Strong effect of PTH (1–34) on regenerating bone

A time sequence study in rats

Ralf Skripitz[1,2], Troels T Andreassen[3] and Per Aspenberg[1]

Departments of [1]Orthopedics, Lund University Hospital, SE- 221 85 Lund, Sweden. Tel +46 46 171526. E-mail: Per.Aspenberg@ort.lu.se; [2]Orthopedics, University Hospital Eppendorf, Hamburg, Germany, [3]Connective Tissue Biology, University of Aarhus, Denmark. Correspondence: Dr. P Aspenberg
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ABSTRACT – This study compares the effects of parathyroid hormone (PTH) treatment on new bone formation and normal baseline remodelling in rats. To study new bone formation we used a titanium bone chamber, and to study normal remodelling we used the femur and vertebrae from the same animals. One titanium bone chamber was inserted in the proximal tibia of each of 37 rats. The rats were randomly assigned to daily injections of human PTH (1–34) 60 μg/kg) or vehicle control and killed after 2, 4 or 6 weeks.

The total distance of bone growth into the chamber was slightly increased by PTH. Body weight was not affected, and there was only a minor increase in trabecular density of the vertebral and femoral cancellous bone after 6 weeks.

The only dramatic effect of PTH was seen in the chambers. In the controls, a marrow cavity formed in the chamber so that the cancellous density decreased from 44% to 24%, and 11% over 2, 4 and 6 weeks. In the PTH-treated animals, a dense network of bone trabeculae was found in the entire bone chamber at all times. The cancellous density increased from 48% to 60%, and 73% at 2, 4 and 6 weeks, respectively.

The results suggest that PTH treatment can reduce the development of a resorption cavity. Thus, PTH in this model had a net antiresorptive effect, probably solely because it stimulated osteoblastic activity. Even though osteoclastic activity was present throughout the PTH specimens, it was not sufficient to resorb all newly formed bone.

Since PTH seemed to have a greater effect on new bone formation in the chamber than on normal bone remodeling, it might become useful for improving the incorporation of orthopedic implants and stimulating fracture repair.

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Parathyroid hormone (PTH) is a multifunctional molecule that can initiate turnover of bone by the stimulation of osteoclasts, resulting in net resorption, or directly activate the formation of bone by initiating osteoblastic activity. Continuous exposure to PTH results in bone resorption, while PTH administration in intermittent doses results in bone formation (Cosman and Lindsay 1998).

Intermittent PTH treatment increases bone formation and bone mass, leading to increased compressive strength (Hirano et al. 1999). This may well be excellent treatment for patients with osteoporosis. However, the proximal femur appears to be less responsive to the anabolic actions of the hormone than, for instance, the spine (Lindsay et al. 1997). The reason for such differences in skeletal response is unclear. One possible contributory factor is the difference in marrow composition at the two sites. The lumbar spine in adults has mainly hematopoietic marrow and a high bone turnover, while the proximal femur has fatty marrow and a relatively low turnover (Li et al. 1999). The question arises whether skeletal sites with an even higher turnover, such as fractures or areas with newly-implanted orthopedic devices, would respond anabolically to PTH to an even greater extent.

Few data exist on the effects of intermittent PTH on areas with high bone turnover. In ovariectomized rats, PTH inhibits the reduction in mechanical strength of healing fractures caused by this operation (Kim et al. 1996). PTH in a dose of 60 μg/kg/d or 200 μg/kg/d can increase the mechanical strength and callus volume of healing fractures in normal adult rats (Andreassen et al. 1999). We studied how intermittent PTH treat-

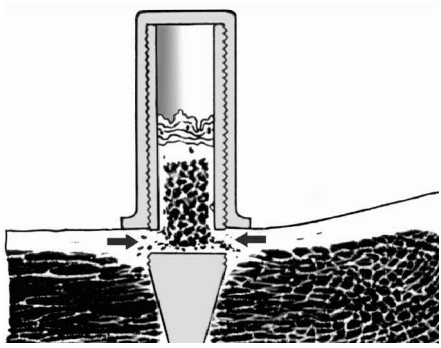

Figure 1. Diagram of the Bone Conduction Chamber. Implant in position at the proximal tibial metaphysis. The holes for tissue ingrowth (arrows) are located in bone.

ment would affect the trabecular density of a forming bone regenerate and how the effects on the remodeling of this regenerate would compare with the effects on normal cancellous bone.

