Plasma Aldosterone Concentrations Are Not Related to the Degree of Angiotensin-Converting Enzyme Inhibition in Essential Hypertensive Patients

Atsuhisa SATO, Yoshiyuki SUZUKI, Hirotaka SHIBATA*, and Takao SARUTA*

There is increasing evidence of important cardiovascular effects of aldosterone via classical mineralocorticoid receptors in the heart. Aldosterone plus excess salt administration has been shown to produce both cardiac hypertrophy and cardiac fibrosis in rats. Various clinical studies have reported that aldosterone plays an important role in cardiac hypertrophy; however, the factors that control plasma aldosterone concentrations during angiotensin-converting enzyme (ACE) inhibitor treatment have still not been established. In the present study, we examined the relationship between plasma aldosterone concentrations and the degree of ACE inhibition in 25 essential hypertensive patients treated with an ACE inhibitor. Blood pressure decreased with treatment and plasma ACE activity, estimated in vitro (by a colorimetric method) and in vivo (by plasma angiotensin II/angiotensin I ratio) assay, was suppressed compared with that of hypertensive patients treated with medication other than ACE inhibitors. No relationship was found between the level of ACE inhibition and plasma aldosterone concentrations, which rose in parallel with the duration of ACE inhibitor treatment. The present study demonstrates that continuous ACE inhibitor therapy produces significant suppression of plasma ACE activity in essential hypertensive patients, but that no relationship exists between plasma aldosterone concentrations and levels of ACE inhibition. Plasma aldosterone concentrations tend to increase with the duration of ACE inhibitor treatment, although this increase did not reflect a reduced inhibition of ACE activity. (Hypertens Res 2000; 23: 25-31)

Key Words: plasma aldosterone concentrations, angiotensin-converting enzyme inhibitor, essential hypertension, angiotensin II, plasma angiotensin-converting enzyme activity

Introduction

Although the classical physiological roles of aldosterone are to promote unidirectional transepithelial sodium transport via epithelial mineralocorticoid receptors (MR) (1), recent studies have revealed major cardiovascular effects of aldosterone via classical MR in nonepithelial tissues such as brain and heart (2, 3). In the heart, aldosterone plus excess salt administration has been shown to produce cardiac hypertrophy and both interstitial and perivascular cardiac fibrosis in rats (4-6), independent of blood pressure (4, 6), and reflecting a direct effect of aldosterone on the heart mediated by cardiac MR. Clinical interest in the cardiac effects of aldosterone has thus grown tremendously in recent years.

Plasma aldosterone concentrations are shown to be elevated in patients with congestive heart failure, and these concentrations represent a marker of poor prognosis in such patients (7). Reduction of plasma aldosterone concentrations with angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin II receptor blockade in recent

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randomized clinical trials has been shown to be effective in reducing the negative effects of elevated plasma aldosterone concentrations in patients with heart failure (8-10). However, continuous ACE inhibitor therapy does not necessarily produce a significant decrease of plasma aldosterone levels, which may remain high or increase eventually during long-term use (aldosterone escape) (11, 12). Recently, in this regard, it was clearly demonstrated in the Randomized Aldactone Evaluation Study (RALES) that adding the MR antagonist spironolactone to the regimen of an ACE inhibitor and loop diuretics with or without digitalis significantly reduced morbidity and mortality in patients with severe congestive heart failure (13).

In selected patients with essential hypertension, as in those with congestive heart failure, plasma aldosterone concentrations have been shown to be higher than those of controls (14-16), with a positive correlation seen between plasma aldosterone concentrations and left ventricular mass index (LVMI) (14). Aldosterone escape has also been reported in patients with essential hypertension treated with ACE inhibitors (17, 18). Recently, we demonstrated a high incidence of aldosterone escape in patients with essential hypertension despite the use of an ACE inhibitor, and that aldosterone escape may play an important role in cardiac hypertrophy in essential hypertensive patients with left ventricular hypertrophy (Sato and Saruta, submitted). In that study, we highlighted the clinical relevance and importance of aldosterone escape, although the mechanisms of aldosterone escape remain to be determined.

