

Pediatric Cardiopulmonary Bypass Adaptations for Long-Term Survival of Baboons Undergoing Pulmonary Artery Replacement

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Abstract: Cardiopulmonary bypass (CPB) protocols of the baboon (*Papio cynocephalus anubis*) are limited to obtaining experimental data without concern for long-term survival. In the evaluation of pulmonary artery tissue engineered heart valves (TEHVs), pediatric CPB methods are adapted to accommodate the animals' unique physiology enabling survival up to 6 months until elective sacrifice. Aortic access was by a 14F arterial cannula and atrial access by a single 24F venous cannula. The CPB circuit includes a 3.3 L/min flow rated oxygenator, ¼" × ⅜" arterial-venous loop, ⅜" raceway, and bubble trap. The prime contains 700 mL Plasma-Lyte, 700 units heparin, 5 mL of 50% dextrose, and 20 mg amiodarone. Heparinization (200 u/kg) targets an activated clotting time of 350 seconds. Normothermic CPB was initiated at a 2.5 L/m²/min cardiac index with a mean arterial pressure of 55–80 mmHg. Weaning was monitored with transthoracic echocardiogram. Post-CPB circuit blood was re-infused. Chest tubes were removed with cessation of bleeding. Extubation was performed upon spontaneous breathing.

The animals were conscious and upright 3 hours post-CPB. Bioprosthetic valves or TEHVs were implanted as pulmonary replacements in 20 baboons: weight = 27.5 ± 5.6 kg, height = 73 ± 7 cm, body surface area = 0.77 m² ± 0.08, mean blood flow = 1.973 ± .254 L/min, core temperature = 37.1 ± .1°C, and CPB time = 60 ± 40 minutes. No acidosis accompanied CPB. Sixteen animals survived, four expired. Three died of right ventricular failure and one of an anaphylactoid reaction. Surviving animals had normally functioning replacement valves and ventricles. Baboon CPB requires modifications to include high systemic blood pressure for adequate perfusion into small coronary arteries, careful CPB weaning to prevent ventricular distention, and drug and fluid interventions to abate variable venous return related to a muscularized spleno-splanchnic venous capacity. **Keywords:** baboon, tissue engineered heart valve, right ventricular outflow tract, pulmonary valve, cardiopulmonary bypass, subhuman primates, homografts, allografts, xenografts. *JECT.* 2010;42:223–231

The baboon (*Papio* sp.) animal model is potentially valuable in comparative cardiovascular research because baboons and humans have significant similarities in physiology, innate and acquired immune systems, rheology, and

resident antigens that are not necessarily captured in other species (1–3). In xenotransplant research, the baboon has been used to evaluate novel sources of transplantable organs from other species to determine human compatibility with these species (4). Much more common in humans is the right ventricle to pulmonary artery valved conduit insertion as a congenital cardiac surgical procedure. These patients represent a significant target population that would benefit from the development of tissue engineered heart valves (TEHVs) (5). Perfecting methods to permit subhuman primate TEHV replacement experimentation would be a significant advance in tissue engineering.

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With regard to cardiopulmonary bypass (CPB), the baboon animal model has been successfully but infrequently used to test surgical robustness, primate physiology, coagulation, complement activation, thrombosis initiated by oxygenators, and the consequences of various pharmacologic and anesthetic techniques (6,7). Baboons have sympathetic neurohumoral responses similar to human responses in addition to profound genetic and anatomical similarities to humans, making baboons an important species for experimentation. Most reported studies derive experimental data from the acute baboon models and very few have attempted longer term valve performance studies (8,9).

Published protocols for the conduct of CPB in the baboon are limited in the literature and most do not describe survival post-CPB. The accounts of baboon CPB in surgical literature describe cannulation, heparinization, protamine reversal, and CPB circuit components and parameters (6,7,10–17). Cardiac outputs and corresponding pump flow rates are reported ranging 50–176 mL/kg/min and mean arterial pressures (MAPs) ranging 30–85 mmHg. The majority of these articles do not report chronic survival with the exception of one reporting a 7 day survival (18).

