Spironolactone Abolishes the Relationship between Aldosterone and Plasminogen Activator Inhibitor-1 in Humans

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Recent studies have defined a link between the renin-angiotensin-aldosterone system and fibrinolysis. The present study tests the hypothesis that endogenous aldosterone regulates plasminogen activator inhibitor-1 (PAI-1) production in humans. Hemodynamic parameters, PAI-1 and tissue-type plasminogen activator (t-PA) antigen, potassium, PRA, angiotensin II, and aldosterone were measured in nine male hypertensive subjects after a 3-wk washout, after 2 wk of hydrochlorothiazide (HCTZ; 25 mg plus 20 mmol KCl/d), and after 2 wk of spironolactone (100 mg/d plus KCl placebo). Spironolactone (P = 0.04), but not HCTZ (P = 0.37 vs. baseline; P = 0.19 vs. HCTZ) treatments. Although both HCTZ (P = 0.004) and spironolactone (P < 0.001 vs. baseline) increased aldosterone, the effect was greater with spironolactone (P < 0.001 vs. HCTZ). HCTZ increased PAI-1 antigen (P = 0.02), but did not alter t-PA antigen. In contrast, there was no effect of spironolactone on PAI-1 antigen (P = 0.28), whereas t-PA antigen was increased (P = 0.01). There was a significant correlation between PAI-1 antigen and serum aldosterone during both baseline and HCTZ study days (r² = 0.57; P = 0.0003); however, treatment with spironolactone abolished this correlation (r² = 0.13; P = 0.23). This study provides evidence that endogenous aldosterone influences PAI-1 production in humans. (J Clin Endocrinol Metab 87: 448–452, 2002)

ACTIVATION OF THE renin-angiotensin-aldosterone system (RAAS) has been associated with an increased risk of ischemic cardiovascular events (1), independent of blood pressure (BP), whereas interruption of the RAAS by angiotensin-converting enzyme (ACE) inhibition reduces cardiovascular mortality (2–4). We have previously proposed that a major component of the vascular toxicity associated with activation of the RAAS derives from the effects of angiotensin II (Ang II) on fibrinolytic balance. Ang II causes a dose-dependent increase in the expression of plasminogen activator inhibitor-1 (PAI-1), the major physiological inhibitor of fibrinolysis in vivo (5, 6). In humans, activation of the RAAS by sodium depletion is associated with increased morning plasma PAI-1 antigen concentrations, whereas ACE inhibition improves fibrinolytic balance (7).

Although these effects of activation and interruption of the RAAS on the fibrinolytic system have been attributed to Ang II, increasing evidence suggests that aldosterone also regulates PAI-1 expression. First, aldosterone interacts with Ang II to increase PAI-1 expression in both vascular smooth muscle cells and endothelial cells (8). In a rat model, aldosterone receptor antagonism attenuates renal PAI-1 expression after radiation injury (9). In humans, plasma PAI-1 antigen concentrations correlate with serum aldosterone concentrations in both salt-depleted normal controls and individuals with primary hyperaldosteronism (7, 8).

The purpose of the present study was to test the hypothesis that endogenous aldosterone regulates PAI-1 expression in humans. To do this, we compared the effect of the aldosterone receptor antagonist spironolactone to the effect of another diuretic, hydrochlorothiazide (HCTZ), on fibrinolytic balance in individuals with essential hypertension.

Subjects and Methods

Subjects

All subjects provided a complete medical history and underwent a physical examination before the investigation. Subjects were defined as hypertensive if they had three or more documented diastolic BP measurements greater than or equal to 90 mm Hg and had had hypertension of at least 6-month duration. Subjects with significant cardiovascular, renal, endocrine, or pulmonary disease or who were taking vasoactive medications were excluded. Written informed consent was obtained, and the study protocol was approved by the Vanderbilt University institutional review board. All procedures followed were in accordance with institutional guidelines.

