Evidence of mitigated calcification of the Mosaic versus Hancock Standard valve xenograft in the mitral position of young sheep

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Objectives: Durability remains the main problem of all bioprosthetic valves, and calcification is the major cause of failure. New tissue treatment processes are expected to reduce mineralization. A comparative animal study was undertaken to evaluate the behavior of a new-generation porcine bioprosthesis in contrast with a first-generation porcine bioprosthesis. The primary goal was to evaluate the efficacy of α -amino-oleic acid as an anticalcification treatment.

Methods: Seventeen Targhee sheep (aged 4.5-7 months) had a mitral valve replacement with a Mosaic or Hancock Standard. The animals were followed up to 20 weeks (144.1 ± 4.0 days vs 144.3 ± 8.2 days) and then euthanized as scheduled. After gross examination, the explants were radiographed for the presence of calcification. The central portions were preserved for histologic examination, and the remainder of the sample was analyzed for quantitative calcium content by atomic absorption spectroscopy.

Results: Four Mosaic sheep were excluded because of perioperative surgical mortality. The remaining 13 were enrolled in the study (9 Mosaic and 4 Hancock Standard). The mean calcium content was 1.97 ± 2.21 $\mu\text{g}/\text{mg}$ tissue weight for Mosaic versus 8.36 ± 4.12 $\mu\text{g}/\text{mg}$ for Hancock Standard valves ($P < .01$). Mild fibrous tissue overgrowth and fibrinous lining were observed regardless the xenograft type.

Conclusions: The low level of calcification in the Mosaic versus Hancock Standard xenografts confirms the efficacy of α -amino-oleic acid treatment in mitigating mineralization. A longer durability is expected with the clinical use of the Mosaic porcine valve.

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This study was supported by an unrestricted research grant from Medtronic, Inc.

Dr Duran reports lecture and consulting fees from Medtronic. Dr Thiene reports lecture fees from Medtronic.

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J Thorac Cardiovasc Surg 2006;132:1137-43
0022-5223/\$32.00

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doi:10.1016/j.jtcvs.2006.06.027

The primary problem of all valve bioprostheses of xenogeneic origin, whether porcine or pericardial, is tissue degeneration with dystrophic calcification.¹⁻³ Structural valve deterioration results in patient morbidity that necessitates reoperation. Considerable effort over many years by the manufacturers of bioprosthetic valves has been directed toward developing tissue treatment, combined with glutaraldehyde fixation, to mitigate the detrimental effects of tissue calcification.⁴⁻⁹ We report the results of a comparative study between the Mosaic and Hancock Standard valve xenografts (both from Medtronic Heart Valve Division, Santa Ana,

Abbreviations and Acronyms

CPB = cardiopulmonary bypass

Calif) in a young sheep model as an accepted means of evaluating the effect of α -amino-oleic acid for the mitigation of calcification in a circulatory model.

Materials and Methods

Design of the Study

Mosaic and Hancock Standard porcine mitral valve prostheses of clinical quality (25-mm annular diameter) were implanted in young Targhee sheep (4.5-7 months of age, approximately 25-45 kg in body weight, and either neutered male or female). The order of valve implantation was determined by random selection. The valve to be implanted on a given day was unknown to the surgeon and the surgical team until the sheep was ready for implantation. According to the Experimental Standard Organization guidelines for in vivo preclinical valve testing,¹⁰ the valves were left in place for a minimum of 20 weeks.

All animals received humane care in accordance with the *Principles of Laboratory Animal Care* formulated by the Animal Welfare Act in the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (publication no. 85-23, revised 1996). The use of the animals for this study was also reviewed and approved by the Institutional Animal Care and Use Committee of the University of Montana.

Surgical Technique

Sheep were evaluated for body temperature, heart rate, and respiratory health (lung sounds and respiratory secretions were checked). Blood was drawn for a complete blood count and serum chemistry profile up to 1 month before surgery. All animals were fasted from food for 24 hours before surgery and from water for at least 16 hours. All animals received ceftiofur sodium (3.0 mg/kg) and gentamicin (80 mg) by intramuscular injection at least 90 minutes before the initiation of cardiopulmonary bypass (CPB). In addition, 80 mg of ceftiofur sodium was added to the CPB prime solution. All surgical procedures were performed by a single surgeon with the same surgical team. A 14-gauge angiocatheter was placed in the external jugular vein, and the animals were premedicated with ketamine 1.0 mg/kg and atropine 0.03 mg/kg, followed by propofol 4.0 mg/kg body weight. The sheep were endotracheally intubated, and artificial ventilation was achieved with a volume respirator (North American Drager, Telford, Pa) supplemented with oxygen at 4 L/min. Anesthesia was maintained with isoflurane (1.5%-2.5%) and intermittent propofol. A left thoracotomy was performed through the fourth intercostal space. Heparin 300 U/kg was injected intravenously as a bolus in preparation for CPB with a target activated clotting time of approximately 480 seconds. The descending aorta was cannulated with a 22F cannula. The right atrium was cannulated with a 29F double-stage venous cannula. The cardioplegia cannula was placed, and CPB was established. Cardioplegia (4:1 blood) was administered, and the heart was arrested. An oblique left atriotomy was per-

