Inhibition of Particulate Debris-Induced Osteolysis by Alendronate in a Rat Model

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Abstract

A rat model was used to study the efficacy of alendronate therapy in inhibition of particle-induced periprosthetic osteolysis. A prosthesis was simulated by inserting a cylindrical polymethylmethacrylate plug into the distal femur of 24 rats allowing the plug to communicate with the joint space. Intra-articular injections of irregularly-shaped ultra-high molecular weight polyethylene particles of 20-200 μm in diameter were administered at 2-week intervals. The rats were randomized into two groups (n=12 each). Group A rats received twice weekly subcutaneous injections of alendronate sodium while group B rats received injections of saline vehicle only. At 10 weeks all rats were sacrificed. The distal femurs were harvested and axial sections were prepared for histologic analysis. Each section was graded on a scale of 1-4, quantifying the degree of osteolysis surrounding the polymethylmethacrylate plug. Microscopic examination showed a significant (P<.0001) difference in the amount of periprosthetic bone. Femurs from group A treated with alendronate demonstrated mostly normal or near-normal periprosthetic trabeculations, whereas femurs from group B treated with saline showed extensive bone resorption. There was no qualitative difference in the inflammatory cellular response between the groups. This study established the ability of alendronate to inhibit the osteoclastic-mediated osteolysis around joint implants.

Periprosthetic osteolysis leading to aseptic loosening is a major cause of failure in cemented and noncemented total hip arthroplasties (THAs). The process of osteolysis begins with the generation of polyethylene, metallic, and polymethylmethacrylate particles. These particles have been shown to stimulate macrophages and giant cells, which release mediators that directly stimulate osteoclasts, leading to periprosthetic bone resorption.

Charnley was the first to recognize significant bone resorption around Teflon and later polyethylene acetabular implants, but did not attribute it to particulate debris. Willert et al first noted that the membrane found between cement and bone was composed of a foreign body granuloma with macrophages, giant cells, and wear particles.

Research focused on reducing osteolysis and improving implant longevity has taken two approaches: improving implant materials and design to reduce the number of wear particles generated, and developing methods to inhibit the biologic processes that cause osteoclast-mediated periprosthetic bone lysis.

Bisphosphonates, drugs used to minimize or prevent bone loss, are currently used to treat osteoporosis, Paget's disease, and metastatic lytic bone lesions. These entities are characterized by high bone turnover and decreased bone mass, similar to that seen in osteolysis with aseptic loosening. Bisphosphonates are thought to act by inhibiting the action of the ruffled border of the osteoclast. Alendronate, the newest bisphosphonate, is more potent than previous agents and does not appear to inhibit mineralization. It has consequently become the treatment of choice for low-bone mass states.

This study determined the success of alendronate in preventing osteolysis...
associated with periprosthetic wear debris in vivo using a rat model of polyethylene-induced periprosthetic osteolysis previously described by Howie et al.9

**MATERIALS AND METHODS**

Polyethylene particles were placed in the distal femur of 24 adult male Sprague-Dawley rats weighing approximately 375 g. Polyethylene implants were premedicated by injecting cement (Simplex; Howmedica, Rutherford, NJ) into the lumens of 16-gauge intravenous catheters. After the cement hardened, the plastic of the catheters was cut away and the rods were cut to 1-cm lengths and sterilized in a standard operating room autoclave.

A lateral arthrotomy was performed on the right knee of each rat. Each rat was given an intramuscular injection of cerfazolin, and then under ketamine/xylazine general anesthesia, the distal femoral articular surface was approached and the intercondylar notch was identified. A 1-cm long cavity was drilled with a 0.062" Kirschner-wire using a high-speed drill and then irrigated with sterile saline solution. A 1.5-cm long × 1.7-mm diameter polyethylene rod was inserted into the distal femur, ensuring that the rod was recessed and not touching the joint surfaces. The capsule and skin were closed separately with absorbable sutures. The experiment was approved by the internal review board of the University of Illinois at Chicago.

Polyethylene solution was then prepared by mixing 3 mL of sterilized ultra-high molecular weight polyethylene powder (DePuy, Warsaw, IN) with 50 mL of normal saline. The powder was the same as that used to manufacture joint prostheses and consisted of irregularly shaped particles between 20 and 200 μm in diameter.

All rats were given intra-articular injections of polyethylene solution at 2-week intervals beginning 2 weeks after polyethylene implantation and continued for the duration of the study (10 weeks) for a total of four injections. This has been demonstrated to induce an osteoclast-mediated osteolytic reaction around the cement plug.9 Twelve rats (group A) were selected to receive twice weekly subcutaneous alendronate sodium injections (Merck, Rahway, NJ) in a dose of 70 μg/kg predissolved in normal saline vehicle10 starting at week 2 postimplantation, while the remaining 12 rats (group B) received injections of saline vehicle only.

