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THE EFFECT OF ALENDRONATE AND INTERMITTENT PTH ON IMPLANT FIXATION IN OVX RATS

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SUMMARY

Intermittent administration of parathyroid hormone (PTH) leads to bone formation by increasing osteoblast numbers and activity. Intermittent PTH improves implant fixation in normal rats. The purpose of this study was to analyze the osseous incorporation of an implant in osteoporotic rats, while the animals were treated with intermittent PTH (1-34) or the bisphosphonate alendronate.

36 ovariectomized (OVX) Wistar rats were randomized into 3 groups. Polymethylmethacrylate cement rods were implanted in one tibia in each rat. The three groups received daily PTH (60 µg/kg BW), alendronate (200 µg/kg BW) or saline (0,5 ml/kg BW). A sham-ovariectomized group (n=12) was treated with saline only. After two weeks, the area around the implanted cemented rods was analyzed by histomorphometry for bone volume density (BVD) and bone implant contact. Bone mineral density (BMD) in the femora was evaluated by DEXA.

Ovarectomy decreased BVD compared to saline OVX. BVD was higher in the specimens treated with PTH compared to the other three groups. The PTH group had 20% higher BVD than the alendronate group ($p = 0.02$) and 50% higher BVD than the saline control ($p < 0.0001$). The PTH group even had 30% higher BVD than the non-OVX controls ($p = 0.03$). The implant bone contact fraction in the PTH group was 54%, approximately three times the contact of the OVX and the controls, and almost double the contact fraction compared to the alendronate group. Alendronate doubled the implant bone contact fraction compared to the OVX and sham groups ($p < 0.0001$), but did not improve BVD or BMD.

These findings confirm that intermittent PTH increases bone formation and thereby enhances implant fixation in osteoporotic bone. Intermittent PTH may be beneficial for early implant fixation in fractures, non-unions, and prosthetic replacements when bone density is decreased.

INTRODUCTION

The vast majority of fractures occur in osteoporotic cancellous bone. Fractures in cancellous bone heal predominantly by metaplastic bone formation. Internal fixation of these fractures depends of the mechanical quality of the bone in which screws and other devices are implanted. The response to the insertion trauma appears similar to metaphyseal fracture repair. If pharmacological treatment could stimulate bone to respond to the trauma of fracture and implant insertion by more vigorous formation of new bone, the stability of the internal fixation might be increased and healing accelerated.

The early fixation of total joint replacements also depends on a fracture response in stable metaphyseal bone. Because the early fixation is coupled to the risk of late loosening, a faster early bone formative response might provide a better long-time prognosis.

Implant fixation can be improved in animals by agents like parathyroid hormone (PTH) or bisphosphonates [1-3]. Whereas intermittent PTH treatment increases callus formation and mechanical strength, continuous exposure to PTH typically results in bone resorption [4-6]. The effects of intermittently administered PTH are not limited to healthy bone, as the impact of PTH on bone has been demonstrated in animals and humans with osteoporosis [7,8]. The anabolic effect seems to be due to early stimulation of proliferation and differentiation of osteoprogenitor cells and increased production of bone matrix proteins.

In rats, the anabolic response to PTH is enhanced at sites undergoing repair, so that a dramatic increase in the amount of bone is seen after one or two weeks, at which time no or minor changes are seen in uninjured bone [2,6,9]. Insertion of an orthopedic implant into the skeletal system constitutes an injury, similar to a fracture, initiating bone repair [10]. Thus, a strong effect of PTH on osseointegration can be expected, as shown in several rat studies [3,11,12].

Bisphosphonates are the clinically most important class of antiresorptive agents available to treat diseases characterized by osteoclast-mediated bone resorption such as osteoporosis, Paget disease, and tumor-associated bone diseases [13]. Bisphosphonates bind to the mineral component of bone exposed by osteoclasts during bone resorption. The osteoclasts resorb and endocytose both the bone and the attached bisphosphonate. Alendronate, a nitrogen containing bisphosphonate, exerts its effects by inhibiting components of the intracellular mevalonate pathway, leading to a loss of osteoclast regulation and induction of apoptosis [14,15].

Bisphosphonates have been shown to improve osseointegration of implants [16-19]. They prevent bone loss and improve implant fixation in osteopenic OVX rats [1,20].

The present study was conducted to investigate the effects of intermittent PTH or alendronate on the osseointegration of a polymethylmethacrylate-implant in OVX rats.