Very recently, Jorde reported that plasma aldosterone concentrations are variable in patients with congestive heart failure treated with maximal doses of ACE inhibitor for prolonged periods, and there is no relation between plasma aldosterone concentrations and the level of ACE inhibition achieved during chronic ACE inhibition (19). Jorde also demonstrated that plasma aldosterone concentrations tended to be higher with increased duration of treatment with an ACE inhibitor (19). In a parallel study, we have evaluated the relationship between plasma aldosterone concentrations and the degree of ACE inhibition in essential hypertensive patients treated with ACE inhibitors. The present data suggest that plasma aldosterone concentrations with ACE inhibitor treatment may be independent of the suppressive effect of ACE inhibitor treatment on ACE levels.

Materials and Methods

Subjects and Study Design

Twenty-five untreated essential hypertensive patients (13 men, 12 women; mean age, 55 ± 13 yr) seen at Mito Red Cross Hospital, Ibaraki, Japan, participated in this study. They were classified as having stage 1 (4 patients), stage 2 (13 patients) or stage 3 (8 patients) essential hypertension based on the sixth report of the Joint National Committee guidelines (20). The subjects underwent routine laboratory studies to exclude patients with coexisting unrelated disease. Blood pressure was measured with a mercury sphygmomanometer at least 10 min of rest in the sedentary position, and it was expressed as the mean of three consecutive measurements, as described previously (21, 22). Heart rate was obtained from the radial pulse over 30 s. None of the patients had received any antihypertensive agent before the study. Medication with an ACE inhibitor [trandolapril (17 patients), enalapril maleate (6 patients), or imidapril hydrochloride (2 patients)] was started at a minimal dose, with blood pressure response assessed every 2 wk and the dose increased gradually. All patients were instructed to practice moderate sodium restriction (about 7-8 g/d), and double-check sodium level at each clinical visit. All patients in this study subsequently underwent monthly follow-up throughout the study period. The therapy duration-, and blood pressure control-matched hypertensive control group (non-ACE inhibitor group; n = 15; 8 men, 7 women; mean age, 52 ± 12 yr) consisted of patients never treated by an ACE inhibitor but who had been treated with a calcium channel blocker (n = 10) or calcium channel blocker plus a1-blocker (n = 5).

Biochemical Determinations

The general biochemical parameters were measured by routine laboratory methods at 3-4 h after drug intake, after the patients were in a supine position for at least 15 min. Plasma renin activity and aldosterone concentrations were measured by commercial radioimmunoassay as previously reported (21, 22). Plasma renin activity was determined by measuring newly synthesized angiotensin I (Ang I) with an angiotensinase inhibitor and PMSF at a sensitivity of 0.1-20 ng/ml/h (Renin Riabead, Dainabot Corporation, Tokyo, Japan). Immediately after drawing blood, we placed it on ice, centrifuged it at 4°C, and confirmed that this method did not affect the value of plasma renin activity. Plasma aldosterone concentrations were determined by radioimmunoassay (SPAC-S Aldosterone Kit, Dai-ichi Radio-isotope, Tokyo, Japan), with a sensitivity of 25-1,600 pg/ml.

Plasma ACE activity was measured in vitro with p-hydroxybenzoyl-glycyl-L-histidy-L-leucine as a substrate by a colorimetric method for ACE-color (ACE color, Fujirebio Inc., Tokyo, Japan), and a range of 8.3-21.4 IU/l/37°C was considered normal. This procedure is time-saving compared to Cushman’s method, and correlation between the ACE color test and Cushman’s method is extremely high (23). ACE activity was also estimated in...
vivo by the plasma angiotensin II (Ang II)/Ang I (Ang II/ Ang I) ratio. Ang I and Ang II were quantitated by radioimmunoassay using the original methods of SRL, Inc., Tokyo, Japan (24). The normal range of Ang I is under 110 pg/ml, and that of Ang II is under 22 pg/ml. The sample was mixed with assay buffer (Tris buffer) containing 100 µl of tracer (125I-Ang I or Ang II) and 100 µl of the primary antibody to Ang I or Ang II, and incubated for 14-18 h at 2-8°C. Then, 100 µl of the secondary antibody was added and the mixture was incubated for 1 h at 2-8°C. After centrifugation at 3,500 rpm for 20 min at 2-8°C, the supernatant was decanted and radioactivity was measured. The level of Ang I or Ang II was determined by extrapolation from the standard curve.

