Journal of Cardiothoracic and Vascular Anesthesia 36 (2022) 2847–2850

Contents lists available at ScienceDirect

Journal of Cardiothoracic and Vascular Anesthesia

journal homepage: www.jcvaonline.com

Editorial

Porcine Orthotopic Cardiac Xenotransplantation: The Role and Perspective of Anesthesiologists

ON JANUARY SEVENTH, 2022, the first genetically modified porcine cardiac xenograft was transplanted into a patient at the University of Maryland Medical Center. As members of the xenotransplant team and division of cardiac anesthesiology at the University of Maryland School of Medicine, the authors here had a role in this historic event. Cardiac xenotransplantation could become a common occurrence if it proves to be a viable answer for the limited supply of donor hearts to treat end-stage heart failure.[1] Early attempts at cardiac xenotransplantation and allotransplantation are well-known.[2-4] The case report[5] written by Dr Ozinsky, the anesthesiologist for the first human allogeneic heart transplant in 1967 at the Groote Schuur Hospital of the University of Cape Town in South Africa, was cited and celebrated in the *Journal of Cardiac and Vascular Anesthesia* 50 years later.[6] Despite the novelty of the surgery in humans, the team back in 1967 had the prior experience of completing 48 heart transplants in a large animal model. Likewise, the authors' cardiac anesthesiology team had considerable clinical experience not only in human heart transplantation but specifically in orthotopic xenotransplantation of genetically modified pig hearts into baboons well before January 2022.

The authors' experience started late in 2017 when an accomplished researcher, Dr Mohiuddin, brought his xenotransplant research to the University of Maryland. Xenotransplantation has numerous obstacles, including immunologic and physiological barriers related to natural antibodies to carbohydrates present on pig endothelium, uncontrolled complement activation, and incompatible procoagulant and anticoagulant proteins or receptors.[7] In addition, porcine xenotransplants have a potential infectious risk of transmitting porcine endogenous retrovirus to humans.[8] These issues add further levels of complexity to an already complex procedure in cardiac transplantation. Anesthesiologists can play a major role in facilitating success in this endeavor by striving to usher cardiac xenotransplantation surgeries through the perioperative period with a consistent and vigilant approach. Collaborative efforts between surgeons and anesthesiologists are vital to the future success of xenotransplantation.

The perioperative management of the first genetically modified pig-to-human cardiac transplant surgery will be presented in a follow-up case report. The overall significance of this transplant is that the cardiac function of the transplanted porcine heart was reliable throughout the perioperative period and allowed the patient to be weaned from and stay off all mechanical support for almost 2 months. The intraoperative course of the xenotransplant surgery was complicated by the development of type A aortic dissection. The need to re-arrest a newly transplanted heart was a significant event, and given the specter of perioperative cardiac xenograft dysfunction (PCXD), such a test for a xenotransplant is uncharted territory. Re-arresting the transplanted xenograft tested the cardiac preservation technique, physiological compatibility, and reserve. The entire procedure was a test of the immunogenic suitability and endurance of the porcine xenograft.

Genetic Engineering Toward the First Genetically Modified Pig-to-Human Cardiac Xenotransplant

An amazing amount of talented physician-researchers have made contributions to the science underlying xenotransplantation.[9,10] In 2014, 1-year cardiac xenograft survival was achieved in a heterotopic pig-heart-to-baboon model with 3 genetic modifications.[11] Acute host antibody-mediated rejection was addressed by knocking out the alpha galactosyltransferase gene (GTKO) combined with transgenic expression of human complement regulatory protein (hCD46) and human thrombomodulin (hTBM), which was added to prevent complications related to microvascular thrombosis.[12] An immunomodulatory treatment regimen, which included an anti-CD40 antibody, also was added.[13] This model initially was used for orthotopic heart transplants starting in late 2017.[14] In the authors' experience, pig-to-baboon xenotransplantation from 2017 to 2019 was associated with instances of PCXD, which manifested as a decrease in cardiac function despite the titration of intravenous (IV) inotropes. The addition of non-ischemic cardiac preservation with continuous ex vivo perfusion of the heart helped to improve PCXD and advance the baboon recipients through the perioperative phase.[15,16] Further genetic

https://doi.org/10.1053/j.jvca.2022.04.004

knockouts of β1,4-N-acetylgalactosyltransferase and cytidine monophosphate-N-acetylneuraminic acid hydroxylase inhibited the production of the carbohydrate antigens SDa blood group antigen (SDa) and N-glycolylneuraminic acid, respectively, and xenotransplant survival extended to 2 to 3 months.[17] Nevertheless, the heart appeared to develop overgrowth and thickening of the myocardium, with diastolic dysfunction resulting in ascites and heart failure.[18] The addition of growth hormone receptor knockout appeared to decrease this myocardial thickening, with diastolic dysfunction and extended survival past 9 months when added to genetic modifications of GTKO, β1,4-N-acetylgalactosyltransferase, hTBM, hCD46, hCD47, humanized heme oxygenase-1, decay-accelerating factor, and endothelial protein C receptor.[18]

