Effectiveness of Aldosterone Blockade in Patients With Diabetic Nephropathy

Atsuhisa Sato, Koichi Hayashi, Mitsuhide Naruse, Takao Saruta

Abstract—it has been reported that continuous ACE inhibitor therapy does not necessarily produce a maintained decrease in plasma aldosterone levels, which may remain high or increase eventually during long-term use (aldosterone escape). We have examined the role of aldosterone escape in 45 patients with type 2 diabetes and early nephropathy treated with an ACE inhibitor for 40 weeks. With treatment, there was a 40% reduction in average urinary albumin excretion, although urinary albumin excretion in patients with aldosterone escape (18 patients) was significantly higher than that in patients without escape (27 patients). In 18 patients with escape, spironolactone (25 mg/d) was added to ACE inhibitor treatment in 13. After a 24-week study period, urinary albumin excretion and left ventricular mass index were significantly reduced without blood pressure change. In conclusion, the present study demonstrates that aldosterone escape is observed in 40% of patients with type 2 diabetes with early nephropathy despite the use of ACE inhibitors. Our study suggests the possibility that aldosterone blockade may represent optimal therapy for patients with early diabetic nephropathy who show aldosterone escape during ACE inhibitor treatment and who no longer show maximal antiproteinuric effects of ACE inhibition. Additional, larger, prospectively randomized, double-blind studies will be needed before adaptation of this strategy. (Hypertension. 2003;41:64-68.)

Key Words: aldosterone □ angiotensin-converting enzyme □ diabetic nephropathy hemodialysis □ hypertrophy □ ventricular function, left

Aldosterone is the principal physiological mineralocorticoid, and its effects on electrolyte transport through epithelial mineralocorticoid receptors (MR) are well characterized.1 Over the past decade, in addition to the action of aldosterone in epithelial target tissues, there is increasing evidence for major cardiovascular effects of aldosterone through classic MR in nonepithelial tissues, such as brain and heart.2,3 In this context, clinical interest in the cardiovascular effects of aldosterone has markedly increased in recent years. The results of the Randomized Aldactone Evaluation Study (RALES) strongly suggest that attenuation of the effects of aldosterone on the heart by antialdosterone therapy may become a new goal for prevention and even regression of heart failure.4

Although a crucial role of aldosterone in the cardiovascular system in patients with essential hypertension remains to be determined, we have recently shown that plasma aldosterone levels tend to increase with duration of an ACE treatment (aldosterone escape)5 and may reverse the beneficial effects of ACE inhibition on left ventricular (LV) hypertrophy in patients with essential hypertension.6 Moreover, we have shown that adding the MR antagonist spironolactone to ACE inhibitor treatment has beneficial effects, which may be explained at least in part by the limitation of extracellular collagen turnover,7 on LV hypertrophy in selected patients with essential hypertension.8 These studies suggest that treatment with an ACE inhibitor to suppress aldosterone synthesis is not adequate and that aldosterone blockade in addition to ACE inhibition has additional benefit in the prevention of organ damage.

There is also recent evidence that the humoral actions of aldosterone have clinical implications for the pathogenesis of progressive renal disease.9 A number of studies have raised the possibility that aldosterone-induced vasculitis may underlie progressive renal disease and indicate that aldosterone may promote deleterious effects on both the cardiovascular system and the kidneys.9,10,11 Diabetic nephropathy has become the leading cause of end-stage renal disease in many countries, and early identification and subsequent renoprotective treatment are thus of utmost importance. In this context, it has been established that ACE inhibitors are of specific benefit not only in reducing proteinuria but in retarding the progression of diabetic nephropathy.12 Recently, however, it has been reported that although the use of ACE inhibitors may be beneficial for patients with nondiabetic renal diseases, approximately half of these patients were improved only at the beginning of treatment and subsequently escaped from antiproteinuric effects of an ACE inhibitor.13

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TABLE 1. Clinical Data of All Patients

