Alendronate did not inhibit instability-induced bone resorption

A study in rats

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Alendronate is a bisphosphonate that can decrease osteoclastic activity. It has been suggested as treatment for periprosthetic osteolysis. We used 48 rats, of which 32 had a plate implant on one tibia, to study the effect of alendronate on bone resorption at an unstable implant-bone interface. The plate has a handle on top, which can be grasped through the skin and turned, to create a sliding motion of a titanium surface against the underlying bone. This is known to result in bone resorption, which was studied by histomorphometry. Osmotic minipumps were used to administer alendronate at 0.063 mg/kg/day or saline. The systemic effect of the treatment was assessed by ashing the proximal metaphyses of the tibia of the contralateral unoperated leg. The ash-weight was increased in the alendronate-treated group by 43% (p = 0.0001), corresponding to histological changes in the metaphyseal bone. There was no inhibition of the instability-induced bone resorption at the test surface by alendronate: bone was being resorbed and replaced by a tissue similar to a loosening membrane.

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The pathogenesis of prosthetic loosening has been debated. Regardless of whether particles, fluid pressure or instability is the main cause, all proposed mechanisms led to periprosthetic bone resorption. Bone resorption is caused mainly by osteoclasts. Osteoclastic activity can be inhibited by bisphoshonates (Fleisch 1997). We investigated whether bisphosphonates can prevent aseptic prosthetic loosening by using a previously described model for instability-induced bone resorption in rats.

Animals and methods

Animals and operations

We used a rat model (Aspenberg and Herbertsson 1996), in which the effects of motion at a bone-metal interface can be studied histologically. 48 male Sprague-Dawley rats with a body weight of 345 (326–363) g were used, following institutional guidelines for the use and care of laboratory animals.

In principle, a tight bone-titanium implant interface is first created. The titanium surface is then made to slide against the bone and the resulting instability-induced bone resorption at the interface is studied.

A 4 × 13 mm commercially pure titanium plate (Figure 1) was fixed onto one proximal rat tibia, using one 1.5 mm screw at each end of the plate. Between the two screw holes, a depression in the tibial cortex was milled out, to correspond to the middle part of the plate. This has a circular area with a diameter of 2.5 mm protruding 0.5 mm into the underlying depression in the bone. This area provided the test surface which, after insertion, faces traumatized bone and hematoma. The bone is then allowed to grow back towards the test surface until there is a fit.

The circular surface can be rotated by a wing nut, protruding into the subcutaneous tissue, so that it can

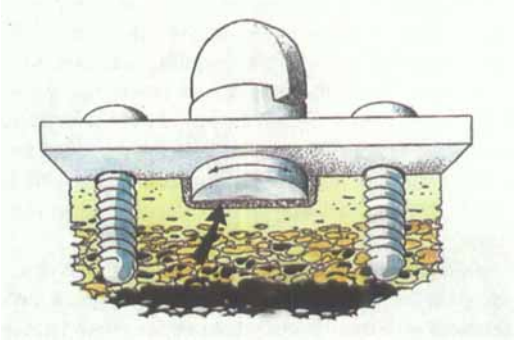


Figure 1. The plate was left without motion for 4 weeks after insertion, to allow close bone-titanium contact. Then daily motion was applied to initiate bone resorption at the interface (fat arrow).

be grasped through the skin. In all rats, rotation of the test surface was started 4 weeks after plate insertion. At this time, the bone formed after the milling at the insertion usually establishes a close bone-to-titanium contact (Aspenberg and Herbertsson 1996). The test surface was rotated 2 × 180° twice a day, 5 days a week for 2 weeks, after which the rats were killed. The rats become used to this procedure after a few days; the surface can be rotated against the underlying tissue, without signs of fear or pain and with no need for anesthesia.

In all 48 rats, osmotic minipumps (ALZET 2002, Alza Corp., Palo Alto, CA, USA) were inserted between the scapulae. The minipumps can be filled with a volume of 0.25 mL, which is released into the extracellular fluid during 14 days at a rate of 0.012 mL/day after implantation. Alendronate in a dose of 0.063 mg/kg/day and corresponding volumes of saline solution for the controls were thus administered.

In 32 rats, the minipump was inserted 4 weeks after plate insertion and at the same time movement of the test surface was started. Of these rats, 16 were treated with alendronate and 16 were controls, chosen at random. The rats were killed after 2 weeks of movement.