In perfusion literature, there are two accounts of using baboons to evaluate low prime circuits for blood conservation in the non-human primate (18,19). In 1993, Sistino et al. (19) described the use of low prime closed circuits for pediatric CPB patients as tested in 13 5–15 kg baboons. The only perfusion parameter reported was a CPB flow of 50 mL/kg/min under deep hypothermia. Survival was not reported. However, the authors conclude that low prime closed CPB circuits resulted in blood conservation and the reduction of foreign surface contact. In 2000, the use of a low prime circuit was reported as a method to perform bloodless heart transplantation in three 5–7 kg primates. Cannulation was achieved with an 8 Fr aortic cannula and bicaval venous cannulae (12 Fr and 14 Fr). Other components included a roller pump, hemoconcentrator, hollow fiber oxygenator, and cardioplegia set. A flow target of 2.2 L/min/m² was used with a MAP > 30 mmHg. The activated clotting time (ACT) was kept greater than 480 seconds. Hyperoxia and modified ultrafiltration were also used. Survival from CPB was noted; however, the animals were sacrificed on the seventh day post-CPB.

Perfusion literature does not describe specific methods to achieve the long-term survival of the baboon model post-CPB. The purpose of this article was to describe perfusion protocols and perioperative management techniques developed by The Children's Mercy Hospital, Cardiac Surgery Research team in collaboration with the Southwest National Primate Research Center (SNPRC) to obtain uncomplicated post-CPB survival of the baboon

until elective sacrifice within 6 months of surgery. The differences and similarities between human pediatric CPB and subhuman primate CPB for right heart pulmonary valve conduit placement were compared. Our model was developed to evaluate the suitability of an old world monkey species to provide different information than that derived from the more usually used ovine preclinical calcification model for biological heart valve evaluations (20,21).

MATERIALS AND METHODS

These experiments were supported by The Children's Mercy Hospital and Clinics of Kansas City, MO, and approved by the Institutional Animal Care and Use Committee of the Southwest Foundation For Biomedical Research and the Southwest National Primate Research Center, San Antonio, TX, protocol number 1199 PC 0, entitled "Pre-clinical In Vivo Primate Model Evaluation of a Tissue Engineered Heart Valve: Right Ventricular Outflow Tract Conduit", with final approval dated October 28, 2008. These experiments were performed as detailed by the Food and Drug Administration Code of Federal Regulations 21CFR58 concerning preclinical trials in animals. All animals received humane care in compliance with the published national guidelines (22).

Data Analysis

All data were recorded in a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA) table. Data were then transferred for analysis to the GraphPad InStat statistical package (version 3.01 for Windows 95/NT, GraphPad Software, Inc., San Diego, CA). Continuous variables are expressed as means and standard deviations. The probability of significance was determined using the unpaired *t* test.

Animal Selection and Preoperative Care

All animals were selected based on the following criteria: surgical naïveté, sex, weight, age, and health status. Initially female animals were considered as surgical candidates for this study but their smaller size precluded their use. Sexually mature male baboons from 6–9 years of age and weighing from 27–32 kg were chosen for this study. All animals were colony born and were surgically naïve. All donor and recipient animals were seronegative for *Trypanosoma cruzi* and received a complete physical exam including electrocardiogram (ECG), complete blood count (CBC), and a routine chemistry panel. Recipient animals also had a coagulation panel performed at this time. All physical examinations were performed approximately 1 month prior to surgery and 100 mg of iron was injected at this time. Once animals were selected for this study they were supplemented daily with a generic oral vitamin containing iron.

Circuit and Priming

To accommodate baboons ranging from 13.4–35.5 kg, a circuit was assembled from components supplied by different manufacturers. These various components also had different surface coatings, depending upon the manufacturer. This circuit was the same circuit used in humans of this size by the cardiac surgery team at The Children's Mercy Hospital. Circuit details were as follows: Dideco Lilliput 2 Oxygenator with Phisio coating (Sorin, Milano, Italy), a Trillium® coated $\frac{1}{4}$ " \times $\frac{3}{8}$ " Arterial-Venous loop (Medtronic, Minneapolis, MN), $\frac{3}{8}$ " Smart coated raceway (Cobe, Arvada, CO), Terumo BT05 bubble trap (Ann Arbor, MI), and Cobe Smart tubing for the ventricular vent and cardiomy suckers (Arvada, CO). The circuit was primed with 700 mL Plasma-Lyte 148 (Baxter, Deerfield, IL), 700 units heparin, 5 mL of 50% dextrose (the animals tended to have low glucose values on CPB without the added dextrose), and 20 mg amiodarone (for prophylactic arrhythmia prevention). Potassium chloride (5–20 mEq) and calcium gluconate (1000 mg) were administered as needed following the initiation of CPB.