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<thead>
<tr>
<th>Characteristic</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
</tr>
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<tbody>
<tr>
<td>Men/women</td>
<td>25/20</td>
<td></td>
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<tr>
<td>Age, y</td>
<td>62±13</td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>150±15</td>
<td>135±9*</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>89±14</td>
<td>83±10*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>74±3</td>
<td>72±2</td>
</tr>
<tr>
<td>Na, mEq/L</td>
<td>142.3±1.4</td>
<td>142.4±1.3</td>
</tr>
<tr>
<td>K, mEq/L</td>
<td>4.2±0.3</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>12.3±2.0</td>
<td>12.8±1.2</td>
</tr>
<tr>
<td>Cr, mg/dL</td>
<td>0.84±0.25</td>
<td>0.89±0.30</td>
</tr>
<tr>
<td>UAE, mg/g Cr</td>
<td>389±109</td>
<td>233±89*</td>
</tr>
<tr>
<td>PRA, ng/mL per hour</td>
<td>1.53±1.00</td>
<td>3.52±2.00*</td>
</tr>
<tr>
<td>PAC, pg/mL</td>
<td>83.7±20.1</td>
<td>85.0±18.4</td>
</tr>
<tr>
<td>HbA1c</td>
<td>7.4±0.4</td>
<td>7.1±0.4</td>
</tr>
<tr>
<td>History of diabetes, y</td>
<td>9.6±1.0</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 vs the value in pretreatment.

In the present study, we have extended our previous studies\(^6,8\) and addressed two issues particular. First was the possibility that aldosterone escape may occur in long-term treatment with an ACE inhibitor in patients with diabetic nephropathy, and, if so, whether such a escape may influence the clinical effects of an ACE inhibitor, and whether escape may play a role in the late escape from the antiproteinuric effect of an ACE inhibitor. Second, we explored the effect of spironolactone in addition to an ACE inhibitor on cardiovascular and renal function in patients with diabetic nephropathy who showed aldosterone escape during ACE inhibitor treatment.

Methods

Subjects and Study Design

Forty-five outpatients with type 2 diabetes and early nephropathy (25 men and 20 women; age, 62±13 years) participated in this study (Table 1). Patient histories and laboratory and ophthalmologic examination largely determined the diagnosis of nephropathy associated with type 2 diabetes. Early nephropathy was defined in this study by the presence of either microalbuminuria with a ratio of urinary albumin to creatinine excretion (UAE) of 30 to 300 mg/g creatinine, or overt proteinuria (UAE>300 mg/g creatinine), with a 24-hour creatinine clearance >60 mL/min. Blood pressure was measured with a mercury sphygmomanometer after at least 15 minutes of rest in the sedentary position and was determined by averaging 3 consecutive measurements. Heart rate was obtained from the radial pulse over a period of 30 seconds. Medication with antihypertensive agents was withdrawn at least 2 weeks before entry into this study. Patients were started on an ACE inhibitor (trandolapril) at a minimal dose, with the dose titrated against the blood pressure response every 2 to 4 weeks, toward a goal for 130/85 mm Hg.\(^14,13\) Spironolactone was added to ACE inhibitor treatment in patients with aldosterone escape at 40 weeks, after obtaining informed consent. Based on earlier reports\(^4\) and our previous studies,\(^7,8\) the dose of spironolactone was fixed (25 mg/d), and treatment with spironolactone and an ACE inhibitor was followed up over the subsequent 24-week study period. The study protocol was approved by the Committee on Medical Research Ethics of Mito Red Cross Hospital.

General biochemical parameters were measured by routine laboratory methods. Plasma renin activity and aldosterone concentrations were measured by commercial radioimmunoassay after the patients were in supine position for at least 30 minutes\(^5,6\); assay sensitivity was 0.1 to 20 ng/mL per hour (Renin Riabead, Dainabot Corporation) and 25 to 1600 pg/mL (SPAC-S Aldosterone Kit, Dai-ichi Radio-isotope). Plasma renin activity and plasma aldosterone concentrations are presented as the average of 2 time points, and aldosterone escape was defined by an increased value compared with the value pretreatment. A random urine sample for determination of protein and creatinine concentrations was also obtained. When properly interpreted, the results of a measurement of protein and creatinine in a single voided urine sample can provide information that for clinical purposes is a satisfactory substitute for the determination of protein excretion in a 24-hour urine collection.\(^16–18\)