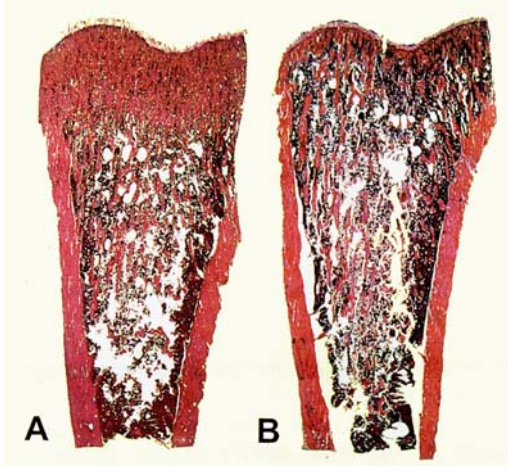
In the remaining 16 rats, minipumps, but no plates were inserted. Among these there were 8 alendronate-treated rats and 8 controls and these rats were killed two weeks after pump insertion.

Evaluation

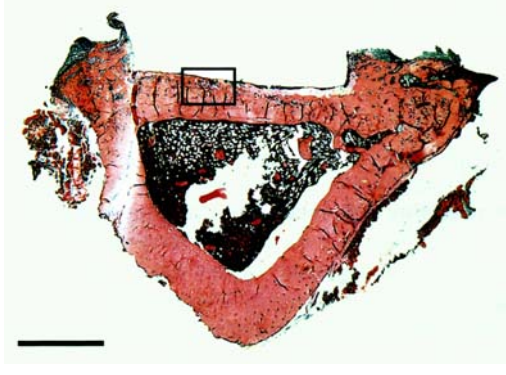
Histology. All handling of specimens and all evaluations were blinded for treatment. The entire tibial segment beneath the test surface was decalcified and prepared by standard histological techniques. Sections were produced at a right angle to the test surface, through the middle of the circular surface, and stained with hematoxylin and eosin. All specimens were examined by using a computerized video system attached to the microscope (Videoplan™ Kontron Bildanalyse, Esching, Germany), by drawing on a digital table with a screen magnification of × 40. Measurements included the length of the total interface surface line, the length of each part of the contact surface line, which did not consist of bone (soft tissue or cartilage), and the area of soft tissue, which had contact with the surface line. From the group without plates, frontal sections of the proximal tibia were made.

Ashweight. Proximal tibial ashweight was determined in the first 16 plated rats (8 controls and 8 alendronate). A 6 mm long segment of the proximal, unoperated tibial metaphyses was cut at a right angle to the long axis of the bone and weighed before and after ashing at 1000 °C for 24 hours. In the group with

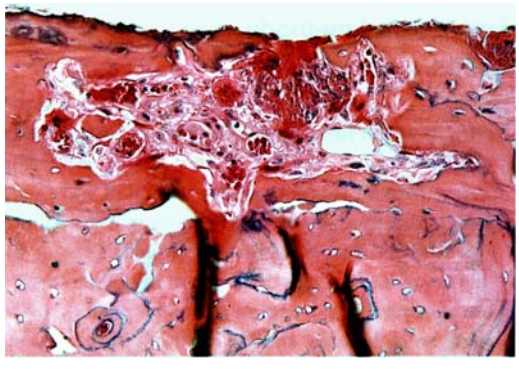
Figure 2.



Proximal tibial metaphyses with growth plate removed. Specimen A: Alendronate treatment. Specimen B: Control. Up to 1 mm broad area of unremodeled primary bone can be seen under the growth plate in treated animals, remodeled bone seen in the corresponding area in the controls.



Alendronate-treated rat. Transverse section of the tibia. Plated area up. The square-shaped depression corresponds to the rotating part of the plate. Hematoxylin eosin, bar length 1 mm.



Framed area in Figure 2 B. A resorption cavity has formed with granulation tissue. Multinucleated giant cells in Howships lacunae are commonly seen in these regions. Hematoxylin eosin.

Table 1. Bone contact, percent

	Min	1st quartile	Median	3rd quartile	Max
Alendronate	0	0.12	0.45	0.53	0.82
Controls	0	0.23	0.49	0.71	0.86

Table 2. Length of total interface surface line, mm

	Min	1st quartile	Median	3rd quartile	Max
Alendronate	2.17	2.69	3.06	3.23	3.41
Controls	2.19	2.72	2.93	3.07	3.22

pumps, but no plates (8 controls and 8 alendronate-treated), one proximal tibia from all rats was ashed and the other examined by histology.

