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Development of an Ovine Model of Pediatric Complete Heart Block

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Abstract

Background—Complete heart block is a significant clinical problem that can limit the quality of life in affected children. To understand the pathophysiology of this condition and provide for development of novel therapies, we sought to establish a large animal model of permanent, pacemaker-dependent atrioventricular block (AVB) that mimics the size and growth characteristics of pediatric patients.

Materials and Methods—We utilized 9 immature lambs weighing 10.5 ± 1.4 kg. After implantation of dual-chamber pacemaker devices with fixed leads, AVB was produced by interrupting His-bundle conduction using radio-frequency ablation at the base of the non-coronary cusp of the aortic valve. Ablations (30 to 60 seconds in duration) were performed under fluoroscopic guidance with electrophysiological monitoring. Interrogation of pacemakers and electrocardiography (ECG) determined the persistence of heart block. Ovine hearts were also examined immunohistochemically for localization of conduction tissue.

Results—AVB was produced in 8 animals using an atypical approach from the left side of the heart. One animal died due to ventricular fibrillation during ablation proximal to the tricuspid annulus and one lamb was sacrificed post-operatively due to stroke. 4 sheep were kept for long-

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term follow-up (109.8 ± 32.9 days) and required continuous ventricular pacing attributable to lasting AVB, despite significant increases in body weight and size.

Conclusions—We have created a large animal model of pediatric complete heart block that is stable and technically practicable. We anticipate that this lamb model will allow for advancement of cell-based and other innovative treatments to repair complete heart block in children.

Keywords

catheter ablation; atrioventricular block; pacemaker implantation; large animal model

INTRODUCTION

Complete atrioventricular conduction block (AVB) can result from iatrogenic, congenital, infectious, ischemic, idiopathic, degenerative, or pharmaceutical-based causes. Regardless of the underlying reason for this potentially life-threatening condition, the conventional medical therapy necessitates placement of a pacemaker generator connected to the heart with pacing leads. While this palliative treatment is frequently successful, it can result in complications such as progressive ventricular dysfunction and dyssynchrony, infection, and the need for numerous surgical or catheter-based interventions to replace pacemaker system components[1,2]. These and other complications are often exacerbated in pediatric patients, as continuous cardiac pacing of small, growing children imposes additional clinical obstacles.

To allow for study of AVB in developing mammals that have a comparable cardiovascular anatomy and size to human infants as well as engaging in translational research aimed at devising alternative treatment strategies to the current standard, we have created a large animal model of heart block. To maintain the clinical-relevance of our experimental system, we have closely replicated procedures commonly employed in humans. These procedures include endocardial radiofrequency ablation as well as fluoroscopically-guided pacemaker generator and fixed lead implantation.

Although a canine model of atrioventricular (AV) node ablation was established in 1981, [3,4] dog models have become less commonly employed in contemporary cardiovascular research. Porcine[5] and ovine[6] models of AV block have also been described; however, a large mammalian model of pediatric complete heart block has yet to be established. Consequently, we have chosen sheep as our model organism as this species demonstrates a rapid growth potential and is amenable to repeated surgical procedures. The growth of the lamb heart and great vessels are comparable to humans[7] and this species is well-established for testing of tissue engineered structures such as heart valves.

MATERIALS AND METHODS

Animals

All procedures were performed according to the “Guide for the Care and Use of Laboratory Animals” published by National Institutes of Health (publication No. 85-23) and approved by the Institutional Animal Care Committee at Children's Hospital Boston. A total of 9 female, just-weaned lambs (Pine Acres Farm) with a body weight of 10.5 ± 1.4 kg were used for experiments. Anesthesia was induced with an intramuscular injection of Ketamine (20 mg/kg), Xylazine (0.1 mg/kg), and Atropine (0.04 mg/kg) followed by endotracheal intubation. Anesthesia was maintained with isoflurane mixed with 100% oxygen (1–3% via inhalation). Surgical sites were prepared using standard sterile procedures and intravenous (IV) access was obtained percutaneously using the saphenous or cephalic vein. Arterial

oxygen (O₂) saturation, end-tidal carbon dioxide output (ETCO₂), surface electrocardiogram (ECG), non-invasive blood pressure levels, and core temperature was monitored throughout these procedures. Cefazolin (25 mg/kg IV) was used as an antibiotic prophylaxis perioperatively. Following the procedures described below, animals were extubated, stabilized, and IV catheters were removed. Fentanyl patches (1–4 µg/kg) were placed on each lamb for 3 days as an analgesic.