All rats were sacrificed at week 10 postimplantation (2 weeks after the last intra-articular injection) using a carbon dioxide euthanasia chamber. After obtaining joint cultures, the distal femurs and knee joints were harvested and placed in 10% neutral buffered formalin. The specimens were then dehydrated in ascending graded ethyl alcohol, cleared in xylene, and infiltrated and imbedded in methylmethacrylate.

Polymerized specimens were sectioned using an EXAKT precision band saw (0.2-mm diamond-coated saw blade). Sectioning was started on the distal femur at the most proximal extent of articular cartilage. Sections were glued to white acrylic slides with a cryoadhesive and ground to a fine finish. The grinding was performed with silicone carbide grit papers and diamond slurry. Slides were surface stained with Paragon stain.

Six microscopic sections were prepared from each femur. One femur in group A had only five sections that were adequate for histologic analysis, two femurs in group B had five sections that were adequate, and one femur in group B had only four sections that were adequate.

The histology of each section was quantified based on the degree of cellular infiltrate surrounding each methylmethacrylate plug. Each section was graded on a scale of 1-4 and the grades were averaged for each femur (Table I and Figures 1-4). The 12 femurs in groups A and B were then averaged to calculate an average and range for each femur. The sections from each group (group A, 69 sections and group B, 71 sections) were then analyzed using a mixed effects ordinal regression model for significance. This model accounts for the different number of sections in some of the femurs.

**RESULTS**

All 24 rats survived the full study period. Of the 12 specimens in group A, the median grade was 1.34 (range: 1-1.83). In group A, 59 of 69 slides (85.5%) showed normal or near normal trabecular patterns (type I or II) of bone in the medullary canal. Ten slides were graded type III, but none of the slides demonstrated complete absence of bone (type IV). The bone seen in the medullary canal was sclerotic and appeared continuous with the more peripheral cortical bone. Further histologic analysis of the specimens revealed predominantly narrow elements in the spaces between the trabeculae. Additionally, very few osteoclasts were seen. A small amount of giant cells and macrophages were seen, representing only a mild inflammatory reaction. The marrow spaces contained unstained voids.
filled with crystalline material, which represented the polyethylene particles.

Of the 12 femurs in group B, the median grade was 3.41 (range: 2.2-3.83). In group B, 63 (89%) of 71 sections in the medullary canal were completely devoid of bony elements or demonstrated only minimal trabeculations (type III or IV). None of the slides showed the medullary canal to be completely surrounded by bone (type I), and only 8 of 71 slides were graded type II. All showed evidence of cellular infiltration with sporadic osteoclasts and giant cells mixed in with the narrow elements. As in group A, voids were noted and areas of crystalline material were present within the matrix. A brownish-yellow hue was observed in the medullary space on some slides that was not evident in group A slides.

None of the slides in either group showed a polymorphonuclear cell response, thus arguing against infection as a cause for osteolysis. The histologic difference in the amount of bony elements present was statistically significant (P<.0001) as determined using a mixed effects, ordinal regression model for multilevel analysis. The difference between the groups is represented in Figure 5.

**DISCUSSION**

The process of bone resorption and osteolysis incited by wear debris is a major cause of bone loss associated with aseptic loosening and failure of total joint prostheses. It is believed that polyethylene and polymethylmethacrylate particulates evoke the greatest response.

Polyethylene particles result from wear of the polyethylene acetabular liner through fatigue wear, third-body wear, or direct abrasions. Material specific behavior associated with polyethylene molecular weight distribution, presence of fusion defects, component aging, and sterilization methods also contribute to the generation of wear particles.

Typically, there are millions of particles produced by these mechanisms, even in hips that have no evidence of lysis. A direct correlation between polyethylene wear and the presence of osteolysis has been demonstrated by several investigators. A retrieval study has documented concentration of wear particles in areas of osteolysis about THA.

In addition to the number of particles, which is a function of rate of production and clearance, particle size plays an important role in the initiation of an inflammatory cell response. Particles >5 μm generally incite a multinucleate giant-cell response, whereas smaller particles produce a macrophage response. These inflammatory cells, unable to digest the foreign particles, release a variety of inflammatory mediators, that in turn activate osteoclasts, which results in bone resorption. These mediators include PGE₂, IL-1β, and IL-6. Whether macrophages resorb bone directly is disputed, but osteoclasts are known as the major cell type responsible for osteolysis.

