Inhibition by diphosphonate compounds of calcification of porcine bioprosthetic heart valve cusps implanted subcutaneously in rats

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ABSTRACT Calcification limits the long-term success of heart valve bioprostheses fabricated from glutaraldehyde cross-linked porcine aortic valves. The pathophysiology of calcification of bioprostheses has been studied experimentally with subcutaneous implants of the valve cusps in rats; in this preparation, the accumulation of calcific deposits is biochemically and morphologically identical to that occurring in clinical specimens. The objective of the present study was to determine whether mineralization of bioprosthetic valve cusps (BC) subcutaneously implanted in 3-week-old male rats could be inhibited through the use of diphosphonate compounds. Ethanehydroxydiphosphonate (EHDP), administered by daily subcutaneous injection (25 mg/kg/24 hr) for 21 days inhibited calcification (BC Ca++ = 4.2 ± 0.7 μg/mg, mean ± SE) compared with control (BC Ca++ = 154.9 ± 4.1), but caused somatic growth retardation and disruption of epiphyseal development. However, local administration of EHDP by osmotic pump (5 mg/kg/24 hr) implanted in direct contact with the cuspal tissue for 14 days prevented BC calcification (BC Ca++ = 4.3 ± 0.7) without adverse effects. Furthermore, EHDP given by osmotic pump had a prolonged effect on reducing calcification, as demonstrated by implants harvested 21 days (BC Ca++ = 12.2 ± 6.4) after the drug supply was exhausted. Finally, BC preincubated in aminopropanehydroxydiphosphonate for 24 hr before 21 day implantation underwent less calcification (Ca++ = 24.2 ± 7.4) than control valves (BC Ca++ = 126.6 ± 7.5) with no adverse effects. We conclude that diphosphonates inhibit BC calcification, and that adverse effects of systemic therapy can be avoided by local administration.


BIOPROSTHETIC cardiac valves (BCs) fabricated from glutaraldehyde-treated porcine aortic valve cusps1-3 or bovine pericardium4-6 have assumed an important role in the surgical management of valvular heart disease, but calcification commonly causes their failure.7-10 The pathophysiology of this disease process has recently been reviewed11 and found to have the following features: The predominant morphology is one of intrinsic calcification, with mineral deposits located deep within the valve cusps in the central spongiosa region of the valve.12-14 These deposits have been localized by electron microscopy in devitalized porcine connective tissue cells13,15 and along collagen fibrils.13-15 The calcium typically cause either cuspal stiffening leading to valve stenosis or a disruption of the tissue planes in the cusps, thereby causing tears and valvular regurgitation.

The pathophysiology of BC calcification has been studied with subcutaneous implants of BCs in rabbits,6,15 rats,17,18 and mice,19 and after 2 to 3 weeks all of these preparations developed intrinsic calcific deposits that were morphologically and biochemically identical to those that have been observed in valves retrieved from humans after long-term implantation.17 The mineral phase involved in both the experimental and clinical calcifications is poorly crystalline hydroxyapatite, the predominant mineral phase of bone.17 The principal accelerators of BC calcification noted in circulatory explants — young age8-10 and mechanical stress14, 19, 20 — also potentiate mineralization of subcutaneous explants.17, 21

The objective of the present study was to determine if mineralization of BCs subcutaneously implanted in 3-week-old male rats could be inhibited through the
use of diphosphonate compounds. Diphosphonates are pyrophosphate analogues that can bind to developing hydroxyapatite crystals, thereby sterically inhibiting further crystal growth, and as a result inhibiting calcification. The effects of ethanehydroxydiphosphonate (EHDP) on subcutaneous BC calcification were studied by comparing effects of systemic administration by daily subcutaneous injection to those of local administration. The local therapy approach was studied to minimize the adverse effects of diphosphonates on bone development and calcium metabolism. Local therapy was administered by either the coimplantation of osmotic drug delivery pumps loaded with EHDP, or by the incorporation of the diphosphonate compound disodium aminopropanehydroxydiphosphonate (APDP) into the BC tissue matrix before implantation.