The general biochemical parameters were measured before and after antihypertensive treatment, and plasma renin activity, aldosterone concentration, plasma ACE activity, Ang I and Ang II were determined only after antihypertensive treatment.

**Echocardiographic Measurement**

Echocardiographic studies were performed by the standard method with an SSA-380A echocardiograph with a 3.0-MHz transducer (Toshiba, Japan), according to the recommendations of the American Society of Echocardiography (25), as previously reported (22, 23). Left ventricular (LV) mass was estimated from the formula of Devereux and Reichek (Penn convention) (26): LV mass (g) = 1.04 × [(LVDD + IVST + PWT)³ - (LVDD)³] - 13.6, where LVDD is LV end-diastolic dimension, IVST is interventricular septal thickness and PWT is posterior wall thickness. The LVMI was calculated for each subject by dividing LV mass by body surface area. To reduce interobserver variability, all tracings were analyzed by a single expert cardiologist who was blinded to the clinical and biochemical data. Echocardiographic studies were performed only at the time of blood sampling, and the relationship between plasma aldosterone concentrations and LVMI was evaluated.

**Statistical Analysis**

Data are expressed as mean ± SD, and statistical significance between two groups is determined by two-tailed, unpaired t-test (Welch’s t-test). Changes in parameters in each group before and after treatment were compared by the two-group paired t-test, with p values of < 0.05 taken as significant. Univariate correlation was established by Pearson’s correlation coefficient.

**Results**

**Clinical and Biological Data of All Patients**

The clinical and biological characteristics of all patients in this study are summarized in Table 1. The median duration of ACE inhibitor or non-ACE inhibitor treatment was 13 ± 7 mo (range, 3 to 25 mo), or 12 ± 8 mo (range, 3 to 24 mo).

**Changes in Blood Pressure, Heart Rate and Electrolytes after Antihypertensive Treatment with an ACE Inhibitor**

Both systolic and diastolic blood pressure decreased after 2 mo of antihypertensive treatment with an ACE inhibitor in most patients. Finally, at the time of blood sam-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ACEI group</th>
<th>Non-ACEI group</th>
</tr>
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<tbody>
<tr>
<td>Men/Women</td>
<td>13/12</td>
<td>8/7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55 ± 13</td>
<td>52 ± 12</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>169 ± 22</td>
<td>167 ± 24</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>96 ± 14</td>
<td>97 ± 13</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>67 ± 6</td>
<td>65 ± 5</td>
</tr>
<tr>
<td>Na (mEq/l)</td>
<td>142.3 ± 1.4</td>
<td>141.3 ± 1.6</td>
</tr>
<tr>
<td>K (mEq/l)</td>
<td>4.2 ± 0.3</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>12.3 ± 0.3</td>
<td>12.1 ± 0.4</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.1</td>
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All values are mean ± SD. BP, blood pressure; ACEI, angiotensin-converting enzyme inhibitor; BUN, blood urea nitrogen.

pling, patient blood pressure was significantly reduced compared with baseline values (before treatment, 167 ± 19/99 ± 16 mmHg; after treatment, 135 ± 9/78 ± 8 mmHg) (Fig. 1). Heart rate (before treatment, 72 ± 10/min; after, 70 ± 8/min) remained unchanged throughout the study period. Serum potassium (before treatment, 4.2 ± 0.3 mEq/l; after, 4.3 ± 0.3 mEq/l) remained unchanged with antihypertensive treatment.

Effects of ACE Inhibitor Treatment on Plasma ACE Activity, Plasma Renin Activity, Angiotensins and Aldosterone Concentrations

Table 2 shows plasma ACE activity, plasma renin activity, angiotensins and aldosterone concentrations in patients in the ACE inhibitor treatment and non-ACE inhibitor treatment groups. Mean values for plasma in vitro ACE activity and the Ang II/Ang I ratio were significantly lower in the ACE inhibitor-treated patients than in the non-ACE inhibitor-treated group. Mean plasma renin activity and Ang I levels were significantly higher in the ACE inhibitor-treated patients. In contrast, no significant difference was observed for plasma aldosterone concentrations and plasma Ang II levels between the two groups.

