Original Article

Low-Dose Losartan Therapy Reduces Proteinuria in Normotensive Patients with Immunoglobulin A Nephropathy

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The present study was designed to assess the antiproteinuric effects of a low dose of an angiotensin II-receptor blocker, losartan, in normotensive patients with immunoglobulin A (IgA) nephropathy. We performed a prospective, controlled trial of losartan (12.5 mg/d) therapy to assess the effects on mild proteinuria and renal function. The study subjects were 18 normotensive and proteinuric patients with IgA nephropathy in the losartan group and 18 IgA nephropathy patients treated with antiplatelet agents in the control group. We prospectively evaluated blood pressure, proteinuria, renal function, and biochemical parameters before and after 12 months of therapy. Blood pressure was kept constant during the 12-month period. Serum creatinine levels and estimated glomerular filtration rate did not significantly change during the 12 months in any of the patients studied. Systolic and diastolic blood pressures did not differ significantly between the losartan and control groups. However, low-dose losartan significantly reduced proteinuria from 0.8 ± 0.5 g/d at baseline to 0.4 ± 0.4 g/d at 12 months ($p=0.006$). Proteinuria was significantly lower at 12 months in the losartan group than in the control group ($p=0.04$). In addition, urinary *N*-acetyl- β -D-glucosaminidase (NAG) levels in the losartan group at 12 months were significantly lower than those in the control group ($p=0.009$). Our data suggest that low-dose losartan therapy for normotensive patients with IgA nephropathy could reduce the amount of urinary protein and NAG excretion without affecting systemic blood pressure. (*Hypertens Res* 2008; 31: 1711–1717)

Key Words: immunoglobulin A nephropathy, normotensive, angiotensin II receptor blocker, proteinuria

Introduction

Immunoglobulin A (IgA) nephropathy is the most common form of glomerulonephritis, a principal cause of end-stage renal disease worldwide (1) and the most prevalent form in Japan (2). Among the clinical and laboratory features, hypertension and proteinuria are important predictors of a poor outcome (3). Proteinuria causes renal damage that accelerates the progression of renal diseases towards end-stage renal failure (4). Angiotensin II plays a critical role in the pathogenesis of proteinuria (5). The role of angiotensin II-receptor blockers

(ARBs) in IgA nephropathy has not yet been completely established, although their positive hemodynamic, renoprotective, and antiproteinuric effects on the kidney appear to be of benefit. In regards to the amelioration of glomerular injury or fibrosis by ARBs, valsartan was shown to be beneficial in animal models of IgA nephropathy (6). Inhibition of angiotensin II action was shown to decrease urinary transforming growth factor β 1 levels in patients with IgA nephropathy, thereby ameliorating extracellular matrix protein accumulation (7, 8). In the Hong Kong Study Using Valsartan in IgA Nephropathy (HKVIN), this ARB decreased proteinuria and slowed renal deterioration in patients with IgA nephropathy

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using other antihypertensive medications (9). Russo *et al.* discovered that therapy with an angiotensin-converting-enzyme (ACE) inhibitor, enalapril, plus an ARB, losartan, exerted additive antiproteinuric effects in hypertensive proteinuric patients (10). In normotensive patients with IgA nephropathy, the use of an ACE inhibitor, temocapril, or losartan both reduced proteinuria (11). However, blood pressure decreased in all patients studied. To examine the clinical benefit of low-dose ARB, *i.e.*, losartan, independently of blood pressure, we conducted a randomized controlled study of 36 Japanese normotensive patients with IgA nephropathy.

Methods

Study Patients

Patients included in the study met the following criteria: 1) biopsy-proven IgA nephropathy; 2) estimated glomerular filtration rate (eGFR) calculated by the formula given by Imai *et al.* (12) of >50 mL/min/1.73 m²; 3) 0.4 g/d or more of persistent proteinuria despite treatment with antiplatelet agents for more than 3 months without ACE inhibitors or ARBs, because proteinuria <0.4 g/d can disappear without medication; and 4) normal BP defined as less than 140/90 mmHg, though the criterion for patients with chronic kidney disease (CKD) is 130/80 mmHg based on the Japanese Society of Hypertension guidelines.

The Research Ethics Board of Tokyo Women's Medical University approved the study protocol. All patients gave written informed consent to participate. Patients who had been treated with steroids, immunosuppressive therapy, and other medications were not included in this study. Patients with active infection, diabetes, lupus erythematosus, chronic liver diseases, renal allografts, and Henoch-Schönlein purpura were also excluded.