MATERIALS AND METHODS

General layout

Three groups of ovariectomized rats (OVX) and one sham-ovariectomized control group were used (n=12 for each group). All rats received implants. The OVX groups received PTH, alendronate or control injections respectively and the sham group received control injections. Bone density adjacent to the implant and bone implant contact were measured after 14 days by histomorphometry.

Animals

Female Wistar rats (16 weeks old; weight: 255 - 410 g) were used in this study. The OVX and the sham operations were performed at Charles River Laboratories, France, 12 weeks prior implantation of implants. The rats were kept at room temperature (19° to 21°C) in 55% humidity, with a circadian light rhythm of 12 hours. Two rats were kept in each cage with free access to laboratory food pellets with a calcium content of 1,1% and water.

The regional animal ethics board, conforming to the laws and regulations of Germany, approved the study. Eleven animals died during anesthesia, but there was no death due to other causes.

Implants

Rods, which were used for establishing bone contact fraction, were made of Palacos R bone-cement (polymethylmethacrylate; PMMA). All rods were 5 mm long with a diameter of 2 mm. The cement rods were formed in Teflon-tubes at a room temperature of 23° C and later sterilized with ethylenoxide. The cement rods had a surface roughness average of 2 μm.

Surgery

Surgical equipment was sterilized in an autoclave. Sterile gloves, theatre caps, gowns and surgical masks were used. The rats were anesthetized with a ketamine injection into the peritoneum. Each rat received a subcutaneous injection of 7 mg oxytetracycline and 0.05 mg of buprenorphine. One of the legs was shaved. The rat was placed in a sterile surgical glove and a hole in the glove was cut through which the shaved leg was carefully pulled out. Sterile tape was wrapped around the paw and the leg cleaned once more with chlorhexidine alcohol.
The medial proximal metaphysis was exposed with a longitudinal incision. The periosteum was reflected proximally up to the epiphysis. An insertion 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 rod was inserted in the hole and pressed down until it stayed in place. The skin was sutured using a 4/0-monofilament nylon suture. The animals bore full weight immediately after awakening from anesthesia.

Pharmaceuticals and Administration

PTH was used as injection by dissolving human PTH (hPTH 1-34; Bachem, Germany) in sodium chloride solution containing 2% inactivated rat-serum. The injection solution containing alendronate (Fosamax®, MSD Sharp and Dome GmbH, Germany) was blended with 2% inactivated rat-serum, as well as the sodium chloride injections (Braun Melsungen AG, Germany) used as vehicle. All injections were applied subcutaneously and daily between 8 and 10 AM. The rats were weighed once a week, and the doses were adjusted accordingly.

The three OVX groups received PTH (60 µg/kg BW), alendronate (200 µg/kg BW) or vehicle alone, whereas the sham-group was treated solely with vehicle.

After implantation of the PMMA-rods, all rats were treated for two weeks, and then sacrificed.

Evaluation

A blinded investigator collected tibiae and femora. All tissue growing on and around the protruding parts of the rods were removed. Both proximal tibiae were fixed in formalin. The segments around the rods were demineralized and prepared by standard histological techniques. The cement rods became dissolved during the preparation. Sections were produced parallel to the axis of the rod, through the middle of the circular hole, and stained with hematoxylin and eosin.

The linear tissue surfaces, which had been in contact with the surface of the rod, could be analyzed. All specimens were given random numbers and then were examined by a third person using a computerized video system (Image Pro Plus Program 4.1, Media Cybernetics, Bethesda, MD, USA) attached to a light microscope. The length of the interface surfaces and the length of each part of the interface surface, in which the bone was not separated from the surface by a layer of non-osseous tissue were measured. The total length of the contact surface, divided by the total length of the interface, constituted a bone contact fraction.

Bone density assessments were again performed in random order with blinded specimens. A Merz grid was used for point counting of the central part of each of the specimens. One side of the grid was placed at the implant-bone contact line. Two visual fields, 0.5 mm x 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 density was expressed as the percentage of points covering bone tissue in relation to the total number of points covering the measured area.

The bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DEXA) using a Hologic QDR-4500A device (Hologic inc.; Waltham, MA; USA). For these measurements the femora of the specimens were used. The bones were placed with the posterior side facing down. The resolution was set at 0,311 x 0,311 mm. A 7,4 mm long segment of the middiaphysis of the femora was chosen as “region of interest” (ROI).

All measurements were tested for significance using one-way analysis of variance (ANOVA) followed by Scheffe’s post-hoc test at the 0.05 significance level.