Random urine samples were taken twice, and values were averaged. Diabetes was relatively well controlled by medical therapy. Ten patients were treated by dietary therapy alone, 30 patients with oral hypoglycemic drugs and/or α-glucosidase inhibitors, and 5 patients with insulin. During treatment, all patients were instructed on dietary therapy for diabetes with appropriate protein restriction (1.0 to 1.2 g/kg per day for patients with microalbuminuria; 0.8 to 1.0 g/kg per day for patients with overt proteinuria) and salt restriction (7 to 8 g/d)

Echocardiographic Measurements

Echocardiographic studies were performed by standard methods with an SSA-380A echocardiograph with a 3.0-MHz transducer (Toshiba), according to the recommendations of the American Society of Echocardiography.\(^19\) LV mass was estimated from the formula of Devereux and Reichek (Penn convention)\(^20\): 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 LV mass index (LVM) was calculated for each subject by dividing LV mass by body surface area.

Statistical Analysis

Data are expressed as mean±SD. Statistical significance was evaluated by 1-way or 2-way ANOVA with repeated measures, as appropriate. Changes in parameters in each group before and after treatment were compared by 2-group, paired t tests, with probability values of <0.05 taken as significant.

Results

Clinical and Biological Data for All Patients Before and After ACE Inhibitor Treatment

The clinical and biological characteristics of all patients are summarized in Table 1. After 40 weeks, both systolic and diastolic blood pressures were significantly reduced compared with baseline values, and plasma renin activity significantly increased. Plasma aldosterone concentrations, serum potassium, renal function, and plasma glucose control remained unchanged. There was an approximate 40% reduction in average UAE (before, 389±109; after, 233±89 mg/g creatinine, \(P<0.05\)).

Clinical Data of Patients With or Without Aldosterone Escape at 40 Weeks

Although overall plasma aldosterone concentrations did not change after treatment with an ACE inhibitor for 40 weeks, they eventually increased in 18 of 45 patients (40%; aldosterone escape), whereas plasma aldosterone concentrations fell in the remaining 27 patients (60%). Because the aim of the present study was to determine whether aldosterone escape
participates in the occurrence of escape from the antiproteinuric effect of an ACE inhibitor, we compared clinical data of patients with aldosterone escape with those of patients without. Age, blood pressure (both systolic and diastolic), renal function, electrolytes, and diabetes control did not differ between the two groups at 40 weeks. In contrast, UAE in patients with aldosterone escape was significantly higher than that in patients without (Table 2). Mean values for LVMI were higher in patients with aldosterone escape than in those without, although the difference was not statistically significant (138 ± 110 vs. 110 ± 17 g/m²). The dose of trandolapril was 1.5 ± 0.4 mg/d in patients with escape and 1.4 ± 0.2 mg/d in patients without, with no significant differences between the groups. Furthermore, we found that aldosterone escape was also observed in patients treated with the maximal dose of trandolapril in this study (2.0 mg/d), which suggests that even higher doses of trandolapril could not eliminate escape phenomenon. Next, dietary sodium and potassium are very important to determine plasma levels of aldosterone. Therefore, first of all, the patients were instructed to follow an appropriate dietary therapy for diabetes, with salt restriction as described previously. Furthermore, to assess dietary sodium and potassium intake and how such intake affects the aldosterone escape phenomenon, we measured urinary sodium and potassium in 24-hour urine after treatment with trandolapril for 40 weeks. As shown in Table 2, there were no significant differences in the urinary sodium and potassium excretion between the two groups.

**Effect of Spironolactone on UAE and LVMI in Patients With Aldosterone Escape**

Among the 18 patients with aldosterone escape, UAE in 3 patients was significantly decreased after ACE inhibitor treatment at 40 weeks but not for other 15. After explaining the purpose of this study, 13 patients (6 men, 7 women) from the remaining 15 patients agreed to take spironolactone in addition to ACE inhibitor treatment, and spironolactone (25 mg/d) was added to the ACE inhibitor treatment. Although blood pressure did not change, LVMI were significantly reduced after a 24-week study period (Figure 1), and UAE was also significantly reduced (Figure 2). Serum potassium remained unchanged throughout the combination therapy (before, 4.2 ± 0.3 mEq/L; after, 4.3 ± 0.2 mEq/L). No patients dropped out of this study.

