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Dose-dependent effect of parathyroid hormone on fracture healing and bone formation in mice

Alexander Milstrey, MD,[a,*,1] *Britta Wieskoetter, MD,*[a,1] *Daniel Hinze, MSc,*[b] *Niklas Grueneweller, MD,*[a] *Richard Stange,*[a] *Thomas Pap,*[b] *Michael Raschke,*[a] *and Patric Garcia, MD*[a]

[a] *Department of Trauma-, Hand- and Reconstructive Surgery, Westfaelische Wilhelms University, Muenster, Germany*

[b] *Institute for Experimental Muscoloskeletal Medicine, Westfaelische Wilhelms University, Muenster, Germany*

ARTICLE INFO

Article history:
Received 29 January 2017
Received in revised form
10 June 2017
Accepted 17 July 2017
Available online xxx

Keywords:
Fracture healing
PTH
Mouse
Dose dependency

ABSTRACT

Background: Parathyroid hormone (PTH) is the only clinically approved osteoanabolic drug for osteoporosis treatment. However, PTH is not established for the treatment of fracture healing, and doses of PTH diverge significantly between different studies. We hypothesized that the effect of PTH on promoting fracture healing and bone formation is dose dependent.

Materials and methods: In vivo, mice were treated with PTH (10, 40, and 200 μg/kg) in a closed femoral fracture model. Fracture healing was analyzed after 4 weeks. The fourth lumbar vertebra was analyzed to assess systemic effects. In addition, osteoblasts from calvaria of mice were treated *in vitro* with PTH doses of 10^{-5}-50 nM, and their differentiation was analyzed after 26 days.

Results: In vivo, PTH dose-dependently stimulated bone formation in the fracture callus and the vertebral body. However, PTH treatment did not increase biomechanical stiffness of the fractured femora in a dose-dependent manner. The increased bone formation in the 200 μg/kg group was associated with a depletion of osteoclasts, indicating diminished bone remodeling. Of interest, *in vitro*, we observed diminished mineralization with the highest doses of PTH in osteoblast cultures.

Conclusions: PTH dose-dependently stimulates bone formation *in vivo*. However, during fracture healing, this did not result in a dose-dependent increase of the mechanical stiffness of the fracture callus. Taken together, our *in vivo* and *in vitro* data indicate that the dose-dependent effects of PTH during fracture healing are based on the actions on multiple cell types, thereby influencing not only bone formation but also osteoclastic callus remodeling.

* *Corresponding author*. Department of Trauma-, Hand- and Reconstructive Surgery, University Hospital Muenster, D-48149 Muenster, Germany. Tel.: +49 251 8356301; fax: + 49 251 8356318.
E-mail address: alexander.milstrey@ukmuenster.de (A. Milstrey).

[1] These authors contributed equally.

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http://dx.doi.org/10.1016/j.jss.2017.07.019

Introduction

Human parathyroid hormone (hPTH) (1-84) or the shortened recombinant equally effective peptide teriparatide (PTH 1-34) regulate calcium metabolism. PTH raises the level of calcium in the serum not only through the absorption of calcium in the kidney and the intestine but also through an osteoclast-dependent release of calcium from bone. However, in contrast to osteoblasts, there is no receptor for PTH on osteoclasts.[1] Physiologically, PTH binds to osteoblasts, which subsequently stimulate osteoclast differentiation and proliferation,[2] leading to osteoclastic bone resorption and calcium release from bone. Continuous high levels of PTH, i.e., in patients with hyperparathyroidism, lead to bone resorption. In contrast, several studies have shown that intermittent PTH treatment stimulates bone formation.[3-6] Nowadays, PTH is well established in the treatment of osteoporosis, as it is the only clinically approved drug with osteoanabolic properties.