Results

Proximal tibial remodeling

In alendronate-treated animals (n 16), the ashweight of the proximal, plateless tibial metaphyses was higher than in the controls (mean 55 mg, SD 3 for controls, mean 80 mg, SD 8 for treated animals). The lowest value in the alendronate-treated group was still higher than the highest value in the controls (Mann-Whitney U-test $p = 0.0001$). In all alendronate-treated rats, histological sections from this area showed a broad band of unremodeled primary bone under the growth plate, whereas all controls showed clearly less, remodeled bone in the corresponding area. This finding was constant, thus making it easy to categorize into treated animals and controls (Figure 2).

Histology under plates

Histological examinations of the test surfaces exposed to 2 weeks of plate movement showed some areas of apparent bone-metal contact, but also many localized areas of soft tissue. This appeared as granulation tissue with abundant fibrous material and local populations of larger, multinuclear cells, most often situated at the bottom of a "pit" in the bone (Figure 2). In some specimens, the fibrous tissue had confluenced to form a continuous fibrous membrane, and in 2 specimens, areas of fibrocartilage could be seen. There was no qualitative difference between alendronate-treated rats and controls. 1 animal of 32 plate-bearing animals was lost due to infection, and 1 was excluded because of local swelling around the implant.

Histomorphometry

There was no difference in the percentage of the contact surface line which had bone contact and there was no difference in the length of the total interface surface line (Tables 1 and 2).

Thus, in spite of a clear effect on bone remodeling as measured by the ashweight of the contralateral tibial metaphyses and histological appearance, there was no obvious inhibition of the motion-induced resorption at the test surface under the plate.

Discussion

Studies on the effects of alendronate on prosthetic loosening have evaluated the effects radiographically or by histological analysis of soft tissue, using arthroplasty models in animals (Shanbhag et al. 1997). These models appear to resemble the clinical situation more closely than ours, but they may also be more complex systems, in which several resorption-activating phenomena may be present. We used a rat model, which allows us to study resorption as initiated by interface shear motion only, excluding, e.g., resorption induced by wear particles. Although this makes the model more specific, it also makes it less clinically relevant, considering the complex instability pattern around loose prostheses, presence of particles, fluid pressure, etc. However, our model allows us to study effects at a histomorphometric level.

We found no evidence of decreased bone resorption under the plates in alendronate-treated animals, even though there was a significant effect on normal remodeling, as evidenced by an increase in ashweight and in unremodeled cancellous bone in the normal proximal tibia in alendronate-treated rats. The alendronate dose was similar to doses that have earlier proven effective in rats (Bikle et al. 1994). We conclude that a systemic effect of alendronate was achieved, reducing osteoclastic activity, but that it did not influence the movement-induced bone resorption at the test surface very much. The most likely explanation would be that the bone resorption process during the remodeling of cancellous bone differs from the resorption process at the unstable bone-metal interface in our model, at least as regards its sensitivity to alendronate. This difference could relate to, e.g., the osteoblastic control of osteoclasts in normal remodeling, which may be less marked at the unstable interface. Thus, we think bone resorption induced by movement at a bone-metal interface could mean a situation different from that in normal bone remodeling. Osteoblastic activity is present during remodeling, and there is evidence of osteoclastic activity being influenced or controlled by osteoblasts (Sahni et al.

1993, Vitte et al. 1996). Movement, on the other hand, could cause osteocyte death near the implant, resulting in necrosis of the bone and a resorption process similar to osteonecrosis, where osteoblastic control of osteoclasts appears disturbed.

We can think of two other possible, but somewhat far-fetched, explanations, both relating to poor bisphosphonate administration to the test surface. First, bisphosphonate might bind to the titanium of the implant, which could diminish the bisphosphonate concentrations in the bone-resorbing areas beneath the test surface. The second is a microcirculatory speculation. The newly-formed bone is vascularized from the deeper bone bed towards the plate. Alendronate could be absorbed and consumed by this newly-formed bone, so that the concentration of alendronate at the end of the capillary bed, which would be at the test surface, is too low to have an effect on osteoclastic activity here. However, we find both these explanations unlikely. It is hard to believe that all compounds necessary for bone matrix production could reach the test surface, but not the alendronate.

The stimulus for osteoclast formation and activity by movement may be stronger than the inhibitory effect of systemic bisphosphonate treatment. This could imply excessive osteoclast formation, each osteoclast being active for only a short period before becoming inactivated by the bisphosponate treatment, thus resulting in substantial bone resorption, in spite of effective treatment. Osteoclastic resistance towards the inhibitory effect of bisphosponates could also explain the findings, although to our knowledge this has not been reported.

Bisphosponates can reduce osteoclastic activity and the idea to use them to reduce periprosthetic bone resorption (Shanbhag et al. 1997) is still promising. However, our results suggest that we cannot take for granted the awaited effects.

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