Bisphosphonates can block the deterioration in implant fixation after withdrawal of intermittent doses of parathyroid hormone

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We have examined the deterioration of implant fixation after withdrawal of parathyroid hormone (PTH) in rats. First, the pull-out force for stainless-steel screws in the proximal tibia was measured at different times after withdrawal. The stimulatory effect of PTH on fixation was lost after 16 days. We then studied whether bisphosphonates could block this withdrawal effect. Mechanical and histomorphometric measurements were conducted for five weeks after implantation. Subcutaneous injections were given daily. Specimens treated with either PTH or saline during the first two weeks showed no difference in the mechanical or histological results (pull-out force 76 N vs 81 N; bone volume density 19% vs 20%). Treatment with PTH for two weeks followed by pamidronate almost doubled the pull-out force (152 N; p < 0.001) and the bone volume density (37%; ANOVA, p < 0.001). Pamidronate alone did not have this effect (89 N and 25%, respectively). Thus, the deterioration can be blocked by bisphosphonates. The clinical implications are discussed.

Continuous exposure to parathyroid hormone (PTH) causes bone resorption, but administration of intermittent doses results in formation of bone by an increase in the number and activity of osteoblasts. Daily injections of PTH post-pone apoptosis of osteoblasts in mice, thereby increasing their number, the rate of formation of bone and the bone mass, but they do not affect the number of osteoclasts. Animal studies have shown that intermittent administration of PTH increases bone mass and bone formation in cancellous and cortical bone, leading to increased compressive strength. The anabolic effect of intermittent PTH on bone can be detected as early as one week after the onset of treatment.

At a dose of 60 µg/kg/day, PTH can effectively enhance the mechanical strength and volume of callus of healing fractures in rats. The response to PTH appears to be more pronounced at sites of bone formation or regeneration than at sites of normal remodelling, since PTH caused a fivefold increase in bone density within a bone chamber, which represented an area of new bone formation, whereas the increase in density in remodelling bones in the same rats was barely detectable.

The insertion of an orthopaedic implant into the skeletal system constitutes an injury, initiating bone repair. Thus, PTH would be expected to have a strong positive effect. We have been able to show that PTH increases the density of regenerating bone and enhances the fixation of steel implants in a dose- and time-dependent manner. Histomorphometric analysis showed a significant increase in contact between the bone and the implant after treatment with PTH for two and four weeks. In another model, a similar but less pronounced improvement in fixation was produced by both systemic and local treatment with bisphosphonate.

However, discontinuation of PTH has been shown to lead to a loss of anabolic effect of PTH on bone in the rat. Our study therefore explores the possible deterioration of the implant-bone stability after withdrawal of PTH, using pull-out force and histomorphometric measurements to determine whether this effect can be counteracted by the subsequent administration of antiresorptive bisphosphonates.

Materials and Methods
We used 138 male Sprague-Dawley rats aged 12 weeks and weighing between 320 g and 350 g. The rats were kept at room temperature (24°C to 25°C) in 55% humidity, with a circadian light rhythm of 12 hours. Two rats were kept in each cage with free access to standard laboratory food pellets and water. One week was allowed for adaptation to these facilities before the experiments began. The regional animal ethics board, conforming to the laws and regulations of Sweden, approved the study.
Table I. Drug administration for the first part of the study. The rats were killed after five weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (mg/kg)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>PTH* (ml/kg)</td>
<td>60</td>
<td>60</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

* PTH, parathyroid hormone

Table II. Drug administration for the second part of the study. The rats were killed after five weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks</th>
<th>1 and 2</th>
<th>3 to 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (n = 10)</td>
<td>0.5 ml/kg</td>
<td>0.5 ml/kg</td>
<td></td>
</tr>
<tr>
<td>PTH* followed by NaCl (n = 11)</td>
<td>60 µg/kg</td>
<td>0.5 ml/kg</td>
<td></td>
</tr>
<tr>
<td>PTH followed by pamidronate (n = 9)</td>
<td>60 µg/kg</td>
<td>500 µg/kg</td>
<td></td>
</tr>
<tr>
<td>NaCl followed by pamidronate (n = 9)</td>
<td>0.5 ml/kg</td>
<td>500 µg/kg</td>
<td></td>
</tr>
</tbody>
</table>

