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The anabolic effect of PTH on bone is attenuated by simultaneous glucocorticoid treatment

H. Oxlund *, G. Ørtoft, J.S. Thomsen, C.C. Danielsen, C. Ejersted, T.T. Andreassen

Department of Connective Tissue Biology, Institute of Anatomy, University of Aarhus, DK-8000 Aarhus C, Denmark

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Abstract

Glucocorticoids (GC) are used for the treatment of a wide spectrum of diseases because of their potent anti-inflammatory and immunosuppressive effects, and they are serious and common causes of secondary osteoporosis. Administration of intermittent parathyroid hormone (PTH) may induce formation of new bone and may counteract the bone loss induced by GC treatment. Effects of simultaneous PTH and GC treatment were investigated on bone biomechanics, static and dynamic histomorphometry, and bone metabolism. Twenty-seven-month-old female rats were divided randomly into the following groups: baseline, vehicle, PTH, GC, and PTH + GC. PTH (1–34) 25 μg/kg and GC (methylprednisolone) 2.5 mg/kg were injected subcutaneously each day for a treatment period of 8 weeks. The rats were labeled with fluorochromes 3 times during the experiment. Bone sections were studied by fluorescence microscopy. The PTH injections resulted in a 5-fold increase in cancellous bone volume. At the proximal tibia, PTH induced a pronounced formation of new cancellous bone which originated from the endocortical bone surfaces and from thin trabeculae. Formation and modeling of connections between trabeculae were observed. Similar but less pronounced structural changes were seen in the PTH + GC group. The compressive strength of the cancellous bone was increased by 6-fold in the PTH group compared with the vehicle group. GC partially inhibited the increase in compressive strength induced by PTH. Concerning cortical bone, PTH induced a pronounced increase in the endocortical bone formation rate (BFR) and a smaller increase in periosteal BFR. The combination of PTH + GC resulted in a partial inhibition of the PTH-induced increase in bone formation. Serum-osteocalcin was increased by 65% in the PTH group and reduced by 39% in the GC group. The pronounced anabolic effect of PTH injections on the endocortical and trabecular bone surfaces and less pronounced anabolic effect on periosteal surfaces were partially inhibited, but not prevented, by simultaneous GC treatment in old rats. Both cortical and cancellous bone possessed full mechanical competence after treatment with PTH + GC.

Keywords: PTH; Glucocorticoid; Bone formation; Bone resorption; Bone biomechanics

Introduction

Glucocorticoids (GC) are used for the treatment of a wide spectrum of diseases because of their potent anti-inflammatory and immunosuppressive effects, although the treatments are serious and common causes of secondary osteoporosis [1,2]. The beneficial effects of intermittent parathyroid hormone (PTH) treatment of postmenopausal women or men with osteoporosis have been shown in randomized, placebo-controlled trials [3–6]. Intermittent PTH treatment increases the turnover of bone by activating the remodeling units in bone which leads to enhancement of bone formation over bone resorption. Therefore, intermittent PTH treatment of GC-induced bone loss is obvious. However, the question is, whether GC counteracts the bone anabolic effects of a simultaneous intermittent treatment with PTH? Lane and colleagues [1] found by quantitative computed tomography that intermittent PTH dramatically increased the bone density in the lumbar spine and the hip of postmenopausal women with GC-induced osteoporosis who underwent hormone replacement therapy, and the maximum effect of the PTH was seen 6–12 months after the PTH treatment was discontinued. In addition, intermittent PTH treatment has been associated with an increase in vertebral cross-sectional area of postmenopausal women with GC-induced osteoporosis [7], and the anabolic effects have been

* Corresponding author. Fax: +45 8613 7539.
E-mail address: ho@ana.au.dk (H. Oxlund).

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demonstrated to be much less pronounced in the hip and forearm than in the spine [1,8].

GC induces a state of low-turnover in rat bone [9]. One animal study has investigated the effects of simultaneous GC and PTH treatment [10]. In that study, 7-month-old female rats with linear growth were injected with prednisolone 2.5 mg/kg/day for 8 weeks, and static histomorphometry demonstrated prednisolone-induced osteopenia by inhibition of both bone formation and bone resorption. Simultaneous intermittent PTH 6 μg/kg/day prevented the low-turnover osteopenia by stimulating bone turnover [10]. Intermittent PTH counteracts GC-induced osteopenia by increasing bone turnover and bone mass, but bone biomechanical competence after combination treatment with GC and intermittent PTH has not yet been reported.

In the present study, the effects of PTH treatment were investigated on bone biomechanics, static and dynamic histomorphometry, and bone metabolism in a model of old rats with GC-induced low-turnover of bone.

Materials and methods

Seventy-five female Wistar rats, 27-month-old at the start of the experiment, were randomly divided into five groups: (1) Baseline control, killed at day 0; (2) vehicle (0.15M saline with 2% heat-inactivated rat serum, pH 5.0), two injections per day; (3) one injection of PTH (1–34) 25 μg/kg/day in vehicle and one injection with vehicle only; (4) one injection of GC (methylprednisolone) 2.5 mg/kg/day in vehicle and one injection with vehicle only; (5) PTH (1–34) 25 μg/kg/day in vehicle + GC (methylprednisolone) 2.5 mg/kg/day in vehicle. The rats were weighed once a week, and the dose of hormones was adjusted in accordance with the actual body weight. The synthetic human PTH fragment (1–34) was purchased from Bachem Inc., Torrance, CA, USA. The methylprednisolone was administered as sodium succinate (Solumedrol, Pfizer). The rats had free access to tap water and pellet food (Altromin diet containing 0.9% calcium and 0.7% phosphorus, Christian Petersen, Ltd., Ringsted, Denmark). All animals were triple labeled with subcutaneous fluorochrome injections: 49 days before killing with alizarin red S (20 mg/kg, Sigma Co., St. Louis, MO), 14 days before killing with calcein (15 mg/kg, Sigma Co.), and 4 days before killing with tetracycline (20 mg/kg, Sigma Co.). After treatment for 56 days, the rats were anesthetized with pentobarbital, 60 mg/kg intraperitoneally, and killed by exsanguination. Blood was withdrawn from the inferior vena cava and centrifuged. Serum was stored at −80°C until analysis. The hind legs were exarticulated at the hip joints and the lumbar vertebrae dissected free and stored airtight at −20°C until examination. The study was approved by the Danish Animal Experiment Inspectorate.

