Original Article

Effect of Renin-Angiotensin-Aldosterone System Triple Blockade on Non-Diabetic Renal Disease: Addition of an Aldosterone Blocker, Spironolactone, to Combination Treatment with an Angiotensin-Converting Enzyme Inhibitor and Angiotensin II Receptor Blocker

Yoshiyuki FURUMATSU[1),2)], Yasuyuki NAGASAWA[2)], Kodo TOMIDA[1),2)], Satoshi MIKAMI[1),2)], Tetsuya KANEKO[1)], Noriyuki OKADA[1)], Yoshiharu TSUBAKIHARA[1)], Enyu IMAI[2)], and Tatsuya SHOJI[1)]

Although dual blockade of the renin-angiotensin-aldosterone system (RAAS) with the combination of an angiotensin-converting enzyme inhibitor (ACE-I) and angiotensin II receptor blocker (ARB) is generally well-established as a treatment for nephropathy, this treatment is not fully effective in some patients. Based on the recent evidence implicating aldosterone in renal disease progression, this study was conducted to examine the efficacy of blockade with three different mechanisms by adding an aldosterone blocker in patients who do not respond adequately to the dual blockade. A 1-year randomized, open-label, multicenter, prospective controlled study was conducted, in which 32 non-diabetic nephropathy patients with proteinuria exceeding 0.5 g/day were enrolled after more than 12 weeks of ACE-I (5 mg enalapril) and ARB (50 mg losartan) combination treatment. These patients were allocated into two groups of 16 patients each: a triple blockade group in which 25 mg of spironolactone daily was added to the ACE-I and ARB combination treatment, and a control group in which 1 mg of trichlormethiazide or 20 mg of furosemide was added to the combination treatment instead of spironolactone depending upon the creatinine level. After 1 year of treatment, the urinary protein level decreased by 58% ($p<0.05$) with the triple blockade but was unchanged in the controls. Furthermore, urinary type IV collagen level decreased by 40% ($p<0.05$) with the triple blockade but was unchanged in the controls. The decreases in urinary protein and urinary type IV collagen were not accompanied by a decrease in blood pressure. Mean serum creatinine, potassium and blood pressure did not change significantly by either treatment. In conclusion, triple blockade of the RAAS was effective for the treatment of proteinuria in patients with non-diabetic nephropathy whose increased urinary protein had not responded sufficiently to a dual blockade. (*Hypertens Res* 2008; 31: 59–67)

***Key Words*: spironolactone, aldosterone, angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, proteinuria**

From the [1)]Department of Kidney Disease and Hypertension, Osaka General Medical Center, Osaka, Japan; [2)]Department of Nephrology, Osaka University Graduate School of Medicine, Suita, Japan.

Address for Reprints: Enyu Imai, M.D., Department of Nephrology, Osaka University Graduate School of Medicine, 2–2 Yamadaoka (A8), Suita 565–0871, Japan. E-mail: imai@medone.med.osaka-u.ac.jp; Tatsuya Shoji, M.D., Department of Kidney Disease and Hypertension, Osaka General Medical Center, 3–1–56 Bandai-Higashi, Sumiyoshi, Osaka 558–8558, Japan. E-mail: tshoji-ind@umin.ac.jp

Received February 14, 2007; Accepted in revised form August 3, 2007.

Introduction

The renin-angiotensin-aldosterone system (RAAS), and particularly angiotensin II (AII), is recognized to be a major contributor to the progression of kidney disease. Solid evidence has established the effectiveness of angiotensin-converting enzyme inhibitors (ACE-Is) or angiotensin II receptor blockers (ARBs) in lessening the contribution of the AII and thereby ameliorating kidney disease. Furthermore, dual blockade with an ACE-I and ARB has been reported to be superior to single blockade both in reducing proteinuria and in slowing the progression of renal disease (*1–3*). However, some patients do not respond adequately to the conventional renin-angiotensin system (RAS) blockade, raising a clinical problem that remains to be solved. This therapeutic inadequacy might be attributable to such putative mechanisms as non-RAAS stimulators (*4*, *5*), aldosterone breakthrough (*6–9*), or a vicious cycle within the RAAS in which aldosterone perpetuates both the expression of ACE and that of AII type 1 receptor (*10–12*).

Recently, not only AII but also aldosterone has been recognized as playing a key role in the RAAS (*5*, *13*). In fact, several clinical studies have demonstrated that monotherapy with an aldosterone blocker (*14–16*) or combination therapy with an aldosterone blocker plus an ACE-I or ARB reduced proteinuria in patients with chronic kidney disease (CKD) (*17–21*). However, Chrysostomou, who first reported the antiproteinuric effect of spironolactone (*17*), could not prove the efficacy of triple blockade with spironolactone plus an ACE-I and ARB in a later report (*22*). To date, therefore, the efficacy of aldosterone blockade, especially in conjunction with an ACE-I and ARB, has not been confirmed in patients with CKD.

We hypothesized that triple blockade of the RAAS with an aldosterone blocker plus an ACE-I and ARB might be more effective than the dual blockade both in reducing proteinuria and in slowing the progression of renal disease, especially in patients whose proteinuria did not respond sufficiently to the dual blockade. Accordingly, we conducted a 1-year randomized study to assess the efficacy of an aldosterone blocker; spironolactone, administered in conjunction with an ACE-I and ARB on proteinuria and renal function in comparison with the additive use of thiazide or loop diuretics along with an ACE-I and ARB.

Methods

Patients

A randomized, open-label, multicenter, prospective, controlled study was conducted at the Osaka General Medical Center and Osaka University Hospital from 2002 to 2004. Japanese patients who had been treated with the combination of an ACE-I and ARB were screened according to the inclusion and exclusion criteria defined below.

