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Bone 42 (2008) 806–818

BONE

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Parathyroid hormone mediates bone growth through the regulation of osteoblast proliferation and differentiation ☆

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Received 27 August 2007; revised 19 November 2007; accepted 26 November 2007
Available online 15 December 2007

Abstract

PTH (1–34) is the only FDA-approved anabolic agent for osteoporosis treatment in the U.S., but its mechanisms are not completely understood. This study investigated PTH effects on osteogenic cells at various stages of differentiation and proliferation using an engineered bone growth model *in vivo*. Ossicles were generated from bone marrow stromal cells (BMSCs) implanted in immunocompromised mice. Three weeks of PTH (40 μg/kg/day) or vehicle treatment initiated 1 day, 1, 2, or 3 weeks after BMSC implantation resulted in an anabolic response in PTH-treated implants (via histomorphometry and microCT) in all treatment groups. A novel *in vivo* tracking strategy with luciferase tagged BMSCs and weekly bioluminescent imaging of ossicles revealed increased donor cell proliferation in PTH-treated ossicles. The greatest increase occurred during the first week, and the activity remained elevated in PTH-treated implants over time. Zoledronic acid (ZA) was combined with PTH to delineate interactive mechanisms of these bone active agents. Combining ZA with PTH treatment reduced the PTH-mediated increase in luciferase BMSC activity, serum osteocalcin, and serum tartrate resistant acid phosphotase-5b (TRAP-5b) but ZA did not reduce the PTH-induced increase in total bone. Since zoledronic acid reduced PTH-induced proliferation without reducing bone volume, these data suggest that combining PTH and bisphosphonate therapy warrants further investigation in the treatment of bone disorders.

Keywords: Parathyroid hormone; Zoledronic acid; Bone marrow stromal cells; Proliferation; Tissue engineering

Introduction

Skeletal integrity is maintained by the process of bone remodeling, which involves the coupling of osteoclast-mediated bone resorption and osteoblast-mediated bone formation. Endogenous parathyroid hormone (PTH) plays a critical role in this process through its action on the skeleton to maintain calcium homeostasis and regulate bone metabolism. Exogenous PTH exerts anabolic or catabolic effects on bone depending on the mode of administration and duration of treatment. It is well accepted that continuous doses of PTH increase bone resorption, whereas intermittent administration of PTH stimulates new bone formation and improves microarchitecture of existing bone. The ability of anabolic doses of PTH to increase bone remodeling and parameters of bone formation has stimulated interest in its current clinical use for the treatment of osteoporosis as well as investigative use for other conditions requiring bone regeneration. Many pharmacologic agents are

☆ **Disclosure Statement**: Dr. Laurie K. McCauley receives clinical research grant support from Eli Lilly. All other authors have nothing to disclose.

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doi:10.1016/j.bone.2007.11.017

