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Modeling and remodeling effects of intermittent administration of teriparatide (parathyroid hormone 1-34) on bone morphogenetic protein-induced bone in a rat spinal fusion model

Takashi Kaito [a,*], Tokimitsu Morimoto [a], Sadaaki Kanayama [a], Satoru Otsuru [b], Masafumi Kashii [a], Takahiro Makino [a], Kazuma Kitaguchi [a], Masayuki Furuya [a], Ryota Chijimatsu [a], Kosuke Ebina [a], Hideki Yoshikawa [a]

[a] Department of Orthopedic Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Center for Childhood Cancer and Blood Disease, The Research Institute at Nationwide Children's Hospital, 700 Chidlren's Drive, Columbus, OH 43205, USA

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ABSTRACT

Background: Bone morphogenetic protein (BMP)-based tissue engineering has focused on inducing new bone efficiently. However, modeling and remodeling of BMP-induced bone have rarely been discussed. Teriparatide (parathyroid hormone [PTH] 1-34) administration initially increases markers of bone formation, followed by an increase in bone resorption markers. This unique activity would be expected to accelerate the modeling and remodeling of new BMP-induced bone.
Methods: Male Sprague-Dawley rats underwent posterolateral spinal fusion surgery and implantation of collagen sponge containing either 50 μg recombinant human (rh)BMP-2 or saline. PTH 1-34 (60 μg/kg, 3 times/week) or saline injections were continued from preoperative week 2 week to postoperative week 12. The volume and quality of newly formed bone were monitored by *in vivo* micro-computed tomography and analyses of bone histomorphometry and serum bone metabolism markers were conducted at postoperative week 12.
Results: Microstructural indices of the newly formed bone were significantly improved by PTH 1-34 administration, which significantly decreased the tissue volumes of the fusion mass at postoperative week 12 compared to that at postoperative week 2. Bone histomorphometry and serum analyses showed that PTH administration significantly increased both bone formation and resorption markers. Analysis of the histomorphometry of cortical bone identified predominant periosteal bone resorption and endosteal bone formation.
Conclusions: Long-term intermittent administration of PTH 1-34 significantly accelerated the modeling and remodeling of new BMP-induced bone.
Clinical relevance: Our results suggest that the combined administration of rhBMP-2 and PTH 1-34 facilitates qualitative and quantitative improvements in bone regeneration, by accelerating bone modeling and remodeling.

1. Introduction

Bone grafting is commonly used to treat skeletal disorders involving bone defects or spinal instability (Steinmann and Herkowitz, 1992). Failure of bony fusion results in pseudarthrosis, pain, and disability (Arrington et al., 1996; Robertson and Wray, 2001; Steinmann and Herkowitz, 1992). Bone morphogenetic protein (BMP) may provide an alternative to bone grafting and is the leading osteoinductive growth factor used clinically in bone-related regenerative medicine today (Boden et al., 2000, 2002; Urist, 1965; Wozney et al., 1988).

Although this therapy is effective, use of high BMP doses can cause complications including inflammatory responses and unintended bone formation; these restrict its widespread application (Shields et al., 2006; Smucker et al., 2006). Improved BMP delivery systems have the potential to produce efficient and spatially controlled bone formation (Kaito et al., 2005). However, modeling and remodeling of BMP-induced bone, processes that occur during normal late-phase fracture healing, have not been investigated thoroughly.

Teriparatide (parathyroid hormone [PTH] 1-34) is the only anabolic agent that has been approved by the U.S. Food and Drug Administration for the treatment of osteoporosis. This hormone has a unique mechanism of action; continuous administration of PTH 1-34 shows a catabolic effect, while intermittent administration demonstrates an anabolic effect (Canalis, 2010; Canalis et al, 2007; Dempster et al., 1993; Jilka, 2007; Tam et al., 1982). Recent studies demonstrated that intermittent PTH 1-34 administration promoted fracture healing (Aspenberg et al., 2010; Gallacher and Dixon, 2010; Komatsubara et al., 2005; Manabe et al., 2007; Neer et al., 2001). Another study found that intermittent

* Corresponding author at: Department of Orthopedic Surgery, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.
E-mail address: takashikaito@ort.med.osaka-u.ac.jp (T. Kaito).

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PTH 1-34 administration significantly increased fusion rates and newly formed bone quality, concluding that combined administration of rhBMP-2 and PTH 1-34 had a synergistic effect in a rat model of rhBMP-2-induced spinal fusion. Interestingly, at postoperative week 6, the newly formed bone tissue volume (TV) in rats treated with rhBMP-2 tended to be lower than in animals treated with PTH 1-34 (Morimoto et al., 2014). These findings suggest the possibility that PTH 1-34 influences modeling and remodeling of newly formed bone. However, it is unclear whether the newly formed bone TV was low from the outset or decreased over time in these rats. The purpose of this study was to elucidate the time course of change in TV induced by the administration of PTH1-34 by determining the dimension of the newly formed bone (TV) using serial *in vivo* micro-computed tomography (CT) and to quantify bone formation and resorption at periosteum and endosteum of the newly formed bone by bone histomorhometry.

