Bone allografts pretreated with a bisphosphonate are not resorbed

Per Aspenberg[1,2] and Jörgen Åstrand[2]

Departments of Orthopedics, [1]Linköping University Hospital, SE-581 85, Linköping, Sweden, [2]Lund University Hospital, SE-221 85 Lund, Sweden. Email: per.aspenberg@ort.lu.se
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ABSTRACT – Bisphosphonates bind to bone surfaces and inactivate osteoclasts when they start to resorb the bone. Therefore, immersion of a bone graft in a bisphosphonate solution before implantation may protect it from resorption.

We implanted frozen cancellous bone allografts into bilateral bone chambers for 6 weeks in 10 rats. One graft in each pair had been immersed in an alendronate solution (1 mg/mL) for 10 minutes, and then rinsed in saline. Controls underwent the same treatment with saline only. Results were evaluated with histomorphometry. Control grafts were almost entirely resorbed, but alendronate-treated grafts seemed intact. In the treated specimens, two thirds of the space behind the bone ingrowth frontier consisted of graft or host bone, but in the controls, only one fifth. Local graft treatment with a bisphosphonate before insertion seems to be risk-free, and may prevent mechanical graft failure due to resorption in patients.

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Resorption of structural bone allografts is a problem, not only in tumor surgery, but also in hip arthroplasties (Gross et al. 1995, Shinar and Harris 1997). In morselized impacted allografts, the role of resorption is unknown, but it may be an important factor causing clinical failures with this method.

Bisphosphonates inhibit bone resorption by adhering closely to bone surfaces and inactivating osteoclasts trying to resorb it. The bisphosphonate is released from the bone mineral by the action of the osteoclast itself, and becomes internalized in the osteoclast as it starts to resorb the bone. Then it interferes with the intracellular metabolism so that the osteoclast dies (Rogers et al. 2000).

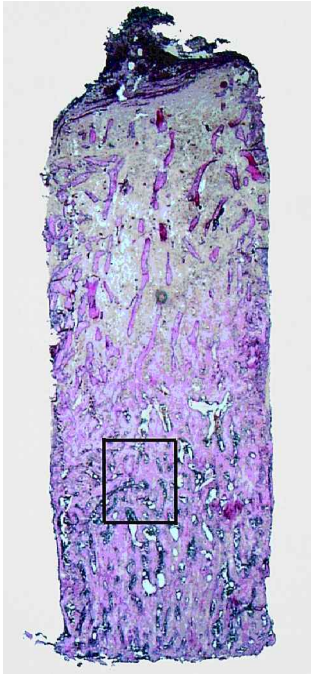
Therefore it may be possible to prevent graft resorption by soaking the grafts in a bisphosphonate solution before implantation. We assessed this method in rats.

Material and methods

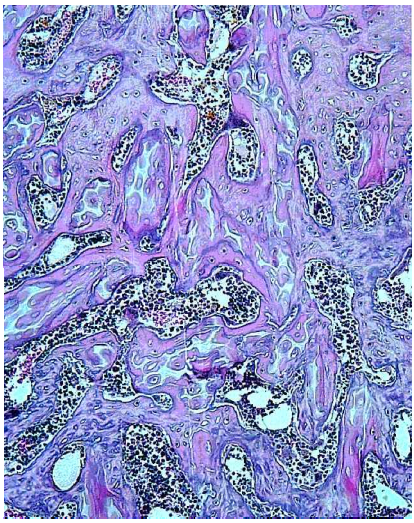
We used the bone conduction chamber (BCC) which is a model for membranous ossification (Tägil et al. 1999). The BCC consists of a titanium screw with a cylindrical interior space. It is made up of two threaded half cylinders held together by a hexagonal closed screw cap. One end of the implant is screwed into the bone. The interior of the chamber has a diameter of 2 mm, and a length of 7 mm. There are two openings for bone ingrowth located at the bone end of the chamber. Thus, the ingrowing tissues enter the cylindrical space from the bone compartment. The chamber space extends far out into the subcutaneous region and the ingrown bone-derived tissue can fill the chamber without competing with other tissues. Ingrowing tissue—most of it bone—will fill part of the chamber within 6 weeks, but will not reach the far end of the cylinder. Thus, the tissue ingrowth distance from the holes towards the other end of the chamber can be used to estimate tissue regeneration. The geometry of the chamber makes it easy to distinguish areas for histomorphometry. The tissue ingrowth distance can be increased by placing an osteoconductive material, such as a bone graft, in the chamber (Wang and Aspenberg 1996). We inserted the chambers bilaterally in rats so that we could compare both sides.