Material and methods

Implants

The bone conduction chamber (BCC) consists of a titanium screw with a cylindrical interior space. When the screw is removed, it can be separated in two half-cylindrical parts to retrieve the specimen (Figure 1). The interior of the chamber has a diameter of 2 mm, and is 7 mm long. The outside diameter is 3.5 mm and the overall length 13 mm.

One end of the chamber has holes for tissue ingrowth and is screwed into the bone. Thus, the ingrown bone-derived tissue can further invade the chamber without competition from other tissues. Due to the size of the chamber, it will not fill completely with tissue. Soft tissue ingrowth is followed by a border of membranous ossification, advancing from the holes towards the other end of the chamber. The ingrowth distance reflects an estimate of bone growth by metaplastic ossification.

Animals and hormone administration

38 male Sprague-Dawley (310–350g) rats were used as recipients of one chamber each. The animals were housed at 22 °C and fed a standard laboratory diet. 2 rats were kept in each cage with free access to food and water and they had a 12-hour light and dark cycle.

After chamber implantation, the rats were randomly divided into 6 groups of 6 or 7 rats each. 3 groups were injected subcutaneously with human PTH (1–34) (Bachem, Bubendorf, Switzerland) in a dose of 60 μg/kg BW/day, dissolved in a vehicle of 0.5 M saline with 2% heat-inactivated rat serum. The 3 remaining groups were injected with vehicle. The injections were given once a day between 8 and 10 a.m. The rats were weighed once a week, and the doses were adjusted to body weight. They were killed after 2, 4, or 6 weeks.

To analyze the bone mineral apposition, injections of Calcein (Sigma, St. Louis, MO) 15mg/kg, Alzarin (Sigma) 25 mg/kg, and Tetracycline (Sigma) 25 mg/kg were given intravenously. Calcein was given on day 14, Alzarin on day 21, and Tetracycline on day 35 (Oxlund et al. 1993).

Surgery

For chamber implantation, the proximal medial aspect of the left tibial metaphysis was exposed with a longitudinal incision, using aseptic technique under general anesthesia (0.6–0.7 mL of a mixture of pentobarbital (15 mg/μL) and diazepam (2.5 mg/μL)). 12.5 mg of streptomycin was given intramuscularly before surgery. The periosteum was removed anterior to the insertion of the medial collateral ligament. The medial cortex was breached with a pinpointed 2.7 mm bone drill, which was carried up through the cortex. Each chamber was screwed into place, so that the pointed end engaged the opposite cortex and the holes for tissue ingrowth were located just below the bone surface. The wound was closed with continuous subcutaneous stitches, using a 4/0-monofilament nylon suture leaving the entire chamber subcutaneous.

Evaluation of results

13 rats, 7 PTH-treated and 6 controls, were killed with an overdose of pentobarbital after 2 weeks. 12 rats, 6 PTH-treated and 6 controls, were killed after 4 and 6 weeks, respectively. The specimens from the chamber were prepared for histology and then rotated and cut at random with sections parallel to the long axis of the chamber. 3 parallel sections from approximately the middle of the specimens, each 200 μm apart, were stained with Masson trichrome, TRAP, or left unstained for fluorochrome-based analysis.

