Striking Increase of Natriuresis by Low-Dose Spironolactone in Congestive Heart Failure Only in Combination With ACE Inhibition

Mechanistic Evidence to Support RALES

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Background—A marked reduction of overall mortality in patients with severe congestive heart failure (CHF) has been demonstrated by addition of the mineralocorticoid receptor antagonist spironolactone to ACE inhibition. The aim of the present study was to examine a hypothesized interaction of spironolactone and ACE inhibitors in renal electrolyte and volume regulation.

Methods and Results—Wistar rats with extensive myocardial infarction or sham operation were treated with either placebo, the ACE inhibitor trandolapril, low-dose spironolactone, or a combination of the 2. Twelve weeks after infarction, rats were housed in metabolic cages. Urinary volume and sodium excretion were significantly increased in CHF rats on a combined treatment with spironolactone and trandolapril (21.2 ± 2.6 mL/d, 2489 ± 320 mmol/d, mean ± SD; \( P < 0.05 \) versus other experimental groups) versus placebo-treated rats (16.7 ± 5.6 mL/d, 1431 ± 458 mmol/d), whereas these parameters were not affected in rats on either spironolactone (16.1 ± 6.6 mL/d, 1153 ± 273 mmol/d) or trandolapril alone (15.9 ± 4.2 mL/d, 1392 ± 294 mmol/d). The effects on natriuresis coincided with a significant reduction of left ventricular end-diastolic pressure (LVEDP) in rats on trandolapril and spironolactone (10.8 ± 8.2 mm Hg; \( P < 0.05 \) versus CHF placebo: 23.3 ± 7.2 mm Hg; sham-operated rats: 5.1 ± 0.9 mm Hg), whereas LVEDP remained elevated in rats treated with either compound alone.

Conclusions—In the present study, we found an unexpected interaction of low-dose spironolactone and the ACE inhibitor trandolapril in experimental CHF leading to marked effects on renal electrolyte and volume regulation that were not apparent by treatment with either drug alone. These findings may explain the beneficial effects of spironolactone in CHF patients. (Circulation. 2000;102:2325-2328.)

Key Words: heart failure ■ kidney ■ angiotensin ■ myocardial infarction

Introduction of ACE inhibitors as standard treatment for patients with moderate to severe CHF in the early 1990s led to a discontinuation of the formerly established spironolactone therapy in those patients. Combination therapy with ACE inhibitors and spironolactone was considered to be relatively contraindicated because of the risk of hyperkalemia and the assumption that ACE inhibitors alone inhibit angiotensin II–mediated aldosterone formation. However, plasma aldosterone levels increase within months in patients on ACE inhibitors, supporting the concept of an “aldosterone escape” phenomenon. Furthermore, increased plasma levels of aldosterone correlate with increased mortality in CHF patients.

The Randomized Aldactone Evaluation Study (RALES) study showed that the mineralocorticoid receptor antagonist spironolactone added to ACE inhibition in patients with severe congestive heart failure (CHF) reduces overall mortality significantly, by ≈30%. The results of the RALES study are convincing and confirm the important pathophysiological role of aldosterone in CHF; however, the mechanisms leading to improved survival by a low daily dose of spironolactone (25 mg/d) remain unclear.

Extrarenal effects of antialdosterone therapy on myocardial fibrosis, sympathoadrenergic stimulation, and neurohumoral dysregulation have been discussed, as well as actions on renal electrolyte and volume regulation. However, the low doses of spironolactone used in the RALES study presumably do not completely block mineralocorticoid receptors. In addition, effective diuretic and antifibrotic actions require higher doses of spironolactone when used as a monotherapy. Because the survival benefit in patients with...
CHF was observed at much lower doses of spironolactone added to ACE inhibition, we hypothesized that there may be an interaction between ACE inhibitors and spironolactone that potentiates the effects of either drug alone. Thus, we investigated the effect of low-dose spironolactone and ACE inhibition either alone or in combination on hemodynamic parameters as well as on renal electrolyte and volume regulation in experimental CHF.

