Alendronate prevents collapse in mechanically loaded osteochondral grafts

A bone chamber study in rats

Magnus Tägil[1], Jörgen Åstrand[1], Lars Westman[1] and Per Aspenberg[2]

Departments of [1]Orthopedics, Lund University Hospital, SE-221 85 Lund, Sweden, [2]Orthopedics and Sports Medicine, Department of Neuroscience and Locomotion, Faculty of Health Science, SE-581 85 Linkoping, Sweden
Correspondence MT: magnus.tagil@ort.lu.se
Submitted 04-01-05. Accepted 04-05-18

Background **Subchondral bone necrosis is important in osteonecrosis, Mb Kienboeck, intraarticular fractures or osteochondral grafting. As revascularization follows, bone resorption may lead to collapse in load bearing areas during the remodeling. Bisphosphonates are potent osteoclast inhibitors. Our hypothesis was that local bisphosphonate treatment of an osteochondral graft, in a high load environment, would protect the subchondral bone from collapse and maintain the joint architecture during remodeling. To investigate this, we used a rat bone chamber model to subject a necrotic osteochondral graft to a large mechanical load during remodeling.**

Method **Cylindrical osteochondral grafts were taken from the patellar groove of rats, one end of the cylinder being the joint surface. The grafts were frozen, thawed and treated with alendronate. The length of the cylinder was measured and the grafts were placed in the chambers, which were inserted into the proximal tibia of rats. The chambers were left to heal in for two weeks to allow establishment of a vascular supply, and then the transplanted osteochondral plugs were mechanically loaded for 4 weeks, once a day with 10 cycles of 2 MPa pressure at 0.16 Hz.**

Results **At harvest, the graft length had decreased during remodeling in 5 of the 6 untreated controls, but only in 2 out of 8 alendronate-treated rats ($p = 0.05$). Histologically, the bone graft in the non-treated controls was resorbed in the remodeled part of the graft, whereas in the alendronate-treated rats a dense trabecular bone was found consisting of both new bone and graft.**

Interpretation **Local treatment of the graft with bisphosphonate diminishes the risk of collapse during revascularization and bone remodeling in a mechanically loaded osteochondral graft. This could be useful in a variety of situations when bone remodeling occurs after a necrosis close to a joint, either spontaneously after osteonecrosis or a fracture, or after surgical procedures such as mosaic-plasty or other osteochondral grafting.**

There are various methods for transfer of cartilage in small or large osteochondral compounds into cartilage defects. In small grafts such as mosaicplasty, the avascular bone within the graft seems to integrate and remodel, whereas the cartilage fails to integrate with the host tissue and sooner or later degenerates (Hurtig et al. 2001). In other situations, remodeling of avascular bone in load-bearing joints may lead to temporary weakening of the subchondral bone. This might lead to subchondral collapse and could be the cause of cartilage degeneration seen in late stages of osteonecrosis or Kienboecks disease, or non-vascularized joint transplants. Also, in a transplanted joint it is important to maintain the joint line architecture and avoid subchondral trabecular collapse leading to incongruity after remodeling.

Bisphosphonates inhibit bone resorption by adhering to the bone mineral and inactivating the osteoclasts once they start to resorb. Since bisphosphonates have a high affinity for bone, grafts can be treated locally by simply soaking the graft in a bisphosphonate solution (Aspenberg and Åstrand

DOI 10.1080/00016470410004157

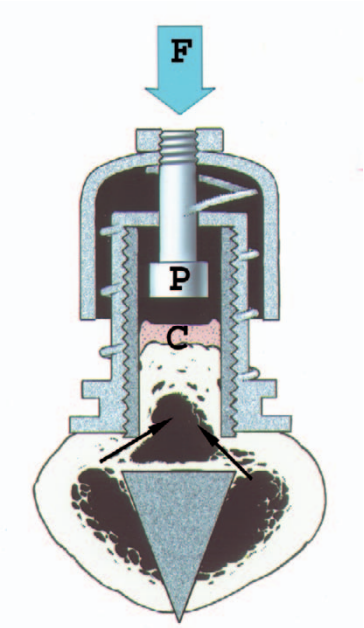

Figure 1. The load chamber (LC). Mesenchymal tissue grows in from the subcortical bone through ingrowth openings at the lower end (arrows) upwards. The loading force (F) is applied from outside the skin by a dynamometer and transferred to the ingrowing tissue (C) via a piston (P). A spring keeps the piston in its upper position when not loaded. (Reproduced with permission from J Orthop Res 1998; 17 (2): 201).

