Published in final edited form as:

Artif Organs. 2009 November ; 33(11): 953–957. doi:10.1111/j.1525-1594.2009.00896.x.

Animal Model Development for the Penn State Pediatric Ventricular Assist Device

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Abstract

In March 2004, the National Heart, Lung, and Blood Institute (NHLBI) awarded five contracts to develop devices providing circulatory support for infants and small children with congenital and acquired cardiac disease. Since 2004, the team at Penn State College of Medicine has developed a pneumatically-actuated ventricular assist device (VAD) with mechanical tilting disk valves. To date, hemodynamic performance, thrombogenesis, and hemolysis have been chronically evaluated in 16 animals, including 4 pygmy goats and 12 sheep. Major complications, mainly respiratory failure, have been encountered and resolved by a multi-disciplinary team. Multi-modal analgesia, appropriate antibiotic therapy, and attentive animal care have contributed to successful outcomes. Time after implant has ranged from 0–40 days. Most recently, a sheep implanted with Version 3 Infant VAD was electively terminated at 35 days post-implant, with no major adverse events. This report describes a successful *in vivo* model for evaluating a pediatric VAD.

Keywords

experimental animal models; *Ovis aries*; pediatrics; ventricle-assist device

Introduction

Penn State has developed a pulsatile pneumatic sac-type Pediatric Ventricular Assist Device based on the design of the adult-sized Pierce-Donachy (Thoratec®) VAD. The Pediatric VAD exists in two sizes: an Infant VAD with a dynamic stroke volume of 12–14 ml, and a Child VAD with a 25–30 ml stroke volume. The primary design objective has been to produce a device with a low thrombogenic risk. We began animal testing early, which gave us time to resolve problems with the animal model, provided early feedback for pump and cannula design, allowed surgeons to gain experience and provide design feedback, and exposed the pump and controller to “near-real-world” hemodynamics.

We are currently using juvenile sheep (20–25 kg) as the animal model for the Infant VAD. Sheep have been used extensively for evaluation of artificial hearts (1,2), ventricular assist

devices (3-5), and acute and chronic studies of prosthetic valves (6-11). Factors influencing the choice of animal model for the Pediatric VAD program include thrombogenicity, hemodynamics, hemolysis, response to implanted components, study duration, and animal size, growth rate, anatomy, and availability (12). Our desire to more closely match the size and cardiac output of an infant/child, as well as the animal's docility, led us to choose the juvenile lamb. The use of a young lamb has not been without challenges, and our design and management of the *in vivo* study is described herein.

Materials and Methods

All animal studies are approved by the Institutional Animal Care and Use Committee at Penn State College of Medicine. The animals are housed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and veterinary care is adherent to The Guide for the Care and Use of Laboratory Animals (13).

Preoperative Procedures

Dorset-Finn cross-breed sheep, weighing 20–25 kg, male or female, are used in our protocol. Each animal is tested for Q-fever and vaccinated for contagious ecthyma and *Clostridium* (8-way) prior to shipment. During the acclimation period (one week), each sheep is given florfenicol every other day for a total of 3 doses as *Mycoplasma* prophylaxis. The animal is placed in a stanchion 2.5 wk prior to surgery and allowed to acclimate to this type of housing. A right external jugular catheter (14 ga × 20 cm polyurethane, Arrow International, Reading, PA) is placed 2 wk prior to surgery to allow for preoperative hematology and thromboelastography (TEG) studies, as well as fluid therapy. Due to concerns about blood volume depletion as a result of multiple collections, we use a venous arterial blood management protection system (VAMP™, Edwards Lifesciences LLC, Irvine, CA) that eliminates waste blood and allows for collection of exact quantities needed. During the week prior to surgery, echocardiography is used to assess the size and position of the heart.

