

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Table 1. Patient Characteristics Before Left Ventricular Assist Device Implantation

	Non-Pulsatile	Pulsatile
Average age	58.4±45.75	47.3±9.481
Men/Women	7 – 2	9 – 0
Days on LVAD	91.9±48.4	129±51.2
Av. Duration HF (years)	6.72±5.36	9.7±6.3
Transplanted/expired	6 – 3	7 – 2
Av. Central venous pressure	12.2±2.8	8.0±3.1
Av. Cardiac index	1.67±0.22	2.08±0.28
Av. Capillary Wedge pressure	25±4.6	20±3.4

HF=heart failure; central venous pressure in mmHg; cardiac index in L/min/m^2; capillary wedge pressure in mmHg.

monitored nonspecific binding by preincubating sections with nonradioactive prazosin before incubating the sections with the fluorescent probe. For BAR typing, we used the generic tag CGHP12177 as previously described [3]. The probe for actin was Bodipy FL Phallicidin (Molecular Probes, Eugene Oregon; product # B607; excitation peak 505nm, emission peak 512nm, and Kd=38 nM). We stained nuclei with DAPI (Molecular Probes/Invitrogen). Filters were set as follows: DAPI-excitation 360nm, band-pass 40nm, emission 457nm, band-pass 50nm; FITC-excitation 490nm, band-pass 20nm, emission 528nm, band-pass 38nm; TxRed-excitation 555nm, band-pass 28nm, emission 617nm, band-pass 73nm.

A primary antibody (SMA Sigma Monoclonal; diluted 1:100) followed by a secondary antibody was used to probe for smooth-muscle actin (AlexaFluor 647 antibody; Molecular Probes; goat anti-mouse; diluted 1:500) or cardiac actin (BODIPY and Texas Red-tagged phallicidin; Molecular Probes, diluted 1:100). We used DAPI (0.1 g/ml; Molecular Probes) to identify nuclei and cell types (fibroblasts, endothelial cells, and myocytes). All images were magnified at 400X. Image acquisition We used an Applied Precision DeltaVision scanning fluorescence microscope (Issaquah, Washington) fitted with an Olympus IX70 microscope (Melville, New York) with deconvolution capabilities. Obtained in a complete pass from tissue bottom to top, the 0.25-µm–thick images were subjected to deconvolution (5 iterations), stacking, and volume-rendition with Imaris software (Bitplane AG, Zurich, Switzerland).

We counted 3 distinct areas of fluorescence per sample to reduce errors in stereology and captured areas of interest as red-green-blue (RGB) files (90×90 µm). The number of pixels in a 60×60-µm area was used to determine the mean receptor density. We selected fields with the least amount of fibrosis to compare receptor number between sections. Receptor numbers were measured by a Corel (Corel Corp., Ottawa, Canada) and a SigmaScan program (SPSS Inc., Plover, Wisconsin) to minimize errors.

RESULTS

AARs and BARs were targeted with fluorescent probes in core biopsy samples of myocardium before and after removal of a pulsatile (n=9) or non-pulsatile (n=9) LVAD. Fluorescent deconvolution microscopy was used to determine the effect of LVAD implantation and subsequent