Thirty-six patients satisfying the criteria were enrolled in the study. Only ethnic Japanese patients were included. The mean age at diagnosis was 35.9 ± 8.2 (range, 17 to 57 years). There were 17 males and 19 females.

Study Design

The study was a prospective randomized parallel-group open-label trial. Eligible patients were randomly assigned to administration of losartan, 12.5 mg/d (losartan group), or to a control group (antiplatelet group) by the trial nephrologists. Randomization was performed using a simple randomization method. The randomization sequence was kept concealed by the secretary until the end of the trial.

The losartan group included 18 patients with a mean age of 36.0 ± 8.5 years (males, 11; females, 7) at the time of renal biopsy, and the control group included 18 patients with a mean age of 35.7 ± 8.1 years (males, 6; females, 12) ($p=0.92$).

Clinical data evaluated were age at the time of renal biopsy and blood pressure (mmHg), as well as laboratory data

including 24 h-urinary protein excretion (U-P, g/d), urinary red blood cell counts (U-RBC, /HPF [high-power field]), serum creatinine (S-Cr, mg/dL), uric acid (S-UA, mg/dL), total cholesterol (TC, mg/dL), high-density-lipoprotein cholesterol (HDL-C, mg/dL), triglyceride (TG, mg/dL), eGFR (mL/min), IgA (mg/dL), C3 (mg/dL), plasma renin activity (PRA, ng/mL/h), plasma aldosterone concentration (PAC, pg/mL), and urinary *N*-acetyl- β -D-glucosaminidase (NAG, U/g Cr) at the time of renal biopsy.

Patients were examined at baseline, namely, the time of renal biopsy, and then every 3, 6, 9, and 12 months. Peripheral PRA and PAC were measured by radioimmunoassay (RIA) (SRL Inc., Tokyo, Japan) before and after 12 months of treatment. We evaluated the glomerular filtration rate after 12 months using the eGFR (12). At each visit, patients were asked about clinical symptoms, possible treatment complications, and drug consumption. Blood pressure was measured, and urinalysis and serum biochemistry tests were performed. The amount of urinary protein excretion per day was estimated at each visit. The same clinician measured blood pressure in the sitting position after at least 15 min of rest, using a standard sphygmomanometer with an appropriately sized cuff. The values obtained from 3 measurements at 3-min intervals were averaged. The first and fifth Korotkoff sounds were taken as the systolic and diastolic blood pressure (SBP and DBP), respectively. Blood pressure was usually maintained at normal levels. If the blood pressure was increased 10% or more than above the baseline after the 1, 3, 6, and 9-month treatment periods, the study medication dosage was doubled (losartan 25 mg/d). If the blood pressure was decreased 10% or more below the baseline after the 1, 3, 6, and 9-month treatment periods, the study medication dosage was halved (losartan 6.25 mg/d).

Histological Examination

All specimens were obtained with the percutaneous needle biopsy method. The specimens were fixed with 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin, and cut into 2 μ m sections. Hematoxylin and eosin, periodic acid Schiff (PAS), silver methenamine, and Masson trichrome stainings were performed for light microscopy.

Each specimen was evaluated for glomerular, interstitial, and vascular changes as previously described (13). Two independent observers with no knowledge of the clinical data scored the changes semiquantitatively. The percentages of glomeruli exhibiting glomerular obsolescence, crescent formation, and glomerular tuft adhesion to Bowman's capsule were estimated. Mesangial cell proliferation in each patient was semiquantitatively graded into four degrees of severity: grade 0, no abnormality; grade 1, mild; grade 2, moderate; and grade 3, severe. The extent of interstitial fibrosis was semiquantitatively graded into four categories according to the proportion of fibrotic lesions to the total cortical area: grade 0, less than 5% of the total cortical area; grade 1, 5–

Table 1. Baseline Clinical Characteristics of Normotensive Patients with IgA Nephropathy

