Original Article

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

Yoshio HORITA, Masato TADOKORO, Koichi TAURA, Naofumi SUYAMA, Takashi TAGUCHI*, Masanobu MIYAZAKI**, and Shigeru KOHNO**

This study investigates the ability of low doses of angiotensin-converting-enzyme inhibitors, in combination with angiotensin II receptor blockers, to exert antiproteinuric effects in normotensive and proteinuric outpatients with immunoglobulin A (IgA) nephropathy confirmed by biopsy. We performed a prospective, randomized, 6-month study of the effects of temocapril 1 mg (n = 10), losartan 12.5 mg (n = 10), and both (n = 11) on mild-to-moderate proteinuria 0.76 ± 0.35 g/day (range, 0.4 to 1.6 g/day) and renal function. The study subjects comprised 31 normotensive and proteinuric outpatients with IgA nephropathy accompanied by normal, or mild-to-moderately reduced but stable renal function (glomerular filtration rate > 50 ml/min) without steroid or immunosuppressive therapy. We prospectively evaluated blood pressure, proteinuria, renal function and biochemical parameters before and after 6 months of therapy. The combination therapy significantly reduced proteinuria (63.2%) compared with either temocapril or losartan alone (41.3% and 36.6%, respectively, \( p < 0.04 \) and \( p < 0.01 \), respectively). Blood pressure was most decreased in the group that received combination therapy. The reduced proteinuria did not correlate with reduced systolic or diastolic blood pressure or mean arterial pressure in any of the groups. The glomerular filtration rate fell during the first 3 months of combined therapy, but became reversible after a further 3 months of therapy. The combination significantly decreased angiotensin II (\( p < 0.01 \)), and this decrease was greater than that by either drug alone. In conclusion, the effectiveness of the combined therapy may have been at least partly due to the greater inhibition of the action of angiotensin II in patients with IgA nephropathy. This strategy apparently reduced mild-to-moderate proteinuria in patients with normotensive IgA nephropathy. (Hypertens Res 2004; 27: 963–970)

Key Words: angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, proteinuria, immunoglobulin A nephropathy

Introduction

Immunoglobulin A (IgA) nephropathy is the most prevalent type of primary glomerulonephritis worldwide, as well as the most prevalent form in Japan (1, 2). Among the clinical and laboratory features, hypertension and proteinuria are important predictors of a poor outcome (3). Proteinuria causes renal damage that leads to the more rapid progression of renal diseases towards end-stage renal failure (4). Because angiotensin II plays a critical role in the pathogenesis of proteinuria, angiotensin-converting-enzyme (ACE) inhibitors are often administered to patients with proteinuric nephropathies (4–8). Clinical studies have shown that angiotensin II receptor blockers (ARBs) have positive hemodynamic, antiproteinuric, and antisclerotic effects on the kidney, even in
the absence of the bradykinin system (9, 10). Long-term therapy with ACE inhibitors does not completely abrogate angiotensin II production (11, 12), as angiotensin II is synthesized by chymase via a pathway that is not regulated by ACE (13). Therefore, one practical use of ACE inhibitors and ARBs might be to decrease angiotensin II production by inhibiting ACE activity and to antagonize the effects of angiotensin II produced via chymase by blocking the receptors. Russo et al. (14, 15) recently discovered that therapy with the ACE inhibitor enalapril plus the ARB losartan exerts additive antiproteinuric effects. However, while a relationship between decreased proteinuria and blood pressure (BP) has been demonstrated in hypertensives, it has not been confirmed in normotensive proteinuric patients (16, 17). 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 hypotension. Under these circumstances, doses of combined ACE inhibitors and ARBs must be reduced even to below the therapeutic range. In addition, dosages of some medications must be minimized from a cost-benefit viewpoint. For all of these reasons, there is need of drugs that can reduce the activity of the renin-angiotensin-aldosterone system at low doses and provide long-term renal protection.

Here, we examined the ability of low doses of an ACE inhibitor, temocapril, in combination with an ARB, losartan, to control BP and to exert antiproteinuric effects in a select group of normotensive and proteinuric outpatients with IgA nephropathy.

Methods

Protocol

We enrolled normotensive patients with IgA nephropathy proven by biopsy. Their urine specimens collected at an outpatient clinic contained 0.4 g/day or more of protein and they had been maintained on antiplatelet agents for more than 3 months without ACE inhibitors or ARBs. Participants were enrolled at Nagasaka Municipal Medical Center between January 2000 and March 2003.

