Effect of Intermittent Systemic Administration of Recombinant Parathyroid Hormone (1-34) on Mandibular Fracture Healing in Rats

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Purpose: To establish a rat mandibular fracture model and investigate the short- and long-term effects of recombinant parathyroid hormone (PTH 1-34) on mandibular fracture healing in rats.

Materials and Methods: A controlled unilateral mandibular fracture was created surgically in 29 male Sprague-Dawley rats and then stabilized using an external fixation device. The rats were divided into 2 groups: 1 group received daily subcutaneous injections of 10 µg/kg of PTH(1-34) and 1 group served as the vehicle control. The rats were killed on postoperative days 7 and 21, and radiographic densitometry and histologic evaluation of new bone formation were performed.

Results: A novel unilateral mandibular fracture model was established that has significant differences from previously published models, both in the location of the osteotomy site and in the rigid external stabilization device. The PTH(1-34) treated rats showed a statistically significant difference (P < .05) in callous formation compared with the control animals. Radiographic densitometry evaluation of the injury site revealed an increase in bone density, apparent at day 7 in the experimental group. Visual inspection of the histologic sections stained with Masson's trichrome blue showed an apparent increase in new bone formation at 21 days in the PTH-treated group compared with the control group.

Conclusions: Intermittent systemic administration of PTH(1-34) might enhance the healing of mandibular fractures in the early phase (7-day period). Long-term administration (21-day period) showed no statistically significant differences between the control and experimental group by radiographic densitometry.

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The mandible is the second most frequently fractured component of the facial skeleton. Hippocrates described the practice of bandaging fractures to stabilize bone segments to allow healing.¹ Twentieth century advanced technology allowed surgeons to combine the principles of immobilization first described by Hippocrates with surgical placement of plates and screws to treat a variety of mandible fractures. Animal models continue to aid in the clinical study of facial skeleton fractures. Many unilateral mandibular osteotomy models found in published studies involve performing the mandibular osteotomy immediately anterior to the first molar, immediately posterior to the last molar, or between the second and third molars.²-⁴ Novel models using alternative locations are needed because of the high risk of damage to the tooth roots and the subsequent increase in oral contamination. Other published techniques have involved a critical-size defect,⁵ which did not achieve the present study’s goal of simulating a displaced mandibular fracture requiring stabilization. Surgically created mandibular fractures that underwent distraction osteogenesis have been reported,⁶-⁷ as have models in which no form of stabilization was used.⁸-⁹ We used an osteotomy model that completely transects the ramus from the sigmoid notch through the inferior border of the mandible, followed by repair with a rigid fixation device that is secured by 2 screws on each side of the osteotomy site.

The treatment of the mandible fracture ranges from maxillomandibular fixation to open reduction with internal fixation. Fracture healing is a complex and sequential process, regulated by multiple inflammatory, growth, and hormonal factors.¹⁰-¹⁵ Currently, no pharmacologic adjuncts are available to enhance the healing of mandibular fractures. In published orthopedic studies, systemic administration of parathyroid hormone (PTH) has demonstrated enhancement of healing in an extremity fracture model using rats¹³,¹⁴,¹⁵; however, it should not be assumed that these results will apply to mandibular fractures, because the mandible differs significantly from long bones in terms of development and force load. The mandible, similar to the flat bones of the skull, develops primarily through the process of intramembranous ossification. In contrast, long bones develop by endochondral ossification. In addition, although the mandible and long bones are subjected to the 3 different types of strain (ie, compression, tension, and shear), the predominant force acting on the mandible during physiologic movement is shear, but the long bones, particularly the femur and tibia (the bones evaluated by most of the studies to date), are primarily subjected to compression forces.¹⁶,¹⁷

