Fluorescence Deconvolution Microscopy and cellular modeling to understand heart repair after ventricular unloading

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A recent publication from the Centers for Disease Control states “that approximately 5.8 million people in the United States have heart failure and about 670,000 people are diagnosed with it each year. About one in five people who have heart failure die within one year from diagnosis and heart failure was a contributing cause of 282,754 deaths in 2006. In 2010, heart failure will cost the United States $39.2 billion, a total that includes the cost of health care services, medications, and lost productivity” [1]. However advances have been made to at least slow the progression to heart failure with drugs such as ACE inhibitors, beta blockers, alpha blockers, cardiac glycosides and calcium channel blockers, drugs that help the heart to ‘take a load off’. However, despite research that has given us a greater understanding of why and how heart failure occurs, many people still suffer, even though they adhere to diet and exercise regimens [2].

Adrenoreceptors are small proteins in heart cell membranes that are intimately involved in the contraction and relaxation of heart muscle, thereby being part of the pathways that control heart rhythm. These receptors are also involved in the contraction of smooth muscle and so they too are involved in the control of blood pressure [3].

Over the years with the development of drugs, a heart failure patients’ quality of life was improved, resulting in better life expectancies and shorter hospital stays. Then along came assist devices. Basically, you put in an assist device to give the heart a rest and lo and behold, via intense research over the last 20-30 years, scientists and clinicians found that in many cases the heart repaired itself [4]. These assist devices, VADS, continue to be developed such that they can sometimes be removed, allowing the patient a return to a reasonably normal life. However, the question begged itself, what repairs occurred and why?

Using many methods, it was shown that fibrosis was reduced (less scar tissue) [5], that cell size was reduced (a need for less energy and better efficiency; ref. 3), and the control of calcium, calcium being of paramount importance in muscle contraction, was returned to normal levels [6]. Then, in 2001 a group at the Cleveland Clinic discovered that the all important beta receptors ‘recovered’ with unloading using a VAD and our group later reported a recovery of alpha adrenergic receptors [7, 8].

It was decided that we study these receptors as not only therapeutic targets in certain stages of heart disease, but also as diagnostic markers in heart failure. Therefore, using deconvolution microscopy and fluorescent probes, we used to visualize adrenoreceptors in left ventricular core/tissue samples taken from patients at the times of surgery for implanting a left ventricular assist device (LVAD), then also took a tissue sample when the device was removed, either due to recovery, or due to the necessity for organ transplantation. This fluorescence microscopy was employed to acquire images and construct 3D models of adrenoreceptors in tissue sections in order to better understand adrenoreceptor changes as a result of ventricular unloading.

We found that adrenoreceptors were seen in the heart muscle as clumps, congregated into little groups in diseased tissue but, after unloading/resting with the LVAD, the adrenoreceptors were redistributed throughout the myocardial muscle fibers, and in a much more homogeneous fashion, suggesting a much better regulation of calcium movements and therefore heart contraction-relaxation. This work gives a comprehensive and detailed view of adrenoreceptor redistributions and regulations and adds to the research that has been previously reported, demonstrating that cardiac recovery with LVAD assisted unloading involves adrenoreceptors and their redistribution and up-regulation, directing us to consider interventions that can increase adrenoreceptor numbers via combined therapeutic strategies.

Keywords Heart; receptors; fluorescence; microscopy

1. Introduction

Previous work has shown the intimate involvement of adrenoreceptors in the surprising ability of the heart to undergo repair and recovery processes [9, 10, 11, 1] and although there is much evidence as to the locations and even relocations of these proteins in damaged and recovering hearts our models, constructed by stacking multiple sections of fluorescently probed tissues from patient core samples before and after surgery, give us a highly detailed and interactive tool in understanding this recovery and directs us to therapeutic approached and targets that will hopefully limit the length of hospital stays for heart patients, will be financially less debilitating and will improve the quality of life for...
both the patient and his/her loved ones. Such has been the success of these new mechanical aides, some patients have had the device removed and returned to a more or less normal life [13, 14]. Despite these advances, not many patients undergo a ‘complete cure’ and still suffer heart damage and symptoms despite LVAD support [15], stressing the fact that our understanding of the whole process, and the need for new therapies and research, are very necessary.

2. Methods and Patients

Approval for these studies was given by the Committee for the Protection of Human Subjects review board, and patients also provided written informed consent at the Texas Heart Institute/St Luke’s Episcopal Hospital, Houston. Localizations of alpha (AAR) and beta (BAR) receptors were compared in tissue samples at the time of LVAD insertion and then again at the time of LVAD removal or at the time organ transplantation if that were necessary. A left ventricular myocardial core was removed from the apex (1-1.5 cm width) for LVAD placement. Tissue was compared with tissue harvested the LVAD was removed. No distinction was made between tissues from patients that had either a pulsatile or a non-pulsatile device. 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 mmHg, a cardiac index (CI) of 1.67 L/min/m², and a central venous pressure (CVP) of 12 mmHg. The pulsatile LVAD patients had an average PCWP of 20 mmHg, a CI of 2.08 L/min/m², and a CVP of 8 mmHg [16].

