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Effects of Low Dose Parathyroid Hormone on Bone Mass, Turnover, and Ectopic Osteoinduction in a Rat Model for Chronic Alcohol Abuse

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Abstract

Parathyroid hormone (PTH) is used clinically in osteoporotic patients to increase bone mass by enhancing bone formation. PTH therapy is not uniformly effective at all skeletal sites and “lifestyle” factors may further modulate the skeletal response to PTH. Alcohol may represent one of those factors. Chronic alcohol abuse is associated with osteoporosis and impaired fracture healing. Therefore, the present study investigated the effects of alcohol on the bone anabolic response to a dose of PTH similar to a human therapeutic dose 1) during normal cancellous and cortical bone growth and turnover, and 2) in a model of demineralized allogeneic bone matrix (DABM)-induced osteoinduction. Three-month-old male Sprague Dawley rats were fed the Lieber-DeCarli liquid diet with 35% of the calories derived from ethanol. The controls were pair-fed an alcohol-free isocaloric diet containing maltose-dextran. Following adaptation to the liquid diets, the rats were implanted subcutaneously with DABM cylinders prepared from cortical bone of rats fed normal chow. The rats were subsequently treated daily with PTH (1 µg/kg/d sc, 5d/wk) or vehicle and measurements on bone and DABM implants performed 6 w later. Total bone mass was evaluated on the day of necropsy using DXA. Tibiae were processed for histomorphometry. Bone mass and architecture in tibial diaphysis and DABM implants was evaluated by µCT. PTH treatment increased whole body bone mineral content (BMC) and bone mineral density (BMD). The hormone also increased bone formation and bone area/tissue area in the proximal tibial metaphysis. In contrast, PTH treatment had no effect on periosteal bone formation and minimal effects on DABM-induced osteoinduction. Alcohol consumption decreased whole body BMC. Alcohol also decreased cancellous as well as cortical bone formation and bone mass in tibia and impaired DABM-mediated osteoinduction. There was no interaction between PTH treatment and alcohol consumption for any of the endpoints evaluated. Our results indicate that the bone anabolic response to a therapeutic dose of PTH in the rat is largely confined to cancellous bone. In contrast, alcohol consumption inhibits bone formation at all sites. Furthermore, alcohol inhibits osteoinduction and reduces periosteal and cancellous bone formation, irrespective of therapeutic PTH administration. Based on the animal model, our findings suggest that alcohol consumption could impair the beneficial effects of PTH therapy in osteoporosis.

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Introduction

Intermittent parathyroid hormone (PTH) is the only FDA approved bone anabolic therapy for treatment of established osteoporosis [1]. PTH has also been investigated for its potential to accelerate fracture healing by increasing bone formation [2,3]. However, PTH is not effective in increasing bone mineral density (BMD) in all patients, suggesting that “life-style” factors may modulate the skeletal response to this bone anabolic therapy [4]. The prevalent use of alcohol may represent one of those factors.

Chronic alcohol abuse inhibits bone growth and turnover, results in a negative bone remodeling balance, decreases bone mass, increases fracture risk and, should a fracture occur, may impair bone healing [5]. Chronic alcohol abuse leads to delayed fracture repair, and a higher incidence of delayed unions and non-unions [6,7]. Overall, fractures in alcoholics are associated with longer hospitalization and increased morbidity and mortality [8,9].

The effects of ongoing alcohol consumption on therapies to treat osteoporosis or accelerate bone healing are uninvestigated in humans and only a small number of studies have been performed in animal models [10,11]. Animal studies to date suggest a detrimental effect of alcohol on the skeletal response to PTH. However, only very high dose rates of the hormone have been modeled. Recent dose response studies suggest that high doses of PTH impair the ability to detect and model “life style” factors that influence the skeletal response to PTH in humans [10,11]. Additional studies that better model the human therapeutic application of PTH are warranted due to the high prevalence of alcohol consumption in countries with high rates of osteoporosis.