PTH is an essential regulator of calcium and phosphate metabolism¹⁸ but has also been shown to have anabolic and catabolic effects on the skeleton.¹⁹ In recent years, PTH has been approved for the treatment of osteoporosis and its anabolic effect has been studied in clinical trials. Although recent studies on the use of recombinant PTH (PTH 1-34) have demonstrated a significant increase in bone density and a reduction in skeletal fractures in postmenopausal women,¹⁹ it is not currently prescribed for fracture repair. From the available data, reason exists to suspect its possible value in such areas. Current research has indicated that PTH enhances both callus formation and remodeling by stimulating the proliferation of osteoprogenitor cells, synthesis of bone matrix proteins, and osteoclastogenesis.¹⁴ In 1932, Selye determined that once-daily administration of PTH had an anabolic effect on the bones of rats, but larger doses resulted in bone resorption. It has since been shown that intermittent administration increases bone deposition but that continuous administration actually decreases bone mass. This is likely a result of the difference in effects of intermittent versus continuous infusion of PTH on osteogenesis and osteoclastic activity. Bone deposition occurs with both forms of administration; however, with continuous infusion, bone resorption offsets deposition.²⁰ Dobniig and Turner²¹ reported that intermittent administration of PTH increased osteoblast numbers and activity, apparently owing to the activation of resting bone lining cells to become osteoblasts. In vitro studies have shown that transient exposure of osteoblasts to PTH stimulates their synthesis of collagen type I but that continuous exposure inhibits its production.²²,²³ Intermittent PTH also postpones osteoblast apoptosis²⁴ but has no effect on osteoclast numbers.²⁵ In contrast, sustained elevation had no effect on osteoblast apoptosis and actually increased osteoclast numbers.²⁶ PTH also affects cortical and cancellous bone differently. It has a greater anabolic effect on cancellous bone than on cortical bone.²⁷ Various studies have indicated a greater effect on bone containing primarily red marrow compared with bone containing yellow marrow.²⁸ Intermittent treatment with PTH has been shown to increase callus formation and mechanical strength in experimental fracture healing.¹⁴,¹⁸,²⁸ A daily dose of 200 μg/kg was more effective in enhancing callus formation and mechanical strength than a dose of 60 μg/kg in rat tibial fractures.²⁸ However, its efficacy, and optimal dosing in treating fractures of the mandible has not been researched. We developed a well-controlled mandible fracture model in rats and examined the hypothesis that daily systemic administration of PTH(1-34) could enhance mandibular fracture healing.
Materials and Methods

SUBJECTS AND HOUSING

A total of 29 adult male Sprague-Dawley rats (weight 550 to 600 g) were pair-housed until surgery with food (standard rat chow) and water ad libitum. The rats were kept on a 12-hour light/dark cycle (lights on from 8:00 AM to 8:00 PM). The Institutional Animal Care and Use Committee reviewed and approved all procedures. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, edition 1996. Of the 29 rats, 15 were randomly assigned to the experimental group, receiving PTH injections, and 14 rats were assigned to the control group.

PREPARATION AND ADMINISTRATION OF PTH

PTH fragment 1-34 (Sigma Chemical, St Louis, MO) was suspended in 1% acetic acid and sterile water and diluted accordingly to create a 10-μg/mL solution, following the manufacturer’s guidelines. The rats were weighed and injected with 10 μg/kg of PTH subcutaneously once daily from the day of surgery until death. The control group received no injections.

SURGICAL PROCEDURE

The rats were premedicated with subcutaneous buprenorphine (0.02 to 0.04 mg/kg), and then anesthesia was induced with a mixture of ketamine (80 mg/kg) and xylazine (4 mg/kg), administered intraperitoneally. Anesthesia was maintained with 1% to 2% isoflurane in oxygen administered by way of a nose cone. No antibiotics were given to any rats before surgery. The rats were placed in a supine position under a surgical microscope (Carl Zeiss, Berlin, Germany). A 1- to 1.5-cm incision was made along the inferior border of the right mandible. Sharp dissection was carried down to the periosteum through the body of the masseter muscle. Subperiosteal dissection was conducted in a superior and lateral direction to expose the sigmoid notch and posterior border of the mandible. Two 5.0-hole plates (OsteoMed, Addison, TX), were placed parallel to the osteotomy, one anterior and one posterior to the sigmoid notch, approximately 4 to 5 mm apart. The plates were secured with 4, 5-mm-long, 1.8 mm diameter screws, and positioned such that the plates extended through the incision. Using a sagittal saw, a vertical osteotomy was created from the sigmoid notch to the inferior border of the mandible. The proximal and distal ends of the fractured mandible were then reapproximated.

