Calcification of Porcine Prosthetic Heart Valves: A Radiographic and Light Microscopic Study

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SUMMARY To determine the incidence and extent of calcification of implanted glutaraldehyde-treated porcine prosthetic heart valves, 82 valves explanted from 73 patients were examined for calcium by radiography and light microscopy. At the time of valve implantation, the patients were 2/4–76 years old. They included 15 children (patients younger than 15 years of age, mean age at time of valve implantation 8.7 ± 4.1 years) and 58 adults (patients older than 15 years, mean age at time of valve implantation 53.5 ± 15.1 years). Valves explanted from children (average time implanted 4.6 ± 1.7 years) included four aortic, five mitral, as well as six right ventricle–pulmonary artery conduits and one left ventricle–abdominal aorta conduit. Valves explanted from adults (average time implanted 3.2 ± 2.5 years) included 32 aortic and 32 mitral, as well as one tricuspid valve and one valve from a right ventricle–pulmonary artery conduit. Calcification of explanted valves was graded from 0 to 4+ based on radiographs. All 16 valves from children were calcified, with grade 3+ or 4+ calcification in each of the aortic and mitral valves. In adult patients, calcification was present in 10 of 33 valves (30%) implanted for less than 3 years (average time implanted 1.0 year), in nine of 11 valves (82%) implanted for 3–5 years (average time implanted 3.7 years) and in 21 of 22 valves (96%) implanted for 5 years or longer (average time implanted 6.2 years). Analysis of variance demonstrated that calcification was strongly related to the duration that valves were implanted (p < 0.001). Age at the time of valve implantation also had a strong effect (p < 0.001) on the amount of valvular calcium. Valves from children showed the most calcification, and the amount did not change when valves were implanted in patients 30 years of age or older. Patient sex and valve position had no effect on the amount of calcification. Calcification occurred at each right- and left-heart valve position, most frequently at sites of commissural attachments.

GLUTARALDEHYDE-TREATED porcine prosthetic heart valves are popular because of their low thrombogenicity, which usually makes systemic anticoagulation unnecessary, and their favorable clinical performance compared with the best available mechanical prosthetic valves.1–3 An estimated 75,000 such bioprosthetic heart valves have been implanted during the past decade,4 and they are currently in widespread use. However, histopathologic changes in explanted porcine valves have raised concerns regarding their durability,4,5 particularly because calcium has been observed in many of these valves when they are explanted.5,6–10 Detailed pathologic studies led Ferrans et al.10 to conclude that the principal sites of calcium deposition in porcine valve prostheses are the connective tissue of the valve cusps and within small thrombi on the surface of the leaflets, and that calcific deposits can spread to other areas from these sites. Relatively few explanted porcine valves have been closely examined. The present study was undertaken to determine the prevalence and extent of calcium in glutaraldehyde-treated porcine heart valves that have been removed during the past 3 years at our hospital.

Materials and Methods

We studied 82 glutaraldehyde-treated porcine heart valves that had been explanted from 73 patients at Stanford University Hospital between August 1978 and October 1980. These included bioprostheses implanted at Stanford University Hospital and elsewhere in the aortic, mitral, and tricuspid valve positions, as well as conduits. Seventy-one of these valves were replaced with new glutaraldehyde-treated porcine valves and 11 valves were obtained at necropsy. Valves explanted because of primary tissue failure included those that had stenosis or regurgitation due to abnormalities of valve leaflets without history of endocarditis.

At the time of valve implantation, the patients were 2/4–76 years old, with 15 children (younger than 15 years of age), 11 boys and four girls, mean age 8.7 ± 4.1 years (± SD), and 58 adults (older than 15 years of age), 32 men and 26 women, mean age 53.5 ± 15.1 years. Valves explanted from children included four aortic, five mitral, six right ventricle–pulmonary artery conduits and one left ventricle–abdominal aorta conduit. Each of the 16 valves from children was explanted because of primary tissue failure, 13 due to stenosis and three due to regurgitation. Valves explanted from adults included 32 aortic and 32 mitral, as well as one tricuspid valve and one right ventricle–pulmonary artery conduit. Twenty-eight of the 66 valves from adults were explanted because of primary tissue failure, nine due to stenoses and 19 due to regurgitation. Among the 38 other valves explanted from adult patients, 14 were explanted because of endocarditis, 11 because of peri-prosthetic regurgitation, 11 at necropsy and two because of systemic emboli.
were explanted after having been implanted for less than 24 hours to 8.5 years, with an average duration of implantation of 4.6 ± 1.7 years (± SD) in children and 3.2 ± 2.5 years in adults. The shorter average time that valves were implanted in adult patients was affected by eight valves obtained at necropsy from adults who died within 1 year of valve operations.

