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Antiproteinuric Effects of Mineralocorticoid Receptor Blockade in Patients With Chronic Renal Disease

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We have recently shown that mineralocorticoid receptor blockade may represent optimal therapy for patients with early diabetic nephropathy who show aldosterone breakthrough during angiotensin-converting enzyme (ACE) inhibitor treatment, and who no longer show the maximal antiproteinuric effects of ACE inhibition. In this study, we explored the effects of the mineralocorticoid receptor antagonist spironolactone on urinary protein excretion in patients with chronic renal disease with proteinuria persistently more than 0.5 g/d, despite maintained blood pressure (BP) control, and including the use of an ACE inhibitor (trandolapril) for at least 10 months. After a 12-week study period of spironolactone treatment (25 mg/d), BP did not change but urinary protein excretion was significantly reduced. The extent of the reduction was on average significantly greater in diabetic patients than in nondiabetics. In patients with diabetic nephropathy, although urinary type IV collagen did not decrease after conventional treatment, it was significantly reduced by spironolactone. None of the patients developed serious hyperkalemia, and no other adverse events were observed. All patients in this study had relatively well preserved renal function. In conclusion, the present study demonstrates that in patients with chronic renal disease with proteinuria persistently more than 0.5 g/d, despite BP control and the use of an ACE inhibitor, adding spironolactone to the conventional treatment produces beneficial effects on urinary protein excretion, particularly in patients with diabetes. Our study suggests that attenuation of mineralocorticoid receptor-mediated effects may become a new goal for patients who escape the antiproteinuric effects of the conventional treatment. Additional, larger, prospective, randomized double-blind studies will be needed for general acceptance of this strategy. Am J Hypertens 2005;18:44–49 © 2005 American Journal of Hypertension, Ltd.

Key Words: Aldosterone, mineralocorticoid receptor blockade, chronic renal disease, diabetic nephropathy, angiotensin-converting enzyme inhibitor.

Aldosterone is synthesized in the outer cortex of the adrenal gland and acts through epithelial mineralocorticoid receptors (MR) to promote unidirectional sodium and water transport.[1] Recently, there has been increasing evidence that aldosterone exerts major cardiovascular effects through classic MR in nonepithelial tissues, such as brain and heart.[2,3] This nonepithelial role of aldosterone has been underscored by the recent Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS).[4,5] Despite the use of angiotensin-converting enzyme (ACE) inhibitors or angiotensin II (Ang II) type 1 receptor blockers (ARB), unblocked aldosterone levels remain an important risk factor for cardiovascular disease progression (aldosterone breakthrough).[6]

There is also recent evidence that the humoral actions of aldosterone have clinical implications for the pathogenesis of progressive renal disease.[7,8] A number of studies have raised the possibility that aldosterone-induced vasculopathy may underlie progressive renal disease, and indicate that aldosterone has deleterious effects on both the cardiovascular system and the kidneys.[9–11] In this regard, we have recently shown that aldosterone blockade may represent optimal therapy for patients with early diabetic nephropathy who show aldosterone breakthrough during ACE inhibitor treatment, and who no longer show maximal antiproteinuric effects of ACE inhibition.[12] In that study, we also demonstrated that such renoprotective effects of aldosterone blockade are independent of changes in blood pressure (BP).

In the present study we have extended our previous

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Table 1. Clinical data of all patients (value in preconventional treatment)