FIGURE 1. Open surgical creation of mandibular fracture that was then stabilized using external fixation device. A,B, Vertical plates surgically placed on mandible. C, Vertical osteotomy was created using sagittal saw. D, Placement of insulin syringe barrel and impression material filling for plate stabilization (black arrow indicates osteotomy site). E, Wound closed, leaving external fixator device extending beyond incision.

using the previously secured plates and held in position. A small portion of the barrel of an insulin syringe was split longitudinally along one side, positioned with the ends of the 2 plates inserted into the barrel through the split, and filled with Fast Set (Blue Mousse-Impression Material Parkell, Farmingdale, NY) to provide rigid external fixation. The mandible was then manipulated to ensure that the 2 segments of the osteotomy were fixated securely (Figs 1A-D).

POSTOPERATIVE CARE

The wound was then closed in layers around the protruding ends of the plates (Fig 1E). The rats were allowed to recover, and buprenorphine at the preanesthetic dose was administered subcutaneously every 8 to 12 hours for 48 hours for postoperative pain management. For the remainder of the experiment, the rats were housed separately and fed a soft diet. The rats were divided into 7- and 21-day groups receiving PTH(1-34) injections, as described previously. The rats were sacrificed on day 7 and 21, and the mandibles were harvested and divided into the right and left sides. The hardware was removed, and the right mandibles underwent radiographic and histologic analysis.

RADIOGRAPHIC DENSITOMETRY ANALYSIS

The right fracture-bearing mandible was radiographed, and the digital images were analyzed with ImageJ (National Institutes of Health, Bethesda, MD) software. The images were inverted to measure darkness within the fracture site. The entire fracture site from the inferior border of the mandible to the sigmoid notch was outlined and the measurement obtained. Next, the filling of the fracture site representing callous formation was outlined and the measurement obtained. The fracture site measurement and the filling measurements were then expressed as a ratio to represent the amount of radiographic callous formation within the fracture site (Fig 2). All sections were analyzed by 2 observers who were unaware of which group was being measured.

HISTOLOGIC PREPARATION AND EXAMINATION

The tissues were fixated in 10% buffered formalin solution, decalcified using standard procedures, and

![Control](image1.png) ![PTH(1-34)](image2.png)

7 days

![21 days](image3.png) ![21 days](image4.png)

**FIGURE 2.** Radiographic images of control at A, 7 days and C, 21 days and PTH-treated groups at B, 7 days and D, 21 days postoperatively. PTH-treated group showed enhanced bone density at 7 days followed by a decrease of bone density at 21 days after surgery.

Control

21 days

PTH

FIGURE 3. Histologic evaluation of specimens showing fractured healing differences in PTH-treated and control groups at 21 days after surgery. Sections stained with Masson's trichrome blue to stain for collagen formation within osteotomy site. Little bone development seen in callus of control group, as denoted by band of light blue collagen. PTH-treated groups, however, showed abundance of bone within osteotomy site (dark blue; original magnification ×60).


