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A combination of bisphosphonate and BMP additives in impacted bone allografts

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ABSTRACT **OP-1 increases bone ingrowth distance of new bone into allografts (Tägil et al. 2000), but the bone density after incorporation may be reduced by an increase in resorption (Höstner et al. 2000). Bisphosphonates inactivate osteoclasts and can be used to increase allograft bone density after incorporation (Aspenberg and Åstrand 2002). A combination of locally-applied bisphosphonate and OP-1 in the graft could therefore be expected to increase both new bone ingrowth and density. We tested this by using a rat bone chamber model.**

OP-1 alone increased the ingrowth distance of bone. Clodronate increased final bone density greatly, but reduced the ingrowth distance of new bone into grafts that were extremely impacted. This reduction was improved by adding OP-1. Regardless of graft density, combinations of OP-1 and clodronate included a high final bone density, but the ingrowth distances were shorter than with OP-1 alone. These data indicate that new bone and tissue ingrowth into a compacted graft depends on resorption and that resorption is a prerequisite for the stimulating effect of OP-1 in this experimental set-up. Although the problems associated with the use of OP-1 in impaction grafting may be solved by adding a bisphosphonate, some of the benefits of OP-1 can be lost.

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Bone grafting is frequently used in orthopedic surgery. The graft should stay strong, but be able to remodel completely. In hip revisions, impaction seems to improve the mechanical properties of the graft, but marked impaction inhibits the ingrowth distance of new bone into the graft (Tägil and Aspenberg 1998). Recent findings suggest that only some parts of impacted morselized bone grafts remodel (Linder 2000).

BMPs stimulate bone formation (Cook et al. 1998) and graft remodeling (Tägil et al. 2000). They can also stimulate the osteoclast lineage (Kanatani et al. 1995, Kaneko et al. 2000). Osteoclast stimulation probably causes some of the negative effects reported (Jeppsson et al 1999, Laursen et al. 1999).

Bisphosphonates adsorb to bone and inactivate osteoclasts when the bone is resorbed (Russell and Rogers 1999). By treating the bone allograft with a bisphosphonate, both the amount of remaining graft after remodeling and the amount of new bone can be increased (Aspenberg and Åstrand 2002). Therefore, the mechanical strength of the graft probably improves. When morselized impacted bone allograft in hip revisions was mixed with a BMP preparation (osteogenic protein, OP-1), resorption and position failure occurred in 2 of 10 patients (Höstner et al. 2000). The OP-1 presumably stimulated not only bone formation but also resorption, which led to a transient period of weakness of the graft, and caused mechanical failure.

We studied two types of graft density and determined whether if treatment with a combination of a bisphosphonate, clodronate, and OP-1 could maintain a high bone density while increasing the ingrowth distance of new bone into the graft.
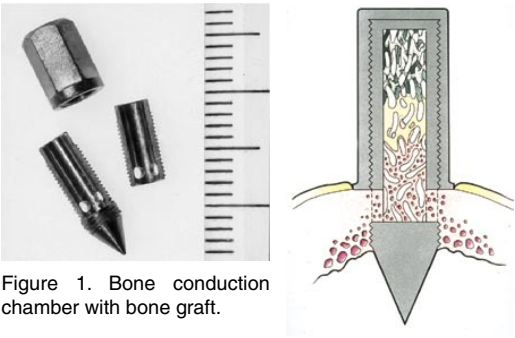
Figure 1. Bone conduction chamber with bone graft.

Table 1. Experimental design group

	No. rats	Graft density	Experimental side	Control side
A	11	moderate	OP-1	untreated
B	11	moderate	OP-1 + clodronate	untreated
C	10	high	clodronate	untreated
D	10	high	OP-1 + clodronate	OP-1

Animals and methods

Experimental design

4 groups, a total of 42 rats, received bilateral bone conduction chambers (Figure 1) loaded with allografts treated according to Table 1. The rats were killed after 6 weeks. We measured the ingrowth distance of new bone and of total tissue as well as bone density on histological examination. The graft density was also measured in 5 high-density and 5 moderate-density grafts that had not been implanted.