Methods

Materials. Analytic grade glutaraldehyde was obtained as a 50% aqueous solution (Eastman-Kodak, Rochester, NY). EHDP was provided as the disodium salt by the Procter and Gamble Company (Cincinnati) and was prepared as a 100 mg/ml aqueous stock solution with the pH adjusted to 7.4. Dilutions of the EHDP solution were made as necessary for the daily injections and osmotic pump loading. APDP was donated by Dr. O. Bijvoet of the University of Leiden and was prepared as an aqueous solution identical to that of the EHDP.

Glutaraldehyde-preserved porcine aortic valves were prepared as previously described and were stored in 0.2% glutaraldehyde in 0.05M HEPES buffer (pH 7.4). All animals used in these studies were male CD strain rats obtained at 3 weeks of age from Charles River Laboratories (Wilmington, MA). The rats were maintained on a diet of Lab Chow (Ralston-Purina, St. Louis). Local drug delivery was carried out with ALZET (Alza Inc., Stanford, CA) osmotic implantation pumps (Model 2002), which are 14 day drug delivery systems. These devices deliver a total volume of 180 μl beginning 6 to 8 hr after biologic implantation, and continue to deliver drug at a well-documented flow rate of 0.5 μl/hr until the reservoir has been exhausted.

Surgical procedures. Implant and retrieval of cusps were done under ether anesthesia. Cusps were either inserted directly into subcutaneous pockets over the rat’s ventral abdominal wall, or they were first attached to an ALZET osmotic pump by the removable pump head, and then inserted. For each group of ALZET implant experiments control experiments were performed in which ALZET pump implants loaded with NaCl at an identical ionic strength as the diphosphonate were used. Blood sampling was done by cardiac puncture at the time the animals were killed and femurs were also routinely removed at this time for assessment of bone morphology.

Protocols for administration of diphosphonate (table 1)

Group 1: systemic EHDP. Group Ia was divided into three subgroups. Group Ia (treated group, high dose) included rats that were implanted subcutaneously (ventral) with BCs and that received daily weight-adjusted subcutaneous (dorsal) injections of EHDP of 25 mg/kg/24 hr beginning in the paraoperative period and continuing for 21 days. Group Ib (treated group, low dose) consisted of rats implanted as above that received daily injections of EHDP of 5 mg/kg/24 hr beginning in the paraoperative period and continuing for 21 days. Group Ic (control group) included control animals with implanted BCs that received NaCl injections at an identical osmotic strength to that of the EHDP used in group Ia.

Group II: local EHDP. Subgroups of group II were as follows: Group IIa (treated group) rats were implanted subcutaneously with BCs and received local EHDP through adjacently implanted ALZET 2002 osmotic pumps (with the cuff attached directly to the head of the pump) as described above under Surgical procedures. The 180 μl drug reservoirs of these devices were loaded with an EHDP solution prepared as above at a concentration of 0.14M. This amount of drug can be calculated to be roughly equivalent to a dose of 5 mg/kg/24 hr based on mean animal weights during the course of the experiment. These devices were left in place for 14 days, at which time implants were harvested. Group IIb (control group) rats were implanted with subcutaneous BC implants and control pumps loaded with NaCl at an identical osmotic strength to the EHDP used in group IIa.

Group III: local EHDP with continued implantation after therapy. Group III contained two subgroups. Group IIIa (EHDP group) rats were implanted with subcutaneous BCs and received local EHDP by ALZET 2002 osmotic pump under the same conditions as in group II, except that the pumps and adjacent BCs were not removed until 35 days after implantation (21 days after the completion of local drug administration). Group IIIb (control group) consisted of BC-implanted animals that received NaCl through ALZET 2002 pumps as described above (group II).