Results

MANDIBULAR FRACTURE MODEL

We established a mandibular fracture model using a technique that involved placement of the osteotomy site caudal to the molars to avoid damaging the tooth roots during the procedure. Also, the fracture was stabilized using an external fixation device anchored by 2 screws on either side of the fracture site for maximum stabilization. Finally, a nonadjustable fixator was used to stabilize the osteotomy site (Figs 1A-E).

embedded in paraffin wax. Serial, 4-μm-thick sections of the entire osteotomized right mandibles were cut parallel to the long axis of the osteotomy site. The sections were stained with Masson's trichrome blue to stain for collagen formation within the osteotomy site. Light microscopic examination was performed at 10× and 60×. Digital photographs were taken and examined using ImageJ (National Institutes of Health) software program. The fracture sites were examined for collagen material filling and new bone formation (Fig 3).

STATISTICAL ANALYSIS

The data were analyzed using statistical analysis computer software (SigmaStat, version 3.0, SigmaPlot, Point Richmond, CA). The 2-way analysis of variance method was used to compare the radiographic measurements between the control and PTH-treated fracture groups. The data are presented as the mean ± standard error of the mean. The measurements were compared, and P less than .05 was chosen as the critical level of statistical significance.

RADIOGRAPHIC DENSITOMETRY FINDINGS

Digital photographic analysis was used to determine the radiographic densitometry in the fracture site at 7 and 21 days postoperatively. Densitometric analysis of the fracture site showed significant differences between the PTH(1-34)-treated and control rats at 7 days postoperatively (Figs 2, 4). The PTH(1-34)-treated rats showed a statistically significant difference in callous formation compared with the control rats. Two-way analysis of variance showed a statistically significant interaction between 7 and 21 day groups and treatment (P < .05). The increase in

FIGURE 4. Histograms representing bone densitometric analysis of PTH(1-34)-treated and control group at 7 and 21 days postoperatively. Optical density measurements were obtained by tracing injury site and normalized using area (density/area). Significant increase seen [P < .05] in bone density in PTH-treated rats compared with controls at day 7. Data shown as mean ratio ± standard error of mean.

radiographic densitometry was evident in the early phase (7 days) of fracture healing; however, as shown in Figure 4, a decrease in optical density had occurred at 21 days after surgery. Moreover, these differences between the control and treated rats on day 21 did not reach statistical significance.

**HISTOLOGIC EXAMINATION FINDINGS**

The effects of PTH(1-34) treatment were examined using light microscopy at 10× to 60× magnification and the ImageJ digital photographic analysis software program (Fig 3). Visual inspection of the histologic sections stained with Masson’s trichrome blue showed a general trend in the collagen deposition and void filling in the early 7- and 21-day PTH-treated experimental group. Masson’s trichrome blue stains dark blue for old bone and light blue for collagen and new bone formation. This finding was consistent in the early 7-day group and 21-day experimental group. Also, subjective evaluation of the slides showed an apparent trend toward increased new bone formation in the osteotomy site in the 21-day PTH-treated group compared with the 21-day controls.

**Discussion**

Postfracture bone healing is a complex process influenced by many molecular, cellular, and biomechanical factors. Recent studies have been conducted to evaluate the effect of several biologic substances on bone fracture healing, including bone morphogenetic protein, transforming growth factor-β, platelet-derived growth factor, and vascular endothelial growth factor. A systemically administered medication that enhances fracture healing could have a dramatically positive effect on the clinical management of fracture treatment. To study novel pharmacologic adjuncts to enhance the healing of mandibular fractures, we established a unique rodent hemimandibular fracture model. Our mandibular fracture model involved the placement of the osteotomy site caudal to the molars to avoid damaging the tooth roots during the procedure. This technique varies considerably from previous studies that used a distraction technique to examine mandibular fractures. The distraction method involved the creation of an osteotomy site that ran between the second and third molars, and the fixation device was attached by implantation of one post on either side of the osteotomy site. The device was also designed to place gradually increasing distraction forces on the osteotomy site to study the effects of such forces on fracture healing.