Pacemaker Implantation

Each lamb was placed in a supine position with limbs gently secured to the procedure table. The right internal jugular vein was isolated and cannulated with an 11 French sheath. Atrial (45 cm) and ventricular (52 cm) CapSureFix Novus steroid-eluting, screw-in leads (Medtronic) were positioned under fluoroscopic guidance using a percutaneous introducer and appropriate stylets (Medtronic). The lead positions were further refined by P or R wave amplitude readings using a programmable ECG analyzer (Model 9790c, Medtronic). Leads were then secured and connected to an EnPulse dual chamber pacemaker generator (Medtronic), which was set to VVI mode at a rate of 80 beats per minute (BPM). The device was then secured in a sub-muscular pocket in the neck of the lamb and the incision was closed in layers using standard surgical procedures.

AV node ablation

The right femoral artery was cut down, cannulated, and a 9 French sheath inserted. Under fluoroscopic guidance, the angiography catheter was advanced in the ascending aorta and Heparin (50 IU/kg IV) was administered. Anterior-posterior and lateral views of the aortic root were visualized using 10 mL of Omnipaque contrast agent (Winthrop Pharmaceuticals) to delineate cardiac anatomy and locate the non-coronary sinus. A Mariner RF ablation catheter (Medtronic) connected to a multi-channel ECG recorder (Bard) was introduced in the arterial sheath and advanced in the aortic root toward the lower portion of the non-coronary sinus. The His-bundle was located using ECG by demonstration of the presence of a His-signal at the tip of the catheter. RF-generated energy was delivered to the tissue for 30–60s so as to not to exceed a temperature of 70°C. After successful ablation and confirmation of complete AV-block, the pacemaker was re-programmed to VDD mode. The pacemaker was interrogated daily and persistence of AVB was confirmed by temporarily changing the settings to VVI mode and lowering the pacing rate.

Immunohistochemistry

At the study end-point for each animal, hearts were explanted and localization of the ablation lesion was confirmed visually. For subsequent histological analyses, heart tissue was fixed in 10% formalin, paraffin-embedded, sectioned, and histologically stained with Masson's trichrome or Hematoxylin & Eosin. The region adjacent to the His bundle between the non-coronary and right-coronary cusp of the aortic valve was identified and adjacent paraffin sections were immunofluorescently stained using either an anti-connexin43 (Cx43 [α1]) monoclonal antibody (Chemicon) or anti-neurofilament 160 (NF-160) monoclonal antibody (Millipore) essentially as previously described[1]. Simultaneously, these sections were stained with an anti-α-actinin-2 polyclonal antibody[1,8]. These antibodies were then detected with AlexaFluor®488-conjugated goat anti-mouse or AlexaFluor®568-conjugated goat anti-rabbit antibodies (Invitrogen) combined with 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Invitrogen). Slides were mounted and visualized as described earlier[1].

RESULTS

Pacemaker implantation

Transvenous dual chamber pacemaker implantation was successfully performed in 9 lambs. Figure 1A is a fluoroscopic image demonstrating the anatomical positions of atrial and ventricular leads. The pacemaker devices allowed for reliable, atrially-triggered ventricular pacing in a sequential manner. Sufficient perfusion and cardiac output was demonstrated by normal activity levels and weight gain throughout the observational period. Chest X-rays (Figure 1B) showed the pacemaker in the neck with an additional loop of pacemaker lead to prevent tension while the animal develops.

