Optimal Dose of Candesartan for Renoprotection in Type 2 Diabetic Patients With Nephropathy

A double-blind randomized cross-over study

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OBJECTIVE — We evaluated the optimal dose of the angiotensin II receptor antagonist candesartan cilexetil for renoprotection as reflected by short-term changes in albuminuria in hypertensive type 2 diabetic patients with nephropathy.

RESEARCH DESIGN AND METHODS — A total of 23 hypertensive patients with type 2 diabetes and nephropathy were enrolled in this double-blind randomized cross-over trial with four treatment periods, each lasting 2 months. Each patient received placebo and candesartan: 8, 16, and 32 mg daily in random order. Antihypertensive medication was discontinued before enrollment, except for long-acting furosemide, which all patients received throughout the study in median (range) doses of 40 (30–160) mg daily. End points were albuminuria (turbidimetry), 24-h blood pressure (BP) (Takeda-TM2420), and glomerular filtration rate (GFR) (^{51}Cr-labeled EDTA plasma clearance technique).

RESULTS — Values obtained during placebo treatment: albuminuria [geometric mean (95% CI)] 700 (486–1,007) mg/24-h, 24-h BP (mean ± SE) 147 ± 4/78 ± 2 mmHg, and GFR 84 ± 6 ml/min/1.73 m^2. All three doses of candesartan significantly reduced albuminuria and 24-h BP compared with placebo. Mean (95% CI) reductions in albuminuria were 33% (21–43), 59% (52–65), and 52% (44–59) with increasing doses of candesartan. Albuminuria was reduced significantly more by the two highest doses than by the lowest dose ($P < 0.01$); 24-h systolic BP was reduced by 9 (2–16), 9 (2–16), and 13 (6–20) mmHg and 24-h diastolic BP was reduced by 5 (2–8), 4 (1–7), and 6 (3–9) mmHg with increasing doses of candesartan. There were no significant differences in the reductions in BP between the three doses. GFR was decreased by ~6 ml/min/1.73 m^2 by all three doses of candesartan ($P < 0.05$ versus placebo).

CONCLUSIONS — The optimal dose of candesartan is 16 mg daily for renoprotection, as reflected by short-term reduction in albuminuria, in hypertensive type 2 diabetic patients with nephropathy.

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Diabetic nephropathy develops in 30–40% of patients with type 2 diabetes and constitutes a serious late complication. It is the most common cause of end-stage renal disease (ESRD) and is also associated with increased cardiovascular morbidity and mortality (1). The worldwide incidence of diabetic nephropathy is expected to rise dramatically due to an increase in the incidence of diabetes; therefore, treatment strategies to prevent the development and progression of diabetic nephropathy are of primary importance.

Several clinical trials have demonstrated the beneficial effects of antihypertensive medication in diabetic patients with proteinuria. Antihypertensive treatment reduces proteinuria, slows the rate of decline in kidney function, decreases the risk of ESRD, and improves survival (2–5).

The evidence suggesting that proteinuria should be reduced as far as possible in diabetic and nondiabetic nephropathies has previously been highlighted (6). First, the initial reduction in proteinuria when patients start antihypertensive treatment predicts the subsequent long-term renoprotection, i.e., the greater initial reduction the better long-term outcome (6–10). Second, the residual proteinuria during treatment with antihypertensive agents is directly proportional to the rate of loss of renal filtration power (8).

Recently, three landmark trials of type 2 diabetic patients with early or advanced renal disease have demonstrated specific renoprotective effects of inhibition of the renin-angiotensin system (RAS) by angiotensin II (AngII) receptor antagonists (11–13). In these trials, the renoprotective effects of AngII receptor antagonists went beyond their blood pressure (BP)-lowering effects and were superior to conventional non-RAS–inhibiting antihypertensive agents in reducing albuminuria and postponing the composite end points of doubling of serum creatinine, ESRD, and death. Accordingly, AngII receptor antagonists are now the initial agents of choice in hypertensive type 2 diabetic patients with microalbuminuria or clinical albuminuria, according to the American Diabetes Association (14).

