Angiotensin II AT1 receptor antagonism prevents detrimental renal actions of acute diuretic therapy in human heart failure

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Chen, Horng H., Margaret M. Redfield, Linda J. Nordstrom, Alessandro Cataliotti, and John C. Burnett, Jr. Angiotensin II AT1 receptor antagonism prevents detrimental renal actions of acute diuretic therapy in human heart failure. Am J Physiol Renal Physiol 284: F1115–F1119, 2003; 10.1152/ajprenal.00337.2002.—Although effective in relieving symptoms of edema in congestive heart failure (CHF), diuretic-induced natriuresis may be associated with reductions in glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), which subsequently may reduce the duration of natriuresis. Moreover, recent studies have reported that the preservation of GFR is an important predictor of survival in human CHF. We hypothesized that the acute detrimental renal hemodynamic and tubular responses to furosemide in symptomatic human CHF will be attenuated by AT1 receptor blockade with losartan. We defined the renal hemodynamic and tubular actions and aldosterone responses to furosemide in 10 subjects with CHF (New York Heart Association II-III) in a double-blind, placebo-controlled crossover study. Furosemide with placebo increased sodium excretion and reduced ERPF and GFR (P < 0.05 vs. baseline). After 4 h, sodium excretion compared with baseline was decreased (P < 0.05). In contrast, furosemide with losartan resulted in a greater increase in sodium excretion but without reductions in ERPF and GFR (P < 0.05 vs. placebo). After 4 h, sodium excretion was greater compared with the placebo group. Importantly, plasma aldosterone tended to increase in the losartan group. These studies underscore the pathophysiological role of the AT1 receptor in mediating detrimental renal and adrenal properties of diuretics in human CHF. AT1 receptor antagonism preserves GFR and renal blood flow and enhances sodium excretion during acute diuretic therapy in addition to inhibiting aldosterone secretion. These findings support the use of AT1 receptor blockade for human CHF requiring acute diuretics to improve renal hemodynamic and tubular function and to suppress aldosterone.

kidney; congestive heart failure; aldosterone; glomerular filtration rate

such as edema and dyspnea. Despite the widespread and long history of use of diuretics in CHF, acute and chronic detrimental actions of diuretics continue to emerge (4, 12). Overdiuresis may lead to excessive preload reduction and systemic hypotension with worsening heart failure. A well-established response to diuretic use is the activation of the renin-angiotensin-aldosterone system (RAAS), which may compromise glomerular filtration rate (GFR) and limit the natriuretic response of the kidney (4).

Recent studies have reported that the preservation of GFR is an important predictor of better long-term survival in human CHF (3, 5). Although increased circulating and tissue ANG II in CHF has been demonstrated, recent studies from our laboratory by Luchner et al. (7) established that throughout the evolution of CHF, renal ANG II concentrations exceed concentrations in other tissues. This observation further supports the importance of ANG II as a major factor in mediating renal vasoconstriction and enhanced sodium reabsorption in CHF. Importantly, angiotensin-converting enzyme inhibitor (ACEI) in CHF in the presence of diuretic therapy results in renal vasodilatation and natriuresis with variable responses in GFR (10). Although a reduction in ANG II generation has been implicated as the mechanism of the renal response to ACEI, the simultaneous accumulation of the renal vasodilating and natriuretic peptide bradykinin makes interpretation of these recent reports difficult and controversial.

Characterization of the importance of ANG II in the control of renal hemodynamic and tubular function in human CHF is now possible with the availability of specific ANG II AT1 receptor antagonists. These pharmacological agents have a high affinity for the AT1 receptor and have no agonistic activity. Among these antagonists, losartan effectively inhibits ANG II bound to specific renal receptors (11). Administration of losartan to renal hypertensive rats results in a dose-dependent decrease in blood pressure. Losartan may also increase renal blood flow and sodium excretion in normotensive and hypertensive dogs (14). In healthy volunteers, losartan abolishes the blood pressure re-
response to exogenous ANG II. Furthermore, sodium status is an important determinant of the therapeutic efficacy of ACEIs, an AT1 receptor antagonist, and renin inhibitors. The correction of volume expansion by dietary sodium restriction, diuretic therapy considered. Renin, ng/ml 38 9