* PTH, parathyroid hormone

Our study was divided into two parts. The first examined the time-dependent properties of fixation of the implant after withdrawal of PTH. A steel screw was placed in the right tibia of 90 rats. After implantation, the rats were randomly divided into two groups which received daily subcutaneous injections of either PTH or saline (NaCl) for two weeks at doses according to the drug administration scheme (Table I). After two weeks, all the rats received NaCl. The injections were adjusted to the weight of the animal and given daily between 8 am and 10 am, beginning on the day of implantation. The rats were killed on days 0, 2, 4, 8 and 16 after withdrawal of PTH to determine changes in mechanical properties. The results of the first and second group from the second part of the study (Table II) were added to the analysis, since these groups had identical treatment to those in the first part and were killed on the 21st day after withdrawal of PTH.

In the second part, 48 rats had a steel screw placed in the right tibia and a steel rod in the left. After implantation, the rats were randomly divided into four equal groups by a lottery according to the drug administration scheme (Table II). All injections were given subcutaneously daily between 8 am and 10 am, starting on the day of surgery. The rats were weighed once a week, and the doses adjusted accordingly. Parathyroid hormone (teriparatide; hPTH 1-34; Lilly Pharma, Bad Homburg, Germany) was administered at a dose of 60 µg/kg body-weight, dissolved in NaCl to 0.5 ml/kg body-weight. NaCl was administered at a dose of 0.5 ml/kg body-weight, whereas pamidronate (Mayne Pharma, Royal Leamington Spa, United Kingdom) was given at a dose of 500 µg/kg body-weight, dissolved in NaCl to 0.5 ml/kg body-weight.

The rats were killed five weeks after implantation. The screws were tested by pull-out in a materials testing machine (100 R; DDL Inc., Eden Prairie, Minnesota) according to the protocol established in our previous studies. The contralateral tibia with the rod was decalcified and prepared for histological examination as previously described. Histomorphometric measurements were obtained using a computer-based image analyser to quantify the bone volume density.

Implants. These were made of stainless steel (SS 2333) and produced in screw or rod form by machining in the technical department of the University Hospital, Lund, Sweden. The screws were 1.7 mm in diameter and 3.0 mm long. The head of the screw had a hole to connect to the mechanical testing device. The rods were 2.0 mm in diameter and 5.0 mm long. The screws were used for measurements of pull-out strength and the rods for histological assessment. All the implants were cleaned in trichloroethylene, rinsed in absolute ethanol in an ultrasonic bath and sterilised in an autoclave before implantation.

Operative technique. The surgical equipment was sterilised in an autoclave. Sterile gloves, theatre caps, gowns and surgical masks were used. The rats were anaesthetised with isoflurane gas. Each received a subcutaneous injection of 7 mg of oxytetracycline and 0.05 mg of buprenorphine. Both legs were shaved. The rat was placed in a sterile surgical glove. A hole was then cut into the glove through which the shaved legs were carefully pulled out. Sterile tape was wrapped around the paw and the legs were cleaned with chlorhexidine alcohol.

The medial proximal metaphysis was exposed by a longitudinal incision. The periosteum was reflected proximally up to the epiphysis. A hole was hand-milled in the cancellous bone approximately 3 mm distal to the epiphysis, using a regular 1 mm injection needle and enlarged using a pinpointed hand drill. Each screw was inserted into the hole and screwed down until its head reached the bone. The rods were inserted into the hole as deeply as possible. The skin was sutured using 4/0-monofilament nylon. The animals bore full weight immediately after waking from anaesthesia. After the operation they received buprenorphine every 12 hours for the first 36 hours for relief from pain.

Evaluation. A blinded investigator (HRJ) collected both tibiae. All tissue growing on and around the screw-head and the protruding parts of the rods was carefully removed. The right tibiae were subjected to pull-out tests immediately. They were fixed by a clamp and traction was applied to those in the first part and were killed on the 21st day after withdrawal of PTH.

The pin was connected to a transducer through a horse-shoe-shaped metal connector. The transducer was connected directly to a computerised materials-testing machine (100 R; DDL Inc.), producing a cross-head speed of 10 mm per minute. The system was calibrated with weights. The
pull-out force was measured as the peak force when the screw became loose from the bone.

