

## Additive effects of PTH and bisphosphonates on the bone healing response to metaphyseal implants in rats

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**Background** When PTH is used to increase the amount of bone in osteoporotic patients, combination with bisphosphonates is known to attenuate the response. This might be explained by the reduced number of remodeling sites after bisphosphonate treatment, which reduces the number of cells able to respond to PTH. However, in a repair situation after trauma, a large number of osteoblasts reside in the wound site. If their activity is no longer coupled to osteoclasts, decreased resorption by bisphosphonates and stimulation of osteoblastic activity by PTH should both (independently) increase bone formation. Thus, we hypothesized that in contrast to the case in osteoporosis treatment, PTH and bisphosphonates have an additive effect in situations involving bone regeneration.

**Material and methods** Stainless steel screws, either coated with bisphosphonates or uncoated, were inserted in 46 rat tibias. This normally elicits a bone repair response, leading to a gradual increase in the strength of screw fixation. Half of the rats also received daily injections of teriparatide (PTH). Thus, there were 4 groups: control, bisphosphonate, PTH, and bisphosphonate plus PTH. Pull-out force and energy were measured after 2 weeks.

**Results** The combined treatment had the strongest effect. It doubled the pull-out force and tripled the pull-out energy, compared to untreated controls. Also, bisphosphonate or PTH alone increased the pull-out force and energy, although less. No treatment cross-dependency was observed.

**Interpretation** Because bisphosphonates mainly influence osteoclasts, and intermittent administration of

PTH mainly influences osteoblasts, our findings indicate that to a large extent these cells work without coupling in this model. It appears that bisphosphonates are unlikely to attenuate the response to PTH during the formation of new bone. ■

Coupling of bone formation to bone resorption is a fundamental concept in bone metabolism. It explains how the amount of bone removed can be replaced by a similar amount of new bone, and is the basis of the remodeling units, which are discrete and localized histological entities. Osteoporosis is caused by disturbances in the number or function of the remodeling units, and the various drugs used to treat osteoporosis mainly exert their effects on them.

Several drugs that are used against osteoporosis can also be used for orthopedic conditions, such as fracture repair, implant fixation, bone grafting, or collapse of osteonecrosis (Skripitz and Aspenberg 2004, Aspenberg 2005). Because our understanding of these drugs is based on research on osteoporosis, i.e. on remodeling units, their potential utility in orthopedic conditions may be misconceived. We claim that bone metabolism in orthopedic conditions often takes place without coupling and remodeling units.

The combination of bisphosphonates and intermittent parathyroid hormone (PTH) treatment appears to be an example of how conclusions from

osteoporosis research may not apply to other orthopedic situations. By and large, PTH causes the remodeling unit to have a positive balance (more bone is produced than resorbed). Bisphosphonates, however, block bone resorption and thereby reduce the number of remodeling units (which is good if they have a negative balance, such as in most cases of osteoporosis). Each drug alone therefore has a positive effect on osteoporosis, but when combined, one would expect that the reduced number of remodeling units would leave fewer sites for the anabolic action of PTH—so that the positive effect of PTH is attenuated. The combined use of PTH and bisphosphonates should therefore be avoided in osteoporosis (Black et al. 2003, Finkelstein et al. 2003, 2006, Ettinger et al. 2004).

If, however, the hypothesis is correct that bone metabolism in an orthopedic process is uncoupled, then PTH and bisphosphonates should have additive instead of antagonizing effects, and could perhaps be recommended in orthopedics. We tested this hypothesis by measuring the fixation of stainless steel screws in cancellous bone—with either drug alone, or in combination. The fixation of the screws is a consequence of the bone-forming response to implantation trauma, which is in turn similar to the fracture-healing response in metaphyseal bone.

## Methods

### Overview

Stainless steel screws were inserted in the proximal tibia of 48 rats, and the pull-out force and energy were measured after 2 weeks. The rats were divided into 4 groups. 2 groups received screws with a bisphosphonate coating and the other 2 groups received uncoated screws. One of each paired group received daily PTH injections and the other received saline.