**METHODS**

Study population. Subjects recruited were limited to men and women, aged 18 yr and above, who had a resting left ventricular ejection fraction of 35% or less with stable symptomatic CHF [New York Heart Association (NYHA) Class II and III] as defined by the criteria outlined by the NYHA classification. Before initiation of the study, patients were stabilized for 1 wk on a 120-meq Na/day diet. A 24-h urine collection was obtained 3 days before the active study day to ensure compliance with the diet. Subjects remained on stable dosing was applied for 2 wk preceding the study. Patients on long-acting ACEIs were switched to equivalent doses of the short-acting ACEI captopril 2 wk before the study, which was withheld 72 h before each study day. Diuretic therapy would result in an improvement in GFR and effective renal plasma flow (ERPF) with suppression of aldosterone (Aldo) and a greater natriuresis. Aldosterone, ng/dl 11 ± 2

<table>
<thead>
<tr>
<th>MAP, mmHg</th>
<th>Losartan Plus</th>
<th>Furosemide Plus</th>
<th>Placebo</th>
</tr>
</thead>
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<tr>
<td>GFR, ml/min</td>
<td>69 ± 7</td>
<td>73 ± 8</td>
<td>66 ± 9</td>
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<tr>
<td>ERPF, ml/min</td>
<td>293 ± 26</td>
<td>303 ± 26</td>
<td>300 ± 25</td>
</tr>
<tr>
<td>Urinary flow, ml/min</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Renin, ng·ml⁻¹·h⁻¹</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.6</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Ang II, pg/ml</td>
<td>38 ± 4</td>
<td>29 ± 5</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>Aldosterone, ng/dl</td>
<td>11 ± 2</td>
<td>10 ± 2</td>
<td>12 ± 3</td>
</tr>
</tbody>
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Values are means ± SE. MAP, mean arterial blood pressure; UNaV, urinary sodium excretion; GFR, glomerular filtration rate; ERPF, effective renal plasma flow.

To date, the role of AT1 receptors in the regulation of renal function in human CHF receiving acute diuretic therapy remains undefined. Therefore, the objective of the present study was to define the role of AT1 receptors in the regulation of renal hemodynamics, tubular function, and in the control of sodium-regulating hormones in humans with CHF receiving acute diuretic therapy. We hypothesized that AT1 receptor antagonism in human CHF subjects receiving acute diuretic therapy would result in an improvement in GFR and effective renal plasma flow (ERPF) with suppression of aldosterone (Aldo) and a greater natriuresis.

Study design and protocol. A total of 10 subjects was studied in a randomized double-blind, placebo-controlled, and crossover design. All subjects received an oral dose of furosemide (40 mg) and were randomized to receive a single dose of losartan (Merck; 50 mg) or placebo. Subjects returned 2 wk later for the crossover study.

After ∼2 wk of the controlled diet, subjects were admitted to the General Clinical Research Center (GCRC) at St. Mary's Hospital, Mayo Clinic and Foundation, Rochester, MN, the evening before the scheduled active study day. On the active study day, they were given their usual medications except captopril, which was held for 72 h before the study day at 6 AM, and they were placed in the supine position for 1 h of equilibration. Standard intravenous heparin lock was placed for infusion and blood sampling. After a priming dose, an intravenous infusion of inulin and para-aminohippurate (PAH) was started to provide a plasma concentration of ∼400 and 20 µg/ml, respectively. Subjects were asked to empty their bladder spontaneously every 30 min throughout the renal clearance protocol. At the end of each period, the patients were asked to drink an amount of water equivalent to the sum of blood losses and urinary volume.