Frozen bone allografts placed for 6 weeks in bone conduction chambers in rats. Tissue has entered at the lower end, and penetrated the entire graft. Ossification has reached about half-way from the bottom towards the top.

A. Control. Below the bone formation frontier, a marrow cavity has formed, and the graft is almost entirely resorbed.



B. Alendronate pretreated graft. Bone ingrowth has reached the same distance, but the graft is not resorbed.



C. Detail of framed area in B. Amalgamation of graft and host bone.

Pairs of structurally intact cancellous bone grafts were obtained from 20 female Sprague Dawley rats (200 g). A cylindrical 2 × 6 mm bone rod was resected in the axial direction from the knee joint with a hole cutter. The epiphysis and the growth plate were excised. The proximal part of the graft had the densest cancellous bone and was later turned towards the ingrowth end of the chamber. The grafts were kept sterile and frozen at –70 °C.

5 similar grafts were taken from another batch of 200 g rats and prepared for histology immediately, without being implanted.

We prepared an alendronate solution by dissolving one 10 mg tablet of alendronate (Fosamax, MSD, Malmö, Sweden) in 10 mL of water for 1 hour while stirring and then passing it through a sterile 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 minutes, then rinsed 3 times for 3 minutes in saline, to remove the unbound alendronate. The other graft in the pair was immersed in water and rinsed as above. Thereafter, the grafts were placed in bone chambers and implanted bilaterally in 10 recipient 350 g male Sprague Dawley rats. After 6 weeks, the rats were killed and the contents of the chambers prepared for decalcified histology with sections parallel to the long axis of the chamber, stained with hematoxylin and eosin. We studied 3 sections, each 0.3 mm apart, in each specimen. All sections were blinded for identity and evaluated in random order.

The evaluation was done by manual point counting of an area of interest from the bottom of the chamber (at the ingrowth end) to the frontier of the advancing new bone formation, but only comprising the central third of the specimen, so that the bone close to the titanium side walls was not included. The total number of points, points covering bone in general and points covering new living bone was counted. We distinguished between graft bone and new bone by evaluating matrix staining (which is paler and more uneven in the graft), pres-

ence of osteocytes and trabecular shape. On average, 366 points were counted per specimen. The point counting was repeated on half of the specimens (chosen by random) by the same person after 1 year. The measurement error (SD) was 6% units for graft bone, 6% units for new bone, and 3% units for bone in general. The bone ingrowth distance was measured by computer-aided methods described elsewhere (Wang and Aspenberg 1996). For all measurements, data from the 3 sections were averaged to form a single value per graft. Statistical analysis was then done with Wilcoxon's signed rank test. Institutional guidelines for the care and treatment of experimental animals were followed.

Point counts. Total number of points counted, number of points covering live (host) bones and dead grafts. Percentages and statistical analysis are given in the text

	Total		Live bone		Dead graft	
Rat	Alen	Cont	Alen	Cont	Alen	Cont
1	418	263	143	42	134	28
2	322	496	99	31	144	17
3	330	251	110	40	110	11
4	261	381	104	69	62	17
5	400	495	181	97	96	51
6	490	405	204	36	146	6
7	453	278	162	37	156	21
8	110	354	20	45	44	30
9	447	533	162	65	131	37
10	452	228	184	34	114	7

Alen = alendronate, Cont = control

Results

Soft tissue had invaded the the whole grafts in all chambers. New bone had formed a bone ingrowth frontier half-way through the graft. In the controls, the grafts appeared entirely resorbed behind this frontier, so that a large marrow cavity had formed behind the frontier, with hematogenous bone marrow. In contrast, the alendronate pretreated grafts seemed to be entirely intact. Further, new bone lined almost all of the graft trabeculae leaving hardly any space for hematogenous marrow (Figure). The difference in bone density was clearly visible to the naked eye.