Bone conduction chamber

The bone conduction chamber (Figure 1) consists of a threaded titanium cylinder, made of two half cylinders held together by a hexagonal closed screw cap. One end of the implant is screwed into the bone. The bone ingrowth space inside the chamber has a diameter of 2 mm and is 7 mm long. There are two bone ingrowth openings at one end of the chamber, situated subcortically.

Animals

We used 42 male and 70 female outbreed Sprague Dawley rats (weight 350–370 g). The female rats were used as graft donors. All animals were obtained from MB, Silkeborg, Denmark. They were kept in our animal facilities for 1 week before the experiments started (22 °C; 2 rats in each cage, free access to food pellets and water). All methods of handling the animals had been approved of by the regional ethics committee.

Surgical procedure

The rats were anesthetized with a 0.7 mL intra-peritoneal injection of a solution containing pentobarbital (15 mg/mL) plus diazepam (2.5 mg/mL) and were killed with an overdose of pentobarbital. Under aseptic conditions, longitudinal incisions were made bilaterally over the anteromedial aspect of the proximal tibial metaphyses. After incising and raising the periosteum, the medial and posterolateral cortices were pierced with a 1 mm awl just anterior to the insertion of the medial collateral ligament. The hole created in the medial cortex was enlarged manually with a 2.7 mm drill. The chambers were then screwed into position so that the bone ingrowth holes were placed just below the periosteum, and the pointed end of the implant was inserted into the opposite cortical bone (Figure 1). Each animal received bilateral chambers containing bone graft treated as shown in Table 1.

Implants and untreated controls

The recombinant OP-1 was a gift from Stryker Biotech (Natick, Massachusetts, USA). It was supplied in the form of a powder and dissolved in 0.02 M acetate buffer, pH 4.5, to make a 1 μg/μL solution and kept at –70 °C. 1 μg of OP-1 was added to each graft. The graft bone density determines how much solution can be absorbed. The impacted grafts can absorb 12 μL and the moderate-density grafts absorb 8 μL. Therefore the OP-1 solution used for these grafts was diluted accordingly. As controls, we used corresponding volumes of a 0.02 M acetate buffer, pH 4.5, in the moderate-density grafts, and NaCl in the high-density grafts. Clodronate was given as a commercially available 60 mg/mL infusion (Bonefos, Astra, Sweden).

Bone grafts were taken from the proximal tibia of female Sprague Dawley rats weighing 200 g. The epiphysis and growth plate were removed and a cylindrical bone rod was taken from the metaphysis in an axial direction, using a hole cutter. The grafts were wet-frozen at –30 °C for at

least 24 hours. After thawing the bone, 50 grafts were taken, but not impacted. These were called moderate-density bone grafts, while the other grafts were packed in a specially designed impactor in which two cancellous bone cylinders from the same animal were compressed into about the size of one. These were referred to as high-density graft. The interior of the impactor was cylindrical, and had the same diameter as the inside of the bone conduction chamber. A piston could then be inserted into the cylinder to impact the graft along its longitudinal axis. It did not fill the entire inner diameter. A constant force of 80 N was maintained on the free end of the piston for 1 minute. During this time, fat and fluid could escape between the piston and the wall of the cylinder. The pressure used was calculated as 25 MPa. The impactor was opened opposite the piston and the graft could be removed as a bone pellet. 5 high-density and 5 moderate-density grafts were used for measurement of immediate histological examination of bone graft density alone. After impaction, all grafts were washed in saline for 10 minutes to remove some of the fat. The grafts treated with clodronate were soaked in the clodronate solution for 10 minutes, and then washed 3 times for 3 minutes in saline to remove excessive unbound clodronate. Their paired untreated controls were treated the same way with saline instead of clodronate. All grafts were then freeze-dried for 24 hours. Just before implantation, they were thawed and rehydrated with the OP-1 or control solution.