	Losartan	Control	<i>p</i> value
Age (years)	36.0±8.5	35.7±8.1	n.s.
Systolic BP (mmHg)	119.3±10.3	122.1±9.6	n.s.
Diastolic BP (mmHg)	74.8±7.5	73.4±12.1	n.s.
U-P (g/d)	0.81±0.52	0.73±0.36	n.s.
Sediment RBC (/HPF)	25.3±27.1	46.2±40.3	n.s.
Serum creatinine (mg/dL)	1.0±0.2	0.9±0.2	n.s.
Serum uric acid (mg/dL)	6.3±1.4	6.2±1.4	n.s.
eGFR (mL/min)	72.0±15.9	75.4±18.1	n.s.
Total cholesterol (mg/dL)	203.8±30.7	199.6±34.3	n.s.
Triglyceride (mg/dL)	132.9±72.2	119.5±80.3	n.s.
HDL-cholesterol (mg/dL)	50.9±11.1	48.5±11.0	n.s.
IgA (mg/dL)	267.3±92.9	334.7±73.6	n.s.
C3 (mg/dL)	93.6±14.8	81.8±11.5	n.s.
CRP (mg/dL)	0.1±0.1	0.1±0.2	n.s.
PRA (ng/mL/h)	1.7±1.0	1.8±0.8	n.s.
PAC (pg/mL)	124.0±23.6	117.3±45.0	n.s.
Urinary NAG (U/g Cr)	5.7±2.4	5.2±2.6	n.s.

Values are expressed as mean±SD. BP, blood pressure; U-P, proteinuria; HPF, high-power field; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IgA, Immunoglobulin A; CRP, C-reactive protein; PRA, plasma renin activity; PAC, plasma aldosterone concentration; NAG, *N*-acetyl- β -D-glucosaminidase; Cr, creatinine; n.s., not significant.

Table 2. Renal Histological Findings at Baseline in Losartan Group and Control Group

	Losartan	Control	<i>p</i> value
Mesangial cell proliferation (grade 0–3)	1.2±0.4	1.4±0.5	n.s.
Glomerular obsolescence (%)	15.2±12.5	12.7±14.0	n.s.
Crescent formation (%)	6.4±7.0	7.3±6.6	n.s.
Glomerular tuft adhesion to Bowman's capsule (%)	20.1±17.9	17.6±16.9	n.s.
Interstitial fibrosis (grade 0–3)	1.2±0.8	1.1±1.0	n.s.
Arterio-arteriosclerotic changes (grade 0–3)	0.5±0.7	0.6±0.8	n.s.

Values are expressed as mean±SD. n.s., not significant.

Fig. 1. Changes in blood pressure in the losartan group and control group. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) did not change significantly in the two groups during the 12-month study period.

20%; grade 2, 20–40%; and grade 3, more than 40%. Arterio-arteriosclerotic changes were also evaluated using a similar grading scale: grade 0, no abnormality; grade 1, mild; grade 2, moderate; and grade 3, severe.

Statistical Analysis

Data are expressed as mean±SD. The differences between groups were examined using Student's *t*-test for nonpaired samples. Changes from baseline to the last follow-up were compared between the losartan and control groups using the *t*-test. Clinical parameters were analyzed in both groups by repeated-measures analysis of variance (ANOVA). Two-way repeated-measures ANOVA was used to compare the proteinuria between the baseline visit and the 3-, 6- and 9-month follow-up visits in each group. When differences could be demonstrated, values were compared with the baseline using a paired-sample *t*-test. Values of *p*<0.05 were considered to

Table 3. Changes in Proteinuria in the Losartan and Control Groups

Month	Losartan (g/d)	<i>p</i> * value	Control (g/d)	<i>p</i> * value	<i>p</i> value
0	0.81±0.52		0.73±0.36		n.s.
3	0.48±0.38	0.02	0.70±0.44	n.s.	n.s.
6	0.45±0.35	0.01	0.63±0.41	n.s.	n.s.
12	0.39±0.42	0.006	0.66±0.41	n.s.	0.31

Values are expressed as mean±SD. n.s., not significant. *p** value: significantly different from baseline; *p* value: significantly different between losartan and control groups.

Table 4. Changes in Clinical Parameters in the Losartan and Control Groups

Parameter	Losartan		Control	
	Baseline	12 months	Baseline	12 months
Sediment RBC (/HPF)	25.3±27.1	18.6±27.4	46.2±40.3	38.4±35.7
Serum creatinine (mg/dL)	1.0±0.2	0.9±0.3	0.9±0.2	0.9±0.4
Serum uric acid (mg/dL)	6.3±1.4	6.3±1.1	6.2±1.4	6.2±1.6
eGFR (ml/min)	72.0±15.9	71.8±17.1	75.4±18.1	76.1±17.3
Total cholesterol (mg/dL)	203.8±30.7	196.1±28.9	199.6±34.3	204.9±36.7
Triglyceride (mg/dL)	132.9±72.2	164.0±95.2	119.5±80.3	145.6±75.4
HDL-cholesterol (mg/dL)	50.9±11.1	47.0±7.1	48.5±11.0	49.2±8.3
IgA (mg/dL)	267.3±92.9	339.0±111.3	334.7±73.6	314.6±71.2
C3 (mg/dL)	93.6±14.8	114.2±23.5	81.8±11.5	95.3±19.5
CRP (mg/dL)	0.1±0.1	0.1±0.1	0.1±0.2	0.1±0.1
PRA (ng/mL/h)	1.7±1.0	1.9±0.8	1.8±0.8	2.0±0.4
PAC (pg/mL)	124.0±23.6	107.0±31.1	117.3±45.0	103.5±42.2
NAG (U/g Cr)	5.7±2.4	3.5±1.3*	5.2±2.6	6.1±1.8