Anesthesia and Surgical Preparation

On the day of surgery, the sheep is given intravenous cefazolin sodium (30 mg/kg) within one hour of anesthetic induction. Intravenous ketamine (7.5 mg/kg) and midazolam (0.4 mg/kg) are administered, and the sheep is moved to the operating room. Isoflurane in 100% oxygen is administered via nose cone, and suction is available to clear the laryngeal area of secretions. The sheep is placed in sternal recumbency and intubated using a 5 mm endotracheal tube (ETT). If necessary, the ETT is suctioned using a catheter <1/2 the diameter of the ETT and vacuum pressure no more than 100 mm Hg. Mechanical ventilation is initiated using the following parameters: (1) peak airway pressure of 25 cm H₂O, adjusted for tidal volume of 8–12 ml/kg, (2) peak end expiratory pressure (PEEP) 5–12 cmH₂O, (3) respiratory rate of 12–25 breaths per minute to maintain end tidal CO₂ 35–40 mm Hg, (4) inspired O₂ concentration >60% with O₂ saturation >90%, and (5) isoflurane concentration of 1.0–1.5%. Albuterol is nebulized via ETT to promote bronchodilation. Use of spirometry (Capnomac Ultima, Datex-Ohmeda, Inc.) allows for breath-by-breath monitoring of the pressure-volume loop, lung compliance, end tidal CO₂, peak end expiratory pressure, tidal volume, and peak inspiratory pressure.

An orogastric tube is placed to prevent rumen bloating and is connected to suction, set at intermittent pressure. Electrocardiographic, non-invasive blood pressure, oxygen saturation, and body temperature monitoring are initiated.

A triple-lumen, long-term intravenous catheter is placed in the left external jugular vein, and continuous rate infusions (CRI) of fentanyl (60–180 mcg/kg/h), lidocaine (1800 mcg/kg/h),

and lactated ringers solution are begun. Central venous pressure (CVP) monitoring is initiated using the left jugular catheter. The left thoracic wall is prepared and draped for surgery. An under-body forced air warming blanket (Bair Hugger®, Arizant Healthcare, Eden Prairie, MN) is placed under the animal to maintain normothermia during the surgery.

Operative Procedure

A lidocaine bolus (1 mg/kg) is administered intravenously, and fentanyl boluses (50–100 mcg) are given intravenously throughout the procedure in response to elevations in blood pressure and heart rate. One unit of donor blood, collected from an adult sheep maintained in our facilities, is ready for transfusion if needed.

A left lateral thoracotomy is performed through the fifth intercostal space. The left internal mammary artery is exposed and cannulated with an arterial monitoring line, and invasive blood pressure monitoring is performed for the remainder of the procedure. Arterial blood gas and hematocrit measurements are taken every 30–45 min. A second incision in the left upper quadrant of the abdominal wall is performed in order to create a pocket to contain the VAD. Two parallel subcostal tunnels that will contain the inlet and outlet cannulae of the VAD are created between the abdominal wall pocket and the mediastinum. The descending thoracic aorta is exposed beyond the left subclavian artery in order to accommodate placement of a side-biting partial occlusion clamp. Heparin (400 U/kg) is given intravenously, and a heparin CRI is initiated at 100 U/kg/h. Five minutes after the heparin bolus, an activated clotting time (ACT) is measured and is repeated every 15 minutes. Heparin is titrated to maintain the ACT >400 sec. The partial occlusion clamp is placed on the aorta, and the distal aorta is palpated to confirm the presence of a pulse. Complete occlusion of the descending thoracic aorta in early animals in this series resulted in renal and hepatic failure. A longitudinal aortotomy is performed, and the 6 mm diameter ringed PTFE outlet graft is sutured end-to-side to the aorta using a running 5–0 Prolene suture. A surgical sealant (Coseal, Baxter, Deerfield, IL) is applied to the anastomosis. The graft is filled with heparinized saline, capped, and secured at the proximal tubing with the tunneling obturator. The side-biting clamp is removed from the aorta, and the area is packed with sponges. The lung is allowed to re-inflate, and multiple deep breaths (not exceeding 30 cm H₂O) are given manually to recruit alveoli.

The pericardium is opened ventral to the left phrenic nerve and suspended with sutures. Four pairs of pledgeted 2–0 Ethibond sutures are placed in a mattress fashion around the apex of the LV. The heart is stabilized, and a ventriculotomy is made into the LV apex with a scalpel, as shown in Figure 1A. The inflow cannula with obturator is inserted into the LV, and sutures are passed through the sewing ring of the cannula and tied down. The obturator is then removed and the cannula is clamped and capped. Two heavy silk ties are used to secure the cannula inside its sheath. Coseal is applied to the ventriculotomy site and inflow cannula.

