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An Experimental Study of Decellularized Xenografts Implanted Into the Aortic Position with 4 Months of Follow Up

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Abstract

This study evaluated decellularized stentless porcine xenografts into the aortic position of seven juvenile sheep. The hemodynamic performance were analyzed by means of echocardiographic examination. Post mortum, specimens were evaluated by gross examination, light microscopy, and immunohistochemical staining. Explantations were performed at up to four months. Echocardiographic evaluation examination showed a maximum flow velocity of 0.94m/s with absence of regurgitation. Gross examination showed smooth and pliable leaflets endothelial cells covered the valve wall and leaflets, deeper layers presented ingrowth of host interstitial cells. There was no evidence for calcification. Our preliminary results showed excellent hemodynamics. Regeneration by host cells and absence of calcifications was observed at 4 months follow-up within the systemic circulation.

Keywords: Juvenile sheep; Tissue-engineering; Remodeling potential; Aortic valve; Systemic circulation

Introduction

Treatment of neonates suffering from critical aortic stenosis is a complex and demanding problem. If biventricular repair is selected in the neonate, surgical and transcatheter balloon valvotomy can be performed. However, both procedures are associated with high mortality, residual valve dysfunction and need for reintervention [1]. The Ross-Konno procedure can enlarge the aortic root, providing excellent hemodynamics without residual valve dysfunction. Furthermore, the pulmonary autograft implanted into the aortic position showed growth potential [2]. Allografts used to reconstruct the right ventricular outflow tract is subject to structural deterioration. It lacks potential of growth, which makes reintervention necessary [3]. Tissue engineering could provide an alternative treatment due to the potential of tissue to regenerate, remodel and grow [4]. Previous studies proved this in the pulmonary position [4-6], however, no data are currently available regarding tissue engineered heart valves implanted in the systemic circulation. This preliminary study presents first results of decellularized porcine xenografts implanted into the systemic circulation.

Materials and Methods

All experiments were performed in accordance with the "Principles of Laboratory Animal Care" prepared by the National Society of Medical Research, and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH, revised 1996). The study was approved by the institutional review and ethical committee of the Pontificia Universidade Catolica de Parana. Proceeding of a decellularized xenograft has been previously published [7].

According to the study protocol, animals were euthanized at one (n=1), two (n=1) and four (n=2) months to allow the exploration of remodeling and regeneration potential.

Surgery

After induction of anesthesia with 10 mg/kg of cetamine chloridrate i.v. (Vetaset®, Fort Dodge Saúde Animal, Campinas – Brazil), a left mini-thoracotomy was performed at the third intercostal space. Heparin was admitted 250 IU/kg intravenously (Liquemine®, Roche Quím. E Farm. S/A, São Paulo – Brazil). The descending aorta was dissected free and cannulated with a 21-Fr aortic tube. A 34-Fr cannula was used for venous return of the right atrium. Normothermic cardiopulmonary bypass and intermittent warm blood cardioplegia was given to both coronary ostia. A running 5-0 polypropylene suture lines was used for the proximal and distal anastomosis to implant a 16 mm decellularized xenograft (Figure 1a). After careful de-airing, cardiopulmonary bypass was discontinued. The chest was closed in layers, as soon as hemostasis was completed. Neither protamin, nor any anticoagulation or antithrombotic therapy was administered postoperatively. Pain medication was given as needed and antibiotic therapy was discontinued after five days.

Examination, explantation and analysis

Transthoracic echocardiography: Transthoracic echocardiography was done by a Hewlett Packard Sonos 5500 (Sunnyvale, CA, USA) with a 7.5 MHz probe to measure the flow velocity, eventually the length and width of the regurgitation jet and the internal diameter of the implanted valve. Examination was performed prior to termination the experiment. All animals were evaluated under general anesthesia. Valves were explanted after application of 250 IU/kg heparin intravenously.

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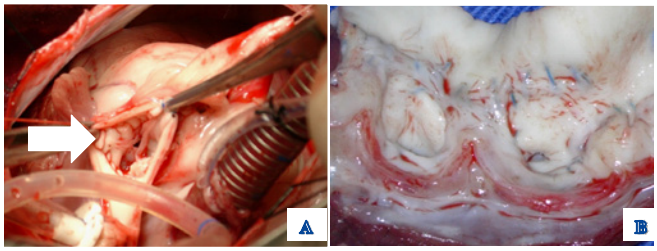

Figure 1: a) Intraoperative situs of the implanted decellularized xenograft (arrow). b) Explantation after 4 months of implantation. Appreciate the smooth and pliable leaflets at explant with absence of pannus formation.

Gross examination: The explanted valves were inspected and color photographs were taken. Especially, leaflets were inspected for fenestrations, retraction, thrombotic material and atheroma or calcification.

Histological examination: was performed from four micrometer thick longitudinally transected valves. Routinely stained with hematoxylin and eosin (HE), Serius red, Gomori, Weigert and von Kossa staining were performed on sections. Immunohistochemical staining was performed with factor VIII-related antigen (DAKO, Hamburg, Germany).

Results

At the end of surgery, three animals were euthanized due to respiratory insufficiency. However, gross anatomy inspection confirmed that the valves were correctly implanted and that the valves showed no abnormalities. In all other animals, no adverse events were noticed during follow up due to the implanted heart valves.