Characteristics	Diabetic	Nondiabetic
Men/women	9/8	8/7
Age (y)	63 ± 2	52 ± 6
Systolic BP (mm Hg)	136 ± 2* (156 ± 5)	135 ± 2* (152 ± 6)
Diastolic BP (mm Hg)	81 ± 2* (93 ± 5)	79 ± 2* (92 ± 4)
Heart rate (/min)	73 ± 3 (73 ± 3)	74 ± 2 (72 ± 3)
Na (mEq/L)	142.3 ± 0.8 (141.6 ± 0.9)	142.4 ± 0.6 (142.0 ± 0.8)
K (mEq/L)	4.2 ± 0.2 (4.1 ± 0.3)	4.3 ± 0.2 (4.2 ± 0.2)
BUN (mg/dL)	15.9 ± 2.0 (14.3 ± 2.0)	14.8 ± 1.2 (14.3 ± 2.2)
Cr (mg/dL)	0.88 ± 0.25 (0.83 ± 0.20)	0.87 ± 0.30 (0.85 ± 0.24)
UPE (mg/d)	1180 ± 101 (1225 ± 149)	1142 ± 203 (1222 ± 188)
PRA (ng/mL/h)	2.93 ± 0.72	2.52 ± 0.82
PAC (pg/mL)	82.7 ± 7.3	89.3 ± 6.1
HbA1c (%)	7.2 ± 0.3* (7.9 ± 0.3)	5.1 ± 0.1
History of diabetes (y)	9.7 ± 0.8	—
Type IV collagen (μg/g Cr)	7.5 ± 0.6 (8.1 ± 0.8)	—
β2-MG (μg/L)	153 ± 45 (143 ± 40)	142 ± 41 (148 ± 49)
NAG (U/L)	6.4 ± 0.5 (7.2 ± 0.8)	6.2 ± 0.9 (6.6 ± 0.7)
24-h Ccr	89.1 ± 3.8 (93.2 ± 4.8)	94.0 ± 3.4 (97.2 ± 4.4)
Antihypertensive drugs		
ACE inhibitor	17 (/17)	15 (/15)
CCB	12	11
Diuretic	7	4
α1-blocker	4	4

All values are mean ± SE. BP = blood pressure; BUN = blood urea nitrogen; CCB = calcium channel blocker; Ccr = creatinine clearance; Cr = creatinine; MG = microglobulin; NAG = *N*-acetyl-glucosaminidase; PAC = plasma aldosterone concentration; PRA = plasma renin activity; UPE = urinary protein excretion.

* <0.05 *v* the value in preconventional treatment.

study[12] and explored the effects of the MR antagonist spironolactone on urinary protein excretion in patients with chronic renal disease with proteinuria persistently more than 0.5 g/d, despite maintained BP control and the use of an ACE inhibitor for at least 10 months.

Methods

Subjects and Study Design

Thirty-two outpatients with diabetic (17 patients) and nondiabetic (15 patients) renal disease participated in this study (Table 1). For those patients with diabetic nephropathy, patient histories plus laboratory and ophthalmologic examination largely determined the diagnosis of nephropathy associated with type 2 diabetes. The diagnosis of nondiabetic renal disease was based on renal biopsy findings (IgA nephropathy, 10 patients; focal segmental glomerulosclerosis, 3 patients; membranous nephropathy, 2 patients), and none of patients had received steroid treatment. Blood pressure was measured with a mercury sphygmomanometer after at least 15 min of rest in a seated position, and was determined by averaging three consecutive measurements.[13,14] Heart rate was obtained from the radial pulse during 30 sec. 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 min[12–14]; assay sensitivity was 0.1 to 20 ng/mL per hour (Renin Riabead, Dainabot Corporation, Tokyo, Japan) and 25 to 1600 pg/mL (SPAC-S Aldosterone Kit, Dai-ichi Radio-isotope, Tokyo, Japan). Urinary protein excretion, *N*-acetyl-glucosaminidase (NAG) and β2-microglobulin (β2-MG) were determined by 24-h urine collection. In patients with diabetic nephropathy, urinary type IV collagen was measured by a random urine sample for protein and creatinine concentrations by an enzyme immunoassay method.[12] NAG and β2-MG were measured by latex agglutination and colorimetry, respectively. Exclusion criteria were more severe renal dysfunction, renal artery stenosis, ischemic heart disease, therapy with steroids or cytotoxic drugs, and other systemic severe diseases.

All 32 patients in this study had persistent proteinuria of more than 0.5 g/d (11 of 17 diabetic patients and 10 of 15 nondiabetic patients were more than 1 g/d) after medical treatment for 10 to 14 months, including an ACE inhibitor (trandolapril) and BP control. Spironolactone was added to the treatment after obtaining informed consent. On the basis of an earlier report[4] and our previous studies,[12,14] the dose of spironolactone was fixed (25 mg/d), and treatment with spironolactone followed up for a subsequent 12-week study period. The study protocol was approved by the Committee on Medical Research Ethics of Mito Red Cross Hospital. Diabetes was controlled by medical therapy in 14 patients with oral hypoglycemic drugs or α-glucosidase inhibitor, and 3 patients with in-

