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Multiscale Imaging of the Human Heart: Building the Foundation for Human Systems Physiology and Translational Medicine

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Abstract

The development of human cardiovascular systems physiology is inhibited by the lack of multiscale functional physiological data, which represents human heart physiology at the molecular, cellular, tissue, organ, and system levels. We have developed an experimental approach to study explanted human hearts in vitro at multiple physiological scales with a wide array of imaging modalities. This approach has already yielded data indicating significant differences between animal models of diseases and actual human heart disease. Our data provides a quantitative foundation for multiscale physiological models of the cardiovascular system and will allow improvement in translation of medical technology and pharmacology from animal models to therapy.

I. Introduction

Heart failure (HF) is a leading cause of mortality and morbidity afflicting tens of millions world-wide. HF management is primarily limited to transplantation and device approaches, due to unsatisfactory efficacy and/or safety of pharmacological therapy. The dominant translational research paradigm used today is based on a long held postulate by Rudolph Virchow that animal models provide direct inferences into the molecular and cellular mechanisms of disease and thus could help in identifying potential therapeutic targets [1]. However, it is becoming increasingly evident that this strategy enjoys only limited success when applied to HF and arrhythmia. Attempts to construct multiscale computer models of human systems physiology have also been hampered by limited human physiology data. We propose to modify Virchow's classical three-step paradigm by adding a new step #3:

1. Identifying clinical determinants of the disease at the bedside.
2. Reproducing the symptoms of the disease in a cell line and/or an animal model and identifying a potential therapy in these models.
3. Testing the functional safety and dose-response of the identified therapy in vitro in viable explanted human organs and tissues donated for research by patients and donors.
4. Evaluating safety and efficacy of the therapy in clinical trials.

Significant genetic, molecular, cellular, anatomical, and systemic differences among species are responsible for the failure of translation from cell lines and animal models to humans. Cardiac rhythm disorders are striking examples of such failures to translate basic science to clinical practice. Despite deep knowledge of the biophysical properties of numerous ion channels, pumps, and exchangers gained over half a century of research conducted at huge

expense, few successful pharmacological therapies are in use to treat arrhythmias. The main reason for this failure is a profound lack of knowledge of the human cardiac physiology at the molecular, cellular, and tissue levels. It is paradoxical, but we know much more about ion channels and action potentials in the mouse, rat, guinea pig, rabbit, and canine as compared to our own species - *Homo sapiens*.

Limited progress in the development of cardiovascular pharmacological therapies indicates that the currently accepted translational paradigm needs improvement. Vulnerability of this translational paradigm is well illustrated by the recent disclosure of cardiovascular side effects of two widely prescribed and highly effective pharmaceuticals Vioxx [2] and Rosiglitazone [3]. It is now clear that cardiovascular safety deserves more attention at the early stages of the development of drugs targeting outside of the cardiovascular system. Preclinical studies assess biochemical and physiological effects in biochemical assays, cell lines, animal and computer models, but do not evaluate them in vitro in the live adult human heart cells and tissues. Human tissue preparations could provide significantly more relevant assessment of safety and efficacy with respect to possible activation of key signaling pathways in the human cardiovascular cells and tissues. Our modified model of translation offers such an opportunity and provides the tremendous advantage of expediting or terminating preclinical studies based on results from step #3: ***“Testing the safety and dose-response of the identified therapy in vitro in explanted functional human organs and tissues donated for research by patients and donors”***.

Addressing this problem, we have developed, refined and extended experimental imaging methodology, which is currently applied only to animal cardiac preparations in basic physiology laboratories, to deepen our understanding of cardiac physiology of *Homo sapiens*. This approach will modify and enhance the currently dominant translational paradigm and provide new important directions of research, which will stimulate and reinvigorate a biomedical research community that has focused significant effort and expenses on investigating animal physiology and has thus hampered effective translation of needed therapies for HF and sudden cardiac death.

II. LOGISTICS

In 2008, 2163 patients received heart transplant in the USA [4]. These 2163 explanted failing hearts are often donated for research, but very few of them are actually used for physiological studies. Most human heart research is limited to molecular investigation of frozen or fixed tissue biopsies and lacks physiological correlates at the cell, tissue, and organ levels. In addition, nearly 6,000 hearts are donated for transplantation and/or research but not used due to logistical hurdles. Thus, nationwide, a precious gift of nearly 8,000 human hearts could be utilized for studies of arrhythmia and heart failure in *Homo sapiens*, instead of focusing entirely on the animal models of these disorders.

