A randomized assessment of an advanced tissue preservation technology in the juvenile sheep model

Willem Flameng, MD, PhD, Hadewich Hermans, MD, Erik Verbeken, MD, PhD, and Bart Meuris, MD, PhD

Background: Despite improved anticalcification technology, bioprosthetic heart valves still cannot be used in younger patients because of progressive structural valve degeneration. A novel advanced tissue preservation technology was developed that uses stable functional group capping and preservation by glycerolization. Valves incorporating this novel technology can be stored in a dry condition and do not require rinsing before use. The aim of the study was to assess the effects of this new technology in terms of valve function and durability in a chronic sheep model of orthotopic implantation.

Methods: Forty-five juvenile sheep were randomized and either a Perimount mitral valve (6900P, control group) or the same valve design incorporating the novel tissue preservation technology (test group) was implanted in the mitral position. All valves were 25 mm. A transthoracic echocardiography was performed at 1 week and at 8 months postoperatively. The animals were then killed, an autopsy was performed, and the valves were examined radiographically (soft tissue radiograph), histologically (hematoxylin and eosin and Von Kossa staining), and chemically (calcium content). Exclusion criteria for analysis included surgical or procedural death, bacterial endocarditis or other diseases leading to premature death.

Results: Thirty-one animals (14 controls and 17 test animals) remained in perfect condition during the 8-month follow-up period. Echocardiography at 1 week showed normal valve function in both groups. At 8 months, cardiac output increased significantly to the same extent in both groups (vs baseline; $P < .01$). The mean transvalvular pressure gradient also increased but significantly more in the control group compared with the test group ($P = .03$). Flow turbulence across the prosthesis was increased in the control valves compared with the test valves. The test valves had significantly less calcium content than the controls (1.9 ± 0.3 vs 6.8 ± 1.6 μg/mg; $P = .002$). This was confirmed by radiographic analysis and histology.

Conclusions: This study demonstrates that the novel tissue preservation technology, when applied to the Perimount mitral valve, significantly improves hemodynamic and anticalcification properties compared with the standard Perimount, a valve currently considered the standard of care. (J Thorac Cardiovasc Surg 2015;149:340-5)

See related commentary on pages 346-7.

Since the Starr-Edwards heart valve was first implanted in 1960, many generations of both mechanical and bioprosthetic valves have emerged. The advantages of bioprosthetic valves include a much lower frequency of thromboembolism and therefore long-term anticoagulation therapy can be avoided.[1] However, structural valve degeneration including calcification remains a major drawback with this type of prosthesis. Clinical data suggest that bovine pericardial valves calcify less than porcine valves.[2] This, however, does not imply that pericardial bioprostheses do not calcify. It has been extensively shown in sheep, as well as in humans, that pericardial prostheses of varying designs and from different manufacturers tend to calcify at different rates.[3-5] Strong predictors of valve calcification include the stress distribution within the valve and the absence of an anticalcification treatment, which can differ considerably among valve types.[6,7] In general, the biological tissue used in the construction of bioprosthetic heart valve substitutes is fixed in glutaraldehyde to cross-link the tissue, thereby preventing early degeneration of the material after implantation. However, glutaraldehyde fixation is also associated with in vivo calcification of tissue. To overcome these detrimental effects, various pre- and postglutaraldehyde fixation tissue treatments have been developed.[8,9] These

From the Laboratory of Experimental Cardiac Surgery, Department of Cardiovascular Diseases, Katholieke Universiteit Leuven, Leuven, Belgium.

Disclosures: For this sheep study, all costs related to animal care, surgery, postoperative follow-up, and postexplant analyses, were sponsored by Edwards LifeSciences, Irvine, Calif. Apart from this, the authors have no financial relationship with Edwards LifeSciences. More specifically, the authors have no relation to the further commercial development of this new tissue preservation technology. B. Meuris has received lecture fees from the Sorin Group.

Received for publication July 13, 2014; revisions received Sept 11, 2014; accepted for publication Sept 21, 2014; available ahead of print Oct 25, 2014.

Address for reprints: Bart Meuris, MD, PhD, University Hospitals Leuven, Cardiac Surgery, Herestraat 49, B-3000 Leuven, Belgium (E-mail: bart.meuris@uzleuven.be).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2014.09.062

Abbreviations and Acronyms
CEP = Carpentier-Edwards Perimount
EO = ethylene oxide

include detergents or surfactants, such as polysorbate 80 (Tween-80). Polysorbate 80 is the major component of the anticalcification technology used in the construction of the Carpentier-Edwards Perimount valve, which, in addition to its design characteristics that optimizes stress distribution and a special pericardial tissue assembling technique, may be responsible for its excellent long-term durability in elderly patients.[4] To be considered feasible in younger patients, however, the anticalcification properties of the leaflet tissue must be enhanced further. Therefore, an innovative advanced tissue preservation technology was developed.

The aim of this study was to compare the anticalcification efficacy of this novel technology with the standard technology in juvenile sheep, which currently represents the best preclinical model to gauge the potential for bioprosthetic heart valve calcification in humans.