Group IV: EHDP and APDP pretreatment. The three subgroups of group IV were as follows: Rats in group IVa (APDP pretreated BC) were implanted with subcutaneous BCs preincubated in 1 ml of APDP (0.14M, pH 7.4) per valve cuff for 24 hr at room temperature. After the pretreatment, cusps were washed exhaustively in saline to remove nonbound APDP, and were then implanted. Rats in group IVb (EHDP-pretreated BC) were implanted for 21 days with BCs preincubated in EHDP at the same concentration and under the same conditions as for the APDP. Pretreated cusps were rinsed in saline as above (group IVa). Control rats in this group (IVc) received subcutaneous BC implants that were preincubated in 0.14M NaCl (0.05M HEPES buffer, pH 7.40) for 24 hr at room temperature.

Unimplanted cusps preincubated in APDP, EHDP, and HEPES buffer were rinsed with copious volumes of saline, and then distilled water, and dried to constant weight in a vacuum desiccator. These cusps were then ashed in porcelain crucibles at 600°C. The ashed specimens were dissolved in 0.01M HCl and then analyzed for phosphorus so that levels of bound diphosphonate could be determined.

Morphologic analyses. A representative portion of each retrieved valve cuff specimen was immediately fixed in cacodylate-buffered 2.5% glutaraldehyde–2% paraformaldehyde solution at pH 7.2. Premiers of control and EHDP-treated animals were dissected free of attached muscle and fixed in 10% neutral buffered formalin. Proximal and distal femurs were processed undecalcified. Specimens of valves and bones were dehydrated in graded ethanol solutions after 24 hr of fixation (valves only), embedded in glycolmethylmethacrylate medium (JB-4, Polysciences, Warrington, PA), sectioned at 2 to 3 μm, and stained with hematoxylin and eosin (for overall morphology) and Von Kossa’s reagent (for calcium phosphate). After light-microscopic examination, selected specimens were examined by transmission electron microscopy.

Biochemical analyses. Retrieved BCs were lyophilized and prepared as liquid nitrogen milled powder. Aliquots of the powders were dried to constant weight in a vacuum desiccator, and then subjected to acid hydrolysis by a previously described method. Aliquots of the hydrolysates were analyzed for calcium (by atomic absorption spectroscopy) and phosphorus.
TABLE 1  
Effects of diphosphonate compounds on subcutaneous bioprosthetic cusp calcification