We have developed the logistics and technology for successful harvesting and functional electromechanical studies of explanted human hearts. In our studies so far, we have obtained 77 hearts from the Washington University School of Medicine and MidAmerica Transplant Services, respectively. Figure 1 shows clinical characteristics of the hearts received thus far. The Institutional Review Board of Washington University School of Medicine has approved the protocol of the study. In close collaboration between several teams of clinical and basic investigators, we have investigated 50 explanted failing human hearts obtained during transplantation and 27 non-failing donor hearts, rejected for transplantation for various reasons, including age, early stage of heart failure, atrial fibrillation, and coronary disease. Donor hearts are being provided by the Mid-America Transplantation Services, which is located within a 10–15 minute drive from our research laboratories. By establishing rigorous

logistics for cardioplegic preservation of the heart and rapid delivery to the laboratory, we have succeeded in reanimation and electrophysiology investigation in each heart donated to us, as well as conducting molecular characterization of these same precise tissue regions or freshly fixed and/or frozen tissues, immediately adjacent to the functionally characterized tissue. During this experience we have developed efficient logistics for (see also Figure 2):

1. At least 3–5 hours advance notification of the basic research team on expected acquisition time of the heart by both the cardiac transplantation team and Mid-America Transplantation Services; this notification allows us to gather the team on call and properly prepare for the functional and molecular experiments;
2. Harvesting and cardioplegic arrest of explanted hearts in the operating room within minutes of crossclamping and removing the heart from the chest of patient or donor;
3. Delivery of each cardioplegically arrested heart to the basic electrophysiology laboratory within 15–20 minutes from either of the clinical operating rooms at the Barnes-Jewish Hospital of Washington University Medical School or Mid-America Transplant services;
4. Harvesting and processing tissue samples for subsequent molecular biology investigation within 30–45 minutes upon harvesting the hearts using various protocols appropriate for high throughput RNA or protein investigation;
5. Dissection and coronary perfusion of various tissue samples (sinus node, AV node, right and left atria, right and left ventricles, wedge preparations, specific tissue for cell isolation, etc), while keeping remaining tissue in cardioplegic arrest until the next sample is used.

These hearts are routinely utilized for electrophysiology and molecular biology studies including studies of the human sino-atrial node, atrio-ventricular node, atria and ventricles [5–11]. We have successfully applied numerous basic science methodologies, which were previously used on animal hearts only, to the human heart, including optical mapping with voltage- and calcium-sensitive dyes and optical coherence tomography. In addition, we used molecular biology methods to characterize gene and protein expression in different anatomical structures and their relation to function, as characterized by imaging. These methods have included: quantitative RT-PCR analysis, immunoblotting, immunohistochemistry, electron microscopy, and other methods.

III. Optical Imaging OF Cardiac Excitation

In order to investigate remodeling of excitation-contraction coupling in heart failure, we have conducted fluorescence imaging using a state-of-art optical imaging system for simultaneous mapping of transmembrane potential and/or intracellular $[Ca^{2+}]$; transients in coronary-perfused preparations from failing and non-failing human hearts, which included: 1) Sinus node (SN), the model is based on the well established and validated canine SN model [12]; 2) Atrio-ventricular junction (AVJ) [5,13]; 3) human right or left atria and ventricles; 4) transmural left ventricular wedge preparation [8]. All these preparations have already been established and studied in 68 hearts in our research laboratory.

In short, human preparations were isolated from the heart, and appropriate branches of the coronary artery(ies) were cannulated to establish perfusion in the entire area of interest. Other tissue outside of the area of interest was trimmed and any large dissected branches of the coronary arteries were sutured. Then, the preparation was optically mapped from the epicardium, endocardium, or cut transmural surface using 1–2 CMOS cameras. Fields of view were adjusted as needed from 5×5 to $30 \times 30 \text{ mm}^2$. We used a dual CMOS camera based

optical system (Ultima-L, SciMedia Ltd., CA) with high spatial (100×100 pixels) and temporal (up to 10,000 frames/sec) resolution. To map the human SAN and AVJ we used the infrared voltage sensitive dye di-ANBDQBS [14], which allows for the visualization of cardiac excitation to a depth of 2–3 mm [7]. We also have developed an optical technique for simultaneous mapping of transmembrane potential V_m and intracellular $[Ca^{2+}]_i$ using fluorescent V_m -sensitive and $[Ca^{2+}]_i$ -sensitive dyes. Isolated coronary-perfused human preparations were double-stained with the dyes RH-237 (V_m) and Rhod-2 dyes ($[Ca^{2+}]_i$). These dyes have overlapping excitation spectra (530/40 nm), allowing simultaneous excitation at the same wavelength range, and separate emission spectra (580/40 nm for Rhod-2 and >700 nm for RH-237) allowing for separation of the fluorescence emission into two components, corresponding to V_m and $[Ca^{2+}]_i$. The emitted fluorescence was collected by the same objective and then passed through the dichroic mirror (615 nm) in the custom-built holder splitting the fluorescent light into 2 beams. These beams were passed through emission filters and directed toward the CMOS cameras (Figure 4,-c).

