Cinacalcet and the Prevention of Secondary Hyperparathyroidism in Rats With Aldosteronism

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ABSTRACT: *Background:* In rats receiving aldosterone/salt treatment (ALDOST), increased Ca^{2+} excretion leads to a fall in plasma-ionized Ca^{2+} and appearance of secondary hyperparathyroidism (SHPT) with parathyroid hormone (PTH)-mediated intracellular Ca^{2+} overloading inducing oxidative stress in diverse tissues. Parathyroidectomy prevents this scenario. Rats with ALDOST were cotreated with cinacalcet (Cina), a calcimimetic that raises the threshold of the parathyroids' Ca^{2+}-sensing receptor. *Methods and Results:* We monitored plasma-ionized $[Ca^{2+}]_o$, PTH, and total Ca^{2+} in heart and peripheral blood mononuclear cells (PBMC), and evidence of oxidative stress in heart, PBMC, and plasma. Cina-treated rats for 4 weeks were compared with 4 weeks of ALDOST alone and with untreated age-/gender-matched controls. In comparison to controls, ALDOST led to a fall ($P < 0.05$) in Ca^{2+} (1.16 ± 0.01 vs 1.03 ± 0.01 mmol/L), which was not prevented by Cina (1.01 ± 0.03 mmol/L); a rise ($P < 0.05$) in plasma PTH (36 ± 7 vs 134 ± 19 pg/mL) that was attenuated by Cina (69 ± 12 pg/mL); increased ($P < 0.05$) cardiac $[Ca^{2+}]$ (3.92 ± 0.25 vs 6.78 ± 0.35 nEq/mg FFDT) and PBMC $[Ca^{2+}]_i$ (29.8 ± 2.3 vs 50.2 ± 2.3 nmol/L), each of which was prevented with Cina (3.65 ± 0.10 nEq/mg FFDT and 32.5 ± 6.0 nmol/L, respectively); increased cardiac MDA (0.56 ± 0.03 vs 0.94±0.07 nmol/mg protein) and PBMC H_2O_2 production (63.5 ± 7.5 vs 154.0 ± 25.2 mcb) and reduced ($P < 0.05$) plasma α_1-AP activity (39.8 ± 0.6 vs 29.6 ± 1.8 mM), each prevented by Cina (0.66 ± 0.04 mmol/mg protein, 58.2 ± 12.7 mcb and 37.0 ± 1.2 mM, respectively). *Conclusions:* PTH-mediated intracellular Ca^{2+} overloading accounts for the induction of oxidative stress in diverse tissues in rats with aldosteronism and which can be prevented by Cina. **KEY INDEXING TERMS:** Aldosteronism; Calcium overloading; Secondary hyperparathyroidism; Oxidative stress; Cinacalcet. **[Am J Med Sci 2008;335(2):105–110.]**

Elevations in plasma parathyroid hormone (PTH) in keeping with secondary hyperparathyroidism (SHPT) have been reported in patients hospitalized with congestive heart failure (CHF), where renin-angiotensin-aldosterone system (RAAS) activation contributes to salt and water retention and consequent signs and symptoms of this clinical syndrome.[1–5] The elevation in plasma PTH found in these patients is associated with reduced serum $[Ca^{2+}]_o$.[6–9] These patients included those with newly diagnosed, untreated CHF, where loop diuretic treatment and associated increase in urinary Ca^{2+} excretion could be excluded, and those previously treated in whom disorders leading to reduced $[Ca^{2+}]_o$ were excluded.[9] Furthermore, calculated creatinine clearance in the decompensated patients was not markedly reduced to account for SHPT.[9] On the other hand, SHPT was not found in outpatients with compensated cardiac failure.[9] Thus, human CHF with secondary aldosteronism is accompanied by SHPT. Moreover, serologic markers of oxidative stress are elevated in these patients, where a proinflammatory phenotype leads to a wasting of soft tissue and bone resorption.[10–12]

Oxidative stress, skeletal muscle wasting, and bone loss are found in rats with chronic aldosteronism and where SHPT is a covariant.[13–17] Plasma aldosterone (ALDO) levels in this model are inappropriately (relative to 1% NaCl in drinking water) elevated to those found in human CHF.[18] Urinary and fecal excretion of Ca^{2+} are increased in these rats and prevented at each site by an ALDO receptor

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antagonist, spironolactone.[16,17] For a fixed dietary intake of Ca^{2+} provided by standard laboratory chow, the heightened excretory losses of Ca^{2+} leads to a fall in plasma-ionized $[Ca^{2+}]_o$, which, in turn, stimulates the parathyroid glands to increase their secretion of PTH.[17] The resultant SHPT promotes bone resorption in an attempt to restore Ca^{2+} homeostasis.[16,17] SHPT can be prevented when the diet of these rats is fortified with Ca^{2+} and supplemental calcitriol (vitamin D).[19]

Despite a reduction in extracellular Ca^{2+} and ongoing PTH-mediated bone resorption, elevations in circulating PTH promote intracellular Ca^{2+} overloading in such diverse tissues as peripheral blood mononuclear cells (PBMC:lymphocytes and monocytes), heart, skeletal muscle, and thymocytes, together with the induction of oxidative stress.[17,20] Surgical removal of the parathyroid glands before initiating aldosteronism prevents SHPT, including this paradoxical Ca^{2+} overloading and oxidative stress.[21] This is also the case for a Ca^{2+}-fortified diet and calcitriol supplement.[19] Hence, an important component of the proinflammatory phenotype that accompanies aldosteronism relates to SHPT and PTH-mediated Ca^{2+} overloading with oxidative stress.