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/24 hr)</th>
<th>Route of dosing</th>
<th>Duration of dosing (days)</th>
<th>n</th>
<th>Valvar levels (µg/mg cusp) Ca</th>
<th>Phosphorus</th>
<th>Serum Ca (mg/dl)</th>
<th>Weight at death (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>25</td>
<td>SCB</td>
<td>21</td>
<td>19</td>
<td>4.2 ± 0.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.7 ± 0.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>10.9 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>157.4 ± 3.2&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ib</td>
<td>5</td>
<td>SCB</td>
<td>21</td>
<td>10</td>
<td>93.2 ± 6.5&lt;sup&gt;  &lt;/sup&gt;</td>
<td>51.2 ± 3.0</td>
<td>9.5 ± 0.2</td>
<td>209.2 ± 7.1</td>
</tr>
<tr>
<td>Ic</td>
<td>—</td>
<td>—</td>
<td>21</td>
<td>19</td>
<td>154.9 ± 4.1</td>
<td>90.0 ± 3.1</td>
<td>9.4 ± 0.2</td>
<td>220.8 ± 4.7</td>
</tr>
<tr>
<td>IIa</td>
<td>5</td>
<td>ALZET</td>
<td>14</td>
<td>8</td>
<td>4.3 ± 0.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>3.0 ± 0.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>10.1 ± 0.2</td>
<td>129.6 ± 3.6</td>
</tr>
<tr>
<td>IIb</td>
<td>—</td>
<td>—</td>
<td>14</td>
<td>12</td>
<td>135.4 ± 6.2</td>
<td>65.9 ± 9.4</td>
<td>10.1 ± 0.2</td>
<td>134.0 ± 4.3</td>
</tr>
<tr>
<td>IIIa</td>
<td>5</td>
<td>ALZET</td>
<td>35&lt;sup&gt;C&lt;/sup&gt;</td>
<td>15</td>
<td>12.2 ± 6.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>11.5 ± 7.0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>9.1 ± 0.4</td>
<td>298.4 ± 8.3</td>
</tr>
<tr>
<td>IIIb</td>
<td>—</td>
<td>—</td>
<td>35</td>
<td>14</td>
<td>130.9 ± 7.4</td>
<td>84.4 ± 5.8</td>
<td>8.4 ± 0.3</td>
<td>320.1 ± 10.0</td>
</tr>
<tr>
<td>IVa</td>
<td>—</td>
<td>Pretreated</td>
<td>21</td>
<td>10</td>
<td>24.2 ± 7.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>15.3 ± 4.0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>9.3 ± 0.3</td>
<td>194.1 ± 3.5</td>
</tr>
<tr>
<td>IVb</td>
<td>—</td>
<td>Pretreated</td>
<td>21</td>
<td>11</td>
<td>114.2 ± 10.4</td>
<td>66.5 ± 5.6</td>
<td>10.1 ± 0.1</td>
<td>218.3 ± 5.7</td>
</tr>
<tr>
<td>IVc</td>
<td>—</td>
<td>—</td>
<td>21</td>
<td>9</td>
<td>150.9 ± 7.3</td>
<td>89.9 ± 4.8</td>
<td>9.5 ± 0.2</td>
<td>196.2 ± 5.0</td>
</tr>
<tr>
<td>Unimplanted cusp</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>2.8 ± 0.7</td>
<td>2.5 ± 0.5</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>A</sup>Plus or minus' means are value ± SEM.  
<sup>B</sup>p<.001 compared with control.  
<sup>C</sup>Daily subcutaneous injection.  
<sup>D</sup>Implant continued for 21 days after depletion of contents of osmotic pump.

Data analysis. Grouped data were analyzed and expressed as the mean ± SEM. Statistical significance of differences between results in treated and control specimens within groups was tested by Student’s t test<sup>28</sup>; level of significance was set at p < .001.

Results

Efficacy of diphosphonates for inhibition of calcification

Chemistry. Calcification was markedly reduced compared with control in all except treatment group IVb (table 1, figure 1). BCs retrieved from group Ia animals were devoid of calcification (table 1). There was no significant calcium uptake by these cusps compared with unimplanted cusps. BC cusps coimplanted with ALZET 2002 osmotic pumps for 14 days (group IIa) also had no significant calcification and the local EHDP therapy administrated to group IIa rats provided lasting protection against calcification (table 1). Cusps pretreated in APDP (group IVa) had significantly less calcification than either control implants or EHDP-pretreated cusp implants (groups IVc and IVb, respectively) (table 1). In contrast, the cusps of animals in group IVb demonstrated severe calcification (table 1).

APDP preincubation resulted in significant cuspal incorporation of this diphosphonate compound compared with control (table 2) according to ash analyses for recoverable phosphorus. EHDP preincubation did not result in a significant accumulation of phosphorus compared with control. By subtracting control phosphorus levels from those measured in the ashed APDP specimens it was calculated that approximately 53.8 nM/mg of cusp of APDP was incorporated.

Cuspal morphology. The morphologic characteristics of the implanted porcine aortic valve cusps are illustrated in figure 1. Valve cusps from animals either not receiving diphosphonates or implanted with EHDP-pretreated cusps had mineral deposits diffusely throughout the tissues that were most prevalent in the valvular spongiosa, which was consistent with previous observations<sup>17</sup>. The morphologic characteristics of cusps removed from diphosphonate-treated animals and those of cusps pretreated with diphosphonate compounds were consistent with the chemical determinations of mineralization. Specifically, no mineral deposits were detectable by light microscopy in cusps from experimental groups Ia, IIa, or IIla. In group IV cusps, no mineral deposits were detectable after pretreatment with APDP; qualitatively less mineralization was noted in EHDP-pretreated cusps compared with control. Likewise, ultrastructural examination of cusps...

