Inducing Valvular Regurgitation in Mice via Thermal Ablation of Cardiac Valves

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Abstract— **This study presents early data in the development of a novel mouse model of heart failure utilizing thermal ablation on cardiac valves to induce valvular regurgitation. Thermal ablation of the valve was achieved through the application of radiofrequency (RF) electrical current. The objective was to apply enough energy to induce valve stiffening and retraction, which was hypothesized to produce valve insufficiency and blood regurgitation in vivo. Preliminary studies were performed to develop a workable energy delivery catheter that could be inserted through the carotid artery to the aortic valve. Catheter position between the aortic valve leaflets was verified by echocardiography. Valve function was evaluated before and after the thermal insult using Doppler measurements near the valve inflow and outflow, and early results demonstrate that the energy delivery catheter could successfully induce acute valve insufficiency. Further study is needed to refine the catheter to provide greater control over the degree of thermal damage and resulting changes in cardiac physiology.**

Keywords— **heart failure, regurgitation, thermal ablation, rodent model, radiofrequency electrical current**

I. INTRODUCTION

Heart failure is the leading cause of hospital admissions in patients over 65 years old. It affects over 5.8 million Americans and over 500,000 new diagnoses are made each year. Readmission rates for such patients exceed 50% in six months, with estimated annual costs exceeding $34 billion in the United States alone [1]. With an aging population and an increasing prevalence of obesity, diabetes, and hypertension, these numbers are likely to increase [2].

Heart failure is characterized by the progressive weakening of muscle in the heart wall. As the condition advances, the heart becomes too weak to eject all the blood from the ventricles, which leads to a decrease in cardiac output and an increase in ventricular filling pressure. Over time, the declining cardiac output will fail to meet the body's metabolic needs for oxygen and the increased pressure will cause fluid to buildup in the lungs and periphery, resulting in dyspnea and edema, respectively [3].

Treatments for heart failure range from medical therapies such as angiotensin converting enzyme inhibitors to device-based therapies utilizing defibrillators and mechanical circulatory support systems [4-5]. However, these options merely provide symptom relief. Cardiac transplantation is the only potentially curative option, but it is available to very few patients due to donor organ availability and cost constraints [6]. Despite all available options, the prognosis of heart failure is still bleak. Hospitalization is often required and quality of life is low, especially for elderly patients.

There are many clinical conditions and risk factors that can lead to heart failure (e.g., coronary and hypertensive heart disease, congestive heart failure, cardiomyopathy, obesity, etc.), but many are characterized by structural or functional changes in the cardiac valves, especially the mitral and tricuspid valves. Small morphological changes in these valves may prevent them from properly opening and/or closing. If the valves fail to close completely, blood will regurgitate; that is, flow backwards through the valve. Regurgitation can cause volume overload in either the atria or ventricles, which ultimately leads to reduced systolic output, ventricular dilation, and eventually heart failure.

Despite the critical clinical significance of heart failure caused by valve regurgitation, there currently is no suitable rodent model that mimics the clinical presentation of mitral or tricuspid regurgitation [3]. There are larger animal models that can produce some characteristics of regurgitation by damaging the aortic valve or bridging the abdominal aorta with the inferior vena cava, but they are limited [7-9]. Overall, the shorter lifespans, genetic versatility, and decreased expenses associated with rodents make them a much more efficient modality to study heart failure, especially when considering the possible age-dependent prevalence of this disease [10].

Our goal in this study was to demonstrate that applied radiofrequency (RF) electrical current could cause sufficient morphological changes in the cardiac valves of mice such that valvular regurgitation was produced in vivo. In addition, we sought to prove the feasibility of this technique being used as a minimally invasive procedure to create a future mouse model of heart failure.

II. METHODS

All animal care and experimental procedures were performed in accordance with the "Guide for Care and Use of Laboratory Animals" and with approval from the Animal Care and Use Committee.

