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Reduction of instability-induced bone resorption using bisphosphonates

High doses are needed in rats

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ABSTRACT – Bone resorption associated with prosthetic loosening can be reduced by giving bisphosphonates since they bind to bone surfaces and inactivate osteoclasts when bisphosphonate-containing bone is resorbed. During loosening, an increase in osteoclastic activity can be triggered by mechanical instability, fluid pressure or wear particles.

We used a rat model in which a titanium surface can be made to slide over a bone surface and cause instability-induced bone resorption. 111 rats were operated on with a plate implant and treated with alendronate or clodronate injections in different doses or saline controls. After 4 weeks of osseointegration, the plate was moved during 2 weeks and the findings evaluated with histomorphometry. The percentage of persisting bone-metal contact and the soft tissue area at the interface were measured to estimate bone loss. Low or intermediate doses of the bisphosphonates increased the ash weight of untraumatized bone, but did not inhibit resorption at the unstable interface. Only rats treated with the highest doses of alendronate or clodronate had more bone-metal contact than controls. Instability-induced bone resorption therefore seems to be reduced by bisphosphonates, but higher doses are needed to obtain this effect than to reduce bone resorption associated with normal remodeling of untraumatized bone.

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Prosthetic loosening is thought to be initiated by wear particles, fluid pressure or mechanical instability (Aspenberg and van der Vis 1998). Studies using radiostereometric analysis (RSA) have shown that early migration of a prosthesis is a strong predictor of loosening (Ryd et al. 1995). Regardless of the cause of the loosening process, it eventually leads to periprosthetic bone resorption. Bone resorption is effected by osteoclasts and can be inhibited by giving bisphosphonates, a treatment in clinical use against osteoporosis.

Bisphosphonates have an affinity for bone mineral, and therefore act specifically on bone. During resorption of bone by osteoclasts, the ingestion of bisphosphonate interferes with specific intracellular processes, which impair osteoclast function and ultimately cause apoptosis or cell death (Russel and Rogers 1999). At least two molecular mechanisms underlying bisphosphonate action have now been described. First, bisphosphonates that resemble pyrophosphate more closely (e.g., clodronate) can be incorporated into non-hydrolysable analogues of ATP that may inhibit ATP-dependent intracellular enzymes. Secondly, nitrogen-containing bisphosphonates (e.g., alendronate) inhibit enzymes in the mevalonate pathway, thereby preventing biosynthesis of essential compounds for protein prenylation and the function of key regulatory proteins. This may explain why nitrogen-containing bisphosphonates reduce osteoclastic activity associated with normal bone remodeling to a greater extent. On the other hand, some studies suggest that the bisphosphonates that resemble the pyrophosphate group have anti-inflammatory effects, while aminobisphosphonates have some pro-inflammatory effects (Makkonen et al. 1996).

The use of bisphosphonates to stop peri-prosthetic bone resorption has been studied by several authors (Shanbhag et al. 1997, Åstrand and Aspenberg 1999). We have previously determined the effects of alendronate on instability-induced bone

resorption in a rat model using a dose similar to that recommended for treatment of osteoporosis (Åstrand and Aspenberg 1999). The bone resorption caused by instability in that model was not affected by such treatment, although normal skeletal remodeling was greatly reduced in the same animals. There seems to be a difference between bone resorption associated with normal, continuous skeletal remodeling and the instability-induced bone resorption in our model. Are higher doses needed to reduce bone resorption induced by instability than to resorb bone during normal remodeling? Are bisphosphonates that resemble pyrophosphate more effective against instability-induced bone resorption than aminobisphosphonates? We studied whether different doses of alendronate or clodronate inhibit bone resorption in this rat model.

Material and methods

Animals and operations

We used 111 male Sprague-Dawley rats, in accordance with institutional guidelines for care of laboratory animals. 96 rats had a mean body weight of 352 (319–388) g. However, by mistake, 15 rats were two weeks older at operation and had a mean body weight of 400 (378–417) g. We used a model (Aspenberg and Herbertsson 1996) in which the effects of motion at a bone-metal interface can be studied histologically.

In principle, after ensuring that the bone-titanium implant interface is tight, the titanium surface can then be 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. A depression in the tibial cortex was milled out between the two screw holes, corresponding 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. The corresponding bone below the implant gradually forms the bony test surface, but immediately after insertion it consists of traumatized bone and hematoma. The bone is then allowed to grow back towards the implant until it fits.