PTH is effective in reducing fractures in postmenopausal osteoporotic women. However, the fracture incidence in postmenopausal women is still much greater than in young women. Therefore, the potential of PTH for accelerating fracture repair is of great interest. Bone healing following a fracture is a complex process. A defect in any one of a number of critical steps could delay or prevent healing. Following a fracture, bone resorption results in the release of osteoinductive growth factors stored in bone matrix. We have recently shown that osteoinduction by demineralized allogeneic bone matrix (DABM) is impaired by alcohol in the rat model for chronic alcohol abuse [12]. PTH is under investigation as a therapy to improve fracture repair [2,3]. The most likely benefit of PTH on bone healing would be increased bone formation at the fracture site, which is in part mediated by matrix-derived growth factors. However, the effects of a therapeutic dose of PTH on the osteoinductive capacity of bone matrix have not been evaluated.

Based on the above considerations, we assessed the effects of PTH on cancellous and cortical bone metabolism and osteoinduction in a rat model for chronic alcohol abuse using a dose rate of the hormone (1 $\mu\text{g/kg/d}$) that closely approximates the human therapeutic dose rate used to treat osteoporosis. We believe that this low dose PTH models the human skeletal response to the hormone much better than the high dose rates commonly used in rodents. We also measured serum levels of insulin-like growth factor-I (IGF-I). IGF-I is a key mediator of PTH action and is decreased by alcohol consumption [13–15]. The results indicate that alcohol consumption and PTH have opposite effects on cancellous bone formation, mass, and architecture in the tibia. They also show that a therapeutic dose of PTH in young adult male rats has minimal effects on cortical bone or ectopic osteoinduction in either the presence or absence of alcohol. In contrast, alcohol consumption inhibits cortical bone formation and osteoinduction.

Methods

Animals

Forty two, 3-month-old, male, Sprague-Dawley rats (body weight, 379 ± 10 ; mean $\pm$ SE) were obtained from Harlan (Indianapolis, IN) and housed in plastic shoebox cages (1 rat/cage) in a temperature- and humidity-controlled room with a 12/12 hour light/dark cycle. Animal care followed the guidelines found in the Guide for Care and Use of Laboratory Animals. The animal experiment was approved by the Institutional Animal Care and Use Committee at Oregon State University.

Experimental Design

After a 1 week period of acclimation, the rats were randomized by weight into four treatment groups (n=10–11/group): 1) control + vehicle, 2) alcohol + vehicle, 3) control + PTH, and 4) alcohol + PTH. Rats in the alcohol-fed group were adapted to a liquid alcohol diet over a 1 week period, as recommended by the manufacturer (Lieber-DeCarli liquid diet, #F1258SP, Bio-Serve, Frenchtown, NJ). At the end of the adaptation period, 35% of caloric intake in these rats was derived from alcohol. Control rats consumed an isocaloric liquid diet with calories from alcohol being replaced with maltose-dextran (BioServ, #F1259SP). The liquid diet contains 1.36% Ca, 1.06% P and 0.13% Mg (v/v). Subcutaneous treatment with synthetic human PTH (1–34) (Bachem, Torrance, CA; 1 μ g/kg/d; 5 d/wk) or vehicle was begun 1 day after completion of adaptation of rats to their liquid diets. Implantation of DABM cylinders was also performed on this day. The rats were weighed weekly and maintained on their respective diets for 6 weeks. Fluorochrome labeling was used to determine active bone mineralization sites and rates of bone formation. Rats were injected subcutaneously with tetracycline (15 mg/kg; Sigma Chemical Co., St. Louis, MO) on the day alcohol treatment was started and calcein (15 mg/kg; Sigma Chemical Co., St. Louis, MO) 8 and 1 days prior to necropsy.

DABM Implant Preparation

The details of preparation of the implants for this study as well as implant surgery have been reported (13).

DXA

Prior to necropsy, total body bone mineral content (BMC) and total body bone area were measured in anesthetized rats and total body BMD calculated from these data using dual-energy x-ray absorptiometry (DXA, Hologic QDR-4500A Elite, Waltham, MA) equipped with small animal software.