2002). Our hypothesis was that local bisphosphonate treatment of an osteochondral graft, in a high load environment, would protect the subchondral bone from collapse and maintain the joint architecture during remodeling. To investigate this, we used a rat bone chamber model to subject an osteochondral graft to a large mechanical load during remodeling.

Animals and methods

We used the load chamber (LC; Figure 1; Tägil and Aspenberg 1999). The chamber consists of a threaded titanium cylinder, formed out of two half-cylinders held together by a mobile cap. One end of the implant is screwed into the bone. There are two bone ingrowth openings at one end, where tissue can grow in from the subcortical cancellous bone. The cap is equipped with a 1.8-mm diameter piston protruding into the chamber, from the subcutaneous end towards the intraosseous end. By applying a known force on the top of the piston, a mechanical load can be transmitted to the tissue within the chamber. When loading is interrupted, the piston returns to its original position by means of a spring and no further mechanical stimuli act upon the tissue within the chamber. The inside diameter of the LC is 2 mm; the distance between the chamber bottom with its ingrowth openings and the piston is 5 mm when the chamber is unloaded, and 1.5 mm when the piston is in its most downward position. The top, with its mobile parts, is covered with a rubber coat to prevent overlying tissues from interfering with the moving parts.

Grafts

Approval of the Institutional Review Board was obtained before the study was started. Osteochondral grafts were taken from the patellar groove of female Sprague Dawley rats, perpendicular to the joint surface. Using a 2.0-mm diameter hole-cutter, a 3–4 mm long cylinder was taken out from both knees and frozen at –70°C. The grafts were kept in pairs. An alendronate solution was prepared by dissolving one 10-mg tablet of alendronate (Fosamax, MSD, Malmö, Sweden) in 10 mL water for 1 hour while stirring and then passing it through a millipore filter (pore size 0.2 µm). After thawing, one graft in each pair was placed in the alendronate solution (1 mg/mL) for 10 min, then rinsed 3 times for 3 min each time in saline to remove the unbound alendronate. The other graft was immersed in water without alendronate and rinsed similarly with saline. Thereafter, the grafts were placed in the chambers with the cartilage surface facing the loading piston. The chamber was assembled and one full push with the loading device was done to seat the graft. The graft was then taken out and its length was measured with a caliper, before reassembly and insertion of the chamber into the rat tibia. After harvest, the length of the cylindrical graft was measured again.

Surgical procedure

16 male Sprague-Dawley rats were operated on with unilateral chambers (361–395 g, Møllegaard, Køge, Denmark). The rats were kept in our animal facilities for 1 week before experiments started (22°C; 2 rats in each cage with free access to food pellets and water). The rats were anesthetized with peritoneal

injections of 0.6–0.7 mL of a solution containing pentobarbital (15 mg/mL) and diazepam (2.5 mg/mL). Under aseptic conditions, a longitudinal incision was made over the anteromedial aspect of the proximal tibial metaphysis. After incising and raising the periosteum, holes were made manually in the medial and posterior lateral cortices using a specially designed chamber-insertion drill, just anterior to the insertion of the medial collateral ligament. The chambers were then screwed into position so that the bone ingrowth holes were situated at the level of the cortical bone, and the pointed end of the implant was engaged through the opposite cortical bone. The wound was closed using a 4/0 monofilament nylon continuous, intracutaneous stitch, leaving the entire chamber subcutaneous.