Evaluation

The specimens were fixed in 4% formalin, decalcified, dehydrated, and embedded in paraffin. They were cut parallel to the long axis of the chamber with a microtome and stained with hematoxylin and eosin. 3 sections from the middle of the specimens, each at 300 μm distance from the other, were used for histological and histomorphometric examinations. All slides in each experiment were examined in random order and blinded, but we could see whether or not the graft had been impacted. The area of the new ingrown bone was measured by circumscribing it on a digitizing table, using the Videoplan equipment at 40 × screen magnification. This area includes marrow cavities and graft remnants that had been surrounded by new bone.

We calculated the mean bone ingrowth distance on each slide by dividing the new bone area by the width of the specimen. In all cases, fibrous tissue had penetrated further into the chamber ahead of the new bone. The total tissue ingrowth distance—i.e., the distance from the ingrowth end of the chamber to the fibrous ingrowth border— was measured in the same way as bone ingrowth.

We measured the bone density by manual point counting of an area of interest ranging from the bottom of the chamber (at the ingrowth end) to the edge of the advancing new bone formation, but comprising only the central third of the bone so that the bone close to the titanium side walls was excluded. Points superimposing new bone or dead graft were counted and recorded as "bone points". The total number of bone points on the slide was divided by the sum of bone points plus remaining points and calculated as a mean for that slide.

The mean of all three slides was then used to determine the final values for each chamber. The paired results were tested for significance, using Wilcoxon's signed rank test, and between group comparisons were tested with Mann-Whitney's test.

Results

Grafts not implanted

The bone density of the 5 moderate-density grafts was 33 (33–34)%, and of the 5 high-density grafts 64 (61– 67)%.

Moderate-density grafts

The bone ingrowth distance (Figure 2a) was 46% more in the OP-1 specimens than in their paired untreated controls (p = 0.02), but mean bone ingrowth distance was 19% less than in the untreated controls when clodronate and OP-1 were combined (p = 0.3; not significant). In an intergroup comparison, the side difference in the group comparing OP-1 versus untreated control (Figures 2a, 3a, 3b) was greater than in the group with OP-1 plus clodronate versus untreated control (p = 0.02). Thus, OP-1 increased ingrowth, but this effect was abolished by clodronate.

The total ingrowth distance (Figure 2b) followed the same pattern with an 11% positive effect of