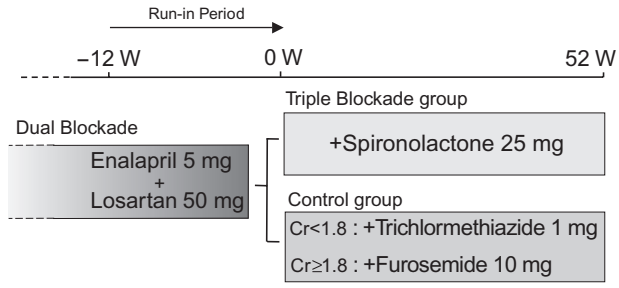

Fig. 1. *Study protocol. W, weeks.*

Inclusion criteria were an age of 20–70 years; controlled blood pressure (BP) below 130/80 mmHg; chronic nephropathy, as defined by serum creatinine concentration below 3.0 mg/dL or calculated creatinine clearance of more than 30 mL/min/1.73 m^2; daily treatment with 5 mg enalapril and 50 mg losartan for 12 weeks or longer; and persistent proteinuria, defined as urinary protein excretion exceeding 0.5 g/day.

Exclusion criteria were diabetes mellitus (HbA1c 5.8% or more); urinary-tract infection; severe renal failure, defined as a serum creatinine concentration more than 3.0 mg/dL; uncontrolled hyperkalemia, defined as a serum potassium concentration more than 5.0 mEq/L; treatment-resistant edema; requirement of treatment with corticosteroids, nonsteroidal anti-inflammatory drugs, or immunosuppressive drugs; proteinuria greater than 5.0 g/g·Cr; hypoalbuminemia less than 2.8 g/dL; renovascular hypertension; malignant hypertension; myocardial infarction; cerebrovascular accident; peripheral vascular disease; heart failure; chronic hepatic disease (rise of serum aminotransferase concentration); connective-tissue disease; obstructive uropathy; cancer; chronic pulmonary disease; drug or alcohol misuse; pregnancy; breast-feeding; and history of allergic reaction to drugs, especially ACE-Is or ARBs.

Protocol

Among the 38 eligible patients, 6 were excluded because they did not excrete urinary protein of more than 0.5 g/day after the dual blockade with an ACE-I and ARB. The remaining 32 subjects were randomly divided into two groups: a triple blockade group ($n=16$) and a control group ($n=16$). In the triple blockade group, we added 25 mg spironolactone to the ongoing treatment with the ACE-I (5 mg enalapril) and ARB (50 mg losartan). Though it has not been clarified whether 25 mg of spironolactone is sufficient for reducing proteinuria, 25 mg was effective in former trials (*17*, *20*, *21*, *23*, *24*). Thus, in this study, we set the dosage of spironolactone to 25 mg. In the control group, we added 1 mg trichlormethiazide or 10 mg furosemide according to the patient's serum creatinine level (trichlormethiazide for subjects whose creatinine was less than 1.8 mg/dL or furosemide for those whose creatinine level

Table 1. Baseline Characteristics of 30 Patients

	Triple blockade (n=15)	Control (n=15)	p
Age (years)	49.8±2.7	53.9±2.7	0.29
Gender (male/female)	10/5	9/6	0.70
Height (cm)	166.1±2.1	162.6±1.8	0.22
Body weight (kg)	70.2±2.8	64.9±2.4	0.16
BMI (kg/m^2)	26.3±1.4	24.4±0.7	0.25
Systolic BP (mmHg)	126.4±2.8	129.6±2.5	0.40
Diastolic BP (mmHg)	79.1±2.2	79.4±2.2	0.92
Mean BP (mmHg)	94.9±2.2	96.1±2.1	0.69
Cr (mg/dL)	1.07±0.11	1.22±0.10	0.33
Ccr (mL/min/1.73 m^2)	91.8±11.8	68.9±7.8	0.12
K (mEq/L)	4.28±0.12	4.28±0.08	0.97
Albumin (mg/dL)	3.73±0.08	3.64±0.09	0.48
T-Cho (mg/dL)	197.0±16.3	172.7±14.7	0.28
Ht (%)	40.3±1.2	39.1±1.3	0.48
PAI-1 (ng/mL)	58.1±13.4	48.5±6.7	0.52
u-Prot/u-Cr (g/g·Cr)	1.42±0.28	1.44±0.28	0.97
Selectivity index	0.264±0.022	0.282±0.027	0.59
u-IV col/u-Cr (μg/g·Cr)	4.80±0.66	6.30±1.18	0.26
Subtypes of renal disease			
IgA nephropathy	6	9	0.63
Benign nephroscrelosis	3	1	
Membranous nephropathy	1	1	
Other renal disease	5	4	

BMI, body mass index; BP, blood pressure; Cr, creatinine; Ccr, creatinine clearance; K, potassium; T-Cho, total cholesterol; Ht, hematocrit; PAI-1, plasminogen activator inhibitor-1; u-Prot, urinary protein; u-Cr, urinary creatinine; u-IV col, urinary type IV collagen. Data are expressed as mean±SEM.

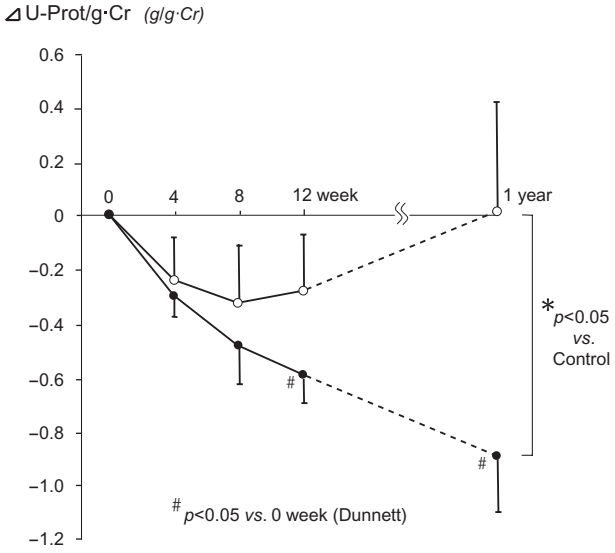

Fig. 2. *Changes in proteinuria. In the triple blockade group (closed circles), the reduction in proteinuria became significant at 12 weeks after initiation of the trial and continued until 1 year, while in the controls (open circles), no significant reduction was found during the study period. Moreover, in the triple blockade group, the reduction in proteinuria at 1 year was more significant than that in the control group. U-Prot, urinary protein; Cr, creatinine. Values are expressed as the means±SEM. $^{\#}p<0.05$ vs. 0 weeks (Dunnett's test). $^{*}p<0.05$ vs. control (Student's t-test).*