Protocol

Subjects participated in a single blind, randomized, cross-over design study. At the beginning of the study, all antihypertensive or vasoactive medications were discontinued or tapered, as appropriate. No subjects took any other medication during the time of the study. After subjects had been off all antihypertensive medications for 1 wk, they were given 10 mmol potassium chloride/d for 2 wk. (The first two subjects studied were not given potassium supplementation during this period.) At the end of these 2 wk and after they had been off antihypertensive medications for 3 wk, subjects were asked to collect all of their urine for 24 h.
for measurements of sodium and potassium excretion. The following morning, subjects were asked to report to the Vanderbilt General Clinical Research Center at 0800 h in the fasting state. An indwelling catheter was placed in an antecubital vein. BP and heart rate were measured at 0900, 1000, 1100, and 1200 h after the subject had been seated for 30 min. After each measurement of BP, blood was drawn through the indwelling catheter for measurement of PAI-1 antigen and tissue-type plasminogen activator (t-PA) antigen. Serum potassium, glucose, and insulin were measured at 0900 h. PRA, Ang II, and aldosterone were measured at 0900 and 1000 h.

After the first study day subjects were randomized to treatment with either 25 mg HCTZ and 20 mmol potassium chloride/d or 100 mg spironolactone and potassium placebo/d for 2 wk. The doses of HCTZ and spironolactone were chosen on the basis of pilot data that suggested equivalent antihypertensive potency. All medications were administered in identical-appearing capsules. Serum potassium was measured every 4 d during active medication. Additional oral potassium supplementation was to be given to any subject who had a serum potassium level of 3.5 mmol/dl or less, but was not required by any subject. At the end of the 2-wk medication period, subjects were again asked to collect a 24-h urine for measurement of sodium and potassium excretion. The following morning they reported to the General Clinical Research Center for repeat study as described above. At the end of the study day, subjects underwent a 2-wk washout period. They were then crossed over to the opposite drug regimen for 2 wk, and the study was repeated.

### Laboratory analysis

Blood samples were collected on ice and centrifuged immediately at 0 C for 20 min. All plasma or serum was separated and stored at –70 C until the time of assay. Blood for measurements of PAI-1 and t-PA antigen was collected in standard Vacutainer tubes (Becton Dickinson and Co., Mountain View, CA) containing 0.105 mmol/liter acidified sodium citrate, and antigen levels were determined using a two-site ELISA (Biopool AB, Umea, Sweden). In prior studies we determined that activation and interruption of the RAAS affect PAI-1 antigen and PAI-1 activity in parallel (7); therefore, PAI-1 activity was not measured. Blood for PRA and aldosterone determinations was drawn into chilled tubes containing EDTA. PRA was measured by RIA for Ang I formation at pH 7.4 and 37 C (10). Aldosterone was measured using a commercially available RIA (Diagnostic Products, Los Angeles, CA) with an extremely low cross-reactivity to either spironolactone (0.06%) or cortisol (below the limit of detection). Blood for Ang II determination was collected in chilled tubes containing a cocktail of protease inhibitors (11). Ang II measurements were made by RIA, as previously described (12, 13). Plasma was extracted on Sep-Pak columns (Waters/Millipore Corp., Milford, MA) activated with 5-ml sequential washes of a mixture of ethanol/water/4% acetic acid (83:13.4, vol/vol/vol), methanol, ultrapure water, and 4% acetic acid. The sample was eluted and reconstituted in assay buffer. The recovery of radioleabeled angiotensin added to the sample and followed through the extraction was 92%. Samples were corrected for recovery. Ang II was measured by RIA with the Nichols Institute Diagnostics RIA (San Juan Capistrano, CA). This antibody shows 67% cross-reactivity with Ang III, 70% with Ang IV, and 91% with Ang (4–8), but less than 0.1% with Ang I. The minimum detectable level of the assays was 4 pg/tube with Ang II. The intraassay coefficient of variation was 12% for Ang II. The plasma glucose concentration was measured with a colorimetric assay (Johnson & Johnson, Raritan, NJ), and serum insulin was measured by immunoassay (Tosoh Medics, Inc., San Francisco, CA).