TABLE 2

Diphosphonate pretreatment of bioprosthetic cusps — studies of bound phosphorus determined by ash analyses

<table>
<thead>
<tr>
<th>Type of pretreatment</th>
<th>n</th>
<th>Phosphorus (µg/mg)cusp&lt;sup&gt;A&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>APDP</td>
<td>3</td>
<td>4.84 ± 0.8&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>EHDP</td>
<td>3</td>
<td>1.90 ± 0.55</td>
</tr>
<tr>
<td>Control (HEPES buffer&lt;sup&gt;C&lt;/sup&gt;)</td>
<td>3</td>
<td>1.18 ± 0.33</td>
</tr>
</tbody>
</table>

<sup>A</sup>Mean ± SEM.  
<sup>B</sup>p<.001 compared with control.  
<sup>C</sup>Cusps preincubated in HEPES buffer, pH 7.4 (see Methods).
Figure 1. Photomicrographs of valve cusp implants from unimplanted, control, and treated groups. A. Unimplanted valve. Note layered architecture, with fibrosa (F), spongiosa (S), and ventricularis (V). Direction of blood flow is indicated by arrow. B. Control implant removed after 21 days. Note diffuse calcification and predominant spongiosa. C through F, Cusps from animals receiving systemic EHDP (C), ALZET osmotic pump-delivered EHDP and pretreated cusps (D) and those pretreated with APDP (E) and pretreated with (F), respectively. Note virtual lack of mineralization in C and D, and partial inhibition of calcification in E and F. A was stained with hematoxylin and eosin and B through F were stained with von Kossa's reagent (calcium phosphate is black). Original magnification of all 200 ×.

Adverse effects of systemic diphosphonate therapy: comparison with local administration. Systemic EHDP caused significant adverse effects, including mild elevation of serum calcium levels (table 1) and a severe growth disturbance affecting both bone growth (figure 2) and body weight in the EHDP-treated animals (group I) compared with control (table I). Use of local therapy, however, avoided these problems. With either local administration of diphosphonate (table 1, groups II and III) or cuspal pretreatment (group IV), the overall somatic growth as measured by the body weight at time of death (table 1) was not affected, and serum calcium levels were also unaffected. Defective ossification of bone osteoid was prominent in femurs from animals receiving daily EHDP injections, but was not present in femurs from animals receiving local therapy (figure 2). APDP pretreatment was not associated with any detectable adverse effects on the animals with implants (table 1). The microscopic features of the growing zones of the femurs in rats receiving systemic and localized EHDP correlated with overall skeletal and somatic maturation (figure 2). Femurs from control animals
demonstrated normal microstructure of the epiphyseal plate: continued maturation, death, and replacement of cartilage on the diaphyseal side of the disk.\textsuperscript{29} Osteogenesis was active at the diaphyseal border of the epiphyseal disk; the cartilaginous disk was firmly cemented to the diaphyseal bone and was formed in association with the calcified intercellular substance of the dying cartilage (figure 2). Femurs from group Ia animals had a striking disturbance of the structure of the growing zones of both proximal and distal portions. Impaired calcification was evident. The hypertrophic zone of cartilage failed to undergo mineralization, and there was defective osteogenesis of the diaphyseal border of the growing zone (figure 2). A broad unmineralized region separated the cartilaginous epiphyseal plate from the calcified metaphyseal trabeculae. In contrast, femurs from animals receiving local EHDP did not have any apparent growth plate disturbance. As seen in figure 2, there was normal mineralization of the hypertrophic cartilage zone and the cartilaginous disk was in close apposition to the calcified metaphyseal trabeculae. Thus, the severe abnormality in skeletal maturation encountered with systemic EHDP was fully prevented in animals receiving localized doses.