was 1.8 mg/dL or higher) (*25*). We chose these diuretics as a control in order to equalize urinary sodium excretion, because increased sodium excretion enhances the effect of dual blockade (*26*). We chose the dose of diuretics according to a comparative study between thiazide diuretics and spironolactone, in which the blood pressure–lowering effect of spironolactone 150 mg was shown to be equivalent to that of trichlormethiazide 4 mg (*27*). Therefore, as a control, we estimated that trichlormethiazide 1 mg would likely be correspondent to spironolactone 25 mg. The types and dosages of the antihypertensive drugs were closely similar between two groups. Long-acting dihydropyridine calcium channel blockers were the most widely prescribed, accounting for 27% of antihypertensives in the triple blockade group and 40% in the control. α- and/or β-blockers were the second most frequently used, accounting for 13% of antihypertensives in the triple blockade group and 7% in the control. This protocol was summarized in Fig. 1. The primary outcome was reduction in proteinuria.

During the study period, diet therapies were maintained. No drug was changed except that in some cases potassium binder or sodium bicarbonate were added. For the safety of this study, addition of potassium binder was permitted when the potassium level exceeded 5.0 mEq/L. Sodium bicarbonate was also allowed when the bicarbonate level dropped below 20 mEq/L. Both the Osaka General Medical Center and the Osaka University Hospital ethics committees approved the trial protocol, and every eligible patient gave written informed consent.

Data Procurement

Office BP, height, body weight, general biochemical parameters (serum creatinine, serum sodium, serum potassium, serum albumin, total cholesterol, hematocrit, transferrin, immunoglobulin G [IgG)]), plasminogen activator inhibitor-1 (PAI-1), plasma renin activity (PRA), AII and plasma aldosterone concentration (PAC) were measured. PRA, AII and PAC were measured after patients had rested in a supine position for at least 30 min. Office BP was measured after at least 15 min of rest.

Twenty-four–hour urine samples were collected to measure urinary excretion of creatinine, urea nitrogen, protein, sodium, potassium, transferrin, IgG, aldosterone and transforming growth factor-β1 (TGF-β1). In evaluating the urinary

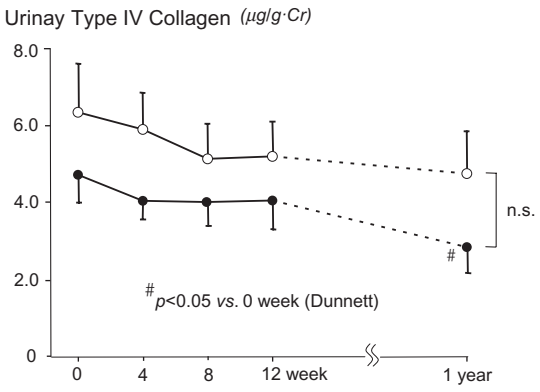

Fig. 3. *Changes in urinary type IV collagen. In the triple blockade group (closed circles), the reduction in urinary type IV collagen became significant at 1 year after the start of the trial, while in the controls (open circles), no significant reduction was observed during the study period. Values are expressed as the means±SEM. #$p<0.05$ vs. 0 weeks (Dunnett's test)*

protein excretion, we calculated the protein to creatinine ratios to rule out the risk of sample collection error (*28–31*). Urinary type IV collagen was measured in a morning urine sample (*32*, *33*).

Based on these parameters, we calculated body mass index (BMI), Cockcroft's creatinine clearance (Ccr) per 1.73 m^2, and selectivity index (*34*).

Statistical Analysis

Data are expressed as the means±SEM, or the median [interquartile range] for normal and non-normal continuous variables, respectively. For between-group analyses of baseline demographic factors and clinical values, Pearson's χ^2 test, Student's *t*-test and Mann-Whitney *U*-test were performed as appropriate. Between-group analyses were examined by Student's *t*-test and Mann-Whitney *U*-test for normal and non-normal variables, respectively. For within-group analyses, the Dunnett's multiple comparison test was applied to identify statistically significant differences *vs*. the baseline. Spearman rank correlation was calculated to assess the correlation between the change in BP and the reduction in proteinuria. *p*-values <0.05 were considered to be statistically significant. Statistical analyses were performed using JMP software (SAS Institute, Cary, USA) for Windows™ (Microsoft Co., Redmond, USA).

Results

The Baseline Characteristics of the Patients

In the triple blockade group, one patient dropped out because of faint, which was probably caused by BP fall. Among the remaining 15 patients, one patient felt faint and another noticed gynecomastia, but both symptoms were considered sufficiently tolerable to continue the trial. In the control group, one patient dropped out because of drug eruption and the remaining 15 continued the trial.

The baseline characteristics of the patients in each group are shown in Table 1; there were no significant differences between the two treatment groups. In our entry criteria, we set the upper limit of serum creatinine as 3.0 mg/dL, although the actual level of every subject enrolled was below 2.0 mg/dL. The mean serum potassium level was 4.28 mEq/L in both groups, and the mean BPs of the triple blockade group and control group were similar (94.9 and 96.1 mmHg, respectively). Urinary protein excretion was also similar between the triple blockade and the control group (1.42 and 1.44 g/g·Cr, respectively).

Twenty-three patients out of 30 (76.7%) underwent renal biopsy. Among them, 15 (50.0%) patients were diagnosed as having IgA nephropathy.

Reduction in Proteinuria

In the triple blockade group, proteinuria began to decline at 4 weeks after the start of the trial, and the reduction was significant at 12 weeks ($p<0.05$ *vs*. 0 weeks) and 1 year ($p<0.05$ *vs*. 0 weeks) (Fig. 2). In the control group, no significant change was observed. After 1 year of treatment, the reduction in proteinuria was significantly greater in the triple blockade group than in the control group ($p<0.05$ *vs*. control).

