Dual Blockade of the Renin-Angiotensin System in Diabetic Nephropathy

A randomized double-blind crossover study

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Objective — Many patients with diabetic nephropathy (DN) have levels of albuminuria >1 g/day and blood pressure ≥ 135/85 mmHg, despite antihypertensive combination therapy, including recommended doses of ACE inhibitors, e.g., lisinopril/enalapril at 20 mg daily. We tested the concept that such patients might benefit from dual blockade of the renin-angiotensin system (RAS).

Research Design and Methods — We performed a randomized double-blind crossover study of 2 months treatment with candesartan cilexetil 8 mg once daily and placebo in addition to previous antihypertensive treatment. We included 18 type 2 diabetic patients with DN fulfilling the above-mentioned criteria. All received recommended doses of ACE inhibitor and, in addition, 15 patients received diuretics, 11 received a calcium channel antagonist, and 3 received a ß-blocker. At the end of each treatment period, we measured the glomerular filtration rate (GFR), 24-h blood pressure, albuminuria, and IgGuria.

Results — The addition of candesartan to usual antihypertensive therapy induced a mean (95% CI) reduction in albuminuria of 25% (2–58), P = 0.036 (geometric mean [95% CI] from 1.76 mg/24 h [1.23–2.50] to 1.33 mg/24 h [0.89–1.99]). It also produced a mean reduction of 35% (9–33) in the fractional clearance of albumin (P = 0.016), a reduction of 32% (1–54) in fractional clearance of IgG (P = 0.046), a reduction in 24-h systolic blood pressure of 10 mmHg (2–18) (P = 0.019) (mean ± SE) from 148 ± 3 to 138 ± 5 mmHg, and a mean reduction in GFR of 5 ml/min·1·1.73 m²-2 (0.1–9) (P = 0.045).

Conclusions — Dual blockade of the RAS reduces albuminuria and blood pressure in type 2 diabetic patients with DN responding insufficiently to previous antihypertensive therapy, including ACE inhibitors in recommended doses.

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nal tract disease (9). A kidney biopsy was performed in patients lacking retinopathy, and only patients with diabetic glomerulopathy were enrolled in the trial. Exclusion criteria were: serum potassium >4.6 mmol/l, pregnancy, age >70 years, alcohol or medicine abuse, inability to understand the patient information, contra-indication to treatment with angiotensin receptor blocker, systolic blood pressure <100 mmHg, and GFR <25 ml/min.

**Design**
We performed a randomized double-blind crossover trial (Fig. 1). Each patient received in random order 2 months treatment with 8 mg candesartan cilexetil once daily and 2 months treatment with one matched placebo tablet daily. Randomization was in blocks of two with concealed computer-generated envelopes. The code was not broken until all data were entered in a database. The study medication was added to the patients’ previous antihypertensive treatment, which was left unchanged throughout the study and which included ACE inhibitors for all patients in daily doses corresponding to 20 mg lisinopril/enalapril or 100 mg captopril daily. None of the patients had their dietary intake of salt or protein restricted.

Patients attended the clinic for a total of five study visits: one screening visit and then visits at 2 and 8 weeks after the start of each treatment period. At the screening visit, albuminuria was determined in three 24-h urine samples, arterial blood pressure was measured three times after 10 min rest, and serum potassium and creatinine were determined. At 14 days after the beginning of each treatment period, blood pressure, serum potassium, and serum creatinine were measured for 14 days for safety reasons. At the end of each treatment period, we assessed clinical end points, including the primary end points albuminuria and 24-h arterial blood pressure and the secondary end point GFR. On the days of the kidney function studies, the patients met fasting at 8:00 A.M., blood samples were taken during the first hour, and then breakfast was given.

Drug compliance was assessed by tablet counts. The local ethical committee approved the study, and all patients gave their informed consent to participate in the study after the nature of the study had been explained.

