Angiotensin II Type 1 Receptor Antagonist Decreases Plasma Levels of Tumor Necrosis Factor Alpha, Interleukin-6 and Soluble Adhesion Molecules in Patients With Chronic Heart Failure

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OBJECTIVES To evaluate the effects of an angiotensin (Ang II) type 1 receptor antagonist on immune markers in patients with congestive heart failure (CHF).

BACKGROUND Ang II stimulates production of immune factors via the Ang II type 1 receptor in vitro, and the long-term effects of Ang II type 1 receptor antagonists on plasma markers of immune activation are unknown in patients with CHF.

METHODS Twenty-three patients with mild to moderate CHF with left ventricular dysfunction were randomly divided into two groups: treatment with Ang II type 1 receptor (candesartan cilexetil) (n = 14) or placebo (n = 9). We measured plasma levels of immune factors such as tumor necrosis factor alpha (TNFalpha), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1). We also measured plasma levels of the neurohumoral factors such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) and cyclic guanosine monophosphate (cGMP), a biological marker of ANP and BNP.

RESULTS Plasma levels of TNFalpha, IL-6, sICAM-1 and sVCAM-1 were increased in the 23 CHF patients compared with normal subjects and significantly decreased after 14 weeks of candesartan cilexetil treatment, but did not change in the placebo group. Plasma levels of BNP, which is a marker of ventricular injury, significantly decreased, and the molar ratio of plasma cGMP to cardiac natriuretic peptides (ANP + BNP) was significantly increased after candesartan cilexetil treatment, but did not change in the placebo group.

CONCLUSIONS These findings suggest that 14 weeks of treatment with an Ang II type 1 receptor antagonist (candesartan cilexetil) decreased plasma levels of the immune markers such as TNFalpha, IL-6, sICAM-1 and sVCAM-1 and that it improved the biological compensatory action of endogenous cardiac natriuretic peptides in patients with mild to moderate CHF. (J Am Coll Cardiol 2000;35:714–21) © 2000 by the American College of Cardiology
ular remodeling (15–21), are increased in plasma in CHF patients (12,22,23). Therefore, therapy for modulation of such cytokines has been tested in CHF patients.

Recently, Ang II, an important component of the renin-angiotensin system, has been shown to stimulate the production of cytokines such as TNFalpha and IL-6 via the Ang II type I receptor, which is present on monocytes, macrophages and vascular smooth muscle cells (24–26). Increased levels of TNFalpha and IL-6 in the plasma are partly derived from production by activation of the Ang II type I receptor. Ang II also directly, or indirectly, stimulates the expression of intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) (27). Therefore, Ang II type I receptor antagonists may improve the immune activation in patients with CHF.

Plasma levels of cardiac natriuretic peptides such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are increased and are useful prognostic predictors in patients with CHF, especially BNP (28–30), which is a ventricular hormone (31). The usefulness of plasma BNP is partly due to the fact that high levels of BNP are significantly correlated with hemodynamic abnormalities such as left ventricular ejection fraction (LVEF) and left ventricular end-diastolic pressure. Moreover, the downregulation of cardiac natriuretic peptide receptors coupled with guanylate cyclase may contribute to the progression of CHF (32–34). Recently, we reported that long-term treatment with an Ang II type 1 receptor (candesartan cilexetil) improved the generation of plasma cyclic guanosine monophosphate (cGMP), a biological marker of ANP and BNP (35), in canine CHF induced by rapid ventricular pacing (36).

In this study, we evaluated the effects of 14 weeks of treatment with candesartan cilexetil on the plasma levels of immune markers such as TNFalpha, IL-6, soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular adhesion molecule-1 (sVCAM-1) and also determined the effects of this treatment on the relationship between the plasma levels of cardiac natriuretic peptides and plasma cGMP, used as their biological markers.