Change in Urinary Type IV Collagen, Serum PAI-1, and Urinary TGF-β1

It has been reported that aldosterone stimulates both type IV collagen synthesis (*35*, *36*) and PAI-1 expression (*37*, *38*), leading to fibrosis caused by TGF-β1 up-regulation (*39*, *40*). In order to elucidate the mechanisms by which triple blockade therapy reduces proteinuria, we measured urinary type IV collagen, PAI-1 and urinary TGF-β1. In the triple blockade group, the reduction in urinary type IV collagen became significant at 1 year ($p<0.05$ *vs*. 0 weeks), while in the control group, it was not significant (Fig. 3). Between the groups, however, no significant difference was observed. As for PAI-1, no change was observed in either group (Table 2). Finally, urinary TGF-β1 could not be properly evaluated because the concentration of urinary TGF-β1 remained under the detection limit (<0.50 ng/mL) in most patients throughout the study period (data not shown).

Change in Renal Function

Renal function was monitored by the serum creatinine level and Cockcroft's estimated Ccr per 1.73 m^2. Throughout the treatment period, neither parameter changed in either group (Table 2). The reduction in proteinuria in the triple blockade

Table 2. Changes in Parameters in Both Groups

	0 week	4 week	8 week	12 week	1 year
Triple blockade group					
Systolic BP (mmHg)	126.4±2.8	121.9±3.2	120.3±3.5	118.6±2.8	119.4±2.9
Diastolic BP (mmHg)	79.1±2.2	75.5±2.5	75.3±1.3	74.6±2.6	77.6±2.3
Mean BP (mmHg)	94.9±2.2	91.0±2.4	90.3±1.9	89.5±2.4	91.5±2.2
Cr (mg/dL)	1.07±0.11	1.15±0.13	1.13±0.11	1.09±0.11	1.18±0.12
Ccr (mL/min/1.73 m^2)	91.8±11.8	85.0±11.6	84.9±10.2	87.1±10.0	79.4±9.9
K (mEq/L)	4.28±0.12	4.57±0.12	4.44±0.08	4.45±0.09	4.43±0.08
u-Na/u-Cr (mEq/g·Cr)	147±10	145±9	149±10	136±11	141±13
u-K/u-Cr (mEq/g·Cr)	32.6±1.6	33.6±1.9	34.2±2.4	31.3±1.7	31.5±2.9
u-UN/u-Cr (g/g·Cr)	6.58±0.35	7.19±0.45	7.12±0.34	6.78±0.27	6.88±0.27
u-Prot/u-Cr (g/g·Cr)	1.42±0.28	0.87±0.17	0.96±0.21	0.84±0.20$^{\#}$	0.60±0.10$^{\#,*}$
u-IV col/u-Cr (μg/g·Cr)	4.80±0.66	4.04±0.43	4.02±0.49	4.18±0.61	2.89±0.38$^{\#}$
PAI-1 (ng/mL)	58.1±13.4	68.5±15.3	58.5±12.6	72.9±12.8	42.0±16.0
PRA (ng/mL/h)	2.8 [1.5–4.8]	5.8 [2.4–13.5]	7.3 [2.9–11.2]	5.4 [1.7–13.0]	7.4 [1.9–15.4]
AII (pg/mL)	9.5 [4.3–13.8]	14.0 [5.5–16.8]	14.0 [7.0–19.5]	12.0 [5.0–23.0]	13.0 [6.0–23.0]
PAC (pg/mL)	81.0 [41.0–97.0]	78.5 [51.0–110.0]	99.0 [50.5–132.5]	69.0 [48.5–92.0]	100.0 [75.0–120.0]
u-Aldo (pg/mL)	3.5 [1.7–4.8]	3.5 [2.5–6.2]	3.5 [2.3–5.8]	3.5 [2.5–5.2]	3.2 [2.6–5.5]
Control group					
Systolic BP (mmHg)	129.6±2.5	130.3±3.3	126.0±4.3	128.5±4.2	125.9±2.8
Diastolic BP (mmHg)	79.4±2.2	80.8±2.2	79.4±1.9	78.7±2.4	78.3±1.8
Mean BP (mmHg)	96.1±2.1	97.3±2.3	94.8±2.5	95.6±2.8	94.1±1.9
Cr (mg/dL)	1.22±0.10	1.34±0.10	1.43±0.12	1.34±0.08	1.39±0.11
Ccr (mL/min/1.73 m^2)	68.9±7.8	61.8±7.7	56.3±8.0	57.7±5.7	61.7±8.4
K (mEq/L)	4.28±0.08	4.44±0.12	4.30±0.12	4.28±0.12	4.22±0.11
u-Na/u-Cr (mEq/g·Cr)	130±14	149±17	154±11	129±16	154±11
u-K/u-Cr (mEq/g·Cr)	35.3±3.0	38.8±3.8	37.5±2.9	33.3±2.6	38.4±4.5
u-UN/u-Cr (g/g·Cr)	6.55±0.30	6.78±0.38	6.38±0.32	6.37±0.47	6.48±0.50
u-Prot/u-Cr (g/g·Cr)	1.44±0.28	1.32±0.38	1.24±0.36	1.15±0.35	1.39±0.56
u-IV col/u-Cr (μg/g·Cr)	6.30±1.18	5.65±0.88	5.11±0.98	5.12±0.90	5.3±1.17
PAI-1 (ng/mL)	48.5±6.7	75.7±23.9	42.6±7.6	73.9±22.6	45.0±20.0
PRA (ng/mL/h)	1.7 [0.8–4.3]	2.5 [0.9–5.2]	2.6 [0.7–5.0]	2.0 [0.6–5.8]	1.6 [0.9–7.5]
AII (pg/mL)	7.5 [4.8–15.3]	7.5 [4.0–20.0]	8.5 [7.0–14.0]	11.0 [9.0–16.8]	10.5 [5.3–14.6]
PAC (pg/mL)	68.0 [54.0–84.2]	67.0 [40.5–107.5]	75.0 [60.3–90.0]	67.8 [58.1–115.0]	94.0 [56.5–116.0]
u-Aldo (pg/mL)	3.3 [2.3–4.4]	3.8 [2.7–5.9]	3.6 [2.1–5.5]	3.8 [2.1–4.8]	2.6 [1.7–4.7]

BP, blood pressure; Cr, creatinine; Ccr, creatinine clearance; K, potassium; u-Na, urinary sodium; u-Cr, urinary creatinine; u-K, urinary potassium; u-UN, urinary urea nitrogen; u-Prot, urinary protein; u-IV col, urinary type IV collagen; PAI-1, plasminogen activator inhibitor-1; PRA, plasma renin activity; AII, angiotensin II; PAC, plasma aldosterone concentration; u-Aldo, urinary aldosterone. Data are expressed as mean±SEM, or median [interquartile range] for normal and non-normal continuous variables, respectively. Significant factors *vs*. 0 weeks in each group are shown in underlined figures. $^{\#}p<0.05$ *vs*. 0 weeks by Dunnett's multiple comparison test. $^{*}p<0.05$ *vs*. control group by Student's *t*-test.

group was not correlated with the change in renal function (Table 3).