Criteria for patient selection were normal BP (<140/90 mmHg; mean arterial pressure <107 mmHg), persistent mild-to-moderate proteinuria of 0.76 to 0.35 g/day (range, 0.4 to 1.6) and normal or mild-to-moderately reduced but stable renal function (glomerular filtration rate >50 ml/min/1.73 m²). Steroid or immunosuppressive therapy and other medications were prohibited throughout the study. Patients with systemic diseases such as diabetes, lupus erythematosus, chronic liver diseases, renal allografts and Henoch-Schönlein purpura were excluded. The 33 patients from our renal clinic who met the inclusion criteria provided written informed consent to participate in the study, which was approved by our institutional ethical committee. Two dropped out, one because of dry cough and the other because of postural hypotension. All statistical calculations were thus made using the data from 31 patients (14 females and 17 males; age, 40.6 ± 10.7; range, 22–61 years), who were randomly assigned to the temocapril, losartan or combined group. Ten patients aged 39.6 ± 10.8 (4 females and 6 males) received 1 mg of temocapril daily. Ten patients aged 42.7 ± 12.0 (6 females and 4 males) received 12.5 mg of losartan daily and 11 patients aged 39.6 ± 10.4 (6 females and 5 males) received a combination of 12.5 mg losartan and 1 mg temocapril daily over a period of 6 months.

The study was a prospective randomized parallel-group open-labeled trial. Patients were instructed to take their medications once upon awakening every morning, and blood samples were tested 3 h later. Baseline laboratory tests included a complete blood count, serum chemistry and 24-h urine collection for protein. Peripheral plasma renin activity, plasma aldosterone and angiotensin II levels were determined immediately prior to and then 2 h after starting the therapy. BP and serum creatinine were tested again 1 week after starting the protocol.

Clinical and Laboratory Procedures

The patients remained on their usual diet (free intake of salt and protein) for the duration of the study. The treatment effects were prospectively assessed once per month. BP and heart rate were measured with the patient seated. Urinary protein excretion was evaluated after 3 and 6 months in 24-h urine samples. Serum and urinary values were measured after 3 and 6 months by routine laboratory methods using a JEOL JCA-BM1650 autoanalyzer (JEOL, Tokyo, Japan). Peripheral plasma renin activity and plasma aldosterone levels were measured by radioimmunoassay (RIA) (SRL Inc., Tokyo, Japan) before and after 6 months of treatment. Plasma angiotensin II levels were determined by RIA (18) before and after 6 months of treatment. To measure angiotensin II, 0.5 ml of plasma was mixed with 2.5 ml of ethanol. After centrifugation at 2,000 × g for 15 min at 4 ºC, the supernatant was dried under nitrogen gas at 37 ºC. The dried sample was resuspended in 50 mmol/L Tris buffer (pH 7.5) for RIA with 125I-labeled angiotensin II and a rabbit anti-angiotensin II antiserum using a kit (SRL Inc.). The cross-reactivity of the antiserum with angiotensin I was 0.037%. Plasma angiotensin II was within the detection range of this assay, since the minimum detectable concentration was 2.5 pg/ml. The intra and interassay coefficients of variation with repeated extractions were 2.9% and 7.2%, respectively. We evaluated the glomerular filtration rate after 3 and 6 months using the Gault-Cockcroft equation (19). The same clinician measured BP every month in the seated patients 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 systolic and diastolic blood pressure (SBP and DBP), respectively. Mean arterial pressure (MAP)
was calculated as the sum of one third of the SBP and two thirds of the DBP.

**Pathological Examination**

Glomerular, interstitial, and vascular lesions were scored by the assessment of light microscopy samples stained with hematoxylin and eosin, periodic acid-Schiff, trichrome, and silver. For each sample of tissue, nine features were assessed: mesangial hypercellularity, mesangial matrix increases, glomerular sclerosis, cellular crescents, fibrous adhesion, tubular atrophy, interstitial infiltrate, interstitial fibrosis, and vascular sclerosis. A severity index (20) was used to assign a semiquantitative score of 0 to 3 for hypercellularity, increase in the mesangial matrix, and glomerular sclerosis for each glomerulus in all tissue samples of each patient examined. Cellular crescents and fibrous adhesion were scored by the percentage of glomeruli having the respective feature, as: absent (0), less than 25% (1), 26% to 50% (2), and greater than 50% (3). Tubular, interstitial, and vascular lesions were scored as: absent (0), focal (1), multifocal (2), or diffuse (3).