The inlet cannula is brought through the medial subcostal tunnel into the abdominal wall pocket, and the outlet cannula is brought through the lateral tunnel (see Figure 1B). A subcutaneous tunnel is then created from the pocket to a selected dorsal site, a stab incision made in the skin, and the pneumatic drive line pulled into the pocket in a dorsal-ventral direction. The pump, which has been pre-filled with heparinized saline, is brought into the surgical field and prepared. The drive line is connected and secured. The inlet cannula is de-aired, connected, and secured to the pump, followed by the outlet cannula (see Figure 1C). Epicardial echocardiography is performed to assess cannulation position in the LV. Pumping is initiated and monitored by the engineering team, and the heparin is discontinued.

Chest closure is commenced. Two round Blake drains are placed through the left pleural space and into the mediastinum, with one ending in the VAD pocket, and the other crossing into the right pleural space (if open). A diffusion catheter (MILA International, Erlanger, KY) for delivery of local anesthetics to the thoracotomy site is placed through a separate stab incision. The pump in the abdominal wall pocket is placed inside an ePTFE felt sack.

As chest closure progresses, the sheep is weaned off fentanyl. Buprenorphine (0.005 mg/kg IV) and flunixin meglumine (1.1 mg/kg IV) are given. Bupivacaine (0.25%, 5 ml) is instilled in the wound catheter. Heparin is *not* reversed. Multiple deep breaths are given manually to re-inflate collapsed alveoli. Isoflurane is discontinued, the orogastric tube is removed, and an Ambu-bag connected to oxygen is used to manually ventilate the sheep during transport to the animal care room.

Postoperative Care

The sheep is placed in a sling within the stanchion and is extubated once the swallowing reflex has returned. Nasal prongs are inserted and oxygen is administered at 2 L/min. Arterial blood gas measurements are taken 30 min after extubation, then in 2 h, then every 4 h during the next 24 h. As the animal becomes more alert, the sling is gently lowered and the sheep positioned in sternal recumbency. Analgesia (buprenorphine and bupivacaine q 8 h and flunixin meglumine q 12 h) is continued for 72 h, at which time the wound diffusion catheter is removed. Buprenorphine and flunixin meglumine are discontinued within the following 24 h, based on assessment of the animal's level of pain, including signs such as bruxism, inappetance, kicking at abdomen, lethargy, and other abnormal behavior. Intravenous fluid therapy is initially given at 2x maintenance (calculated as $(BW \text{ (kg)} \times 60)/24 \text{ h}$) and gradually decreased as the animal begins to drink. Rumen function is supported by oral administration of probiotic (Durvet, Blue Springs, MO) and free choice grass hay and grain. Antibiotic therapy (cefazolin sodium 30 mg/kg IV q 6 h or ciprofloxacin 10 mg/kg IV q 12 h) is continued for 10 days. All incision, catheter, and chest tube sites are cleaned and coated with antibiotic ointment on a daily basis. The jugular catheters placed pre- and peri-operatively are maintained for the length of the study and only changed if they are not patent. An animal care technician is present with the sheep around the clock for the duration of the study, and a veterinarian is on-call 24 h per day. Figure 2 shows a lamb on post-operative day 1.

Hematologic studies (complete blood count with differential, serum chemistries, PTT, fibrinogen, anti-Xa, and platelet aggregation) are performed twice weekly. Unfractionated heparin is administered IV to maintain a clotting time 2x normal, as measured by TEG, and a PTT of 1.5x normal.