**METHODS**

**Patients.** We studied 23 patients with stable symptomatic CHF (New York Heart Association [NYHA] functional classification of II and III) and LVEF < 45%. There were 19 men and 4 women, and the mean age was 58 years. The cause of heart failure was dilated cardiomyopathy in 15 patients, and 8 patients had suffered a myocardial infarction more than six months before the study. Patients with angina pectoris, renal failure or liver dysfunction were excluded. Informed consent was obtained from all patients before participation in the study, and the protocol was approved by the Human Investigations Committee of our institution. Fifteen patients were classified according to the standards of the NYHA as functional class II, and eight patients as class III. At entry to the study, 20 patients were treated with diuretics, 11 with ACE inhibitors, 18 with digitalis and 4 with beta-blockers. Angiotensin-converting enzyme inhibitors were discontinued at least two weeks before the study.

**Study protocol.** All patients were in stable condition for at least six months, and ACE inhibitors were not given for at least two weeks before the study. Eleven patients had received ACE inhibitors before the study, and these were withheld for at least two weeks before the study. The 23 patients with mild to moderate symptomatic left ventricular dysfunction were randomly divided into two groups: treatment with candesartan cilexetil (n = 14) or placebo (n = 9). Candesartan cilexetil was administered orally after a placebo run-in period of more than two weeks. The initial dosage of candesartan cilexetil was 2 mg once daily, which could be increased up to a maximum of 8 mg once daily.

Blood samples used to measure the plasma levels of neurohumoral factors and immune factors were collected from the antecubital vein after the supine position for at least 30 min before and after 14 weeks of treatment with candesartan cilexetil or placebo. Blood samples were collected 3 h after the oral administration on the morning of medications such as digitalis and diuretics, before the treatment with candesartan cilexetil or placebo. After 14 weeks of treatment with candesartan cilexetil or placebo, blood samples were also collected 3 h after administration on the morning of oral medications such as digitalis and diuretics, without candesartan cilexetil or placebo. M-mode echocardiography was also performed with two-dimensional monitoring using a Sonolayer phased-array sector scanner (model SSH-160A, Toshiba Co., Tokyo, Japan) before and after 14 weeks of treatment with candesartan cilexetil or placebo. Left ventricular volumes were calculated using Teichholtz’s formula, and LVEF was determined.
Measurement of neurohumoral factors and immune factors. Blood for the measurement of the plasma levels of ANP, BNP, endothelin-1 (ET-1), IL-6, TNFalpha, sICAM-1 and sVCAM-1 was transferred to a chilled tube containing EDTA (1 mg/ml) and aprotinin (500 kallikrein inactivator units/ml) and then centrifuged at 3,000 rpm for 15 min at 4°C. The plasma thus obtained was stored at 2-30°C until assayed. Plasma ANP concentrations were measured with a specific immunoradiometric assay for alpha-human ANP using a commercial kit (Shionoria, Osaka, Japan) as previously reported (30). Plasma BNP concentrations were measured with a specific immunoradiometric assay for human BNP using a commercial kit (Shionoria) as previously reported (30). The plasma ET-1 level was determined using an antibody directed against synthetic ET-1 (Peninsula Laboratories, Inc., Belmont, California) and 125I ET-1 (Amersham Japan, Tokyo, Japan) as previously reported (37). Plasma levels of IL-6, TNFalpha, sICAM-1 and sVCAM-1 were measured using commercially available immunoassay kits (Quantikine HS, R&D Systems, Minneapolis, Minnesota) as previously reported (23,38). In age-matched normal subjects (n = 13), the plasma levels of IL-6, TNFalpha, sICAM-1 and sVCAM-1 were 1.2 ± 0.2 pg/mL, 2.7 ± 0.4 pg/mL, 149 ± 7 ng/mL and 530 ± 38 ng/mL, respectively.

Blood specimens for the assay of plasma cGMP concentrations were transferred to a chilled disposable tube containing 5 mmol/L EDTA. Aliquots of plasma were measured by radioimmunoassay with a commercial kit (Yamasa Shoyu Co. Ltd., Tokyo, Japan) as previously reported (33).