33% bone allografts

a)   **Bone ingrowth distance, mm**

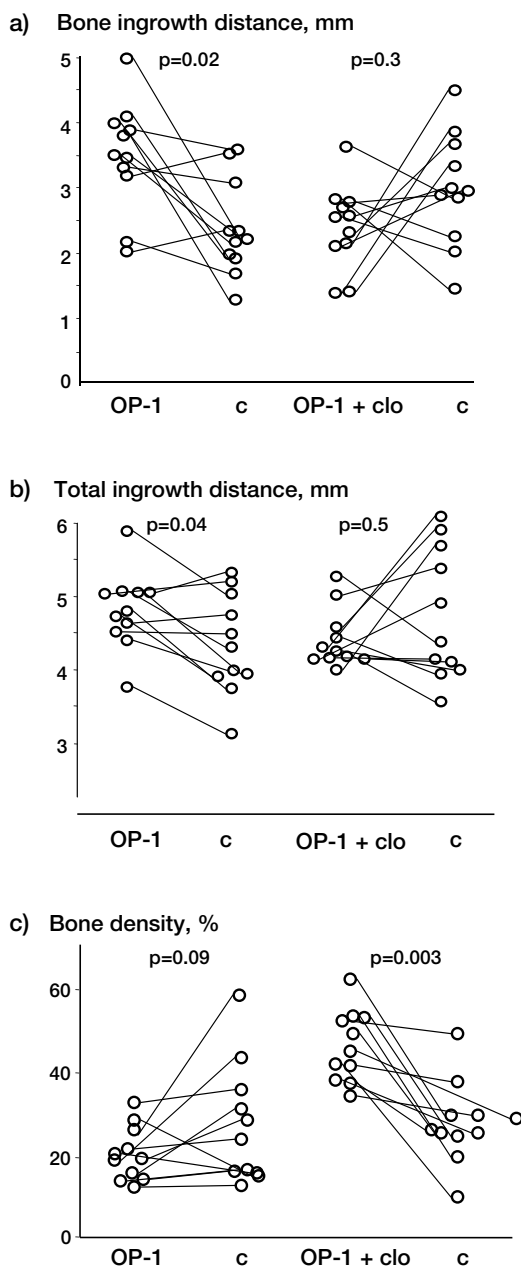b)   **Total ingrowth distance, mm**

c)   **Bone density, %**

Figure 2. Moderate-density grafts. Paired results connected by line.

OP-1 (p = 0.04) and a 7% reduction by OP-1 and clodronate (p = 0.5; not significant). Again, there was also a difference in the effect between the groups (p = 0.05).

No statistically significant difference in ingrowth distance was found between the untreated controls in the 2 groups.

The bone density was increased by 64% in the clodronate plus OP-1-treated specimens (Figures 2c, 3c), as compared to the untreated controls (p = 0.003). OP-1 alone had no effect on bone density.

High-density bone grafts

The bone ingrowth distance (Figure 4a) was 38% less in clodronate-treated grafts than in their paired untreated controls (p = 0.05), but with clodronate and OP-1 combined, the bone ingrowth distance was not significantly reduced, as compared to OP-1 treatment alone (p = 0.2). In an intergroup comparison, we found no significant difference in bone ingrowth between OP-1 plus clodronate versus clodronate alone.

The total tissue ingrowth (Figure 4b) distance was 30% less in clodronate-treated grafts than in their paired untreated controls (p = 0.02), and with clodronate and OP-1 combined, the total tissue ingrowth distance was 32% less than in the OP-1 treated groups alone (p = 0.02). In an intergroup comparison, there was no statistically significant difference between clodronate plus OP-1 versus clodronate alone.

The bone density (Figure 4c) was 80% greater in the clodronate-treated specimens than in their paired untreated controls (p = 0.01), and 93% greater in the clodronate plus OP-1-treated specimens than in OP-1-treated specimens alone (p = 0.005).