A prototype energy delivery catheter suited to the mouse anatomy was constructed. Due to its superior flexibility and already proven ability to reach the mouse heart through insertion via the vascular network, a 1.2F pressure catheter was modified to be compatible with the Aaron BOVIE A1250 electrosurgical generator, utilizing a mono-polar handle (Figure 1).

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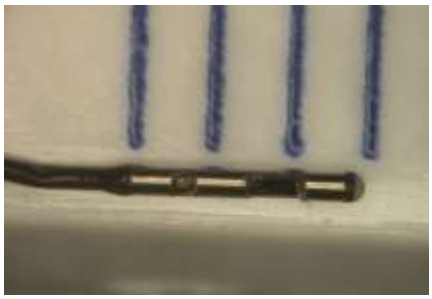

Figure 1: Magnified image of the prototype catheter tip: 3.0 mm length by 0.39 mm (1.2F) width, with a single electrode.

A. *Ex Vivo Testing*

In a preliminary study, RF electrical current was applied to ex vivo bovine liver tissue. Current from the electrosurgical source was applied at various output levels from the electrosurgical unit through the catheter, and delivered onto the surface of the tissue as it rested on the dispersive electrode. This procedure was used to determine the approximate power settings necessary to deliver a thermal insult to the liver tissue directly in contact with the catheter tip, but minimize thermal effects to the surrounding tissue.

B. *Open chest procedure*

After determining a range of plausible energy delivery parameters, one female mouse was anesthetized with isoflurane. The back and chest were shaved and treated with chemical hair remover to minimize ultrasound attenuation, as well as to provide better contact between the animal's back and the dispersive electrode. The heart was exposed through an anterolateral thoracotomy and a sternectomy. A small incision was then made in the right ventricle to permit the introduction of the RF catheter. The catheter was inserted near the pulmonary semilunar valve by visual inspection. Echocardiography was then used to fine tune the placement of the catheter and position it against the valve leaflets. The valve was ablated using coagulation mode at level 2 (~2 W) for approximately one second. The Visualsonics Vevo 770 high frequency ultrasound system was then used to measure the velocity on either side of the valve. Since no changes were seen in the Doppler signal, an additional one-second ablation was applied using the coagulation level 3 (~3 W) setting.

C. *Intravascular procedure*

After the developmental open-chest study, we aimed to evaluate the catheter using an intravascular approach. Four male mice were anesthetized with isoflurane, and their hair was removed as described previously. The right carotid artery was exposed by blunt dissection, and the catheter was inserted until it was as close to the aortic semilunar valve as possible with visual inspection. Echocardiography was then used to fine tune the placement of the catheter and position it against the valve leaflets. The valve was ablated using various coagulation levels and velocity changes on either side of the valve were recorded with Doppler. The hearts were then excised and stored in formalin.

III. RESULTS

A. *Ex Vivo Testing*

Thermal damage was visually assessed in the bovine liver tissue by identifying areas of blanching, which corresponds with histological evidence of thermal damage and morphological shrinking in collagenous structures [11-12] (Figure 2). Given enough time, these changes were observed in the tissue at the lowest power levels of the electrosurgical unit. However, there were notable differences seen in the resulting ablation when the time, power, as well as mode type were changed. As expected, increasing the power level and the ablation time both resulted in larger ablation zone (a larger area that was effectively blanched), which expanded both in the axial and radial directions in relation to the catheter tip. For a fixed ablation time and power level, the coagulation mode produced a much larger ablation zone when compared to the cut mode, which seemed to sink into and separate the tissue more than induce a color and stiffness change. Since we were interested in inducing morphological changes within the valves and not cutting off individual valve leaflets, the coagulation mode was used for the remaining experiments.

Using the coagulation mode, power levels 1 and 2 (~1-2 W) resulted in minimal ablation and there was only a slight radial expansion of the ablation zone with prolonged application. On the other hand, a prolonged application (approximately 2 seconds) at level 4 (~4 W) resulted in irreparable damage to the catheter tip.