**Methods**
GFR was measured after a single intravenous injection of 3.7 MBq $^{51}$Cr-EDTA at 8:00 A.M. by determining the radioactivity in venous blood samples taken 180, 200, 220, and 240 min after the injection (10,11). Extra renal loss was accounted for by subtracting 3.7 ml/min (12). The small underestimation (10%) of $^{51}$Cr-EDTA renal clearance versus renal clearance of inulin was corrected by multiplying the EDTA clearance by 1.10 (12). The results were standardized for $1.73 \text{ m}^2$ body surface area. The mean day-to-day coefficient of variation in GFR is 4% in our laboratory.

Arterial blood pressure values were measured by 24-h ambulatory blood pressure measurements using a Takeda TM2420 device (A&D Medical, Tokyo). A measurement of the blood pressure was performed every 15 min in the time period from 7:00 A.M. to 11:00 P.M. and every 30 min from 11:00 P.M. to 7:00 A.M. To determine mean day- and night-time blood pressures, patients were asked what time they went to sleep and what time they woke up. Values were averaged for each hour before calculating the mean 24-h, day, and night arterial blood pressures.

Albuminuria was determined as the geometric mean of three consecutive 24-h urine collections, completed immediately.
before each visit at the end of each treatment period (using turbidimetry performed with a Cobas Mira Plus [Roche, Montclair, NJ]). During the clearance procedure, urine was collected quantitatively to determine albuminuria and IgGuria (using enzyme-linked immunosorbent assay) (13). In addition, sodium, urea, creatinine, and carbamid excretion in the urine were determined (Cobas Mira Plus; Roche). The excretion of urea was used to calculate the protein intake from the nitrogen content of the urea and an estimated value of nonurea nitrogen of 31 mg/kg.

Table 1—Results of 2 months additional treatment with candesartan cilexetil 8 mg daily in 17 type 2 diabetic patients with DN responding insufficiently to conventional antihypertensive treatment including recommended doses of ACE inhibitor

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Candesartan 8 mg daily</th>
<th>Mean difference (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>24-h</td>
<td>148 ± 3</td>
<td>138 ± 5</td>
<td>10 (2–18)</td>
<td>0.019</td>
</tr>
<tr>
<td>Day</td>
<td>153 ± 3</td>
<td>142 ± 5</td>
<td>11 (1–20)</td>
<td>0.03</td>
</tr>
<tr>
<td>Night</td>
<td>139 ± 5</td>
<td>130 ± 5</td>
<td>9 (2–16)</td>
<td>0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h</td>
<td>74 ± 2</td>
<td>71 ± 2</td>
<td>3 (–1 to 6)</td>
<td>0.17</td>
</tr>
<tr>
<td>Day</td>
<td>77 ± 2</td>
<td>75 ± 2</td>
<td>3 (–1 to 7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Night</td>
<td>66 ± 2</td>
<td>65 ± 2</td>
<td>1 (–4 to 7)</td>
<td>0.63</td>
</tr>
<tr>
<td>Albuminuria (mg/24 h)*</td>
<td>1,764 (1,225–2,540)</td>
<td>1,334 (890–1,998)</td>
<td>24% (2–58)</td>
<td>0.036</td>
</tr>
<tr>
<td>θ Ab. (×10⁻⁷)</td>
<td>476 (272–832)</td>
<td>310 (167–577)</td>
<td>35% (9–53)</td>
<td>0.016</td>
</tr>
<tr>
<td>θ IgG (×10⁻⁷)</td>
<td>124 (58–266)</td>
<td>84 (40–180)</td>
<td>32% (1–54)</td>
<td>0.046</td>
</tr>
<tr>
<td>GFR (ml · min⁻¹ · 1.73 m⁻²)</td>
<td>74 ± 7</td>
<td>69 ± 6</td>
<td>5 (0.1–9)</td>
<td>0.045</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>113 ± 8</td>
<td>119 ± 10</td>
<td>–5 (–5 to 15)</td>
<td>0.31</td>
</tr>
<tr>
<td>Serum potassium (mmol/l)</td>
<td>4.2 ± 0.4</td>
<td>4.4 ± 0.1</td>
<td>–0.1 (–0.20 to 0.02)</td>
<td>0.09</td>
</tr>
<tr>
<td>Hba1c (%)</td>
<td>8.6 ± 0.3</td>
<td>8.7 ± 0.4</td>
<td>–0.04 (–0.04 to 0.3)</td>
<td>0.68</td>
</tr>
<tr>
<td>Serum angiotensin II (pmol/l)</td>
<td>9.2 ± 2.1</td>
<td>13.8 ± 4.7</td>
<td>–4.6 (–4.1 to 13.2)</td>
<td>0.28</td>
</tr>
<tr>
<td>Urinary sodium (mmol/24 h)</td>
<td>217 ± 17</td>
<td>223 ± 27</td>
<td>–7 (–43 to 30)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Statistical analysis