Relationship between Plasma Aldosterone Concentrations and ACE Activity in Patients Treated with an ACE Inhibitor

The degree of ACE inhibition, estimated by Ang II/Ang I, and ACE activities measured in vitro were variable during ACE inhibitor treatment (Fig. 2, upper and lower panel). Taken together with data from comparisons with non-ACE treatment patients (Table 2), these results suggest that ACE activity in essential hypertensive patients treated with an ACE inhibitor in this study was suppressed throughout the study period. No relationship existed between plasma aldosterone concentration and the level of ACE inhibition assessed by either the Ang II/Ang I ratio (R = 0.06, p = 0.76) or ACE activity in vitro (R = 0.10, p = 0.65) during ACE inhibition. However, plasma aldosterone concentrations were higher with increased duration of ACE inhibitor treatment (R = 0.46, p = 0.02, Fig. 3). At the time of blood sampling, neither blood pressure (both systolic and diastolic) nor LVMI (144 ± 38 g/m²; R = 0.10, p = 0.65) was correlated with plasma aldosterone concentrations. In the non-ACE inhibitor-treated group, neither ACE activities (Ang II/Ang I; R = 0.02, p = 0.93, ACE activity in vitro; R = 0.14, p = 0.56)
nor plasma aldosterone concentrations ($R = 0.08$, $p = 0.79$) was correlated with the duration of antihypertensive treatment.

**Discussion**

This study shows that continuous ACE inhibitor therapy in essential hypertensive patients produces a significant suppression of plasma ACE activity compared with patients on non-ACE treatment, estimated both by ACE activity in vitro and by the Ang II/Ang I ratio in vivo. However, no relationship exists between plasma aldosterone concentrations and the level of ACE inhibition. This is potentially an important finding in that plasma aldosterone concentrations are eventually independent of ACE activity in such patients. Moreover, this is the first report to show that plasma aldosterone concentrations are higher with longer duration of ACE inhibitor treatment. Further studies will be needed to establish which factors modulate plasma aldosterone concentrations in essential hypertensive patients treated with an ACE inhibitor and to determine the clinical relevance of increased aldosterone concentrations in such patients on long duration ACE inhibitor therapy.

Accurate determination of ACE activity in patients treated with an ACE inhibitor is generally difficult. ACE activity measured by an in vitro assay using a synthetic substrate has been reported to depend on the type of substrate and the assay condition used (27), and the results are evidence of a large range of interindividual variation, partly because of genetic polymorphism of this enzyme (28, 29). Moreover, because extravascular renin-angiotensin systems (tissue ACE) are inhibited to various degrees by ACE inhibitors, ACE activity in vitro does not accurately reflect suppression of Ang II or the hemodynamic effects of treatment (30, 31). In this regard, Azizi et al. have recently reported that the plasma level of N-acetylseryl-aspartyl-lysyl-proline (Ac-SDKP) is a better marker of in vivo ACE inhibition than are the standard methods of assessment (32). This method is, however, not yet commonly used in the clinical setting. In this respect, plasma Ang II/Ang I ratio is currently considered to be the optimal index of plasma ACE inhibition in the presence of an ACE inhibitor, and the ratio is believed to more closely reflect the in vivo plasma and endothelial inhibition of ACE (27, 33). However, it has been shown that plasma ACE in vitro also closely reflects in vivo ACE activity, and plasma ACE thus provides a useful marker of ACE activity (34). For that reason, we determined plasma ACE activity both in vitro and in vivo in this study. Although ACE activity has been reported to be induced by ACE inhibitors (35), the present study clearly demonstrates that continuous ACE inhibitor therapy in essential hypertensive patients produces a significant suppression of plasma ACE activity. In this regard, a sufficient hypotensive effect was observed in all patients throughout the study, and plasma renin activity and plasma Ang I concentration remained elevated.

