Antiproteinuric Effects of Combined Antihypertensive Therapies in Patients with Overt Type 2 Diabetic Nephropathy

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Combined antihypertensive therapy plays a crucial role in achieving targeted blood pressure reductions and renoprotection. We therefore compared the antihypertensive and antiproteinuric effects of combined therapy with either a calcium channel blocker (CCB) plus an angiotensin II receptor blocker (ARB) or an angiotensin converting enzyme inhibitor (ACE-I) plus an ARB in patients with type 2 diabetes mellitus complicated by overt nephropathy and mild to moderate hypertension. After a 12-week dietary control period, diabetic patients with mildly to moderately impaired renal function were randomly assigned to either a CCB (amlodipine 5 mg once daily) or an ACE-I (temocapril 2 mg once daily) for 12 weeks (monotherapy period). Both groups then received add-on therapy with an ARB (candesartan 4 mg once daily) for an additional 12 weeks. During the monotherapy period, blood pressure was decreased equally well in both groups. Daily urinary protein excretion remained unchanged in the CCB-treated group (control period, 4.0 ± 1.8 g/day vs. CCB period, 4.1 ± 1.9 g/day; ns; n = 8), but decreased in the ACE-I-treated group (control period, 4.3 ± 1.8 g/day vs. ACE-I period, 3.5 ± 1.7 g/day; p < 0.05; n = 9). After the combined therapy period, blood pressure was decreased to the same degree in both groups. Although ARB plus CCB significantly reduced urinary protein excretion (to 3.5 ± 1.5 g/day; p < 0.05 vs. control period; n = 8), a more profound reduction was achieved with ARB plus ACE-I (to 2.6 ± 1.3 g/day; p < 0.01 vs. control period; n = 9). Monotherapy with the ACE-I increased the serum potassium concentration, and this elevation was sustained after addition of the ARB. In contrast, the serum potassium concentration was not influenced by monotherapy with the CCB, but was significantly increased after addition of the ARB. A decreased hematocrit was observed in the ARB plus ACE-I group. The present study suggests that combined antihypertensive therapy with either a CCB plus an ARB or an ACE-I plus an ARB exerts an antiproteinuric effect in patients with type 2 diabetic nephropathy with mildly impaired renal function. Although the latter combination had a more profound effect, it was associated with an increased serum potassium concentration and worsening of renal anemia. Thus, the combination of a CCB and an ARB should be the first line antihypertensive therapy in those with overt diabetic nephropathy. The long-term efficacy of these combined antihypertensive therapies will need to be further addressed in a future study. (Hypertens Res 2002; 25: 849–855)

Key Words: angiotensin converting enzyme inhibitor, angiotensin II receptor blocker, calcium channel blocker, hypertension, diabetic nephropathy
**Introduction**

Proteinuria is not only an important clinical manifestation of renal disease but is also a strong predictor of the renal prognosis (1, 2). Importantly, hypertension and proteinuria frequently coexist in patients with renal disease, and this coexistence is associated with the most rapid progression of chronic renal disease to end-stage chronic renal failure (CRF) (3–5). Therapy should therefore be directed at decreasing both blood pressure and proteinuria. There is now agreement that antihypertensive therapies which reduce daily protein excretion may retard the progression of renal impairment and produce a more favorable prognosis (6).

With overwhelming evidence that earlier and more intensive blood pressure control is renoprotective in patients with hypertension, preferably achieving a blood pressure of less than 130/85 mmHg (7–9), it is clear that these patients will require multiple medications to forestall the development of cardiovascular complications (10). Recently, a clear general consensus has been reached regarding the usefulness of angiotensin converting enzyme inhibitors (ACE-Is) in progressive renal disease (11–13), since these agents exert a substantial renoprotective effect independent of any reduction in systemic blood pressure. Clinical studies have also shown that angiotensin II receptor blockers (ARBs) lower blood pressure as effectively as ACE-Is, and that both of these classes of antihypertensive agent are equally effective in slowing the progression of kidney disease to end-stage CRF (14, 15). Thus, to ensure renal protection, any combined therapy for progressive renal disease must include either an ACE-I or an ARB, or both.