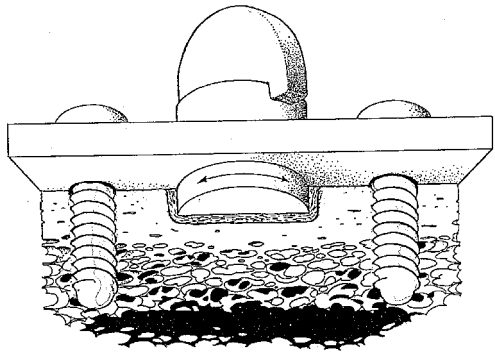


Figure 1. The plate was not moved for 4 weeks after insertion, to ensure close bone–titanium contact. Then daily motion was applied to induce bone resorption at the interface.

The circular surface can be rotated by a wing nut, protruding into the subcutaneous tissue, so that it can be gripped through the skin. In all rats, rotation of the smooth test surface was started 4 weeks after inserting the plate. At this time, the bone formed after the insertion has usually established 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 became used to this procedure after a few days, showed no signs of fear or pain and did not need anesthesia.

Alendronate was given to 3 groups of rats in different doses—i.e., 3.8 μg/kg/day to 8 rats, 21 μg/kg/day to 16 rats, 205 μg/kg/day to 8 rats. For each dose we had a control group of an equal number of rats, given corresponding volumes of saline. Clodronate was given to 2 groups of rats, 0.12 mg/kg/day to 8 rats and 21 mg/kg/day to 16 rats. Similarly, for each dose there was a control group of an equal number of rats given corresponding volumes of saline. The bisphosphonates were injected subcutaneously 3 times a week, starting one week after the first operation. All rats were killed after 2 weeks of movement—i.e., after 5 weeks of bisphosphonate treatment. The groups were operated on at different times. Therefore, all groups had their own controls, operated on at the same time to correct for any differences between animal batches.

The bisphosphonate solutions were made by dissolving one tablet of alendronate (T. Fosamax 10

Number of rats in the various groups

Treatment	No. of rats	No. of rats remaining for analysis
Alendronate		
low dose + control	16	12
intermediate dose + control	16	10
intermediate dose + control	16	11
high dose + control	16	12
Clodronate		
low dose + control	15	13
high dose + control	16	8
high dose + control	16	9
Total	111	75

mg, Merck Sharp & Dome) or clodronate (T. Bonefoss 400 mg, Leiras) in sterile water. The solutions, stirred for 2 hours and sterile filtrated with a filter (Schleicher & Schuell, Dassel, Germany), were then diluted with saline to appropriate concentrations to obtain the daily dose as above, and given 3 times a week in a volume of 0.5 mL per injection. The weight of the rats was approximated to 1/3 kg.

Evaluation

Histology. All handling of specimens and all evaluations were blinded for treatment. After removing the implant, the entire tibial segment below the test surface was decalcified and prepared by standard histological methods and embedding was done in paraffin. Sections were made 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 with a computerized video system attached to the microscope (Videoplan™ Kontron Bildanalyse, Esching, Germany), and by drawing on a digital table at a screen magnification of ×40. Measurements included the length of the total interface surface line and the length of each part of the contact surface line consisting of soft tissue. The percentage of bone-metal contact along the total interface was calculated. We also measured the areas of soft tissue, which had contact with the surface line. A few specimens had cartilage at the interface. That part of the interface surface line was disregarded in all measurements, because according to various criteria, the cartilage had been there from the start of motion, and would resist motion-induced resorption differently.

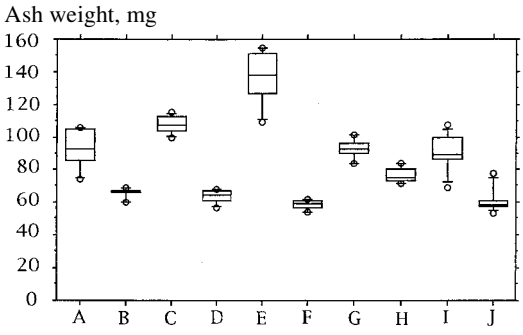


Figure 2. Ash weight (mg) of the unoperated proximal tibia (box plot)
A. alendronate low dose
B. alendronate low dose - control
C. alendronate intermediate dose
D. alendronate intermediate dose - control
E. alendronate high dose
F. alendronate high dose - control
G. clodronate low dose
H. clodronate low dose - control
I. clodronate high dose
J. clodronate high dose - control.

