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Initial experience with a juvenile sheep model for evaluation of the pediatric intracorporeal ventricular assist devices

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Abstract

Background—There is a scarcity of source material available in animal models appropriate to test pediatric-size blood pump design for assisting the left ventricle. A juvenile ovine animal model was developed to evaluate two pediatric ventricular assist devices (VAD). The child size Jarvik 2000 and CircuLite VADs were tested with this model. The 33 in vivo experiments were retrospectively studied to evaluate the reliability of the animal model.

Methods—Dorset hybrid juvenile sheep (20~36 kg) were used for evaluation of the small pediatric VADs. Under general anesthesia, a left lateral thoracotomy was performed through the fifth intercostal space. The devices were implanted between the left ventricle apex and the descending aorta without cardiopulmonary bypass. Heparin was continuously infused for anticoagulation therapy.

Results—Support duration averaged 26.7±19.6 days. 75.7% (25/33) experiments were completed as intended. Animals were docile and did not require sitters beyond the immediate operative period. Complication includes leg injury, graft infection, gastrointestinal bleeding, intravenous line disconnection, weight loss, renal failure, red urine (1 for each) and pulmonary failure in two. The activated clotting time (ACT) was adjusted via continuous intravenous heparin to 150-200 second. Reliable hemodynamics and biocompatibility data were collected for evaluate the pediatric intracorporeal VADs in the animal model.

Conclusion—The juvenile sheep model is a reliable, reproducible, and translatable for testing the Pediatric VADs. These experiments provided vital information for improvement of the devices and for clinical application in the future.

Introduction

The development for pediatric mechanical circulatory support systems is necessary and indispensable. ¹⁻³ Evaluation a newly developed circulatory support device by an in vivo

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animal study is a required component of pre-clinical testing. For the pediatric devices, the choice of animal model is especially important and challenging.^{4,5} The National Heart, Lung, and Blood Institute (NHLBI) convened a meeting of NIH contractors funded under the Pediatric Circulatory Support program in 2008 and summarized the findings of the working group on animal models.⁶ This specific report outlined the important factors to consider in designing the animal study and discussed important general study objectives, issues, and methods, which provided a guide to develop the animal model for in vivo evaluation of the pediatric VADs. Under this funding, a juvenile ovine animal model for testing the pediatric VADs was developed over the past 6 years at the University of Maryland. Two intracorporeal VADs, the child size Jarvik 2000 heart and pediatric CircuLite VADs, were tested with this animal model. In the present report, we summarized the experiences of the 33 in vivo studies and evaluated the reliability of the animal model for evaluation of the intracorporeal pediatric VADs.

Methods

All the surgical procedure and post-operative care were carried out according to the approved protocol by the Institutional Animal Care and Use Committee (IACUC) of the University Of Maryland School Of Medicine. During the course of the animal experiments, all animals received humane care in accordance with the Guide for Care and Use of Laboratory Animals (NIH publication 86-23, revised 1996).

Preparation of animals

Thirty-three Dorset hybrid juvenile sheep (20~36 kg) bred for laboratory use (Thomas Morris, Reisterstown, MD) were used for evaluation of the pediatric VADs in this study. Each animal was tested for Q-fever and vaccinated for contagious ecthyma and clostridium prior to shipment. A pre-operative health check was performed on each sheep to establish baseline health parameters prior to the start of the study after the animal had completed the mandatory 48-hour acclimation period. The animal was fasted 12 hours prior to the surgery with free access to water.

Anesthesia

On the day of surgery the animal was weighed and pre-anesthetized with Atropine (0.02 mg/kg, IM) and Glycopyrrolate (0.01 mg/kg, IM) followed by Ketamine (10-15 mg/kg, IM) for sedation. The left side of the neck was shaved for jugular catheter placement. After sterilization by alternating scrubs of 8-12% betadine and 70% isopropyl alcohol rinse, an intravenous catheter (16 gauge, Abbocath-T, US) was placed in the jugular vein. Anesthesia was induced by using Propofol (2-4 mg/kg, IV). Inhaled anesthesia was maintained with 1-4% isoflurane after intubation. The animal was mechanically ventilated with a tidal volume of 10-20mL/kg at a rate of 12-20 breaths per minute with 3-5mm Hg positive end-expiratory pressure (PEEP).