The right femur and the L-5 vertebra of the two 6-week groups were also dissected free. The femur and the vertebra were prepared for decalcified histology. Transverse sections of the femur (7 μm thick) were taken 10 mm proximal to the distal joint plane and stained with H&E. Longitudinal sections (7 μm thick) from the middle of the vertebral bodies were cut and also stained with H&E.

Histological and histomorphometric assessments were performed in random order with blinded specimens. A Merz grid was used for point counting of the central part of each of the specimens to measure the bone density in this area. The bone density was expressed as the percentage of points covering bone tissue in relation to the total number of points covering the measured area. The three different sections from each chamber specimen were measured and their mean value was used for analysis.

In the chamber specimens, the area of the bone compartment, and the total tissue area were measured. The bone compartment includes marrow cavities. The areas were circumscribed on a digitizing table, using Videoplan equipment at a magnification of 10×. The ingrowth distances were then calculated by dividing the total or the bone area by the width of the specimen. Statistical differences between the groups were evaluated with Kruskal-Wallis non-parametric Anova, followed by the Mann-Whitney U-test. Differences were considered significant at p-values less than 0.05.

Results

All implants were clinically stable, without discoloration or swelling around them. The PTH treatment did not affect the body weight of the rats.

Morphology

In all cases and at all times, soft tissue had reached the longest distance into the chamber, followed by an advancing border of metaplastic bone-formation. In the controls at 4 and 6 weeks, the bone behind the border of ossification was resorbed and replaced by a marrow cavity. With intermittent PTH treatment at all times this marrow cavity was almost absent, and a dense network of bone trabeculae was found in the entire chamber behind the ossification border (Figure 2). In the controls, TRAP staining revealed osteoclastic activity solely close behind the ingrowth border, but in the specimens from the treated animals, osteoclasts were found at the trabeculae throughout the specimens.

In the 6-week controls, only the last labeling with Tetracycline could normally be seen in fluorescent light, but all 3 different labels were found in the PTH treated animals for 6 weeks. The early labels were found mostly in the area close to the ingrowth holes. In this area, the 2-week label could be seen in scattered places, usually in the middle of the trabeculae. A band from the 3-week label was found between this and the 5-week label. The latter was found at almost all trabecular surfaces of the PTH specimens, more or less in the same parts of the specimens where osteoclasts could be seen with TRAP-staining (Figure 2).

Histomorphometry

In both the PTH group and the controls, the ingrowth distances increased with time, but there was only a slight insignificant effect of PTH treatment on ingrowth distances. The effect of PTH treatment on cancellous bone density from intact femurs and vertebrae after 6 weeks was also minor (Table).

In the chamber, PTH treatment caused a substantial increase in bone density with time (Figure 3). As early as at 2 weeks, the bone was denser in the chambers of PTH-treated rats ($p = 0.02$). In the vehicle group, bone density declined with time from median 44 (32–52)% at 2 weeks, 24 (7–37)% at 4 weeks, to 11 (2–22)% at 6 weeks. This figure was determined in the middle of the bone compartment, and is therefore unaffected by the amount of bone surrounding the marrow cavity. With 60 μg PTH /kg/day, the density was median 48 (44–64)% at 2 weeks, 60 (47–74)% at 4 weeks, and 73 (61–82)% at 6 weeks. The last measurement shows an almost homogeneous distribution of bone in the bone compartment (Table, Figure 3).