Ash weight. Proximal tibial ash weight of the unoperated leg was determined in all 111 rats. The most proximal 6 mm of the tibia was cut off at a right angle to the long axis of the bone and weighed before and after ashing at 1000 °C for 24 hours. This evaluation was also blinded for treatment.

Statistics

The treatment groups were compared with their controls using the Mann-Whitney U-test.

Results

Of 111 plate-bearing animals, 36 were excluded. 4 animals died during the experiment, 10 were excluded because of local swelling that interfered with motion, 19 because of poor positioning of the implant and 3 because of poor histological preparations (Table). Exclusions due to plate position and poor histological preparations were done from blinded sections.

Ash weight. In all bisphosphonate-treated groups, the ash weight of the proximal, plateless tibia was greater than that of controls (Figure 2).

Histology below the plates. The controls had only a few areas with apparent bone-metal contact. The dominant feature was the formation of local-

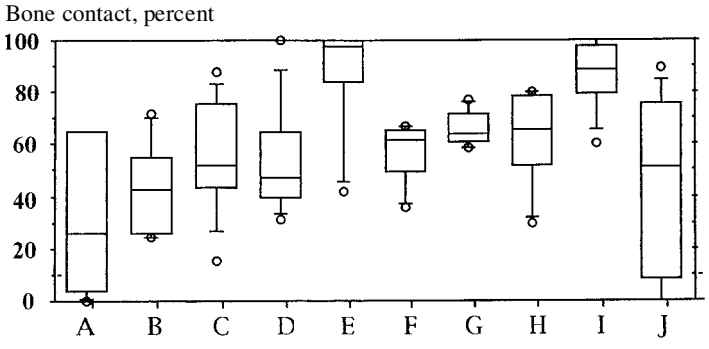
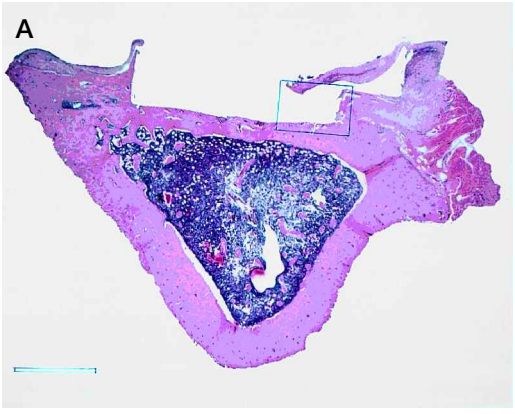


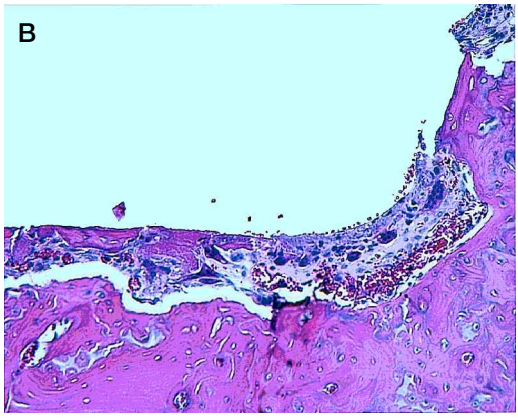
Figure 3. Bone contact, percent of total interface line (box plot). A–J, see Figure 2.

ized areas of soft tissue along the contact surface which resembled granulation tissue with abundant fibrous material and local collections of larger, multi-nucleated cells usually at the bottom of a "pit" in the bone. In some specimens the localized areas of soft tissue had fused to form a continuous fibrous membrane. The groups treated with the highest doses of alendronate and clodronate were, with one exception, the only ones where bone-metal contact was sometimes entirely intact, despite 2 weeks of implant movement (Figure 4).

Histomorphometry. No difference was seen in the length of the total interface surface line. As regards bone-metal contact, the rats treated with the highest doses of alendronate or the highest dose of clodronate differed from their controls, $p = 0.04$ and $p = 0.004$, respectively, but those given the lower doses showed no obvious effect (Figure 3).

The group treated with the highest dose of alendronate or clodronate had a lower soft tissue area in contact with the surface line than their controls ($p = 0.05$ and $p = 0.02$, respectively). Similarly, no obvious effects were seen in rats treated with lower doses of alendronate or clodronate.