**Discussion**

In the first part of this study, we showed that UAE in patients with aldosterone escape is significantly higher than that in

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**TABLE 2. Clinical Data of Patients With or Without Aldosterone Escape**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Escape (−)</th>
<th>Escape (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (men/women)</td>
<td>27 (15/12)</td>
<td>18 (10/8)</td>
</tr>
<tr>
<td>Age, y</td>
<td>64±12</td>
<td>61±10</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>136±13</td>
<td>135±12</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>84±11</td>
<td>83±10</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>73±3</td>
<td>72±3</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>4.3±0.2</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>13.5±1.8</td>
<td>12.8±1.3</td>
</tr>
<tr>
<td>Cr, mg/dL</td>
<td>0.88±0.25</td>
<td>0.90±0.20</td>
</tr>
<tr>
<td>UAE, mg/g Cr</td>
<td>119±95</td>
<td>368±142*</td>
</tr>
<tr>
<td>PRA, ng/mL per hour</td>
<td>3.98±1.66</td>
<td>3.15±1.58</td>
</tr>
<tr>
<td>PAC, pg/mL</td>
<td>53.2±15.1</td>
<td>112.0±18.7*</td>
</tr>
<tr>
<td>HbA1c</td>
<td>7.2±0.4</td>
<td>7.1±0.3</td>
</tr>
<tr>
<td>Urinary sodium excretion, mEq/d</td>
<td>158±33</td>
<td>149±34</td>
</tr>
<tr>
<td>Urinary potassium excretion, mEq/d</td>
<td>44±8</td>
<td>42±10</td>
</tr>
</tbody>
</table>

All values are mean ± SD. (−) indicates group without aldosterone escape; (+), group with aldosterone escape.

*P < 0.05 vs the value in the group without aldosterone escape.
patients without. In the second part, although our data were obtained in a small sample, we demonstrated that adding spironolactone to treatment of with an ACE inhibitor is clinically useful and safe for patients with aldosterone escape.

Three aspects of this study are worthy of analysis. One is that aldosterone escape was detected in 40% of patients with early diabetic nephropathy. Escape of aldosterone production despite ACE inhibition has been shown in patients with hypertension, chronic heart failure, and those with acute myocardial infarction. We have previously shown that aldosterone escape during ACE inhibition treatment occurred in 46% of patients with essential hypertension and to a very similar extent to that in the present study. In terms of the mechanisms of aldosterone escape, we previously reported that changes in blood pressure, electrolytes, and plasma renin activity during treatment with an ACE inhibitor did not differ between patients with and without escape, suggesting that such breakthrough might occur independent of blood pressure control or plasma renin activity.

Moreover, we directly demonstrated in a subsequent study that plasma aldosterone concentrations are not related to the degree of ACE inhibition in patients with essential hypertension. In this regard, Tang et al demonstrated that even at maximal doses of enalapril, elevated plasma aldosterone level was frequently observed despite a dose-dependent reduction in serum ACE activity in patients with chronic heart failure. Given that the dose of trandolapril was similar between the patients with and without escape in this study, aldosterone escape phenomenon is not due to incomplete suppression of ACE activity. In contrast, Ciccoira et al recently reported that failure of aldosterone suppression despite ACE inhibitor administration in patients with chronic heart failure is associated with ACE DD genotype and concluded that different ACE genotypes might partially account for the different degree of aldosterone suppression during long-term ACE inhibitor therapy. Nevertheless, definition of aldosterone escape in their study was quite different from ours and thus only 10% of patients with aldosterone escape. In this study, we also assessed whether dietary sodium and potassium may affect the aldosterone escape phenomenon. Although we found some interpatient variation in urinary sodium and potassium excretion, there was no significant difference between plasma and urinary electrolyte concentrations. Further studies, both clinical and fundamental, are needed to determine the mechanisms.