After 1 h of equilibration, a 60-min baseline period was observed. At the end of the baseline period, the subjects were given an oral dose of 40 mg of furosemide and randomized to receive a single dose of losartan (50 mg) or placebo. Thereafter, 60-min clearance periods were repeated for 4 h and a final clearance was done at 8 h post-losartan/placebo administration. During each clearance period, urinary, hormonal, and hemodynamic measurements were obtained and averaged for analysis. Blood pressure was measured at 10-min intervals by using automatic blood pressure and heart rate continuously monitored by electrocardiographic lead II. Urinary samples for determination of volume, sodium, PAH, and insulin were obtained at the end of each clearance period. Venous blood samples for PAH, inulin, sodium, renin, and Aldo were obtained at the middle of each clearance period.

**Fig. 1. Change from baseline (BL, abaseline) in sodium excretion and urinary flow at 60, 120, 140, 180, and 480 min after the administration of furosemide and losartan (broken line) or furosemide and placebo (solid line). *P < 0.05 vs. BL.**

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The patients underwent a 2-wk washout period, after which they returned to the GCRC for the crossover study.

**Analytic methods.** GFR was calculated from the clearance of inulin; ERPF was determined from the clearance of PAH. The method of determination of ERPF with PAH is based on previous studies performed in subjects in the supine position. Plasma and urine were analyzed for inulin by the anthrone method, for PAH by the method of Harvey and Brothers, and for sodium with the Beckman ion-selective analyzer. Plasma renin activity and Aldo were measured by appropriate radioimmunoassays as previously described (2).

**Statistical analysis.** Values will be expressed as means ± SE. Comparison within a group was assessed by repeated-measures ANOVA and post hoc Dunnett's test before. Comparison between losartan and placebo was assessed by two-way ANOVA test for simultaneous multiple comparisons. A statistically significant difference will be considered to be present when P < 0.05.

**RESULTS**

**Clinical characteristics.** A total of 10 subjects with stable NYHA class II-III CHF was studied, eight were men and two were women with a mean age of 64 ± 5 yr. Three subjects had idiopathic dilated cardiomyopathy, and the remaining seven had ischemic cardiomyopathy. The mean ejection fraction was 24 ± 6%, and mean plasma creatinine was 1.2 ± 0.1 mg/dl. All subjects were on stable doses of diuretics, digoxin and ACEI for 2 wk before enrollment, and seven were receiving β-blocker therapy. The mean dose of furosemide that the patients were taking before the study was 40 ± 8 mg daily. No patient had serum sodium of less than 135 meq/l. All the subjects tolerated the study well without any adverse events. Baseline renal and neurohumoral function are reported in Table 1.

**Renal function.** The responses in urinary sodium excretion (UNaV) and urinary flow from baseline after the administration of furosemide in combination with losartan or placebo are illustrated in Fig. 1. The increase of UNaV with furosemide plus losartan was greater compared with furosemide plus placebo (P < 0.05, 2-way ANOVA). More importantly, at 480 min after administration of furosemide plus placebo, UNaV was less compared with baseline, whereas with furosemide plus losartan this delayed sodium retention was abolished. The increase in urinary flow was greater with furosemide plus losartan compared with furosemide plus placebo (P < 0.05, 2-way ANOVA).

The responses in GFR, ERPF, and mean arterial blood pressure (MAP) from baseline after administration of furosemide in combination with losartan or placebo are illustrated in Fig. 2. Furosemide plus losartan resulted in a greater decrease in MAP compared with furosemide plus placebo (P < 0.05, 2-way ANOVA). Both GFR and ERPF decreased with furosemide plus placebo, whereas GFR and ERPF were preserved with furosemide plus losartan (P < 0.05, 2-way ANOVA).

**Neurohormonal function.** The response in plasma Aldo from baseline after administration of furosemide in combination with losartan or placebo is illustrated in Fig. 3. There is a trend for plasma Aldo to increase within 120 min after furosemide plus placebo but this
did not reach statistical significance. However, with furosemide plus losartan, there is a significant suppression of plasma Aldo, and plasma Aldo remained significantly lower compared with furosemide plus placebo ($P < 0.05$, 2-way ANOVA). Plasma renin activity (1.2 ± 0.4 to 1.3 ± 0.4 ng·ml$^{-1}$·h$^{-1}$) remained unchanged, whereas there was a trend for ANG II (30 ± 5 to 33 ± 5 pg/ml) to increase within 60 min after furosemide and placebo. However, this did not reach statistical significance. On the other hand, with furosemide and losartan, both plasma renin (1.3 ± 0.4 and 3.0 ± 0.4 ng·ml$^{-1}$·h$^{-1}$, $P < 0.05$) and ANG II (38 ± 5 and 59 ± 9 pg/ml, $P < 0.05$) increased after 4 h.

