Bone Loss in Rats with Aldosteronism

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ABSTRACT: *Objective:* We hypothesized that aldosteronism is accompanied by hypercalciuria and hypermagnesuria that lead to bone loss, which could be rescued by hydrochlorothiazide and spironolactone. *Methods:* We monitored 24-hour urinary Ca^{2+} and Mg^{2+} excretion; plasma ionized $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ and plasma K^+; and bone mineral density of the femur. The following groups (n = 5 in each group) were studied: age- and gender-matched, untreated controls; controls + 4 weeks hydrochlorothiazide; 4 weeks aldosterone/salt treatment (ALDOST, 0.75 μg/h and dietary 1% NaCl/0.4% KCl); 4 weeks ALDOST + hydrochlorothiazide (50 mg/kg in prepared food); and 4 weeks ALDOST + hydrochlorothiazide + spironolactone (200 mg/kg day in divided doses by twice-daily gavage). *Results:* ALDOST increased ($P < 0.05$) urinary Ca^{2+} and Mg^{2+} excretion four- and twofold, respectively; hydrochlorothiazide cotreatment attenuated ($P < 0.05$) Ca^{2+} excretion in controls and during ALDOST without affecting augmented Mg^{2+} excretion whereas hydrochlorothiazide + spironolactone normalized Ca^{2+} and reduced Mg^{2+} excretion ($P < 0.05$). Compared with controls, plasma $[Ca^{2+}]_o$ at 4 weeks of ALDOST was reduced (0.89 ± 0.02 versus 0.83 ± 0.03 mmol/L; $P < 0.05$) but remained no different from levels in controls with hydrochlorothiazide and hydrochlorothiazide + spironolactone (0.88 ± 0.04 and 0.97 ± 0.03 mmol/L, respectively). Plasma $[Mg^{2+}]_o$ fell ($P < 0.05$) with ALDOST + hydrochlorothiazide (0.23 ± 0.01 versus 0.34 ± 0.01 mmol/L) and was prevented with spironolactone co-treatment (0.33 ± 0.01 mmol/dL). Hypokalemia (2.9 ± 0.2 mmol/L) occurred in rats with ALDOST + hydrochlorothiazide but not with spironolactone co-treatment. At 4 weeks of ALDOST, plasma parathyroid hormone was increased (30 ± 4 versus 11 ± 3 pg/mL; $P < 0.05$) and bone mineral density was reduced (0.153 ± 0.006 versus 0.170 ± 0.002 g/cm^2; $P < 0.05$). Co-treatments with either hydrochlorothiazide or hydrochlorothiazide + spironolactone each prevented bone loss. *Conclusions:* Hypercalciuria and hypermagnesuria accompany aldosteronism and account for a decline in their plasma ionized concentrations and secondary hyperparathyroidism with bone resorption. Attenuation of bone loss in aldosteronism can be achieved with hydrochlorothiazide; however, mono- and divalent cation homeostasis, together with bone integrity, are each preserved with the combination hydrochlorothiazide + spironolactone. **KEY INDEXING TERMS:** Aldosterone; Calcium; Magnesium; Parathyroid hormone; Bone loss. **[Am J Med Sci 2005;330(1):1–7.]**

Congestive heart failure (CHF), a clinical syndrome whose origins are rooted in neurohormonal activation that includes effector hormones of the circulating renin-angiotensin-aldosterone system, is accompanied by a systemic illness. Pathophysiologic features include oxi/nitrosative stress in blood and such diverse tissues as skin, skeletal muscle, immune cells, and heart[1-5] and a proinflammatory phenotype with elevated plasma levels of chemokines and cytokines and a catabolic state with loss of soft tissues and bone that eventuate in a wasting syndrome termed *cardiac cachexia*.[6-12] Pathogenic mechanisms involved in the appearance of this illness are under investigation.

An animal model of aldosteronism has been used to represent one aspect of the CHF neurohormonal profile. Uninephrectomized rats receive 1% NaCl in drinking water and aldosterone (ALDO) by minipump to inappropriately (relative to dietary Na^+ intake) raise its plasma levels to those seen in cases of human CHF. Referred to as *aldosterone/salt treatment (ALDOST)*, this regimen is accompanied by a proinflammatory phenotype induced by the marked and persistent excretion of Ca^{2+} and Mg^{2+} in both urine and feces and which adversely influences extra- and intracellular concentrations of these divalent cations.[13-18] Pathophysiologic responses accompany the negative balance of Ca^{2+} and Mg^{2+}. They include parathyroid

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hormone–mediated loss of bone mineral density (and bone strength) and intracellular Ca^{2+} loading with induction of oxi/nitrosative stress in diverse cells and an immunostimulatory state with activation of peripheral blood mononuclear cells that invade the coronary and systemic circulations to create a proinflammatory vascular phenotype.[13-22]