Loading

The chambers were left unloaded for 2 weeks, allowing them to heal in and the revascularization and remodeling of the graft to start. The grafts were then loaded for 4 weeks. Pressure was applied by hand using a specially designed dynamometer. This was held to the top of the chamber, outside the intact skin, for 3 sec followed by an unloaded interval of a further 3 sec. This 6-sec cycle was repeated 10 times, once a day. The loading device consisted of a metal rod, a spring and a metal cylinder that could glide over the rod, at the same time compressing the spring. By holding only the cylinder in the hand and pushing it down to a premarked level, a known force could be transferred to the rod and thereby to the top of the chamber. The resistance of the rubber coating and the spring was measured in bench tests, showing that with an external loading force of 8 N, the force loading the tissue in the chamber was 4 N. The pressure exerted on the tissue beneath the piston was calculated to be about 2 MPa.

Evaluation

The rats were killed with peritoneal injections of pentobarbital. The chambers were harvested, disassembled and the specimens measured with a caliper while the investigator was unaware of specimen treatment. In some specimens an increased length was found at harvest compared to at insertion, probably relating to a fibrous tissue layer added on top of the cartilage. Collapse was defined to have occurred when the specimens were shorter at harvest. After fixation in formalin and decalcification in EDTA, the specimens were embedded in paraffin and cut parallel to the longitudinal axis of the chamber with a microtome. Three sections from the middle of the specimens, each at 300 µm distance from the other, were put on separate slides and stained with hematoxylin and eosin for histology and histomorphometry.

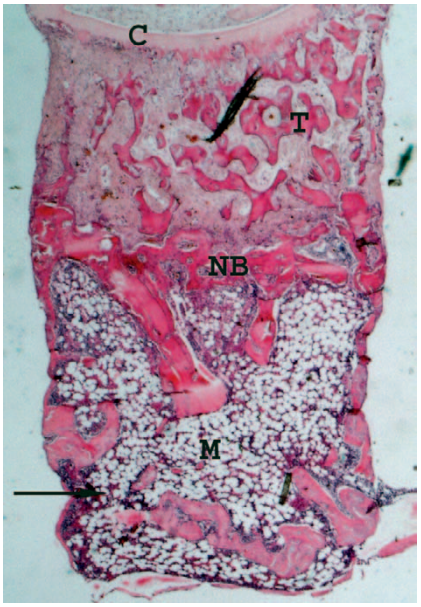

Figure 2. Control specimen after 4 weeks of loading. At the bottom, next to the ingrowth openings (arrow), a marrow cavity (M) is formed and the graft here has been largely resorbed. Above this, the invading frontier of new ingrown bone can be seen (NB). At the top, facing the loading piston, the transplanted cartilage (C) covers the end of the specimen. Beneath the cartilage, a vascularized fibrotic marrow is seen with unremodeled bone trabeculas (T). At the left, the subchondral bone is starting to resorb and fibrous tissue is forming (F) (hematoxylin-eosin × 20).

Results (Table)

1 rat was killed because the chamber loosened and 1 rat was found dead in the cage after the second day of loading; both were in the control group. No wound infection occurred. At harvest, 5 of the 6 remaining control grafts had collapsed, i.e. the graft length had decreased, whereas this occurred in only 2 of 8 alendronate-treated grafts (Fischer exact test, $p = 0.05$).

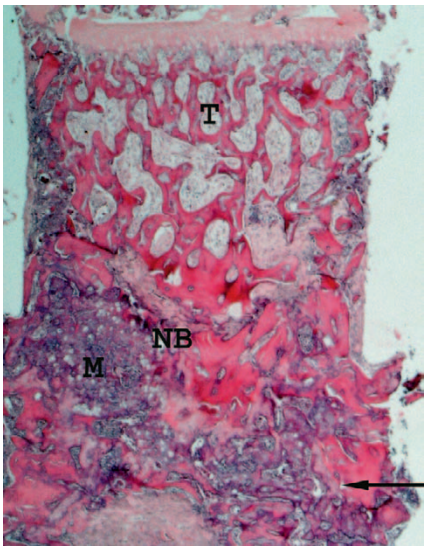

Figure 3. Alendronate-treated specimen after 4 weeks of loading. No marrow cavity can be found at the bottom, but instead there is a dense network of new bone and graft. Above the new bone ingrowth frontier, the specimen looks similar to the controls, with intact graft bone trabeculas in a fibrotic stroma. This specimen retained its original height during remodeling (hematoxylin-eosin × 20).