RESULTS

Patient characteristics. There was no difference of age, gender, NYHA functional class, etiology of heart failure, LVEF or medications at entry into the study between the candesartan group and the placebo group (Table 1).

Comparison of hemodynamics, neurohumoral factors and immune factors before treatment between candesartan cilexetil group and placebo group (Table 2). There was no difference in heart rate, mean blood pressure or left ventricular volume before treatment between the candesartan cilexetil group and placebo group (Table 2), and there was no difference in neurohumoral factors such as ANP,
BNP, cGMP, ET-1, NE, PARC, Ang II or ALD before treatment between the two groups. There was also no difference in immune markers such as IL-6, TNFalpha, sICAM-1 and sVCAM-1 before treatment between the two groups, but the levels of these immune factors were significantly increased compared with the age-matched normal subjects.

**Chronic effects of candesartan cilexetil on hemodynamics, neurohumoral factors and the immune factors.** The mean dose of candesartan cilexetil was 6.3 ± 0.5 mg. In the placebo group, there was no significant change of NYHA functional class, heart rate, mean blood pressure or LVEF after 14 weeks. There was also no significant change in neurohumoral factors such as ANP, BNP, cGMP, ET-1, PARC, Ang II or ALD or immune markers such as IL-6, TNFalpha, sICAM-1 and sVCAM-1 after 14 weeks. In the candesartan cilexetil group, the mean blood pressure was significantly decreased without a change of heart rate, and LVEF was significantly increased with the improvement of functional class (2.4 ± 0.17 vs. 1.9 ± 0.16, p < 0.01) after 14 weeks (Fig. 1). Plasma active renin concentration and Ang II levels were significantly increased; plasma ALD was slightly decreased, and plasma NE was slightly decreased in spite of the significant decrease of mean blood pressure (Fig. 2). The plasma levels of ANP, cGMP and ET-1 were not changed, but plasma BNP was significantly decreased (Fig. 3). The plasma levels of IL-6, TNFalpha, sICAM-1 and sVCAM-1 were significantly decreased (Fig. 4).

**Table 2. Comparison of Hemodynamics, Neurohumoral Factors and Immune Factors Before Treatment in 23 CHF Patients Divided Into Two Groups: Candesartan Cilexetil Treatment or Placebo**

<table>
<thead>
<tr>
<th></th>
<th>Candesartan Cilexetil (n = 14)</th>
<th>Placebo (n = 9)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>77.9 ± 5.7</td>
<td>66.8 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>88.7 ± 3.4</td>
<td>87.8 ± 3.5</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDV (mL)</td>
<td>285 ± 21</td>
<td>265 ± 26</td>
<td>NS</td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>204 ± 23</td>
<td>180 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>ANP (pg/mL)</td>
<td>69.2 ± 15.3</td>
<td>79.2 ± 23.9</td>
<td>NS</td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>163.1 ± 49.4</td>
<td>113.2 ± 26.2</td>
<td>NS</td>
</tr>
<tr>
<td>cGMP (pmol/mL)</td>
<td>6.4 ± 1.1</td>
<td>6.7 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>PARC (pg/mL)</td>
<td>57 ± 17</td>
<td>60 ± 19</td>
<td>NS</td>
</tr>
<tr>
<td>Ang II (pg/mL)</td>
<td>41 ± 8.4</td>
<td>45 ± 9.3</td>
<td>NS</td>
</tr>
<tr>
<td>ALD (pg/mL)</td>
<td>138 ± 29</td>
<td>132 ± 62</td>
<td>NS</td>
</tr>
<tr>
<td>NE (pg/mL)</td>
<td>399 ± 44</td>
<td>284 ± 45</td>
<td>NS</td>
</tr>
<tr>
<td>ET-1 (pg/mL)</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>4.1 ± 0.6</td>
<td>2.6 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>TNF alpha (pg/mL)</td>
<td>5.4 ± 0.4</td>
<td>7.2 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>sICAM-1 (ng/mL)</td>
<td>322 ± 31</td>
<td>236 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>sVCAM-1 (ng/mL)</td>
<td>865 ± 77</td>
<td>734 ± 89</td>
<td>NS</td>
</tr>
</tbody>
</table>