The arterial line was established by puncture of the femoral artery and followed with placement of a 5 Fr catheter in the vessel. The previously placed 16 gauge jugular catheter was replaced with an 8 Fr catheter to enable placement of a Swan-ganz catheter for monitoring pulmonary arterial pressure, wedge pressure, and CVP during the surgery. The vital signs were monitored using the pulse oximeter attached to a shaved area of the ear or at the edge of the mouth, visual signs of anesthetic depth (jaw tightness or laxity, movement, limb muscle tone, tachycardia and/or hypertension), electrocardiograph (ECG), and body temperature (via rectal thermometer probe). An orogastric tube was placed to prevent the buildup of gasses in the stomach.

Operative procedure

A left lateral thoracotomy was performed through the fifth intercostal space. The fifth rib was removed and the pericardium was opened. A 14-mm transonic flow probe was placed around the pulmonary artery for monitoring cardiac output (Systems). The peri-vascular space in the descending aorta was dissected with a flow meter (T401; Transonic and the hemiazygos vein across the aorta was ligated to accommodate placement of a partially occluding vascular clamp. Heparin (1000 U/ml) was administered via IV at a loading dose of 100-300 U/kg in order to increase the activated clotting time (ACT) to above 200 seconds. The vascular clamp was placed no deeper on the aorta than a loss of distal pulse and pressure would permit. An 8-mm-diameter polytetrafluoroethylene (PTFE) outlet graft (CircuLite pump) or Dacron outflow graft (Jarvik pump) was sutured in an end-to-side fashion to the aorta using a 5-0 Prolene suture.

For the intraventricular child Jarvik pump, a fixation cuff was sewn to the apex of the left ventricle with 4-6 3-0 prolene pledgeted sutures. An 11 surgical blade was used to make a small cross incision within the inner boundary of the cuff on the ventricular apex. A coring device was then inserted into the LV. A cylinder of myocardium was removed from the apex. As the coring device was removed, the pump was directly positioned into the left ventricle and hemostatically fixed on the cuff via umbilical-type 7xp. For the extra-ventricular CircuLite VAD, an inflow cannula was inserted into the left ventricle via the apex and connected to the pump. A newly designed child cannula and delivery system was used to puncture through the left ventricular Apex and advance the inflow cannula through the sewing ring. The cannula placement was verified through echocardiography. The proximal end of the cannula was connected to the pump and the outflow graft was connected to the descending aorta via a graft. Epicardial echocardiography was performed to assess the position of the pump or the inflow cannula in the LV. Lidocaine was administered (10-30 µg/kg/min) prophylactically to suppress ventricular arrhythmia. Internal cardioversion was used to treat ventricular fibrillation and sustain tachycardia when occurring.

After connecting all components of the mechanical circulatory support systems, the devices and conduits were de-aired through the grafts and the pumps were turned on to start operation. The electrical cables from the VAD and flow probes were tunneled to a point at the back of the animal. The system was positioned above the muscle and the left lung was inflated before chest closure. The pump speed was set according to the research purposes.

Chest closure—A chest drainage tube was placed and exited laterally on caudal aspect of skin incision. The chest tube, flow probe line, and electrical control cables for the VAD were tunneled and secured subcutaneously. The intercostal block procedures were performed using 0.5% bupivacaine 10ml previously used prior to closing the thoracotomy incision. The regular IV catheter was changed with a double lumen catheter using the Seldinger technique. The catheter was percutaneously tunneled to exit the skin at the middle of the back neck which will help prevent infection during following postoperative care. The femoral arterial line was removed and pressure was imposed on the puncture site for 20 min to avoid bleeding. Once the animal became conscious and could breathe on its own, the animal was extubated and transported in the specially designed stanchion to the preparation room for recovery.

Postoperative care

Supplemental oxygen was delivered to the sheep via nose cone to ensure blood oxygen saturation greater than 95%. Adequate recovery from anesthesia was accomplished when the

animal was awake and comfortable and able to maintain greater than 95% oxygen saturation on room air.