**Methods**

**Myocardial Infarction and Study Protocol**

Left coronary artery ligation were performed in adult male Wistar rats (250 to 300 g, Charles River, Sulzfeld, Germany) as previously described. Briefly, the thorax was opened under ether anesthesia, the heart was exteriorized, and a ligature was placed around the proximal part of the left coronary artery. The heart was returned to its normal position and the thorax closed. Sham-operated rats were treated similarly except that the operative procedure did not produce a detectable myocardial infarction (MI). On day 7 after surgery, surviving rats were randomized to placebo, the ACE inhibitor trandolapril (0.3 mg kg body wt\(^{-1}\cdot d\(^{-1}\)), spironolactone alone (SP, 10 mg kg body wt\(^{-1}\cdot d\(^{-1}\)), or a combination of trandolapril and spironolactone. Sham indicates sham operation; LVSP, left ventricular systolic pressure; and LVEDP, left ventricular end-diastolic pressure. Values are mean\(\pm\)SD. Animals were treated with either placebo, trandolapril alone (TR, 0.3 mg kg body wt\(^{-1}\cdot d\(^{-1}\), spironolactone alone (SP, 10 mg kg body wt\(^{-1}\cdot d\(^{-1}\)), or a combination of trandolapril and spironolactone. Sham indicates sham operation; LVSP, left ventricular systolic pressure; and LVEDP, left ventricular end-diastolic pressure. Values are mean\(\pm\)SD. Animals were treated with either placebo, trandolapril alone (TR, 0.3 mg kg body wt\(^{-1}\cdot d\(^{-1}\)), spironolactone alone (SP, 10 mg kg body wt\(^{-1}\cdot d\(^{-1}\)), or a combination of trandolapril and spironolactone. Sham indicates sham operation; LVSP, left ventricular systolic pressure; and LVEDP, left ventricular end-diastolic pressure. Values are mean\(\pm\)SD.

**Determination of Blood and Urinary Parameters**

Twelve weeks after coronary artery ligation and before the hemodynamic measurements were conducted, rats were housed in metabolic cages for 24 hours. At the end of this period, animals were weighed and urine volumes determined gravimetrically. Creatinine concentrations in blood and urinary samples were determined by routine procedures (Hitachi-Autoanalyser, Boehringer Mannheim). Endogenous creatinine clearance and fractional sodium excretion was calculated by use of standard formulas and expressed relative to body weight. Sodium and potassium concentrations in serum and urine were measured photometrically (FLM-3; Radiometer).

**Hemodynamic Measurements, Determination of Infarct Size**

Hemodynamic studies were performed under barbiturate anesthesia and controlled respiration. Saline-filled catheters were advanced from the right carotid artery into the left ventricle and connected to a Statham transducer. After measurement of the left ventricular pressure, the catheter was withdrawn for the measurement of aortic pressure. Histological slices (5 \(\mu\)m) of the middle part of the left ventricle were stained with Sirius red. The boundary lengths of the infarcted and noninfarcted endocardial and epicardial surfaces were traced with a planimeter digital image analyzer. Infarct size (fraction of the infarcted left ventricle) was expressed as a percentage of length, and only rats with extensive infarcts (\(\geq 40\%\)) were included in the study.

**Statistics**

Values are mean\(\pm\)SD. Statistical comparisons were done by ANOVA followed by Fisher’s post hoc test with StatView 5.0 for the Apple MacIntosh.