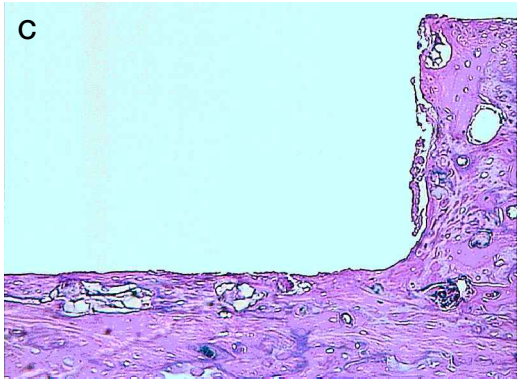


Figure 4.
A. Rat treated with a high dose of alendronate. Transverse section of the tibia. Plated area up. The square-shaped depression corresponds to the rotating part of the plate. Hematoxylin and eosin, bar length 1 mm.
B. Rat treated with an intermediate dose of alendronate. Area corresponding to framed area in Figure 4A. Note resorption cavity formed of granulation tissue containing multi-nucleated cells. Hematoxylin and eosin.
C. Rat treated with a high dose of alendronate. Area corresponding to framed area in Figure 4A. Bone-metal contact persists after two weeks of implant movement. Hematoxylin and eosin.

The group treated with a high dose of alendronate and its control was used to evaluate the intra-individual variation. A second histological section, parallel to the first one, was cut 0.5 mm deeper into the paraffin blocks. The original and the new sections were all blinded and analyzed for bone-metal contact as above. The re-measurement was done one year after the first evaluation. The Spearman rank correlation between the two sections 0.5 mm apart was 0.93 and between the first and the second measurements of the first analyzed sections, it was 0.83. This correlation was slightly lower because of one section that was probably misinterpreted in the first evaluation. The effect of a high dose of alendronate on the bone-metal contact became more evident on the second evaluation, using the mean of the two sections (Mann-Whitney U-test, $p = 0.004$). However, we regard the first evaluation as the correct one to report, as has been done above.

Thus, all doses of alendronate and clodronate had a definite effect on bone remodeling, as evidenced by the increase in ash weight of the contralateral tibia. In contrast, only the highest dose of alendronate or clodronate reduced the bone resorption caused by motion at the test surface below the plate.

Discussion

Instability has been suggested as initiator of periprosthetic bone resorption. We have addressed this problem in a rat model to determine whether bisphosphonates inhibit this type of bone resorption. In a previous study (Åstrand and Aspenberg 1999), we gave alendronate via subcutaneously placed osmotic minipumps, implanted 4 weeks after inserting the implant, at the same time when implant movement was started. We found no reduction, and therefore no inhibition of osteoclastic activity at the unstable site, but normal skeletal remodeling was greatly affected. In this study, we gave subcutaneous injections of bisphosphonates 1 week after implant insertion, when the abraded bone below the implant was re-forming. When we used the doses recommended for treatment of osteoporosis, no reduction occurred in the instability-induced bone resorption in this model, but when we increased them about 50-fold, inhibition was noted.

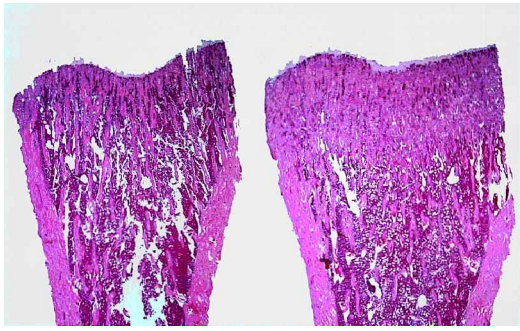


Figure 5. Specimens from previous study (Åstrand and Aspenberg 1999). Proximal tibial metaphyses with growth plate removed. Left: control, right: treated with alendronate 0.063 mg/kg/day for 2 weeks. Up to 1 mm broad band of unremodeled primary bone can be seen below the growth plate in treated animals; remodeled bone is visible in the corresponding area in controls. Hematoxylin and eosin.

We conclude that higher doses of bisphosphonates are needed to inhibit the instability-induced bone resorption in this model than to inhibition of normal remodeling. The dose recommended for treatment of osteoporosis in humans is 10 mg/day o alendronate. Due to low gastrointestinal uptake, about 0.7% is distributed systemically. Hence, the dose distributed after oral administration is about 1 μg/kg/day. We gave 3.8 μg/kg/day as our lowest dose, which was not effective. Clodronate is about 50 times less effective than alendronate (Muehlbauer et al. 1991). We therefore gave 115 μg/kg/day as a low dose, and 21,000 μg/kg/day as a high dose—i.e., about 2 μg/kg/day and 430 μg/kg/day of alendronate, respectively.