Echocardiography

Transthoracic echocardiography showed a valve diameter at four months of 17.0 mm in one and 20.8 mm in the other sheep. The maximum flow velocity was 0.94 and 0.83 m/s, respectively and a medium flow velocity of 0.53 and 0.57 m/s, respectively. Freedom of regurgitation was found in both valves.

Gross examination

At all time intervals the implanted decellularized xenografts were free of any of hematomas, vegetations, or thrombotic material. No signs of tearing, perforation, fibrous tissue overgrowth, cusp deformation, retraction or hardness were detected at the leaflets (Figure 1b).

Histology

Initially, islands of endothelial like cells were detected, however four months after implantation, a monolayer of endothelial-like cells were seen at the inner surface of the decellularized xenogenic valve (Figure 2a). Endothelial cell origin was confirmed by Factor VIII staining (Figure 2b). Von Kossa staining showed absence of calcification at four months of implantation (Figure 2b). Fibroblasts were identified in the deeper layers, which increased during time (Figure 2c), and the collagen structures were well preserved (Figure 2d).

Discussion

Our previous work was focused on the implantation of decellularized xenografts to reconstruct the right ventricular outflow tract (RVOT). After yielding promising results during animal experiments, first implants were made in human [11]. In neonates, infants and children this prosthesis is used to reconstruct the RVOT in complex congenital heart diseases or during the Ross procedure. In adult patients, decellularized xenografts are used to replace the pulmonary valve during the Ross procedure or to replace allograft with structural deterioration. Since these decellularized heart valves have been implanted in more than 1,000 patients, including patients suffering from pulmonary hypertension, the interest increased to develop a decellularized xenograft, suitable for implantation in the systemic circulation.

Initially, we felt more comfortable to implant the decellularized xenografts in a subcoronary position as this results in decreased wall-stress of the decellularized valve wall. The tissue mainly is stressed at the leaflets and valve commisures. The disadvantage during implantation is the demanding handling, however in the setting of the animal experiments performing a root replacement is also a difficult task since no protamine was given to neutralize heparin and blood transfusion was not available.

Echocardiography showed low transvalvular flow velocities and absence of regurgitation. There was also no retraction of the valve wall and leaflets observed, moreover there was a slight increase in the valve's annulus diameter noticed. Due to the fact of the limited number of experiments at four month of implantation, no conclusion can yet be made of any growth potential. Gross examination showed no tissue abnormality. This was confirmed by histological examination. In addition, there was after four months in-vivo no tearing or destruction of the extracellular matrix at systemic circulation. Confluent monolayers of endothelial cells were recognized at the leaflets as well as the wall tissue of the decellularized xenograft. Pankajakshan and Krishnan [12] studied the importance of non-synthetic scaffolds which has a crucial impact on supporting substantial signals for cell growth and tissue development in tissue engineering. His findings support again the importunacy of choosing a sufficient scaffold for cell ingrowth independent of pulmonary or systemic circulation circumstances. Comparing these results with earlier results of repopulation under low

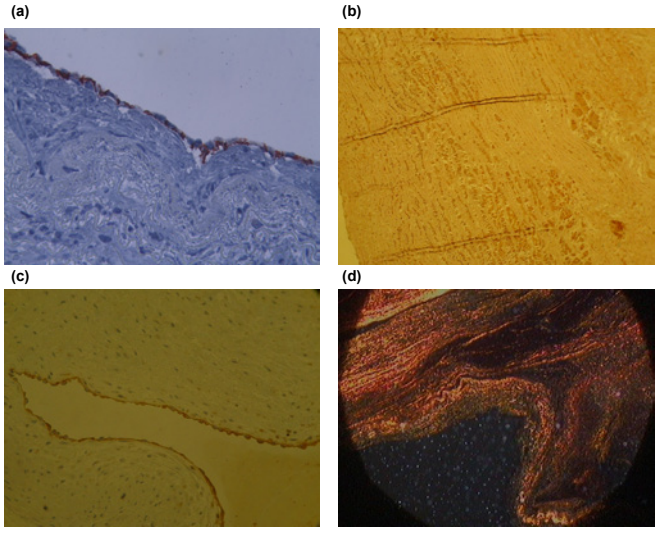

Figure 2: A represantative sample of an implanted decellularized xenograft after 4 months of implantation. a) CD 31 staining demonstrates a confluent monolayer of endothelial cells at the wall of the decellularized xenograft. b) Factor VIII staining showed a monolayer of endothelial cells at the leaflets. c) Von Kossa shows a lack of calcification of the decellularized xenograft. d) Serius red staining demonstrates the remodeling potential of the collagen.

pressure circumstances, our data suggest an earlier remodeling in the high pressure system. Further investigations are necessary to support this hypothesis. Von Kossa staining demonstrated lack of calcification which was previously reported in RVOT experiences [4,7] confirmed by earlier experiments of Thiene et al. [13] who was able to show similar calcification intensities in the right and left side.

In summary, these preliminary experiences showed excellent hemodynamics, earlier remodeling and regeneration potential of the implanted decellularized xenografts under systemic circulation circumstances. We believe that the ongoing experiments with increased number of animals as well as longer follow-up periods, will prove and rectify future use of decellularized valves implanted in the human systemic circulation.

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