**Results**

Body weights of rats as well as areas of MI were comparable among the experimental groups (Table). As a sign of pulmonary congestion, right ventricular weight was significantly increased in rats with chronic MI compared with sham-operated animals (0.48\(\pm\)0.02 versus 0.25\(\pm\)0.02 g; \(P<0.05\)). Plasma levels of sodium and potassium and creatinine clearance (Table) did not differ significantly among the experimental groups. However, there was a trend toward an increased urinary Na\(^+\)/K\(^+\) ratio and a significant increase of urinary sodium and volume excretion in CHF rats treated with a combination of spironolactone and trandolapril, whereas these parameters were not affected in rats on either drug alone (Figure). The fractional sodium excretion did not differ between CHF and sham-operated rats (0.26\(\pm\)0.02\% versus 0.30\(\pm\)0.04\%). Fractional sodium excretion was significantly increased in CHF rats on a combination of trandolapril and spironolactone (0.46\(\pm\)0.04\%; \(P<0.05\) versus all other groups), whereas treatment with trandolapril or spironolactone alone did not change fractional sodium excretion (trandolapril, 0.32\(\pm\)0.03\%; spironolactone, 0.28\(\pm\)0.06\%). Left ventricular systolic pressure was reduced in CHF rats treated with placebo compared with sham-operated animals and was further decreased in all active treatment groups, reflecting afterload reduction. However, only in the CHF rats treated with the combination of spironolactone and trandolapril was left ventricular end-diastolic pressure, an index of left ventricular preload, significantly decreased (Table).

**Discussion**

Our data show that the addition of low-dose spironolactone to ACE inhibition in CHF significantly increases urinary so-
Animals with CHF were treated with either placebo, trandolapril alone (TR, 0.3 mg · kg body wt\(^{-1} \cdot d^{-1}\)), spironolactone alone (SP, 10 mg · kg body wt\(^{-1} \cdot d^{-1}\)), or a combination of trandolapril and spironolactone (TR-SP). Values are mean±SD of urinary Na\(^+\)/K\(^+\) ratio (top), urinary sodium excretion (middle), and volume excretion (bottom) of rats housed in metabolic cages for 24 hours. n=6 to 10 per group; \(P<0.01\) and \(P<0.05\) vs other experimental groups.

Urinary parameters of rats with heart failure (CHF) 12 weeks after myocardial infarction vs sham-operated animals (sham). Animals with CHF were treated with either placebo, trandolapril alone (TR, 0.3 mg · kg body wt\(^{-1} \cdot d^{-1}\)), spironolactone alone (SP, 10 mg · kg body wt\(^{-1} \cdot d^{-1}\)), or a combination of trandolapril and spironolactone (TR-SP). Values are mean±SD of urinary Na\(^+\)/K\(^+\) ratio (top), urinary sodium excretion (middle), and volume excretion (bottom) of rats housed in metabolic cages for 24 hours. n=6 to 10 per group; \(P<0.01\) and \(P<0.05\) vs other experimental groups.

Urine potassium excretion exceeds that of sodium, leading to a urine Na\(^+\)/K\(^+\) ratio <1 in all groups of rats in our study, which is in contrast to findings in humans\(^{10}\) and Sprague-Dawley rats.\(^{15}\) Low urine Na\(^+\)/K\(^+\) ratios in Wistar rats have also been reported recently,\(^{16}\) pointing to a different intestinal absorption of electrolytes in different rat strains. Thus, our finding of a therapy-induced change of urinary electrolyte excretion at a Na\(^+\)/K\(^+\) ratio <1 reflects the situation of a standard diet in normal Wistar rats. The increase of fractional sodium excretion points to saluretic actions of a combined treatment with spironolactone and trandolapril in CHF rats. Values do not indicate that extensive MI or either drug treatment induced some structural damage to the nephron.

In our study, the absolute oral dose of spironolactone per body weight (10 mg · kg\(^{-1} \cdot d^{-1}\)) is higher than that used in humans (0.35 mg · kg\(^{-1} \cdot d^{-1}\) in patients with CHF).\(^{5}\) The dosage of drugs given in rat models of experimental CHF are usually much higher than the dosages used in CHF patients because of a different drug metabolism and efficacy of the compounds. The therapeutic relevance of our investigation is supported by other studies in this rat model, in which beneficial effects of ACE inhibitors have been reported with captopril at a dose of \(\approx 50\) mg · kg\(^{-1} \cdot d^{-1}\),\(^{17}\) whereas much lower doses of captopril were used in humans (\(\approx 2.1\) mg · kg\(^{-1} \cdot d^{-1}\)).\(^{1}\) The spironolactone dosage used in our study is even lower than the low-dose group described recently in a rat model of experimental MI (20 mg · kg\(^{-1} \cdot d^{-1}\)),\(^{18}\) and subcutaneous dosages of up to 200 mg · kg\(^{-1} \cdot d^{-1}\) were applied in studies on myocardial fibrosis and blood pressure.\(^{7}\) Finally, neither treatment alone influenced renal electrolyte and volume regulation, whereas spironolactone added to ACE inhibition showed clear effects.