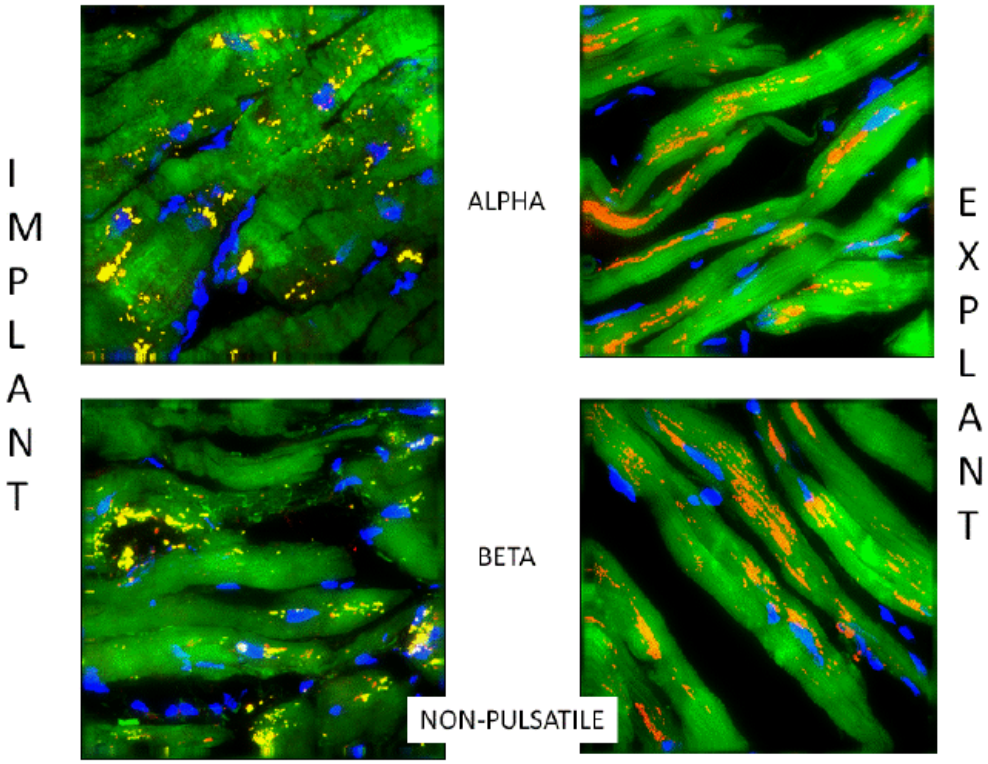

Fig. (1). Alpha-and beta-adrenoreceptor expression before (at implant) and after (at explant) a pulsatile left ventricular assist device was implanted. Green, cardiac actin; blue, nuclei (DAPI); and yellow/red, adrenoreceptors. Magnification 400X.

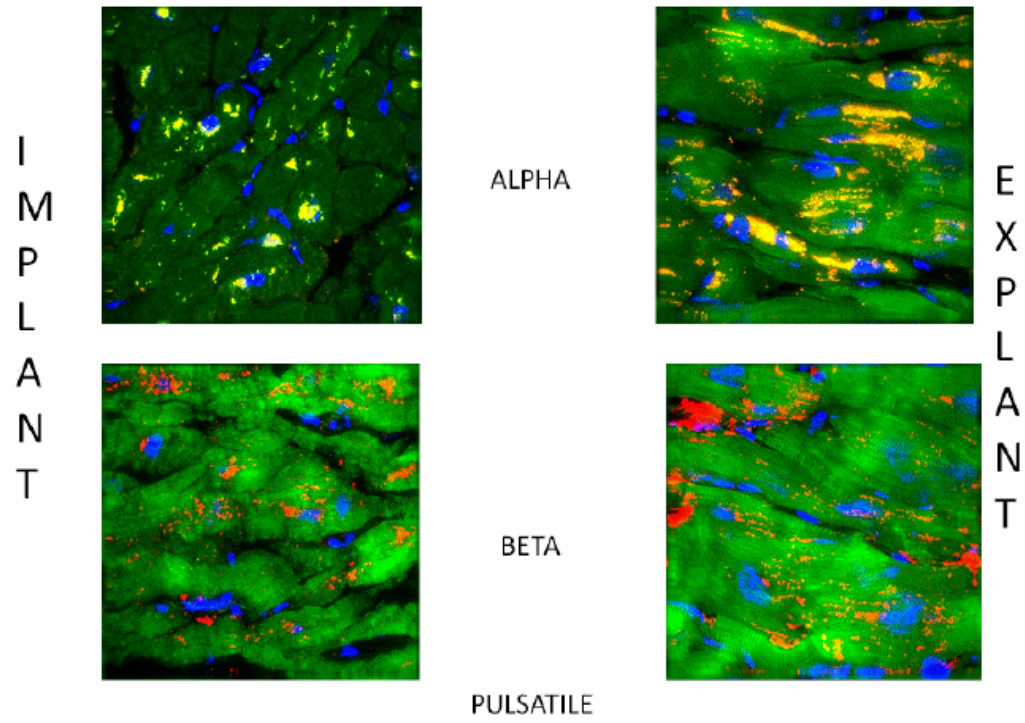

Fig. (2). Beta-adrenoreceptor expression before (at implant) and after (at explant) a pulsatile left ventricular assist device was implanted. The number of receptors is not increased by ventricular unloading nor does myocyte structure improve. Green, cardiac actin; blue, nuclei (DAPI); and yellow/red, adrenoreceptors. Magnification 400X.

