Increases in callus formation and mechanical strength of healing fractures in old rats treated with parathyroid hormone

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ABSTRACT – We studied the effects of intermittent administration of parathyroid hormone (PTH(1-34)) on callus formation and mechanical strength of tibial fractures in 27-month-old rats after 3 and 8 weeks of healing. 200 µg PTH(1-34)/kg was administered daily during both periods of healing, and control animals with fractures were given vehicle. At 3 weeks, PTH treatment increased maximum load and external callus volume by 160% and 208%; at 8 weeks, by 270% and 135%. It also enhanced callus bone mineral content (BMC) by 190% and 388% (3 and 8 weeks). From week 3 to week 8, callus BMC increased by 60% in the vehicle-injected animals, and by 169% in the PTH-treated animals.

In the contralateral intact tibia, PTH treatment increased BMC by 18% and 21% (3 and 8 weeks). No differences in body weight were found between the vehicle-injected and the PTH-treated animals during the experiment. In conclusion, PTH treatment enhances fracture strength, callus volume and callus BMC after 3 and 8 weeks of healing.

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Intermittent administration of parathyroid hormone (PTH) has an anabolic effect on cortical bone in young and old rats, and thereby increases bone strength (Ejersted et al. 1993, Wronski et al. 1993, Mosekilde et al. 1995, Andreassen and Oxlund 2000). Recent experiments have shown that PTH treatment enhances callus formation and strength of healing fractures in young rats (Andreassen et al. 1999, Holzer et al. 1999, Kim and Jahng 1999), although PTH only induces a modest periosteal bone formation in intact diaphysial bones (Ejersted et al. 1998, Andreassen and Oxlund 2000).

Since bone cell activity is impaired in old rats (Liang et al. 1992, Roholl et al. 1994, Quarto et al. 1995, Fleet et al. 1996), we have now studied the effect of PTH treatment on fracture healing in such rats.

Animals and methods

27-month-old female Wistar rats were randomly divided into 4 groups. The fractures were tested after 21 and 56 days of healing, and at each healing period, two groups were tested: rats injected daily with vehicle, and rats injected daily with 200 µg of PTH(1-34)/kg. Human parathyroid hormone (1-34) (hPTH(1-34), Bachem, Bubendorf, Switzerland) was dissolved in a vehicle consisting of 0.15 M saline with 2% heat-inactivated rat serum. The injections were given subcutaneously from the day of fracture, and each week the rats were weighed and the dose adjusted to their actual body weight. They had free access to tap water and pellet food (Altromin diet 1324, Chr. Petersen, Ltd, Ringsted, Denmark; 0.9% calcium, 0.7% phosphorus).

Fracture technique

Using halothane anesthesia, a unilateral standardized closed tibial fracture was produced by three-point bending above the right tibiofibular junction. Under sterile conditions, closed medullary nailing was performed with a Kirschner wire. Radiographs were taken and unprotected weight-bearing was allowed. The experiment was approved by the

Danish Animal Experiment Inspectorate.

The rats were killed with pentobarbital (150 mg/kg intraperitoneally). Both tibiae were freed, and bilateral radiographs were taken. Then the bones were stored in buffered Ringer's solution (4 °C, pH 7.4).

External callus volume and dimensions

Total volumes of both tibiae were gauged (Archimedes' principle), and external callus volume calculated as the volume of the fractured tibia minus the volume of the intact tibia (Andreassen et al. 1999). Then the external medial-lateral and anterior-posterior diameters at the fracture line were measured.

