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Mineralocorticoid receptor function in bone metabolism and its role in glucocorticoid-induced osteopenia

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ABSTRACT

Although the mineralocorticoid receptor (MR) is expressed in osteoblasts and osteocytes and frequently co-localizes with the glucocorticoid receptors (GR), its pathophysiological functions in bone remain elusive. We report here that pharmacologic inhibition of MR function with eplerenone resulted in increased bone mass, with stimulation of bone formation and suppression of resorption, while specific genetic deletion of MR in osteoblast lineage cells had no effect. Further, treatment with eplerenone as well as specific deletion of MR in osteocytes ameliorated the cortical bone thinning caused by slow-release prednisolone pellets. Thus, MR may be involved in the deleterious effects of glucocorticoid excess on cortical bone.

1. Introduction

Hypertension and osteoporosis are among the most prevalent disorders associated with aging, and emerging evidence suggests that the renin–angiotensin–aldosterone system (RAAS), which plays a central role in the regulation of fluid and electrolyte balance as well as blood pressure, is closely linked to bone metabolism. We have previously demonstrated that activation of RAAS in double transgenic mice expressing both the human renin and angiotensinogen genes induces osteopenia due to excessive bone resorption [1], while genetic deletion of the angiotensin II receptor subtype AT1a in mice results in a high bone mass phenotype in association with elevated bone formation [2].

In primary aldosteronism, the most common cause of secondary hypertension, aldosterone excess has been associated with cardiovascular and renal injury as well as disordered calcium and bone metabolism [3]. In fact, in a rat model mimicking aldosteronism by means of chronic aldosterone infusion and salt loading, increased urinary calcium excretion and elevated PTH levels were observed along with a concomitantly compromised bone strength, all of which were reversed by treatment with spironolactone, a mineralocorticoid receptor (MR) antagonist [3,4]. These observations led to the hypothesis that renal calcium leak and secondary hyperparathyroidism underlie the bone abnormalities associated with aldosterone excess. While MR was detected in bone samples taken from human specimens, especially osteoblasts and osteocytes [5], the pathophysiological functions of MR in bone remain to be elucidated.

Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis, and glucocorticoids are believed to impact calcium and bone metabolism through the glucocorticoid receptor (GR) in bone, intestine and kidney [6–8]. Glucocorticoid activity is locally controlled by the activity of the 11β-hydroxysteroid dehydrogenase (11β-HSD) isoenzymes 11β-HSD type 1 (11β-HSD1) and type 2 (11β-HSD2), which catalyze the interconversion of active glucocorticoids (cortisol in humans, corticosterone in rodents) and the inert 11-keto forms (cortisone and 11-dehydrocorticosterone or 11-DOC) [9,10]. 11β-HSD1 is widely distributed and has been suggested to regenerate active glucocorticoids from circulating inert 11-keto steroids, thereby amplifying glucocorticoid activity locally [10], while 11β-HSD2 is expressed in aldosterone target organs, such as distal nephron, colon and sweat glands, where it protects the MR from becoming occupied by glucocorticoids, thereby allowing aldosterone-selective access to the otherwise nonselective MR in target cells [9,11]. In view of the fact that glucocorticoids bind to the MR with high affinity [11], there exists the possibility that at least a portion of the glucocorticoid effect on bone is mediated through the MR, although this has never been reported. In the present study we have examined the

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pathophysiological functions of MR in bone metabolism using pharmacologic as well as genetic approaches. In doing so, we have focused on the functional interaction between the MR and GR in the setting of glucocorticoid excess.

2. Materials and methods

2.1. Reagents

Eplerenone was provided by Pfizer, Inc. (Groton, CT). 60-day release pellets respectively containing 5 mg prednisolone and placebo were purchased from Innovative Research of America (Sarasota, FL). Calcein and tetracycline for bone labeling were purchased from Sigma (St. Louis, MO).

2.2. Mice

MR flox mice have been described [12]. Mice hemizygous for the *Osterix-GFP::Cre* transgene were obtained from The Jackson Laboratory (Bar Harbor, ME) [13]. A transgenic cassette carrying a 7.4 kb mouse DMP1 gene promoter, including intron 1, Cre, IRES and an enhanced GFP (eGFP), was constructed. The transgenic construct was excised and purified using standard techniques. Founder DMP1-Cre mice were generated by microinjection of the DNA into fertilized eggs of C57BL/6 mice. Three independent transgenic founders were mated to wild-type C57BL/6 mice, and the F1 offspring were analyzed. All three lines produced pups at the expected ratio. These pups appeared normal, grew indistinguishably from wild-type mice, and were fertile. The results presented here are from line #2. RT-PCR with the RNA extracted from various tissues revealed that cre mRNA was expressed only in the bone of DMP1-Cre mice (Fig. 4A). Integration of the transgene was screened by PCR and then confirmed by Southern analysis of genomic DNA extracted from the tail. CAG-CAT-Z reporter mice were kindly provided by Dr. Junichi Miyazaki (Osaka Univ.) [14].