Figure 2: Bovine liver tissue ablation using prototype catheter for 3 seconds at coagulation power level 3 (~3 W). The tissue is white and shrunken in comparison to the surrounding tissue, indicative of thermal insult on collagenous structures.

B. *Open chest procedure*

There was no change in the Doppler velocity after the first ablation. Immediately after the second ablation was stopped, there was a slight increase in the velocity of backward flow. The amount of regurgitation continued to progress without any additional applied stimuli, until eventually the velocity of the backwards flow exceeded that of forward flow (Figure 3). These findings verified the feasibility of using an RF ablation catheter to induce thermal damage to, and regurgitation in, the pulmonary semilunar valve.

C. *Intravenous procedure*

The intravenous approach through the carotid artery to the aortic semilunar valve under the guidance of ultrasound was successful in all four animals (Figure 4). Due to constraints in

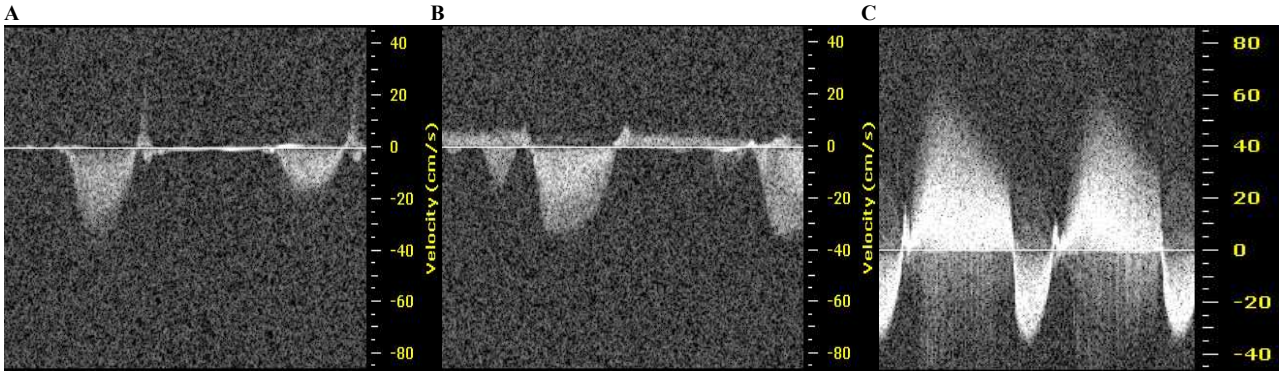

Figure 3: Doppler ultrasound profiles of the flow through the pulmonary valve. Velocity in the downwards direction is forward flow (towards the lungs) and velocity in the upward direction is regurgitation (flow back into the right ventricle): **A)** before ablation, **B)** 5 minutes after the application of a one second ablation, **C)** 30 minutes after the applied ablation.

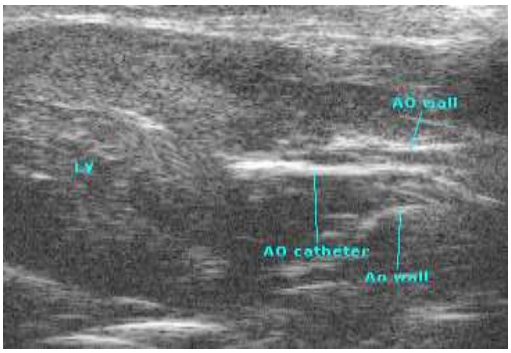

Figure 4: 2-dimensional echocardiogram showing catheter placement.

