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Anabolic actions of PTH (1-34): Use of a novel tissue engineering model to investigate temporal effects on bone

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Abstract

PTH is in clinical use for the treatment of osteoporosis and is under intensive investigation for its potential in applications of tissue engineering, fracture healing, and implant integration. However, the mechanisms of its action to stimulate bone formation are still unclear. A novel bone tissue engineering model was used to elucidate basic mechanisms of PTH anabolic actions. Ectopic ossicles containing cortical bone, trabecular bone, and a hematopoietic marrow were generated from implanted bone marrow stromal cells (BMSC). One week after implantation, nude mice were administered PTH or vehicle for 1 week (group 1), 3 weeks (group 2), or 7 weeks (group 3). Another group was also treated for 3 weeks, initiated 12 weeks after implantation (group 4). Micro-radiography and histomorphometry revealed increased marrow cellularity in group 1 PTH-treated ossicles, increased bone in group 2 PTH-treated ossicles, and similar amounts of bone in both group 3 and 4 ossicles regardless of treatment. Incidence of phosphate mineral and phosphate mineral to hydroxyproline ratio via Raman spectroscopy were significantly higher after 3 weeks versus 1 week of PTH treatment, but there was no difference between PTH- and vehicle-treated ossicles. Early events of PTH action in group 1 ossicles and the effects of a single injection of PTH on 1- and 2-week-old ossicles were evaluated by Northern blot analysis. Osteocalcin (OC) mRNA was increased after 1 week of intermittent PTH treatment in ossicles and calvaria but an acute injection did not alter OC mRNA. In contrast, a single injection of PTH increased matrix γ-carboxyglutamic acid protein (MGP) mRNA in 2-week-old ossicles. Differential and temporal-dependent effects of PTH on OC and MGP suggest at the molecular level, that PTH acts to inhibit osteoblast mineralization. However, this does not translate into tissue level alterations. These data indicate that anabolic actions of PTH in ectopic ossicles are temporally dependent on the BMSC implanted and suggest that cell implantation strategies are particularly responsive to PTH.

Keywords: Parathyroid hormone; Ectopic ossicle; Mineralization; Bone marrow stromal cells; Tissue engineering

Introduction

Parathyroid hormone (PTH) is a peptide hormone that is involved in calcium homeostasis. PTH has been found to increase bone mass when administered intermittently and promote bone resorption when administered in a continuous fashion [16]. PTH has been used extensively via systemic administration to evaluate its potential for treating osteoporosis. In contrast, there are fewer studies outlining its use to treat localized defects in bone. Still, there are several reports of the beneficial use of systemic PTH to address osseous defects by increasing callus formation and mechanical strength during fracture healing in young and old rats [1,2,17,20], for the promotion of implant

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integration [40], and in association with a bone chamber model [39]. Furthermore, local delivery strategies are emerging that could expand the application of PTH to positively impact tissue engineering of bone [43]. However, much remains unclear about the mechanisms involved in the anabolic actions of PTH. An innovative in vivo model system was used in this study to gain a better understanding of the temporal effects of intermittent PTH treatment on bone.

A number of studies have demonstrated that the anabolic responses of bone to PTH depend on the time and duration of treatment [10,31,36]. It has been shown that intermittent administration of PTH stimulates bone formation, increases cancellous bone volume, architecture, cortical width, and biomechanical properties of bone in rodents, rabbits, primates, and humans [6,9,15,27,35,38,45]. Once daily subcutaneous administration of PTH also results in a substantial increase in bone mineral density (BMD) depending on both the dosage and treatment period. Nishida et al. reported that treatment with PTH for 1 week did not increase BMD at the femoral metaphysis of rats, whereas the same treatment for 3 weeks resulted in a significant increase [32]. In addition, it has also been shown that the anabolic action of PTH is skeletal-site specific in mice [19].

In the present study, an innovative in vivo tissue engineering model was utilized to investigate the anabolic actions of PTH on bone formation over the course of time. Ectopic ossicles were generated from the differentiation of implanted bone marrow stromal cells (BMSC) in immunocompromised mice. These ossicles have been well characterized and include cortical bone, trabecular bone, and a hematopoietic marrow component [25]. We have recently demonstrated that ectopic BMSC-derived tissue engineered ossicles respond to hormonal cues [37]. It has been documented that PTH is anabolic during bone growth [8,29], but few studies have been conducted to compare the stimulatory effects of PTH on modeling and remodeling bone in the same animal. The anabolic effects of PTH on growing bone at various stages of development can be analyzed with this tissue engineering model and also compared with endogenous bone of recipient animals.

The effect of intermittent PTH treatment on the expression of bone-related extracellular matrix (ECM) proteins such as osteocalcin (OC), matrix γ-carboxyglutamic acid protein (MGP), and the PTH/parathyroid hormone-related protein receptor (PTH-1R) were also evaluated in this study. An intermittent PTH dosing regimen stimulates increases in OC mRNA expression. It has also been shown that the actions of PTH on mineralization are partly due to its ability to upregulate MGP when administered acutely [14]. We investigated alterations in the expression of these genes in bone treated with PTH for 1 week and in bones from animals where an acute injection of PTH was administered 8 h prior to sacrifice. These data will provide more insight into how PTH affects the mineralization of bone and the differentiation of osteogenic cells in tissue engineered bone.

Materials and methods

Isolation of bone marrow stromal cells (BMSC)

Four- to 8-week-old C57BL/6 mice were used to isolate BMSC as previously described [37]. Briefly, bone marrow was flushed with α-modified minimum essential medium (α-MEM) (Invitrogen, Grand Island, NY) from the femoral, tibial, and humeral cavities. The whole marrow was plated into a 75-cm^2 culture flask in 30 ml of growth medium (α-MEM, 2 mM glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin sulfate) supplemented with 20% fetal bovine serum (HyClone, Provo, UT) and 10^{-8} M dexamethasone (Sigma, St. Louis, MO). Cells were maintained at 37°C in an atmosphere of 100% humidity and 5% carbon dioxide. The first passage was carried out when a confluent adherent cell layer was observed. The adherent cell layers were harvested using two washes with Hanks' balanced salt solution (Invitrogen) followed by incubation with 1× trypsin-EDTA (Invitrogen) for 10 min at 37°C. Cells were centrifuged at 1000 rpm for 5 min, the cell pellet resuspended in growth medium and plated into a 75-cm^2 or 150-cm^2 culture flask. These cultures were maintained for 5–7 days before implantation. All animal protocols were performed in compliance with the University of Michigan Committee for the Use and Care of Animals.

Surgical implantation of BMSC

BMSC implantation was performed as previously described [37]. BMSC (passage 2, $2–3 \times 10^6$ cells) were resuspended in 1 ml growth medium, centrifuged at 1000 rpm for 5 min, and the supernatant was decanted. BMSC pellets were incorporated into pre-soaked gelatin sponges that were 3–5 mm in diameter (Gelfoam®Upjohn, Kalamazoo, MI) by capillary action. Immunocompromised 4- to 6-week-old male nude mice (NIH III Nude; Charles River Laboratories, Wilmington, MA) were used as implant recipients. Following anesthesia with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (5 mg/kg), two midlongitudinal skin incisions of approximately 1 cm in length were made on the dorsal surface of each mouse. Blunt dissection was used to form subcutaneous pouches and each animal received four implants.

In vivo injection of PTH (1-34)

Animals were randomly assigned to one of four groups of animals (4–6 animals per group). Each was given daily subcutaneous injections of either recombinant human PTH (1–34) (40 μg/kg) (Bachem, Torrance, CA) or vehicle