Surgical Preparation, Anesthesia, Monitoring, and Pain Control

The procedures were carried out with the following goal in mind. Subsequent to the surgery, the animal must be arousable, sedated, and with sufficient analgesia, yet still capable of maintaining adequate spontaneous ventilation and oxygenation. The postoperative goal was to remove all lines and tubes prior to returning the animals to their individual cages in the recovery room. Thus, the animal must be essentially fully recovered and able to survive without any traditional postoperative intensive care unit treatment.

All animals were fasted from food approximately 14 hours prior to surgery, but allowed free access to water. The innate aggressiveness of baboons required heavy sedation for safety in all out-of-cage interactions. Out-of-operating room (OR) sedation was used to provide adequate chemical restraint to permit the safe transport of the animal to the OR. On the day of surgery each animal was sedated with ketamine (5–10 mg/kg) and given glycopyrrolate (.01–.02 mg/kg) to decrease secretions (e.g., saliva or phlegm) in the mouth and throat. Crown-to-rump measurements and weights were obtained. The animals were shaved, two peripheral intravenous catheters were placed, the trachea was intubated, and the urinary bladder was catheterized. Usually no additional medications were required prior to endotracheal intubation in a spontaneously breathing animal, but on occasion, propofol (2–4 mg/kg) was given to facilitate intubation. Muscle relaxants were never given prior to successful intubation of the trachea.

Animals were positioned on the OR table in dorsal recumbence and ventilated with a mechanical ventilator. Induction of general anesthesia was initiated by inhalation

of isoflurane (Forane) at 1.0–2.0%. Ventilation was initiated at a tidal volume of 10 mL/kg and the frequency was adjusted to maintain an end-tidal CO₂ below 36–38 mmHg. Lung ventilation with a positive end-expiratory pressure of 5 cmH₂O was required to avoid atelectasis. Blood was drawn for preoperative CBC and routine chemistry.

Monitored parameters included arterial blood pressure, heart rate, pulse oximetry, capnography, inhalation agent concentration analysis, body temperature, urine output, and ECG. A 4 Fr 12 cm femoral arterial catheter was placed percutaneously to obtain accurate aortic pressures. Attempts at using shorter catheters resulted in a dampened waveform, probably due to the thick arterial walls of the iliac and femoral artery structures. No central venous catheter or monitoring was used. An adult sized Hewlett Packard (Palo Alto, CA) transesophageal echo (TEE) probe was placed preincision and used for cardiac assessment and for monitoring fluid management by evaluating cardiac chamber volumes. The TEE probe was maintained in place during the entire procedure.

General anesthesia was maintained during surgery with 1.0–1.5% isoflurane (1 MAC = 1.28%) with air/oxygen mixture. This was supplemented with fentanyl given as a loading bolus of 10 mcg/kg just prior to incision and then by continuous infusion at 10–20 mcg/kg/h until closure of skin. Muscle relaxation was maintained with atracurium (Tracrium) .5–.6 mg/kg prior to start of surgery and subsequent dosing (.25–.3 mg/kg) given every 30 minutes thereafter until the start of surgical closure. During CPB, general anesthesia was maintained with intravenous propofol infusion at 100–200 mcg/kg/min as the extracorporeal circuit did not have an attached anesthetic vaporizer. Propofol infusion was discontinued and inhalation anesthesia resumed upon weaning from CPB.

Surgical and CPB Procedures

Table 1 lists the additional variables of the 20 baboons undergoing surgery. The body surface area (BSA) was calculated using the Haycock formula: $BSA = \text{kg weight}^{0.5378} \times \text{cm height}^{0.3964} \times .024265$. The Haycock formula was selected as it assumes a smaller body mass contribution from the lower body which is true for human children and also for these adult baboons whose reliance on the upper body for locomotion results in a massively enhanced relative contribution to lean body mass. For the combined population the average weight = 27.5 ± 5.6 kg, the average height = 73 ± 7 cm, the average BSA = $.77 \text{ m}^2 \pm .08$, the average calculated CPB blood flow = $1.97 \pm .25$ L/min, and the average CPB time = 60 ± 40 minutes.

The skin was prepared by shaving and washing with soapy antiseptic followed by alcohol and a 10% povidone iodine solution. After sterile drapes were applied, the appropriate equipment and CPB tubing area was secured. The routine perioperative antibiotic prophylaxis regimen

Table 1. Baboon valve recipients: Survivors versus expired (means and standard deviations).