FIGURE 2. Photomicrographs of undecalcified sections of epiphyseal growth plate of distal femurs from control rats (A and B), rats receiving systemic EHDP (C and D), and those given EHDP by ALZET 2002 osmotic pump (E and F). Compared with controls, femurs from animals treated with systemic EHDP (25 mg/kg/24 hr) had a striking disturbance of the developing epiphyseal growth plate. Note broad, poorly mineralized trabeculae. In contrast, growth plates of femurs from animals receiving localized minipump injections of EHDP releasing 5 mg/kg/24 hr showed no growth plate disturbances as growth plates closely resemble those from control animals. A, C, and E were stained with hematoxylin and cosin; B, D, and F were stained with von Kossa’s reagent. Original magnifications of all 150×.
Discussion

Bioprosthetic cardiac valve substitutes have, as advantages over mechanical prostheses, generally superior hemodynamic performance and a low associated incidence of thromboembolism without long-term anticoagulation.2, 7, 11 However, calcific degeneration leading to valve failure occurs after 5 years8-10 in over 50% of children receiving bioprosthetic valve replacements and 5% to 10% or more of implants in adults fail after 5 years.7 The pathophysiology of this disease process has been reviewed,11 and involves host factors such as young age,11, 17 and the presence of renal disease,12 as well as implant factors such as glutaraldehyde pretreatment13 and physical stress.17, 19, 20 To date, no satisfactory solution for this problem has been found. Work by others has concentrated on preventive maneuvers involving detergent pretreatment of BCs to prevent calcification.10-32 This approach has given contradictory results, with some investigators reporting severe BC calcification despite detergent pretreatment,21, 32 and with others reporting diminished mineral deposition.30 The results of the present study suggest that diphosphonate compounds may offer an attractive means of inhibiting BC calcification. Circulatory bioprosthetic valve replacement experiments involving a similar pharmacologic approach to that used in this study should be the next step in pursuing this issue fully.

Adverse diphosphonate effects and drug turnover: advantages of local administration. Diphosphonates, when administered systemically, are well-known inhibitors of calcification that have significant retarding effects on bone development and overall somatic growth.22, 23 The most prominent features of diphosphonate bone toxicity, namely deficient mineralization in the hypertrophic cartilage and formation of wide osteoid seams in the metaphysis,23 were present in the femurs from the systemically treated animals, but were not noted in the femurs from the animals receiving local diphosphonate therapy, either by ALZET (with EHDP) or APDP preincubation. The results of the present study clearly demonstrate that diphosphonates given locally are efficacious for the inhibition of BC calcification at a relatively low total body dose compared with that necessary for effective systemic therapy. Furthermore, local administration can be achieved either by a drug delivery device or by incorporation into the prosthetic material.

A limited course of local therapy was shown to have a relatively long-term postadministration inhibitory effect on BC calcification. Diphosphonate therapy might be expected to provide long-lasting posttherapy protection against cuspal calcification, since the calcific deposits that occur in this disease process are thought to be resistant to dissolution or active removal and are localized to a fixed number of potential mineralization sites. No remodeling of existing BC calcifications has ever been noted,14, 17 nor is there thought to be biosynthesis of any new calcifiable matrix in this disease process. Furthermore, since no cuspal deposits were morphologically demonstrated by transmission electron microscopy in EHDP-treated animals, the suggestion is that these compounds either interfere with crystal nucleation or arrest growth at a very early stage. It is very likely that in the cuspal calcifications, hydroxyapatite-bound diphosphonate will not be subject to metabolic removal, as is the case in bone,22, 23 and this may also explain the long-term protective benefits noted in the present study (see data from group III, table I).

