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Effects of daily treatment with parathyroid hormone 1–84 for 16 months on density, architecture and biomechanical properties of cortical bone in adult ovariectomized rhesus monkeys

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Abstract

Treatment with parathyroid hormone 1–84 (PTH) or teriparatide increases osteonal remodeling and decreases bone mineral density (BMD) at cortical (Ct) bone sites but may also increase bone size. Decreases in BMD and increases in size exert opposing effects on bone strength. In adult ovariectomized (OVX) rhesus monkeys, we assessed the effects of daily PTH treatment (5, 10 or 25 μg/kg) for 16 months on BMD at the radial, tibial and femoral diaphyses, and on biomechanical properties (3-point bending) of radial cortical bone and the femoral diaphysis. PTH treatment did not affect areal BMD measured by dual-energy X-ray absorptiometry at the tibial diaphysis but caused a rapid, dose-related decrease at the distal radial diaphysis. Peripheral quantitative computed tomography at the radial and femoral diaphyses confirmed a significant PTH dose-related decrease in volumetric Ct.BMD caused primarily by increased cortical area. Significant increases in cortical thickness were the result of nonsignificant increases in periosteal length and decreases in endocortical length. Histomorphometry revealed increased endocortical bone formation at the tibial diaphysis and rib, higher Haversian remodeling at the rib and increased cortical porosity at the rib and tibia. Biomechanical testing at the femoral diaphysis showed that PTH treatment had no effect on peak load, but significantly decreased stiffness and increased work-to-failure (the energy required to break the bone). Similar changes occurred in radial cortical beams but only stiffness was changed significantly. Thus, PTH treatment of OVX rhesus monkeys decreased BMD and stiffness of cortical bone but did not affect peak load, likely because of increased bone size. However, PTH treatment increased the energy required to break the femur making it more resistant to fracture.

Keywords: Osteoporosis treatment; Ovariectomized rhesus monkey; Parathyroid hormone; Cortical bone; Biomechanical testing

Introduction

Parathyroid hormone 1–84 (PTH) and N-terminal fragments and analogs of PTH and PTH-related protein are potent anabolic agents in the skeleton of animals and humans, particularly at skeletal sites such as the spine that are rich in cancellous bone [1–11]. The increase in bone mineral density (BMD) at the spine is associated with a large reduction in vertebral fracture incidence in postmenopausal women with osteoporosis [10,11]. Treatment with PTH peptides also increases bone mass and strength of cortical bone in rodents by adding new bone to both the periosteal and endocortical surfaces [12,13]. However, unlike humans, rodents do not exhibit Haversian remodeling in response to PTH except under extreme conditions, such as severe secondary hyperparathyroidism [14]. Because of increased intracortical remodeling and possibly because of increased bone size, treatment of humans with PTH or PTH(1–34) (teriparatide) decreases cortical BMD at the hip and distal radius [10,15–18]. Since decreases in BMD and increases in size exert opposing effects on strength [19], cortical bone strength may still be increased by PTH treatment, despite the

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lower BMD. However, it is not possible to determine bone strength directly in humans.

The effects of treatment with PTH peptides on cortical bone have been evaluated in rabbits and in dogs in which Haversian remodeling occurs [20,21], but ovariectomized (OVX) monkeys are a better model of human estrogen-deficiency bone loss [22,23]. However, there is very little information available on the effects of treatment with PTH peptides in monkeys [6,24–30] and most published papers are from a single study with teriparatide in cynomolgus monkeys in which treatment was initiated immediately after OVX [24–29]. In that study, daily teriparatide treatment for 18 months increased cortical porosity at the humerus and hip but did not affect the biomechanical properties of bone at the humerus, most likely because the porosity was localized primarily to the inner one-third of the cortex, a region less important biomechanically [6,19,28].

We have previously reported that daily treatment with human PTH(1–84) for 16 months increases bone turnover, BMD, and improves trabecular architecture and biomechanical properties of vertebral bodies at the lumbar spine of skeletally mature OVX rhesus monkeys [30]. This paper reports the effects of PTH treatment at four cortical bone sites in the same animals.

Materials and methods

This study was conducted at Charles River Laboratories Preclinical Services Montréal in accordance with the "Guidelines for the Preclinical and Clinical Evaluation of Agents used in the Prevention or Treatment of Postmenopausal Osteoporosis" [31] and the Good Laboratory Practice regulations of the United States Food and Drug Administration (FDA).

Animals

Complete details of the animals, diets, and housing conditions have been described previously [30]. Briefly, 12- to 17-year-old female rhesus monkeys with confirmed closed epiphyses were obtained from the Texas Primate Center (Alice, TX), and housed for 9 months prior to surgery. They were divided into 7 groups (n=6–10/group) that were matched by whole body bone mineral content (BMC) derived from dual-energy X-ray absorptiometry (DXA) scans. The groups were verified to be homogeneous with respect to age, body weight and whole body BMC. Five groups of animals were OVX and two groups underwent sham surgery, which was followed by a 9-month bone depletion period, at the end of which one group of sham and one group of OVX monkeys were killed as baseline controls. Three groups of OVX animals received daily subcutaneous injections of recombinant human PTH(1–84) at doses of 5, 10, or 25 μg/kg. These doses were based on the results of a preliminary 9-month dose-ranging study in OVX rhesus monkeys [32], conformed with the FDA guidelines [31], and resulted in plasma PTH exposures that were, respectively, 2.1-, 4.1-, and 13.2-fold higher than that of a 100 μg dose in postmenopausal women (unpublished results). The remaining groups of sham and OVX monkeys received injections of vehicle (sodium citrate, 10 mM; mannitol, 50 mg/mL; pH 5.5). Following 16 months of treatment, animals were anesthetized with intravenous sodium pentobarbital and euthanized through exsanguination by incision of the axillary or femoral arteries.