ALD = aldosterone; Ang II = angiotensin II; ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; cGMP = cyclic guanosine monophosphate; ET-1 = endothelin-1; HR = heart rate; IL-6 = interleukin-6; LVEDV = left ventricular end-diastolic volume; LVESV = left ventricular end-systolic volume; MBP = mean blood pressure; NE = norepinephrine; PARC = plasma active renin concentration; sICAM-1 = soluble intercellular adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecule-1; TNF = tumor necrosis factor.

**Relationship between plasma levels of cardiac natriuretic peptides and cyclic GMP, a second messenger of cardiac natriuretic peptides, before and after treatment with candesartan cilexetil.** There were significant positive correlations between the plasma levels of cardiac natriuretic peptides (ANP + BNP) and plasma cGMP levels before and after the treatment with candesartan cilexetil or placebo. The molar ratio of plasma cGMP to cardiac natriuretic peptides (ANP + BNP) was significantly increased after candesartan cilexetil treatment, but did not change in the placebo group (Fig. 5).

**DISCUSSION**

We demonstrated that 14 weeks of treatment with an Ang II type 1 receptor antagonist (candesartan cilexetil) significantly decreased immune markers such as plasma levels of TNFalpha, IL-6, sICAM-1 and sVCAM-1 in patients with mild to moderate CHF. Recently, Ang II, an important component of the renin-angiotensin system, was shown to stimulate the production of cytokines such as TNFalpha and IL-6 via the Ang II type I receptor, which is present on monocytes, macrophages and vascular smooth muscle cells (24–26). Ang II also stimulates the expression of ICAM-1 and VCAM-1 in vivo (27). Therefore, these decreases of the immune markers may be direct effects of the Ang II type I receptor antagonism of candesartan cilexetil.

We previously reported that the possibility of downregulation of cardiac natriuretic peptides receptor coupled with
guanylate cyclase may contribute to the progression of CHF (30,32–34). In this study, we showed that the molar ratio of plasma cGMP to cardiac natriuretic peptides was significantly increased after candesartan cilexetil treatment, suggesting that candesartan cilexetil improved the biological compensatory action of endogenous cardiac natriuretic peptides in patients with mild to moderate CHF, as previously shown in canine CHF (36). The mechanism of the improvement of the relationship between cardiac natriuretic peptides and cGMP after candesartan cilexetil treatment is partly due to the fact that Ang II reduces cGMP accumulation produced by cardiac natriuretic peptides in target cells by the activation of phosphodiesterase (39).

Recently, losartan has been shown to produce an unexpectedly lower risk of mortality than ACE inhibitors in older patients with CHF, suggesting the usefulness of Ang II receptor antagonists (4). Considering this along with our findings, it might be useful to determine in future studies the relationship of changes in cytokines and adhesion molecules to the effects of Ang II inhibition on ventricular remodeling and prognosis. We have reported that plasma levels of sICAM-1 are increased with the severity of CHF and can provide prognostic information in patients with CHF (38). The evaluation of the levels of adhesion molecules may yield important information about the activation of the immune system, since molecules such as ICAM-1 and VCAM-1 affect the biological activity of leukocytes and various cytokines which stimulate the expression of ICAM-1 or VCAM-1 in target cells. The mechanism of

Figure 1. Hemodynamic parameters and functional class before and after 14 weeks of treatment with candesartan cilexetil in 14 patients with mild to moderate congestive heart failure. HR = heart rate; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; MBP = mean blood pressure; NYHA = New York Heart Association. *p < 0.05; **p < 0.01 vs. before.

Figure 2. Plasma levels of active renin concentration (PARC), angiotensin (Ang II), aldosterone (ALD) and norepinephrine (NE) before and after 14 weeks of treatment with candesartan cilexetil in 14 patients with mild to moderate congestive heart failure. *p < 0.05; **p < 0.01 vs. before.
the decrease of sICAM-1 and sVCAM-1 after candesartan cilexetil treatment is mainly due to the decreases of TNF-\(\alpha\) and IL-6, which stimulate the expression of sICAM-1 and sVCAM-1 in vitro.