AV node ablation

AV node ablation was successfully performed in 8 lambs (Table 1). Figure 1A indicates the anatomy of the aortic root using contrast agent injected via a catheter. A right atrial approach was only used in the first animal, in which we were unable to reliably detect a His electrographic signal or even establish complete heart block, despite 10 ablation attempts. This animal died at the time of procedure, due to ventricular fibrillation. Following autopsy of this lamb, we decided to subsequently employ a left-sided (*i.e.* systemic) approach due to apparent proximity of conduction tissue to the membranous septum of the aortic non-coronary sinus. A regular surface ECG is obtained before the procedure indicating normal sinus rhythm (Figure 2A). Recordings from the ablation catheter on the left side of the heart demonstrated a readily-detectable His-deflection (Figure 2B and 2C) and RF ablation of AV conduction at a site near the non-coronary sinus in 8 out of 8 cases was successfully performed. Unfortunately, one animal died during recovery due to a stroke. A total number of 7 early survivors showed either consistent high-grade 2nd degree (Figure 2D) or complete AVB with no escape rhythm (Figure 2E). Two mortalities were caused by infection and/or malnutrition in animals that were of small size (6.7 and 8.7 kg). These lambs had an immature digestive tract and received a sub-optimal feeding regimen. Another animal died during a second surgical procedure not related to the AV node ablation described (*i.e.* a right-sided thoracotomy). Other than this one instance, any secondary procedures, surgical or otherwise, had no influence on the outcome of the animals or establishment of pediatric complete heart block. All surviving animals (N = 4) showed persistent AV conduction block requiring constant pacing in VDD mode during an overall observational period of 109.8 ± 32.9 days. For pacemaker interrogations, the rate in each animal was gradually lowered to 30 BPM (Figure 2F).

Pathology Results

The lesion that is depicted in Figure 1C is located between the non-coronary and right-coronary sinus of the aortic valve, extending to the membranous septum. RF ablation of tissue at the base of the non-coronary sinus established heart block. The heart was sectioned perpendicular to the septum and left ventricular outflow tract (LVOT) following an antero-lateral to postero-lateral direction. Figure 3A indicates the area of the atrioventricular node and the aortic wall on the right hand side of the slide. Figure 3B and 3C depict the His bundle at higher magnifications. These areas were positive for neurofilament 160 (NF-160) staining using a monoclonal antibody (Figure 3D) and negative for gap junction protein connexin 43 (Cx43 [$\alpha 1$]) monoclonal antibody (Figure 3E), which is located in the working myocardium[9].

DISCUSSION

In the present study, we established that a catheter-based, closed-chest AV node ablation procedure is both feasible and safe in young lambs. Consequently, we have produced a large

animal model of persistent atrioventricular block (AVB) that mimics the size and growth characteristics of pediatric patients. To perform these experiments, we approached the His bundle from the left side of the heart and applied RF current to the site that demonstrated the largest His-deflection. This procedure resulted in reliable AVB requiring pacemaker support. The anatomical position of AV conduction tissue near the NCC was verified by histological and immunohistochemical staining (Figure 2).

In an earlier study, Bru *et al.*[6] demonstrated the feasibility of AVN ablation in adult sheep involving the delivery of very high RF (27 MHz) current to the tricuspid annulus. The animals did not require permanent pacemaker support due to efficient escape rhythm, although complete heart block was demonstrated. For our purpose and to allow eventual translation of our earlier research findings into clinical practice[1] we wanted to establish an animal model of constant pacemaker-dependent complete AV block in growing lambs. This particular species was chosen because they grow rapidly within 2 months[10] and are amenable to multiple invasive procedures. The mean observational period of all animals included in our study was 68.9 days. During this time frame the animals demonstrated a mean growth of 9.5 ± 2.3 kg ($N = 4$). In addition, lambs have heart rates, cavitory pressures, and a cardiovascular anatomy that closely resembles that of children. Here, we show that these animals rapidly increase in size throughout the average study period despite AVB and pacemaker dependency. Using conventional ablation frequencies (500 kHz), we found consistent ablation at the tricuspid valve annulus was not possible.