**Data Analysis**

All data are expressed as the means ± SD. Test data for the groups were analyzed by repeated measures ANOVA. Fisher’s PLSD post hoc test identified significant differences among mean values. Logistic regression analysis was also used to examine changes (decrease or increase) in proteinuria as a dependent variable with other factors as independent variables. An unpaired Student’s t-test was used to determine the significance of differences among the three treatment groups. Discrete data were examined using the $\chi^2$ analysis. Values of $p<0.05$ were considered to indicate statistical significance.

**Results**

Primary end points could be analyzed in 31 of the 33 patients at the end of the 6-month period. One patient in the temocapril group developed a dry cough; another who received the combined therapy developed postural hypotension. Table 1 shows the characteristics of all groups. The gender distribution, age, baseline BP, urinary protein excretion and renal function were similar, and routine laboratory values for cholesterol triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), electrolytes and urine sodium excretion did not vary significantly throughout the study period. The average values for the histological features in each group are shown in Table 2. There were no significant differences in mesangial cell proliferation, mesangial matrix increase, glomerular sclerosis, cellular crescents, fibrous adhesion, tubular atrophy, interstitial infiltrate and fibrosis, or vascular lesions among the groups. Proteinuria, measured as 24-h urinary protein excretion, did not differ significantly among the three groups. Proteinuria decreased significantly after 6 months in all groups (Table 3). Proteinuria was diminished by 41.3% (baseline, 0.75 ± 0.30 g/day; after 6 months, 0.44 ± 0.31 g/day) on temocapril and by 36.6% (baseline, 0.81 ± 0.44 g/day; after 6 months, 0.55 ± 0.38 g/day) on losartan. However, the magnitude of the decrease in proteinuria did not differ significantly between the group that received temocapril and the group that received losartan. Combined therapy induced a more remarkable reduction of proteinuria (63.2%; baseline, 0.75 ± 0.30 g/day; after 6

<p>| Table 1. General Characteristics of Proteinuric Patients with Normotensive IgA Nephropathy |
|---------------------------------------------|-----------------|-----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temocapril (n = 10)</th>
<th>Losartan (n = 10)</th>
<th>Combined (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>4/6</td>
<td>5/5</td>
<td>5/6</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>39.6 ± 10.8</td>
<td>42.7 ± 12.0</td>
<td>39.6 ± 10.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.7 ± 3.2</td>
<td>20.4 ± 2.7</td>
<td>19.7 ± 2.0</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>55.5 ± 11.5</td>
<td>56.4 ± 8.4</td>
<td>52.4 ± 5.2</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>0.73 ± 0.36</td>
<td>0.81 ± 0.44</td>
<td>0.75 ± 0.30</td>
</tr>
<tr>
<td>Serum total protein (g/dl)</td>
<td>7.09 ± 0.41</td>
<td>6.87 ± 0.35</td>
<td>6.75 ± 0.62</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.85 ± 0.21</td>
<td>0.88 ± 0.17</td>
<td>0.83 ± 0.19</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>11.1 ± 0.28</td>
<td>10.4 ± 0.24</td>
<td>10.2 ± 0.33</td>
</tr>
<tr>
<td>Serum potassium (mEq/l)</td>
<td>4.37 ± 0.34</td>
<td>4.47 ± 0.31</td>
<td>4.39 ± 0.34</td>
</tr>
<tr>
<td>Urine salt (g/day)</td>
<td>11.3 ± 1.7</td>
<td>11.1 ± 1.9</td>
<td>10.0 ± 2.2</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>92.5 ± 17.2</td>
<td>88.3 ± 19.8</td>
<td>91.5 ± 246</td>
</tr>
<tr>
<td>Office SBP (mmHg)</td>
<td>121 ± 8</td>
<td>124 ± 5</td>
<td>121 ± 9</td>
</tr>
<tr>
<td>Office DBP (mmHg)</td>
<td>78 ± 8</td>
<td>80 ± 5</td>
<td>73 ± 11</td>
</tr>
<tr>
<td>Office MAP (mmHg)</td>
<td>92 ± 8</td>
<td>94 ± 5</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>74 ± 6</td>
<td>75 ± 6</td>
<td>78 ± 6</td>
</tr>
</tbody>
</table>

IgA, immunoglobulin A; Urine salt, urinary salt excretion; GFR, glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
months, 0.28 $\pm$ 0.20 g/day, $p<0.01$) than either drug administered alone ($p = 0.04$ vs. temocapril monotherapy; $p = 0.01$ vs. losartan monotherapy) (Fig. 1).