At the time of explantation, the valves were placed in 10% buffered formalin. After radiography of the whole mounted valve using a Fexitron x-ray unit (30 kV), the valve leaflets were carefully removed and x-rayed separately. After studying the radiographs, sections for light microscopy were made through the valve leaflets to include calcified areas demonstrated by radiography. These sections of tissue were then decalcified and processed by conventional methods for paraffin embedding and sectioning. All sections were stained with hematoxylin-eosin, Masson’s trichrome and von Kossa’s method for calcium, and they were examined by light microscopy. The presence or absence of calcium observed microscopically was correlated with the corresponding radiographs.

Calcification of these valves was graded from 0 to 4+, based on the amount of calcium observed by radiography. Valve leaflets were divided into four regions: base, body, free edge and commissural attachment (fig. 1). Calcification in each of these four regions counted as one grade of calcification, irrespective of how many of the three leaflets were involved. Therefore, using this system of grading, calcification would be 4+ if all four regions of a single leaflet were calcified while it would be 1+ if the same region of all three leaflets were calcified.

The data were analyzed to assess the amount (i.e., radiographic grade) of valvular calcification as a function of the duration that valves were implanted, patient age at valve implantation, the reason for valve removal (stenosis, regurgitation, periprosthetic regurgitation, endocarditis, systemic emboli or at necropsy), patient sex and the position of the valve. The following model for the analysis of variance gave a satisfactory fit for the data from these 82 explanted valves:

\[
\text{amount of calcification} = \mu + \beta(\text{age}) + t_i + \text{error},
\]

where \(\mu\) = the grand mean or the average amount of calcification for these 82 valves, \(\beta\) = the coefficient of patient age in years at time of valve implantation, and \(t_i\) = the effect of duration implanted for \(i\) years. The "error" term in the model is equivalent to the unexplained variability in the data that has not been accounted for by the other parts of the model. More complicated models that accounted for the reason for valve removal, patient sex and valve position did not improve the model fit.

**Results**

Calcium at each of the valve sites that was indicated by radiography was confirmed by light microscopy. Examples of each grade of valve calcification are illustrated in figure 2. Calcium was observed at the base of the valve leaflet in 43 valves, in the body of the valve cusp in 31, near the free edge of the valve leaflet in 31, and at commissural attachments in 55. Light micrographs from valves with no calcification, focal calcification and diffuse calcification are shown in figure 3.

The duration that valves were implanted and the grade of calcification observed radiographically are summarized in figure 4 for children and in figure 5 for adults. Each of the 16 valves from children showed calcification, with 3+ or 4+ calcification in the nine valves from the aortic and mitral position and 1+ to 3+ calcification in the seven valves from conduits. From adult patients, calcification was present in 10 of 33 valves (30%) implanted for less than 3 years (average time implanted 1.0 year), in nine of 11 valves (82%) implanted for 3–5 years (average time implanted 3.7 years), and in 21 of 22 valves (96%) implanted for 5 years or longer (average time implanted 6.2 years). Forty-two of 44 valves explanted because of primary tissue failure and 16 of the remaining 38 explanted valves contained calcium.

The duration that valves were implanted had a significant effect \((p < 0.001)\) on calcification, with the amount of calcium increasing steadily for 6 years after implantation. The effect of patient age at valve implantation was also significant \((p < 0.001)\). Children had the most calcification. Maximal calcification was observed when age at valve implantation was near 15 years, and no change in the amount of valvular calcium was observed in patients 30 years or older. There was no relationship between the duration that valves were implanted and patient age at time of implantation that affected the amount of valvular calcification. The incidence of primary tissue failure increased the longer the valves were implanted; however, 13 valves without primary tissue failure were calcified. The reason for valve removal did not affect the amount of calcification, except that significantly less calcium \((p < 0.05)\) was present in valves that were replaced because of periprosthetic regurgitation. Patient sex and the position of the valve did not affect the amount of valvular calcification.
FIGURE 2. Radiographs of explanted porcine valves with 0 to 4+ calcification. (A) 0 calcification; size 31 valve from a 70-year-old man, explanted from the mitral position after 14 months because of dehiscence of sutures and 4+ mitral regurgitation. (B and C) 1+ calcification; size 33 valve from 36-year-old woman, explanted from the mitral position after 28 months because of dehiscence of sutures and 1+ mitral regurgitation. Small amounts of calcium are present at commissural attachments (arrows, panel C). (D) 2+ calcification; size 29 valve from a 41-year-old man, explanted from the mitral position after 68 months because of mitral regurgitation. Note calcium at the commissural attachments (black arrowheads) and at the base of one valve leaflet (white arrow). (E) 3+ calcification; size 20 valve from an 11-year-old boy with a right ventricle-pulmonary artery conduit, explanted after 61 months because of right ventricular outflow obstruction. Note the heavy deposits of calcium at the commissural attachments (black arrowheads), free-edge and base of the valve leaflet. (F) 4+ calcification; size 23 valve from an 11-year-old boy, explanted from the aortic position after 51 months because of aortic regurgitation. Note calcification at the commissural attachments, free-edge, body and base of the valve leaflets.