### Statistical analysis

Data are presented as the mean ± sem. The effects of treatment on BP, endocrine and electrolyte parameters, and fibrinolytic balance were analyzed using a general linear model in which the within-subject variables were time and drug. F statistics and P values derived from the general linear model analysis are presented in the text unless otherwise specified. Post-hoc comparisons were made using paired t test. A two-tailed P < 0.05 was the criterion for statistical significance. Linear regression was used to assess the relationship between PAI-1 antigen and various endocrine parameters.

### Results

#### Subjects

Nine male subjects (mean age, 49.4 ± 4.1 yr; range, 34–68 yr) were studied. Six subjects were white, and three were black. Two subjects had a low renin-sodium profile, and seven had normal to high renin hypertension (1). The mean body mass index was 30.0 ± 2.0 kg/m² (range, 20.0–39.3 kg/m²). The mean serum cholesterol concentration was 5.1 ± 0.2 mmol/liter, whereas the mean serum triglyceride concentration was 1.6 ± 0.2 mmol/liter.

#### Hemodynamic parameters

Treatment with spironolactone (F = 5.7; P = 0.04), but not HCTZ (F = 0.4; P = 0.57), significantly lowered systolic BP compared with the baseline (Table 1). There was no significant difference between systolic BP measured during HCTZ and that measured during spironolactone (F = 3.6; P = 0.10). Neither drug lowered diastolic BP (for HCTZ: F < 0.01; P = 0.95; for spironolactone: F = 3.9; P = 0.09). There was no effect of either spironolactone or HCTZ on heart rate (P > 0.1 for all comparisons).

Despite the fact that subjects were given potassium supplementation at baseline and during HCTZ treatment, the serum potassium concentration was significantly decreased during treatment with HCTZ compared with baseline (Table 1; P < 0.001, by t test) and significantly increased during treatment with spironolactone (P = 0.02 vs. baseline; P < 0.001 vs. HCTZ). Urinary potassium excretion was significantly lower during spironolactone treatment compared with that during HCTZ treatment (P = 0.001), but was not

### Table 1. Effect of treatment on hemodynamic and electrolyte parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>HCTZ</th>
<th>Spironolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average systolic BP (mm Hg)</td>
<td>131.8 ± 3.8</td>
<td>129.8 ± 3.9</td>
<td>125.0 ± 2.8*</td>
</tr>
<tr>
<td>Average diastolic BP (mm Hg)</td>
<td>94.0 ± 1.4</td>
<td>94.0 ± 1.3</td>
<td>91.0 ± 1.3</td>
</tr>
<tr>
<td>Average heart rate (beats/min)</td>
<td>67.4 ± 2.8</td>
<td>68.6 ± 2.5</td>
<td>70.6 ± 2.3</td>
</tr>
<tr>
<td>Serum potassium (mmol/dl)</td>
<td>4.2 ± 0.1</td>
<td>3.8 ± 0.1³</td>
<td>4.5 ± 0.1³</td>
</tr>
<tr>
<td>24-h urinary vol (ml)</td>
<td>1736 ± 219</td>
<td>1736 ± 219</td>
<td>1995 ± 267</td>
</tr>
<tr>
<td>24-h urinary sodium (mmol/d)</td>
<td>73 ± 13</td>
<td>75 ± 7</td>
<td>61 ± 8⁴</td>
</tr>
<tr>
<td>Fasting glucose (mmol/liter)</td>
<td>4.7 ± 0.3</td>
<td>5.2 ± 0.5</td>
<td>5.5 ± 0.2⁵</td>
</tr>
<tr>
<td>Fasting insulin (pmol/liter)</td>
<td>78.9 ± 16.6</td>
<td>85.0 ± 21.2</td>
<td>76.6 ± 25.6</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. baseline.
³ P < 0.001 vs. baseline.
⁴ P < 0.001 vs. HCTZ.
⁵ P < 0.001 vs. baseline.
significantly different from the baseline. There was no effect of treatment on urinary sodium excretion or volume, as expected in subjects with normal baseline aldosterone concentrations (14).