Recently, combined therapy with an ACE-I and an ARB has been shown to provide a greater degree of renoprotection than monotherapy with either of these agents in patients with immunoglobulin A (IgA) nephropathy (16, 17). This also holds true for type 2 diabetic nephropathy (18). However, the potential hazards of ARB plus ACE-I combinations must also be taken into consideration: such combinations often produce worsening of hyperkalemia (19, 20), and may be associated with a decline in the hematocrit in CRF patients with renal anemia (21, 22). Therefore, the use of a calcium channel blocker (CCB) plus an ARB may be preferable in order to avoid these adverse effects. In addition, because CCBs per se yield a reliable reduction in blood pressure, while ARBs offer an assured antiproteinuric effect, such combinations may be potentially useful for providing renoprotection. To date, however, few comparative studies on the pharmacological efficacy of combined therapy with a CCB plus an ARB and/or an ACE-I plus an ARB have been performed. We therefore conducted a pilot study to explore the effectiveness of these two combined antihypertensive regimens, focusing on patients with diabetic nephropathy, who are particularly likely to progress to terminal renal failure.

**Patients and Methods**

**Patient Selection**

Seventeen patients with type 2 diabetes mellitus complicated by overt nephropathy were enrolled in the study. All had mild to moderate hypertension which had not previously been treated, and were suitable for antihypertensive therapy. The subjects were between 45 and 70 years of age and were receiving outpatient treatment for their diabetes. Their serum creatinine (Cr) concentrations were within the range 2–4 mg/dl. Patients with serious diabetic complications such as myocardial infarction and cerebral vascular disease or who were receiving insulin therapy were excluded from the study. Informed consent was obtained from each patient before starting the study.

**Study Treatment**

The patients underwent an initial 12-week dietary titration period before starting antihypertensive therapy (control period). They were then alternatively assigned to receive either a CCB (amlodipine 5 mg once a day; Group A) or an ACE-I (temocapril 2 mg once a day; Group B) for 12 weeks (monotherapy period), after which both groups were given an ARB (candesartan 4 mg once a day) as an add-on therapy for an additional 12 weeks (combination period). Daily protein excretion, NaCl and uric acid and endogenous Cr clearance (Ccr) were measured from 24-h urine collections, which were repeated every 4 weeks. Throughout the control, monotherapy and combination periods, the patients were required to maintain a daily calorie intake of 31–33 kcal/kg, a daily protein intake of 0.6–0.8 g/kg and a daily salt intake of 7 g. Adherence to this dietary restriction was evaluated by calculating the estimated protein and salt intake from the urinary urea nitrogen and Na concentrations, respectively, in the 24-h urine collections. Blood pressure was measured in a sitting position in the morning (10:00 to 11:00 AM) every 4 weeks. We followed all American Heart Association Recommendations published in 1988 (23), including use of a 47 cm bladder and 24 cm cuff to avoid cuff hypertension. The cuff was strictly positioned 2 cm above the antecubital crease to obtain a similarly leveled compression of the brachial artery. Blood pressure was measured in a sitting position every 2 weeks, and all blood pressures were averages of 2 measurements. All blood pressure values were expressed as the average of two measurements obtained at the same time-point.

**Statistical Analyses**

The paired and unpaired Student’s t-test, χ² test and step-wise regression analysis were applied using SAS system software (SAS Institute, Cary, USA). The computer used for
the analysis was a Dynabook Satellite 2590X (TOSHIBA, Tokyo, Japan). All data are presented as the means ± SD unless otherwise indicated.

**Results**

Table 1 shows the baseline characteristics of the patients enrolled in this study. There were no differences in any of the parameters between the two groups. The severity of diabetes mellitus and the blood pressure readings were also comparable.

The changes in blood pressure occurring in response to the various antihypertensive therapy regimens are shown in Fig. 1. During the monotherapy period, blood pressure was significantly reduced to the same degree by both the CCB and the ACE-I. During the following combination period, both Group A (CCB plus ARB) and Group B (ACE-I plus ARB) showed further reductions in blood pressure; the magnitude of these extra reductions was also comparable.

Figure 2 shows the effects of the antihypertensive therapy regimens on daily urinary protein excretion in individual patients. In Group A, monotherapy with the CCB produced an increase in daily urinary protein excretion in 4 patients, a decrease in 3 patients and no change in one patient. However, after addition of the ARB, there was a decrease in 6 patients and an increase in only one patient. In contrast, in Group B, monotherapy with the ACE-I reduced daily urinary protein excretion in all 9 patients, and addition of the ARB further enhanced these antiproteinuric effects. The insets in Fig. 2 depict the daily excretion of urinary protein. In Group A, the CCB alone did not change urinary protein excretion (control period, 4.0 ± 1.8 g/day vs. CCB period, 4.1 ± 1.9 g/day; ns). However, addition of the ARB led to a significant reduction (to 3.5 ± 1.5 g/day; *p < 0.05* vs. control period paired *t*-test; *n = 8*). In Group B, the ACE-I alone significantly decreased urinary protein excretion (control period, 4.3 ± 1.8 g/day vs. ACE-I period, 3.5 ± 1.7 g/day; *p < 0.05*, paired *t*-test; *n = 9*). Moreover, addition of the ARB to the ACE-I produced a further decrease (to 2.6 ± 1.3 g/day; *p < 0.01* vs. control period, paired *t*-test; *n = 9*). Calculations of the percentage reduc-

