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Corticosterone selectively targets endo-cortical surfaces by an osteoblast-dependent mechanism☆

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ABSTRACT

Background: The pathogenesis of glucocorticoid-induced osteoporosis remains ill defined. In this study, we examined the role of the osteoblast in mediating the effects of exogenous glucocorticoids on cortical and trabecular bone, employing the Col2.3-11βHSD2 transgenic mouse model of osteoblast-targeted disruption of glucocorticoid signalling.

Methods: Eight week-old male transgenic (tg) and wild-type (WT) mice ($n = 20–23$/group) were treated with either 1.5 mg corticosterone (CS) or placebo for 4 weeks. Serum tartrate-resistant acid phosphatase 5b (TRAP5b) and osteocalcin (OCN) were measured throughout the study. Tibiae and lumbar vertebrae were analysed by micro-CT and histomorphometry at endpoint.

Results: CS suppressed serum OCN levels in WT and tg mice, although they remained higher in tg animals at all time points ($p<0.05$). Serum TRAP5b levels increased in WT mice only. The effect of CS on cortical bone differed by site: At the endosteal surface, exposure to CS significantly increased bone resorption and reduced bone formation, resulting in a larger bone marrow cavity cross-sectional area ($p<0.01$). In contrast, at the pericortical surface bone resorption was significantly decreased accompanied with a significant increase in pericortical cross-sectional area ($p<0.05$) while bone formation remained unaffected. Vertebral cortical thickness and area were reduced in CS treatment mice. Tg mice were partially protected from the effects of exogenous CS, both on a cellular and structural level. At the CS doses used in this study, trabecular bone remained largely unaffected.

Conclusion: Endocortical osteoblasts appear to be particularly sensitive to the detrimental actions of exogenous glucocorticoids. The increase in tibial pericortical cross-sectional area and the according changes in pericortical circumference suggest an anabolic bone response to GC treatment at this site. The protection of tg mice from these effects indicates that both catabolic and anabolic action of glucocorticoids are, at least in part, mediated by osteoblasts.

Introduction

Glucocorticoids (GCs) have potent anti-inflammatory, anti-proliferative and immuno-modulatory effects when used at pharmacological doses. However, their clinical benefits are often marred by serious adverse effects such as glucose intolerance [1,2], skin atrophy [3,4], sarcopenia [5] and osteoporosis [6]. While the detrimental effects of exogenous GCs on *trabecular* bone mass and structure are well documented [7,8], their action on cortical bone is less clear. Indeed, recent data indicate that GCs at pharmacological doses may have divergent effects on cortical and trabecular bone [9,10]. A recent observational study found that the bone mineral

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content of children treated with GCs for non-inflammatory conditions was increased [9]. In a follow-up study, Wetzsteon et al. could further establish an anabolic effect of exogenous GCs specifically in cortical bone, while confirming that trabecular bone was adversely affected by GC treatment [10]. Moreover, the catabolic effects of excess GCs are in contrast to their known anabolic actions at lower concentrations. Thus, endogenous GCs are required for the normal development of numerous tissues, including the pancreas, lungs and the skeleton [11–13]. In bone, osteoblast differentiation and maturation [14–16] as well as skeletal development and maintenance [17] depend upon intact GC signalling.

The disparities in the apparent effects of endogenous and exogenous GCs on bone and its specific compartments signify that the actions of GCs on the skeleton in general, and on cortical bone in particular are poorly understood. Given the significance of cortical bone structure for bone strength and fracture risk [18,19], we aimed to determine, in mice, the effects of exogenous GCs on cortical bone, and the cellular compartment that would mediate these actions. Specifically, we hypothesised that GCs would induce site-specific changes in bone metabolism and structure, and that these effects would be primarily mediated through the osteoblast or osteocyte. To this aim, we made use of a transgenic mouse model in which the expression of a GC inactivating enzyme, 11ß-hydroxysteroid-dehydrogenase Type 2 (11β-HSD2), has been targeted exclusively to osteoblasts using the osteoblast-specific 2.3 kb collagen type Ia1 promoter (Col2.3-11βHSD2 tg mice). Targeted overexpression of 11β-HSD2 represents a highly specific means of disrupting GC signaling in osteoblasts [13,14,17].

In our previous studies using this transgenic mouse model, we have shown that endogenous GC signalling in mature osteoblasts is necessary for proper intramembranous bone development in the calvaria [13]. At the adult stage, we found that transgenic mice were characterised by lower bone volume, lower trabecular number and higher trabecular separation in tibial trabecular bone, as well as lower tibial cortical bone area and periosteal and endosteal perimeters. These changes resulted in a marked decrease in mechanical bone strength and stiffness [17]. Taken together, we have demonstrated that endogenous glucocorticoids are required for normal bone development and maintenance while high dose exogenous GCs are known to adversely affect both trabecular and cortical bone. Fracture risk predominantly depends on cortical bone strength [18,20]. As the Col2.3-11βHSD2 tg model allows us to dissect the primary target cell of endogenous or exogenous GCs, we were particularly interested in investigating the effects of low-doses of exogenous GCs on cortical bone, and bone structure in general.