RESULTS

Bone Volume Density

Bone volume density around the implant differed between groups. Intermittent PTH led to almost 20% higher BVD compared to the alendronate group ($p = 0.017$) and 50% higher BVD compared to the control-group ($p < 0.0001$). PTH also led to a 30% higher BVD than the sham-operated controls ($p = 0.030$). Alendronate did not improve BVD significantly (Table 1).

Group	Mean BVD (%)	SD
OVX (n=9)	58.7	11.0
Alendronate (n=11)	72.9	15.4
PTH (n=8)	88.9	10.3
Sham op (n=12)	68.8	17.6

Table 1: Bone Volume Density adjacent to the implant after two weeks

Implant Bone Contact

The results show significant differences between all groups, except between the OVX-group and the sham-operated controls. Alendronate approximately doubled the implant bone contact fraction (IBC) compared to the OVX and sham groups ($p < 0.0001$). PTH established an implant bone contact of 54% – approximately three times the contact fraction of the OVX group, and almost double the contact fraction of the alendronate group ($p < 0.0001$) (Table 2).

Group	Mean Implant Bone Contact (%)	SD
OVX (n=9)	19.9	5.5
Alendronate (n=11)	33.4	4.6
PTH (n=8)	54.4	7.0
Sham op (n=12)	17.6	5.1

Table 2: Implant Bone Contact after two weeks

Bone Mineral Density

The bone mineral density as measured by DEXA showed a significant increase in the specimens treated with intermittent PTH ($p = 0.02$) compared to the OVX-group. The other groups did not differ (Table 3).

Group	**Mean BMD (mg/cm^2)**	**SD**
OVX (n=12)	158	31
Alendronate (n=12)	176	22
PTH (n=11)	190	11
Control (n=12)	163	25

Table 3: Bone Mineral Density after two weeks

DISCUSSION

The results indicate that previous findings with both PTH and bisphosphonates in this model did not require normal bone metabolism, but could be repeated in osteoporotic bone. The doses were chosen to correspond to the previous studies and do not allow a direct comparison between the two drugs. The alendronate was given at a dose corresponding to osteoporosis treatment and PTH about 100 times higher than the osteoporosis dose, as based per body weight. A dose-response relation for bisphosphonates has been shown in a related model, suggesting that a stronger response to alendronate might be possible. PTH has proven efficacious in a rat model with only 5 μg/kg BW [21]. This is still higher than the approved human dose, but might perhaps correspond to human treatment if differences in metabolic rate are taken into consideration.

This study was based on a study design previously used by our group [22]. The model was established to assess the implant bone interface. In earlier studies with a similar model screws had been used [3], making morphometric assessment of tissue contact more difficult. However, in this model, coating the screw with bisphosponate doubled the adjacent bone density at eight weeks. Reference: Reference: Wermelin et al. Bone. 2008 Feb;42(2):365-71.

The PMMA-rods develop a straight area of contact, allowing easier morphometric assessment. There were several limitations to this study, such as the high dosage of PTH compared to the doses in humans, the short study duration, and implants that were not weight loaded. Nevertheless, our model for implant fixation in rats [3], has now been established also in osteoporotic animals. OVX evidently leads to decreased bone mass and altered microarchitecture, as defined for osteoporosis.

In a dog study alendronate enhanced shear strength of press-fit implants, bone ongrowth, and periprosthetic bone after ten weeks of treatment at a dose of 0.5 mg/kg BW [23]. Two weeks of systemic or local ibandronate treatment improved the mechanical implant fixation in a model similar to the presently used one. Yet, in that study, no effects on bone contact or BVD could be shown [19,24,25]. In another rat model, with intramedullary rods, four weeks of low-antiosteoporotic doses of ibandronate (0.25 mg/kg BW) could improve the histologic osseointegration in osteoporotic bone [1].

The comparatively modest effect of alendronate in this study could be due to the rather low

dose, but also the shorted duration than in most other studies [1,20,23]. Short duration studies usually showed mechanical, but no histomorphometric improvements [19]. From a clinical point of view, the early response appears more important. The histological appearance suggests that in our rat model, two weeks correspond to the end of the proliferative, and the beginning of the remodeling, phase.

Only few studies have been conducted investigating the effects of PTH on implant fixation in osteoporotic bone. In ovariectomized rats, intermittent high dosage treatment (150 µg/kg BW) improved fracture healing mechanically and morphometrically [26]. The present study confirms these results, as intermittent PTH improves early implant fixation in osteoporotic bone. The clinical significance of these findings might be that application of intermittent PTH may be beneficial for implant fixation in fractures, non-unions, and prosthetic replacements even in osteoporotic bone.

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