Several animal studies have shown that PTH is also able to accelerate fracture healing.[7-11] Despite anecdotal reports on the actions of PTH during fracture healing in humans, only three studies have analyzed the effect of PTH on fracture healing in randomized double-blind placebo-controlled trials.[12-14] Aspenberg *et al.* compared the time to radiological healing in 102 postmenopausal women with a distal radial fracture. Patients were treated with 20 or 40 μg of PTH 1-34 daily compared to placebo. The lower dose of 20 μg significantly reduced the time to radiological union compared to placebo and the 40 μg group.[12] Bhandari *et al.* could not observe an effect of 20 μg/day PTH 1-34 for 6 months on consolidation of femoral neck fractures in 159 patients after 2 years,[14] whereas Peichl *et al.* showed that daily administration of 100 μg hPTH (1-84) significantly accelerated fracture healing in 65 postmenopausal women with pelvic ring fractures.[13]

Analogous to these studies, animal studies have also demonstrated different effects of PTH on fracture healing depending on the dose. In the last 2 decades, several studies with doses ranging 5-200 μg/kg of PTH showed accelerated bone healing and an increase in biomechanical strength, bone mineral content, and callus volume.[7-10,15] However, an ideal dose for stimulation of bone healing has not even been established in animal models. Therefore, the aim of the present study was to analyze the effect of different doses of PTH on bone formation during fracture healing *in vivo*. To analyze the dose-dependent effect of PTH on osteoblasts, we additionally performed experiments in osteoblast cultures *in vitro*.

Materials and methods

In vivo

Study design

Fracture healing was analyzed in 10 (±1)-week-old female C57/Bl6 mice (20 ± 2 g) using a closed femoral fracture model as described previously.[16] Animals were treated subcutaneously with PTH 1-34 in NaCl (Bachem, Bubendorf, Switzerland) at doses of 10 ($n = 6$), 40 ($n = 8$), and 200 μg/kg ($n = 8$) once daily for 28 days. Controls ($n = 8$) were treated with NaCl (B. Braun, Melsungen, Germany) only. After 28 days, animals were euthanatized for micro computed tomography (micro-CT), biomechanical, and histomorphometric analysis. In addition, to analyze systematic effects of PTH treatment, the fourth lumbar vertebral body was analyzed by micro-CT and biomechanical analysis.

All animal care and experimental procedures were performed in adherence to governmental guidelines for the use of experimental animals and approved by the German legislation on the protection of animals (84-02.04.2013.A011).

Surgical procedure

Mice were anesthetized by an intraperitoneal application of 25 mg/kg xylazine and 75 mg/kg ketamine. The right femur was fractured with a 3-point bending device and fixed with an intramedullary MouseScrew (ResearchImplantSystems, Switzerland) as described previously.[16] The fracture repair and the configuration of the femoral fracture were determined immediately postoperatively by radiography. Only those animals with a simple closed transverse fracture and adequate implant placement and fracture repair were included in the study.

μ-CT analysis

After 28 days, animals were euthanized, both femora were resected carefully, and the intramedullary screw was removed. Then, the fractured right femur was scanned with a microtomographic imaging system (Bruker SkyScan 1172, Kontich, Belgium) using 70 kV, 1.2-mm aluminum filter and a 9-μm resolution. The two-dimensional images were reconstructed using SkyScans proprietary software (NRecon and DataViewer). The volume of interest was defined by the axial and radial extension of the healing callus excluding the femoral cortex and bone marrow as described previously.[17] The CT images were segmented into bone and unmineralized regions by applying a visually chosen gray value threshold for all samples (range 68-74 of maximal 256 gray scale value). Bone volume (BV), tissue volume (TV), bone volume/tissue volume (BV/TV), trabecular thickness, trabecular number, and trabecular separation were calculated.

To evaluate a systemic effect of intermittent PTH treatment, we scanned the fourth lumbal vertebral body. The volume of interest was defined by 50 consecutive slices in the coronal plane in the ventral direction, starting from the merge of the pedicle and the body as described previously.[18] The μ-CT parameters mentioned previously were calculated.

Biomechanical analysis

The stiffness of the right femur was measured with a nondestructive three-point bending test, and the left femur served as a paired control. The femora were mounted on the testing device with the lateral part of the femur facing upward and a working gauge length of 6 mm (LR5Kplus, Lloyd Instruments, Meerbusch, Germany). Bones were loaded with a constant speed of 1 mm/min, and measurement was stopped when a linear load deformation curve was reached. The