Zoledronic acid causes enhancement of bone growth into porous implants

The effect of zoledronic acid on bone ingrowth was examined in an animal model in which porous tantalum implants were placed bilaterally within the ulnae of seven dogs. Zoledronic acid in saline was administered via a single post-operative intravenous injection at a dose of 0.1 mg/kg. The ulnae were harvested six weeks after surgery. Undecalcified transverse histological sections of the implant-bone interfaces were imaged with backscattered scanning electron microscopy and the percentage of available pore space that was filled with new bone was calculated. The mean extent of bone ingrowth was 6.6% for the control implants and 12.2% for the zoledronic acid-treated implants, an absolute difference of 5.6% (95% confidence interval, 1.2 to 10.1) and a relative difference of 85% which was statistically significant. Individual islands of new bone formation within the implant pores were similar in number in both groups but were 69% larger in the zoledronic acid-treated group. The bisphosphonate zoledronic acid should be further investigated for use in accelerating or enhancing the biological fixation of implants to bone.

Porous materials have proven to be a very effective method for attaching prosthetic implants to the bony skeleton. The incidence of radiographic signs of bone ingrowth in total hip arthroplasty (THA) is reportedly 95% in uncomplicated primary hips, regardless of the type of porous coating or implant design. There remains a need to develop effective method for attaching prosthetic implants to the bony skeleton. This is confirmed in studies of retrieved THAs. There remains a need to develop techniques which can enhance biological fixation. The more rapid the bone ingrowth, the faster the implant becomes protected against the disruptive forces of load bearing. This is particularly important in situations where bone healing is compromised or initial implant stability is more tenuous. Tissue ingrowth may protect the bone-implant interface against wear particle-induced osteolysis.

Various methods have been investigated to improve bone ingrowth into porous implants, with varying degrees of success. These include the use of autograft and allograft, demineralised bone matrix, fibrin glue, calcium phosphate granules, collagen, periosteal activation agent, tricalcium phosphate coating, hydroxyapatite coating, prostaglandin F2α, TGF-β2, electrical stimulation, ultrasound stimulation and morphogenetic proteins.

Bisphosphonates have been shown to increase the cellular response in both mature and healing bone. This has led to the use of oral bisphosphonate therapy to mitigate the osteolytic effects of wear debris. Bisphosphonates have been used to reduce peri-prosthetic bone loss through stress shielding mechanisms. In experimental studies, Little et al. have shown that a single post-operative dose of pamidronate decreased the disuse osteopenia normally associated with bone lengthening and increased the amount and density of the regenerated bone. In a further study, they showed that one or two doses of the more potent zoledronic acid abolished osteopenia and increased the quality of the regenerate.

We hypothesised that bisphosphonates could have a positive effect on bone ingrowth into porous implants. The purpose of this study was therefore to investigate the potential of zoledronic acid to enhance bone ingrowth in a canine model using porous intramedullary implants.

Materials and Methods

Surgical Implantation. Porous implants were inserted into the intramedullary canal of the ulna of skeletally mature, mongrel dogs weighing between 25 and 35 kg. The implants were cylindrical (50-mm long, 5-mm diameter) and fabricated from a porous tantalum biomaterial (Impex Corp., Allendale, New Jersey). The
implants had a mean pore size of 430 µm (95% confidence interval (CI), 413 to 447) and a volume porosity of approximately 75% (Fig. 1). The porous tantalum material has been studied in various animal models and used clinically.37,38

The surgical procedure was performed under general anaesthetic in sterile conditions. A 2-cm incision was made over the olecranon process and the triceps tendon was split by sharp dissection down to bone. Under fluoroscopic guidance, a 5-mm drill was orientated along the long axis of the ulna in line with the intramedullary canal for 5.5 cm. The porous implant was then tapped down the intramedullary canal with a punch (Fig. 2). The implant was countersunk to avoid irritation of the triceps tendon. The wound was irrigated and closed in a standard fashion. The procedure was repeated on the contralateral side. The positioning of the implants varied in depth of insertion within the canal and in orientation. This, together with differences in ulnar size, resulted in variability of the relationship of different parts of an implant to endosteal cortical bone.

Immediately after surgery seven test dogs with 14 ulnar implants were given a single intravenous dose of 0.1 mg/kg zoledronic acid (Novartis Pharma AG, Basel, Switzerland). Because of the systemic exposure of the test dogs to zoledronic acid it was necessary to use control data from an earlier experiment in which zoledronic acid was not used.22 Previous canine ingrowth studies have demonstrated that bone ingrowth into porous-coated implants is maximal at six to eight weeks.7,22,37 As the purpose of our study was to determine the effect of zoledronic acid on the early progression of bone ingrowth into intramedullary porous implants, the treatment period chosen was six weeks.

**Histological examination.** The bones were harvested, stripped of soft tissue, radiographed and processed for undecalcified hard-section histology. This involved dehydration in ascending solutions of ethanol, defatting in ether and acetone, and embedding in methylmethacrylate. Each implant was sectioned transversely into five sections at 1 cm intervals (Fig. 3). The sections were radiographed, polished, sputter-coated with gold-palladium and imaged with back-scattered scanning electron microscopy. Bone that was observed around the implants, but not within the pores, was not quantified because of the uncertainty of the difference between new and pre-existing bone. For each section, computerised image analysis, based on gray level discrimination, was used to identify islands of bone within the implant pores and to generate quantitative information on the extent of bone ingrowth, defined as the percentage of the available porosity that was filled with new bone. Also tabulated was the area of each bone island within the implant pores in each histological section as well as the total number of bone islands. This enabled a calculation of the mean bone island size and number of bone islands. The quantitative analysis was performed by an independent observer who was blind to the implant groups. The computer-aided method for quantifying new bone formation was determined to have an intra-observer repeatability of ± 2%.