Endocrine parameters

There was no significant effect of either HCTZ (F = 0.7; P = 0.44) or spironolactone (F = 2.4; P = 0.16) on PRA (Fig. 1 and Table 2). However, treatment with both HCTZ (F = 8.7; P = 0.02) and spironolactone (F = 9.4; P = 0.02) significantly increased Ang II concentrations, and the magnitude of the effect was similar during treatment with the two drugs (F = 2.2; P = 0.19). In addition, treatment with both HCTZ (F = 15.7; P = 0.004) and spironolactone (F = 73.3; P < 0.001) significantly increased the serum aldosterone concentration.

Serum aldosterone was significantly greater during treatment with the aldosterone receptor antagonist spironolactone than during treatment with HCTZ (F = 35.4; P < 0.001). There was no effect of treatment with either HCTZ (P = 0.71, by t test) or spironolactone (P = 0.94) on insulin concentrations. Serum glucose was increased compared with the baseline during spironolactone (P = 0.01), but not during HCTZ (P = 0.12), treatment.

Fibrinolytic balance

Treatment with HCTZ was associated with a significant increase in PAI-1 antigen (F = 8.5; P = 0.02), whereas t-PA

**TABLE 2.** Effect of treatment on renin-angiotensin-aldosterone and fibrinolytic systems

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>HCTZ</th>
<th>Spironolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (ng Ang I/ml-h)</td>
<td>1.5 ± 0.3</td>
<td>1.9 ± 0.4</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Ang II (pmol/liter)</td>
<td>21.5 ± 2.6</td>
<td>30.2 ± 4.2</td>
<td>39.4 ± 6.8</td>
</tr>
<tr>
<td>Aldosterone (pmol/liter)</td>
<td>244 ± 26</td>
<td>351 ± 31</td>
<td>642 ± 55</td>
</tr>
<tr>
<td>PAI-1 antigen (ng/ml)</td>
<td>15.9 ± 5.2</td>
<td>23.3 ± 6.6</td>
<td>18.1 ± 5.6</td>
</tr>
<tr>
<td>T-PA antigen (ng/ml)</td>
<td>11.0 ± 1.4</td>
<td>12.2 ± 2.0</td>
<td>12.8 ± 1.4</td>
</tr>
<tr>
<td>Molar ratio PAI-1:t-PA</td>
<td>1.9 ± 0.5</td>
<td>2.6 ± 0.7</td>
<td>2.0 ± 0.6</td>
</tr>
</tbody>
</table>

For PRA, Ang II, and aldosterone, data presented are the average of two samples drawn at 0900 and 1000 h. For PAI-1 and t-PA antigen, data presented are the average of four samples.

* P < 0.05 vs. baseline.

* P < 0.01 vs. baseline.

* P < 0.001 vs. baseline.

* P < 0.001 vs. HCTZ.

