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Original article

Angiogenesis inhibitor attenuates parathyroid hormone-induced anabolic effect☆

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Abstract

In vivo osteogenic responses to anabolic stimuli are expected to be accompanied by angiogenesis as well as in the process of remodeling of bone. Consequently, angiogenesis might play an important role in mediating bone forming stimulating effect of parathyroid hormone (PTH). To investigate this relationship, we used actively growing young Sprague–Dawley rats and CKD-732, one of the angiogenesis inhibitor (AI) to reveal the relationship of angiogenesis in the effect of PTH. The groups were divided as (1) vehicle [VEH group], (2) PTH(1–84) [PTH group], (3) AI alone [AI group], (4) PTH(1–84) + AI concomitance [PTH-AI group] and were treated for 6 weeks. The bone mineral density (BMD) of PTH group was higher than VEH group and the gain of bone mass was attenuated in PTH-AI group. The maximal failure load in PTH group was higher than VEH group, but it was definitely attenuated by concurrent use of AI. Moreover, the toughness showed similar significant deterioration in PTH-AI group. General bone turnover was also significantly decreased in PTH-AI group as shown by the absence of increase in osteocalcin and β-crosslaps and by decrease in metaphyseal length. The BMD or the biomechanic data of AI only group were similar to the VEH group, suggesting the minimal effect of AI itself on the normal modeling phase of the growing rats. In conclusion, the angiogenesis seemed to contribute to completing the anabolic effect of PTH especially for bone strength.

Keywords: Parathyroid hormone; Angiogenesis inhibitor; Bone strength; Bone formation

1. Introduction

It is now well established that parathyroid hormone (PTH) has the ability to stimulate bone formation and is critical in bone metabolism. Many studies have revealed that PTH given by subcutaneous injection leads to increase in bone mass and can compensate estrogen deficiency-induced bone loss in animals and humans [1,2]. In experimental fracture healing, intermittent treatment with PTH also promotes osteogenesis and increases mechanical strength [3]. Moreover, PTH induces a large cartilaginous callus with increase in the recruitment of mesenchymal cells and the differentiation into the chondrocyte lineage [4].

Angiogenesis is known to be important for bone homeostasis and osteogenesis. Increased bone formation is parallel with increase of bone capillaries during electrical stimulation [5]. Reduction of bone mass or bone necrosis could be caused

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by impairment of vascular supply [6]. The relationship between osteogenesis and angiogenesis has been searched and several factors such as vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, bone morphogenetic proteins (BMP) and parathyroid hormone-related protein (PTHrP) are disclosed to involve in bone angiogenesis [7–9].

Previous studies showed that osteoblasts have PTH receptor and consequently *VEGF* gene expression and protein production are increased by PTH stimulation [10–12]. In addition, osteoblasts line the terminal capillary wall of the sprouting capillaries which is important for invading the epiphyseal plate during endochondral bone formation [13]. However, the mechanisms of interaction between angiogenesis and bone formation induced by PTH are still unclear and there is no available data verifying PTH effect from the viewpoint on bone angiogenesis *in vivo*.

We hypothesized that PTH has anabolic effect related to bone angiogenesis and this effect would be impeded by angiogenesis inhibitor (AI). To analyze the relationship between the pharmacological osteoanabolic stimuli and angiogenesis, we selected the growing animal model in a critical condition for vascularization of growing tissue and assessed the effects of the analogue PTH(1–84) and the CKD-732 on bone mineral density (BMD) and bone quality.

2. Materials and methods

2.1. Animals

Twenty-eight, 5-week-old, virgin female Sprague–Dawley rats were purchased from the Department of Laboratory Animal Medicine at Yonsei Medical Research Center (Seoul, Korea). They were housed in a room maintained at 22.2 °C with 12:12 h light:dark cycle. The animals were fed freely with Purina laboratory rodent chow (Hagribrand Purina Korea Co., Kunsan, Korea), which contained 1.17% calcium, 0.77% phosphorous and 2.6 IU vitamin D per gram. All animals were treated in accordance with the guidelines and regulations for the use and care of animals of Yonsei University, Seoul, Korea.

2.2. Administered materials

The recombinant human PTH(1–84), donated by the Mogam Biotechnology Research Institute (Kyungi, Korea) was dissolved in 0.9% normal saline. A synthetic TNP-470 analog, CKD-732 [6-*O*-(4-dimethylaminoethoxy) cinnamoyl fumagillol hemioxalate], was provided by Chong Kun Dang Pharmaceutical, Korea. CKD-732 is known to be 1000 times more active than TNP-470 *in vitro*. Moreover, CKD-732 has been proven to be more effective in inhibition of human umbilical vein endothelial cells (HUVEC) growth and in induction of cell cycle arrest in HUVEC compared with TNP-470. CKD-732 also significantly suppressed tumor growth *in vivo* and its efficacy was much higher than TNP-470 [14].

2.3. Experimental design

The animals were randomized into the following four groups at the age of 4 weeks, with seven rats per group, and two rats were housed per cage. The first group was treated with a vehicle for 6 weeks from the age of 5 weeks [VEH group]. The second group was maintained on PTH(1–84) [PTH group], and another was given AI only for whole 6 weeks [AI group]. The last group was administered both PTH(1–84) and AI [PTH-AI group]. The PTH was administered daily for 5 days per week with intermittent subcutaneous injections of 80 μg/kg per day. The AI was administered subcutaneously, 1 mg/kg per day at the same frequency. The VEH group was injected subcutaneously with an equivalent volume of 0.9% normal saline at the same frequency. At the end of the 6 weeks of treatment, the rats were sacrificed. The femurs and vertebrae were harvested and stored in saline-soaked gauze at −20 °C until required for analysis. Right tibiae were stored in the formalin to analyze the growth plate. The blood was centrifuged and the serum was stored at −70 °C until required for analysis.

2.4. Bone densitometry

The BMD of the excised right femurs and lumbar spine were measured by dual energy X-ray absorptiometry (QDR-4500A, Hologic, Waltham, MA, USA). The femurs and 1st to 4th lumbar spine were scanned at a resolution of 0.5 mm with a scanning speed of 2 mm/s. The region was analyzed for bone mineral content (BMC), projected bone area and BMD. The triplicate determinations of the five different femora, with repositioning, showed a coefficient of variation at 0.59%.

2.5. Microcomputed tomography (μCT)

The μCT and software used for this experiment were from Skyscan (Antwerpen, Belgium). The computer system was adapted to allow a simultaneous acquisition step on one sample and an analysis step on another sample as previously described [15]. Using this technique, the static parameters were measured and included the trabecular bone volume (BV), volume fraction (Vol. F), trabecular thickness (Tb. Th.), trabecular separation (Tb. Sp.), trabecular number (Tb. N.), degree of anisotropy (DOA) and structural model index (SMI).

2.6. Biomechanical analyses

The femurs were thawed prior to testing, and the bone strength of the intact femurs was measured using a three-point bending test. A load was applied midway between two supports that were 15 mm apart. The femurs were positioned so the loading point was 7.5 mm proximal from the distal popliteal space, and bending occurred about the medial–lateral axis. Load-displacement curves were recorded at a crosshead speed of 1 mm/s using a servo-hydraulic materials testing