Tissue Collection

All rats were anesthetized with isoflurane prior to tissue collection. Death was induced by exsanguination from the heart, followed by cardiac excision. Serum was stored at -70°C until assay. Tibiae and DABM implants were excised and placed in 70% ethanol for bone histomorphometry and/or μ CT evaluation.

Serum

Serum IGF-I levels were measured by radioimmunoassay using a kit (IGF-R21) as described by the manufacturer (American Lab Products Company, Windham, NH).

Quantitative Bone Histomorphometry

For histomorphometric evaluation of cancellous bone, proximal tibiae were dehydrated in graded ethanols and xylene, and embedded undecalcified in modified methyl methacrylate [16]. Longitudinal sections (4µm thick) were cut with a vertical bed microtome (Leica 2065) and affixed to slides precoated with a 1% gelatin solution. Histomorphometric data were collected in one tibial section per animal using the OsteoMeasure System (OsteoMetrics, Inc., Atlanta, GA).

The measurement area consisted of secondary spongiosa at distances greater than 0.5 mm from the growth plate. On average, 3.2 mm² of cancellous bone tissue (including marrow) was measured in each section. The following histomorphometric data are reported using standard nomenclature: cancellous bone area/tissue area (B.Ar/T.Ar, %), trabecular thickness (Tb.Th, µm), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, µm), mineralizing perimeter (M.Pm, double labeled perimeter/bone perimeter, %), mineral apposition rate (MAR, µm/d), and bone formation rate (BFR/B.Pm, M.Pm × MAR, µm²/µm/d). Calcein labels 1 and 2 were used for measuring dynamic cancellous bone histomorphometry.

The measurement area in the tibial diaphysis consisted of cortical bone located approximately 1 mm proximal to the tibio-fibular synostosis. A low speed saw (Buehler, Rosemont, IL) was used to cut a 150 µm cross section through each tibial diaphysis. The section was polished between a roughened ground glass plate and cork and then illuminated with UV light to measure the fluorochrome labels. M.Pm/B.Pm, MAR, and BFR/B.Pm were determined as described [10,11].

µCT analysis

µCT was used to measure cortical bone architecture. The tibial diaphysis was scanned at a voxel size of 16 × 16 × 16 µm using a Scanco Medical µCT 40 (Scanco Medical AG, Basserdorf, Switzerland). The threshold for analysis was determined empirically and set at 276 (0–1,000 range) for all bones. Ten slices (200 µm) 1 mm proximal to the tibio-fibular junction were analyzed for cross-sectional volume (mm³), cortical bone volume (mm³), marrow volume (mm³), and cortical thickness (µm).

Whole implants (4/rat) were scanned at a voxel size of 12 × 12 × 12 µm and analyzed at a threshold of 265. Bone volume (BV, mm³) was determined for each implant. The BV for the 4 implants/rat was averaged to obtain a mean endpoint value for each animal. The implants were ashed and BMC was determined as the product of BV and ash density [12].

Statistical Analysis

The effects of diet (with two levels, alcohol and control), PTH treatment (with two levels, PTH and vehicle), and their interaction were analyzed using a two-way analysis of variance (SPSS 13.0, SPSS Inc., Chicago, IL). Differences were considered significant at $P < 0.05$. All data are expressed as mean ± SE.

Results

Effects of Alcohol and PTH on Serum IGF-1, Total Body Bone Mass and Density, and Tibial Cancellous and Cortical Architecture

All animals gained weight and differences in weight gain were not detected among any of the treatment groups during the 6 week duration of study.

The effects of alcohol consumption and PTH treatment on serum IGF-I levels and whole body BMC, bone area, and BMD are shown in Table 1. Alcohol consumption resulted in lower serum

IGF-I levels, whereas PTH treatment had no effect on serum IGF-I. Total body BMC and bone area were lower in alcohol-fed compared to control-fed rats. Total body BMC and BMD were higher in rats treated with PTH compared to vehicle-treated control rats. Significant interactions between alcohol consumption and PTH treatment were not detected for any of the endpoints evaluated.