The chest tube was usually removed on the first day post-surgery, when the bleeding was deemed to be minimal (chest drainage below 20 ml/hour), free air could not be aspirated from the chest tube for at least 2 hours, and the overall respiratory status of the animal indicated full re-expansion of the left lung. Analgesic therapy was initiated to attain good pain relief as assessed by respiratory rate, heart rate, and animal behavior post-operatively and continued for five days post-operatively. A Banamine dose of 2.2-mg/kg initially and thereafter 1.1-mg/kg twice daily via IV is administered after surgery. Buprenorphine was used (0.005-0.01 mg/kg IV) to relieve pain associated with the surgical procedure if the above analgesia was not sufficient to relieve post-operative pain. Enrofloxacin (5 mg/kg IV, BID) was administered for 5 days to prevent infections.

Chronic / Prolonged Restraint

Since the VAD was connected to the electrical control system and power supply via an electric cable, the study animals were restrained with a head halter and leads securely attached to the front of a study stanchion. All the infusion lines and cables were connected to an adjustable cable to the back of the animal so that they could move with the animal in the limited space, but they were placed securely out of reach to prevent accidental chewing on the lines (Figure 1).

Anticoagulation and ACT monitoring

For the first six chronic studies, sheep received aspirin (325 mg by mouth) and clopidogrel bisulfate (75 mg by mouth) daily. Anticoagulation was not monitored for these experiments. For the rest of the 28 chronic experiments, anticoagulation was achieved by heparin infusion via an indwelling catheter to maintain a target ACT range between 160 to 200 seconds. An ACT level is checked every six hours during the initial 24 hours post heparin therapy initiation until heparin dose was stabilized. The blood samples were also sent to an outside laboratory for measurements of blood chemistry and complete blood count (CBC) (Antech Diagnostics, Lake Success, NY).

Necropsy Study

At the study end-point, a non-survival surgery was performed. The study animal was brought to the operating room and prepared in the similar manner described above. The chest was opened through the previous left thoracotomy. A series of measurements (echocardiogram, hemodynamics, flow measurement) were performed to assess pump performance and cardiac function at the study end-point. Once the required measurements were completed, Heparin (400 U/kg IV) was administered. After a 5-min interval, the animal was euthanized by exsanguination under general anesthesia. The animal heart was excised for tissue sampling. Other vital organs and tissues (liver, lungs, spleen, brain and kidneys) were collected for gross and histological analysis. The implanted components were retrieved for analysis for thrombotic events.

Results

General information

The general information of the device implantation and support in this animal model are summarized in Table 1. These animal studies were carried for the in vivo evaluation of the two pediatric devices - Jarvik 2000 and CircuLite. The requirements for the device support duration are different for different intentions, including acute fitting non-survival surgery and 14-day, 30-day, and 60-day chronic survival studies. Two bearing designs of the child

size Jarvik 2000 heart have been evaluated with this animal model: one with original pin and the newer cone-type bearings.

Average cardiac output of the study animals is 3.2 ± 1.3 L/min after surgery. The pump flow was set between 1.0-2.6 L/min according to the different study purpose. The in vivo experiments with the child-size Jarvik 2000 heart with pin bearings highlighted the excellent hemodynamic performance and acceptable biocompatibility of the device. However, it was found that the pin-shaped bearings were the site of thrombi formation and thus the major cause of mechanical failure in most cases. A set of novel conical bearings were developed after the early experiments to address this problem. The recent experiments with the modified child-size pediatric Jarvik heart are encouraging showing anatomic fit, partial to complete circulatory support, and good biocompatibility. The utilization of the conical bearings had mitigated the thromboembolic phenomenon and bearing seizure. We have not experienced device clotting events or pump malfunction since the modifications in the bearings were made.

The ongoing studies for CircuLite pump were also evaluated successfully and reliably by this juvenile ovine animal model; 87.5 % experiments (7/8) are completed as intended.