Anesthetic Care for Pig-to-Baboon Cardiac Xenotransplants

The authors' group (ES, PO, and BW) was involved in the anesthetic care for 41 pig-to-baboon orthotopic cardiac xenotransplants, and the experience gained from these surgeries educated their approach to the first human transplant. Although many of the details of the authors' anesthetic care for these surgeries are not directly translatable or pertinent to human cardiac surgery, these experiences should be reported because they explain their approach to the first human xenotransplant and are useful for future reference.

The baboons weighed 15 to 30 kg, and after premedication with intramuscular ketamine 10 mg/kg, the baboon was shaved and scrubbed with chlorhexidine. Inhalation induction with sevoflurane 2.0% to 2.5% was performed prior to orotracheal tube placement, which varied in size from 6.5 to 8.0. Sevoflurane provided a predictable and easily titratable maintenance anesthetic, which facilitated emergence at the end of surgery. Rocuronium (20-30 mg) was administered in most cases after intubation. Baboons have suitable veins for 18-gauge catheters, which typically were placed in each forearm. All baboons had a tunneled 10F triple-lumen catheter surgically inserted into the jugular vein prior to the day of surgery, and this catheter was transduced to monitor central venous pressure but not typically used for intraoperative medication or fluid administration since it would be excluded from circulation with the heart explanted. A 12 cm 20-gauge catheter was inserted into the femoral artery and transduced for systemic pressure. A human adult-sized transesophageal echocardiography (TEE) probe X7-2t (Philips Medical Systems, Andover, MA) was inserted without difficulty in all baboons, and an iE33 machine (Philips Medical Systems) was used for imaging. Useful TEE images were obtained with difficulty as the sizes of the hearts, and the left atria (LA) were comparatively smaller and narrower than in humans, which provides a small imaging window. Post-xenotransplant TEE images, including the images of the orifice created by the LA suture-line, which joins the native and xenotransplant LA, are shown in video 1. A point-of-care blood gas and activated clotting time analyzer were used for all cases. Intraoperative immunosuppression medications were administered at various time points and consisted of infusions of rituximab (300 mL), ganciclovir (50 mL), tocilizumab (50 mL), and antiCD40 (150 mL), in addition to bolus dosing of solumedrol and etanercept. The total volume of these infusions (550 mL) was noteworthy, especially for smaller baboons (15 kg). Additional fluid administration was minimized whilst hemoconcentration was maximized on cardiopulmonary bypass (CPB). Ceftriaxone (50 mg/kg) was given for antimicrobial prophylaxis. Heparin was administered for CPB anticoagulation, with a goal activated clotting time above 480 seconds. Intraoperative pain control was addressed with IV fentanyl boluses (total 50-100 μg) early in the xenotransplant experience, but post-CPB boluses resulted in episodes of hypotension and delayed extubation. Buprenorphine IV (0.3-0.6 mg total) was used for pain control in more recent procedures, with a buprenorphine patch (10 μg/h) applied post-extubation. After separation from CPB, the heparin anticoagulation was reversed with a small bolus of protamine (20-40 mg). Minimal postoperative bleeding or coagulopathy was encountered throughout the experience, perhaps because the baboon sternum appeared to be less vascularized with less bleeding from needle holes and bone marrow compared to humans.

An article detailing the transplant surgery procedure and management of CPB is available in preprint.[19] Anecdotally, the porcine heart was exquisitely sensitive to inotropes immediately after separation from CPB. Epinephrine (0.01-0.04 μg/kg/min) and dobutamine (1.0-2.5 μg/kg/min) administration usually caused an initial overshoot in heart rate (>110) and systemic blood pressure (mean arterial pressure >70) with hyperdynamic function seen on TEE (Video 2). If the systolic function and systemic pressure decreased in the late intraoperative or early postoperative phase, inotropes could be started without such overshoot, but the need for inotropic support was usually a potential sign of pending PCXD. Overall, inotropes were avoided unless the right or left ventricular function was seen to be significantly depressed on TEE (Video 3).