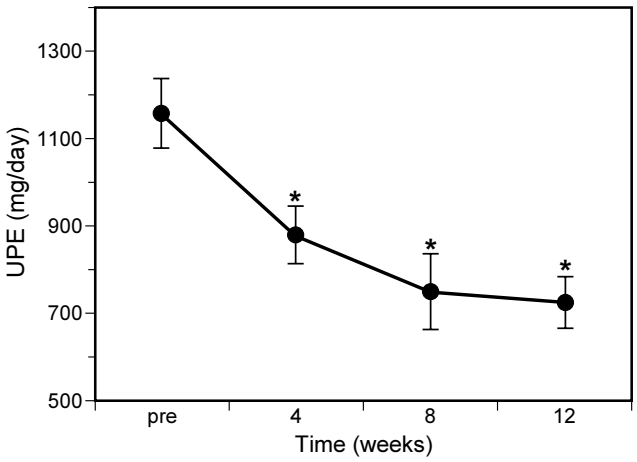

FIG. 1 Change in urinary protein excretion (UPE) during treatment with spironolactone. Data represent the mean ± SE. *$P < .05$ *v* baseline value.

sulin. During treatment, all patients were instructed on dietary therapy with appropriate protein restriction (0.8 to 1.0 g/kg per day) and salt restriction (7 to 8 g/d).

Statistical Analysis

Data are expressed as mean ± SE. Statistical significance was evaluated by one-way or two-way ANOVA with repeated measures, as appropriate. Changes in parameters in each group before and after treatment were compared by two-group paired *t* tests, with *P* values of $< .05$ taken as significant.

Results

Clinical and Biological Data for all Patients Before Spironolactone Treatment

The clinical and biological characteristics of all patients before spironolactone treatment are summarized in Table 1. After at least 10 months of ACE inihibitor-based treatment, both systolic and diastolic BPs were significantly reduced compared with baseline. Nevertheless, all 32 patients in this study had persistent proteinuria levels of more than 0.5 g/d, and no longer showed the maximal antiproteinuric effects of such treatment. The mean estimate glomerular filtration rate was 93 ± 5 mL/min, with no patient having less than 85 mL/min. Baseline usage of antihypertensive drugs is also shown in Table 1, and final average doses of trandolapril used were 1.8 ± 0.2 mg/d in the diabetic group and 1.7 ± 0.2 mg/d in nondiabetic group. No patients were on β-blocker. Glucose control for diabetes has been shown to be important in preventing the progression of diabetic nephropathy, and glucose levels were relatively well controlled in the patients studied.

Effects of Spironolactone on Urinary Protein Excretion and BP

After the purpose of this study was explained, and informed consent obtained, spironolactone (25 mg/d) was added to the current medical treatment. After a 12-week study period, although BP did not change, urinary protein excretions were significantly reduced (before spironolactone treatment: 1162 ± 77 mg/d; after: 722 ± 58 mg/day; $P < .05$). The mean reduction of urinary protein excretion was significantly greater in the diabetic group (46% ± 7%) than that in nondiabetics (29% ± 8%). Fig. 1 shows the time course changes in urinary protein excretions, and the beneficial effects of spironolactone were seen after only 4 weeks of treatment, again without BP reduction, and with further improvement during the subsequent 8 weeks. Glomerular filtration rate (estimated by 24-h creatinine clearance), NAG, β2-MG (data not shown), and serum potassium remained unchanged throughout spironolactone therapy (before spironolactone treatment: 4.2 ± 0.2 mEq/L; after: 4.3 ± 0.3 mEq/L), and all patients had relatively well preserved renal function. In patients with diabetic nephropathy, although urinary type IV collagen did not decrease after at least 10 months of medical therapy (Table 1), it was significantly reduced after the treatment with spironolactone (Fig. 2).

Adverse Events

None of the patients developed serious hyperkalemia (>6.0 mEq/L) after treatment with spironolactone. No other adverse events, including gynecomastia, hyponatremia, or arrhythmia were observed, and no patients dropped out of this study.