To immobilize the preparation and prevent motion artifacts in optical recordings, we used the excitation-contraction uncoupler Blebbistatin (10 μ M) [8]. We have shown in our previous studies in animal models (canine, rabbit, rat, mouse, etc) and in the human heart preparation that Blebbistatin does not significantly change AP morphology [8]. The acquired fluorescent signal was digitized at 1,000–2,000 frames/second, amplified, and visualized using SciMedia software. A custom-written Matlab computer program was used to analyze the optical signals in the SAN, AVJ, atria, and ventricles as previously described in detail [8]. Our optical mapping studies also were supplemented with microelectrode recordings and electrograms from the coronary-perfused human preparations. Membrane potential was measured with 3 M KCl-filled glass microelectrodes of 15–25 M Ω resistance. The signals were acquired by a computer at 5 kHz sampling rate.

In addition to the functional electrophysiology experiments described above, we have also simultaneously developed protocols for the analysis of protein and mRNA. Freshly isolated tissues from the same hearts that are functionally characterized are immediately preserved for such molecular analyses as immunoblotting, immunohistochemistry, and quantitative RT-PCR. Figure 4d shows an example in which we have used protein analysis to complement the results of an optical mapping experiment characterizing the transmembrane potential and intracellular calcium concentration. Western blot analysis of SERCA2A shows a difference in expression across the LV transmural wall between non-failing and failing hearts. We also have designed high-throughput screening techniques to probe gene expression differences across gender and disease state using a Taqman custom-designed gene array with 96 target assays. Preliminary results have shown no significant gender differences at the level of ion channels and calcium handling proteins between non-failing male and female hearts and significant remodeling of specific calcium and potassium channels in ischemic cardiomyopathy.

IV. Conclusion

We have developed logistics and experimental methodologies to acquire, preserve, and study live human hearts from patients with heart failure and donors, whose hearts were not accepted for transplantation. In our studies, we have characterized molecular, cellular, tissue, and organ level physiological remodeling of excitation-contraction coupling, including (1) identification of 87 genes, which are differentially expressed in failing versus non-failing hearts [10]; (2) transmural gradient in repolarization and its remodeling during heart failure [8]; (3) transmural gradient in calcium handling and its remodeling in heart failure [14].

Our experimental data provides for the first time functional and molecular characterization of excitation-contraction coupling in the failing and non-failing human heart. It provides a quantitative framework for future development of accurate mathematical models of the human heart, based on human data, rather than extrapolating from animal models. It also provides a basis for comparison of many animal models to actual human disease and extends our ability to understand cardiovascular physiology of *Homo sapiens* at the systems level.

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Figure 1.
Clinical characteristics of the human hearts (n=77) acquired and studied in this project.

Human Heart Physiology Study Flowchart

Figure 2.

Flowchart of tissue harvesting, dissection, processing, and functional studies at different scales: molecular, cellular, tissue and organ. Resulting data will be collecting in overarching bioinformatics database, which will be available to all participants.

Figure 3.

Optical mapping of activation and repolarization in the transmural section of a non-failing human heart. Evidence of transmural gradient of repolarization. Optical mapping was conducted in a wedge preparation dissected from the left ventricular free wall of a nondiseased human heart, rejected for transplantation. Action potential duration was measured at slow heart rate of 30 beats per minute in order to expose presence of M-cells. Map of action potential duration (APD) shows a distinct subendocardial population of cells with APD reaching 560 ms.

Figure 4.

Optical imaging of transmembrane potential and intracellular calcium concentration from the transmural section of the human heart with ischemic cardiomyopathy. A: Representative example of an action potential (blue) and calcium transient (red) recorded from one location out of 10,000 at the dissected transmural section of the human heart. B: Reconstructed maps of activation of action potentials (AP) and calcium transients (CaT), and maps of action potential duration and calcium transient duration measured at 80 relaxation of the amplitude. C: Representative example of measurements of relaxation rate of calcium transient, which illustrates a difference in calcium reuptake between endocardium and epicardium of the human heart. D: Western blots showing a difference in expression of SERCA2A across the wall and between non-failing and failing hearts [15].