The reaction to polymethylmethacrylate may also involve other mechanisms. There may be an immunological response to proteins bound to polymethylmethacrylate that contribute to the inflammatory reaction. This response may vary, making some individuals more susceptible to loosening.

Alendronate is a second-generation bisphosphonate that has greater potency than older agents at inhibiting osteoclast-mediated bone resorption without impairing bone mineralization. Its mechanism of action is not yet completely understood but is dependent on the unique structure of its side chain.
and involves osteoclast inhibition at the bone surface. Evidence also supports its ability to inhibit the osteoclastic cytokine IL-6.

Because of the success in treating bone diseases with high bone turnover and bone loss such as osteoporosis, Paget's disease, and metastatic lesions, interest has been generated to determine whether the effectiveness of alendronate can be translated to treatment of bone loss with periprosthetic osteolysis. Shanbhag et al demonstrated in a canine THA model that alendronate inhibited wear debris mediated osteolysis. They also showed that while alendronate was effective in preventing osteoclastic bone resorption histologically and biochemically, it did not reduce the underlying particle-induced inflammatory response that concurs with other literature describing alendronate's mechanism of action.

In this study, a proven model of particle-induced osteolysis was used to evaluate the ability of alendronate to inhibit osteoclastic bone resorption in vivo. We improved the power of the model by developing a histologic classification system to quantify the results and thereby derived statistical significance. We noted a significant (P<.0001) difference in periprosthetic bone between treated and untreated animals. No rats in the treatment group exhibited complete loss of periprosthetic bone, while 89% of untreated rats demonstrated total or near-total bone loss. Because the only variable between the two groups was the administration of alendronate, we conclude that the prevention of osteolysis was due to the alendronate.

Although alendronate protected against bone lysis, it was not expected to suppress the associated particle-induced immune or inflammatory response. The tissues in our specimens exhibited a giant-cell and macrophage response, although none of these responses would be characterized as intense, as seen in other tissue studies. This may have been the result of the polyethylene particles clearing from the joint space and thus decreasing the particulate load exposed to the periprosthetic tissues. Additionally, the degree of reaction to the debris may be inherent to the organism and may vary among strains, species, or individual organisms. Additionally, the particles may have been slightly larger than those generated in the actual clinical situation.

There were, however, no differences noted overall between the two groups with respect to the magnitude of tissue response. Thus, our findings are consistent with previous data that showed alendronate to act on the osteoclast at the bone's surface and did not have an effect on the inflammatory response.

For future use of this model, synovial and periprosthetic tissues could be examined histologically, and tissue samples could be cultured and assayed for presence of inflammatory mediators to quantify the reaction.

Several limitations of the study are recognized. Polyethylene was the only material introduced into the joint, whereas in actual hip implants, metallic and polymethylmethacrylate particles also are generated. However, as it is likely that the number and size of particles are more critical in eliciting a cellular response than the type of particle, our model would appear to be a reasonable simulation of wear-induced osteolysis despite injecting debris of only one type into the joint.

The polymethylmethacrylate plug inserted into the distal femur is not a true "prosthesis." It is not a load-bearing implant nor does it completely fill the medullary cavity as would a cemented or uncemented femoral stem. Although it is composed of actual bone cement, its insertion does not simulate that of the actual human process, where the periprosthetic bone is subjected to the intense heat caused by polymerization of the cement. This subject can be addressed with future use of the model.

The strengths of this model include the relatively low cost and ease with which trials are performed. It has been

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<td>Average Histologic Pattern for Each Rat</td>
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Figure 5: Distribution of histologic cell type by treatment group.
demonstrated to be reproducible, and the advantages of in vivo testing include the complex tissue and systemic interactions that cannot be duplicated in cell cultures. The continuous, albeit intermittent administration of wear debris closely simulates the clinical environment in which particles are constantly generated throughout the life of the prosthesis.

The results of this study prove that alendronate is a potentially useful agent in the prevention of wear debris induced osteolysis associated with aseptic loosening of total joint prostheses. The rat model is a reasonable simulator of the clinical situation and histological and biochemical analyses can be performed.

Further research is necessary to determine if alendronate can arrest or reverse the changes of osteolysis. The ideal time for starting alendronate (at time of surgery versus when early changes of osteolysis occur) must be evaluated in light of the long-term effects of alendronate. Alendronate has been shown to affect the gastric and esophageal mucosa and to potentiate the ulcerogenic effects of nonsteroidal anti-inflammatory agents. The appropriate dosing and duration of administration must be determined for the purpose of reversing osteolysis. Furthermore, development of a pharmacologic protocol to inhibit the cytokine response as well as the osteoclastic response might also prove beneficial.

REFERENCES