On the other hand, left-sided (*i.e.* systemic) ablation procedures can cause arterio-arterial or ventriculo-arterial emboli[6]. For instance, one lamb died post-operatively due to a stroke. Thereafter, we administered IV Heparin during the procedure to minimize risk of a recurrent episode. Furthermore, vascular perforation or ventricular perforation was not observed and none of the animals showed pericardial effusion. For the novel ablation approach used here, it was not necessary to introduce the catheter in the left ventricle as the target site is located above the aortic valve annulus; thereby, minimizing side-effects such as ventricular tachycardia and/or fibrillation.

We anticipate that our animal model will be utilized for investigations directed at understanding the long-term effect of congenital cardiac conduction abnormalities and chronic pacing therapy in children. Specifically, alterations in cardiac function and remodeling of the heart chambers can be studied in a growing animal with a cardiac physiology similar to that found in humans. For example, as ventricular pacing increases the risk of atrial fibrillation and mortality in patients with chronic heart failure[11], the underlying mechanisms of these consequences could be explored. This model will also allow for further development of cell-based strategies for re-establishing AV electrical conduction[1,2,5,].

The systemic approach for ablation of the AVN in lambs is feasible and results in a high success rate for complete heart block after ablation. This technique has a low risk of ventricular arrhythmias or arterial emboli. The clinical implications of complete heart block and future therapeutic regimens will be available to be studied in a pediatric animal model.

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Figure 1.

A - Characterization of the aortic anatomy by fluoroscopy. The ascending aorta and the aortic root are indicated by injected contrast agent. The atrial and ventricular pacemaker leads are depicted. **B** – Chest X-ray. **C** - Gross anatomy of an explanted lamb heart. This view depicts the LVOT and aortic root. An ablation lesion is indicated by the arrow in the non-coronary cusp (NCC).

Figure 2.

A – A representative surface electrocardiogram (ECG) depicting a normal sinus rhythm under anesthesia. **B** - Bipolar electrogram from the ablation catheter distal pair showing a His-deflection (150 BPM). **C** - Bipolar electrogram from the ablation catheter proximal pair showing another His-deflection. **D** - A representative surface ECG of an animal with high-grade 2nd degree heart block AVB (ventricular rate of 45 BPM). **E** - A representative surface ECG depicting ventricular pacing (VVI/ 120 BPM) and recording during no pacing indicating atrial rate (90 BPM), no ventricular escape rhythm. **F** - A representative surface ECG acquired during pacemaker interrogation in the post-operative period showing AVB with ventricular pacing (VVI/30 BPM).

Figure 3.

Histological (Masson's trichrome as well as Hematoxylin and Eosin) staining of 5 μm thick tissue sections (Figures **A–C**). **A** - The atrial and ventricular septum is indicated and the aortic wall adjacent to the non-coronary sinus is located at the right hand side of the slide. The scale bar indicates 100 μm . The region of interest is marked with a box and depicted at higher magnification (Figures **B** and **C**). **D** – Immunohistochemical staining of tissue sections. NF-160 staining is indicated in green, nuclei are stained blue, and α -actinin-2 staining is shown in red. **E** – Cx43 ($\alpha 1$) staining is indicated in green, nuclei are stained blue, and α -actinin-2 staining is shown in red. The arrowheads indicate gap junctions, whereas the arrows point toward highly auto-fluorescent erythrocytes. The scale bar in 2B to 2E represents 50 μm .

Table 1

Results of Radio-frequency Ablation Experiments in Lambs

Animal	Ablation Site	Perioperative Complications	Postoperative Complications	Follow up (days)	Final cardiac rhythm	Cause of Death
1	Tricuspid Annulus	VF Death	--	--	--	--
2	Aortic Root	None	None	132	AV block III	SAC
3	Aortic Root	None	None	12	AV block III	Malnutrition
4	Aortic Root	None	None	16	AV block III	Infection
5	Aortic Root	None	None	15	AV block III	Surgical Procedure
6	Aortic Root	None	None	142	AV block III	SAC
7	Aortic Root	Stroke/Death	--	--	--	--
8	Aortic Root	None	None	44	AV block III	SAC
9	Aortic Root	None	None	121	AV block III	SAC