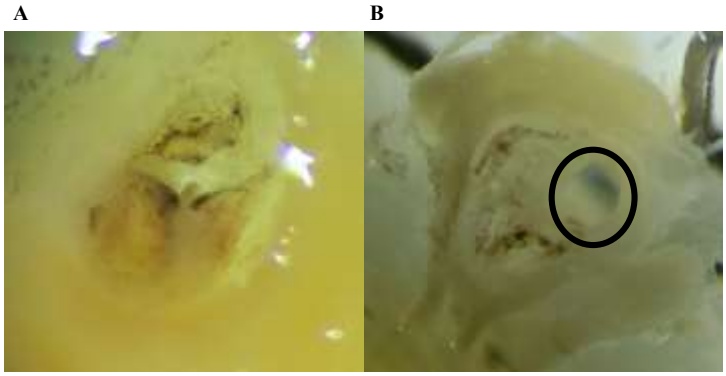

Figure 5: Stereoscopic images: **A)** control aortic valve, **B)** ablated valve.

spatial resolution and slight anatomical differences between the mice, it was difficult to determine the exact positioning of the catheter on the leaflets, but we attempted to place the catheter tip near the center of the valve.

Several subsequent ablations with increasing power levels were applied to the valve, but minimal (if any) changes were seen in blood flow velocity. Once the chest cavities were open, it was discovered that two of the mice had developed small holes in the walls of the aorta. Despite the minimal changes seen in blood flow velocity, stereoscopic analysis of the preserved hearts showed that the thermal insult was successfully applied to the valve, which produced a hole in one of the valve leaflets (Figure 5).

IV. Discussion

The ex vivo testing showed differences in the ablation patterns produced by the coagulation and cut modes when the ablation level and the ablation time were kept constant. This is most likely due to differences in the voltage and current waveform being applied during each mode; the coagulation mode provides more time for thermal diffusion from each voltage pulse, while the cutting mode is designed to create intracellular steam with minimal thermal spread into the surrounding tissue [13]. Since the desired outcome of this study was to induce shrinking and stiffening changes in the cardiac valve, the coagulation mode selected for subsequent studies. The ex-vivo testing also showed an operational power range—low power levels produced very little evidence of thermal damage, while excessively high power levels led to catheter damage. Due to the presence of flowing blood in the remaining studies, which rapidly dissipates heat, the risk of catheter damage was assumed to be very low compared to ex vivo experiments.

In vivo testing demonstrated that RF electrical current is capable of inducing enough morphological change in a heart valve to cause regurgitation, and that the amount of damage may be controlled by regulating the applied power level or time of exposure. It also demonstrated that the resulting damage is location dependent; Doppler showed significant velocity changes when the ablation was performed on the pulmonary valve, while little change was seen in the aortic valve despite the production of large holes.

One major limitation of this method is the inability to see changes in valve structure by ultrasound imaging. For very small ablation applications, it is possible that the catheter itself obstructs the Doppler from showing any signs of regurgitation. Without moving the catheter, it is difficult to ensure that the applied thermal insult resulted in significant changes. Refinement in the catheter delivery technique and imaging protocol may yield improved results in future studies. In addition, histological analysis may provide greater insight into cellular changes occurring from the thermal insult. It may also help determine the optimal power settings necessary to produce leaflet damage, while ensuring the surrounding vascular wall is left undamaged. Longer-term follow-up is

also necessary to fully evaluate the efficacy of the RF ablation approach. It is possible that low power settings may not cause enough damage to show immediate regurgitation on Doppler but, as noted even in the open chest study, regurgitation would develop over time.

V. Conclusion

We demonstrated that radiofrequency electrical current could be successfully applied to mouse heart valves in vivo, using both open surgical and intravascular approaches. Thermal damage led to measurable morphological changes and, in some cases, acute regurgitation. We also demonstrated that by changing the ablation time and power level, we could regulate the amount of thermal damage that is produced. Additional study appears warranted to optimize the catheter design, treatment protocol, and determine the longer-term effects of such thermal damage, with the eventual goal of a controlled mouse model of heart failure via valve sufficiency.

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