The segments of the left proximal tibiae containing the rods were demineralised and prepared by standard histological techniques. The rods were carefully removed after demineralisation. Sections were made parallel to the axis of the rod through the middle of the circular hole and stained with haematoxylin and eosin. The tissue surfaces which had been in contact with the surface of the rod were analysed. All the specimens were given random numbers and were then examined by a third blinded person (HRJ) using a computerised video system (Olympus DP-Soft 3.2 analySIS image processing: Media Cybernetics, Bethesda, Maryland) attached to a light microscope (Olympus BX-51).

The sections were used for measurements of bone volume density. A Merz grid, placed directly on the edge of the former implant, was used for point counting to measure the bone volume density in the area in direct proximity to it. Two visual fields, 0.5 mm × 0.5 mm, were evaluated at each side of the implant. Ten sections per specimen were randomly assessed and a mean value obtained from the 40 measurements from each specimen. The bone volume density was expressed as the percentage of points covering bone tissue in relation to the total number of points covering the measured area.

**Statistical analysis.** The pull-out results and the histomorphometric measurements were tested for significance using one-way analysis of variance (ANOVA) followed by Scheffe’s post hoc test at a significance level of \( p < 0.05 \).

**Results**

In the first part, seven animals were excluded because of failure of the implant in six and death in one from the anaesthesia. Failure of the implant was attributed to misimplantation in four rats and infection in two. The exclusions were made blindly before mechanical evaluation. All the other implants were clinically stable, without signs of displacement or swelling around them.

On the first day after withdrawal of PTH there was a difference in the pull-out force between the two groups \( (p = 0.0009) \). During the first two days after withdrawal, the mechanical properties of the PTH group still improved, reaching a plateau on the fourth day, at which time they remained different from those of the control group \( (p < 0.001) \). On the eighth day, the differences between the groups started to decline, but still remained significant \( (p < 0.001) \). On the 16th day, the difference was no longer statistically significant \( (p = 0.0557) \), and by the 21st day they were indistinguishable \( (p = 0.6445) \) (Table III, Fig. 1).

In the second part of the study, nine animals were excluded as a result of failure of the implant because of misimplantation in four and infection in one, and death in four. In the latter, death was caused by the anaesthesia in three and post-operative heart failure in one. All the other implants were clinically stable, without displacement or swelling around the implants.

At the end of the five weeks, the mechanical properties were identical in the tibiae treated by PTH for two weeks and those given NaCl \( (p = 0.90) \). However, injections of PTH for two weeks followed by pamidronate for three weeks resulted in a dramatic increase in the pull-out force \( (p < 0.001) \) (Table IV).

The histomorphometric results showed that the combination of PTH and pamidronate almost doubled the bone volume density adjacent to the implant as compared with the other groups \( (p < 0.001) \), which did not differ significantly (Fig. 2; Table IV).

**Table III.** Mechnical properties after withdrawal of parathyroid hormone (PTH) with p-values expanded with the results of the first and second groups from the first part of the study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>Mean (sd) pull-out force (N)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>0</td>
<td>87  (19)</td>
<td>0.0009</td>
</tr>
<tr>
<td>NaCl</td>
<td>0</td>
<td>47  (12)</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>2</td>
<td>108 (28)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NaCl</td>
<td>2</td>
<td>39  (12)</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>4</td>
<td>110 (20)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NaCl</td>
<td>4</td>
<td>42  (13)</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>8</td>
<td>99  (35)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NaCl</td>
<td>8</td>
<td>48  (29)</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>16</td>
<td>90  (41)</td>
<td>0.0557</td>
</tr>
<tr>
<td>NaCl</td>
<td>16</td>
<td>67  (18)</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>21</td>
<td>76  (21)</td>
<td>0.6445</td>
</tr>
<tr>
<td>NaCl</td>
<td>21</td>
<td>80  (23)</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

In previous studies, we found that intermittent dosage with PTH led to a significant increase in bone implant contact and in the pull-out force after a short period of time in models identical or similar to those in this investigation.\(^5\)\(^-\)\(^8\),\(^13\) Other studies have shown the effectiveness of this treatment on implant fixation.\(^4\),\(^7\),\(^14\) The first part of the current study showed that withdrawal of PTH induced a rapid deterioration in implant fixation and the second part demonstrated that the deterioration could be prevented by subsequent treatment with a bisphosphonate.