The authors encountered episodes of ventricular arrhythmias resistant to cardioversion, which could be related to the phenomenon of PCXD; however, anatomic and physiological factors could have played a role. With the potential of using transgenic pigs for human cardiac transplants, Crick et al (*Journal of Anatomy*, 1999) studied the autonomic innervation of the pig heart with immunohistochemical and histochemical techniques, which showed important differences from human hearts. Pig hearts were shown to have an extensive innervation and an intrinsic supply of neural ganglia, which would be capable of functioning without efferent control.[20,21] The pig heart is known to develop ventricular arrhythmia, particularly in the setting of cardiac surgery with cardioplegia.[22] To mitigate perioperative arrhythmias, a combination of amiodarone (0.25-0.5 mg/min) and lidocaine (1-2 mg/kg/h) were started prior to porcine heart implantation. In addition, an infusion of dexmedetomidine (0.5-1.0 μg/kg/h) was administered to decrease surges of endogenous catecholamines and possibly decrease arrhythmia. A secondary cause of refractory arrhythmia could be intracardiac thrombus formation, which was common in xenografts without the hTBM gene modification.[23] Although instances of intracardiac thrombi were not recorded

with TEE, clots were seen on gross examination immediately after cardiac arrest. As the experience progressed with more genetic modifications and ex-vivo perfusion of the heart, the occurrence of ventricular arrhythmias diminished. Epicardial pacing was occasionally required before weaning from CPB, but the intrinsic rhythm was sufficient and preferred in most cases.

Early in the authors' experience, they observed that the porcine heart function and hemodynamics were overly sensitive to calcium chloride boluses (50-100 μg). Serum ionized calcium would predictably decrease below 1.00 mmol/L in the absence of regular calcium supplementation after CPB. This hypocalcemia may be related to immunosuppression medications, particularly anti-CD40. T-cell expression of CD40 ligand sensitizes bone marrow stroma cells to the parathyroid hormone for the induction of osteoclast formation, which would break down cortical bone and release calcium.[24] The anti-CD40 medication may produce a functional state of hypoparathyroidism, thereby causing this observed calcium-seeking physiology. Calcium chloride infusions (100-200 mg/h) provided stable hemodynamics and helped avoid unexpected declines in cardiac function after transplantation. The authors avoided calcium chloride bolus administration. Early postoperative extubation became a common goal as their experience progressed. Low rates of bleeding and improved cardiac function with the ex-vivo perfusion and improved genetic modifications facilitated this goal. Immediately after closure, a chest x-ray was taken to assess the lung fields for consolidation, effusion, and pneumothorax. Buprenorphine IV was administered close to the end of surgery, and a buprenorphine patch was applied as fentanyl was difficult to titrate and typically caused systemic hypotension and unpredictable respiratory depression. Dexmedetomidine was stopped while infusions of amiodarone, calcium chloride, lidocaine, and occasionally inotropic medications were continued. Sugammadex (2 mg/kg) was administered in some cases if rocuronium had been redosed. Once the baboons spontaneously began ventilating, they were transported to their cage while still intubated. With the door closed, the oxygen level in the cage could be increased. Each baboon was fitted into a restraining jacket, which held the mediastinal drains on bulb suction and protected the infusion tubing connected to their 10F tunneled central venous catheter. The external portion of the infusion tubing had metal armor, which was connected to infusion pumps outside of the cage. Intramuscular injections of ketamine (10-20 mg) facilitated this transition if the baboon emerged from anesthesia before the process could be complete. Vital signs were monitored by direct observation and with a surgically implanted hemodynamic telemetric monitoring device.[25]

Lessons Learned Before the First Pig-to-Human Cardiac Xenotransplant

Multiple years of experience with orthotopic cardiac xenotransplantation guided the authors' decision-making and prepared them for the clinical challenge of a porcine cardiac transplant into a human patient. Prior knowledge and experience in allograft transplantation, along with medication regimens and exposure to PCXD in the pig-to-baboon model, were brought to the operating room on January 7. The decision to administer amiodarone, dexmedetomidine, lidocaine, and calcium chloride infusions stemmed from this previous experience. Additionally, inotropes and other vasoactive agents, which commonly are used for allogeneic heart transplants, were avoided because of the observation of increased sensitivity of the porcine heart. Other potential issues related to size mismatch and small LA orifice area were anticipated well in advance by the surgeon, Dr Bartley Griffith, because of his previous experience in the pig-to-baboon model. Issues related to fluid management, CPB, and cardioplegia for the xenograft were well understood and anticipated by the perfusion staff because they also were involved in the pig-to-baboon surgeries. Team cohesion was facilitated by prior experiences and played a role in the management of the unexpected intraoperative aortic dissection. Early formation of a collaborative multidisciplinary cardiac surgery team made this translational research effort possible.

Conflict of Interest

None.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.jvca.2022.04.004.

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