Table 1. Mechanical properties of fractured tibiae after 3 and 8 weeks of healing [a]

	Vehicle	PTH(1-34) 200 μg/kg/day	P-value
21 days of healing			
Number	5	5	
Fractured tibia			
Ultimate load (N)	6.8 (2.8)	17.7 (2.0)	0.03
Ultimate stiffness (N/mm)	41 (31)	40.0 (13)	0.3
Deflection at ultimate load (mm)	0.69 (0.15)	1.01 (0.29)	0.4
56 days of healing			
Number	9	7	
Fractured tibia			
Ultimate load (N)	37 (5.9)	137 (30)	<0.01
Ultimate stiffness (N/mm)	189 (33)	435 (54)	<0.01
Deflection at ultimate load (mm)	0.32 (0.06)	0.46 (0.05)	0.1

[a] Mean value (SEM)

Mechanical testing

The Kirschner wire was removed and, using a three-point-bending procedure, fracture strength was measured in a materials testing machine (Alwetron 250, Lorentzen and Wettre, Stockholm, Sweden). The healing tibia was placed on two rounded bars (distance between bars 15 mm; fracture line halfway between bars), and bent until failure by lowering a third bar onto the fracture line. Load and deflection were recorded continuously, and ultimate load, ultimate stiffness, and deflection at ultimate load were calculated (Andreassen et al. 1999).

Peripheral quantitative computed tomography (pQCT)

All fractures broke at the fracture line without any of the callus/bone material coming loose. The two ends of the fracture were realigned at their original position, fixed with surgical tape, and frozen for later measurement of bone mineral content (BMC). Tomographic measurements of total BMC were performed by pQCT (XCT Research SA^{TM}, Stratec Medizintechnik, Pforzheim, Germany; settings: voxel size 0.150 × 0.150 × 0.750 mm, cortical bone threshold 730 mg/cm^3, cancellous bone threshold 214 mg/cm^3, contour mode 1, peel mode 20, cortical mode 1). In the fractured tibia, BMC was determined in five 0.75 mm-thick cross-sectional segments (position: fracture line, 1.5 mm and 3.0 mm above fracture line, 1.5 mm and 3.0 mm below fracture line). The intact tibia BMC was measured in the same way. Total BMC was calculated as the sum of the 5 segments. Callus BMC was calculated as total BMC of the fractured segments minus total BMC of the intact segments.

Statistics

The Mann-Whitney U-test was used and $p < 0.05$ (two-tailed) was considered statistically significant (SPSS-10, SPSS Inc., Chicago, IL, USA).

Results

After 21 days of healing, the ultimate load of the fractures was increased by 160% in the PTH(1-34)-treated group. After 56 days of healing, ultimate load was increased by 270%, and ultimate stiffness by 130% in the PTH(1-34) group (Table 1). No differences in deflection at ultimate load were found after 21 or 56 days of healing.

PTH(1-34) treatment for 21 days increased external callus volume (208%), callus dimensions at the fracture line (anterior-posterior 43%, medial-lateral 41%) and callus pQCT-BMC (190%) (Table 2). PTH increased the diaphysial pQCT-BMCs in the fractured and contralateral intact tibiae (67% and 18%).

Table 2. Dimensions, volume and BMC of fractured and contralateral intact tibae afte 3 weeks of healing [a]

	Vehicle	PTH(1-34) 200 µg/kg/day	P-value
21 days of healing			
Number	5	5	
Fractured tibia			
External callus dimensions			
Anterior-posterior (mm)	4.2 (0.2)	6.0 (0.1)	0.01
Medial-lateral (mm)	3.7 (0.1)	5.2 (0.3)	0.01
External callus volume (mm^3)	63 (14)	194 (15)	0.01
Callus pQCT-BMC [b] (mg)	10 (1)	29 (2)	0.01
Tibia volume (mm^3)	408 (9)	543 (16)	0.01
Diaphysial pQCT-BMC [b] (mg)	33 (1)	55 (3)	0.01
Contralateral intact tibia			
External bone dimensions at locations corresponding to callus measurements			
Anterior-posterior (mm)	2.63 (0.08)	2.60 (0.05)	0.8
Medial-lateral (mm)	2.13 (0.04)	2.15 (0.03)	0.4
Tibia volume (mm^3)	345 (7)	350 (7)	0.8
Diaphysial pQCT-BMC [b] (mg)	22 (0.4)	26 (0.5)	0.01
Body weight at			
Operation (g)	290 (11)	297 (15)	1.0
3 weeks of healing (g)	268 (15)	286 (18)	0.8

BMC, bone mineral content. pQCT, peripheral quantitative computed tomography.