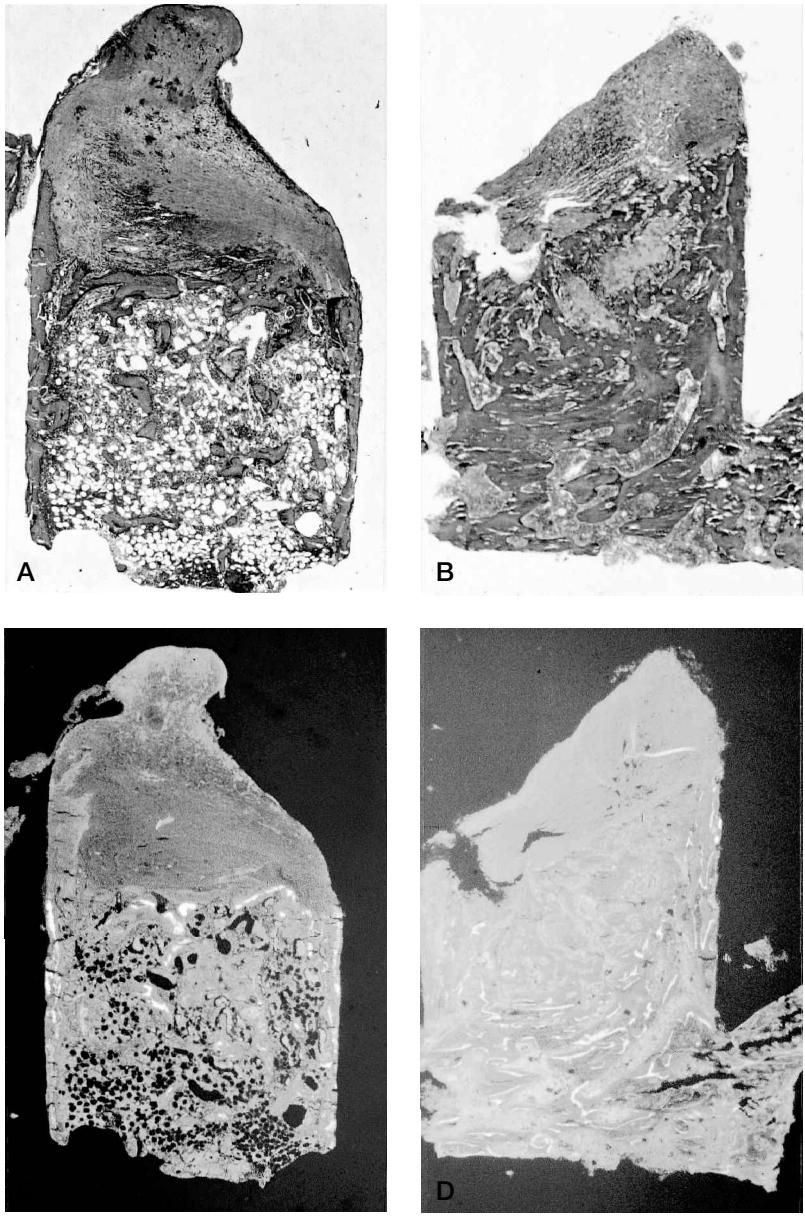

Figure 2. Specimens at 6 weeks. Growth direction is vertical from bottom to top. (A) and (C) are controls treated with vehicle. (B) and (D) are PTH-treated. (A) and (B) are stained with Masson trichrome. (C) and (D) are labeled with Tetracycline at 5 week (unstained with fluorescent light). Note the Tetracycline labeling almost all over the specimen from a treated animal, with the exception of the bone formed within the 6th week. The Calcein (2 weeks) and Alzarin (3 weeks) labels are not seen, due to the filter used. Original magnification of all 4 specimens, ×4.

Discussion

In this study, intermittent PTH treatment caused a 5-fold increase in the trabecular density in an area of high bone turnover. Resuls are similar to those of a previous dose-response study using the same model (Skripitz et al. 2000). Although the rate of bone growth was not significantly increased, PTH treatment inhibited the bone resorption, which otherwise occurred behind the ossification border,

Bone density (in min., median, and max. percent units) located in the central parts of cancellous compartments at 6 weeks

	Control			PTH			P-value
Chamber	2	11	22	61	73	82	0.004
Vertebra	35	50	64	46	56	71	0.06
Femur	3	10	14	6	14	17	0.2

and thus blocked the formation of a marrow cavity. One can therefore say that from a practical standpoint, PTH is not only anabolic, but also has a net antiresorptive effect. In the PTH-treated group, the density not only remained unchanged, but even increased.