APDP pretreatment and mechanisms of glutaraldehyde potentiation of BC calcification. APDP significantly inhibited BC implant mineralization when used as a pretreatment. This observation raises a number of questions about the localization of action of APDP under the experimental conditions used in this study. APDP was chosen as a reactive compound based on the hypothesis that residual aldehyde groups remaining in the cusps after glutaraldehyde preparation procedures would covalently react with APDP via Schiff base formation.33, 34 Cuspal ashing and phosphorous analyses of the preimplanted APDP-pretreated valves revealed relatively high levels of bound drug compared with those in control valves, and in view of the technical difficulties involved in ashing soft tissue, the actual amount could be considerably higher if representative recoveries could be quantitated. If one assumes, based on published data,34 that the cuspal protein composition is about 90% collagen, then roughly five molecules of APDP might be bound per molecule of collagen. This figure serves to emphasize the relatively high levels of bound drug, since there must be possible binding sites for APDP other than collagen or those created by residual aldehyde functions. Clearly, since APDP pretreatment appears to be an effective means of inhibiting bioprosthetic cuspal calcification, the nature of the binding of APDP to the cuspal material and its stability in vivo needs to be completely understood. Previous work from our laboratory has established that glutaraldehyde pretreatment is a prerequisite for the ultimate calcification of cuspal implants.17 The APDP results suggest that the residual aldehyde groups may be important loci for calcification, and may explain, in part, the effects of glutaraldehyde pretreatment in pro-
moting calcification. In any case the APDP results are of direct importance because they suggest an approach that eventually might be applicable in the clinical setting.

Clinical implications. The results of the present study suggest a potential role for diphosphonate compounds in the prevention of BC calcification and therapy of patients with cardiac valve bioprostheses. In the rat preparation of accelerated BC calcification in the present study levels of calcification in the control implants were comparable to those noted after 1 or more years of clinical implantation.31, 35 Thus, the amount of diphosphonate compound required to inhibit the cuspal calcification process in the rat implants is probably comparable to a total dose that would be needed to inhibit calcification in a clinically implanted cusp.

Local diphosphonate therapy represents a potentially important approach that circumvents the adverse systemic effects of diphosphonates. This would be of particular importance in children, since this patient group experiences accelerated valve calcification, and would experience more severe side effects of systemic diphosphonate therapy as well. The local therapy strategy could be applied to BC valve replacement by the development of a diphosphonate drug delivery system in which there is copolymerization of these drugs with suitable biocompatible polymers.36, 37 The copolymer containing slowly releasing diphosphonate could then be incorporated into the structure of the bioprosthesis, perhaps as a coating of the stent, or impregnated into the fabric swing ring. Implant calcification complicates a wide range of biomaterial applications,38, 39 and local administration of diphosphonates eventually may be efficacious in this setting as well. Furthermore, diphosphonate compounds have been demonstrated to inhibit early events in the process of atherogenesis40, 41 as well as formation of late calcific atherosclerotic lesions.42 It is conceivable that implants that could release locally effective diphosphonates might be of value in the treatment of advanced atherosclerosis as well as in the prophylactic inhibition of atheromata in native vessels.

Cusp pretreatment with APDP or a related compound could also provide an efficacious approach to the prevention of BC calcific failure. APDP or related compounds may also be more desirable drugs to incorporate into time-release copolymers (see above) since they could theoretically combine with both hydroxyapatite and the products of residual aldehyde functions.

The results of these investigations illustrate a new concept in the prevention of BC calcification that may have wider applicability. The data from the present study also suggest that a limited course of diphosphonate therapy may provide lasting protective benefit (table 1). This ‘pulse’ therapy could be initiated either with early postoperative intervention or, possibly, later in the clinical course. This may eventually have important implications for patients with existing bioprosthetic valve replacements, since it may be possible that a limited course of systemic therapy in this setting could arrest preexisting bioprosthetic cusp calcification at its current level.

It is concluded that diphosphonate therapy effectively inhibits the pathologic calcification of BCs implanted subcutaneously in young rats. EHDP is effective used either systemically or locally. Adverse effects encountered with systemic administration were avoided with local administration. Locally administered EHDP also has a prolonged protective effect against calcification. APDP is effective in inhibiting calcification when used as a cuspal pretreatment, and is also without adverse effects. This pharmacologic approach should be tested in experimental circulatory valve replacements.

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