Given this sufficient suppression of ACE activity, the most important finding in this study is that plasma aldosterone concentrations do not predict the degree of ACE inhibition in essential hypertensive patients. It has been generally assumed that ACE inhibitors effectively block generation of both Ang II and aldosterone, and that plasma aldosterone concentrations may be suppressed during ACE inhibitor treatment. However, plasma aldosterone concentrations were variable in this study, and there was no relation between plasma aldosterone concentrations and the level of ACE inhibition. The present study suggests that currently used, clinically effective antihypertensive doses of ACE inhibitors may not regulate plasma aldosterone concentrations, i.e., plasma aldosterone concentrations can be controlled independently by ACE inhibition. Therefore, our study also raises an important question regarding which factors control plasma aldosterone concentrations in essential hypertensive patients treated with ACE inhibitors.

As a clue to help answer this question, we have shown here that no significant difference was observed for plasma Ang II levels, which is considered to be the most potent aldosterone stimulator, between patients in the ACE inhibitor treatment and non-ACE inhibitor treatment groups. Moreover, we have shown that plasma aldosterone concentrations tended to be higher with longer durations of ACE inhibitor therapy. Very recently, similar findings were reported by Jorde, who demonstrated that there is no relation between plasma aldosterone concentrations and the level of ACE inhibition in patients with congestive heart failure treated with maximal doses of ACE inhibitor, and that plasma aldosterone concentrations tended to be higher with longer duration of ACE inhibitor treatment (19). There are reports which indicate that ACE participates in the metabolism of peptides other
than Ang I and bradykinin in vivo (36). Therefore, one possible hypothesis is that other biologically active peptides that are generated by ACE but that not dependent on ACE activity in the chronic phase increased in the course of chronic ACE inhibitor therapy, and such peptides may increase plasma aldosterone concentrations.

In this study, because plasma aldosterone concentrations were not determined in all patients before treatment, we could not compare their pre- and post-treatment value in terms of aldosterone concentrations. Therefore, although plasma aldosterone concentrations tend to be higher with increased duration of therapy with ACE inhibitor, it is possible that there are patients whose plasma aldosterone concentrations remain well suppressed under ACE inhibitor treatment. Moreover, because ACE activities and plasma aldosterone concentrations were also not measured in all patients before treatment, our data did not reflect the changes in ACE activities and plasma aldosterone concentrations in each patient. An additional study will be needed to determine the precise relationship between duration of ACE inhibitor therapy and plasma aldosterone concentrations.

In several studies plasma aldosterone levels in patients with essential hypertension have been reported to be higher than in controls (14), with increased levels of LV hypertrophy (15, 16). Moreover, LV hypertrophy has been shown to be more prominent in patients with primary aldosteronism than in patients with other types of secondary hypertension, and a positive correlation has been found between LVMI and plasma aldosterone concentrations in these patients (37). Finally, LV hypertrophy has been shown to precede other target-organ damage in patients with primary aldosteronism (38). The results of these clinical studies, and of experimental studies on animals given aldosterone with excess salt (4-6), indicate it is very likely that elevated aldosterone is primarily involved in the regulation of cardiac hypertrophy via cardiac MR. Recently, we have demonstrated that aldosterone escape during ACE inhibitor therapy may also play an important role in cardiac hypertrophy in essential hypertensive patients with left ventricular hypertrophy (Sato and Saruta, paper submitted). Therefore, it is important to determine how plasma aldosterone concentrations are controlled during ACE inhibitor therapy. Given the results of the present study, further studies will be needed to establish which factors modulate plasma aldosterone concentrations in essential hypertensive patients given an ACE inhibitor, and also to determine the clinical relevance of increased aldosterone concentrations with duration of ACE inhibitor therapy in such patients.

In conclusion, the present study has demonstrated that continuous ACE inhibitor therapy produces a significant suppression of plasma ACE activity in essential hypertensive patients, but that no relationship exists between plasma aldosterone concentrations and the level of ACE inhibition. Plasma aldosterone concentrations are higher with longer duration of ACE inhibitor treatment, which is, however, not due to reduced inhibition of ACE activity.

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