The recent RALES trial clearly showed the clinical relevance and benefit of the blockade of the effects of aldosterone by the MR antagonist in patients with congestive heart failure. The second aspect of the present study is the demonstration that LVMI and UAE were significantly reduced after a 24-week treatment with spironolactone and an ACE inhibitor in patients with diabetic nephropathy and aldosterone escape during ACE inhibitor therapy. Recently, Chrysostomou and Becker published compelling data showing that spironolactone in addition to an ACE inhibitor reduced proteinuria in patients with chronic renal diseases, including diabetic nephropathy. They evaluated 8 patients whose proteinuria was persistently over 1 g/d despite treatment with enalapril for >12 months. They prescribed them spironolactone at the dose of 25 mg/d in addition to enalapril and after 4 weeks, they observed a 54% reduction of protein excretion. They suggested that spironolactone therapy might be useful for patients with proteinuria and renal impairment who still have proteinuria after treatment with an ACE inhibitor. Because they showed neither the renin-angiotensin-aldosterone profile nor the reason they administered enalapril for 12 months, the participation of aldosterone escape was uncertain. Arutyunov et al also demonstrated that combination therapy with an angiotensin II receptor antagonist and spironolactone showed a potent naphroprotective effect as compared with that of the angiotensin II receptor antagonist alone.

It has also been shown that, in experimental models of diabetes, spironolactone reduced blood pressure and partially reversed the decrease in expression and activity of renal 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). In this regard, we have previously demonstrated that high glucose levels potentiate the effects of aldosterone on leucine incorporation by neonatal rat cardiomyocytes in culture, indicating that the effects of aldosterone on the heart may be augmented under hyperglycemic conditions. It is possible that aldosterone receptor blockade may have particular clinical efficacy in terms of prevention of organ damage in patients with hyperglycemia.

The third aspect concerns the dose of spironolactone used in the present study. In experimental studies, cardiac effects of aldosterone, those mediated by non-epithelial MR, have been shown to be completely blocked by concomitant administration of the MR antagonist at a dose that only modestly lowers blood pressure. Given the absence from heart of 11β-HSD2, cardiac MR in vivo are presumably overwhelmingly occupied by glucocorticoids. Nevertheless, occupancy by aldosterone of such unprotected MR causes cardiac fibrosis and hypertrophy in rats. It is therefore possible that lower doses of MR antagonist to block aldosterone binding to such a small percentage of unprotected MR may arrest or reverse deleterious cardiac effects of aldosterone. We previously demonstrated that 25 mg daily spironolactone may have beneficial effects on LV hypertrophy in selected patients with essential hypertension. In contrast, classic effects of aldosterone such as ion transport and salt/water balance are thought to be mediated by epithelial MR. This study shows that 25 mg spironolactone daily reduces proteinuria, although we did not perform an accurate dose-dependent study. Whether this beneficial effect of spironolactone concerning renal protection mediated blocking either epithelial or non-epithelial MR in the kidneys awaits further studies.

Finally, our study has several limitations (small sample size, lack of randomization, or blinded design). In addition, in terms of statistical power to determine a significant difference in UAE or LVMI in this study, it would have been preferable to set a control group that showed aldosterone escape after a 40-week treatment with trandolapril and without spironolactone. Therefore, additional, larger, prospectively randomized, double-blind studies will be needed before adaptation of this strategy.

In conclusion, adding spironolactone to ACE inhibitor therapy may have beneficial effects in patients with diabetic
nephropathy. Our study suggests the possibility that attenuation of the aldosterone effects may become a new goal for patients with early diabetic nephropathy who show aldosterone escape during ACE inhibitor treatment and had escaped antiproteinuric effect of an ACE inhibitor.

Perspectives
Although our study has several limitations, we showed that UAE in patients with aldosterone escape is significantly higher than that in patients without. Moreover, we demonstrated that adding spironolactone to ACE inhibitor treatment is clinically useful and safe for patients with early diabetic nephropathy who show aldosterone escape during ACE inhibitor treatment and who no longer show maximal antiproteinuric effects of ACE inhibition. Whether this beneficial effect of spironolactone concerning renal protection mediated blocking either epithelial or nonepithelial MR in the kidneys awaits further studies. Additional, larger, prospectively randomized, double-blind studies will be needed before adaptation of this strategy.

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References
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