Discussion

In this study, we demonstrated that adding spironolactone to the conventional treatment of diabetic and nondiabetic

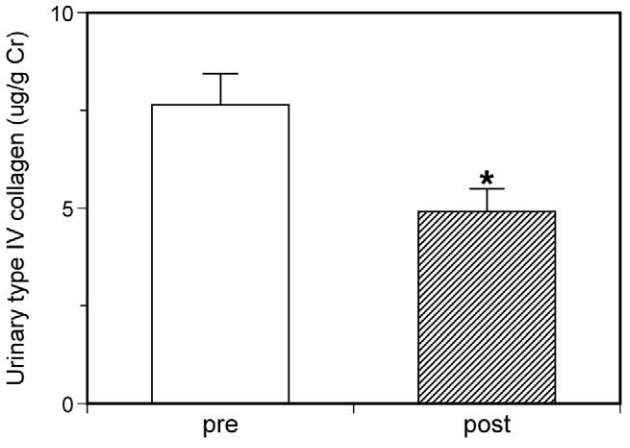

FIG. 2 Changes in urinary type IV collagen in patients with diabetic nephropathy pretreatment and post-treatment with spironolactone for 12 weeks. Data represent the mean ± SE. *$P < .05$ *v* baseline value.

renal disease is clinically useful and safe for patients who no longer show the maximal antiproteinuric effects of such a treatment.

It is well known that decreasing proteinuria, regardless of its cause, is beneficial in slowing the progressive loss of the glomerular filtration rate and in reducing the risk of terminal renal failure, and therefore, early renoprotective treatment is of utmost importance. It has been established that ACE inhibitors are of a specific benefit not only in reducing proteinuria but also in retarding the progression of both diabetic[15,16] and nondiabetic renal disease.[17,18] However, the effects of ACE inhibitors during long-term therapy have not always been optimal. It has been reported that although ACE inhibitors may be beneficial for patients with nondiabetic renal disease, approximately half of such patients are improved only at the beginning of treatment, and subsequently escape from the antiproteinuric effect and ACE inhibition,[19] suggesting that further strategies need to be investigated.

There is considerable experimental and clinical evidence that aldosterone can contribute to the development of nephrosclerosis and renal fibrosis in models of diabetes and hypertension.[7–12] Aldosterone-induced vasculopathy may underlie progressive renal disease, and that aldosterone excess can produce deleterious effects in both the cardiovascular system and the kidneys, without any change in BP. Failure of either the ACE inhibitor or ARB to suppress aldosterone production during long term often results in no amelioration of the direct cardiovascular and renal effects of aldosterone,[4–6,12,13,20] therefore aldosterone breakthrough may become extremely significant from the standpoint of end organ protection, even during treatment with an ACE inhibitor or ARB. Although the combination therapy with ACE inhibitor and ARB has been reported to retard progression of nondiabetic renal disease, there were still patients whose renal function aggravated.[21] Taken together with our previous study,[12] adding an MR antagonist to conventional treatment will provide additional benefits in the prevention of renal disease, even in those patients with optimal BP control.

Moreover, we found that although hyperkalemia has been frequently associated with the combination therapy of an ACE inhibitor plus spironolactone, there was no evidence of severe hyperkalemia in the present study. It is reported that the side effects of spironolactone are concentration dependent, and especially, in hyperkalemia, the dose, severity of renal dysfunction, and age are regarded as very important. In this study, using a small dose of 25 mg/d of spironolactone was considered significant. In the RALES study using this dose, no significant differences of serum potassium levels were found in the placebo group.[4] Moreover, we speculated glomerular filtration rate for our subjects in this study, and started the treatment after it was confirmed to be 93 $\pm$ 5 mL/min on average and 85 mL/min or higher at the minimum. Moreover, the mean ages of the subjects were relatively young, 63 $\pm$ 2 years for diabetic nephropathy and 52 $\pm$ 6 years for nondiabetic renal disease, with very advanced aged subjects not being included. These may be the greatest factors that caused the no-hyperkalemia results. Therefore, we need to underscore that this study may not apply to patients with more advanced kidney disease.

In this study, we have confirmed the effectiveness of aldosterone blockade in selective patients with diabetic nephropathy, as shown in our previous study.[12] We previously demonstrated that high glucose levels potentiate the effects of aldosterone on leucine incorporation by neonatal rat cardiomyocytes in culture,[22] indicating that the effects of aldosterone on the heart may be augmented with hyperglycemic conditions. It is thus possible that MR blockade may have particular clinical efficacy in terms of the prevention of organ damage in patients with hyperglycemia. The exact mechanism for the antiproteinuric effect of spironolactone in our study is unknown. Although spironolactone is a diuretic, BP did not decrease after spironolactone treatment in this study, and the low dose of spironolactone (25 mg/d) used suggests that the beneficial effects of MR blockade were not due mainly to its hemodynamic effects. In this study, the antiproteinuric effect of spironolactone was seen after only 4 weeks of treatment (Fig. 2), without any changes in BP. Therefore, one of the plausible mechanisms of the beneficial effects of spironolactone may be to decrease glomerular capillary pressure, while not changing systemic BP, by decreasing the efferent arteriolar tone and augmentating the effects of the ACE inhibitor.[23]