2. Materials & methods

2.1. Animal treatments

A total of 37 male, 8-week-old, Sprague-Dawley rats with an initial average body weight of 305.9 g (range, 280–330 g) were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and used in the present study. All animal procedures were conducted in accordance with the guidelines of the Regulations on Animal Experimentation at Osaka University. The study groups are shown in Table 1.

2.1.1. Administration of PTH 1-34

Rats were treated with subcutaneous injections of either PTH 1-34 (60 μg/kg) 3 times a week (180 μg/kg/week) or 0.9% saline vehicle. Injections commenced 2 weeks prior to surgery, in order to enhance the anabolic effect of PTH 1-34 at the time of surgery, and continued until the animals were euthanized. Animals were weighed every 7–10 days, and injection volumes were adjusted accordingly.

2.1.2. Surgery

Rats were anesthetized with a combination of 0.15 mg/kg of medetomidine (Domitol; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), 2 mg/kg of midazolam (Dormicum; Astellas Pharma Inc., Tokyo, Japan), and 2.5 mg/kg of butorphanol (Vetorphale; Meiji Seika, Ltd., Tokyo, Japan). In addition, preoperative antibiotic (20,000 U/kg penicillin G; Meiji Seika) was administered subcutaneously. We then surgically induced posterolateral lumbar fusion (Kaito et al., 2013; Morimoto et al., 2014; Wang et al., 2003). A posterior midline skin incision was made, followed by 2 separate paramedian incisions in the lumbar fascia 3 mm from the midline, through which the transverse processes were exposed. The L4 and L5 transverse processes were decorticated using a high-speed burr.

A commercially available absorbable collagen sponge (CollaCote; Zimmer Dental Inc., *CA*, USA) was cut into 5 × 10 mm fragments and placed in a sterile tube. Phosphate-buffered saline (PBS) or rhBMP-2 (50 μg in PBS) was applied to the sponge just before implantation on each side of the spine. We chose 50 μg rhBMP-2 because this corresponded to a high clinical dose (1500 μg/mL) (Morimoto et al., 2014). The fascia and skin incisions were closed using a 4-0 absorbable suture. The rats were housed in separate cages and allowed to eat and drink *ad libitum* while their condition was monitored daily.

Table 1
Treatment groups.

	Implanted material	Injected material
Group A (n = 9)	Collagen carrier	Saline
Group B (n = 9)		PTH1-34
Group C (n = 10)	Collagen carrier + 50 μg rhBMP-2	Saline
Group D (n = 9)		PTH1-34

2.1.3. Calcein double labeling for bone histomorphometry

All rats were injected subcutaneously with 10 mg/kg calcein (Dojindo Laboratories, Kumamoto, Japan) at 5 and 2 days before they were euthanized.

2.1.4. Euthanasia and tissue collection

Immediately prior to euthanizing the rats by anesthetic overdose 12 weeks after surgery, blood samples were collected for analysis of bone metabolism markers and stored at −80 °C. Spinal segments and femurs were harvested and fixed with 10% formalin or 70% ethanol for further assessments.

2.2. Serial bone TV measurement by in vivo micro-CT

The TV of the L4-L5 fusion segments (from the bottom of the L5 transverse process cranially to the top of L4 end plate) was measured *in vivo* at a resolution of 59 μm at time 0 (just before surgery) and at 2, 6, 8, and 12 weeks after surgery.

2.3. Assessment of L4-L5 fusion

L4-L5 fusion was assessed using the two methods described below. Each was performed in a blinded manner by three independent observers and unanimous agreement was required before these were considered to be fused.

2.3.1. Micro-CT

The spines were scanned using high-resolution micro-CT (R_mCT; Rigaku Mechatronics, Tokyo, Japan) at 90 kV and 200 μA. Visualization and data reconstruction were performed using the TRI/3D-BON (RATOC System Engineering, Tokyo, Japan). Coronal and sagittal L4-L5 images at a resolution of 40 μm/voxel were evaluated for clear evidence of bridging bone formation with cortical continuity between the L4 and L5 transverse processes.

2.3.2. Manual assessment

The explanted lumbar spines were manually tested for intersegmental motion. Any motion detected in the anterior-posterior and left-right direction was considered to indicate a failure of fusion, while the absence of motion was considered to indicate successful fusion.

2.4. Microstructural analysis

The quality of the newly formed fusion mass between the transverse processes was analyzed as described previously (Morimoto et al., 2014). Fusion mass scanning was initiated from the lower endplate level of the L4 vertebral body and continued cranially at 2.0-mm increments (fifty slices) at a resolution of 40 μm/voxel on each side. The bone volume (BV)/TV, trabecular thickness (Tb·Th), trabecular number (Tb·N), trabecular separation (Tb·Sp), thickness of cortical bone (Ct), and cortical bone ratio (Cv/Av) were determined.

2.5. Analysis of the systemic effects of PTH 1-34

The BV/TV ratios of the distal femoral epiphysis and L6 vertebral body were analyzed at 40 μm/voxel. Scanning of the distal femur was initiated at 1.5 mm proximal to the growth plate and continued at 3.0-mm increments (75 slices) in order to exclude the primary spongiosa of the femur. Scanning of the L6 vertebral body was initiated at 1.0 mm cranial to the lower growth plate and continued at 3.2-mm increments (80 slices) in order to exclude the primary spongiosa of the vertebrae.