Histologically, the whole graft was revascularized and invaded by fibrous tissue replacing the original bone marrow. New bone had invaded the graft, but did not reach all the way up to the cartilage in any specimen. A clear ossification front was seen in all grafts. In the remodeled area of the controls, the graft was almost totally resorbed and replaced by bone marrow (Figure 2). In the alendronate-treated specimens, the graft prevailed in the remodeled area and the graft trabeculas were lined with new bone (Figure 3). The increased bone density was obvious to the naked eye, and histomorphometrically a bone density of 42% (SD 12) was found in the remodeled areas of the experiments versus 20% (SD 10) in the untreated controls. In 2 of the controls and in 1 alendronate specimen, a fracture of the cartilage layer was seen, and the cartilage looked necrotic and was undergoing resorption. In these specimens, the cartilage layer had sunk down and was embedded in fibrous tissue.

Discussion

We have shown here that local treatment of an osteochondral graft with a bisphosphonate diminishes the risk of collapse during revascularization in a high-load environment. This may help to maintain a congruent joint line during remodeling. Since 2 rats in the control group died, the number of animals in the study became small and with a p-value just reaching 0.05, the results must be interpreted with some caution. Also, in the experimental group one specimen showed a collapse with a large decrease in height, and an absolute protection is not supplied by the treatment.

Difference in graft length before and after loading and remodeling in bisphosphonate-treated grafts and controls

Graft	Graft length (Δ mm)	
	Experiment	Control
1	0	0.2
2	0	–0.6
3	–0.2	–0.5
4	0.3	–1.1
5	0.25	–0.25
6	–0.9	–0.1
7	0.3	–
8	0.4	–

Avascular necrosis is an infarction of the bone, i.e. all cells die but the inorganic trabecular network remains intact. Once the bone is necrotic, cells from the surrounding living tissue start to invade the necrotic zone and, once revascularized, osteoblasts and osteoclasts start to remodel the dead bone. If the necrosis and the remodeling that follows are taking place in a low-stress part of the bone, the lesion may heal uneventfully. On the other hand, if the remodeling occurs in a highly loaded area such as in the subchondral bone of a joint, the mechanical strength of the remodeling bone may be temporarily decreased so that the bone collapses.

By treating a transplanted bone plug, an osteonecrosis lesion or a load-bearing fracture fragment with a bone resorption inhibitor, the collapse during remodeling in high-stress environments may be avoided and the architecture of the joint line maintained. Instead, new bone will cover the remaining graft trabeculas and increase the bone volume fraction substantially. The remodeling will then lead to an immediate increase in the strength of the subchondral bone, without the transient weakening that normally occurs.

Fluid pressure is a potent inducer of bone resorption (Van der Vis et al. 1998, Skripitz and Aspenberg 2001) and joint cartilage may have an important function in protecting the subchondral bone from the hydrostatic pressure within a joint by hindering massive flow of fluid (Freund 1940, Schmalzried et al. 1997). Direct communication of joint fluid with bone, causing cavitary bone resorption, may have an important role in an osteoarthritic joint, where the intracapsular pressure is elevated due to effusion and decreased capsular compliance (Landells 1953).

In previous chamber studies using the present model (Tägil and Aspenberg 1999, Aspenberg et al. 2000, de Roijj et al. 2001) we have loaded tissue that has formed spontaneously in the chamber, and not grafts as in the present one. In principle, loading in those experiments led to two different outcomes. Either the fluid pressure led to bone necrosis and resorption with gross deformation, or the undifferentiated tissue adjacent to the loading piston differentiated into a sealing cartilage layer, protecting the underlying newly formed bone from fluid pressure and resorption. By qualitative comparison with previous experiments, we have gained the impression that in the present experiment, the transplanted cartilage protected the underlying bone from pressure-induced resorption. In an experimentally induced arthritis study on animals, bisphosphonate was shown to have an effect on subchondral bone thickness and volume, with less focal breaks in the osteochondral barrier. A role for biphosphonate in protection of cartilage, at least partially, was discussed (Podworny et al. 1999).