formed starting at the roof of the atrium and continuing through the left appendage to the atrioventricular groove. Duran sizors (Medtronic, Inc, Minneapolis, Minn) were used to determine the size of the mitral valve annulus. Eleven to 13 2-0 pledgeted and double-edged Ethibond (Ethicon, Inc, Somerville, NJ) sutures were placed all around the annulus. Sutures were then passed through the sewing ring of the bioprosthesis so that the largest intercommissural portion corresponded to the left outflow tract, thus assuring that each valve was placed in each animal in an identical position. The native leaflets and chordal structures were preserved in each case. The prosthesis was descended and tied at the annulus level. The left atriotomy was closed, and the heart was defibrillated as needed. Lidocaine and magnesium were administered if necessary. The sheep were weaned from CPB, and hemodynamics were allowed to stabilize before epicardial echocardiography. Protamine sulfate was administered intravenously. The aorta was decannulated, and hemostasis was checked. A chest tube was placed, and bupivacaine was administered along the third, fourth, and fifth intercostal nerves during closing. The muscles and skin were closed in 3 layers with 2-0 Vicryl (Ethicon) running sutures. After a minimum of 5 days of postoperative care, the sheep were returned to the farm.

Explant Analysis

On the scheduled date of termination, sheep were anesthetized as described for surgery. After all hemodynamic data were recorded as described previously, the sheep were euthanized, the gross pathology of the heart and lungs was observed, and any abnormalities were noted. The hearts were excised, the mitral valves were dissected from the heart, and photographs were taken of both the atrial and ventricular aspects. The condition of the valve was scored by the surgeon on a scale of 0 to 4 (with 0 representing the absence of a problem), for the following parameters: stiffness, leaflet insertion dehiscence, tissue overgrowth, thrombi, cuspal hematomas, calcifications, vegetations, fenestration or tears, stretching, abrasion, suture interactions, and structural relationships. The valves were placed in 10% neutral buffered formalin.

Radiography

Leaflets were removed from the stent and labeled anterior, left, and right according to the orientation of the valve in situ. Radiographs were taken with mammography equipment (LORAD M III; Bedford, Mass). Calcification was scored on a scale of 0 to 4 for each leaflet: 1, pinpoint single; 2, single more than 2 mm or multiple pinpoint; 3, multiple more than 2 mm; and 4, massive calcification. The overall mean score of the 3 leaflets was calculated.

Histology

The fixed samples were processed by cutting a 2- to 3-mm-wide radial section through the middle of each cusp along with the associated segment of sinus wall followed by dehydration and embedding in PolyFin wax (Polysciences, Inc, Warrington, Pa). The samples were sectioned at 5 μ m and collected on SuperFrost microscope slides (Fisher Scientific, Hampton, NH). All sections were stained with hematoxylin and eosin for general tissue morphology, Heidenhain trichrome for connective tissue, and Von Kossa for the presence of calcification. The thickness of fibrous tissue overgrowth was measured with a micrometer.

Quantitative Calcium Analysis

After the histology section was removed from each cusp, the remainders of each leaflet, including the aortic wall and base, were all placed into an appropriately labeled container and shipped to NAMSA (Northwood, Ohio) for analysis by inductively coupled plasma mass spectrometry. Calcium content was expressed as micrograms per milligram of tissue (sample weight).

Statistical Analysis

All numeric data are presented as mean $\pm$ SD. All statistical analyses were performed with SPSS (SPSS, Inc, Chicago, Ill). Differences between groups were determined by unpaired Student *t* tests for the radiography score. Normal probability plots showed that the quantitative calcium data had a nonnormal distribution. Evaluation of the quantitative calcium data found a single outlier in each valve group (Mosaic #330 cusp A and Hancock #311 cusp R). The Wilcoxon 2-sample test was used to compare the quantitative calcium data from the 2 valve groups.