After 6 months of treatment, the BP declined in all the groups. SBP decreased significantly during the administration of losartan (124 $\pm$ 5 to 116 $\pm$ 5 mmHg; $p = 0.01$) and during the combination therapy (121 $\pm$ 9 to 106 $\pm$ 7 mmHg; $p<0.01$), and decreased slightly, but not significantly, during the administration of temocapril. Diastolic blood pressure (DBP) and MAP did not change significantly in response to any of the treatments.
The glomerular filtration rate (GFR) measured using the Gault-Cockroft equation (19) did not change significantly in any of the groups over the 6-month treatment period. Body weight, serum total protein, serum creatinine, serum potassium, urinary salt excretion and GFR did not change throughout the study (Table 4 and Fig. 3).

After 6 months, the hormones of the renin-angiotensin-aldosterone system and the plasma renin activities were significantly increased in all groups (temocapril: 1.56 ± 1.01 to 5.56 ± 5.04 ng/ml/h, p < 0.02; losartan: 1.19 ± 1.06 to 4.04 ± 3.01 ng/ml/h, p < 0.01; combined therapy: 1.12 ± 0.84 to 3.3 ± 1.64 ng/ml/h, p < 0.01) (Fig. 4), and only angiotensin II levels were significantly decreased in the group given combined therapy (13.73 ± 4.75 to 6.55 ± 3.72 pg/ml; p < 0.01) (Fig. 5), but plasma aldosterone levels did not change significantly in any of the groups (Fig. 6).

## Discussion

The present findings indicated that low-dose combination therapy with temocapril and losartan reduced proteinuria in normotensive patients with proteinuric IgA nephropathy. This process was independent of changes in GFR. Therefore, the finding that the reduction in proteinuria by combination therapy was greater than that by either monotherapy was probably not a consequence of a reduction in the renal function of normotensive patients with proteinuric IgA nephropathy. Many studies support the notion that ACE inhibitors are superior to other antihypertensives in terms of protecting against deteriorating renal function and reducing proteinuria (4–8). The ACE inhibitor temocapril might provide renal protection by reducing urinary microalbumin even in hypertensive patients with no evidence of renal impairment (21).
Similar results have been achieved with ARBs (9, 10, 22). The combined use of ACE inhibitors and ARBs has been shown to have pronounced antiproteinuric effects in normotensive patients with IgA nephropathy (14, 15).

Although proteinuria was reduced in all three groups, whether this was due to the effects of ACE inhibitors and ARBs on systemic hypertension, or to inhibiting the effects of angiotensin II on intrarenal hemodynamics (23), or to glomerular barrier size-permeability (24, 25) remains unknown. Russo et al. (14) pointed out that the effect of reduced BP on the antiproteinuric outcome of combined therapy could not be completely excluded in their study, since seated BP was recorded only in the morning, 24 h after taking the antihypertensive agents. In the present study, low-dose combination therapy significantly decreased seated BP when compared with the baseline values (Table 3). Thus, the antiproteinuric effect of the combination of ACE inhibitors and ARBs in this study can be partly explained by the reduced BP. The present study did not aim to estimate the impact of the examined drugs on BP. However, BP was decreased in all participants. On the other hand, there was no significant correlation between BP and reduced proteinuria in any group. Russo et al. (14, 15) obtained similar results in patients with proteinuric IgA nephropathy. The limitations of the present study with respect to measuring the advantages of the combined therapy must be addressed. The antiproteinuric activity of the combined treatment was significantly more pronounced compared with that by temocapril or losartan monotherapy. Moreover, we used doses of losartan and temocapril that were on the low border of the therapeutic range. This makes it difficult to compare our results with those of studies performed using maximal therapeutic doses with proven antiproteinuric effects. On the other hand, the more pronounced reduction of BP in patients given both drugs could have had an important impact on the more enhanced antiproteinuric effect observed by this therapy, despite the absence of a correlation between the BP reduction and the proteinuria reduction in this group. These limitations should be considered when planning future studies.