MATERIALS AND METHODS

Animals

All animals were cared for by a veterinarian in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH publication 85-23, revised 1985). The Ethics Committee of the Katholieke Universiteit Leuven approved the study.

Forty-five female juvenile sheep, all less than 6 months of age and weighing between 22 and 38 kg, were obtained from the Zoötechnical Center, Katholieke Universiteit Leuven, and were quarantined at the animalium facility of the Medanex Clinic (Webbekom, Belgium) before undergoing surgery. After surgery, they were kept at this facility for 8 months.

Description of the Device

The Carpentier-Edwards Perimount (CEP) mitral valve (6900P) was used in the control group and the Perimount mitral valve (6900P) incorporating the novel tissue preservation technology was used in the test group (Figure 1). All valves used were 25 mm in size. The leaflets of the CEP 6900P mitral valve are composed of bovine pericardium. During manufacture, the pericardial tissue thickness is measured and leaflet deflection testing is conducted to characterize each leaflet with regard to elasticity. All 3 leaflets are matched for similar thickness and elasticity, after which each leaflet is mounted onto a wireform frame to minimize commissural stress points. Bovine pericardium was selected because of its superior intrinsic properties for valve manufacture, notably in terms of collagen content and tolerance to high bending curvatures.[2] The novel tissue preservation technology introduces several new approaches to preservation of tissue integrity: functional group capping, glycerolization, and a new terminal ethylene oxide (EO) sterilization process. Capping is used to reduce functional groups (eg, aldehydes) present in the glutaraldehyde-fixed tissue to prevent oxidation of the tissue and to mitigate tissue calcification. There are several ethanol rinse steps between capping and glycerolization that lower the amount of residual chemicals to acceptable levels. The other novel aspect of the technology is that the valves undergo treatment with a glycerol and ethanol mix, which displaces most of the water present in the pericardial tissue and replaces it with glycerol. As a result of glycerolization, the valves can be packaged and stored dry, without the need for any liquid-based storage solution such as glutaraldehyde. The valves are sterilized via 100% EO. Rinsing is not required before use.

Surgery

The animals were operated under general anesthesia. The animals were premedicated with ketamine (10-20 mg/kg body weight, intramuscularly). Anesthesia was induced with increasing concentrations of isoflurane (Isoba, Schering-Plough Animal Health, Middlesex, United Kingdom) in oxygen. Anesthesia was maintained with halothane and N_2O. After endotracheal intubation, mechanical ventilation was started. All ventilation parameters were adjusted to keep the arterial blood gasses and pH within the physiologic range. A large-bore orogastric tube was placed in the rumen, and allowed to drain by gravity to prevent rumenal distention. Electrocardiographic limb leads were connected and monitored. A maintenance intravenous drip of Ringer solution was started. Gentamycin (6.6 mg/kg, Genta-Kel 10%, Hoogstraten, Belgium) and benzylpenicillinum natricum (40,000 U/kg, penicillin, 1,000,000 units, Kela, Hoogstraten, Belgium) was administered venously. A left thoracotomy was carried out, then the animals were placed on cardiopulmonary bypass and mitral valve replacement was performed as described previously.[3] The animal was placed on the operating table in the right lateral recumbent position. The sheep was surgically scrubbed and draped to expose the distal left cervical region. An arterial pressure monitoring line was placed in the right central ear artery. A baseline blood sample was drawn. The left carotid artery and the jugular vein were freed from surrounding tissue. Afterwards, the left chest was prepped and draped, and a left thoracotomy was performed in the third interspace. The ribs were retracted for adequate exposure of the heart and the pericardium was incised in a T-shaped fashion anterior to the phrenic nerve. The heart was suspended in a pericardial cradle by suspension of the pericardial flaps. After administration of heparin (3 mg/kg), the carotid artery and the jugular vein were cannulated with an arterial cannula and a 2-stage venous cannula of appropriate size and connected to the heart-lung machine. Extracorporeal circulation was started and maintained at an adequate flow rate without cooling. Then, the heart was electrically fibrillated and the auricle of the left atrium was incised. The mitral valve was exposed and the left ventricle was emptied by suction through the mitral valve. The valve was implanted similar to a clinical situation (with separate, pledgeted, 2/0 Premicron sutures). After implantation of the valve, the left atrium was closed and the heart defibrillated. When the hemodynamics were stable, the heart-lung machine was stopped. The cannulas were removed and the vessels ligated. The wound in the neck was closed in layers and careful hemostasis was performed. The chest was closed in layers with a chest drain in the left pleural space. The valve was chosen immediately before surgery by picking an envelope by the study assistant.

Postoperative Care

The animal was weaned from the ventilator as soon as there was spontaneous respiration with adequate tidal volumes and stable hemodynamics. The animal was carefully observed during the immediate postoperative period for up to 7 days. The chest drain was removed after 3 hours when possible. The animals receive analgesics (piritramide) for the first 3 days on regular schemes as well as diuretics. Albipen LA and enoxaparine (20 mg twice daily) were administered for 3 days. Afterwards, the animals returned to the controlled animal facility where the general health of the sheep was checked daily.