Herein we focused on the bone loss seen with aldosteronism,[13] which far exceeds that seen solely with the hypercalciuria accompanying a high-NaCl diet alone.[23] We hypothesized that pharmacologic intervention could attenuate the urinary loss of these divalent cations seen with ALDOST and thereby would prevent bone loss. To uncouple hypercalciuria and hypermagnesuria during ALDOST, we selected hydrochlorothiazide, to promote urinary Ca^{2+} resorption and compared this intervention to the combination of hydrochlorothiazide and spironolactone, an ALDO receptor antagonist, to reduce both Ca^{2+} and Mg^{2+} excretion. Age- and gender-matched, unoperated, untreated rats served as controls. Accordingly, we monitored 24-hour urinary Ca^{2+} and Mg^{2+} excretion, plasma ionized $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$, plasma K^+, and femur mineral density.

Methods

Animal Model

Male, 8- to 12-week-old Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used in this study, approved by the institution's Animal Care and Use Committee. There were five groups with five rats in each group, unless otherwise specified. Age- and gender-matched, unoperated, untreated rats served as controls. As previously reported, ALDOST consists of uninephrectomized rats who receive ALDO (0.75 μg/h) by implanted minipump (Alzet, Cupertino, CA) together with a 1% NaCl/0.4% KCl drinking water and standard laboratory chow (Harlan Tekland 2215 Rodent Diet) containing 1.13% Ca^{2+}.[24] Separate groups of animals received ALDOST plus hydrochlorothiazide or hydrochlorothiazide alone (treated controls). Hydrochlorothiazide was mixed into powdered standard chow (50 mg/kg body weight) and water was added. The mixture was placed in a pan and cut into small squares and air dried. We did not monitor daily food intake. Yet another group received ALDOST and hydrochlorothiazide plus spironolactone (200 mg/kg/day) in divided doses by twice-daily gavage. At 4 weeks of treatment, the animals were anesthetized and killed, and blood and femur samples were harvested.

Urinary Ca^{2+} and Mg^{2+} Excretion

On the day of the metabolic study, food was withheld but water with 1% NaCl was provided. Animals were "bathed" in distilled water to remove any feces or food that could contaminate collected urine. Animals were then placed in a cleaned, minerally decontaminated and distilled-deionized, water-rinsed metabolic cage. Urine was collected over 24 hours and kept frozen for Ca^{2+} and Mg^{2+} assay. After each use, cages were manually cleaned with deionized water; all nonmetallic parts were washed with diluted hydrochloric acid (3N), rinsed three times with deionized water, and finally rinsed twice with distilled-deionized water for future use as previously reported.[13,14]

Urinary Ca^{2+} and Mg^{2+} concentrations were determined as reported elsewhere[13,14] using an atomic absorption spectrophotometer. Urinary Ca^{2+} and Mg^{2+} excretion rates were calculated from the product of their concentration (μg/mL) by the 24-hour urine volume (mL/24 hours) and expressed as μg/24 hours.

Plasma Ionized $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ and Plasma K^+

The concentrations of plasma ionized $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ and K^+ were determined by the direct ion-selective electrode technique using a Nova 8 Analyzer (Nova Biomedical, Waltham, MA) and expressed in mmol/L.

Parathyroid Hormone

Plasma parathyroid hormone (PTH) was measured by the intact PTH immunoassay (IRMA) using a commercial kit (Nichols Institute Diagnostics, San Clemente, CA). IRMA is a two-site immunoradiometric assay for the measurement of the biologically intact 84 amino acid chain of PTH molecule. Blood (2 mL) was collected from the rat heart into a chilled EDTA tube and immediately centrifuged ($1600\times g$) for 15 minutes. Plasma was then separated and kept at -80° C. For IRMA, each plasma sample (200 μL) was added to the tube containing 100 μL of the ^{125}I-PTH antibody solution and PTH antibody-coated beads and incubated for 24 hours. Beads were then washed twice with washing solution and each test tube was counted with a gamma counter for 1 minute. A standard curve was generated using prepared intact PTH standards and plasma PTH values and expressed as pg/mL plasma.

Bone Mineral Density

Bone mineral density was determined for excised, manually cleaned femurs by peripheral dual-energy x-ray absorptiometry using GE Lunar PIXImus2 (GE Healthcare, Fairfield, CT). Quality control and calibration were carried out within 24 hours of each scanning period. This method has been validated for rat long bones.[25] We have previously reported on the equivalence of this noninvasive assessment of tibia and femur bone mineral density with their total concentrations of Mg^{2+} and Ca^{2+} determined by atomic absorption spectrophotometry.[13]