The HeartMate II shown below is a high-speed, axial flow, rotary blood pump. As an axial flow device, the HeartMate II produces no pulsatile action. Weighing 12 ounces (about 375 grams) and measuring about 1.5 inches (4 cm) in diameter and 2.5 inches (6 cm) long, it is significantly smaller than other currently approved devices. As such, it may be suitable for a wider range of patients, including small adults and children.

2.1 Tissue preparation and staining

Core samples of fresh cardiac tissue are embedded in OCT compound (10.24% polyvinyl alcohol, 4.2% polyethylene glycol, and 85.5% sucrose from Tissue-Tek (Torrance, California.) for processing, then frozen on dry ice. Sections of tissue are cut on a cryostat (Reichert HistoSTAT) to a thickness of 10 ± 3 µm at 4°C. The sections are placed on glass coverslips, soaked in 3.7% paraformaldehyde for 5 minutes at room temperature, and then stained with fluorescent probes to show the proteins and organelles of interest using Elvanol (DuPont Antifade, Wilmington, Delaware) to protect against fading. To localize the AARs, we used a Bodipy 558/568-tagged prazosin probe (Invitrogen/Molecular Probes Inc., Carlsbad, California), prazosin being a common agent used to treat high blood pressure, making sure that the binding was specific via preincubation of sections with cold prazosin prior to labeling with fluorescent probe. BAR typing was by tagging with the generic tag CGP12177 as previously described [9]. The muscle protein actin and cell nuclei were also stained with DAPI (Molecular Probes/Invitrogen) and images were acquired.

2.2 Image acquisition

Samples were acquired with an Applied Precision DeltaVision scanning fluorescence microscope (Issaquah, Washington) fitted with an Olympus IX70 microscope (Melville, New York) and deconvolution capabilities. Images were acquired at a thickness of 0.25 µm in a complete pass from the bottom to the top of the tissue, and then they were deconvoluted (5 iterations), stacked, and volume-rendered with Imaris software (Bitplane AG, Zurich, Switzerland).
Deconvolution is a software-based process for ‘refocusing’ an out of focus image and is applied after image acquisition using near-neighbor algorithms to extract information out of blurred regions of an image to clean up these regions. Three distinct areas of fluorescence were counted for adrenoreceptor densities (pixel numbers) and areas of interest were captured as red-green-blue (RGB) files (90×90 µm). The number of pixels in a 60×60-µm area indicated mean receptor density. Image fields, with minimal or no fibrosis, were selected so that the most number of muscle cells were included. Receptor numbers were measured by both a Corel (Corel Corp., Ottawa, Canada) and a SigmaScan program (SPSS Inc., Plover, Wisconsin) to minimize errors.

3. Results

Figure 1
Adrenoreceptors, shown here in red, should be found throughout the heart muscle as is seen in the left panel. What is surprising, is that this tissue was taken from a patient that underwent ventricular unloading with an LVAD. The tissue on the right is from the pre-LVAD sore sample and shows few adrenoreceptors, probably indicating a poor cardiac function. Also in the left panel can be seen striations and intercalated discs, both of which are necessary for regular contraction to occur and are not as apparent in the heart failure tissue on the right.

Figure 2
Although these distribution findings were evidence for a return to normalcy of unloaded heart tissue, this work did, to a large extent, add data and observations to previous reports. However, using deconvolution microscopy we were able to show that the receptors were not only reduced in number, by migrated to the blood vessels and became associated with the nuclei of cells away from the muscle. The picture on the left shows the clumping of cells along the areas between the muscle, associated with small blood vessels, while the right panel is a model that revealed the receptors to be closely associated with the nucleus (blue) of these cells.
4. Discussion

Heart failure is still a major drain on the health care system and in 2010 the Centers for Disease Control estimate that the cost will be 39.2 billion dollars. That’s billions of dollars! [17, 18]. It is therefore beneficial to both patients and physicians, to have treatments that shorten the hospital stay, eliminate most of the bed rest, and allow for a productive return of individuals to the workforce. This fluorescence microscopy study adds further insights into the abilities of the heart to recover and directs us to targets for therapeutic interventions that will greatly enhance ventricular unloading with the inevitable introduction of newer, smaller devices. Restoring a full quota of receptors while the heart is recovering will result in a better outcome, and now that we know the receptors relocalize and down-regulate, probably in a protective manner, we can focus and define further research.

References

[3] University of Texas Medical School at Houston, Department of Pathology and Laboratory Medicine (http://www.uth.tmc.edu/pathology/research/corelab/heart.html. Accessed April 1st, 2010