**Statistical analysis.** The quantitative histological data from the six control and 14 test implants were statistically analysed using a two-level hierarchical model. At the first level of the model, the set of results from the limbs of each dog was assumed to follow a normal distribution with dog-specific means and a global variance parameter. At the second level of the model, the means from each dog in each group from the first level followed a second normal distribution, with the mean representing the overall mean for the
treatment or control groups and the variance representing the variability within the group.

A similar statistical model was also run where the results for each dog were allowed to vary with the distance along the limb. As these results were virtually identical to those from the model without this extra variable, only results from the simple hierarchical model are presented. The mean values for overall extent of bone ingrowth, number of bone islands within the implant pores and bone island size were analysed similarly with 95% CIs.

Results
A total of 28 histological sections from the external control implants and 67 sections from the zoledronic acid-treated implants (two control and three zoledronic acid-treated sections were lost due to preparation error) were examined. It was common to observe varying degrees of new bone formation within the intramedullary canal around the implants, probably resulting from the stimulus caused by reaming and implantation (Fig. 4). This new bone tended to be more dense where the implant was closer to endosteal bone and less dense in metaphyseal sections. New bone formation within the pores of the tantalum implants was observed in all sections to varying degrees (Fig. 4). There was a general tendency for more bone ingrowth at the implant periphery than in the centre. Small islands of bone were observed throughout many of the implant cross-sections.

The quantitative histological data are listed in Tables I and II. The mean extent of bone ingrowth for the six external control implants was 6.6% (95% CI, 3.2 to 10.0) while the mean extent of bone ingrowth for the 14 zoledronic acid-treated implants was 12.2% (95% CI, 9.2 to 15.2). The 5.6% difference of the means was significant (95% CI, 1.2 to 10.1). In relative terms, there was a mean of 85% more bone growth into the zoledronic acid-treated implants compared with controls.
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The mean number of bone islands within the implant pores was 117 for the control (95% CI, 94 to 142) and 118 for the zoledronic acid-treated group (95% CI, 98 to 138). The size of the bone islands differed significantly between the groups, with a mean of 0.010 mm² (95% CI, 0.009 to 0.012) for the control implants and a mean of 0.017 mm² (95% CI, 0.015 to 0.019) for the zoledronic acid-treated implants. The difference of the means was 0.007 mm², a relative difference of 69% that was statistically significant (95% CI, 0.004 to 0.010).

Discussion

A simple, controlled canine implant model was used to assess the effect of administering intravenous zoledronic acid on bone growth into porous intramedullary implants. The model had clinical relevance in that it resembled the period of early post-operative non-loading which is commonly recommended after uncemented arthroplasty. The dogs treated with a single post-operative dose of zoledronic acid showed almost twice as much net bone ingrowth six weeks after surgery compared with external controls. This is sufficiently encouraging to warrant further studies to determine the zoledronic acid dose response at different time periods and perhaps with different porous materials in both unloaded and loaded models.

Many different methods have been investigated for their potential to augment the rate and extent of bone ingrowth into porous implants. One of the more notable effects was reported by Tanzer et al using the identical model in which porous tantalum implants were subjected to daily 20-minute treatments of non-invasive low intensity ultrasound for six weeks. A mean increase in bone ingrowth of 119% was obtained with ultrasound treatment compared with controls. Sumner et al recently showed, in a gap model using porous-coated rods, that local delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) to the implant site enhanced bone ingrowth by a factor of 3.5 compared with controls four weeks after surgery. Substantial increases in bone ingrowth have also been reported by Bragdon et al and Barrack et al using recombinant morphogenetic proteins in canine acetabular gap models using porous-coated cups.

The increased potency of zoledronic acid compared with other bisphosphonates makes it a logical choice to enhance net bone ingrowth. The quantitative data on the bone islands that formed within the implant pores revealed that the mean number of sites of new bone formation within both implant groups was essentially the same but that the size of each site was greater with exposure to zoledronic acid. This finding is consistent with the documented suppression of osteoclastic remodelling with bisphosphonate therapy and the study of Smith et al suggesting that osteoblastic activity is not increased in the presence of bisphosphonates.

The marked enhancement of bone ingrowth afforded by this simple therapy could have very important benefits in any clinical application in which implants require mechanical attachment to the skeleton. The positive effects of bisphosphonates will have to be compared with those recently described for bone morphogenetic proteins. Bone morphogenetic proteins, which increase bone turnover, are unlikely to offer protection in the longer term against stress-shielding osteopenia or osteolysis, whereas potent bisphosphonate therapy could do so. In a recent study of impaction arthroplasty in a sheep model, Howie et al noted rapid resorption of bone graft and one stem subsidence after administration of OP-1 (bone morphogenetic proteins). Although the power of that study was insufficient...
to make strong conclusions, it highlights the theoretical risk of approaches which increase bone turnover in the presence of stress shielding. The relative merits of increasing or decreasing bone turnover in these situations remain unclear as both approaches can result in increases in bone mass.

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References