Normally distributed variables are expressed as the mean ± SD and otherwise as median (range). Values for albuminuria, θ Ab, and θ IgG clearance were logarithmically transformed and are expressed as the geometric mean (95% CI) because of their positively skewed distribution. Changes in variables between visits are expressed as the mean (95% CI). All comparisons of normally or log normally distributed parameters were performed with a paired Student’s t test. Data were tested for a period effect and a treatment-period interaction with a two-sample t test. Before the present study, we calculated the SD (log scale 0.1771) of the mean difference in urinary albumin excretion rate in three consecutive 24-h urine samples collected twice within 3 months in 36 diabetic patients with DN. Based on these data, a sample-size calculation revealed a necessary minimum of 16 patients to detect a 25% difference in change in the urinary albumin excretion rate (α = 0.05 and β = 0.8).

The difference between the two examinations was transformed into absolute changes in arterial blood pressure and relative changes in albuminuria and GFR. Linear regression analysis was used to analyze for correlations between the change in arterial blood pressure and the relative change in GFR (%). Spearman’s rank correlation was used to estimate the correlation between the change in arterial blood pressure and the change in albuminuria.

P < 0.05 was considered significant (two-tailed test). Data were evaluated using SPSS version 10.0 (SPSS, Chicago, IL).

RESULTS—One patient was excluded because he discontinued active treatment after 2 weeks because of nausea and stomach upset. No other adverse effects were reported. Altogether, 17 patients completed the study (Fig. 1). The median (range) duration of each treatment period was 63 days (57–74), and compliance as assessed by tablet count was 100% (95–100) during both placebo and candesartan treatment. We found no evidence of carryover or sequence effects in the treatment periods in the statistical analysis.

At entry to the study, the mean (±SD) known duration of diabetes and DN was 13 ± 6 and 8 ± 5 years, respectively. The mean age of the patients was 58 ± 8 years, 13 of 17 were male, and the mean BMI was 32 ± 4 kg/m². Seven patients had nonproliferative diabetic retinopathy and six had proliferative diabetic retinopathy. Four patients showed no signs of diabetic retinopathy. In these patients, the diagnosis of diabetic glomerulopathy was verified by a renal biopsy. The patients suffered primarily isolated systolic hypertension, with a sitting office blood
pressure of 159 ± 16 mmHg systolic and a blood pressure of 85 ± 7 mmHg diastolic, and all had highly elevated albuminuria, with a geometric mean (95%CI) of 1,781 mg/24 h (1,326–2,391), despite antihypertensive treatment. The median number of antihypertensive agents was 3 (range 2–4). All patients received ACE inhibitors in daily doses corresponding to 20 mg lisinopril and enalapril (n = 5 and 9, respectively) or 100 mg captopril (n = 4). In addition to ACE inhibition, nearly all patients received diuretics (n = 15), 11 patients received a calcium channel antagonist (10 mg amlodipine once daily), and 3 were treated with a β-blocker.

Values for 24-h systolic blood pressure, albuminuria, θ Alb, θ IgG, and GFR were significantly lower during dual blockade of the RAS, when candesartan cilexetil 8 mg was added to conventional ACE inhibitor treatment, as compared with blockade using ACE inhibitors alone (Table 1).

In 16 patients, 24-h recordings of the arterial blood pressure were available. In these patients, the mean difference (95%CI) for the 24-h systolic blood pressure decreased by 10 mmHg (2–18) during the addition of 8 mg candesartan cilexetil. The reduction in systolic blood pressure was sustained during both the day and night (Table 1). The decline in 24-h diastolic blood pressure of 3 mmHg (–2 to 6) during candesartan treatment was not statistically significant, and no significant changes were found in the 24-h mean heart rate.