### Implants

Stainless steel screws 1.7 mm in diameter (type M 1.7) and 3 mm in length were used. The screws were designed with a hole in the head, so that they could be fastened to a hook in a materials testing machine. This type of screw has been used previously in several similar experiments (Skripitz

and Aspenberg 2001b, Tengvall et al. 2004). The screws were etched for 2 min in 40% hydrogen fluoride (HF), cleaned twice in a basic hydrogen peroxide solution for 5 min, and finally cleaned in an acidic hydrogen peroxide solution at room temperature for 1 min. After each cleaning step, the screws were rinsed extensively in distilled water. Half the number of screws was dried in a flow of N<sub>2</sub>, placed in plastic tubes filled with N<sub>2</sub>, sealed with a plastic film, and stored until insertion into the rats.

Thereafter, the screws were coated with bisphosphonates as described in detail elsewhere (Tengvall et al. 2004). Briefly, glutaraldehyde was bound to the screws to serve as an anchor for fibrinogen attachment. 10 layers of fibrinogen were immobilized by ethyl-dimethyl-aminopropylcarbodiimide/N-hydroxysuccinimide (EDS/NHS) coupling techniques, followed by chemical binding of pamidronate (Aredia; Novartis, Sweden) by the same technique. Finally, ibandronate was weakly bound to the screws during an overnight incubation (Bondronate; Roche, Switzerland). The screws were rinsed in water, dried in flowing N<sub>2</sub> and placed in plastic tubes filled with N<sub>2</sub>, sealed with a plastic film, and stored until insertion into rats.

Ellipsometric film thickness measurements indicated that approximately 300 ng/cm<sup>2</sup> (25 Å) pamidronate and 340 ng/cm<sup>2</sup> ibandronate (28 Å) was bound to the screws.

### Rats and operations

The protocol was approved by the regional animal ethics committee. Forty-eight 11-week-old male Sprague-Dawley rats weighing 432 (295–481) g were operated under sterile conditions. 2 rats were excluded due to confusion about their identity. The rats were anesthetized with isoflourane gas. Each rat received a subcutaneous injection of 7 mg oxytetracycline and 0.05 mg buprenorphine. One of the legs was shaved.

A 5–6-mm longitudinal incision was made along the medial aspect of the tibia. The periosteum was reflected dorsally to the physis. A hole, 1 mm in diameter, was drilled, approximately 4 mm from the proximal tibial metaphysis. A screw was inserted, and the skin was sutured.

The treatment was blinded for the operating surgeon and randomized by lottery.

### Evaluation

All analyses were performed while blinded. The rats were killed after 2 weeks and the tibia harvested. The screws were tested for pull-out strength in a computerized materials testing machine (100 R; DDL Inc., Eden Prairie, MN), at a cross-head speed of 0.2 mm/s. The tibias were mounted so that the head of the screw pointed out through a hole (diameter 3.5 mm) in a metal plate, and the head was fixed by a metal pin passing through the hole in the screw head and a horseshoe-shaped connector. The machine recorded the maximum force and the energy uptake until the force had dropped to 90% of maximum.

### Statistics

The primary effect variable was pull-out force. The data appeared roughly normally distributed on graphical representations, and variances were not significantly different between groups (Statview 5.01 for Windows). The main hypothesis was that PTH and bisphosphonates in combination would yield a better fixation than either of them alone. This was tested by 1-way ANOVA followed by post hoc test according to Bonferroni-Dunn. The most important post hoc test was the comparison between combined treatment and bisphosphonates alone, which tested the hypothesis that PTH had a positive effect also in the presence of bisphosphonates. We used 2-way ANOVA to look for dependence in the response of one treatment on the other.

### Results

There were no complications, but 2 rats had to be excluded because of unclear ear marking (1 with PTH and 1 with combined treatment).

The combined treatment doubled the pull-out force and tripled the pull-out energy, compared to untreated controls (Tables 1 and 2).

Table 1. Pull-out force (N)

Group	Treatment	n	Mean	Significant difference versus group	SD
a	Control	12	45	b,c,d	14
b	Control	12	67	a,d	15
c	PTH	11	87	a	22
d	PTH	11	96	a,b	19

Table 2. Pull-out energy (Nmm)

Group	Treatment	n	Mean	Significant difference versus group	SD
a	Control	12	20	b,c,d	7
b	Control	12	34	a,c,d	11
c	PTH	11	46	a,b,d	11
d	PTH	11	59	a,b,c	13

By 2-way ANOVA, both PTH and bisphosphonate increased pull-out force and energy (p-values for force: bisphosphate 0.005, PTH 0.001; p-values for energy: bisphosphate 0.001, PTH 0.001). There was no significant interference between the two treatments (variance ratio (dependence/residual) was 1.4 and 0.07, respectively).