Bone densitometry

The monkeys were anesthetized by an intramuscular injection of glycopyrrolate, ketamine, and xylazine prior to scanning by DXA. Areal BMD was measured at the right 1/3 distal radius and at the right proximal tibial diaphysis using QDR-2000 *plus* bone densitometers (Hologic, Bedford, MA). For both sites, the region of interest was 20 mm long, the midpoint of which was one-third the bone length measured from the distal end of the radius and the proximal end of the tibia. Scans were performed every 3 to 5 months throughout the bone depletion and treatment phases of the study.

The right radius and right femur were removed and stored at −20 °C. Prior to scanning by peripheral quantitative computed tomography (pQCT) and biomechanical testing, the bones were thawed overnight at 4 °C and immersed in physiological saline. Scans of the femoral diaphysis were performed at the expected breaking site in biomechanical testing (41 mm below the femoral condyles) and at the radial diaphysis using an XCT Research SA bone scanner (Stratec Medizintechnik, Pforzheim, Germany) using software version 5.40. Volumetric (v) measures of cortical (Ct) BMD, BMC and bone area (BMA) and geometric properties [periosteal (Ps) and endocortical (Ec) length (Le), cortical thickness (Ct.Th)], and cross-sectional moment of inertia (CSMI) were obtained at the femoral and radial diaphysis using threshold analysis (X-ray attenuation coefficient of 0.800 l/cm).

Histomorphometry

Fluorochrome markers were injected at 15 days and again at 5 days prior to bone collection to label bone-forming surfaces. Calcein (8 mg/kg, i.v.), oxytetracycline (40 mg/kg, i.v.) and xylenol orange (90 mg/kg, s.c.) were administered at baseline, and at months 6 and 16, respectively. The right central tibia and a 1-cm-long piece from the right central seventh (baseline) or ninth rib (month 16) were isolated, fixed in 10% neutral buffered formalin, and transferred to the Creighton University Osteoporosis Center in 70% ethanol for histomorphometric analysis. Measurements were made using an interactive image analysis system (Bioquant R&M Biometrics, Nashville, TN). Histomorphometric variables were measured, calculated, and abbreviated as described by

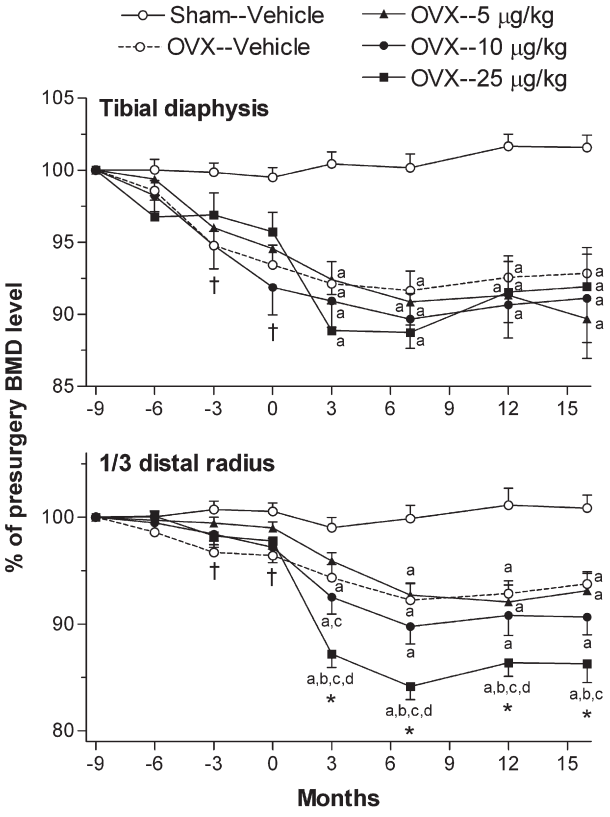

Fig. 1. Effects of daily s.c. injection of vehicle or PTH (5, 10, or 25 μg/kg) for 16 months on BMD measured by DXA at the tibial diaphysis and 1/3 distal radius of sham or OVX rhesus monkeys. Ovariectomy or sham surgery occurred at month −9 and treatment started at month 0. Values are mean±SE, n=8–10/group. †Pooled OVX animals significantly different from sham (p<0.05). $^{a,b,c,d}p$<0.05=significance of difference from Sham–Vehicle, OVX–Vehicle, OVX–5 μg/kg, and OVX–10 μg/kg groups, respectively. *Significant linear dose-related trend across dose levels (p<0.05).