Change in BP

The average values of systolic, diastolic and mean BP are shown in Table 2. There were no significant BP falls during the observation period in either group. The relationship between the change in BP and the change in proteinuria in the triple blockade group is shown in Table 3 and Fig. 4. We could not find any correlation between the decrease in proteinuria and the change in BP. These results indicated that the antiproteinuric effect by the addition of spironolactone could not be explained by the BP change. In addition, the decrease in urinary type IV collagen was not correlated with the change in BP (data not shown).

Change in Serum Potassium

We watched carefully for the possibility of hyperkalemia,

Table 3. Univariate Analysis on Factors Potentially Influencing the Change in Urinary Protein

	r	*p*
ΔSBP (mmHg)	−0.03	0.92
ΔDBP (mmHg)	−0.11	0.70
ΔMBP (mmHg)	−0.10	0.71
ΔCr (mg/dL)	−0.34	0.24
ΔCcr (mL/min/1.73 m^2)	0.10	0.72

SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; Cr, creatinine; Ccr, creatinine clearance.

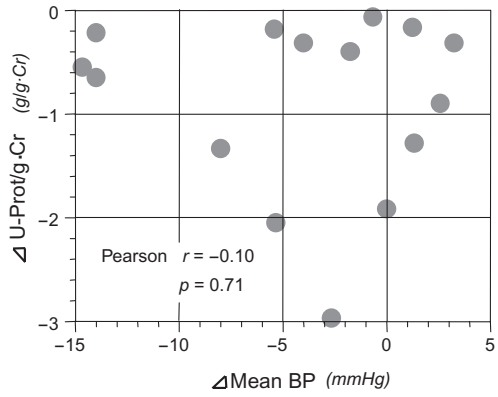

Fig. 4. *Relationship between the changes in mean blood pressure and urinary protein. U-prot, urinary protein; BP, blood pressure. No correlation was observed among these parameters (r = −0.10, p = 0.71).*

especially in the triple blockade group. After the addition of spironolactone, we added potassium binder for two patients because their potassium levels exceeded 5.0 mEq/L. In another two patients, sodium bicarbonate was added because their bicarbonate levels dropped below 20.0 mEq/L. These drugs helped us to prevent fatal hyperkalemia (K≥6.0 mEq/L). As a result, we could maintain the serum potassium level throughout the study period.

Change in RAAS Activity

No significant change was found in PRA, AII, PAC or urinary aldosterone from baseline through 1 year in either group (Table 2). Over the course of 1 year of triple blockade therapy, we could not find any significant change in systemic RAAS activity.

Change in Other Parameters

Changes in urinary sodium excretion and urinary urea nitrogen excretion are shown in Table 2. No significant change from baseline was observed throughout the year in either group. In addition, no difference was observed between the two groups at each measurement point.

Discussion

In this randomized, controlled study, it was shown that triple blockade of the RAAS had a more beneficial effect on proteinuria than dual blockade in patients with non-diabetic nephropathy (Fig. 2). This result indicated that, in those who had not responded sufficiently to treatment with dual blockade using an ACE-I and ARB, additional blockade of aldosterone proved important.

Several investigators have reported the beneficial effects of adding an aldosterone blocker for patients treated with an ACE-I (*41*, *42*). Chrysostomou *et al*. first reported that 4-week spironolactone treatment (25 mg/day) resulted in an average 54% reduction in urinary protein excretion without an accompanying antipressor effect in mostly type 2 diabetic patients who had persistent proteinuria (>1 g/day) under ACE-I treatment for more than 1 year (*17*). In the 4E-Left Ventricular Hypertrophy Study (*14*), subjects were treated with monotherapy of either eplerenone (200 mg/day) or enalapril (40 mg/day) or a combined therapy of eplerenone (200 mg/day) and enalapril (10 mg/day). After 36-week treatments, the reduction rates of urinary albumin excretion were 24.9%, 37.4%, and 52.6%, respectively, indicating both the synergistic effect of added aldosterone blocker and the inadequacy of ACE-I monotherapy.

Recently, aldosterone breakthrough has been considered as one of the possible reasons for the inadequacy of ACE-I or ARB monotherapy (*43*). Sato *et al*. first investigated the relationship between aldosterone breakthrough and antiproteinuric effects (*24*). After 40-week treatments with trandolapril in patients with early diabetic nephropathy, aldosterone breakthrough developed in 46% of patients, and an antiproteinuric effect of ACE-I was blunted in patients with aldosterone breakthrough, while the significant reduction in urinary albumin excretion lasted in patients without aldosterone breakthrough. In their subsequent report, in patients with aldosterone breakthrough, the addition of spironolactone (25 mg/day) resulted in a 30% decrease in urinary albumin excretion (*24*). Other studies have suggested that a single blockade of RAS might have its own limits because of the development of aldosterone breakthrough (*44*, *45*).

In order to achieve a more powerful blockade of the RAAS, the combined use of an ACE-I, an ARB and an aldosterone blocker has drawn attention as a new treatment strategy. Antiproteinuric effects have been reported by the addition of spironolactone to treatment with an ACE-I and/or ARB, but these reports did not confirm the superiority of triple blockade to dual blockade, because patients were not always treated with an ACE-I and ARB in combination. There has been only one trial designed to confirm the advantage of triple blockade over dual blockade (*22*); this study was conducted by Chry-

sostomou *et al*., who were also the first to report the beneficial effects of adding spironolactone to ACE-I therapy (*17*). Unfortunately, in their later report (*22*), no significant antiproteinuric effect was observed by the addition of spironolactone; the reduction in proteinuria achieved by ACE-I, ARB and spironolactone was the same as that obtained by ACE-I and spironolactone.