Complications—Complications occurring in the chronic survival surgery studies are summarized in Table 2. The mechanical complications of the mechanical circulatory support systems, such as pump stop and leakage from the device were not included. Most complications occurred within the first two years of conducting these experiments. The most recent nine study cases had no animal-related complications. One animal unexpectedly died on the third post-operative day due to sudden cardiac arrhythmia. The necropsy examination indicated that the pump was not appropriately positioned in the left ventricle. The pump was pointed toward the ventricular septum and likely caused suction, indicated by an endocardial bruise. One sheep was euthanized on post-operative day 9 secondary to a non-device related leg injury. One animal died due to bleeding of gastrointestinal tract on post-operative day 54. One animal was terminated on the 41st post-operative day due to infection, but during necropsy it was discovered that the outflow graft was completely blocked due to thrombosis in the graft while the impeller was still rotating. One sheep died due to loss of blood because the IV line disconnected during the night. One sheep was electively terminated due to weight loss from 30 kg to 22 kg. Four animals were euthanized for complications, 1 for renal failure, 1 for hematuria, and 2 for pulmonary dysfunctions according to the requirements of the university Veterinary Resources

Anticoagulation—Two devices explanted from the animals anticoagulated with aspirin and clopidogrel bisulfate showed a small amount of thrombosis at the origin of the outflow graft site and around the bearing site. As a result, continuous heparin infusion was adopted for the last 24 chronic experiments. It was observed that heparin doses needed to be increased for a significant period following surgery but eventually stabilized and could even be decreased by the third week post operatively (see Figure 2). We have found that the need to escalate the heparin dose to maintain the targeted ACT corresponds with the observed increase of the platelet counts (Figure 3) following initial implantation surgery. This increase in platelet count is possibly not device-related because of the increase of the platelet counts also observed in sham sheep. The white blood cell (WBC) count was in the normal range after surgery and the antibiotics medication was discontinued after 5 days post operation unless infection occurred (Figure 4). Hemoglobin was stable throughout the experiment (Figure 5).

Discussion

Animal species used during VAD implantation are listed in order of frequency: calves, sheep, lambs, dogs, pigs, goats, and horses.⁷ Calves are the most frequently used animal species in the current era of preclinical device testing. However, the heart size of the calf is too big to test pediatric mechanical assist devices.^{8,9} The canine model are regarded as the less appropriate model in which to test a VAD due to their higher incidence of thromboembolic complications compared with other species.¹⁰ Sheep have hemostatic, anatomic, and physiologic properties similar to those of humans. Several studies have favored the sheep or lamb model as an acceptable animal model for testing the blood compatibility of devices.^{7,10} From our initial experience, 75.7% of the proposed animal studies were completed as intended and reliable data for the performance of these devices were collected from this animal model. With the animal model, the child-size Jarvik 2000 VADs were successfully evaluated and improved previously.^{11,12} This animal model was also improved from these early experiences, which resulted in successful in vivo evaluation of the pediatric CircuLite VADs recently. Surgical implantation is still the most pivotal point for the success of the study. Mechanical myocardial damage during device implantation, incorrect pump position, significant blood loss, and the duration of surgery are the points identified as causes for failure in early experiments. Correct positioning of the intra-ventricular pump depends on the direction of inserting the coring device, as it is difficult to adjust the pump position if the opening through which it is inserted is in any way misplaced. One of our early failed experiments was a case where the pump was positioned toward the LV septum and caused suction of the pump and failure of the experiment due to incorrect positioning of the coring device. To correct this, we determined that the coring device should be inserted toward the mitral valve and the myocardium cut completely which made pump insertion easy and decreased blood loss, which was another complication we faced. Furthermore, we checked for correct pump position by utilizing epicardial echocardiography, which was especially helpful for determining the insertion depth of the pump.

We also met challenges managing respiratory function in juvenile sheep similar to those faced by the Penn State group.¹³ The inflation of the lung before closing the chest and careful surgical cauterization are helpful in maintaining adequate respiratory function after surgery. Secondly, the graft of the pump should not be cut too short to avoid suppression of the lung by the graft.

Careful attention to pain management post-operatively is important for the recovery of the animal. Adequate post-operative pain management has resulted in better respiration and prevents atelectasis. Sheep often indicate pain first through a lack of appetite that leads to significant weight loss, a factor requiring alternative endpoint euthanasia per the university Veterinary Resources. In our study, we used a two-pronged approach by first using Bupivacaine locally in the area of surgical incisions after surgery to block pain both prior to incision and during the wound closure. This was followed with a combination of Banamine, both pre-surgically and post-operatively, and Buprenorphine used daily to relieve pain associated with the surgical procedure.

