High Serum Level of Procollagen Type III Amino-Terminal Peptide Contributes to the Efficacy of Spironolactone and Angiotensin-Converting Enzyme Inhibitor Therapy on Left Ventricular Hypertrophy in Essential Hypertensive Patients

Atsuhisa SATO, Hiroshi TAKANE, and Takao SARUTA*

We recently demonstrated that spironolactone may have beneficial effects on left ventricular hypertrophy in selected patients with essential hypertension undergoing treatment with an angiotensin-converting enzyme (ACE) inhibitor. To clarify the possible mechanisms by which spironolactone improves cardiac hypertrophy, we investigated the change in serum procollagen type III amino-terminal peptide (PⅢNⅠP) in 11 patients with essential hypertension treated with spironolactone and an ACE inhibitor for 24 weeks. Both blood pressure and serum PⅢNⅠP levels were significantly decreased by treatment. There was a statistical significant correlation between the changes in LVMI and those in PⅢNⅠP. The reduction in PⅢNⅠP was significant in patients whose initial serum PⅢNⅠP levels were above the normal range. Before treatment, there were no statistically significant correlations between serum PⅢNⅠP levels and either LVMI, blood pressure, or plasma aldosterone concentration. Essential hypertensive patients matched in terms of duration of therapy, blood pressure and LVMI and treated with an ACE inhibitor alone showed no change in serum PⅢNⅠP levels. In conclusion, the results of the present study demonstrate that patients with essential hypertension and high serum levels of PⅢNⅠP are particularly responsive to MR blockade in terms of left ventricular hypertrophy. Moreover, these results suggest that spironolactone limits cardiac collagen turnover in such patients. Larger studies may provide definitive evidence for the involvement of aldosterone in left ventricular hypertrophy in patients with abnormally high PⅢNⅠP levels.

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Key Words: mineralocorticoid receptor antagonist, angiotensin-converting enzyme inhibitor, essential hypertension, cardiac fibrosis, serum PⅢNⅠP.

Introduction

In addition to the experimental studies which demonstrated that aldosterone with salt imbalance causes cardiac hypertrophy, and both interstitial and perivascular cardiac fibrosis (1-5), recent clinical studies have shown major effects of aldosterone on the heart (6-8). The Randomized Aldactone Evaluation Study (RALES) clearly demonstrated that administration of the mineralocorticoid receptor (MR) antagonist spironolactone conferred a 30% survival advantage to patients with congestive heart failure (9). Despite angiotensin-converting enzyme (ACE) inhibitor therapy, substantial levels of aldosterone can
often be demonstrated, and RALES showed the potentially harmful effects of residual aldosterone even with ACE inhibitor treatment.

In this regard, we recently demonstrated that spironolactone may have beneficial effects on left ventricular hypertrophy in selected patients with essential hypertension undergoing treatment with an ACE inhibitor (10), although we did not address the possible mechanisms by which spironolactone reversed cardiac hypertrophy. Because a number of studies have shown that fibrosis is the main cardiac effect of administration of aldosterone with excess salt (1-5), we here focused on serum procollagen type III amino-terminal peptide (PIIINP), which is believed to reflect collagen type III synthesis and thus to provide indirect diagnostic information on myocardial fibrosis (11-13), in an attempt to clarify the possible mechanisms through which spironolactone ameliorates cardiac hypertrophy.

Materials and Methods

Subjects and Study Design

The study population consisted of 11 patients with essential hypertension (5 men and 6 women; mean age, 52±8 years), of whom 6 were among the subjects examined in our previous study (10). Blood pressure and heart rate were measured as described previously (7, 8, 10, 14). All patients were withdrawn from medication at least 2 weeks prior to their entry to this study. After obtaining their informed consent, they were started on spironolactone plus an ACE inhibitor (trandolapril). During this study, the dose of spironolactone was constant (25 mg/day), and the dose of the ACE inhibitor was titrated based on changes in blood pressure, with all patients undergoing monthly follow-up throughout the study period (24 weeks). The therapy duration-, blood pressure-, and LVMi-matched hypertensive control group consisted of 8 patients treated with an ACE inhibitor alone (trandolapril).

Biochemical Determinations

General biochemical parameters were measured by routine laboratory methods 3 to 4 h after the daily dosing, and after the patients were allowed to rest in the supine position for at least 30 min. Plasma renin activity and plasma aldosterone concentration were measured with commercially available radioimmunoassay kits as previously reported (Renin Riahead, sensitivity of 0.1-20 ng/ml/h, Dainabot Corporation, Tokyo, Japan; and SPAC-S Aldosterone Kit, sensitivity of 25-1,600 pg/ml, Dai-ichi Radio-isotope, Tokyo) (7, 8, 10, 14). Serum PIIINP was also measured with a commercially available immunoradiometric assay kit that employs a monoclonal antibody to PIIINP with a normal range of 0.3-0.8 U/ml (RIA-ghost PIIIP, CIS Diagnostic Corp., Chiba, Japan).