The effects of alcohol and PTH treatment on static bone histomorphometry, measured at the proximal tibial metaphysis, are shown in Table 2. Rats consuming alcohol had lower cancellous B.Ar/T.Ar and Tb.N and greater Tb.Sp compared to control-fed rats. Differences in Tb.Th were not detected with alcohol treatment. Treatment with PTH resulted in higher cancellous B.Ar/T.Ar, Tb.Th, and Tb.N and lower Tb.Sp compared to treatment with vehicle. Significant interactions between alcohol consumption and PTH treatment were not detected for any of the endpoints evaluated.

The effects of alcohol and PTH on dynamic bone histomorphometry at the proximal tibial metaphysis are shown in Figure 1. Alcohol and PTH had independent but opposite effects on mineralizing perimeter. Alcohol treatment resulted in lower M.Pm/B.Pm whereas PTH treatment resulted in higher M.Pm/B.Pm. MAR was higher in PTH-treated rats, irrespective of alcohol treatment. BFR/B.Pm was lower in alcohol-fed and higher in PTH-treated rats compared to the appropriate controls.

The effects of alcohol consumption and PTH treatment on cortical bone architecture and dynamic histomorphometry in the tibial diaphysis are shown in Table 2 and Figure 2, respectively. Alcohol resulted in lower tibial cortical bone volume and tended to result in lower cross-sectional volume ($P = 0.09$) and cortical thickness ($P = 0.06$). These changes in architecture were associated with lower periosteal M.Pm/B.Pm, MAR and BFR/B.Pm in rats fed alcohol compared to rats fed control diet. PTH had no effect on cortical bone architecture or indices of bone formation and there were no interactions between alcohol and PTH on cortical bone. We did not measure the effects of alcohol or PTH on endocortical bone formation because very little fluorochrome was deposited at this site in any of the treatment groups, indicating a very low level of bone formation. Differences in volume of the medullary cavity were not detected among groups, suggesting that bone resorption was not altered at the endocortical surface of the tibial diaphysis by either alcohol or PTH. Taken together, these findings show that neither alcohol nor PTH altered endocortical bone turnover.

Osteoinduction

Alcohol consumption resulted in lower implant BMC, whereas PTH treatment had no significant effect on BMC of DABM implants (Figure 3).

Discussion

Alcohol consumption decreased total body bone area and BMC. Cancellous bone was decreased at the proximal tibial metaphysis and the resulting osteopenia was due, at least in part, to a decrease in BFR. Alcohol consumption decreased cortical bone by inhibiting periosteal bone formation. Alcohol also impaired DABM-induced ectopic osteoinduction. Treatment with low-dose PTH increased total body BMC and cancellous bone and cancellous BFR in the proximal tibia. In contrast, PTH had minimal effects on cortical bone and DABM-induced osteoinduction in rats fed control or alcohol diet.

PTH increases bone formation in rats and humans [17–20]. Transient increases in PTH (intermittent PTH therapy) promote a net increase in bone formation, in part by modulating the activity of terminally differentiated osteoblasts [21]. In both species, the resulting increase in bone mass is bone compartment specific. The majority of human studies have reported the

most pronounced anabolic response to PTH to occur at predominantly cancellous sites [22–24]. Thus, low dose PTH treatment in the rat results in site specific increases in bone formation similar to the therapeutic use of the hormone in humans. This finding contrasts with high dose PTH treatment in rats which results in a pronounced bone anabolic response in cortical bone [10,11].