Parameter	Survivors (<i>n</i> = 16)	Expired (<i>n</i> = 4)	<i>t</i> test <i>p</i>
Weight (kg)	28.0 ± 5.0	24.5 ± 8.3	.43
Height (cm)	72 ± 5	77 ± 12	.25
BSA* m ²	.77 ± .08	.76 ± .12	.74
Calculated flow (mL/min)	2000 ± 236	1865 ± 332	.36
CPB time (min)	47 ± 8	112 ± 74	.0015
Post-CPB temp (°C)	37 ± 0	37 ± 1	.44
Crystalloid added (mL/kg)	10 ± 11	37 ± 30	.0099
U/O (mL/kg/h)	15 ± 17	3 ± 3	.1760
Pre-CPB Hct (%)	41 ± 4	40 ± 3	.60
On-CPB Hct (%)	30 ± 4	27 ± 5	.30
Post-CPB Hct (%)	31 ± 4	24 ± 4	.0172
Post-CPB ABG			
pH	7.48 ± .08	7.42 ± .01	.25
pCO ₂ (mmHg)	41 ± 6	40 ± 11	.86
pO ₂ (mmHg)	364 ± 147	481 ± 4	.31
Base balance	7 ± 4	1 ± 7	.10
Post-CPB electrolytes			
Potassium (mEq/L)	3.6 ± .2	3.7 ± 1.1	.60
Ionized calcium (mmol/L)	1.18 ± .11	1.01 ± .01	.0567

Significant observations between surviving and expiring animals included the CPB time, crystalloid added, urine output, post-CPB hematocrit, base balance, and ionized calcium.

consisted of cephazolin or ampicillin and amikacin in the appropriate doses. The median sternotomy was performed and the chest entered. The pericardium was opened and secured. Aortic and venous cannulae were placed and connected to the CPB circuit. CPB was initiated and the heart drained of blood. Aortic access was obtained with a 12–14 Fr BioMedicus® Arterial cannula (Medtronic, Minneapolis, MN) and atrial access through a single 24–28 Fr DLP venous cannula (Medtronic, Minneapolis, MN), depending on the size of the animal. Baseline ACT tests were performed. The baseline average was 113 ± 16 seconds. Subsequently, 200 units per kilogram of heparin were administered as a loading dose prior to the initiation of CPB with a targeted ACT of 350 seconds.

CPB was initiated with a target CI = 2.5 L/m²/min and an MAP of 60–80 mmHg. The anatomy of the right coronary orifice and artery of the baboon was quite small and requires higher arterial pressure to maintain adequate perfusion of the right heart. In contrast, the left heart (and consequently the left coronary artery) was large and robust implying the ability to provide rapid increases in cardiac output for bursts of physical activity and also for maintaining a higher than human systemic blood pressure. Normal baboon blood pressure is 168/119 mmHg with a MAP of 137 mmHg (23). A phenylephrine bolus (1–2 mcg/kg) followed by a continuous infusion (.5–2 mcg/kg/min) was usually required to compensate for the hypotension induced by the hemodilution of crystalloid priming of the extracorporeal circuit. Hypotension was aggressively treated with increased flow and/or phenylephrine during the period of

CPB. Normothermic CPB is used in all cases. Rectal temperature averaged 37°C ± 1 before and after CPB as compared to the published normal temperature for baboons of 38.5°C (24).

Right ventricle to pulmonary artery valved conduit insertion is a common congenital cardiac surgical procedure and such pediatric patients are a target patient population for the development of tissue engineered heart valves (5). For this reason, methods to permit primate pulmonary valve replacements were developed. The pulmonary artery was mobilized and a vascular clamp applied proximal to the bifurcation. The native pulmonary valve and artery were excised and replaced with bioengineered pulmonary valves (*n* = 18) as controls, or commercially available clinical grade stentless porcine bioprosthetic (*n* = 2) valve conduits (Medtronic Freestyle®, Minneapolis, MN). The biological test valves were derived from either baboon or human donor pulmonary valves. The human valves were derived from discarded (out of date) cryopreserved human clinical allografts whose donors had approved both transplantation and research use of their gifted tissues (LifeNet Health, Virginia Beach, VA). The explanted native pulmonary valves were placed in a sterile culture media and retained for later experimentation. The animal was weaned from CPB and blood and fluid from the chest cavities were removed and chest tubes inserted. The sternum was approximated and the muscle, fascia, and skin were closed using absorbable sutures.