Values are expressed as mean±SD. **p*<0.05. HPF, high-power field; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IgA, Immunoglobulin A; CRP, C-reactive protein; PRA, plasma renin activity; PAC, plasma aldosterone concentration; NAG, *N*-acetyl-β-D-glucosaminidase; Cr, creatinine.

indicate statistical significance.

Results

Primary end points could be analyzed in all patients at the end of the 12-month period. There were no significant differences in any parameters, including gender, average age, body weight, body mass index, systolic blood pressure, diastolic blood pressure, heart rate, U-P, U-RBC, S-Cr, S-UA, TC, HDLC, TG, eGFR, IgA, C3, PRA, PAC, and urinary NAG levels, between the losartan and control groups at the time of renal biopsy (Table 1).

The average values for histological features of the two groups are shown in Table 2. There were no significant differences in mesangial cell proliferation, mesangial matrix increase, glomerular obsolescence, crescent formation, glomerular tuft adhesion to Bowman's capsule, interstitial fibrosis, or arterio-arteriosclerotic changes between the two groups.

Blood pressure was kept constant during the 12-month period. Blood pressure was stable not only in the control group (SBP: 122.1±9.6 to 120.5±9.5 mmHg, *p*=0.31, DPB:

73.4±12.1 to 70.4±9.0 mmHg, *p*=0.21) but also in the losartan group (SBP: 119.3±10.3 to 114.9±11.2 mmHg, *p*=0.10, DPB: 76.1±6.6 to 71.8±9.7 mmHg, *p*=0.07) (Fig. 1). In the losartan group, all 18 patients were administered losartan 12.5 mg/d, with no dosage changes during the 12-month period. There were no major adverse events.

The proteinuria profile throughout the study period is presented in Table 3. In the losartan group, proteinuria decreased from 0.81±0.52 g/d at baseline to 0.39±0.42 g/d at 12 months (*p*=0.006), showing a normal distribution. Conversely, there was no significant change in proteinuria in the control group, from 0.73±0.36 g/d at baseline to 0.66±0.41 g/d at 12 months (*p*=0.31). In addition, proteinuria at 12 months was significantly lower in the losartan group than in the control group (*p*=0.04). A correlation analysis was conducted between the changes in blood pressure and the changes in proteinuria in the losartan group (for systolic blood pressure [*p*=0.194, *r*=0.14] and for diastolic blood pressure [*p*=0.08, *r*=0.31] [data not shown]).

The U-RBC, S-Cr, S-UA, TC, HDLC, TG, eGFR, and urinary NAG were compared between the time of renal biopsy and during the 12-month period. The comparison revealed

that the two groups had similar characteristics, except for a significant decrease in urinary NAG levels in the losartan group (5.7 ± 2.4 to 3.5 ± 1.3 U/g Cr, $p=0.02$) as compared to the control group (5.2 ± 2.6 to 6.1 ± 1.8 U/g Cr, $p=0.25$) (Table 4). In addition, urinary NAG levels in the losartan group at 12 months were significantly lower than those in the control group ($p=0.009$). We found a correlation between the reduction in proteinuria and the change in urinary NAG ($p=0.03$, $r=0.48$) (data not shown).

After 12 months, the PAC (from 124.0 ± 23.6 to 107.0 ± 31.1 pg/mL, $p=0.34$) and the PRA (from 1.7 ± 1.0 to 1.9 ± 0.8 ng/mL/h, $p=0.25$) had not changed significantly in either group.