The need for a high dose of bisphosphonates to reduce the instability-induced bone resorption in this model may be species-related. However, research on bisphosphonates, including the establishment of relevant doses in humans for the treatment of osteoporosis has been done on rats, with an estimated correlation of 10 times higher dose needed in humans than in rats (Muehlbauer et al. 1991).

As in our previous study, the ash weight of the proximal tibia was greatly increased in all bisphosphonate-treated animals. We have shown that this increase corresponds to histological changes due to a reduction of remodeling in the metaphyses close to the growth zone (Åstrand and Aspenberg 1999;

Figure 5). As regards alendronate, the ash weight showed a dose-response relationship (Figure 2), but this cannot be said of clodronate. The group of rats treated with the low dose of clodronate was older at the initial operation, as evidenced by an average of 9 mg greater weight. Hence, the larger size of the proximal tibias precluded comparison with the group of rats treated with a high dose of clodronate.

The stimuli for resorptive activity appear to be stronger in the case of instability-induced bone resorption compared to the situation with normal remodeling. This would suggest a higher rate of bone resorption by the individual osteoclast, or the recruitment of more osteoclasts. Considering that ingested bisphosphonates impair cell function, it is unlikely that an individual osteoclast triggered by a stronger stimulus can resorb more bone before being inactivated. It seems more likely that a limited amount of bone can be resorbed—i.e., since only a certain amount of bisphosphonate will be ingested before the cell is inactivated. If more osteoclasts are recruited at the unstable site than at a normal remodeling site, all these osteoclasts may be active for some time before they become inactivated by ingested bisphosphonates. This could cause substantial bone resorption despite effective bisphosphonate treatment if there are enough activated osteoclasts. An increase in the dose of bisphosphonates or even treatment for a longer period might then be necessary to reduce the required amount of bone matrix to be resorbed before the osteoclast is rendered inactive.

Some data show that the distribution of bisphosphonates to bone is not homogeneous (Davi et al. 1999). Bisphosphonates bind preferentially to regions with higher turnover of bone, such as the trabecular bone in the proximal tibia in rats, as opposed to the cortical bone in the same region. Therefore, our need for a higher dose to reduce bone resorption below the implant compared to the effect on the ash weight of the proximal tibia may be influenced by a difference in the distribution of bisphosphonates. On the other hand, we abrade the cortical bone during insertion of the implant. When we start moving the implant 4 weeks after surgery, bone-metal contact is usually established (Aspenberg and Herbertsson 1996). Thus, a high local bone turnover has been present below the implant during the 3 weeks of bisphosphonate treatment, and therefore one would assume that the local amount of bisphosphonates is comparatively high. More studies will address the question of bisphosphonate distribution in similar situations.

We used 2 ×180° turns of the rotating plate. This might seem a large movement compared to what may take place around even a very loose prosthetic implant. However, we did some trials with a similar plate, mechanically limiting the movement to 15°, and bone resorption of similar degree occurred (data unpublished). Therefore we plan to continue using the 2 × 180° turns to give the manipulator a better and more standardized control of movements during the experiment.

The mechanisms by which clodronate and alendronate affect the osteoclast differ, and some studies suggest that clodronate has an anti-inflammatory effect, but alendronate has some pro-inflammatory effects (Makkonen et al. 1996). However, our findings with this model do not permit us to draw any conclusions regarding such differences.

A recent clinical study with radiostereometric analysis (RSA) of migration of total knee prostheses showed a reduction in early migration after oral treatment with clodronate in doses corresponding to those used to treat osteoporosis (Hilding et al. 2000). Some data indicate that the early migration rate, as mentioned above, is related to later loosening (Ryd et al. 1995). Therefore, our findings suggest that the risk of later loosening, at least as regards knee prostheses, may be reduced with bisphosphonate treatment. However, the migration of the knee prostheses was reduced, but not entirely inhibited, by giving clodronate in a dose of 1.6 g/day. A higher dose may be even better.

Bisphosphonates reduce bone resorption specifically, and it seems reasonable to use them to reduce periprosthetic bone resorption. However, our findings suggest that high doses may be needed to inhibit bone resorption adjacent to an implant which has become unstable. Since the oral dose used for treatment of osteoporosis can hardly be exceeded without side effects, such high doses are hardly suitable for use in humans. Other methods of distribution of the bisphosphonate, for example, local administration, should be evaluated.

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