In our report, however, the triple blockade reduced proteinuria more prominently than treatment with the dual blockade plus thiazide or loop diuretics. There are several possible reasons why we observed a beneficial effect of triple blockade, while the previous report failed (*22*). First, our protocol had a more than 12-week run-in period under dual blockade with an ACE-I and ARB (Fig. 1). Aldosterone breakthrough has been reported to emerge around 6 months after starting an ACE-I or ARB (*8*). Most of our patients were treated with an ACE-I and ARB for more than 6 months, and their persistent proteinuria might suggest the occurrence of aldosterone breakthrough (*20*). In our study patients, the harmful effects of aldosterone breakthrough may have been eliminated by spironolactone, which resulted in a significant reduction in proteinuria. Second, our study had a 1-year observation period without any change in RAAS blockers or diuretics, although the protocol in the previous report (*22*) had a 6-month observation period with a reduction of ACE-I in some cases. When comparing the effectiveness of RAAS blockers, it is important not to change these drugs. In addition, 6 months may not have been a sufficient period to detect a significant effect of aldosterone blockade in such a small sample. Third, none of our patients had diabetic nephropathy, although 67% of those in the previous report did (*22*). Because, in diabetic nephropathy, intra-renal RAAS is usually elevated though systemic RAAS is suppressed (*46*, *47*), simultaneous coadministration of an ACE-I might have had too strong an impact for confirming the antiproteinuric effect of spironolactone addition.

In our study, the triple blockade reduced urinary type IV collagen as well (Table 2, Fig. 3). Type IV collagen is a major structural component of basement membrane and mesangial matrix; and thus an increase in urinary type IV collagen is considered to reflect acceleration of fibrosis in the kidney. It has been reported that the more diabetic nephropathy progresses, the higher the concentration of urinary type IV collagen becomes (*48–50*). Moreover, increase in urinary type IV collagen has been shown to be ameliorated by an ACE-I, indicating that ACE-Is have an antifibrotic effect (*51*). In our study, the addition of spironolactone reduced urinary type IV collagen in 1 year, which might also indicate an antifibrotic effect of spironolactone.

RAAS stimulates PAI-1 synthesis (*13*), which leads to fibrosis in the kidney. ACE-Is, ARBs, and aldosterone blockers have all been reported to reduce the concentration of PAI-1 (*37*, *52*, *53*). Contrary to these reports, we did not observe a reduction in PAI-1 in the triple blockade group (Table 2).

Both the reduction in proteinuria and that in urinary type IV collagen were independent of BP change (Table 2, Fig. 4). This fact suggested that, in this study setting, the major antiproteinuric effect might not have been caused by BP fall but by the aldosterone blockade itself. It has been reported that the addition of diuretics, such as thiazide or loop diuretics, increases sodium excretion and thereby augments the effects of ACE-Is and ARBs (*54*). In the present study, it was anticipated that spironolactone would enhance the effect of the ACE-I and ARB by increasing sodium excretion (*55*). If we had chosen just a placebo as a control instead of thiazide or loop diuretics, the difference in BP between the two groups may have become significant. In that case, it would have been difficult to determine which mechanism had the greater impact on the antiproteinuric effect, BP reduction or aldosterone blockade itself. Therefore, in our study design, we chose diuretics as a control. In fact, urinary sodium excretion was comparable between the groups. Though no difference was observed in BP or urinary sodium excretion, the antiproteinuric effect was more prominent in the triple blockade group than in the controls. This may have been attributable to one of several mechanisms: non-RAAS stimulators (*4*, *5*), aldosterone breakthrough (*6–9*), or a vicious cycle within the RAAS (*10–12*).

The RALES trial reported that the serum potassium elevation induced by spironolactone was only 0.3 mEq/L (*23*), and the EPHESUS trial reported that a serious potassium elevation was induced by eplerenone in 1.6% of treated patients (*56*). After the RALES report, the increased use of combination treatment with spironolactone and an ACE-I caused an increase in hospitalization and death due to hyperkalemia (*57*). However, in our study, there was neither a significant elevation in serum potassium nor any serious problems caused by hyperkalemia, because of the active control of potassium. In addition, there was no difference in serum potassium level between the triple blockade group and the control group (Table 2).

When blocking mineralocorticoid receptor by spironolactone, a small rise in potassium and PAC is often observed (*18*, *19*). In our trial, the same tendency was observed but it remained insignificant in the triple blockade group (Table 2). Since elevation of potassium increases PAC *via* a "non-RAAS pathway," active control of potassium might inhibit the elevation of PAC. Control of potassium is important both in preventing the serious problems caused by hyperkalemia and in inhibiting the PAC elevation caused by potassium elevation.

One limitation of our study was the lack of multivariate analysis, which we were unable to perform due to the small number of subjects in our cohort. Additional larger studies will be needed for general acceptance of this strategy.

In conclusion, we observed a significant reduction in proteinuria as a result of triple blockade of the RAAS. Triple blockade is thus confirmed to be more effective than dual blockade in reducing proteinuria. At the same time, while triple blockade appears to be the strongest strategy for RAAS

blockade, care should be taken due to the potential risk of hyperkalemia.