Discussion

Clinical results are excellent with the "Ling-Slooff" technique (Slooff et al. 1984, Gie et al. 1993), using impacted morzelised bone allograft in hip revision cases. Many of these grafts seem to remodel completely on radiographs (Gie et al. 1993), but despite these findings, recent histological studies suggest that the allograft may not remodel very much (Tägil et al. 1999, Linder 2000). Ingrown fibrous tissue usually supports the graft fragments and forms a mechanically stable composite which, after a brief initial period of remodeling, remains inert (Linder 2000). This fibrous tissue cover con-
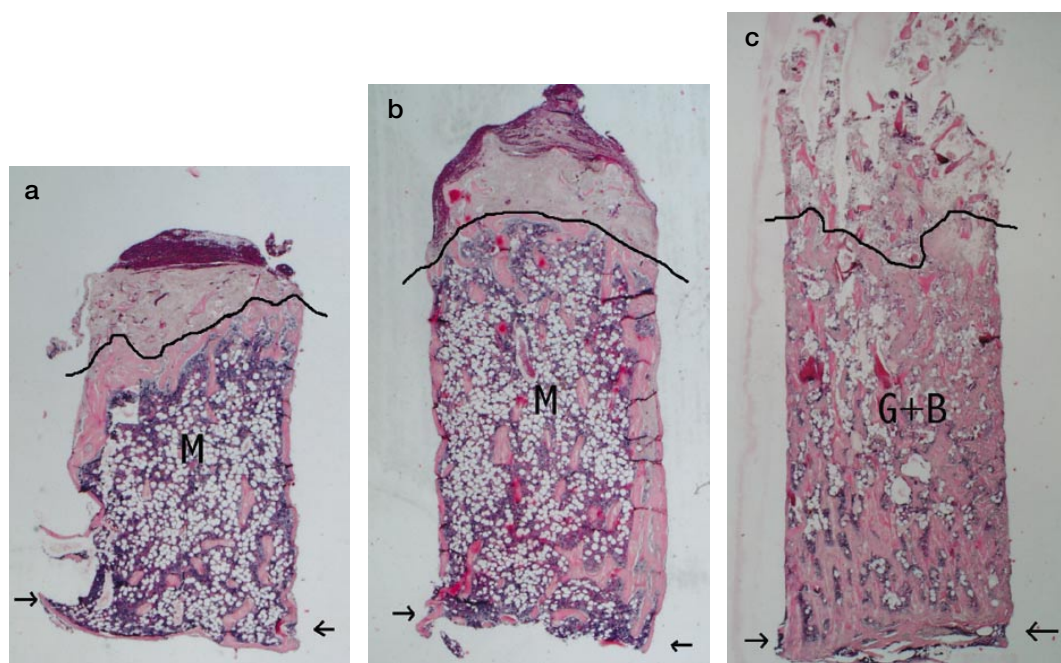
Figure 3. Histological findings in moderate-density grafts. Marrow (M) behind bone ingrowth edge (line). Ingrowth openings (arrow). Newly formed bone (B) and graft (G).
a) untreated control
b) OP-1 treatment
c) OP-1 and clodronate treatment.

tributes to the mechanical properties of the graft (Tägil and Aspenberg 2001).

Bisphosphonates adsorb to bone and inactivate osteoclasts when the bone is resorbed (Russell and Rogers 1999). One way to reduce further the resorption of a morselized graft would be to treat it with a bisphosphonate, and we have found that the final bone density in a rat chamber increases greatly if the graft is pretreated with clodronate (Aspenberg and Åstrand 2002). The impairment of the bone ingrowth into the graft by bisphosphonates depends partly on the original graft density. With a bone density of 38%, the new bone ingrowth distance is not affected by alendronate treatment in the used chamber model currently (Aspenberg and Åstrand 2002), but in this study, with an original graft density of 64%, clodronate reduced the new bone ingrowth distance. There is probably a physical obstacle that prevents cells from entering the graft, if the graft is greatly impacted. It would therefore be of considerable interest to know the bone density of impacted morselized allograft in clinical cases. We measured the bone density by point counting in three histological specimens from Linder's (2000) series and the bone density in areas that had not remodelled was less than 30%. Thus, the most clinically relevant part of our results would be those with a graft density of about 33%, although these grafts were not impacted.

Marked impaction of allograft impairs new bone ingrowth in our model (Tägil and Aspenberg 1998), but OP-1 treatment of the graft can still lead to more than normal ingrowth (Tägil et al. 2000). Behind the new bone ingrowth border in high-density OP-1-treated grafts, the bone quality resembles marrow. This may be caused by simultaneous stimulation of the osteoclast lineage (Kaneko et al. 2000). An increase in osteoclastic activity may also explain the early failure in cases of hip revision, where the morselized bone graft was mixed with OP-1 (Höstner et al. 2000) or in vertebral compression fractures that lost correction after OP-1 was placed in the bone defect (Laursen et al. 1999).