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**Table 1. Baseline Characteristics of the Two Patient Groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (CCB + ARB)</th>
<th>Group B (ACE-I + ARB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.5 ± 6.0</td>
<td>50.2 ± 9.6</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5 / 3</td>
<td>4 / 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 1.9</td>
<td>22.8 ± 2.5</td>
</tr>
<tr>
<td>HbA₁c</td>
<td>7.9 ± 1.4</td>
<td>7.5 ± 1.6</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>189 ± 22</td>
<td>172 ± 40</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td>4.2 ± 0.5</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>s-Cr (mg/dl)</td>
<td>2.2 ± 0.6</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>s-K (mEq/l)</td>
<td>4.5 ± 0.4</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>36.7 ± 3.6</td>
<td>35.0 ± 4.2</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.2 ± 1.2</td>
<td>11.6 ± 1.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>156 ± 11</td>
<td>150 ± 12</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>93 ± 5</td>
<td>89 ± 7</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td>28.0 ± 16.9</td>
<td>30.5 ± 8.2</td>
</tr>
<tr>
<td>U₉₀-V (mEq/day)</td>
<td>158 ± 40</td>
<td>168 ± 52</td>
</tr>
<tr>
<td>U₆₉-V (mg/day)</td>
<td>750 ± 210</td>
<td>610 ± 180</td>
</tr>
<tr>
<td>U₉₀-V (g/day)</td>
<td>3.8 ± 1.9</td>
<td>4.5 ± 2.2</td>
</tr>
</tbody>
</table>

M, male; F, female; BMI, body mass index; TC, total cholesterol; Alb, serum albumin concentration; s-Cr, serum creatinine concentration; s-K, serum potassium concentration; SBP, systolic blood pressure; DBP, diastolic blood pressure; Ccr, endogenous creatinine clearance; U₉₀-V, daily excretion of salt; U₆₉-V, daily uric acid excretion; U₉₀-V, daily excretion of protein.
tions in daily urinary protein excretion (Fig. 3) revealed that, in Group A, the CCB alone increased excretion by 11%, whereas the CCB plus the ARB reduced it by 10% compared with the control value. In Group B, monotherapy with the ACE-I reduced urinary protein excretion by 20%, and addition of the ARB reduced it by 43%. The reduction obtained with this combination was significantly larger than that obtained with ACE-I monotherapy.

Table 2 shows changes in the various other measured parameters occurring in response to the various antihypertensive treatments. In both Group A and Group B, the serum Cr concentration and the Ccr were unchanged throughout the trial period. On the other hand, in Group B, the hematocrit (Ht) tended to be lowered during monotherapy with the ACE-I. This trend became statistically significant with the addition of the ARB (control period, 35.0 ± 4.2% vs. ACE-I plus ARB period, 30.0 ± 4.6%; p < 0.01 paired t-test; n = 9). Similar to the result for Ht, hemoglobin (Hb) was also reduced in patients treated with the ACE-I plus ARB compared to either the control or monotherapy with the ACE-I. Glycosylated hemoglobin (HbA1c) levels remained unchanged throughout the study period in both groups.

The changes in serum potassium concentrations are depicted in Fig. 4. In Group A, the serum potassium concentration remained unchanged with CCB alone, but was significantly increased with CCB plus ARB (control period, 4.5 ± 0.4 mEq/l vs. CCB plus ARB period, 4.8 ± 0.4 mEq/l; p < 0.05, paired t-test; n = 8). In contrast, in Group B, the serum potassium concentration was significantly increased with the ACE-I alone (control period, 4.6 ± 0.6 mEq/l vs. ACE-I period, 4.8 ± 0.5 mEq/l; p < 0.05, paired t-test; n = 8), and this effect was further reinforced by addition of the ARB (control period, 4.6 ± 0.6 mEq/l vs. ACE-I plus ARB period, 4.9 ± 0.6 mEq/l; p < 0.05, paired t-test; n = 8). Whether or not patients adhered well to the designated diet was evaluat-}

ed by urinary salt excretion. The calculated daily salt excretion was 8–9 g/day throughout the study period, which was about 20% larger than the expected excretion (7 g/day).