The treatment with PTH during the first two weeks appeared to be more important than the subsequent bisphosphonate treatment, since the group receiving NaCl for the first two weeks, followed by bisphosphonate for three weeks, did not show a significant change in either the mechanical or histomorphometric measurements.

Withdrawal of PTH appeared to lead to resorption of the bone formed under its influence because of a residual stimulation of osteoclasts after withdrawal of PTH. This may have been caused by a change in the balance of osteoprotegerin (OPG) and the receptor activator of nuclear factor (NF)-kappaB ligand (RANKL).\(^15\) The subsequent treatment with bisphosphonate protected against this excess resorption. Pamidronate is a nitrogen-containing bisphosphonate which leads to a loss of regulation of osteoclasts, including control of cell morphology, altered membrane trafficking, loss of membrane ruffling and induction of apoptosis.\(^16\)

Intermittent administration of PTH enhances bone formation by increasing the number and activity of osteoblasts. After infusion of \(^3\)H-thymidine to label all cells progressing through mitosis during treatment with PTH, it was found that almost all the osteoblasts induced by PTH treatment in 16-month-old rats were unlabelled. Thus, it is believed that the increased number of osteoblasts is a result of activation of resting bone lining cells to become osteoblasts.\(^1\) Intermittent dosage with PTH initiates modelling and postpones apoptosis of osteoblasts,\(^17\) while not affecting the number of osteoclasts.\(^2\) However, markers of both bone formation and resorption increase during intermittent treatment with PTH,\(^18\) but continuous

Table IV. Differences in mechanical properties (N) and bone volume density (%) five weeks after implantation

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD) pull-out force (N)</th>
<th>Mean (SD) bone volume density (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>81 (25)</td>
<td>20 (3)</td>
</tr>
<tr>
<td>PTH followed by NaCl</td>
<td>76 (21)</td>
<td>19 (3)</td>
</tr>
<tr>
<td>PTH followed by pamidronate</td>
<td>152 (24)</td>
<td>37 (2)</td>
</tr>
<tr>
<td>NaCl followed by pamidronate</td>
<td>89 (23)</td>
<td>25 (2)</td>
</tr>
</tbody>
</table>

* PTH, parathyroid hormone
elevation of PTH does not affect apoptosis of osteoclasts but increases the number of osteoblasts.19

The cellular mechanism responsible for the anabolic effects of intermittent treatment with PTH is not fully known. The genes and pathways which are regulated by intermittent and continuous PTH have been delineated. Both types of treatment coregulate 22 genes, including those known to influence bone formation. Intermittent PTH regulates 19 additional genes while continuous treatment regulates 173 additional genes.20

Bone resorption appears to be mainly controlled by the balance of OPG and RANKL. Every modification in the OPG/RANKL ratio can induce either excessive bone resorption when RANKL is up-regulated, or decreased resorption when OPG is up-regulated.21 Parathyroid hormone controls resorption primarily through modulations of this system.22 Continuous infusion of PTH shifts the OPG/RANKL ratio in favour of RANKL,23 whereas intermittent PTH at first does not show any alterations in this ratio.24

Intermittent administration of PTH is associated with an initial increase in the markers of osteoblast activity followed by an increase in those of osteoclast activity.25,26 These biochemical markers demonstrate that rapid bone formation occurs at first, but over time changes in the OPG/RANKL ratio occur, leading to increased bone resorption.15 However, the effects of intermittently administered PTH remain anabolic. After long-term intermittent treatment with PTH, the OPG/RANKL ratio remains shifted for a certain period of time.15 This led to our conclusion that after withdrawal of PTH its anabolic effects disappear quickly, whereas the increased osteoclast activity remains for a longer period leading to bone resorption. This would also explain how inhibition of osteoclasts by bisphosphonates could prevent this effect.

The risk of loosening of total joint prostheses is determined, to a large extent, during the first few months after operation.27 The effects of PTH appear to be greater on repairing cancellous, loaded bone, since osteoblasts are more abundant at a repair site than in the rest of the skeleton.5 The clinical implication of our findings is that intermittent treatment with PTH followed by bisphosphonates may be as meaningful in orthopaedic surgery, as has been shown in the treatment of osteoporosis.28 It might be reasonable for orthopaedic patients to receive daily injections of PTH for some weeks, followed by some months of treatment with bisphosphonates. This might improve the osseous incorporation of implants, thereby postponing late aseptic loosening.

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References