Muscle mass and mechanical three-point bending of cortical bone in the tibia diaphysis

The anterior tibialis and extensor digitorum longus muscles were dissected free from the hind limbs. The tendinous tissue was removed and the muscular tissue defatted in acetone for 7 days and freeze dried for 7 days, and the dry defatted weight was measured. All remaining soft tissues were removed from the tibias and the length determined by the use of an electronic sliding calliper.

Both tibias were then analyzed in a materials testing machine (Alwetron 250, Lorentzen and Wettre, Stockholm, Sweden) using a three-point bending procedure [11–13]. The tibias were placed on the lateral surface on two rounded supporting bars with a distance of 15.1 mm between the bars. The load was applied at the medial surface of the diaphysis by lowering a third rounded bar at a point which was 2 mm proximally to the tibia–fibular junction. The tibia was deflected at a constant deflection rate of 2 mm/min until fracture, and load–deflection curves were registered continuously on an *x–y* recorder by transducers coupled to measuring bridges. The load–deflection curves were scanned into a computer, and the following mechanical parameters were calculated from the curves by means of the Sigma scan software (Jandel Scientific Software, St. Rafael, CA, USA): bending strength (load at fracture) and bending stiffness (maximum slope of the load–deflection curve). Two 300-μm-thick sections were cut 1.0 mm distally to the loading point with a precision parallel saw (Exact-Apparatebau, Otto Herrmann, Norderstedt, Germany). The first section was placed on a glass slide and projected onto a screen with a projection microscope (magnification ×200). The periosteal and endocortical circumferences and diameters were drawn and scanned into the computer, and bone cross-sectional areas, medullary area, and diameters were calculated.

Tibia mid-diaphyseal cortical bone formation rate

The other mid-diaphyseal section was embedded in Pertex (Histolab Ab, Göteborg, Sweden) on a glass slide and used for determination of mineral apposition rates (MAR) and bone formation rates (BFR) using an epifluorescence microscope (Leitz DMRBE, Leica, Wetzlar, Germany) [14]. A translucent star plastic grid was placed on top of each section with the center of the star in the mid-point of the marrow cavity and with 16 lines radiating out from the center point. Since only the center of the star was fixed, the 16 radiating lines intersected the periosteal and endocortical surfaces randomly. At the point of intersection, the distance between the middle of the tetracycline label and the middle of the alizarin red S label was measured at a magnification of ×200. An aspect with no labeling was classified as a point of no mineral apposition. No distance between the tetracycline and calcein labels was classified as a point of no measurable mineral apposition, i.e., zero mineral apposition. The MAR was calculated by dividing the distance between the two labels by the interlabeling period in days. Similarly, the BFR was determined by multiplying MAR by the percentage mineralizing part of the circumference of a 1-mm-thick section [14,20].

Determination of cancellous bone volume fraction (BV/TV) in the proximal tibia metaphysis

The proximal tibia metaphysis was fixed in 70% ethanol at 4°C for undecalcified bone processing. The metaphysis was dehydrated with sequential changes of ethanol and embedded in methyl metacrylate. Mid-sagittal sections (10 μm thick) were cut using a microtome (model 2050, Jung, Heidelberg, Germany). The mounted unstained sections were then placed in the epifluorescence microscope, magnification ×200, and the cancellous bone volume was determined by point counting in a 0.5-mm-high zone, 1.5 mm distally to the growth plate. Furthermore, the sections were studied by fluorescence microscopy (Fig. 3). Formation of new trabeculae and connections between trabeculae were studied by means of the three labels, alizarin red S at start of the experimental period, and calcein and tetracycline towards the end. Resorption pits and remodeling of the trabecular structures were observed.

Mechanical compression analysis of cancellous bone in the left proximal tibia metaphysis

A two-millimeter-high specimen with plano-parallel surfaces was cut transversely from the proximal tibia metaphysis 1.5 mm distally to the epiphyseal growth plate, and a compression analysis was performed on the cancellous bone [15]. Two 300-μm-thick transverse sections were cut just proximally and distally to this bone specimen, and the cancellous bone core diameters within the cortical rim were determined by use of the epifluorescence microscope. Cancellous bone was separated from adjacent endocortical bone using the definition described by Parfitt [16]. The cancellous bone of the 2.0-mm-high specimens was then placed between two platens in the materials testing machine. The diameter of the platens used corresponded to the transverse diameter of the cancellous bone minus endocortical bone. The upper platen was lowered at a constant strain rate of 0.50 per minute, compressing the cancellous bone between the upper and lower platens until fracture. The load–deformation curves obtained were scanned into a computer, and the ultimate compressive load was determined. Ultimate compressive stress was calculated by normalizing the ultimate compressive load to the cross-sectional area of the cancellous bone compartment.