Laboratory Testing

Most blood testing during the surgical procedure was performed using the i-STAT's hand-held point-of-care system (Abbott Point of Care Inc., Princeton, NJ), providing real time pH, pCO₂, pO₂, Na, K, iCa, glucose, and ACT. Centrifuged blood samples were used to determine the hematocrits.

Potassium supplementation was required in two of the animals while on CPB. The ionized calcium tended to drop below normal towards the end of CPB in all animals. Calcium gluconate was given as supplementation. Ten of the animals received additional heparin during CPB after measured ACT fell below 350 seconds.

The published normal hematocrit for baboons is about 40% (24). The pre-CPB hematocrit averaged 41% ± 4. The hematocrit during CPB averaged 29% ± 4. The post-CPB hematocrit averaged 30% ± 4.

Conduct of Perfusion, Fluid Management, and Urine Output on CPB

A sweep gas of 100% oxygen flow was maintained in the ratio of 1:1 with the blood flow rate. Normothermia was maintained through the use of a Sarns Dual Cooler-Heater (Sarns 3M Health Care, Ann Arbor, MI). Mean arterial blood pressure was maintained at 60–85 mmHg through increases in CPB flow with fluid volume added if needed to

maintain a safe venous reservoir level. Phenylephrine was also administered by the anesthesiologist. Lower pressure frequently resulted in cardiac arrhythmias, which responded to increased MAP. No cardioplegia or fibrillation was used. Cardiotomy suckers were used to return blood loss to the circuit. No ventricular vent was used. No visually observable third spacing of fluid occurred. However, volume shifts often required the addition of crystalloid (16 ± 19 mL/kg) to the circulating volume on CPB to maintain pressures within the defined parameters. Urine output on CPB averaged 13 ± 16 mL/kg/h.

CPB Weaning

CPB weaning was managed with the aid of TEE. The baboons' right heart muscle wall seemed to have little systolic reserve, being very sensitive to volume overloading and/or acute increases in pulmonary vascular resistance. This easily resulted in frank right ventricle (RV) failure. While utilizing a TEE four chamber view, the venous cannula was clamped and the right heart was slowly given volume from the CPB circuit until the RV minor axis was approximately 2/3 that of the left ventricle. The animal was then terminated from CPB. If the RV over-distended with volume, the TEE showed a more spherical shaped RV with grossly hypokinetic wall motion. In this situation, volume was immediately withdrawn by unclamping the venous cannula, restoring the RV contractility. The occurrence of this phenomenon prompted a loading dose of milrinone (50 mcg/kg). This improved the RV contractility. Weaning from CPB was then resumed with continued real-time assessment of contractility by TEE. Animals that could not be readily weaned with these methods were administered epinephrine (.03–1.00 μ g/kg/min as needed) intravenously.

Heparin Reversal and Bleeding Management

The heparin was reversed post-CPB with .75–1.0 mg/kg of protamine until an ACT near the baseline value was achieved. Following the cessation of CPB, the CPB circuit volume was transferred to an infusion bag for direct intravenous (IV) administration. None of the animals received a non-autologous blood transfusion. Post-CPB, bloody chest drainage was captured in the cardiotomy reservoir, which functioned as a chest bottle under a slight vacuum. This drainage could have been used for reinfusion but the necessity never arose. When bleeding ceased, the chest tubes were removed before the animal regained consciousness.

Postoperative Care

Prior to wound closure, intercostal nerve blocks were performed with bupivacaine .25% in the area of the sternotomy incision and chest tubes. No muscle relaxant reversal agents were used because of atracurium's predictable non-metabolic breakdown and a planned post-surgical period of at least 90–120 minutes during which time the animal

was maintained on a lower concentration of isoflurane (.5–.7%) to monitor hemodynamic status and drainage from the mediastinal tubes. Prior to removal from gas anesthesia, ketorolac (1.0–1.5 mg/kg) was given IV for management of pain. Buprenorphine (.01–.02 mg/kg) was given intramuscular as well for immediate postoperative pain control. The gas anesthetic was discontinued and the animals were weaned from the ventilator until the establishment of spontaneous breathing. Extubation was performed after hemodynamic stability had been achieved and maintained for 90 minutes and chest tube drainage had remained minimal for 1 hour, permitting chest tube removal. All monitoring and intravenous lines were then removed.

