Calcification of allograft and stentless xenograft valves for right ventricular outflow tract reconstruction: An experimental study in adolescent sheep

Willem Flameng, MD, PhD,[a] Ramadan Jashari, MD,[b] Geofrey De Visscher, PhD,[a] Lindsay Mesure, MSc,[a] and Bart Meuris, MD, PhD[a]

Objective: Aortic homografts were compared with pulmonary homografts in the setting of right ventricular outflow tract reconstruction in adolescent sheep. Furthermore, clinically available stentless porcine and bovine xenografts were studied as an alternative to homografts.

Methods: In 51 adolescent sheep cryopreserved aortic and pulmonary (ovine) homografts, as well as 6 different types of clinically available stentless bioprostheses (Prima Plus, Toronto SPV, Toronto BiLinx, Freestyle, Pericarbon Stentless, and Contegra) were implanted in the pulmonary position. After 5 to 6 months, the valves were explanted and studied for structural valve degeneration by means of radiographic analysis, histology, and calcium content determination.

Results: Pulmonary homografts calcified significantly less than aortic homografts in the wall portion. Leaflet calcification was mild, hardly detectable on radiographic analysis, and comparable between aortic and pulmonary homografts. Stentless porcine xenografts showed severe calcification in the aortic wall portion, irrespective of the antimineralization treatment. Leaflet calcification was mild and in the range of that seen in homografts. Pannus formation was present but never induced leaflet retraction or cusp immobilization. Calcification was absent in the stentless Pericarbon valve implants, but all valves showed extensive pannus overgrowth, leaflet retraction, and cusp immobilization. The Contegra valves showed wall calcification, but the leaflets were completely free of calcification and pannus.

Conclusions: For right ventricular outflow tract reconstruction, the pulmonary homograft remains the first choice. All xenografts result in either calcific degeneration or cusp immobilization. (J Thorac Cardiovasc Surg 2011;141:1513-21)

Valved homografts have become the most commonly used valved conduits for reconstruction of the right ventricular outflow tract (RVOT) because stented xenografts develop calcification, cusp tears, pannus formation, and typical cusp immobilization.[1-4] Recently, there is increasing evidence in congenital heart surgery that aortic homografts also calcify but faster and more significantly compared with their pulmonary counterparts. Therefore pulmonary homografts have become the conduit of choice for RVOT reconstruction, especially in young children.[4,5] However, given the relative shortage of small pulmonary homografts, it was suggested that aortic homografts still can be used in infants and older patients without compromising long-term results.[4]

It is the first goal of the present study to compare the calcification potential of aortic versus pulmonary homografts in an experimental setting in which growth and outgrowth of the allografts do not play a role. We included a series of aortic (ovine) homografts and a series of pulmonary homografts (originating from the same donor as their aortic homograft counterparts) in our adolescent sheep model experiments.[6]

On the other hand, more recently, several new types of heterografts have been developed and promoted as alternatives to homografts and stented xenografts. Compared with the older stented porcine heterografts mounted in a Dacron conduit, stentless design and antimineralization treatment render these new xenografts theoretically more suitable candidates to replace homografts than the older generation of conduits.[6] Different models of stentless xenografts are commercially available and clinically used. We tested 6 of them in our adolescent sheep model: 4 of them are glutaraldehyde-fixed porcine roots, and the other 2 are glutaraldehyde-fixed bovine tissue, either pericardium or valved jugular vein. Several of these valves are treated with an antimineralization treatment, although others are not. It was the second goal of this study to compare the calcification potential of these stentless

From the Laboratory for Experimental Cardiac Surgery,[a] Department of Cardiovascular Diseases, Katholieke Universiteit Leuven, Leuven, Belgium; and the European Homograft Bank (EHB) International Association,[b] Brussels, Belgium.

Disclosures: Authors have nothing to disclose with regard to commercial support.
Received for publication May 25, 2010; revisions received Aug 3, 2010; accepted for publication Aug 29, 2010; available ahead of print Nov 22, 2010.

Address for reprints: Willem Flameng, MD, PhD, Cardiac Surgery, University Clinic Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium (E-mail: willem.flameng@med.kuleuven.be).

0022-5223/$36.00

doi:10.1016/j.jtcvs.2010.08.082

ET/BS

Abbreviations and Acronyms
DW = dry weight
RVOT = right ventricular outflow tract

xenografts with that of (ovine) homografts as RVOT implants.

MATERIALS AND METHODS

Model

Fifty-eight Lovenaar sheep between 8 and 11 months of age (weighing 44.9 ± 6.2 kg) were used in the study. The animals were bred at a special unit of the Katholieke Universiteit Leuven and procured for research through the Animalarium Katholieke Universiteit Leuven. The experiments were approved by the Ethics Committee of the Katholieke Universiteit Leuven.