No serious side effects, such as an increase in serum potassium concentration exceeding 5.5 mEq/l, were observed in either Group A or Group B during the study periods.

**Discussion**

The finding by Lewis et al. that addition of an ACE-I to standard treatment reduced the progression of renal failure to a greater extent than addition of a placebo in type 1 diabetic patients with hypertension (11) inspired numerous clinical studies on the renoprotective effects of blockade of the renin-
Table 2. Effects of Monotherapeutic and Combined Antihypertensive Regimens on Various Clinical Parameters in Patients with Type 2 Diabetic Nephropathy

<table>
<thead>
<tr>
<th></th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>sCr (mg/dl)</th>
<th>sK (mEq/L)</th>
<th>Ht (%)</th>
<th>Hb (g/dl)</th>
<th>Ccr (ml/min)</th>
<th>*GFR (mEq/day)</th>
<th>*GFR (mg/day)</th>
<th>*GFR (%)</th>
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<tbody>
<tr>
<td><strong>Group A</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>150±11</td>
<td>90±5</td>
<td>2.2±0.6</td>
<td>4.0±0.4</td>
<td>115±5</td>
<td>8.5±0.9</td>
<td>34.8±3.4</td>
<td>150±16</td>
<td>100±10</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>CCB</td>
<td>142±10*</td>
<td>88±10*</td>
<td>2.4±0.7</td>
<td>4.6±0.5</td>
<td>119±15</td>
<td>8.4±0.4</td>
<td>33.1±1.5</td>
<td>140±15</td>
<td>100±15</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>CCB + ARB</td>
<td>130±16*</td>
<td>85±8</td>
<td>2.6±0.6</td>
<td>4.8±0.6</td>
<td>114±14</td>
<td>8.3±0.4</td>
<td>31.0±1.5</td>
<td>130±16</td>
<td>100±16</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>150±12</td>
<td>90±7</td>
<td>2.4±0.7</td>
<td>4.6±0.6</td>
<td>116±16</td>
<td>8.5±0.4</td>
<td>35.0±4.7</td>
<td>150±16</td>
<td>100±16</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>ACE-I</td>
<td>143±8*</td>
<td>85±8</td>
<td>2.6±0.7</td>
<td>4.8±0.5</td>
<td>111±16</td>
<td>8.4±0.4</td>
<td>33.2±1.5</td>
<td>140±15</td>
<td>100±15</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>ACE-I + ARB</td>
<td>134±8*</td>
<td>85±8</td>
<td>2.8±0.9</td>
<td>4.9±0.6</td>
<td>107±15</td>
<td>8.5±0.4</td>
<td>30.0±4.6</td>
<td>130±16</td>
<td>100±16</td>
<td>1.0±0.5</td>
</tr>
</tbody>
</table>

Abbreviations are listed in Table 1.

*p < 0.05 compared to the respective control.

*p < 0.05 compared to the monotherapy of the respective group.

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Angiotensin system (RAS). Following this initial observation, the specific effects of ACE-Is and ARBs on diabetic nephropathy have been extensively investigated in patients with type 2 diabetes (24–27). The results have consistently shown a beneficial renoprotective effect of ACE-Is and ARBs in diabetic nephropathy. In addition, a large body of clinical evidence now indicates that patients with moderate to severe hypertension require multiple antihypertensive medications to produce more intensive blood pressure reductions and achieve target levels (10). Such therapeutic strategies are especially applicable to patients with chronic renal diseases. Patients with proteinuria and hypertension, which frequently coexist in diabetes, have a strikingly high mortality risk (28), and their treatment must therefore be directed towards decreasing daily protein excretion as well as lowering blood pressure.

In the present study, we have focused on combined antihypertensive therapy, which has been proven very effective for these purposes. There are two main findings of this study. First, combined therapies based on either an ACE-I plus an ARB or a CCB plus an ARB exert a substantial antiproteinuric effect, although the former appears to be more effective than the latter. However, our second major finding was that therapy with an ACE-I alone or in combination with an ARB was associated with hyperkalemia and/or worsening of renal anemia.