All animals received additional pain medication 6–8 hours after they were returned to their cages the day of surgery. The postoperative analgesics used were ketorolac 15–30 mg/kg twice a day (b.i.d.) and buprenorphine .01 mg/kg b.i.d., both given parenterally for up to 5 days. All animals received gentamycin 20 mg b.i.d. and amoxicillin 330 mg b.i.d., parenterally for 7 days. All animals were offered fruit and oral electrolyte replacement fluid approximately 24 hours after surgery. Solid and liquid intake and output were monitored during the first post-op week and the animals were observed for any signs of bleeding, respiratory distress, and wound infection or dehiscence at least twice a day. All animals were observed twice daily for attitude, food consumption, urine, stool production, and incision sites. All animals were sedated weekly for 10 weeks and thereafter every 4 weeks for a physical exam, CBC, and immune panel until elective euthanasia.

RESULTS

Sixteen animals survived and four expired perioperatively. There was no metabolic acidosis during CPB in those animals that survived or expired. The surviving animals were conscious, upright, and unencumbered by any invasive monitors or lines within 3 hours post CPB. Expired animals tended to be on CPB longer (due to difficulty in weaning), and required the addition of more crystalloid. Expired animals also had less urine output, lower hematocrit values, and less base excess after CPB (Table 1). Of the four animals that died, three developed RV failure after CPB and expired within 3–6 hours of separation from CPB. The fourth animal succumbed to an acute anaphylactoid reaction from an inadvertent rapid infusion of antibiotics (ampicillin and amikacin). Two of the RV failure deaths were a consequence of pressure overload due to intentional downsizing of the selected RV conduit relative to recipient BSA. The third RV failure was due to volume overload (acute RV dilatation) after an infundibular incision (analogous to the standard Tetralogy of Fallot operative approach) impaired RV systolic function. Six animals

required inotropic support with epinephrine for successful separation from CPB, only two of these survived. Thirteen of the 16 surviving animals had no significant early or late postoperative complications and appeared healthy (vital signs normal, robust activity, and eating) until elective sacrifice (up to 6 months). Three animals experienced complications in the late postoperative period. One developed increased bronchial sounds at week 5 post surgery. Radiographs demonstrated a collapsed left lung, which had resolved on its own by the time this animal was scheduled for a thoracocentesis. No other problems were observed in this animal and it completed the study uneventfully. A second animal was found to be losing weight despite a voracious appetite. On physical examination a large abdominal mass was palpated in the stomach region. Subsequent radiographs demonstrated a large mass presumed to be a trichobezoar. A gastrotomy was performed and two large trichobezoars were removed from the stomach. This animal recovered well and completed the study as planned. The third animal developed tachycardia, lethargy, poor appetite, and discoordination several days after surgery. Examination revealed congestive heart failure (CHF). This animal had received a downsized donor valve (1 mm smaller than recipient) and required substantial epinephrine to wean from CPB. The CHF was treated with a loading dose of .5 mg once a day digoxin, which was subsequently tapered to .125 mg per day and this resulted in marked improvement. The digoxin was discontinued after 6 weeks without clinical or functional evidence of CHF. These three episodes are examples of the robustness of the baboon model for performing CPB for testing novel cardiovascular devices and biologics.

The average donor annulus internal diameter was 20.18 ± 2.5 mm and the average recipient annulus was 16.35 ± 2.3 mm. Five animals, however, received valves with smaller or equal diameters to the recipient annulus; of these, two died of RV failure, one developed transient CHF requiring digoxin, and one required an outflow enlargement during the surgery. One had an annulus of 17.0 mm and received a 17.00 mm valve; this animal required epinephrine to wean from CPB, but ultimately survived without chronic RV CHF.

All incisions healed well and no animals developed any infections. Most animals developed a mild regenerative anemia that resolved 4–6 weeks after surgery. Many had auscultable flow murmurs that waxed and waned during the monitoring period.

CONCLUSION

Pulmonary valve replacement using normothermic CPB in the baboon for the long term, pre-clinical evaluation of bioengineered heart valves was possible without blood

transfusion, excessive bleeding, CPB related complications, infection, or anesthesia related mortalities. Rapid recovery within 3 hours from the end of CPB to full upright unencumbered consciousness was consistently achieved. The baboons proved to be a robust, subhuman primate, open heart surgical model, especially suitable for testing primate tissue derived biological valve substitutes. However, the baboon hearts had a thinner RV, a far smaller right coronary artery, and much less contractile reserve than human hearts. On CPB, the animals required a relatively high perfusion pressure, were prone to cardiac arrhythmias, sequestered volume in their spleno-splanchnic circulation, tended toward hypoglycemia, and had abnormally high base excess values. In baboons, the technical methods must enhance right coronary perfusion and avoid myocardial injury from ventricular distention or pressure overload (due to outflow restriction) even at levels that would typically be well tolerated by the human heart.