Echocardiographic Measurement

Echocardiographic studies were performed by the standard method using an SSA-380A echocardiograph with a 3.0 MHz transducer (Toshiba, Japan) according to the recommendations of the American Society of Echocardiography (15) as previously reported (7, 8, 10, 14). Left ventricular (LV) mass was estimated from the formula of Devereux and Reichek (Penn convention) (16): 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.

Statistical Analysis

Data are expressed as the mean±SD, and statistical significance between groups was investigated 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 before and after Treatment with an ACE Inhibitor Plus Spironolactone

The clinical and biological characteristics of all patients are summarized in Table 1. At the end of treatment, both systolic and diastolic blood pressure were significantly reduced compared with baseline values. Plasma renin activity increased significantly after treatment. Plasma aldosterone and serum potassium concentration remained unchanged throughout the study period.

Changes in LVMi and Effects of Spironolactone on Serum PIIINP Levels

After 24 weeks of treatment, LVMi decreased significantly (pretreatment, 132±33 g/m²; post, 108±19 g/m²) (Fig. 1A). IVST and PWT also decreased significantly after treatment, whereas LVDD did not change (data not shown). Cardiac index and ejection fraction did not change (data not shown). Serum PIIINP significantly decreased from 0.67±0.24 to 0.50±0.12 U/ml (Fig. 1B). There was a statistically significant correlation between the changes in LVMi and PIIINP (r=0.63, p=0.04) (Fig. 1C). There was a significant reduction in PIIINP in patients whose initial serum PIIINP levels were above the
Table 1. Clinical and Biological Data of All Patients Treated with an Angiotensin-Converting Enzyme Inhibitor plus Spironolactone

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pre</th>
<th>Post</th>
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<tr>
<td>Men/Women</td>
<td>5/6</td>
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<tr>
<td>Age (years)</td>
<td>52±8</td>
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</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>163±10</td>
<td>137±7*</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>100±21</td>
<td>78±10*</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>74±10</td>
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<tr>
<td>PRA (ng/ml/h)</td>
<td>1.88±1.07</td>
<td>3.61±2.38*</td>
</tr>
<tr>
<td>PAC (pg/ml)</td>
<td>78±24</td>
<td>93±18</td>
</tr>
<tr>
<td>Na (mEq/l)</td>
<td>142.5±1.8</td>
<td>142.3±1.6</td>
</tr>
<tr>
<td>K (mEq/l)</td>
<td>4.1±0.3</td>
<td>4.2±0.3</td>
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<tr>
<td>BUN (mg/dl)</td>
<td>12.5±0.4</td>
<td>12.8±0.5</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.0±0.2</td>
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All values are mean±SD. BP, blood pressure; PRA, plasma renin activity; PAC, plasma aldosterone concentration; BUN, blood urea nitrogen. *, p<0.05 vs. the value before treatment.

normal range (Fig. 2). Before treatment, there were no correlations between serum PIINP levels and either LVMl (r=0.15, p=0.67), blood pressure (systolic; r=0.08, p=0.82, diastolic; r=0.16, p=0.64), or plasma aldosterone concentration (r=0.05, p=0.87). These results suggest that collagen metabolism is one of the major factors contributing to cardiac hypertrophy, but not the only factor to reflect it. Table 2 shows data for the essential hypertensive control patients; these patients received an ACE inhibitor alone and were matched with the experimental subjects in terms of therapy duration, blood pressure, and LVMl. Although blood pressure and LVMl significantly decreased after treatment, the change in serum PIINP level was not significant (Fig. 3A, B).

Discussion

Our results strongly suggest that the beneficial effects of spironolactone shown in our previous study (10) may be explained, at least in part, by the limitation of extracellular collagen turnover in patients with essential hypertension. Moreover, this study showed that patients with initial high serum levels of PIINP appeared to benefit more from MR blockade in terms of left ventricular hypertrophy. This assessment may be clinically important, since at present there are no available data for helping to determine which patients with essential hypertension should be given spironolactone to halt or reverse cardiac hypertrophy.

We have previously reported that adding a low dose of spironolactone to an ACE inhibitor in patients with essential hypertension reversed left ventricular hypertrophy more effectively than treatment with an ACE inhibitor alone (10). We also demonstrated that this effect of spironolactone occurred independently of changes in blood pressure, although the mechanism underlying the additional efficacy of spironolactone remained to be clarified. Spironolactone is a very effective diuretic, and it is possible that spironolactone acts by improving natriuresis and reducing cardiac pre- and afterload, and in this way improving cardiac hypertrophy. However, the results of our previous study (10) showed that IVST and PWT decreased significantly whereas LVd did not change, suggesting that the diuretic effects of spironolactone were not significant for the heart. Moreover, the low dose of spironolactone (25 mg/day) used also suggests that the beneficial effect of MR blockade was not due to a hemodynamic effect.

Aldosterone has been shown to cause progressive car-
Table 2. Clinical and Biological Data of Patients Treated with an Angiotensin-Converting Enzyme Inhibitor Alone

<table>
<thead>
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<td>Men/Women</td>
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<tr>
<td>Age (years)</td>
<td>54±9</td>
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</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>160±12</td>
<td>136±10*</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>101±18</td>
<td>81±10*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>72±9</td>
<td>71±8</td>
</tr>
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</table>

All values are mean ± SD. BP, blood pressure. *, p<0.05 vs. the value before treatment.