Altogether, seven patients had a decline in 24-h systolic blood pressure of >10 mmHg. These patients were characterized by higher baseline (placebo) values for 24-h diastolic blood pressure (77 ± 6 vs. 70 ± 6 mmHg; P = 0.011) and plasma angiotensin II levels (11 ± 1.4 vs. 4.5 ± 1.2 pmol/l; P = 0.02). There was a significant positive correlation between the level of angiotensin II during placebo treatment and the absolute decrease in arterial blood pressure during addition of candesartan (r = 0.66; P = 0.004).

Albuminuria was reduced from placebo values by 25% (2–58) during dual blockade of the RAS. θ Alb was reduced by 35% (9–58), and θ IgG was reduced by 32% (1–54). There was a significant correlation between changes in systolic blood pressure and albuminuria (correlation coefficient 0.53; P = 0.03) (Fig. 2).

GFR was reduced by 5 ml/min · 1.73 m² (0.1–9) (placebo – candesartan) during the addition of candesartan treatment (P < 0.05). The relative decline (in percent) in GFR during candesartan treatment was positively correlated with the decline in systolic blood pressure (r = 0.58; P = 0.01). Serum potassium, sodium, creatinine, HbA1c, and angiotensin II remained unchanged, as did urinary sodium excretion (Table 1).

CONCLUSIONS — In our randomized double-blind crossover trial, we demonstrated that dual blockade of the RAS induced a reduction in 24-h systolic blood pressure, albuminuria, θ Alb, and θ IgG in type 2 diabetic patients with DN responding insufficiently to antihypertensive combination therapy. All received ACE inhibition corresponding to 20 mg enalapril (n = 9)/lisinopril (n = 5) and 100 mg captopril (n = 4) daily. Symbols indicate the number of additional antihypertensive agents. ■ = 1; □ = 2; ▲ = 3. All patients received diuretics except two (▲).

We found a slight decrease in GFR during treatment with candesartan. This is probably explained by the reduced blood pressure, and is most likely fully reversible when treatment is discontinued, as previously suggested (18). In patients who received candesartan in the first treatment period, the GFR increased by 5 ml · min⁻¹ · 1.73 m² during subsequent treatment with placebo. This suggests a reversible effect of candesartan on GFR.

There was a great variability in the response to treatment. Three patients did not have a reduction in either blood pressure or albuminuria. Two of these patients did not receive diuretics, which are known to potentiate agents blocking the RAS. Conversely, seven patients had a >10 mmHg decline in 24-h systolic blood pressure. These patients were characterized by higher baseline (placebo) values of diastolic blood pressure and plasma angiotensin II levels as compared with the rest of the patients.

The additional effect obtained by dual blockade in our study indicates an incomplete inhibition of the RAS during ACE inhibitor treatment. This may be caused by underdosing of the ACE inhibitor or the “ACE-escape” phenomenon (6), or it may reflect a true additive effect of dual blockade. In the present study, patients received doses of ACE inhibition equal to or higher than that previously used in
long-term studies documenting a renoprotective effect in DN (2–5,19,20). The dose of 20 mg lisinopril/enalapril corresponds to the dose inducing the maximal blood pressure–lowering effect in patients with moderate-to-severe essential hypertension (21–24). In these trials no additional significant reduction in blood pressure was demonstrated, despite a dose escalation of enalapril/lisinopril to 80 mg daily or an increase of captopril to 600 mg daily (21). A true additive effect of dual blockade on blood pressure is suggested by our finding of a significant reduction in systolic blood pressure.

The optimal dose of ACE inhibitors with respect to a maximal reduction in albuminuria has never been established in Caucasian patients with DN. However, recently it was reported that the addition of 50 mg losartan daily to a regimen of 40 mg lisinopril daily had no additional effect on arterial blood pressure and proteinuria in a small group of hypertensive proteinuric African-Americans with advanced renal failure of different origin (25). Surprisingly, this study showed a lowered plasma renin activity and enhanced GFR during treatment with 50 mg losartan daily. Nevertheless, these findings cannot be extrapolated to other ethnic groups because suppressed RAS activity and reduced effect of blocking the action/formation of angiotensin II have been demonstrated in African Americans suffering from DN (26) or left ventricular dysfunction (27). Consequently, future studies in Caucasians must evaluate whether additional effects on albuminuria can be obtained with increased doses of ACE inhibition (e.g., ≥40 mg enalapril/lisinopril daily).