Apart from the pressure-induced bone resorption caused by direct communication with the joint, two further reasons for subchondral bone collapse can be suggested and may be addressed by bisphosphonate treatment. In a large necrosis, trabecular fatigue fractures may occur in the avascular bone, which would then lack a system of repair. Alternatively, the collapse may occur in the border-zone between dead and living bone, as a consequence of the ongoing repair. In both scenarios, the cartilage might be undermined, leading to joint level collapse. This process may be responsible for the osteochondral collapse in osteochondritis dissecans, osteonecrosis, Kienboeck and Preiser disease, and for some of the problems associated with mosaic-plasty or other osteochondral grafting techniques.

In terms of cartilage repair, the present model using an osteochondral plug as a graft inside the load chamber best resembles the situation in small osteochondral grafts such as mosaic plasty (Matsusue et al. 1993). In this method, the osseous compartment is immediately sealed by the transplant. However, just as in all other methods of transferring cartilage, the integration between the cartilaginous part of the graft and the host cartilage tissue is incomplete. If the gap between the host and graft cartilage is not sealed immediately, or if it starts to leak later due to cartilage degeneration, the fluid under pressure in the joint is able to penetrate into the osseous compartment. A cyst might form around the graft, which might disintegrate—possibly even though the bone part has already been integrated—making the osteochondral plug unstable. No long-term histological follow-up of mosaic-plasty has been described, and there have only been a few animal models. In one equine model, the cartilaginous part of the graft survived for only about 6 months (Hurtig et al. 2001). Perhaps the cartilaginous deterioration is secondary to resorption of the subchondral bone.

The authors thank Inger Mårtensson for technical assistance. This project was supported by the Swedish Medical Research Council (project 2031) and the Medical Faculty of Lund.

Aspenberg P, Åstrand J. Bone allografts pretreated with a bisphosphonate are not resorbed. Acta Orthop Scand 2002; 73: 20-3.

Aspenberg P, Basic N, Tagil M, Vukicevic S. Reduced expression of BMP-3 due to mechanical loading: a link between mechanical stimuli and tissue differentiation. Acta Orthop Scand 2000; 71: 558-62.

Freund E. The pathologic significance of intra-articular pressure. Edinburgh Med J 1940; 47: 192-203.

Hurtig M, Pearce S, Warren S, Kalra M, Miniaci A. Arthroscopic mosaic arthroplasty in the equine third carpal bone. Vet Surg 2001; 30: 228-39.

Landells J W. The bone cysts of osteoarthritis. J Bone Joint Surg (Br) 1953; 35: 643-9.

Matsusue Y, Yamamuro T, Hama H. Arthroscopic multiple osteochondral transplantation to the chondral defect in the knee associated with anterior cruciate ligament disruption. Arthroscopy 1993; 9: 318-21.

Podworny N V, Kandel R A, Renlund R C, Grynpas M D. Partial chondroprotective effect of zoledronate in a rabbit model of inflammatory arthritis. J Rheumatol 1999; 26: 1972-82.

de Rooij P P, Siebrecht M A, Tagil M, Aspenberg P. The fate of mechanically induced cartilage in an unloaded environment. J Biomech 2001; 34: 961-6.

Schmalzried T P, Akizuki K H, Fedenko A N, Mirra J. The role of access of joint fluid to bone in periarticular osteolysis. A report of four cases. J Bone Joint Surg (Am) 1997; 79: 447-52.

Skripitz R, Aspenberg P. Pressure-induced periprosthetic osteolysis: a rat model. J Orthop Res 2000; 18: 481-4.

Tägil M, Aspenberg P. Cartilage induction by controlled mechanical stimulation in vivo. J Orthop Res 1999; 17: 200-4.

Van der Vis H M, Aspenberg P, Marti R K, Tigchelaar W, Van Noorden C J. Fluid pressure causes bone resorption in a rabbit model of prosthetic loosening. Clin Orthop 1998; (350): 201-8.