Fig. 2. Changes in serum procollagen type III amino-terminal peptide (PIIINP) level before and after antihypertensive treatment in patients whose initial serum PIIINP levels were within the normal range (closed circles) and those in whom they were above the normal range (open circles).

Cardiac fibrosis with elevation of both type III and I procollagen in both ventricles (3). Fibrillar type III and I collagen constitute the majority of total collagen found in the myocardium (17). In experimental studies, it has been clearly demonstrated that MR antagonists halved the increase in blood pressure and cardiac hypertrophy and completely reversed the aldosterone-induced increase of cardiac collagen (4, 5). Given these findings, it is possible that spironolactone reduces cardiac collagen turnover, and thus causes reduction in LVMi in patients with essential hypertension. To demonstrate this clinically, it is preferable to develop noninvasive methods indicating the presence of myocardial fibrosis. Serum PIIINP levels have been proposed as a useful marker of collagen type III synthesis (11-13), and a close correlation between serum PIIINP level and the amount of myocardial collagen type III has been shown in cardiac biopsy specimens from patients with heart failure (17). In patients with essential hypertension, serum PIIINP levels are reported to be elevated (12, 18), and our present study shows for the first time that aldosterone, either systemically or locally, may be involved in the elevation of serum PIIINP levels and left ventricular hypertrophy in patients with essential hypertension. A number of studies have suggested that aldosterone does not exert a direct effect on collagen synthesis in cardiac fibroblast (19, 20); therefore, spironolactone may have inhibited the indirect effects of aldosterone in terms of cardiac fibrosis in the present study.

Angiotensin II (Ang II) has been shown to be an important mediator of cardiovascular hypertrophy (21), and ACE inhibitors have been reported to reverse cardiac hypertrophy (22). Nevertheless, animal studies from several laboratories have clearly shown that a component of cardiac fibrosis is mineralocorticoid specific (2, 4), with one study showing independent effects of Ang II and aldosterone on cardiovascular hypertrophy in rats (23). It is of interest that, in agreement with our present results, two previous studies reported that the change in serum

Fig. 3. Changes in left ventricular mass index (LVMi) (A) and serum procollagen type III amino-terminal peptide (PIIINP) level (B) before and after antihypertensive treatment with an angiotensin-converting enzyme inhibitor alone in patients with essential hypertension. Data represent the mean ± SD. *, p<0.05 vs. baseline value.
PIIINP level was not significant in patients treated with an ACE inhibitor alone (24, 25), even though ACE inhibitors have been shown to reverse cardiac hypertrophy. The present studies strongly suggest that even if an ACE inhibitor produces a significant decrease in plasma Ang II levels, this may not play a major clinical role in the cardiac fibrosis seen in patients with essential hypertension or heart failure, and Ang II and aldosterone may participate in cardiac hypertrophy in a very different manner. Therefore, it seems important to block aldosterone-induced effects on the heart in patients with essential hypertension who are receiving an ACE inhibitor. The present study showed that treatment with spironolactone was associated with a reduction in serum levels of PIIINP and a decrease of LVMI. Considered together, these results suggest that, in patients with essential hypertension, major inhibition of extracellular collagen turnover can be attained by adding spironolactone to the treatment protocol.

We have previously shown that high glucose levels potentiate the effects of aldosterone on cultured rat cardiomyocytes (26), suggesting that even if plasma aldosterone levels are only marginally elevated, the effects of aldosterone on the heart may be augmented under certain conditions. Recently, Delacaye et al. (27) demonstrated the production of aldosterone in rat hearts, and in their subsequent study they showed that spironolactone effectively attenuates reactive fibrosis in the viable myocardium of the postinfarcted left ventricle of rats (28), an important finding because that model is not characterized by elevated plasma aldosterone levels. Benetos et al. (29) also showed that spironolactone could reduce aortic fibrosis and improve vascular compliance in the SHR with normoaldosteronemia, demonstrating that aldosterone can play a pathophysiological role even when plasma aldosterone levels are in the normal range. Given these findings, it is conceivable that the effect of the low dose of spironolactone on the cardiac collagen turnover observed in this study primarily reflects blockade of aldosterone of cardiac origin. In previous study plasma aldosterone levels have been reported to be higher in patients with essential hypertension than in controls (6), whereas in most patients with essential hypertension plasma aldosterone levels are only marginally elevated or within the normal range. Nevertheless, considering recent studies which have suggested the importance of cardiac rather than circulating aldosterone, attenuation of the effects of cardiac aldosterone in patients with essential hypertension whose plasma aldosterone levels are within the normal range may become a new goal for the prevention and regression of cardiac fibrosis.

In conclusion, the results of the present study demonstrated that patients with essential hypertension and high serum levels of PIIINP are particularly responsive to MR blockade in terms of left ventricular hypertrophy. These results also suggest that spironolactone limits cardiac collagen turnover in such patients. Larger studies may provide even more definitive evidence for the involvement of aldosterone in left ventricular hypertrophy in patients with abnormally high PIIINP levels.

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References