Regarding the efficacy of combined therapy with ACE-Is and ARBs, Russo et al. demonstrated that a combination of losartan and enalapril produced a more marked reduction in proteinuria compared to either agent alone in patients with IgA glomerulonephritis with normal renal function, suggesting a substantial advantage of the combination therapy (16, 17). Combined therapy with candesartan and lisinopril has also been found to reduce protein excretion more successfully than either agent alone in type 2 diabetic nephropathy (18), and similar additive effects have been reported by other investigators (29, 30). These previous data along with our present findings show that the combination of an ARB and an ACE-I is more efficacious than monotherapy with either of the components in ameliorating proteinuria in diabetic nephropathy. However, the present finding that combination therapy with ACE-I and ARB runs a risk of increased renal anemia and increased serum potassium concentrations, which is in good agreement with the previous reports, presents a problem that must be addressed (19–22). Hyperkalemia is especially likely to occur when ARB/ACE-I combinations are used in patients with impaired renal function. Thus, one must be alert to changes in serum potassium concentration and Ht or Hb when the two RAS blockers are combined.

Clinical evidence for the additive effects of CCB and ACE-I combinations is scanty. Initial reports of short-term and long-term combination therapy in type 1 and type 2 diabetic patients with nephropathy appear very promising. In type 2 diabetic nephropathy, urinary albumin excretion
(UAE) was reduced by approximately 50% in groups treated with either nicardipine or captopril alone, whereas combined therapy with these two drugs produced a more profound decline of approximately 70% (31). Similar results were obtained in a study involving Japanese type 2 diabetic patients, in which combinations of amlodipine and ACE-Is reduced UAE to a greater extent than the ACE-Is alone (32). Our present study using patients with type 2 diabetic nephropathy showed that monotherapy with CCB did not alter urinary protein excretion, yet CCB and ARB in combination substantially reduced it. The additive effect might have been observed if the decrease in blood pressure had been more profound. An additive effect was observed in a previous study using a CCB and an ACE-I to treat type 1 diabetic nephropathy (33). Moreover, Bakris et al. not only demonstrated the beneficial antiproteinuric effect of combined therapy with an ACE-I and a CCB, but also suggested that a combination of reduced-dose ACE-I and CCB produced fewer side effects than either agent alone at high doses (34). In animal models, verapamil alone elicited modest efferent arteriolar vasodilation, and in rats pretreated with an ACE-I, the efferent arteriolar constriction induced by angiotensin II was attenuated by either verapamil or diltiazem (35). Moreover, a study in a chemically induced diabetic model demonstrated that the combination of an ACE-I and a CCB had an additive antiproteinuric effect (36). Such combinations have also been noted to have greater beneficial effects on proteinuria, intraglomerular pressure and histological parameters in subtotally nephrectomized rats compared with the respective monotherapies (37). Interestingly, Muller et al. showed that, even at non-hypotensive doses, the combination of verapamil and trandolapril retarded the development of glomerular sclerosis more effectively than either agent alone in stroke-prone spontaneously hypertensive rats (38).

Clearly, a number of arguments could be advanced for combining ACE-Is or ARBs with CCBs. The CCBs have come under fire in recent years due to their potential for promoting ischemic events and reports of their insufficient renoprotective action (39, 40); however, these agents have been definitively proven to reduce blood pressure, and their blood pressure reduction would be expected to make a major contribution to renoprotection. Moreover, the use of a long-acting CCB such as amlodipine is unlikely to be associated with the reflex increase in heart rate due to sympathetic nerve activation reported with short-acting CCBs, which are known to exert insufficient renoprotection.

One important limitation of the present study was the small number of subjects recruited. A small cohort was employed because many of our patients, who were basically asymptomatic, were reluctant to switch from one therapeutic regimen to another, and therefore either refused to participate or revoked their consent during a later phase of the study. Despite this flaw, we believe that our results have worthwhile clinical relevance in the field of combined antihypertensive therapy research, although there is no doubt that a larger study, preferably with a double-blind crossover, multi-center design, will be required to confirm our findings. It would also be of great interest to investigate whether these combinations are suitable for treating other forms of progressive renal disease, such as chronic glomerulonephritis or nephrosclerosis due to essential hypertension.

In summary, the results of the present study suggest that combined therapy with an ARB and a CCB has a potentially useful antiproteinuric effect in patients with type 2 diabetic nephropathy, even when their renal function is reduced. This combination may be worth trying for patients with progressive renal disease who do not respond well to monotherapy with a CCB or an ARB. Although treatment with an ARB plus an ACE-I has a greater antiproteinuric effect, such combinations may be associated with potential hazards, including worsening of renal anemia and increased serum potassium concentrations, especially in patients whose kidney function is mildly to moderately impaired. The long-term efficacy and safety of these combination therapies must, however, be further addressed in a large-scale prospective clinical study.

References