Seven animals were male and used as donors for allografts. They were premedicated with ketamine (10–20 mg/kg administered intramuscularly), and anesthesia was induced with increasing concentrations of isoflurane in oxygen. After heparinization (3 mg/kg), they were killed with an overdose of pentobarbital (Nembutal; Ovation Pharmaceuticals, Inc, Deerfield, Ill) and KCl intravenously. The pulmonary and aortic roots were excised from every sheep and cryopreserved as described below.[7]

Fifty-one animals were female and used as recipients for allografts or xenografts. The animals were premedicated with ketamine (10–20 mg/kg administered intramuscularly), and anesthesia was induced with increasing concentrations of isoflurane in oxygen. Anesthesia was maintained with isoflurane in 5 L/min O_2 and 2 L/min N_2O. A left thoracotomy was performed, and after administration of heparin (3 mg/kg), the animal was started on cardiopulmonary bypass. The main pulmonary artery was cross-clamped, the native pulmonary valve was excised, and the pulmonary root was replaced with either the allograft or the xenograft. After weaning from bypass, the chest and surgical wounds were closed, mechanical ventilation was discontinued, and the animal was allowed to recover.

At 5 to 6 months after surgical intervention, according to the protocol, the animals were again anesthetized and killed after heparinization (3 mg/kg) with an overdose of pentobarbital and KCl administered intravenously. The implanted bioprosthesis was excised and analyzed as described below.

Bioprostheses

Two different types of allografts and 6 different types of xenografts were implanted. The characteristics of these valves in terms of brand name, manufacturer, number of implants, donor tissue, antimineralization treatment, and tissue treatment are given in Table 1.

Allografts. The pulmonary and aortic roots from 7 donor sheep were processed by the qualified personnel of the European Homograft Bank (Brussels, Belgium).[7] Exactly the same procedure of cryopreservation was followed as is used for clinically available human homografts.[7] The allografts were sterilized in the antibiotic cocktail (Lincocin, Vancocin and Polymixin B) over 20 to 48 hours and cryopreserved by using liquid nitrogen with 10% dimethyl sulfoxide in Hanks' solution as a cryoprotectant, with controlled-rate freezing at 1°C per minute down to –40°C and 5°C per minute down to –100°C. They were stored in the vapors of liquid nitrogen at a temperature of less than –150°C. Just before implantation, they were thawed by means of rapid temperature increase according to the European Homograft Bank protocol, and the dimethyl sulfoxide dilution was performed with isotonic solution (saline) in 4 steps of 1 minute each, decreasing the dimethyl sulfoxide concentration from 10% to less than 1%.

Xenografts. Six different types of clinically available stentless bioprostheses were used (Table 1). All valves were cross-linked with glutaraldehyde. Four types (Prima Plus, Toronto SPV, Toronto BiLinx, and Freestyle) are porcine roots. The other 2 types were made from bovine tissue (ie, bovine pericardium [Pericarbon] or bovine jugular vein [Contegra]).

Explant Examination

After careful rinsing, gross examination of the explanted specimen was performed, with special attention to vegetations, cuspal hematoma, thrombosis, stiffness of the wall portion, visible calcifications, cusp retraction, tissue overgrowth (pannus), and leaflet tears and perforations.

Radiographic examinations were performed (Faxitron X-Ray, Lincolnshire, Ill), first in a horizontal plane perpendicular to the inflow–outflow axis showing the leaflets. The root was then longitudinally sectioned through the commissures, providing 3 wall portions, each containing one leaflet. The 3 sections were unrolled in a single plane, and radiographs were obtained.

Half of every section was used for calcium determination, and the rest was used for histology and further Faxitron analysis. This means that a longitudinal section (thickness of 1 cm) was made, including leaflet and inflow and outflow wall portions. Because this section and the histologic sections were taken adjacently, comparison between radiographic and histologic localization of calcifications became possible (Figure 1). For histology, 5-μm-thick sections were prepared, showing the entire wall and the leaflet of the bioprosthesis (Figure 1). The sections were embedded in paraffin and stained with hematoxylin and eosin, Masson's trichrome stain for collagen, Von Giesson stain for elastin, phosphotungstic acid–hematoxylin for fibrin, and Von Kossa stain for calcium.

For quantitative calcium determination, half of every segment was used, which means that 50% of the valve was analyzed for calcium content. The tissue was divided into conduits and leaflets. The conduit wall was divided into outflow and inflow portions. For quantification of the calcium content,

TABLE 1. Valve types and characteristics

	Valve type	Manufacturer	No. of implants	Antimineralization treatment	Tissue treatment
Ovine homograft	Aortic homograft	European Homograft Bank, Brussels, Belgium	7	None	Cryopreservation
	Pulmonary homograft	European Homograft Bank, Brussels, Belgium	6	None	Cryopreservation
Porcine xenograft	Prima Plus	Edwards Lifesciences, Irvine, Calif	8	Tween-80	Glutaraldehyde
	Toronto SPV	St Jude Medical, Inc, St Paul, Minn	5	None	Glutaraldehyde
	Toronto BiLinx	St Jude Medical, Inc, St Paul, Minn	8	Ethanol (leaflets); aluminum (wall)	Glutaraldehyde
	Freestyle	Medtronic, Inc, Minneapolis, Minn	7	α-Amino oleic acid	Glutaraldehyde
Bovine xenograft	Pericarbon	Sorin Biomedica, Saluggia, Italy	5	None	Glutaraldehyde
	Contegra	Medtronic, Inc, Minneapolis, Minn	5	None	Glutaraldehyde

ET/BS