To further address the role of PTH-mediated intracellular Ca^{2+} overloading in the appearance of oxidative stress, a nonsurgical approach to prevent SHPT was undertaken in rats with aldosteronism. Cinacalcet raises the threshold of the extracellular Ca^{2+}-sensing receptor of the parathyroid glands' chief cells to circulating levels of $[Ca^{2+}]_o$.[22,23] This G-protein–coupled receptor is the molecular mechanism by which these cells detect and respond to even modest changes in blood $[Ca^{2+}]_o$. In resetting the Ca^{2+}-sensing receptor, this calcimimetic reduces PTH secretion and to an extent greater than that seen with conventional vitamin D therapy while improving bone strength and histology without the risk of hypercalcemia or tissue calcification.[23–25] Herein, we hypothesized cinacalcet cotreatment in these rats would prevent SHPT together with PTH-mediated rise in intracellular Ca^{2+} and the subsequent induction of oxidative stress.

Methods

Animal Model

Eight-week-old male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used in this study approved by the institution's Animal Care and Use Committee. Unoperated, untreated, age-matched rats served as controls. As previously reported,[13–17,21,26] ALDOST consists of uninephrectomized rats who receive ALDO (0.75 μg/h) by implanted minipump (Alzet; Cupertino, CA) together with 1% NaCl/ 0.4% KCl in drinking water, and standard laboratory chow (Harlan Teklad 2215 Rodent Diet; Madison, WI) containing 1.13% Ca^{2+} and 0.24% Mg^{2+} for 4 weeks. A separate group of rats received ALDOST plus cinacalcet (5 mg/kg body weight by daily gavage) for 4 weeks. Animals were anesthetized and killed, and blood and hearts were harvested for the isolation of PBMC and other studies (vide infra). Each experimental group and controls consisted of 6 rats.

Plasma-Ionized $[Ca^{2+}]_o$

Plasma $[Ca^{2+}]_o$ was determined by the direct ion-selective electrode technique using a Nova 8 Analyzer (Nova Biomedical; Waltham, MA) and expressed as mmol/L as previously reported by us.[17,21]

Plasma Parathyroid Hormone

Plasma PTH was measured by the intact PTH immunoassay (Immutopics; San Clemente, CA) and expressed as pg/mL.[17,21]

Peripheral Blood Mononuclear Cell Cytosolic-Free $[Ca^{2+}]_i$

PBMC were isolated and their cytosolic-free $[Ca^{2+}]_i$ measured using a ratiometric method and fluorescent molecular probe fura-2 (Molecular Probes; Eugene, OR), as previously reported by us.[14,15,17] PBMC $[Ca^{2+}]_i$ levels are expressed as nmol/L.

Cardiac Tissue Ca^{2+} Concentrations

Microdetermination for Ca^{2+} concentrations in ventricular tissue was carried out in 12- to 15-mg demoisturized, defatted specimens after complete digestion in 5 mL of 0.75 M Ultrex quality nitric acid (J. T. Baker Chemical Co) for 15 hours at 68°C. This procedure extracts >99% of Ca^{2+} from dry, defatted tissue. Tissue Ca^{2+} levels are expressed as nEq/mg of fat-free dry tissue (FFDT).

Malondialdehyde in Heart Tissue

To measure ventricular malondialdehyde (MDA), tissue was homogenized (1:10 wt:vol) in ice-cold 20 mM Tris buffer (pH 7.4) containing 5 mM butylated hydroxytoluene (BHT) and sonicated. After centrifugation at 3000*g* for 10 minutes, supernatant MDA concentration was determined colorimetrically according to a modification of the method of Esterbauer et al,[27] using a commercially available kit (Oxis Research; Portland, OR). Briefly, duplicate aliquots of supernatant were incubated at 45°C with 7.7 mM N-methyl-2-phenylindole in acetonitrile:methanol (75:25, vol:vol) and 15.4 mM methanesulfonic acid. After clarification by centrifugation at 15,000*g*, absorbance was measured at 586 nm using 1,1,3,3-tetramethoxypropane as a standard. Ventricular MDA levels are expressed as nmol/mg total protein.

Lymphocyte H_2O_2 Production

H_2O_2 production by PBMC was measured fluorometrically with 2′,7′-dichloroflourescein diacetate using a FACS Caliber flow cytometer (Becton, Dickinson & Co; Franklin Lakes, NJ) and reported as mean channel brightness (mcb) as previously reported.[14,15,17]

Plasma α_1-Antiproteinase Activity

As previously reported,[13] plasma α_1-AP was assessed by using the α_1AP-410 assay system (Oxis Research: Portland, OR) and expressed in μmol/L.

Statistical Analysis

Values are presented as mean ± SEM (number of rats). Data were analyzed using an analysis of variance. Significant differences between individual means were determined using the post hoc Bonferroni multiple comparisons test. Significance was assigned to a value of $P < 0.05$.

Results

Plasma-Ionized $[Ca^{2+}]_o$

In untreated, unoperated, age-matched control rats, plasma $[Ca^{2+}]_o$ was 1.16 ± 0.01 mmol/L. At 4 weeks of ALDOST, plasma $[Ca^{2+}]_o$ was reduced ($P < 0.05$) to 1.03 ± 0.01 mmol/L. Ionized hypocalcemia was also seen (1.01 ± 0.03 mmol/L) at week 4 of ALDOST, during which time cinacalcet was provided as cotreatment. We have previously reported