References

1. Russo D, Pisani A, Balletta MM, *et al*: Additive antiproteinuric effect of converting enzyme inhibitor and losartan in normotensive patients with IgA nephropathy. *Am J Kidney Dis* 1999; **33**: 851–856.
2. Russo D, Minutolo R, Pisani A, *et al*: Coadministration of losartan and enalapril exerts additive antiproteinuric effect in IgA nephropathy. *Am J Kidney Dis* 2001; **38**: 18–25.
3. Nakao N, Yoshimura A, Morita H, Takada M, Kayano T, Ideura T: Combination treatment of angiotensin-II receptor blocker and angiotensin-converting–enzyme inhibitor in non-diabetic renal disease (COOPERATE): a randomised controlled trial. *Lancet* 2003; **361**: 117–124.
4. Okubo S, Niimura F, Nishimura H, *et al*: Angiotensin-independent mechanism for aldosterone synthesis during chronic extracellular fluid volume depletion. *J Clin Invest* 1997; **99**: 855–860.
5. Epstein M: Aldosterone as a mediator of progressive renal disease: pathogenetic and clinical implications. *Am J Kidney Dis* 2001; **37**: 677–688.
6. Borghi C, Boschi S, Ambrosioni E, Melandri G, Branzi A, Magnani B: Evidence of a partial escape of renin-angiotensin-aldosterone blockade in patients with acute myocardial infarction treated with ACE inhibitors. *J Clin Pharmacol* 1993; **33**: 40–45.
7. Pitt B: "Escape" of aldosterone production in patients with left ventricular dysfunction treated with an angiotensin converting enzyme inhibitor: implications for therapy. *Cardiovasc Drugs Ther* 1995; **9**: 145–149.
8. Sato A, Saruta T: Aldosterone escape during angiotensin-converting enzyme inhibitor therapy in essential hypertensive patients with left ventricular hypertrophy. *J Int Med Res* 2001; **29**: 13–21.
9. Naruse M, Tanabe A, Sato A, *et al*: Aldosterone breakthrough during angiotensin II receptor antagonist therapy in stroke-prone spontaneously hypertensive rats. *Hypertension* 2002; **40**: 28–33.
10. Robert V, Heymes C, Silvestre JS, Sabri A, Swynghedauw B, Delcayre C: Angiotensin AT1 receptor subtype as a cardiac target of aldosterone: role in aldosterone-salt–induced fibrosis. *Hypertension* 1999; **33**: 981–986.
11. Harada E, Yoshimura M, Yasue H, *et al*: Aldosterone induces angiotensin-converting–enzyme gene expression in cultured neonatal rat cardiocytes. *Circulation* 2001; **104**: 137–139.
12. Sugiyama T, Yoshimoto T, Tsuchiya K, *et al*: Aldosterone induces angiotensin converting enzyme gene expression *via* a JAK2-dependent pathway in rat endothelial cells. *Endocrinology* 2005; **146**: 3900–3906.
13. Epstein M: Aldosterone as a mediator of progressive renal dysfunction: evolving perspectives. *Intern Med* 2001; **40**: 573–583.
14. Pitt B, Reichek N, Willenbrock R, *et al*: Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: the 4E-left ventricular hypertrophy study. *Circulation* 2003; **108**: 1831–1838.
15. White WB, Duprez D, St Hillaire R, *et al*: Effects of the selective aldosterone blocker eplerenone *versus* the calcium antagonist amlodipine in systolic hypertension. *Hypertension* 2003; **41**: 1021–1026.
16. Takeda Y: Pleiotropic actions of aldosterone and the effects of eplerenone, a selective mineralocorticoid receptor antagonist. *Hypertens Res* 2004; **27**: 781–789.
17. Chrysostomou A, Becker G: Spironolactone in addition to ACE inhibition to reduce proteinuria in patients with chronic renal disease. *N Engl J Med* 2001; **345**: 925–926.
18. Bianchi S, Bigazzi R, Campese VM: Antagonists of aldosterone and proteinuria in patients with CKD: an uncontrolled pilot study. *Am J Kidney Dis* 2005; **46**: 45–51.
19. Schjoedt KJ, Rossing K, Juhl TR, *et al*: Beneficial impact of spironolactone in diabetic nephropathy. *Kidney Int* 2005; **68**: 2829–2836.
20. Bianchi S, Bigazzi R, Campese VM: Long-term effects of spironolactone on proteinuria and kidney function in patients with chronic kidney disease. *Kidney Int* 2006; **70**: 2116–2123.
21. Schjoedt KJ, Rossing K, Juhl TR, *et al*: Beneficial impact of spironolactone on nephrotic range albuminuria in diabetic nephropathy. *Kidney Int* 2006; **70**: 536–542.
22. Chrysostomou A, Pedagogos E, MacGregor L, Becker G: Double-blind, placebo-controlled study on the effect of the aldosterone receptor antagonist spironolactone in patients who have persistent proteinuria and are on long-term angiotensin-converting enzyme inhibitor therapy, with or wihtout an angiotensin II receptor blocker. *Clin J Am Soc Nephrol* 2006; **1**: 256–262.
23. Pitt B, Zannad F, Remme WJ, *et al*, Randomized Aldactone Evaluation Study Investigators: The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *N Engl J Med* 1999; **341**: 709–717.
24. Sato A, Hayashi K, Naruse M, Saruta T: Effectiveness of aldosterone blockade in patients with diabetic nephropathy. *Hypertension* 2003; **41**: 64–68.
25. Bakris GL, Williams M, Dworkin L, *et al*: Preserving renal function in adults with hypertension and diabetes: a consensus approach. National Kidney Foundation Hypertension and Diabetes Executive Committees Working Group. *Am J Kidney Dis* 2000; **36**: 646–661.
26. Buter H, Hemmelder MH, Navis G, de Jong PE, de Zeeuw D: The blunting of the antiproteinuric efficacy of ACE inhibition by high sodium intake can be restored by hydrochlorothiazide. *Nephrol Dial Transplant* 1998; **13**: 1682–1685.
27. Shimizu N, Miyashita H, Koide K, Yoshikawa J: Antipressor effect of spironolactone or thyazide diuretics for essential hypertension: double blind comparative study. *Horumon To Rinsho* 1977; **25**: 167–174 (in Japanese).
28. Ginsberg JM, Chang BS, Matarese RA, Garella S: Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med* 1983; **309**: 1543–1546.
29. Schwab SJ, Christensen RL, Dougherty K, Klahr S: Quantitation of proteinuria by the use of protein-to-creatinine ratios in single urine samples. *Arch Intern Med* 1987; **147**: 943–944.
30. Kawasaki T, Ueno M, Uezono K, *et al*: Average urinary