The GFR fell slightly during the first 3 months of combined therapy, and this was reversible after a further 3 months of therapy. Apperloo et al. (26) reported that the initial fall in GFR after starting enalapril in patients with mildly to moderately impaired renal function is reversible even after years of treatment, suggesting that the origin of this phenomenon is hemodynamic and not structural. The GFR might be reversible after 6 months of treatment with low doses of temocapril and losartan. This initial fall in GFR associated with a later decrease in response to ACE inhibitors reflects renal protection (26). The effects were similar in normotensive patients with proteinuric IgA nephropathy treated with combined therapy. This finding suggests that the combination of temocapril and losartan can be safely administered to patients with normotensive IgA nephropathy accompanied by mild-to-moderate proteinuria. In addition, the combined therapy decreased BP and effectively retarded the aggravation of renal insufficiency for 6 months in these patients. Nakao et al. (27) recently confirmed the safety and antihypertensive value of ACE inhibitors combined with ARBs in patients with non-diabetic renal insufficiency.

The additive antiproteinuric effect of the combined use of ACE inhibitors and ARBs, which could be the result of a more marked reduction of angiotensin II production, still did not completely block proteinuria in the present study, both because reactive hyperreninemia develops during therapy with ACE inhibitors (28), and because there is a close linear relationship between the ratio of plasma angiotensin II and
the amount of administered angiotensin I (29). Angiotensin II is also synthesized by chymase, a pathway that is not regulated by ACE (11). In a study using in situ hybridization, Miyake et al. (30) found that angiotensin II is largely produced via the chymase pathway in patients with IgA nephropathy. Therefore, ACE inhibitors combined with ARBs should have more pronounced effects than either drug alone. In the present study, the plasma renin activity and angiotensin II levels were changed significantly from the baseline. Navis et al. (31) showed that an angiotensin II infusion offsets the acute effect of ACE inhibitors on renal hemodynamics. Heeg et al. (32) also revealed that even after 2 months of treatment with ACE inhibitors, the renal hemodynamic effects could still be offset by an angiotensin II infusion, indicating that the renal hemodynamic effect of continued ACE inhibitor treatment remains dependent on angiotensin II inhibition. Thus, angiotensin II levels might be related to changes in renal hemodynamics, which would explain the antiproteinuric effects seen during prolonged treatment. We believe that systemic angiotensin II levels were maximally decreased by combined therapy, and remained stable thereafter, whereas the onset of the antiproteinuric effect was slow. This confirms the notion that the renoprotective and antiproteinuric effects of inhibiting the renin-angiotensin-aldosterone system are independent of the action of antihypertensive agents. Our results indicate that combination therapy with low doses of temocapril and losartan is beneficial even in patients with IgA nephropathy and mild-to-moderate proteinuria.

We evaluated renal function as the glomerular filtration rate and by using the Gault-Cockroft equation. The National Kidney Foundation published glomerular filtration rate estimations and assessed proteinuria in their Kidney Disease Outcomes Quality Initiative (K/DOQI) Clinical Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification (33). These guidelines state that the GFR level should be estimated from predictive equations that include the serum creatinine concentration and some or all of the following variables: age, gender, race and body size; and useful GFR estimates in adults are given in the Modification of Diet in Renal Disease (MDRD) Study, which used the Cockroft-Gault equations for its estimations (19).

In conclusion, the results of this study indicated that combination therapy with even low doses of temocapril and losartan reduced mild-to-moderate proteinuria in normotensive patients with IgA nephropathy. The antiproteinuric effect of the combined treatment was significantly greater than that by either agent alone. The ultimate goal of reducing proteinuria by combining an ACE inhibitor with an ARB is to preserve renal function. However, our results suggested that the combination of an ACE inhibitor and ARB was also superior to other antihypertensive combinations in regard to nephroprotection and long-term survival, and this relation should be investigated in larger trials in the future.

Acknowledgements

The authors are grateful to Dr. Shoko Tsukasaki, Dr. Masaya Miyazaki, and Dr. Sunao Atogami, Department of Internal Medicine, Nagasaki Municipal Medical Center.

References