Secondary, urinary type IV collagen decreased in patients with diabetic nephropathy (Fig. 2). Aldosterone has been shown to stimulate type IV collagen in cultured rat mesangial cells,[24] and has also been shown to cause progressive renal fibrosis. Because type IV collagen is the principal component of the glomerular basement membrane and mesangial matrix, the levels of urinary type IV collagen may reflect the rate of its turnover.[25] We recently reported that urinary type IV collagen excretion was not significantly decreased by ACE inhibitor treatment alone.[26] Taken together, the beneficial effects of spironolactone in patients with diabetic nephropathy may also be explained, at least in part, by the limitation of extracellular collagen turnover, which seems to be incomplete with ACE inhibitor treatment alone. In terms of collagen turnover, it has been demonstrated that despite maximal ACE inhibition, proteinuric patients with renal failure have increased urinary levels of TGF (transforming growth factor)-β1, a profibrotic cytokine that activates extra cellular matrix protein synthesis.[27] It was also reported that ACE inhibitors fail to suppress overexpression of TGF-β1 in diabetic rat glomeruli.[28] Given that spironolactone completely prevented the decrease in renal function in experimental chronic cyclosporin A nephrotoxicity, a characteristic of which is the upregulation of TGF-β1 expression,[29] it is possible that MR blockade may be preferable to ACE inhibition in terms of prevention of TGF-β1 action in renal disease. However, further studies,

both clinical and basic, are needed to determine the potential effect of spironolactone on TGF-β1 in the kidney.

Finally, the beneficial effects of the combination therapy with ACE inhibitor and MR antagonist again need to be considered. Addition of spironolactone or the selective MR antagonist eplerenone to ACE inhibitor treatment has been shown to improve endothelial dysfunction in rats[30] and humans[31] with chronic heart failure, and to strikingly increase natriuresis only in such combination.[32] It has been also demonstrated that platelet activation shown in chronic heart failure was completely rescued to basal levels only by the combination therapy with eplerenone and ACE inhibitor.[33] Recently, Epstein et al[34] reported that the combination therapy of eplerenone and ACE inhibitor showed more antiproteinuric effect than each monotherapy in hypertensive patients with diabetes and proteinuria. Taken together, there may be promising interaction of low-dose MR antagonist and ACE inhibitor from the standpoint of preventing end organ damage.

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 urinary protein excretion, it would have been preferable to have a control group that showed persistent proteinuria with conventional treatment without spironolactone. Therefore, there is no direct evidence that the antiproteinuric effect found during the study period is due to spironolactone. However, it is reported that the antiproteinuric effect of ACE inhibitors has been demonstrated in 2 weeks, occurring earliest by the correction of hyperfiltration. In addition, the blood concentration of trandolapril usually reaches a plateau on day 7. These findings indicate that it is unlikely that the accumulated action of trandolapril appears after 10 months. Thus, we assume that the antiproteinuric effect seen after a short period (3 months in this study), results from an additionally administered spironolactone. Additional, larger, prospective, randomized double-blind studies will be needed before general adaptation of this strategy can be recommended as standard of care.

In conclusion, in patients with chronic renal disease and proteinuria persistently at more than 0.5 g/d, despite appropriate BP control and the use an ACE inhibitor, adding spironolactone to the conventional treatment may have beneficial effects on urinary protein excretion. Our study suggests that attenuation of the aldosterone effects may become a new goal for patients who have escaped the antiproteinuric effect of conventional treatment for renal disease.

Perspectives

We have shown the clinical effectiveness and safety of the MR antagonist spironolactone on urinary protein excretion in patients with diabetic and nondiabetic renal disease and proteinuria persistently more than 0.5 g/d, despite maintained BP control and use of an ACE inhibitor for at least 10 months. Whether these effects of spironolactone conferring renal protection are mediated by blocking epithelial or nonepithelial MR in the kidney awaits further studies. Additional, larger, prospective, randomized double-blind studies will be needed before widespread adaptation of this strategy as clinical standard of care.

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