DISCUSSION

This pilot experimental series of 20 animals was designed to evaluate the tolerance limits of the proposed model. Therefore, biological test valves were derived from both human and baboon sources which ranged in size from donors smaller than the recipients to significant upsizing consistent with the clinical practice of homograft valve surgery in children. The average weight of full grown female baboons was approximately 18 kg. Full grown males weighed approximately 28 kg. When valved conduits from female or smaller male donors were selected for implantation into larger males, the valve annuli or distal pulmonary artery conduits were 1.0–3.0 mm smaller than the recipient. The pressure overload was poorly tolerated by the recipient and resulted in acute right ventricular failure. TEE imaging demonstrated significant pressure gradients across these smaller implanted valves, peaking as high as 50–60 mmHg with mean gradients ≥ 30 mmHg, which often proved to be fatal in this species. A strategy evolved to consistently obtain valves from larger male baboons (optimally 4 kg larger) for implantation into smaller males (mean upsizing of donor to recipient annulus = 4.7 mm). This was uniformly successful and is analogous to the standard upsizing used when implanting cryopreserved pulmonary valve conduits into small children. For example, surgical techniques such as proximal annulus V-plasty (a 'V' shaped incision across the annulus made to increase the pulmonary valve annulus diameter) and distal anastomosis spatulation have been frequently used in humans to allow significant over sizing of the conduit (5).

The Freestyle[®] porcine glutaraldehyde stentless valves implanted were 21 mm which resulted in a significant upsizing over the native valves of the recipients; mean annual

internal diameter = 16.4 ± 2.3 mm by Hegar dilator calibration and TEE annulus measurements. There was no geometric obstruction despite being a stiffer prosthetic valve.

In one case, an incision was made across the annulus of the native valve after excision of the native leaflets to assess the suitability of the baboon right ventricle for Tetralogy of Fallot surgical models. This resulted in RV dysfunction, difficult weaning from CPB and death within 180 minutes of cessation of mechanical support. The incision was only a 1 cm extension from the annulus into the substance of the infundibulum and did not cross any significant coronary arteries. This animal's reaction to this minimal Tetralogy-type of surgical incision best demonstrated the extreme sensitivity of the baboon right ventricle to such injury.

Also notable was the inotropic response to both sympathomimetic amines and phosphodiesterase inhibitors. While inotropic responses to both classes of drugs were documented by increased wall thickening as visualized on TEE imaging in the right and left ventricles, the chronotropic responses were relatively low suggesting a smaller level of β_1 receptor activity. Given the ability to rapidly augment preload with the contractile mesenteric venous capacitance reserve, the baboon heart seemed to be more similar to well-trained athletes (unlike children) in which a recruitable stroke volume reserve plays an important role augmenting heart rate responses for enhancement of cardiac output. The left ventricular dominance seemed to reflect a lower pulmonary resistance or a transthoracic pleural "pump" augmentation of RV output by upper body ambulation resulting in lower stroke work demands on the RV myocardium. In retrospect, all four acute deaths may have been preventable based on the knowledge learned during this model development pilot study with the exception of the animal with the acute anaphylactoid response.

Clinically analogous TEE can be accomplished during the same surgical anesthetic and perhaps indefinitely post-operatively using serial general anesthetic episodes with spontaneous ventilation. These results enhanced the confidence that mortality and morbidity due solely to technical perioperative factors, anesthesia, and conduct of CPB supported cardiac surgery can be kept very low, thus enhancing the efficiency and pertinence of baboon models for testing novel cardiovascular devices and biologics.

Normal pediatric CPB primes often add bicarbonate to buffer the crystalloid to prevent a dilutional base deficit. Crystalloid solutions with bicarbonate substrates (lactate, gluconate, acetate) still require the addition of bicarbonate to accommodate the child's inability to quickly convert the substrates to bicarbonate. No bicarbonate needed to be added to the circuit prime or during CPB in any of the animals. Pre-CPB alkalosis was commonly observed in the baboon and base excesses averaged 6 ± 5 mEq/L after CPB. This alkalosis may have been secondary to the dietary restrictions and the nothing by mouth status pre-CPB.