The molecular mechanisms that mediate the bone anabolic response to PTH are poorly understood but appear to require IGF-I signaling. Animals with low circulating levels of IGF-I have deficient bone formation [15]. Also, bone in IGF-I knockout mice is insensitive to PTH, suggesting that IGF-I is essential for the bone anabolic effects of the hormone [25,26]. Acute administration of alcohol decreases IGF-I mRNA levels in liver and bone [13,14]. Alcohol also reduces the circulating level of IGF-I. Thus, alcohol abuse could decrease the skeletal response to PTH by reducing systemic and/or locally produced IGF-I or, alternatively, by inducing an end organ resistance to IGF-I signaling [27]. PTH increases mRNA levels for IGF-I in skeletal tissue in rats [28] and the hormone has been shown to be effective in increasing cancellous bone formation in hypophysectomized (HYPOX) rats [29]. Since HYPOX rats have very low serum IGF-I levels, locally generated IGF-I may be sufficient for the bone anabolic effects of PTH [30]. However, other studies suggest that systemic IGF-I is critically important for the bone anabolic response to the hormone [31]. Thus, PTH-induced IGF-I in bone cells may compensate for the reduced circulating levels of the growth factor in alcohol-fed rats. Regardless of the relative importance of locally generated versus systemically derived IGF-I, PTH increases cancellous bone formation in alcohol-fed rats without the requirement for restoring normal serum IGF-I levels.

Allogeneic bone particles are commonly used to facilitate bone healing in orthopedic procedures [32,33]. In the current study, PTH treatment failed to enhance osteoinduction by DABM, irrespective of alcohol consumption. Also, PTH had minimal effects on implant architecture; the hormone resulted in a slight (+6%, $P<0.02$) increase in Tb.Th, but BV, Tb.N, connectivity density, Tb.Sp and structure module index were unchanged (data not shown). Our negative findings, using a model for the clinical application of DABM, contrast with reports of PTH enhancing bone fracture repair [3,34–37]. The reason for the divergent results is not entirely clear but could be related to differences in the dose rate of PTH or fundamental differences among the various bone-healing models.

In the present study, the PTH dose used approximates the human therapeutic dose rate. In contrast, the studies reporting positive results of intermittent PTH administration on fracture healing models used dose rates 10–60 times higher than our dose. These dose rates also typically increase cortical bone mass in rat long bones, a finding that is generally not observed in the femur in PTH-treated women [23]. In another study, we administered PTH at 80 $\mu\text{g/kg/d}$ for 3 weeks to rats implanted sc with DABM (Turner RT, unpublished results). PTH treatment significantly increased double fluorochrome labeled perimeter. From these findings, we conclude that DABM-induced osteoinduction can be enhanced by PTH. However, the dose rate needed is higher than that required to increase cancellous bone formation and is much higher than a human therapeutic dose rate. Our findings suggest that therapeutic PTH may not increase osteoinduction by implanted bone matrix in patients whereas alcohol consumption may impair such osteoinduction.

We previously observed a modest increase in periosteal bone formation in skeletally mature rats treated with low dose PTH [38]. The failure to observe a PTH effect on cortical bone in the current study may be related to age of the animals. The earlier studies showing positive effects on bone were performed in older rats which have lower baseline levels of bone formation. The percent bone surface covered with quiescent bone lining cells, a putative target cell population for PTH, increases with age. This increase provides a plausible explanation for

the recently reported increased sensitivity of the aged skeleton to intermittent PTH therapy [38].

In contrast to the lack of an effect of PTH, alcohol had a distinctly negative effect on cortical bone. The detrimental effects on bone metabolism that we observed are consistent with earlier studies [39–42]. Alcohol is a potent inhibitor of bone growth in rats and decreases peak bone mass [39–42]. In addition to reducing the accumulation of bone during growth, chronic alcohol consumption results in bone loss in skeletally mature rats [43,44]. Thus, alcohol abuse could contribute to osteoporosis by decreasing peak cancellous and cortical bone mass and accelerating cancellous bone loss during aging.