Results

Survival Data

Seventeen young sheep (average weight, 37.2 ± 6.0 kg; average age, 6.2 ± 1.0 months) underwent a mitral valve replacement with a Mosaic or a Hancock Standard valve. Four sheep that received a Mosaic valve were excluded because of perioperative surgical mortality. Two animals died on the operating table of arrhythmia: one before cannulation and the other after bypass. Two sheep died after surgery: one at day 4 and one at day 5 of unknown causes. At necropsy, there was no hemothorax, and the valves were well seated without paravalvular leaks or thrombus. The remaining 13 were enrolled in the study and included 9 Mosaic and 4 Hancock Standard valves. All Mosaic and Hancock Standard sheep reached the planned sacrifice time of at least 140 days, for an average survival of 144.1 ± 4.0 days and 144.3 ± 8.2 days, respectively.

Echocardiographic Evaluation

At implantation, all valves were competent, gradients were normal, and mitral regurgitation was absent. Not all echocardiographic data at sacrifice were available, but mitral regurgitation, if present, was trivial for both valve types.

Macroscopic Evaluation

No valve showed evidence of coarse calcification or vegetation, leaflet tears, stenotic pannus, commissural dehiscence, paravalvular leak, or suture looping (average score: Mosaic, 0.11 ± 0.32 ; Hancock, 0.14 ± 0.35). Tiny cuspal hematomas were observed on 2 of the Hancock Standard valves. Small thrombus vegetations (<2 mm) were evident on 5 of 9 Mosaic valves and 2 of 4 Hancock Standard valves. Calcific nodules were seen by the naked eye in only 1 Hancock Standard (Figure 1, a).

Figure 1. Hancock Standard explant in place in the mitral position for 142 days (calcium content: $10.87 \mu\text{g}/\text{mg}$ tissue weight). **a**, Gross view of the cusps: the arrow indicates a calcific nodule in the left cusp close to the commissure. **b**, Radiograph: calcific deposits are mainly located at the commissures and aortic wall (mean score, 2.33). A, Anterior; L, left; R, right.

Radiograph Data

The mean radiograph score for the Mosaic valves was 1.15 ± 0.51 versus 2.58 ± 0.51 for the Hancock Standard ($P < .01$). Values for individual valves are shown in Table 1. In the Hancock Standard, calcification was focused in the aortic wall and commissures (Figures 1, b, and 2, a, and Table 1) and occasionally at the base of the cusps (Figure 3, a, and Table 1). Mineralization of the Mosaic valve spared the cusps and, when present, involved the aortic wall (Figure 4, a, and Table 1). Three of 9 Mosaic valves had no detectable calcification.

Histologic Analysis

Regardless of the valve type, the endothelial lining appeared mostly detached, and small thrombus vegetations were frequently seen on the atrial side of the cusps. The Hancock Standard xenograft showed a loose spongiosa and flattened collagen fibers. In contrast, the Mosaic xenograft showed a mostly preserved ground substance and wavy collagen in the fibrosa (Figure 4, b). Intracuspal hemorrhage was an occasional finding in both types of valves.

Host fibrous tissue overgrowth was observed mostly on the atrial surface, and it covered the basal part of 1 or more cusps. No difference in thickness was found between the

Table 1. Summary of major findings for individual cases

Sheep no.	Time in place (d)	Radiograph score (mean A, L, R)	Calcium content (mean A, L, R; $\mu\text{g}/\text{mg}$ tissue)	Site of calcification
Mosaic				
309	140	0.33	0.08	Aortic wall
315	141	1.7	2.4	Aortic wall
316	140	2	3.6	Aortic wall
321	149	0	0.04	None
314	148	2.7	3.6	Aortic wall
323	148	1	0.15	Aortic wall
326	148	0	0.03	None
330	141	0	2.71*	None
334	142	2.7	5.4	Aortic wall
Hancock Standard				
311	142	2.3	10.87*	Aortic wall and cusps
317	153	2.7	10.5	Aortic wall
333	148	2.7	5.9	Aortic wall and cusps
338	134	2.7	7.0	Aortic wall and cusps

A, Anterior; L, left; R, right. *Average of 2 leaflets.

Mosaic and Hancock Standard ($493 \pm 184 \mu\text{m}$ and $400 \pm 151 \mu\text{m}$, respectively).

In the aortic wall, calcium deposits most often occurred close to the commissures (Figure 2, b). A node of calcification was observed only in the free margin of a Hancock Standard cusp. When the base of the Hancock Standard cusp was mineralized, the muscle shelves appeared to be involved (Figure 3, b).