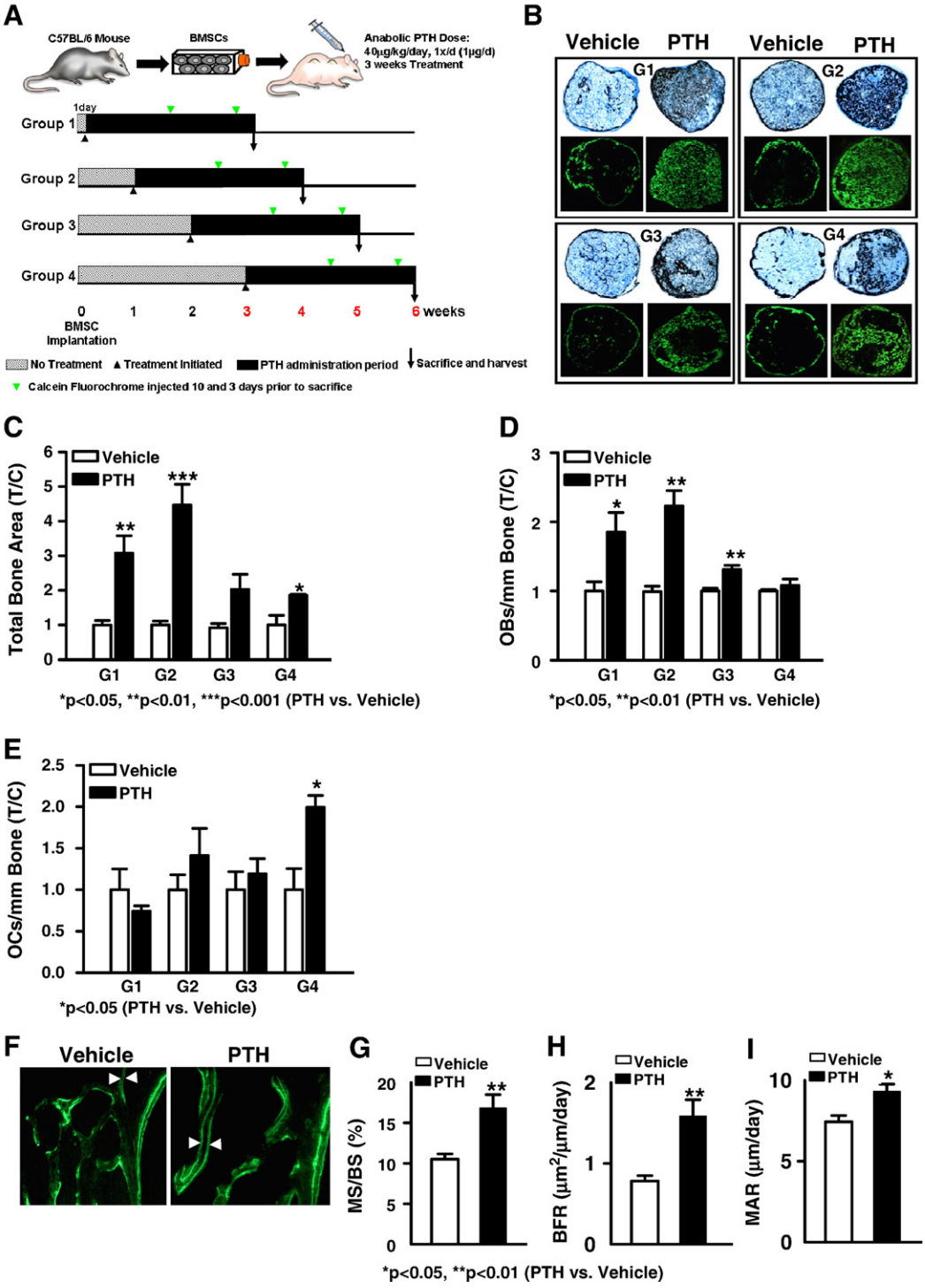

Fig. 1. Experimental design and histomorphometric analysis of undecalcified ossicles. Bone marrow stromal cells (BMSCs) were isolated from C57BL/6 mice, cultured as described, then implanted into athymic mice. (A) 3 weeks (21 days) of anabolic PTH (1–34) or vehicle (0.9% saline) treatment was administered to mice 1 day (group 1), 1 week (7 days) (group 2), 2 weeks (14 days) (group 3), or 3 weeks (21 days) (group 4) after BMSC implantation. (B) Representative tetrachrome stained and calcein fluorochrome labeled sections of ossicles treated with PTH or vehicle for 3 weeks, initiated 1 day (G1), 1 week (7 days) (G2), 2 weeks (14 days) (G3), and 3 weeks (21 days) (G4) after implanting BMSCs. (C) Total bone area was significantly increased in the tetrachrome stained sections for groups 1, 2, and 4 PTH-treated ossicles. (D) PTH significantly increased osteoblasts/mm bone in groups 1–3 implants (n=4/treatment). (E) TRAP-positive osteoclasts/mm bone were significantly increased in PTH-treated implants in group 4 (n=3–4/treatment). (F) Histomorphometric examination of double calcein labeling of vertebrae harvested from mice in group 2 treated with vehicle or PTH for 3 weeks (21 days) (n=4/treatment). (G) Mineralizing surface/bone surface (MS/BS), (H) bone formation rate (BFR), and (I) mineral apposition rate (MAR) were quantitatively increased in PTH-treated vertebrae. Data expressed as mean±SEM.