Therapeutic PTH is effective in reversing cancellous bone loss associated with menopause [1]. Although alcohol abuse can result in cancellous bone loss, it also antagonizes periosteal bone formation. Periosteal bone formation is solely responsible for cortical bone expansion which plays an important adaptive role in compensating for age-related endocortical bone loss [45]. Our results suggest that the inhibitory effects of alcohol on periosteal bone formation may negate the beneficial effects of the hormone on endosteal bone formation in osteoporotic patients. PTH treatment was effective in increasing cancellous but not cortical bone formation in alcohol-fed rats. The present studies were performed in a rat model for chronic alcohol abuse. However, we have shown that low to moderate alcohol consumption also reduced bone formation in the rat model [44]

In summary, the present studies, although performed in normal rats, suggest that by reducing the baseline level of bone formation, alcohol consumption has the potential to decrease the efficacy of therapeutic PTH to restore bone in an osteopenic skeleton. Alcohol may have additional independent detrimental effects by 1) inhibiting bone formation at skeletal sites such as the periosteum that are already resistant to the anabolic actions of PTH, and, 2) should a fracture occur by impairing bone healing.

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Figure 1.

Effects of alcohol consumption and PTH treatment on dynamic bone histomorphometry at the proximal tibial metaphysis. (A) Mineralizing perimeter (M.Pm./B.Pm). (B) Mineral apposition rate (MAR). (C) Bone formation rate (BFR/B.Pm). Values are mean $\pm$ SE (n=9–11 rats/group)

Figure 2.

Effects of alcohol consumption and PTH treatment on dynamic bone histomorphometry at the cortical periosteum 1 mm proximal to the tibio-fibular joint. (A) Mineralizing perimeter (M.Pm./B.Pm). (B) Mineral apposition rate (MAR). (C) Bone formation rate (BFR/B.Pm). Values are mean $\pm$ SE (n=9–11 rats/group)

Figure 3.
Effects of alcohol consumption and PTH treatment on implant bone mineral content.

Effects of alcohol consumption and low-dose PTH treatment on serum IGF-1 levels and whole body bone densitometry.

Table 1

Endpoint	Control	Alcohol	Control+PTH	Alcohol+PTH	ANOVA		
					Alcohol (P<)	PTH (P<)	Interaction (P<)
Serum							
IGF-1 (ng/ml)	616 ± 14	522 ± 24	566 ± 18	534 ± 30	0.009	NS	NS
Bone mineral content and density							
BMC (g)	13.9 ± 0.2	13.5 ± 0.1	14.6 ± 0.2	14.0 ± 0.2	0.003	0.002	NS
Area (cm ²)	72.6 ± 0.9	69.7 ± 0.8	73.7 ± 0.5	70.9 ± 0.9	0.001	NS	NS
BMD (g/cm ²)	0.191 ± 0.003	0.193 ± 0.001	0.197 ± 0.002	0.197 ± 0.002	NS	0.018	NS

Values are mean ± SE; n=10–11 rats
NS, not significant

Table 2

Effects of alcohol consumption and low-dose PTH treatment on static bone histomorphometry at the proximal tibial metaphysis and tibial diaphysis.

Endpoint	Control	Alcohol	Control+PTH	Alcohol+PTH	ANOVA		
					Alcohol (P<)	PTH (P<)	Interaction (P<)
Tibial metaphysis (cancellous bone)							
Bone area/Tissue area (%)	9.6 ± 1.7	5.3 ± 0.5	13.2 ± 1.5	10.0 ± 1.2	0.007	0.003	NS
Trabecular number (1/mm)	1.9 ± 0.3	1.1 ± 0.1	2.1 ± 0.2	1.6 ± 0.1	0.002	NS	NS
Trabecular thickness (µm)	52 ± 4	51 ± 4	63 ± 4	63 ± 3	NS	0.005	NS
Trabecular separation (µm)	642 ± 139	1016 ± 133	457 ± 45	638 ± 71	0.009	0.009	NS
Tibial diaphysis (cortical bone)							
Cross-sectional volume (mm ³)	1.11 ± .02	1.07 ± .02	1.11 ± .02	1.09 ± .02	NS	NS	NS
Cortical Volume (mm ³)	0.92 ± 0.02	0.89 ± 0.02	0.93 ± 0.02	0.89 ± 0.02	0.043	NS	NS
Marrow volume (mm ³)	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	NS	NS	NS
Cortical Thickness (µm)	842 ± 11	824 ± 13	863 ± 10	837 ± 11	NS	NS	NS

Values are mean ± SE; n=10–11 rats

NS, not significant