With morphometry, no difference was seen in how much further the bone ingrowth frontier had advanced (control 2.9, SD 0.9, alendronate 3.2, SD 0.9). In alendronate-treated specimens, 70% (SD 8), but in the controls, only 22% (SD 10) of the space behind the bone ingrowth frontier consisted of graft or host bone ($p = 0.0007$). More than half of this bone in the alendronate-treated specimens seemed to be new living bone that had formed on the surfaces of the remaining graft trabecula. Thus, host bone comprised 38% (SD 9) of the total volume. In the controls it, was 16% (SD 8) ($p = 0.0007$; Table).

The bone content of the grafts that were not implanted was 38% (SD 10). This is similar to the graft content of the alendronate-treated specimens, which was 32% (SD 7). The controls contained 6% (SD 3) ($p = 0.0007$)

Discussion

Local pretreatment of a graft with a bisphosphonate solution appears to be a harmless procedure. The total amount of bisphosphonate in the solution needed to pretreat grafts for a single patient would be less than is accepted for intravenous injection in patients with hypercalcemia. Of this amount, only a fraction will bind to the bone, and the rest will be rinsed away before implantation. If a fraction of the bisphosphonate is spontaneously released from the graft after implantation, most of it will probably bind to adjacent bone. Therefore, only a minor part of the amount that can already be tolerated will reach the circulation.

Our study shows that local bisphosphonate treatment can protect the graft from resorption at least during the early postoperative period. Nonviable bone mean an increased risk of resorption, but once the graft surface has been covered by host bone, it seems to be protected against resorption at least until normal remodeling occurs later on. At that time, such remodeling is unlikely to lead to mechanical failure.

The bone surface of the graft is covered by lining cells and a thin osteoid layer, which may prevent the bisphosphonate from reaching and binding to bone mineral. At least after freezing and thawing, this did not seem to be a problem in our present model.

Another theoretical problem in the clinical use is that bisphosphonate-coated graft surfaces give short-lived protection only against resorption, because after many osteoclasts have resorbed a little bone each and then died, the protective layer will finally disappear. At least in our model, this did not seem to occur, probably because local treatment yields very high local concentrations, so that many osteoclasts would be needed to break through the protective layer. Preliminary experiments in our laboratory indicate a dose-response relationship, and normal systemic treatment does not give high enough local concentrations to protect against the strong osteoclastic activity that dead bone may encounter, but this experiment showed that local treatment does. Moreover, only vascularized bone will be reached by systemic treatment. Because revascularization and resorption of an allograft usually occur together, systemic treatment would therefore have to be prolonged and be continued.

In conclusion, local graft treatment with a bisphosphonate before implantation seems risk-free, and may prevent mechanical graft failure due to resorption in patients.

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Gross A, Hutchison C, Alexeeff M, Mahomed N, Leitch K, Morsi E. Proximal femoral allografts for reconstruction of bone stock in revision arthroplasty of the hip. Clin Orthop 1995; 319: 151-8.

Rogers M J, Gordon S, Benford H L, Coxon F P, Luckman S P, Monkkonen J, Frith J C. Cellular and molecular mechanisms of action of bisphosphonates. Cancer (Suppl 12) 2000; 88 : 2961-78.

Shinar A A, Harris W H. Bulk structural autogenous grafts and allografts for reconstruction of the acetabulum in total hip arthroplasty. J Bone Joint Surg (Am) 1997; 79 (2): 159-68.

Tägil M, Johnsson R, Strömqvist B, Aspenberg P. Incomplete incorporation of morselized and impacted autologous bone graft: a histological study in 4 intracorporally-grafted lumbar fractures. Acta Orthop Scand 1999; 70 (6): 555-8.

Wang J S, Aspenberg P. Basic fibroblast growth factor enhances bone-graft incorporation: dose- and time-dependence in rats. J Orthop Res 1996; 14 (2): 316-23.