In this study, the plasma Ang II level was significantly increased after candesartan cilexetil treatment, which is consistent with the reports of previous studies. Recent studies have suggested that the Ang II type 1 receptor is downregulated and the Ang II type 2 receptor is upregulated in the failing human ventricle (40). The pathophysiological role of Ang II type 2 receptors has not been fully clarified; the increase of plasma Ang II after treatment with Ang II type 1 receptor antagonists may inhibit interstitial fibrosis via upregulated Ang II type 2 receptors in fibroblasts and, thereby, have beneficial effects on ventricular remodeling (40). In this study, the significant decrease of mean blood pressure and left ventricular end-systolic volume was probably due to the vasodilatory action via the Ang II type 1 receptor. The significant improvement of LVEF with a slight decrease of left ventricular end-diastolic volume was mainly due to vasodilatory action, suggesting that improvement of left ventricular remodeling requires more long-term treatments. Moreover, the increase of plasma Ang II after Ang II type 1 receptor antagonist treatment may suppress the production of cytokines such as TNF-\(\alpha\) and IL-6 via effects on the Ang II type 2 receptors on human monocytes (41).

**Study limitations.** We cannot rule out the possibility that the decrease of the plasma levels of TNF-\(\alpha\), IL-6, sICAM-1 and sVCAM-1 were due to the improvement of hemodynamic parameters and symptoms. In general, there were wide ranges of plasma levels of TNF-\(\alpha\) and IL-6 in patients with CHF, and the levels were significantly increased in patients with moderate to severe CHF. Moreover, there were no or poor correlations between plasma

Figure 3. Plasma levels of endothelin-1 (ET-1), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and cyclic guanosine monophosphate (cGMP) before and after 14 weeks of treatment with candesartan cilexetil in 14 patients with mild to moderate congestive heart failure. *\(p < 0.05\) vs. before.

Figure 4. Plasma levels of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-\(\alpha\)), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular adhesion molecule-1 (sVCAM-1) before and after 14 weeks of treatment with candesartan cilexetil in 14 patients with mild to moderate congestive heart failure. *\(p < 0.05\) vs. before.

Figure 5. The molar ratio of plasma cGMP (cyclic guanosine monophosphate) to cardiac natriuretic peptides before and after 14 weeks of treatment with candesartan cilexetil (closed columns) or placebo (open columns). ANOVA = analysis of variance; ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide. *\(p < 0.05\) vs. before with a two-way ANOVA by Scheffé F test.
cytokines and hemodynamic parameters in CHF patients. We believe that the significant decreases of the plasma levels of TNF-α, IL-6, sICAM-1 and sVCAM-1 in a small number of patients were mainly due to the direct action of Ang II type 1 receptor antagonist because other neurohumoral factors, except plasma BNP, did not change significantly. However, future studies will be needed in a larger number of patients over a longer follow-up period to determine whether these changes are persistent and are found in other Ang II type 1 receptor antagonists.

Conclusions. Plasma levels of immune markers such as TNF-α, IL-6, sICAM-1 and sVCAM-1 were significantly decreased after 14 weeks of candesartan cilexetil treatment but not after treatment with placebo. We also showed that plasma levels of BNP, which are a useful marker of prognosis, significantly decreased, and the molar ratio of plasma cGMP to cardiac natriuretic peptides (ANP + BNP) was significantly increased after candesartan cilexetil treatment but not after treatment in placebo, suggesting that candesartan cilexetil improved the biological compensatory action of endogenous cardiac natriuretic peptides in patients with mild to moderate CHF. These findings suggest that long-term treatment with the Ang II type 1 receptor antagonist (candesartan cilexetil) improved the activation of the immune system and improved the biological compensatory action of endogenous cardiac natriuretic peptides in patients with mild to moderate CHF.

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