Daily subcutaneous injections of PTH (10 µg/kg) were used with our unique rodent mandibular fracture model as a pharmacologic adjunct to enhance the healing of mandibular fractures. PTH is produced by the chief cells of the parathyroid gland. It is an 84-amino acid polypeptide that physiologically regulates serum calcium and phosphate levels by increasing bone resorption, increasing gastrointestinal calcium absorption, and increasing renal calcium and phosphate reabsorption. The continuous administration of PTH results in net bone resorption; however, intermittent administration causes a net increase in bone deposition. Because of the very short half-life of PTH, approximately 1 hour in humans after subcutaneous injection, daily dosing such as was conducted in the present experiment would constitute intermittent administration. Andreassen et al showed that intermittent administration of high (200 µg/kg) doses of PTH(1-34) significantly increased fracture callus formation in a rat model. In addition, PTH(1-34) has been administered to patients with osteoporosis, with dosages of 10 to 40 µg and has been shown to effectively increase bone mass after a 21-month clinical trial. We chose the low 10-µg/kg dosage to avoid the side effects of high-dose administration of PTH, and because it is comparable to the proven clinical dose given for the treatment of osteoporosis. Additionally, Nakajima et al demonstrated that dosages as low as 10 µg/kg in a rat fracture model increased both mechanical strength and bone mass of callus formation in a rat femur fracture model.

In the present study, the radiographic densitometry analysis results revealed that at 7 days after fracture, an increase occurred in callus and density in the PTH-treated group compared with the control group. However, although the optical density levels appeared to decline with time, the levels were not significantly different from those of the controls at 21 days. Nakajima et al showed that daily subcutaneous injection of 10 µg/kg of PTH(1-34) induced a significant increase in the osteoclast index in the hard callus and an increase in insulin-like growth factor-I, type I collagen (COL1A1), osteonectin, and osteocalcin mRNA expression at 7 days after a femoral fracture. These cellular and molecular events are perhaps indicative of the enhancement of fracture healing using low-dose PTH(1-34) during the early stages of femoral bone healing. Our data have also indicated that PTH(1-34) has enhancement properties in the early stages of mandibular fracture healing. At days 14 and 21, Nakajima et al observed mature trabeculae in the PTH-treated group but no significant difference in the osteoclast index. Although in their study they did not measure the bone mineral density at 21 days, they clearly showed a significant increase at 28 and 42 days after femoral fracture. In our study, we found a decrease—although not significant—in the radiographic optical density in the PTH-treated rats compared with the control group. This is consistent with the finding of Alkhia et al, in which they compared the effect...
of PTH(1-34) daily subcutaneous injections on femoral bone fracture healing. They found that at 21 days after fracture, the animals treated with the lower dose of 5 µg/kg PTH(1-34) did not show any differences in bone mineral density compared with the control animals. However, they observed a significant difference in bone mineral density with the lower dose at 35 days after fracture.

Visual inspection of the histologic sections stained with Masson's trichrome blue also suggested that administration of PTH might play a role in overall cartilage remodeling and bone formation. Even at a low dosage of 10 µg/kg, these histologic sections showed a general trend in collagen deposition and void filling in the PTH-treated group. On visual inspection at 60× magnification, a trend also appeared toward an increase in bone fill in the PTH group compared with the controls at 21 days. In our study, subjective analysis of the 21-day PTH-treated group displayed an increase in staining and callus maturity compared with the control group.

In conclusion, we were able to show evidence that a low dosage of PTH administration might enhance the healing process in the early phase of a mandibular fracture model in rats. Although low-dose PTH administration showed evidence of enhanced bone healing, additional studies are needed to quantify these results. In addition, additional research is needed to determine the parathyroid hormone receptor activity within the bone after fracture to establish the optimal time of drug administration in relation to the fracture healing mechanism. Furthermore, the effects of different dosages of PTH on our fracture model should be examined at different intervals. Biomechanical testing to evaluate the strength of the callus formation is an area of future interest. These tests might prove that PTH is the first systemic drug to enhance the fracture repair of patients with mandible fracture.

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References