Mice were raised under standard laboratory conditions at 24 ± 2 °C and 50–60% humidity, and were allowed free access to tap water and standard rodent chow (CE-2, Clea Japan) containing 1.20% calcium, 1.08% phosphate and vitamin D_3 (240 IU per 100 g). For the p.o. administration of eplerenone (approximately 400 mg/kg/day by calculation), AIN-76A chow containing eplerenone (2.5 g/kg) obtained from the ESG Co., Ltd. (Tokyo, Japan) was used. Slow-release prednisolone pellets were implanted s.c. (2.8 mg/kg/day for 2 months) into 6-month-old male C57BL/6 mice according to the manufacturer's instructions. Control mice were implanted with placebo pellets from the same supplier. Experiments were performed on male mice unless indicated otherwise. All experiments were performed in accordance with NCGG ethical guidelines for animal care, and the experimental protocols were approved by the animal care committee.

2.3. Gene expression analysis

Total RNA was isolated with TRIzol reagent, purified with an RNeasy Mini Kit (Qiagen, Valencia, CA) and subjected to RT using a high-capacity cDNA RT kit (Applied Biosystems, Carlsbad, CA). Quantitative PCR analysis was performed using PowerSYBR Green PCR master mix and an ABI7300 real-time PCR system (Applied Biosystems). Semiquantitative RT-PCR was conducted using GoTaq (Promega, Madison, WI). RNAs from osteoblast- and osteocyte-rich fractions were isolated from the tibia and femur, as described previously [15]. In brief, after the bone marrow was flushed out with PBS, the diaphysis was cut longitudinally and the cells on the endocortical surface were collected in TRIzol reagent as the osteoblast-rich fraction. The residual bone pieces were crushed in liquid nitrogen to yield osteocyte-rich fraction, from which total RNA was isolated with the use of TRIzol. The sequences of the PCR primers (forward and reverse, respectively) were as follows: *Cre*, 5′-AGGTTCGTTCACTCATGGA-3′ and 5′-TCGACCA GTTTAGTTACCC-3′; *Gapdh*, 5′-ACTTTGTCAAGCTCATTTCC-3′ and

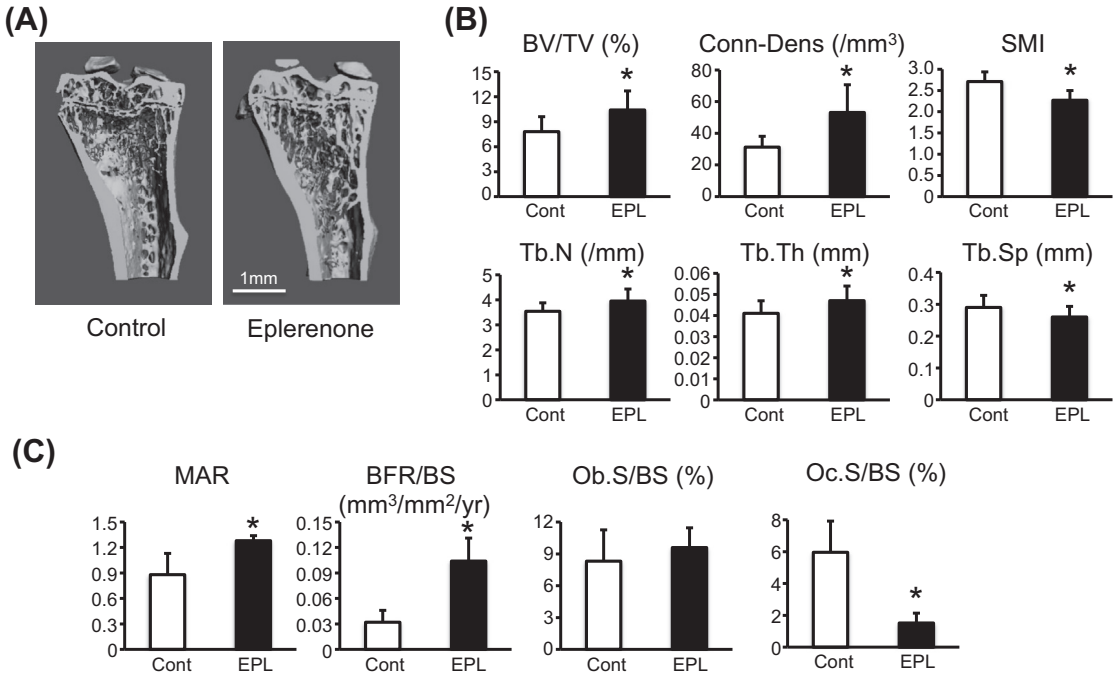

Fig. 1. Pharmacological inhibition of MR function with epleronone. (A) Representative micro-CT images of the tibia in control and eplerenone-treated mice. (B) Quantitation of the 3D bone volume fraction (BV/TV) and trabecular structure by micro-CT imaging as in (A). Conn-Dens, connectivity density; SMI, structure model index; Tb.N, Tb.Th, and Tb.Sp, trabecular number, thickness, and separation, respectively. Data are means ± SD for 9–10 mice of each group. $^*P < 0.05$. (C) Histomorphometric analysis of the tibial metaphysis of the control and eplerenone-treated mice. Mineral apposition rate (MAR), bone formation rate (BFR), osteoblast surface (Ob.S) and osteoclast surface (Oc.S) were corrected for the bone surface (BS). Data are means ± SD for 4 mice of each group. $^*P < 0.05$.