* P < 0.05 vs. HCTZ.
antigen was unchanged \((F = 1.2; P = 0.32; \text{Fig. 2 and Table } 2)\). Hence, the molar ratio of PAI-1 antigen to t-PA antigen was significantly increased during treatment with HCTZ \((F = 13.0; P = 0.007)\). In contrast, there was no effect of spironolactone on PAI-1 antigen \((F = 1.4; P = 0.28)\), but treatment with spironolactone was associated with a significant increase in t-PA antigen \((F = 10.8; P = 0.01)\). Thus, the molar ratio of PAI-1 antigen to t-PA antigen was significantly lower during treatment with spironolactone than during treatment with HCTZ \((F = 6.3; P = 0.04)\). Because serum potassium concentrations were significantly different among treatment arms, we examined the relationship between serum potassium and PAI-1 antigen concentrations. There was no relationship between serum potassium and plasma PAI-1 antigen \((r^2 = 0.01; P = 0.62)\). There was also no relationship between change in systolic BP in response to drug and plasma PAI-1 antigen \((r^2 = 0.11; P = 0.18)\). On the other hand, there was a significant correlation between the average serum aldosterone concentration and the average plasma PAI-1 level during both baseline \([\text{PAI-1, } 0.14(\text{aldosterone}) = 18.5; \ r^2 = 0.50; P = 0.03]\) and treatment with HCTZ \([\text{PAI-1, } 0.18(\text{aldosterone}) = 39.9; \ r^2 = 0.69; P = 0.0053]\). The slopes and intercepts of the relationship were similar on baseline and HCTZ study days \((P = 0.58\) for a difference between the slopes of the lines; \(P = 0.14\) for a difference between the intercepts); therefore, the relationship between aldosterone and PAI-1 for the combined baseline and HCTZ data \([\text{PAI-1, } 0.14(\text{aldosterone}) = 20.6; \ r^2 = 0.57; P = 0.0003]\) is presented in Fig. 3. Notably, treatment with spironolactone abolished the relationship between serum aldosterone and plasma PAI-1 antigen \((r^2 = 0.13; P = 0.33)\). There was no significant relationship between Ang II and PAI-1 \((r^2 = 0.07; P = 0.19)\).

**Discussion**

Previous studies have demonstrated that activation of the RAAS through either sodium depletion or diuresis increases PAI-1 antigen in healthy normotensive volunteers \((7, 15)\). The present study demonstrates that activation of the RAAS by treatment with HCTZ exerts a deleterious effect on fibrinolytic balance in individuals with essential hypertension, increasing PAI-1 antigen without increasing t-PA antigen. More importantly, to the extent that treatment with the aldosterone receptor antagonist spironolactone attenuated the effect of increased Ang II and aldosterone on PAI-1 antigen, the study suggests that activation of the RAAS increases PAI-1 through endogenous aldosterone.

The magnitude of the effect of HCTZ on fibrinolytic balance observed in this study of patients with essential hypertension was somewhat greater than that observed in healthy volunteers by Lottermoser et al. \((15)\). In that study treatment of normotensive subjects with 25 mg HCTZ for 2 wk resulted in a 27% increase in morning PAI-1 antigen from \(21.4 \pm 3.2\) to \(26.8 \pm 5.8 \text{ ng/ml}\) without a change in t-PA antigen. In the current study treatment with 25 mg HCTZ increased PAI-1 antigen by 64% in subjects with hypertension. Given that the hypertensive patients studied were heavier \((\text{mean body mass index, } 30.0 \pm 2.0 \text{ vs. } <25 \text{ kg/m}^2)\), this may reflect some underlying insulin resistance in the hypertensive subjects studied. On the other hand, the absolute PAI-1 antigen concentrations measured during HCTZ were remarkably similar in the previous study of normotensives and the present study.

Like HCTZ, spironolactone increased both Ang II and aldosterone. In fact, the effect of spironolactone on aldosterone concentration was greater than that of HCTZ. Previous investigators have reported that aldosterone receptor antagonism may increase aldosterone synthesis \((16)\). Significantly, despite activation of the RAAS, treatment with spironolactone did not increase plasma PAI-1 antigen. This differential effect of spironolactone and HCTZ on PAI-1 antigen cannot be attributed to metabolic effects of the drugs, because there was no significant difference in glucose or insulin concentrations between HCTZ and spironolactone treatment days.

In vitro, aldosterone interacts with Ang II to increase PAI-1 expression in human endothelial cells through a MR-dependent mechanism \((8)\). In the present study aldosterone receptor antagonism abolished the relationship between serum aldosterone and plasma PAI-1 antigen concentrations. Taken together, these data suggest that endogenous aldosterone regulates PAI-1 expression in humans.