TRAP staining showed abundant osteoclasts in the controls and the PTH-treated animals. In the latter group, the fluorescent labeling showed that this osteoclastic activity was not even strong enough to resorb all the bone formed during the first 2 weeks.

PTH treatment had a strong effect in the bone conduction chamber, an area of new bone formation with a high turnover. A less dramatic response to PTH was found in areas such as the vertebra and the femur, which most probably have a lower basic turnover rate. Thus it seems that the effect of PTH is somewhat selective in areas with higher turnover such as fracture repair sites. However, it is important to note that the rat has a stronger bone-anabolic response to PTH than humans and our results may not predict intraskeletal variations in the response of adult human bone to the hormone (Li et al. 1999).

The mechanism underlying the anabolic effect of PTH treatment is not fully understood. Although PTH increases both the number of osteoblasts and activity, the resulting increase in cancellous bone density is probably due primarily to a dramatic increase in the number of osteoblasts (Li et al. 1999, Watson et al. 1999). Since PTH increases bone turnover, the observation of more bone-forming surfaces with active osteoblasts is expected. The origin of these new osteoblasts is unclear. Some authors have shown that the PTH-induced increase in the number of osteoblasts is due mainly to modulation of lining cells to express the osteoblast phenotype (Dobnig and

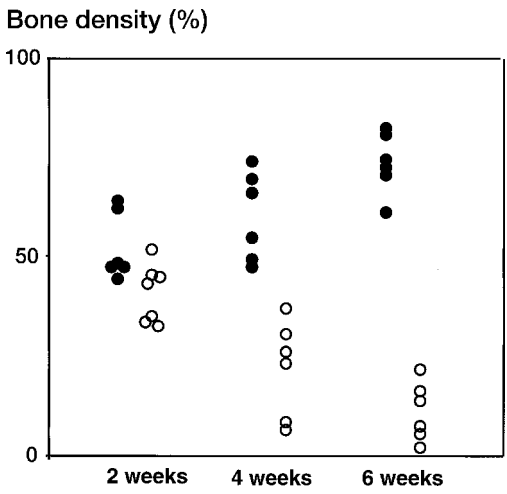

Figure 3. Bone density (in percent over time) after daily PTH (60 μg PTH/kg/d) (●) or vehicle (●) treatment.

Turner 1995, Leaffer et al. 1995). Others have suggested that the increased osteoblast population in rats treated with PTH was due to enhanced proliferation and differentiation of osteoprogenitor cells in the bone marrow (Nishida et al. 1994).

It has been shown that PTH can stimulate both formation and resorption on trabecular surfaces, but cancellous bone volume did not increase significantly at a low dose in beagles (Inoue 1985). Concerning callus formation, Kim et al. (1996) found, however, that the reduced strength of fractures in ovariectomized rats could be prevented partly by giving them 175 μg PTH (1–84)/kg/day. Due to the different molecular weights, this dose corresponds to the 60 μg PTH (1–34)/kg/day used in our experiment. PTH administration to normal adult rats in a dose of 60 μg or 200 μg/kg/day increased the amount of callus and the strength of the fractures after 40 days of healing (Andreassen et al. 1999).

Bone formation in this chamber model takes place solely by metaplastic (membranous) ossification (Tägil and Aspenberg 1998), which is a specific component of the more complex process of fracture repair. Such bone formation also is responsible for the incorporation of stable orthopedic implants. By standardizing the shape of the newly formed bone, the chamber facilitates measurement of bone density, which should reflect the strength of the bone supporting an orthopedic implant. The anabolic effect of PTH can block the

effect of a resorption cavity in our model. This indicates, that PTH treatment may increase bone density in a fracture callus and might therefore be considered as a possible drug to enhance incorporation of orthopedic implants and fracture repair.

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