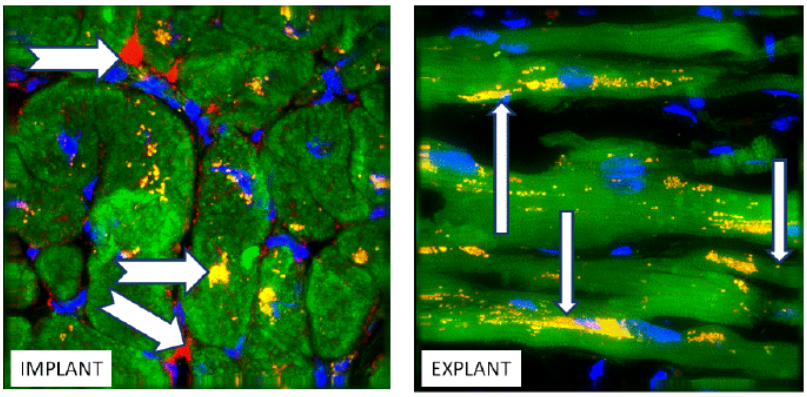

Fig. (3). Alpha-and beta-adrenoreceptor expression before (at implant) and after (at explant) a non-pulsatile left ventricular assist device was implanted. Green, cardiac actin; blue, nuclei (DAPI); and yellow/red, adrenoreceptors. Magnification 400X.

explantation on the density and distribution of adrenoreceptors. The number of BARs decreased from LVAD implant to explant in tissues from patients supported with a pulsatile device (Table **2**). These values are somewhat skewed because not all patients had high levels of receptors at the time of device implantation or responded to the LVAD with an increase in receptor number [12]. Figs. (**1**, **2**) present representative implant and explant tissue samples from patients in whom ventricular unloading did not increase the number of BARs, which are localized at the periphery of the myocytes and in the interstitial spaces of the explant (red clusters). In contrast, in these same patients, AARs increased in number after LVAD implantation (Table **2**). Fig. (**1**) shows a tissue sample from a patient with a pulsatile LVAD. At implant, the receptors formed clusters in the intra-fibrillar area (yellow); after implant, these clusters increased in number and became more homogeneously distributed. Fig. (**3**) shows a representative tissue sample from a patient with a non-pulsatile LVAD. Both AARs and BARs (yellow/orange-red) increased in number after device implantation (Table **2**). In addition, the myofibrillar structure improved at explant, and the AARs and BARs showed a more widespread, intra-fibrillar distribution. Fig. (**4**) shows another image of the changes in BARs before and after implant of the non-pulsatile device. Both interstitial/perivascular (red) and intra-fibrillar (yellow) clusters of BARs are apparent at implant. At explant, the intra-fibrillar receptors are more widely distributed, and the clusters of interstitial/perivascular receptors are absent. In both patient groups, ventricular unloading caused the adrenoreceptors to increase in number and form a more homogeneous distribution, with the exception of BARs in the pulsatile group. The BARs in this group decreased in number and were mostly distributed in the interstitial spaces.

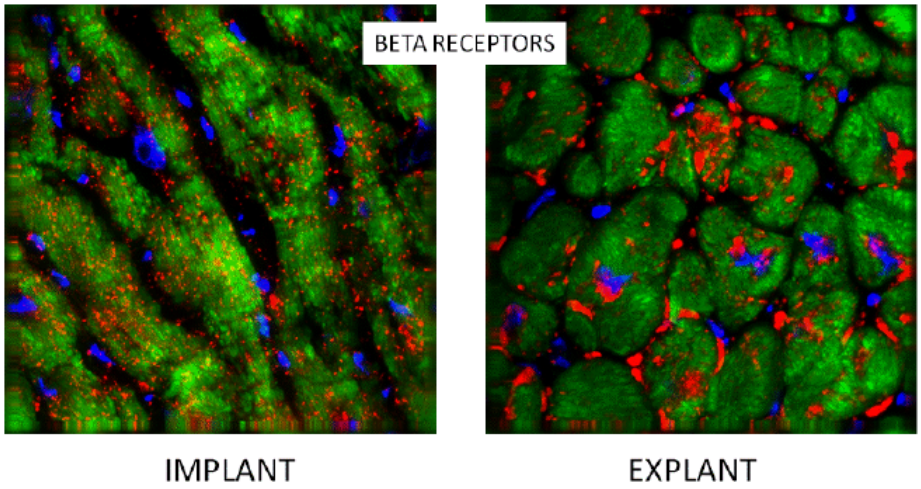

Fig. (4). Beta-adrenoreceptor expression before (at implant) and after (at explant) a non-pulsatile left ventricular assist device was implanted. Large arrows in the image on the left indicate the clumped and interstitial clusters of beta receptors before ventricular unloading. The arrows on the right indicate the widely distributed clusters of beta-adrenoreceptors after ventricular unloading. Green, cardiac actin; blue, nuclei (DAPI); and yellow/red, adrenoreceptors. Magnification 400X.