Leg injury was another common complication we faced in our animal studies, with one study animal unable to stand up postoperatively. There are three main causes, and subsequent preventative measures, for leg injury and post-operative inability to stand. The first is a result of poor circulation to the legs during surgery. This is resolved by placing thick support pads with extra padding cranial and caudal to the right scapula to prevent pressure trauma as much as possible to the dependent leg. A similar problem was found regarding the removal of the arterial line in the femoral artery, especially if adequate

pressure was not applied to the area after removal of the catheter. Thirdly, we use sling support to treat leg lameness and improve the circulation to the leg when the animal is too weak to stand in the first several days. The use of a sling when an animal is not willing to stand allows the legs to straighten out from the natural flexed position that restricts blood flow when the animal lies.

Since the VAD is connected to the electrical control system and power supply via an electric cable it is necessary to restrain the study animals through the use of a halter and leads connected to the front of a study stanchion. If not properly restrained in this manner, the animal can easily turn around to chew the IV line and electrical cable or strain the cable. For behavioral purposes, we often house a second sheep or two study sheep together in order to decrease stress caused by isolation of an animal used to a large herd. Over the last four years, we have been using this approach to successfully host the sheep implanted with various VADs as shown in figure 2.

The anticoagulation and ACT monitoring are a critical issue for reliably evaluating the performance of the devices. When using the anticoagulation with aspirin and clopidogrel bisulfate, we met thrombogenicity problems in our early experiments. After using heparin as anticoagulation and ACT monitoring, which emulate the expected clinical protocol, our experiments had more stable results. It is necessary to create a hypercoagulation status in the study animals in the first two weeks post-operatively to prevent early formation of thrombocytes within the device. In order to achieve that, intravenous heparin titration should be increased by 200 U/day and monitored intensively via ACT.

Conclusion

The juvenile sheep model is a reliable, reproducible animal model for testing pediatric VADs. With this animal model, two kinds of axial pump, the Jarvik 2000 and the CircuLite pediatric pump, were evaluated reliably. The results from these experiments provided vital information for improvement of the devices and for clinical application in the future.

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Figure 1.

A sheep on the postoperative day 1. The electric cable, the flow probe cable, and the infusion line are tunneled from the dorsal of the animal and connected to an adjustable cable. The electrical control system, flow monitor and UPS power are fixed on the top of the stanchion.

Figure 2.
The average dosage of heparin infusion after implantation of the VAD

Figure 3.
The average platelet counts post operatively in animals with VAD and in sham sheep

Figure 4.
The average WBC counts post operatively in animals with VAD and in sham sheep

Figure 5.
The average hemoglobin post operatively in animals with VAD and in sham sheep

Table 1**General Information**

	Numbers
Number of animals	33
Gender Male/female	22/11
Mean Body weights (kg, SD, range)	27.3 (4.5), 19.1-36.9
Average cardiac output (L/min, SD, range)	3.2 (1.3), 2.2-5.1
Average pump flow (L/min, SD, range)	1.5 (0.7), 1.0-2.6
Averaged Device support duration (days, SD, range)	26.7 (19.6), 1-73
Acute fitting study (number, days, SD)	5, 0.6 (0.5)
14-day study (number, days, SD)	8, 14 (1.7)
30-day study (number, days, SD)	4, 31 (7.3)
60-day study (number, days, SD)	16, 40.5 (22.3)
Types	
Jarvik 2000	25
CircuLite pediatric	8
Completion as intended (percentage, number)	75.7% (25/33)
Coagulation	
Aspirin and clopidogel	9
Heparin	24

Table 2

Complications in chronic studies (n=33)

Complications	Number	Occurrence day (POD)
Died due to failed surgery	1	1
Leg injury	1	1
Graft Infection	1	41
GI bleeding	1	54
IV line disconnection	1	2
Weight loss	1	37
Renal failure	1	7
Pulmonary failure	2	13/ 18
Hematuria	1	7
Total	10	