Atomic Absorption Spectroscopy

The mean calcium content for the Mosaic valve was $1.97 \pm 2.21 \mu\text{g}/\text{mg}$ tissue weight, compared with $8.36 \pm 4.12 \mu\text{g}/\text{mg}$ for the Hancock Standard group ($P < .0001$; Figure 5). Two data points were removed from the analysis on the basis of a statistical test for outliers: the value for cusp A from sheep 330 (Mosaic) and that for cusp R from sheep 311 (Hancock).

Discussion

Dystrophic calcification of glutaraldehyde-treated cardiac valve bioprostheses remains the main threat of long-term durability.¹⁻³ Major efforts have been made to develop different anticalcification strategies to prevent or mitigate the calcification process and improve long-term durability.⁴⁻¹⁰

Postfixation treatments that neutralize the toxicity of the aldehyde group residuals from the cross-linking have been used as possible anticalcification strategies.^{11,12} In this respect, the use of α -amino-oleic acid as a successful anticalcification agent for glutaraldehyde cross-linked tissue valves was first reported in a young sheep model in 1992 by Gott and colleagues,⁵ and a refined technique was described by the same group in 1997.¹³ Chen and colleagues^{6,14}

Figure 2. Hancock Standard explant in place in the mitral position for 134 days (calcium content: $7.00 \mu\text{g}/\text{mg}$ tissue weight). a, Radiograph: calcification mostly involves the aortic wall (mean score, 2.66). b, Histology of the aortic wall of the left cusp: note the calcific deposit involving the aortic tunica media (stain, Von Kossa; original magnification, $12\times$). A, Anterior; L, left; R, right.

a

b

Figure 3. Hancock Standard explant in place in the mitral position for 148 days (calcium content: $5.90 \mu\text{g}/\text{mg}$ tissue weight). **a**, Radiograph: the calcific deposits also involve the base of the leaflets (mean score, 2.66). **b**, Histologic panoramic view of the left cusp showing a large spot of calcium deposit on the muscle shelf of the leaflet (stain, Von Kossa; original magnification, $3\times$). A, Anterior; L, left; R, right.

showed that the mechanism of α -amino-oleic acid as an anticalcification agent may be multifactorial; besides bonding the free aldehyde groups, it greatly reduces calcium ion influx into glutaraldehyde-fixed bioprosthetic tissue, thus retarding the kinetics of crystalline calcium phosphate formation. A comparative study in the rat subcutaneous model of 5 commercially available bioprosthetic valves demonstrated the efficacy of α -amino-oleic acid (Medtronic Mosaic and Freestyle) and sodium dodecyl sulphate (Medtronic Hancock II) for the inhibition of tissue calcification compared with no special treatment (St Jude Toronto SPV; St Jude Medical, Inc, St Paul, Minn) or treatment with toluidine blue (Medtronic Intact).¹⁵ In a clinical report by Gerosa and associates¹⁶ that compared the Hancock Standard with the Hancock II bioprosthesis (which is fixed under low pressure and treated with sodium dodecyl sulphate), the

a

b

Figure 4. Mosaic explant in place in the mitral position for 140 days (calcium content: $0.08 \mu\text{g}/\text{mg}$ tissue weight). **a**, Radiograph with pinpoint calcification (arrow) at 1 commissure (mean score, 0.33). **b**, Histologic view of the left cusp showing the integrity of the cusp tissue, particularly the spongiosa (stain, Heidenhain trichrome; original magnification, $8\times$).

actuarial freedom from structural valve deterioration at 15 years was 50% in patients with the Hancock Standard bioprosthesis and 88% in those with the Hancock II bioprosthesis.

The Mosaic valve is a third-generation stented porcine bioprosthesis. Its improved design includes zero-pressure differential fixation of the glutaraldehyde-treated tissue, the addition of an α -amino-oleic acid postfixation treatment to retard mineralization, and the use of a flexible polymer low-profile stent. The results of the first clinical trials showed very satisfactory mid-term performance. In a study by Fradet and associates,¹⁷ freedom from structural valve deterioration was found to be 100% at 7 years in both the mitral and the aortic positions. The multicenter European experience reported by Eichinger and colleagues¹⁸ endorsed these findings, reporting freedom from structural valve deterioration in the aortic position at 5 years and in the mitral position at 4 years of 98.8% and 100%, respectively.

Figure 5. Calcium content by atomic absorption spectroscopy: $1.97 \pm 2.21 \mu\text{g/mg}$ tissue weight for the Mosaic versus $8.36 \pm 4.12 \mu\text{g/mg}$ tissue weight for the Hancock Standard (mean $\pm$ SD; * $P < .01$).