Study Termination

On the 30th day of the study (or later as determined by the principal investigator), the sheep is anesthetized, intubated, and ventilated. The pump pocket is opened, and the pump is freed from the pouch. An incision is made along the skin overlying the driveline. Heparin (400 U/kg IV) is given, and after 5 min elapses, clamps are placed loosely around the inlet and outlet grafts. A needle is placed into the inlet cannula 2 cm from the inlet connector, and normal saline is infused under pressure as the inlet cannula is clamped to prevent an inflow of blood. After initiating the saline infusion, the outlet cannula is clamped and cut 2 cm from the outlet connector. The pump is allowed to run clear and then is stopped. The inlet cannula is cut, and the pump is positioned upright and filled with saline to allow for further investigation. The animal is euthanized under full anesthesia with an overdose of sodium pentobarbital ($> 100 \text{ mg/kg IV}$). A complete necropsy is then performed.

Results

We have completed 16 chronic studies with the Infant VAD. The intended duration of the chronic studies is 4–6 weeks. The animal results throughout the series have been generally positive in regard to pump performance and minimal thrombogenicity. However, sheep in the early implants experienced severe, diffuse, irreversible atelectasis intra-operatively, followed by pleural effusion and respiratory acidosis. Experiments #8–11 were performed in goats, since we had previous success with that animal model. At the same time, we extensively reviewed and modified the protocol, especially respiratory management (14). Beginning with Experiment 12, we have been able to achieve repeatable and healthy survival in lambs. Most recently (January 2009), a male sheep implanted with version 3 Infant VAD was electively terminated at 35 days post-implant. Pump performance was excellent, thrombogenicity was low (no infarcts noted at necropsy), and the animal thrived during the post-operative period.

Discussion

Incorporating existing and emerging technologies into mini-devices intended to support infants and children with complex cardiovascular disease has presented challenges, not the least of which is selecting an appropriate and viable animal model (15). Repeated, successful *in vivo* testing of the VAD is critical to obtain the investigational device exemption (IDE) that allows the pump to move forward into clinical trials.

The problems we have faced, particularly respiratory compromise, are not unique to our program. During annual NHLBI contractor meetings, most of the participants using sheep as an animal model have shared similar accounts of adverse events. Our approach to solving these problems, namely drawing on the expertise of our clinical colleagues at Penn State Hershey Medical Center, has allowed us to move forward with design and testing.

In spite of many years of experience implanting VADs in calves in our facility, we faced new challenges managing respiratory function in sheep. Use of pressure-limited ventilation, in which gas is delivered to the patient during inspiration until a preset pressure develops in the bellows, followed by the expiratory phase, has been key (16). The acquisition of new equipment for ventilation monitoring enhanced our ability to respond quickly to changes in the pressure-volume loop and lung compliance.

Careful attention to pain management post-operatively has allowed our animals to recover faster and resume eating and drinking within 24 h after surgery. This is an important factor in ruminants, since prolonged atony of the rumen will lead to the demise of the animal. Although the analgesic potency of opioids in sheep has been questioned (17–19), our clinical impression is that buprenorphine (a centrally-acting analgesic), combined with a peripherally-acting non-steroidal anti-inflammatory and local analgesic results in animals showing little to no post-operative pain and resuming normal respiratory patterns more quickly.

Conclusions

Animal model development for the Penn State Pediatric VAD has progressed alongside device development. We have established a reliable, reproducible juvenile sheep model for testing the VAD. Additional chronic studies are planned to further refine anticoagulation protocols and assess thrombogenic risk.

Acknowledgments

This research was funded by the National Heart, Lung, and Blood Institute Contract N01-HV-48191, the Fondation Coeur Artificiel Charles Hahn - Dr. Radovanovic, and the Donald B. and Dorothy L. Stabler Foundation. The authors wish to thank Tigran Khalapyan, MD and John Connell, MD, MPH for their efforts in early development of the model, as well as the team of animal/veterinary technicians, veterinarians, and engineers who support this program on a daily basis.

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Figure 1.

A) Preparation for insertion of the left ventricular apical cannulae. B) The outlet cannula prior to tunneling through the diaphragm. C) The VAD is placed in a pocket in the left upper quadrant of the abdominal wall.

Figure 2. Lamb on post-operative day 1. The Pediatric VAD is implanted in the abdomen and the driveline is tunneled to the percutaneous exit site as shown. Also shown is the blood management device (VAMP™, Edwards Lifesciences LLC, Irvine, CA).