In a previous study of salt-depleted normotensive volunteers, PAI-1 antigen correlated with serum cortisol as well as aldosterone \((7)\). In addition, dexamethasone induces PAI-1 expression in adipose tissue and vascular smooth muscle cells \((8, 17)\). Because spironolactone can act as a weak GR antagonist as well as a MR antagonist, we cannot exclude the possibility that spironolactone prevented the increase in PAI-1 antigen associated with diuresis through a GR-mediated mechanism. However, the antiguclucocorticoid activity of spironolactone is approximately 100-fold lower than its antimineralocorticoid activity \((18)\).

The finding that endogenous aldosterone contributes to the effect of activation of the RAAS on PAI-1 antigen has important clinical implications. Accumulating data indicate that aldosterone causes myocardial, vascular, and renal fibrosis in animal models \((19–22)\) and that hyperaldosteronism is associated with vascular dysfunction in humans \((23)\). The finding that endogenous aldosterone regulates PAI-1 in humans suggests that aldosterone may contribute to vascular toxicity and fibrosis through effects on PAI-1. Local PAI-1 overproduction at sites of injury or inflammation appears to contribute to the accumulation of provisional matrix \((24)\).
addition, coadministration of an aldosterone receptor antagonist has been shown to decrease mortality in ACE inhibitor-treated patients with congestive heart failure (25). The present study suggests a mechanism by which aldosterone receptor antagonism could reduce death due to thrombotic events.

There are two potential limitations to the present study. First, although both HCTZ and spironolactone increased Ang II and aldosterone concentrations, only spironolactone decreased BP. The differential effects of spironolactone and HCTZ on PAI-1 antigen are unlikely to be explained by differences in the blood pressure response. First, there was no relationship between the change in systolic BP and PAI-1 antigen in this study. Second, we observed a similar effect of HCTZ on PAI-1 antigen in hypertensive subjects in whom HCTZ significantly lowered blood pressure (26). The lack of effect of HCTZ on BP in the present study may reflect a preponderance of individuals with a normal to high renin/sodium index in the study group. Moreover, to the extent that the effect of activation of the RAAS by salt depletion on fibrinolytic balance is greater in individuals with normal to high renin hypertension compared with those with low renin hypertension (27), this study may overestimate the effect of HCTZ on fibrinolytic balance in a hypertensive population at large. Studies are needed to compare the effect of HCTZ on fibrinolytic balance in normal to high and low renin hypertensives.

Second, the fact that potassium concentrations differed among the treatment groups raises the possibility that serum potassium influences fibrinolytic balance. Several lines of evidence contradict the hypothesis that changes in potassium underlie the differential effects of HCTZ and spironolactone on fibrinolytic balance. In particular, there was no correlation between serum potassium concentration and plasma PAI-1 antigen concentration. Although serum magnesium was not measured in the present study, a previous study indicated that magnesium does not affect PAI-1 antigen in patients with acute myocardial infarction (28). More importantly, the finding that PAI-1 antigen correlated significantly with aldosterone concentrations and that spironolactone abolished this relationship provides solid evidence that spironolactone alters the effect of activation of the RAAS on fibrinolytic balance directly through its effects at the aldosterone receptor rather than through alterations in serum potassium.

In summary, activation of the RAAS by HCTZ increased PAI-1 antigen in individuals with essential hypertension. In contrast, spironolactone given at an equipotent dose with respect to activation of the RAAS did not increase the PAI-1 antigen concentration and abolished the relationship between PAI-1 antigen and the serum aldosterone concentration. This study provides evidence that endogenous aldosterone regulates PAI-1 production in humans. It suggests a mechanism by which aldosterone receptor antagonism may reduce the risk of vascular thrombotic events.

Acknowledgments

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