Within recommended doses, ACE inhibitors and angiotensin receptor blockers are equally effective in reducing blood pressure and albuminuria (17,28). Consequently, the results in our study could probably not be obtained simply by substituting the ACE inhibitor with an angiotensin II receptor antagonist. Our patients received equipotent doses of different types of ACE inhibitors (29).

The Candesartan and Lisinopril Microalbuminuria (CALM) study, which included patients with type 2 diabetes, microalbuminuria, and hypertension, demonstrated an enhanced reduction in blood pressure by dual blockade (16 mg candesartan cilexetil and 20 mg lisinopril) compared with therapy with either agent alone (17). The reduction obtained in sitting systolic blood pressure on monotherapy using either drug alone was ~15 mmHg. The additional effect of combination therapy was a further reduction in systolic blood pressure of 10 mmHg corresponding to the decrease obtained in our study. In the CALM study, there was a tendency for a more pronounced antiproteinuric effect of dual blockade, as documented in our study dealing with overt DN.

Albuminuria and hypertension are strong predictors of poor renal and cardiovascular outcome in diabetic patients, and numerous studies have demonstrated that by reducing these risk factors, antihypertensive treatment slows the progression of DN, as reviewed by Parving (30). Furthermore, the magnitude of the initial reduction in albuminuria induced by the initiation of ACE inhibitor treatment has been shown to predict the long-term rate of decline in GFR in the succeeding years, i.e., the greater the reduction in proteinuria, the less the decline in kidney function (31–33). Consequently, the short-term reduction in albuminuria demonstrated in the present study during dual blockade may suggest a long-term renoprotective effect of such a treatment strategy. The long-term renoprotective effect of dual blockade of the RAS remains to be established. However, recent studies (26,34) with angiotensin II receptor antagonists suggest a progressive beneficial effect on urinary albumin excretion as compared with conventional antihypertensive treatment in micro- and macroalbuminuric type 2 diabetic patients.

Furthermore, the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study documented a greater beneficial effect of angiotensin II receptor antagonists as compared with conventional antihypertensive agents on the combined end point of doubling of baseline serum creatinine, end-stage renal disease, or death in type 2 patients with diabetes and proteinuria (26). This additional renoprotective effect of the receptor antagonists was obtained with nearly identical blood pressure levels.

The correlation between blood pressure and albuminuria found in our study has been demonstrated in several other studies, as reviewed by Parving (30). The correlation suggests that the renoprotective effect is due at least in part to changes in systemic and local hemodynamics, with a reduction in glomerular capillary hydraulic pressure.

The reduction in albuminuria might also be attributable to structural changes such as improved size-selective properties of the glomerular capillary membrane, as demonstrated in patients with type 1 diabetes (35). Our study did not suggest such a mechanism because the reduction in fractional clearance of the larger molecule IgG paralleled the reduction in fractional clearance of albumin.

In the U.K. Prospective Diabetes Study (UKPDS), tight blood pressure control led to a reduction in arterial blood pressure of 10 mmHg systolic and 5 mmHg diastolic when compared with less tight control. This blood pressure reduction was associated with a risk reduction of 32% in deaths related to diabetes, 37% in microvascular complications, 44% in stroke, and 56% in heart failure (36). The reduction in blood pressure was 10 mmHg systolic and 3 mmHg diastolic in our long-standing complicated type 2 diabetic patients with DN. Because the absolute risk of cardiovascular events is much higher in our patients than in the UKPDS study, such patients may benefit even more if the blood pressure reduction can be sustained for years.

In conclusion, dual blockade of the RAS is well tolerated and reduces albuminuria and blood pressure in patients with type 2 diabetes and DN responding insufficiently to antihypertensive combination therapy, including the recommended dose of ACE inhibitor. However, the long-term renoprotective effects remain to be evaluated.

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References


Dual blockade in diabetic nephropathy

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