excretion of sodium in 24 hours can be estimated from a spot-urine specimen. *Jpn Circ J* 1982; **46**: 948–953.
31. Kawamura M, Kusano Y, Takahashi T, Owada M, Sugawara T: Effectiveness of a spot urine method in evaluating daily salt intake in hypertensive patients taking oral antihypertensive drugs. *Hypertens Res* 2006; **29**: 397–402.
32. Obata K, Iwata K, Ichida T, *et al*: One step sandwich enzyme immunoassay for human type IV collagen using monoclonal antibodies. *Clin Chim Acta* 1989; **181**: 293–303.
33. Donovan KL, Coles GA, Williams JD: An ELISA for the detection of type IV collagen in human urine—application to patients with glomerulonephritis. *Kidney Int* 1994; **46**: 1431–1437.
34. Cameron JS, Blandford G: The simple assessment of selectivity in heavy proteinuria. *Lancet* 1966; **2**: 242–247.
35. Sato A, Hayashi K, Saruta T: Antiproteinuric effects of mineralocorticoid receptor blockade in patients with chronic renal disease. *Am J Hypertens* 2005; **18**: 44–49.
36. Han KH, Kang YS, Han SY, *et al*: Spironolactone ameliorates renal injury and connective tissue growth factor expression in type II diabetic rats. *Kidney Int* 2006; **70**: 111–120.
37. Sawathiparnich P, Kumar S, Vaughan DE, Brown NJ: Spironolactone abolishes the relationship between aldosterone and plasminogen activator inhibitor-1 in humans. *J Clin Endocrinol Metab* 2002; **87**: 448–452.
38. Brown NJ, Vaughan DE, Fogo AB: The renin-angiotensin-aldosterone system and fibrinolysis in progressive renal disease. *Semin Nephrol* 2002; **22**: 399–406.
39. Goumenos DS, Tsakas S, El Nahas AM, *et al*: Transforming growth factor-beta(1) in the kidney and urine of patients with glomerular disease and proteinuria. *Nephrol Dial Transplant* 2002; **17**: 2145–2152.
40. Agarwal R, Siva S, Dunn SR, Sharma K: Add-on angiotensin II receptor blockade lowers urinary transforming growth factor-beta levels. *Am J Kidney Dis* 2002; **39**: 486–492.
41. Sato A, Suzuki Y, Saruta T: Effects of spironolactone and angiotensin-converting enzyme inhibitor on left ventricular hypertrophy in patients with essential hypertension. *Hypertens Res* 1999; **22**: 17–22.
42. Asai M, Monkawa T, Marumo T, *et al*: Spironolactone in combination with cilazapril ameliorates proteinuria and renal interstitial fibrosis in rats with anti-Thy-1 irreversible nephritis. *Hypertens Res* 2004; **27**: 971–978.
43. Sato A, Saruta T, Funder JW: Combination therapy with aldosterone blockade and renin-angiotensin inhibitors confers organ protection. *Hypertens Res* 2006; **29**: 211–216.
44. McKelvie RS, Yusuf S, Pericak D, *et al*: Comparison of candesartan, enalapril, and their combination in congestive heart failure: Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) pilot study. The RESOLVD Pilot Study Investigators. *Circulation* 1999; **100**: 1056–1064.
45. Tang WH, Vagelos RH, Yee YG, *et al*: Neurohormonal and clinical responses to high- *versus* low-dose enalapril therapy in chronic heart failure. *J Am Coll Cardiol* 2002; **39**: 70–78.
46. Price DA, Porter LE, Gordon M, *et al*: The paradox of the low-renin state in diabetic nephropathy. *J Am Soc Nephrol* 1999; **10**: 2382–2391.
47. Hollenberg NK, Price DA, Fisher ND, *et al*: Glomerular hemodynamics and the renin-angiotensin system in patients with type 1 diabetes mellitus. *Kidney Int* 2003; **63**: 172–178.
48. Watanabe H, Sanada H, Shigetomi S, Katoh T, Watanabe T: Urinary excretion of type IV collagen as a specific indicator of the progression of diabetic nephropathy. *Nephron* 2000; **86**: 27–35.
49. Okonogi H, Nishimura M, Utsunomiya Y, *et al*: Urinary type IV collagen excretion reflects renal morphological alterations and type IV collagen expression in patients with type 2 diabetes mellitus. *Clin Nephrol* 2001; **55**: 357–364.
50. Tomino Y, Suzuki S, Azushima C, *et al*: Asian multicenter trials on urinary type IV collagen in patients with diabetic nephropathy. *J Clin Lab Anal* 2001; **15**: 188–192.
51. Nishimura M, Sasaki T, Ohishi A, *et al*: Angiotensin-converting enzyme inhibitors and probucol suppress the time-dependent increase in urinary Type IV collagen excretion of Type II diabetes mellitus patients with early diabetic nephropathy. *Clin Nephrol* 2001; **56**: 96–103.
52. Goodfield NE, Newby DE, Ludlam CA, Flapan AD: Effects of acute angiotensin II type 1 receptor antagonism and angiotensin converting enzyme inhibition on plasma fibrinolytic parameters in patients with heart failure. *Circulation* 1999; **99**: 2983–2985.
53. Fogari R, Zoppi A, Preti P, Fogari E, Malamani G, Mugellini A: Differential effects of ACE-inhibition and angiotensin II antagonism on fibrinolysis and insulin sensitivity in hypertensive postmenopausal women. *Am J Hypertens* 2001; **14**: 921–926.
54. Uzu T, Harada T, Namba T, *et al*: Thiazide diuretics enhance nocturnal blood pressure fall and reduce proteinuria in immunoglobulin A nephropathy treated with angiotensin II modulators. *J Hypertens* 2005; **23**: 861–865.
55. Sato A, Saruta T: Aldosterone-induced organ damage: plasma aldosterone level and inappropriate salt status. *Hypertens Res* 2004; **27**: 303–310.
56. Pitt B, Williams G, Remme W, *et al*: The EPHESUS trial: eplerenone in patients with heart failure due to systolic dysfunction complicating acute myocardial infarction. Eplerenone Post-AMI Heart Failure Efficacy and Survival Study. *Cardiovasc Drugs Ther* 2001; **15**: 79–87.
57. Juurlink DN, Mamdani MM, Lee DS, *et al*: Rates of hyperkalemia after publication of the Randomized Aldactone Evaluation Study. *N Engl J Med* 2004; **351**: 543–551.