In parallel to the clinical situation in patients with CHF, the renin-angiotensin-aldosterone system is markedly activated in the rat model of experimental CHF.\(^{13}\) One may assume that elevated levels of angiotensin II increase sodium reabsorption in the proximal tubule, leading to a marked reduction of luminal sodium supply in the distal tubules of the kidney.\(^{19}\) Thus, mineralocorticoid receptor blockade in the distal renal tubule, the primary site of aldosterone action, may have only minor effects on urinary sodium excretion. In contrast, attenuation of sodium reabsorption in the proximal tubule by an ACE inhibitor will be outbalanced by an increased sodium reabsorption in more distal sites of the nephron. However, therapeutic modulation of sodium reabsorption in both the proximal and distal parts of the renal tubule by a combination of an ACE inhibitor and a mineralocorticoid receptor antagonist may lead to additive effects on renal sodium and volume excretion. Our interpretation is supported by observations in patients with liver cirrhosis showing a pronounced increase of a previously blunted diuresis by a combination of spironolactone and captopril.\(^{20}\)

Comparable interactions on electrolyte and volume excretion should be expected by a combination of ACE inhibitors and loop diuretics, a regimen currently used in clinical practice. However, diuretic and natriuretic actions of loop diuretics are obviously not modulated by ACE inhibition in humans\(^{21,22}\) and in rats,\(^{23}\) and investigations in the rat model of experimental CHF are lacking. Although we did not investigate the effect of spironolactone in addition to ACE inhibitors and loop diuretics, our data conform to the hypothesis that the reduction of intravascular volume (as suggested by the reduction in plasma atrial natriuretic peptide levels and increase in plasma renin activity) by low-dose spironolactone observed in the RALES dose-ranging study\(^{10}\) may depend on the interaction of spironolactone and ACE inhibitors in addition to the diuretic effects of loop diuretics. Nevertheless,
previous dosing requirements with larger doses of spironolactone to promote a saluresis may reflect (1) absent loop diuretic, (2) absent ACE inhibitor, and (3) absence of the combinations of medications. Thus, we cannot exclude the possibility that electrolyte and volume excretion may be further increased in CHF patients when spironolactone is added to loop diuretics and ACE inhibitors. It is currently not possible to draw firm conclusions about the relative contribution of either effect in the clinical situation; however, the enhancing effects of an ACE inhibitor and spironolactone on renal electrolyte and volume excretion may even reduce the need for loop diuretics and thus attenuate the cardiovascular jeopardy related to hypokalemia and hypomagnesemia induced by these agents.

Furthermore, increased natriuresis in rats may be explained by an interaction of ACE inhibitors and spironolactone at the cellular or molecular level. It is tempting to speculate that the binding affinity of spironolactone to the mineralocorticoid receptor or its effect on renal tubular epithelial sodium reabsorption or both are modulated by ACE inhibitors. Such a hypothesis is supported by reports of ACE inhibitor–modulated bradykinin signaling in endothelial cells by interference with receptor sequestration and the modulation of the binding characteristics of the vasopressin receptor in the collecting tubule by ACE inhibitors. Thus, ACE inhibitors may sensitize the renal tubule to be more susceptible to the natriuretic actions of the mineralocorticoid receptor antagonist spironolactone.

In summary, low-dose spironolactone added to an ACE inhibitor in experimental CHF induced a striking increase of urinary sodium (and volume) excretion, leading to improved left ventricular hemodynamics, whereas neither compound alone modulated renal electrolyte regulation. Our results may at least in part explain the beneficial effects of spironolactone added to ACE inhibition in patients with severe CHF.

References


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