The young, growing sheep is a well-known model for accelerated calcification of bioprosthetic valves, and it is well accepted for the comparison of anticalcification treatments in circulatory implants.^{19,20} In our experiment, the calcification that regularly occurred in the Hancock Standard was mostly confined to the commissures, aortic wall, and muscle shelves, thus confirming that the young sheep model was effective in the 20-week span.¹⁰

The Mosaic porcine valves showed a sharp difference in calcification in radiographic (mean score of 1.15 ± 0.51 for Mosaic vs 2.58 ± 0.51 for Hancock Standard) and atomic absorption spectroscopy (mean calcium content of $1.97 \pm 2.21 \mu\text{g/mg}$ tissue weight for Mosaic vs $8.36 \pm 4.12 \mu\text{g/mg}$ tissue weight for Hancock Standard) measurements. In this regard, it is quite significant that 3 cases with the Mosaic bioprosthesis showed no evidence of calcification 20 weeks after implantation.

Our results support the findings of an earlier study by Duarte and colleagues,²¹ who reported that tissue calcification was significantly reduced in Mosaic valves placed in the mitral position of juvenile sheep when compared with Hancock Standard valves. In Duarte and associates' study, valves were radiographed in toto. Although this method makes it easier to view cusp calcification, it is impossible to evaluate calcification of the commissures and base of the valve because of the presence of the stent. In our experiment, valves were dissected from the stent before radiography, thus allowing the precise localization of all radiopaque calcium deposits. The reported radiograph score of calcification is representative of the total valve. Of note is that no calcification was localized in the cusps of the Mosaic valves, whereas evidence of cusp calcification was seen in the Hancock Standard valves. The absence of calcific deposits in the cusps of the Mosaic valves confirms the more effective anticalcification influence of α -amino-oleic acid on cusp tissue than on the aortic wall.^{6,14}

In Duarte and associates' study,²¹ in which cuspal calcium levels were analyzed separately from those of the aortic wall, the mineralization was $9.6 \pm 13.9 \mu\text{g/mg}$ for the Mosaic versus $130.8 \pm 43.2 \mu\text{g/mg}$ dry weight for Hancock Standard valves for cusps and $60.8 \pm 43.1 \mu\text{g/mg}$ for the Mosaic versus $149.4 \pm 43.6 \mu\text{g/mg}$ dry weight for the Hancock Standard in the aortic wall. The levels of quantitative calcium that we detected were much lower than those reported in the Duarte study, a finding that may be explained by the fact that the average age of the sheep at implantation in the Duarte study was 3 to 5 months, versus 4.5 to 7 months in our experiment. In contrast with the Duarte study, in which a sharp difference of survival was present in the Hancock Standard versus the Mosaic group (82.3 ± 35 days vs 139.9 ± 4 days, respectively), our experiment showed comparable survival between the 2 groups (144.3 ± 8.2 days vs 144.1 ± 4.0 days). The difference in survival is most likely attributable to increased calcification in the younger sheep resulting in excessive calcification and valve stenosis. This information is important in the planning of any study designed to distinguish anticalcification treatments of bioprosthetic valves.

Our results are also supported by a study by Flameng and associates²² that evaluated the Medtronic Mosaic valve along with the Epic (St Jude Medical, St Paul, Minn) Hancock II, and Labcor (Sulzer Carbomedics, Austin, Tex) valves in juvenile sheep for 3 and 6 months. Although this study did not compare the Hancock Standard valve and valves were implanted in the pulmonary position, thus resulting in less calcification, the Medtronic Mosaic valve clearly had significantly less calcium content, as measured by atomic absorption spectroscopy, than the Hancock II.

The limitations of our study include the small sample size in the control group and the fact that the study design did not consider differentiation between cusp and aortic wall calcification by quantitative methods, although our method of radiograph analysis allowed superior localization of calcification.

In conclusion, the low level of calcification in the Mosaic bioprosthesis (measured both by radiography and by atomic absorption spectroscopy) confirms the efficacy of α -amino-oleic acid treatment in retarding mineralization. A longer durability is expected with the clinical use of the Mosaic porcine valve.

We express our gratitude for the skillful collaboration given during this investigation by Emily Dove, Leslie Trail, Billi Billington, Holly Meskimen, Matthew Hiro, Mauro Pagetta, and Agostino Leorin. The authors also thank Katherine Johnson and Jorge Solis, MD, for their assistance with the statistical analysis.

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