A notable difference between pediatric patients and baboons was the necessity for higher MAPs to maintain perfusion into the baboons' small right coronary artery. No cardiac arrhythmias (e.g., ventricular tachycardia) were encountered when MAPs were maintained from 60–85 mmHg through increases in CPB flow and phenylephrine administration. Arrhythmias were more common when the MAP dropped below 55 mmHg. These arrhythmias generally dissipated with higher perfusion pressures, suggesting a linkage to the very small size of the baboons' right coronary artery.

On CPB, these animals exhibited an exceedingly variable venous return, requiring as much as 1800 mL additional volume above the 700 mL circuit prime to maintain a safe operating level. This was likely a consequence of the muscularized spleno-splanchnic venous capacity of the baboon resulting in large and sudden changes in circulating volume during CPB. Variable sequestration of blood may have caused the need for additional volume administration and alpha agonist infusions to abate dilation of the venous system (25). Urine output was 15 ± 17 mL/kg/h in the surviving animals, which exceeded the volume additions needed to maintain CPB flow (10 ± 11 mL/kg). By comparison, the non-survivors had a urine output of only 3 ± 3 mL/kg/h, and required more crystalloid supplementation to maintain CPB flow (37 ± 30 mL/kg). Due to this unstable volume variation, gradual and careful separation from CPB was needed to avoid sudden venous return fluctuations. To prevent RV over distention and to control rapid volume shifts, it was found most efficient to use a mechanical venous line occluder and wean slowly under the supervision of both the surgeon's direct vision and the anesthesiologist who used the TEE for real-time assessment of cardiac chamber volumes.

The right ventricle is a critical performance variable in congenital heart disease and can be a limiting survival factor (26). The lack of RV functional reserve in these animals was intriguing and suggested that a chronic Fontan model might potentially be feasible based on this essential feature of the baboon heart.

An integral part of the surgical model development plan was to create a traveling cardiac surgery research team derived from clinical staff to perform surgeries in the baboon operating room facilities in conjunction with the resident veterinary staff of the SNPRC who are all primate specialists. Although this was a busy and experienced surgical primate center, recovery cardiac surgery had not been frequently or consistently performed. The Children's Mercy Hospital cardiac surgery program had experience with the logistics of traveling abroad to hospitals without an open heart surgery program with the intention to train and supply the locals to initiate open heart surgery and recovery in humans. These skills were transferable to a traveling "Cardiac Surgery Research Team." Unique

features of animal husbandry were required for the preoperative, perioperative, postoperative, and long-term recovery in this subhuman primate model and suggested that bringing the cardiac surgery research team to the primate facility was a better strategy than the reverse. This clearly was supported by this initial series of 20 animals.

An additional value of a facility such as the SNPRC was that there are a large number of animals available for selection as both donors and recipients thereby making accurate size matching feasible and assuring nonconsanguineous tissue transplants. The surviving subjects rapidly returned to normal physical, dietary, and social activities indistinguishable from their peers. The two animals that were electively allowed to survive beyond the 10 week interval to 6 months exhibited no limitation or symptoms.

As surgical strategies evolve using the various methods of tissue engineering, multiple models for testing viable tissue engineered cardiovascular implants will be required (5,20,27). Such novel cardiovascular replacement parts are without precedence and their development will benefit from the availability of biologically active subhuman primate chronic recovery models to bridge the gap from food-stock animals (e.g., sheep, goats, pig, and bovine) to Phase I human trials (20,28,29). Satisfactory validation of the safety and efficacy of such constructs will require various endpoints to be assessed including biological consequences, chronic hemodynamics, performance measurements, and immune/inflammatory issues that are novel and more complex than typically assessed for evaluation of the more familiar manufactured glutaraldehyde cross linked treated bioprosthetic valves. By definition these viable "replacement parts" for reconstructive cardiac surgeries should be capable of growth and matrix remodeling. Consequently, having a reproducible, high yield, open heart experimental model based on a semi-upright subhuman primate species will be an important research and development tool for testing biological materials of human origin.

The baboon model may be less valuable for testing future bioengineered bioprosthetic/biologic valves with scaffolds derived from lower mammals (e.g., porcine, bovine) due to the presence of xenoantigens not found in either hominoids or cercopithecoids (28). The fragility of the RV suggested that cardioplegic arrest may not be well tolerated by the baboon heart. Anatomically, the thick walled baboon aorta and narrow left ventricular outflow may make orthotopic aortic or mitral valve replacement models challenging—but this is not a problem on the right side.

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