**DISCUSSION**

This study underscores the pathophysiological role of the AT$_1$ receptor in mediating the detrimental renal and adrenal properties of acute diuretic therapy in human CHF. Specifically, AT$_1$ receptor antagonism preserved GFR and ERPF and enhanced sodium excretion during acute diuretic therapy in addition to inhibiting Aldo secretion. Therefore, this study supports the strategy of ANG II blockade with acute diuretic therapy in human CHF to preserve renal hemodynamic and tubular function and suppress Aldo.

The kidney and adrenals play a fundamental role in the pathophysiology of CHF, serving as a mechanism for the increase in cardiac preload secondary to sodium retention and by releasing renin and activating the RAAS with direct actions of ANG II and Aldo on the cardiac myocyte and fibroblast (13). Supporting a seminal role for ANG II in the kidney is the abundance of ANG II AT$_1$ receptors in the renal vasculature, glomeruli, proximal and distal tubules, and in renomedullary interstitial cells (6). The predominant receptor subtype is the AT$_1$ receptor, which when activated mediates mesangial contraction, renal vasoconstriction, and increased tubular sodium reabsorption (8). AT$_1$ receptors in the adrenal glands when activated result in the release of Aldo, leading to sodium retention. Experimental studies have shown that exogenous ANG II administration results in marked decreases in GFR, whereas antagonism of the AT$_1$ receptors with losartan increases GFR (1).

The mechanisms by which AT$_1$ receptor antagonism enhances GFR are several and include increasing renal blood flow and transglomerular hydrostatic pressure with attenuation of the tubular glomerular feedback (13). In the present placebo-controlled, double-blind crossover investigation, the detrimental actions of furosemide in reducing GFR and ERPF were abolished with the AT$_1$ receptor antagonist losartan. Furthermore, the efficacy of natriuresis and diuresis was also augmented by AT$_1$ blockade, which may be explained by enhancing sodium delivery in the presence of preserved GFR and by tubular actions. Underscoring a tubular effect is the presence of reduction of Aldo observed in the presence of losartan. Previous studies showed that a high dose of acute diuretic administration activates ANG II and Aldo. However, in the present study, there was a trend for ANG II and Aldo to increase within 60 min of the administration of furosemide and placebo. A possible explanation for this may be due to the fact that the dose of furosemide given in this study was similar to the chronic dose taken by the patients. Furthermore, the patients’ baseline ANG II and Aldo levels were already elevated to begin with, which may be due to the fact that withdrawal from chronic ACE inhibition may lead to heightened neurohormonal activation and response to AT$_1$ receptor blockade.

These findings may have important clinical implications and would suggest that acute diuretic therapy may benefit by antagonism of ANG II so as to potentiate the natriuretic actions of the diuretics and preserve renal hemodynamics in addition to suppression of Aldo. One of the limitations of the present study is that it was done in a state of ACE inhibition withdrawal, which may affect its clinical implications. Therefore, further studies evaluating the long-term effects of AT$_1$ blockade on renal function in the presence of diuretics are warranted.

In summary, this study underscores the pathophysiological role of the AT$_1$ receptor in mediating the detrimental renal and adrenal properties of acute diuretic therapy in human CHF. Specifically, AT$_1$ receptor antagonism preserved GFR and ERPF and enhanced sodium excretion during acute diuretic therapy in addition to inhibiting Aldo secretion. Therefore, this study supports the strategy of ANG II blockade with acute diuretic therapy in human CHF to preserve renal hemodynamic and tubular function and suppress Aldo.

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