Post hoc test following 1-way ANOVA showed that both drugs alone significantly increased pull-out force and energy compared to untreated controls (Tables 1 and 2). The combined treatment increased pull-out force significantly relative to bisphosphonates alone, and it increased energy compared to each of the single treatments.

### Discussion

As expected from previous studies, both single treatments were effective. No cross-dependence in the responses could be detected, i.e. there was no sign that the bisphosphonates inhibited the response to PTH. On the contrary, the combined treatment was the most effective, although improvement over PTH alone could only be shown for pull-out energy.

Our results indicate that inhibition of the formation of remodeling units by a bisphosphonate

does not ameliorate the effect of PTH, which in turn shows that bone formation and resorption are at least partly independent in this orthopedic condition. One could perhaps argue that because the bisphosphonate was applied only at the screw interface, and the PTH was given systemically, they had exerted their effects in different regions. This is probably not the case, however, because in pull-out tests, the bone does not fail at the implant-to-bone interface, but at the edges of the thread, and histology of bisphosphonate-coated implants has shown that the bisphosphonates increase bone density not only within the threads, but further out in the surrounding tissue (Wermelin et al. 2007). Similarly, PTH induces an increased bone density around the screws (Skripitz and Aspenberg 2001a). Thus, both drugs exert their effects in more or less the same region.

The results are in accordance with some recent studies in which other anabolic substances were mostly used. A bone morphogenetic protein (BMP) in combination with a local bisphosphonate was found to increase bone formation within a bone allograft in a bone chamber model. However, if the bone graft was extremely compacted, bisphosphonates reduced bone ingrowth, and the BMP was not able to reverse this effect (Jeppsson et al. 2003). This is logical, considering that in this situation compact bone has to be resorbed to leave space for new bone. The combination of a BMP and a bisphosphonate was superior to BMP alone as regards bone mass and strength in a rat fracture model (Little et al. 2005).

To what extent may these rat data be relevant to humans? We believe that the tissue surrounding the newly inserted screw produces a basic bone repair response, which is similar in all mammalian species. Bone remodeling, on the other hand, differs between species: rat cortical bone shows little remodeling and there are very few osteons. Still, pretreatment with bisphosphonates attenuates the response to PTH in ovariectomized rats—with respect to bone mass and strength (Gasser et al. 2000). These findings may depend on the rat model, however (Ma et al. 2003). In ovariectomized mice, an additive effect of PTH and a bisphosphonate on femoral strength has been shown (Samadfam et al. 2007). However, in these animals, bone modeling and remodeling were occurring simultane-

ously, and it is possible that the results demonstrate effects on modeling, rather than remodeling.

The idea that osteoblast-osteoclast coupling characterizes all phases of fracture repair has led to a fear of introducing secondary prevention with bisphosphonates shortly after an osteoporotic fracture. The reason would be that if fracture repair were regarded merely as locally increased remodeling, bisphosphonates would slow down the healing process, as they do with the increased remodeling of osteoporosis. There is no clinical evidence for such an effect—and some evidence for the contrary (Adolphson et al. 2000)—and animal data speak against it (Little et al. 2005). Our present finding of independent drug effects upon osteoblastic and osteoclastic activity in bone modeling supports the use of immediate secondary prevention. However, later stages of fracture repair—when mechanical demands have already been met—are indeed characterized by remodeling, and it has been well described that this remodeling is reduced by bisphosphonates, leading to a larger and more immature but strong callus (Adolphson et al. 2000). This also seems to be the case in our screw model. Histological examination has shown a wide area of newly formed bone with an immature appearance, around bisphosphonate-coated screws (Wermelin et al. 2007). Still, the fixation was stronger.

#### *Contributions of authors*

PA envisaged the study, analyzed the data, and wrote the manuscript. KW responsible for screw coating, surgery, and mechanical testing. PT supervised the coating and invented the method. AF planned the study in detail and assisted in mechanical analysis, data handling, and writing.

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