Discussion

Explanted glutaraldehyde-treated porcine heart valves contained calcium in direct relation to the duration that valves were implanted. While calcification of four of the 18 valves that were implanted in adults for less than a year is unusual, it is in keeping with the few cases of rapidly calcifying porcine valves that have been recorded.5, 9, 12, 13 None of the four patients in whom the valves were implanted had risk factors, such as renal failure,9 that may have predisposed them to early valve calcification. The significantly greater incidence of calcified valves (30 of 33, 91%) for valves implanted 3–8.5 years in our adult patients compared with previous pathologic studies in which calcium was detected in 34%11 and nearly 50%9 of porcine valves that had been implanted for an average of slightly more than 3 years, is probably due to our greater cumulative follow-up interval and to the radiographic method used, which is particularly well suited for localizing calcium.9

Valvular calcification was related to patient age at the time of valve implantation. The high incidence of calcified valves (16 of 16) and the usual presence of extensive calcification (grade 3+ or 4+) in porcine valves explanted from children confirms similar reports by other investigators.6–8, 13, 14 The predisposition to clinically significant valvular calcification in children is based on the pathologic confirmation of extensive calcification in seven of 14 porcine aortic or mitral valves implanted in children at our hospital that have required reoperation. We suspect that two other valves were calcified, but we did not have the opportunity to study them pathologically. The greatest amount of calcification was in valves implanted in patients near the age of 15 years, and no change in the amount of calcium was observed when valves were implanted in patients 30 years of age or older. We could not determine that valves calcified at an accelerated rate in adults younger than 35 years of age, as reported by other investigators.2 Although calcification of porcine valves has been associated with childhood,13, 14 chronic renal failure5, 9 and the presence of the calcium-binding
CALCIFIED PORCINE VALVES/Cipriano et al. 1103

**Figure 3.** Histologic sections of explanted porcine prosthetic heart valves that have (A) normal leaflet morphology, (B) focal calcification (arrows) with disruption of the morphology of the middle of the leaflet, and (C) diffuse calcification (dark areas) with disruption of the morphology of the entire length of the valve leaflet. Masson's trichrome stain; magnification × 7.

Amino acid gamma carboxyglutamic acid on the valve leaflets, the cause of calcification of glutaraldehyde-treated porcine valves is unknown.

We could not find a correlation between the propensity of a bioprosthesis to calcify and either valve position or patient sex, which confirms what other investigators have observed. The presence of calcification of porcine valves in all six of our right-heart conduits from children concurs with the recent demonstration of clinically significant obstruction to right ventricular outflow in conduits at the valve, which necessitated revision of the conduit. Valves removed for peri-prosthetic regurgitation were less calcified than those removed for valvular stenosis, valvular regurgitation, endocarditis, systemic emboli or at necropsy.

**Figure 4.** The grade of valve calcification observed by radiography and the duration valves were implanted for the 10 valves from patients younger than 15 years of age at the time of implantation. The diagonal line through valve symbol indicates primary tissue failure; □ = aortic position; ○ = mitral position; ▲ = right ventricle–pulmonary artery conduit. Asterisk indicates left ventricle–abdominal aorta conduit.
FIGURE 5. The grade of valve calcification observed by radiography and the duration valves were implanted for the 46 valves from patients older than 15 years of age at implantation. Diagonal line through valve symbol indicates primary tissue failure. ● = aortic position; ○ = mitral position; ▲ = right ventricle to pulmonary artery conduit; □ = tricuspid position.

The most frequent site of calcification in porcine valves was at commissural attachments, as reported previously, with calcification occurring less frequently in the base, body and near the free edge of the valve leaflets. Commisural attachments may be sites of high mechanical stress and tissue damage due to flexure compression; however, whether this contributes to the predilection of calcification in these areas in porcine valves is unproved.

The decrease in actuarial probability of freedom from valve failure among adult patients with porcine valve replacement at our hospital between the fifth and sixth years after valve implantation is a sign of accelerated primary tissue failure that appears to be related to valve calcification. Calcification of 16 valves that were explanted without demonstrable primary tissue failure suggests that progressive calcification antedates, and probably contributes to, eventual tissue failure. Thus, calcification represents a feature of the ongoing degenerative process of the presently used porcine valves.

Addendum
Since this study was completed, we have radiographed an additional 45 gluteraldehyde-treated porcine prosthetic heart valves (23 aortic and 22 mitral) explanted at the time of valve replacement. Thirty-eight of the 45 explanted valves (84%) contained grade 1+ to 4+ calcium (8, 1+; 11, 2+; 11, 3+; and 8, 4+). Although the clinical data have not been completely analyzed, these findings support the observation that calcium is frequently present in explanted prosthetic heart valves of this type.

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