Materials and methods

Transgenic animal model

The Col2.3-11βHSD2 tg mice used in this study were generated and characterised as previously described [14] and were provided as a gift by Dr Barbara Kream, University of Connecticut, USA. Animals were kept at the animal facility of the ANZAC Research Institute, in accordance with Institutional Animal Welfare Guidelines. The mice were maintained on a 12:12 h light–dark cycle with free access to standard chow and water.

Study design

Eight-week-old male Col2.3-11βHSD2 transgenic (tg) mice ($n=42$) and their wildtype (wt) littermates ($n=44$) were subcutaneously implanted with slow-release pellets containing either corticosterone 1.5 mg or placebo (Innovative Research of America, Sarasota, USA). Following a previously described protocol [21], pellets were implanted at days 0, 7, 14 and 21 in order to achieve continuous suppression of osteoblast function (as indicated by a sustained reduction in serum osteocalcin levels) [21]. At day 1 after implantation, serum corticosterone levels peaked at 536 ± 85 and 489 ± 79 nmol/L in corticosterone treated WT and tg mice While serum corticosterone levels remained low in *placebo*-treated WT and tg animals, at 149 ± 43 and 171 ± 37 nmol/L respectively. Serum corticosterone was measured by stable isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described previously [22]. All studies were approved by the institutional Animal Ethics Committee.

Tissue collection and sample preparation

Blood was collected at days 0, 7, 14 and 21 by retro-bulbar puncture and at day 28 by cardiac puncture, at which time animals were sacrificed. Left tibiae were harvested for biomechanical assessment while the right tibia and the lumbar spine were fixed in 4% paraformaldehyde/PBS. Following analysis by micro-CT, tibia and lumbar spine were decalcified in 10% EDTA for 2–3 weeks and embedded in paraffin. Serial 5-µm sections were prepared and stained with hematoxylin and eosin (H&E) and Safranin O for histological evaluation [23]. Additional sections were stained for tartrate-resistant acid phosphatase (TRAP) and alkaline phosphatase (ALP). TRAP staining was performed using naphthol-AS-BI phosphate as substrate and fast red violet LB salt (Sigma, Germany) as the

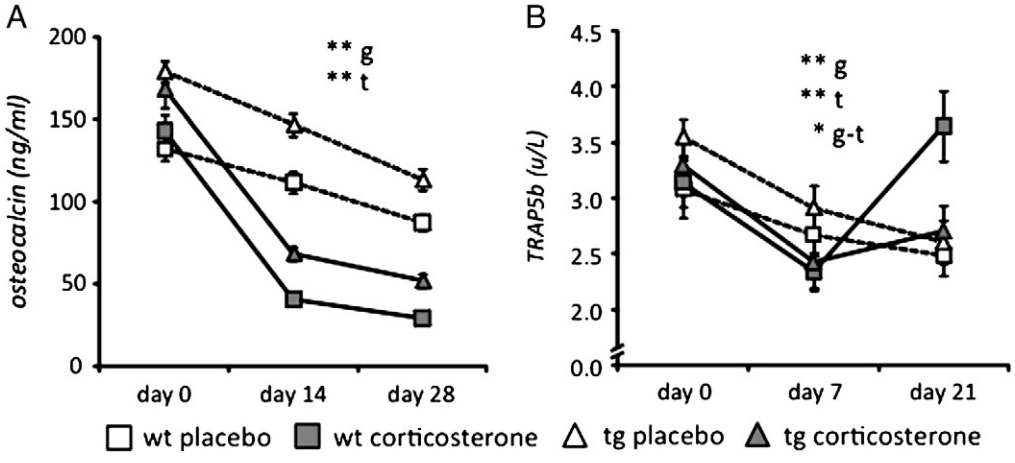

Fig. 1. Effects of corticosterone treatment on markers of bone turnover. Serum concentrations of osteocalcin (A) and mouse tartrate-resistant acid phosphatase 5b (TRAP5b) (B) show the age-related decline in placebo treated WT and tg mice. While serum osteocalcin levels decrease in WT and tg mice after corticosterone exposure, tg mice maintain higher osteocalcin concentrations at all time points ($p<0.01$). At day 21 the serum level of TRAP5b increases above baseline in corticosterone-treated WT mice only. *p*-Values shown are within subject effects from a generalised linear model with repeated measures analysis, hence influences on development over time. $N=9$–10 mice per group; $^{*}p<0.05$; $^{**}p<0.01$; g = genotype (WT *v* tg); t = treatment (WT-placebo *v* WT-corticosterone); g-t = WT-corticosterone *v* tg-corticosterone. Baseline weight has been identified as a confounder with significant influence on the development of TRAP5b ($p=0.034$), thus was accounted for during analysis.