By combining OP-1 and clodronate, we aimed to stimulate the osteoblast lineage and inactivate
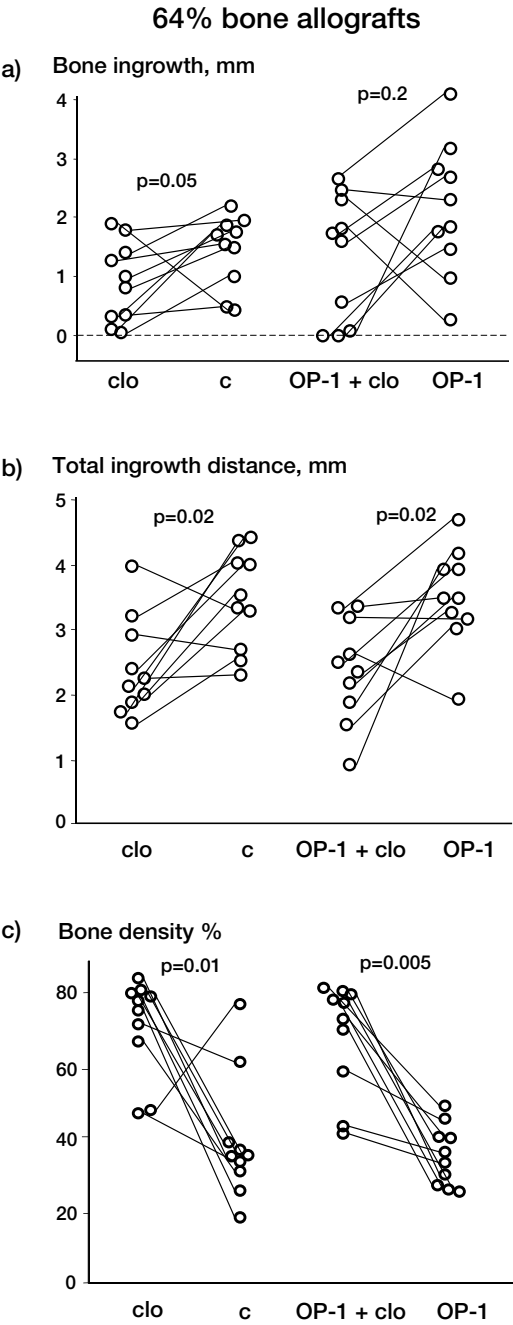
64% bone allografts

a) **Bone ingrowth, mm**

b) **Total ingrowth distance, mm**

c) **Bone density %**

Figure 4. High-density grafts. Paired results connected by line.

the osteoclast linage, thereby increasing new bone ingrowth while maintaining resistance to deformation of the graft. In a previous series (Aspenberg and Åstrand 2002) using bone grafts with a density

Table 2. General interpretation of OP-1 and bisphosphonate effects in this model, based on present and previous results (Aspenberg and Åstrand 2002 [a]; Tägil et al. 2000 [b])

	OP-1	Bisphos	Bisphos+OP-1
Moderate-density graft			
Ingrowth distance	+	0 [a]	0
Density	0	+ [a]	+
High-density graft			
Ingrowth distance	+ [b]	–	0
Density	0 [b]	+	+

similar to clinically impacted morselized allograft, the final bone density was greatly increased by a bisphosphonate, but new bone ingrowth was not affected. In the present study, OP-1 increased new bone ingrowth into similar grafts. When combining OP-1 with clodronate the final bone density was still much greater than in untreated controls, but the OP-1-stimulation of new bone ingrowth was lost. These findings show that clodronate seems to counteract the positive effect on bone ingrowth distance by OP-1 on these grafts, yet there may be a smaller positive effect that would need larger series to be detected.

On the other hand, if the initial graft bone density is much greater, 65%, OP-1 treatment increases new bone ingrowth (Tägil et al. 2000). We now find that the addition of OP-1 to clodronate-treated similar grafts neutralized the impairment in new bone ingrowth distance caused by clodronate, while maintaining the great increased bone density.

In conclusion, the effects on the graft by OP-1, clodronate, or combinations of both, seem to depend partly on the bone density of the graft (Table 2). The decision to treat a graft has to be based on the requirements of the graft. If one wishes to stimulate new bone formation, OP-1 can be useful. In situations where the end result depends on the resistance of the graft to transient resorption, bisphosphonate treatment may be of value.

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