[a] Mean value (SEM).

[b] Value of 3.75 mm-high segment (cumulated from five 0.75 mm-high segments).

PTH treatment for 56 days increased external callus volume (135%), callus dimensions at the fracture line (anterior-posterior 35%, medial-lateral 60%) and callus pQCT-BMC (388%) (Table 3). PTH increased the diaphysial pQCT-BMCs in both the fractured and contralateral intact tibiae (168% and 21%).

No differences in body weight were observed between the vehicle-injected group and the PTH(1-34)-treated group, neither after 21 days (Table 2) nor after 56 days of healing (Table 3).

Discussion

PTH treatment increases callus formation and mechanical strength of healing fractures in 3-month-old rats (Andreassen et al. 1999). In the present study, a similar PTH dose enhanced fracture healing also in old rats. However, the healing pattern in old rats differs from that in young rats, and this applies to control fractures and PTH-treated fractures. In old controls, callus production is slow with little callus volume at 21 days, but young controls have a larger callus volume at 20 days. Still, in old controls, the callus volume continues to increase, and after 56 days of healing, their callus volume is similar to that in young controls at 20 days. In the latter, however, the callus volume declines after 20 days of healing. Old and young PTH-treated animals have similar callus volumes at days 21 and 20, respectively. In old PTH-treated rats, however, callus volume remains unchanged from day 21 to day 56, whereas in young PTH-treated rats, callus volume declines after 20 days of healing.

At present, only a few experiments have studied the effects of PTH treatment on healing fractures. The reduced fracture strength in ovariectomized rats can be counteracted by PTH treatment (Kim and Jahng 1999), and treatment with a PTH-related protein has been shown to prevent the corticosteroid-induced impaired bone healing in rabbits (Bostrom et al. 2000). In both experiments, an increase in callus amount was observed in relation to the treatment.

Using a titanium chamber implanted in rats, as a model of orthopedic implants, a dose-response study recently found a positive correlation between chamber bone density and increasing doses of PTH(1-34) (Skripitz et al. 2000).

Since PTH increases callus deposition and mechanical strength, the treatment may positively influence the management of impaired healing, particularly in situations with reduced callus formation.

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Table 3. Dimensions, volume and BMC of fractured and contralateral intact tibiae after 8 weeks of healing [a]

	Vehicle	PTH(1-34) 200 μg/kg/day	P-value
56 days of healing			
Number	9	7	
Fractured tibia			
External callus dimensions			
Anterior-posterior (mm)	4.9 (0.2)	6.6 (0.6)	0.04
Medial-lateral (mm)	3.5 (0.1)	5.6 (0.7)	0.03
External callus volume (mm^3)	96 (10)	226 (54)	0.06
Callus pQCT-BMC [b] (mg)	16 (2)	78 (19)	<0.01
Tibia volume (mm^3)	447 (13)	594 (68)	0.11
Diaphysial pQCT-BMC [b] (mg)	40 (2)	107 (20)	<0.01
Contralateral intact tibia			
External bone dimensions at locations corresponding to callus measurements			
Anterior-posterior (mm)	2.62 (0.02)	2.66 (0.04)	0.6
Medial-lateral (mm)	2.21 (0.01)	2.21 (0.04)	0.4
Tibia volume (mm^3)	351 (7)	368 (18)	0.4
Diaphysial pQCT-BMC [b] (mg)	24 (0.4)	29 (1.0)	<0.01
Body weight at			
Operation (g)	309 (10)	305 (9)	0.6
8 weeks of healing (g)	295 (10)	289 (16)	0.7

BMC, bone mineral content. pQCT, peripheral quantitative computed tomography.

[a] Mean value (SEM).

[b] Value of 3.75 mm-high segment (cumulated from five 0.75 mm-high segments).

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