Table 2. The Number of Alpha- and Beta-Adrenoreceptors in Patients with Either a non-Pulsatile or Pulsatile Ventricular Unloading Measurements were Made Before (Pre-LVAD) and After (Post-LVAD) the Device was Implanted. AR = Adrenoreceptor

Adrenoreceptor Type	Pulsatile		Non-Pulsatile	
	Pre-LVAD	Post-LVAD	Pre-LVAD	Post-LVAD
	N=9	N=9	N=9	N=9
Beta	8272±3042	9751±4680	8716±4791	5721±1475
Alpha	5390±1793	7953±3225	4656±1304	7415±5418

DISCUSSION

Our previous studies have shown that LVAD assistance is a viable, long-term treatment option for patients with heart failure. LVADs reportedly reverse cardiac damage, hypertrophy, and fibrosis [6,13], accomplished possibly by restoring AAR and BAR levels, which play an important role in maintaining calcium homeostasis in the heart [3,4,12]. Failure to control calcium homeostasis in the heart leads to loss of myocardial contractile function and membrane disruption [4]. Other studies have shown similar results of improvement at the cellular level following ventricular unloading, in a rat model using a beta-2 antagonist [14], in a multicenter group report [15] and also the "sequestration" of beta-receptors and subsequent recovery in human ventricular samples, similar to our study [16]. We recently studied whether AAR and BAR subtypes undergo similar upregulation and redistribution in human myocardium after implantation of a Jarvik or Thoratec LVAD [12]. Our current objective was to determine whether pulsatile and non-pulsatile ventricular unloading devices differentially affect the number and distribution of AARs and BARs in the heart. Understanding the cellular mechanisms that are initiated during LVAD use is important because particular unloading devices and pharmacologic support may be better suited for specific ailments [17].

This particular collaboration suggests that not only do beta adrenergic receptors become 'dormant' and then respond to ventricular unloading, but that alpha adrenergic receptors respond in a similar manner, if not to such a great extent, possibly initiating a mechanism to avert inotropic changes [18], be part of the repair and restoration process [19], or become a pharmacologic treatment target [20].

Indeed, over 20 years ago alpha-adrenergic targeting drugs were considered as an important part of the physician's arsenal for the treatment of high, blood pressure, hypertension and heart failure [21] and our findings do show extra-myocytic receptors, possibly indicating an association with the microvasculature, but also implicating these proteins in the control of ionic equilibrium and contraction-relaxation well being.

CONCLUSION

Our studies suggest that ventricular unloading is beneficial regardless of the LVAD type; however, the degree of repair and recovery may correlate with the patient's pre-LVAD number of AARs and BARs. Results from this study and our previous study indicate that the presence of high numbers of adrenoreceptors at the time of LVAD implantation was predictive of an increase in adrenoreceptor number at explantation [22]. We have also shown that there are a great many variables that lead to a successful recovery and patients often do not respond as expected on the cellular level. This study is limited by the fact that it is observational and descriptive and by the relatively small numbers of patients. In addition, patients who received the non-pulsatile pump were selected based on the strict protocol associated with the investigational device, whereas patients who received the pulsatile pump were chosen based on size. It was also difficult to engage in a 'follow-up' study due to personnel changes, reduced funding and the requirement for the removal of patient identifiers before tissue preparation and fluorescent imaging. Nine percent of the patients were

explanted in the work group study [15]. The work group study also concluded that "this study has a number of limitations. Because no uniformly accepted criteria exist for device explantation for recovery, criteria for explantation were determined by clinicians at the individual centers and not prescribed in the study protocol. In addition, the observational nature of this study precludes us from ascertaining the recovery rate without LVAD implantation", and these limitations therefore apply to our cellular research. Fortunately, last year a pump was approved by the Federal Drug Administration for permanent placement. (http://www.texasheart.org/AboutUs/News/2010-01-21news_FDAapprove.cfm).

However, these studies suggest that ventricular unloading is beneficial regardless of the type of LVAD; however, the degree of repair and recovery may correlate with the patient's level of ventricular dysfunction at implant and the pre-LVAD number of adrenoreceptors. This work, as do a number of others, strongly suggests that cardiac unloading, by whatever method, is beneficial to the heart failure patient and should be implemented whenever possible. This study also points to alpha-adrenergic receptor involvement in cardiac muscle contraction, a finding that might warrant more consideration regarding heart failure mechanisms, particularly those initiated to allow for later repair and recovery.

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