Discussion

The present findings indicate that low-dose losartan therapy reduces proteinuria without changing blood pressure in normotensive patients with proteinuric IgA nephropathy. Many studies support the findings that ACE inhibitors are superior to other antihypertensives in terms of protecting against deteriorating renal function and reducing proteinuria (4, 5, 14, 15). Similar results have been achieved with ARBs (16, 17). Recent studies compared the effect of an ACE inhibitor, enalapril, and an ARB, losartan, on blood pressure, proteinuria, renal blood flow, GFR, PRA, and angiotensin II levels and found no significant differences (18, 19). On the other hand, different results were found when the effects of losartan were compared with those of a calcium channel blocker, amlodipine, in non-diabetic proteinuric renal diseases (20). Although amlodipine did not produce significant variations in proteinuria levels in patients with non-diabetic proteinuric renal disease, moderate hypertension, and a serum creatinine <2.5 mg/dL, losartan markedly decreased levels of proteinuria. Other similar studies, including a small number of patients, had previously investigated the effects of losartan on proteinuria levels in patients with chronic non-diabetic renal diseases (21–23). The antiproteinuric activity of losartan was significantly more pronounced than that of calcium channel blockers. Taking these observations together, the antiproteinuric effects of ARBs such as losartan were due to their effects not only on systemic hypertension but also on other clinical features. However, whether that was due to inhibition of the effects of angiotensin II on intrarenal hemodynamics (24) or to glomerular barrier size-permeability (25, 26) or to anti-inflammation (27) remains unknown. Increased synthesis of angiotensin II by the kidney plays a central role in these events, activating transcription factor NF- κ B (nuclear factor- κ B) and increasing the expressions of several cytokines, chemoattractants, cell adhesion molecules, and growth factors (28–30). A recent study showed that ARB ameliorated high levels of proteinuria by preventing a reduction in the expression of slit diaphragm functional molecules (31). In glomerular mesangial cells, enhanced gene expression for angiotensin II is detected in IgA nephropathy. Hale *et al.* sug-

gested the presence of local angiotensin II receptor down-regulation despite proliferation of the glomerular mesangium in IgA nephropathy (32). Moreover, mesangial cells in culture to which angiotensin II had been exogenously added showed sclerotic changes within the glomerulus independent of changes brought about by increased glomerular pressure (33). While many clinical studies have demonstrated a relationship between reduced proteinuria and blood pressure in hypertensive subjects, this relationship has not been confirmed in normotensive proteinuric patients (34, 35). In addition, clinical practice has shown that some patients with renal injury who are normotensive cannot tolerate even moderate doses of antihypertensive agents because of systemic hypertension (9). In a recent study, losartan reduced mild-moderate proteinuria in normotensive patients with IgA nephropathy (11). However, blood pressure decreased after therapy.

In our study, we were not able to observe nocturnal blood pressure changes, though we showed that patients remained normotensive during visit. The consistency of circadian blood pressure variation seems to be poor in CKD. The lack of circadian rhythm in blood pressure was observed in about 20% of normotensive IgA nephropathy patients (36). In CKD patients, urinary protein excretion increases were greater in the non-dipper blood pressure than in the dipper blood pressure (37). A previous study demonstrated a difference in nocturnal drop in blood pressure between non-sodium-sensitive and sodium-sensitive patients (38). Changes in dietary sodium intake modified the circadian rhythms of both blood pressure and the urinary albumin excretion rate in non-dipper blood pressure. Renal sodium handling may contribute to determining the circadian rhythm of blood pressure, and, furthermore, an elevated glomerular capillary hydraulic pressure during the night may enhance the nocturnal albumin excretion in non-dipper blood pressure (39). There is a possibility that the nocturnal drop in blood pressure might contribute to decreased urinary protein excretion.

In addition, we assessed the influence of losartan therapy on urinary NAG levels, as has been previously reported (33). We found a significant decrease in urinary NAG after 12 months of losartan therapy. As previously reported, urinary NAG, which is derived mainly from proximal tubular cells and serves as a marker of tubular damage, showed a significant correlation with proteinuria. Moreover, the scores for both glomerular obsolescence and interstitial fibrosis correlated positively with urinary NAG levels. These results indicate that urinary NAG measurement may be a highly beneficial clinical marker of the severity of IgA nephropathy (40, 41). The pathogenic mechanisms through which proteinuria induces the appearance of interstitial infiltrates and progressive tubulointerstitial fibrosis have been partially clarified.

In conclusion, we have demonstrated that low-dose losartan therapy significantly reduced proteinuria without changing blood pressure in normotensive patients with proteinuric IgA nephropathy. Studies with a longer follow-up period are

required in order to evaluate whether or not these effects are accompanied by a long-term renoprotective action of losartan in IgA nephropathy.

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