Previously, investigators have chosen doses of AngII receptor antagonists by measurement of maximal beneficial effects on BP. However, dose-response re-

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Abbreviations: AngII, angiotensin II; BP, blood pressure; ESRD, end-stage renal disease; GFR, glomerular filtration rate; RAS, renin-angiotensin system; RENAAL, Reduction of End Points in Type 2 Diabetes With the Angiotensin II Antagonist Losartan.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

lationships for AngII receptor antagonists and their renoprotective effect, as assessed by measuring reduction in albuminuria, have never been established in type 2 diabetic patients with nephropathy.

Consequently, the aim of this study was to evaluate the short-term renoprotective effects, as assessed by reduction in albuminuria, of increasing doses of candesartan in hypertensive type 2 diabetic patients with nephropathy.

RESEARCH DESIGN AND METHODS

Subjects

From the Steno Diabetes Center, we included 24 consecutive Caucasian type 2 diabetic patients, based on World Health Organization criteria (15), with nephropathy (persistent albuminuria >300 mg/24-h) and elevated BP (systolic BP >135 mmHg and/or diastolic BP >85 mmHg).

Exclusion criteria included the following: known nondiabetic kidney or renal tract disease, serum potassium values >4.6 mmol/l, age >70 years, glomerular filtration rate (GFR) <25 ml/min, and systolic BP <100 mmHg or systolic BP persistently >160 mmHg and/or diastolic BP >100 mmHg upon discontinuation of previous antihypertensive treatment.

Design

This study was a randomized, double-blind, double-dummy cross-over trial consisting of four treatment periods lasting 2 months each. In random order, each patient received treatment with candesartan cilexetil 8, 16, and 32 mg once daily and matching placebo tablets. Randomization was concealed with computer-generated envelopes. The code was not broken until all data were entered into a database, which was locked for editing. Drug compliance was assessed by tablet counts. Previous antihypertensive medication was discontinued at least 2 weeks before enrollment in the study. During withdrawal of the antihypertensive medication, BP was monitored at home with an automatic BP-measuring device supplied by the Steno Diabetes Center. Patients were instructed to measure BP three times daily at home to ensure that the BP did not exceed the safety limits of the study (>160/100 mmHg). In addition to the study medication, all patients received loop diuretics throughout the study to prevent edema. The dose of the diuretic was left unchanged throughout the study. None of the patients had restrictions for dietary intake of salt or protein.

The patients attended the clinic for a total of nine study visits: one screening visit and, subsequently, 2 and 8 weeks after the beginning of each of the four treatment periods. At the screening visit, albuminuria was determined in three 24-h urine samples, BP was measured three times after 10 min rest, and serum potassium and serum creatinine levels were determined.

BP, serum potassium, and serum creatinine were measured 14 days after the beginning of each treatment period for safety reasons.

At the end of each treatment period, the primary end point, which was 24-h urinary albumin excretion, and the secondary end points, including 24-h BP and GFR, were determined. GFR was assessed at 8:00 A.M. after an overnight fast, and breakfast was given after the initial blood samples had been collected at ~8:30 A.M.

The local ethical committee approved the study, and all patients gave their informed consent to participate after the nature of the study had been explained. The study was performed in accordance with the Helsinki Declaration.

Laboratory procedures

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 (turbidimetry, Cobas Mira Plus; Roche, Montclair, NJ). During the clearance procedure, urine was collected quantitatively to determine sodium, urea, creatinine, and carbamid excretion in the urine (Cobas Mira Plus). Excretion of urea was used to calculate the protein intake (16). In addition, urinary excretion of albumin, IgG, and IgG_4, determined by enzyme-linked immunosorbent assay (17,18), was determined during the clearance procedure to asses fractional clearance of albumin (θ_{Alb}), IgG (θ_{IgG}), and IgG_4 (θ_{IgG4}). Fractional clearances were determined as [urinary protein excretion]/([serum protein concentration] × GFR).

BP was measured using a 24-h ambulatory BP device (Takeda TM2421; A&D Medical, Tokyo, Japan). BP was measured every 15 min during the day (7:00 A.M. to 11:00 P.M.) and every 30 min during the night (11:00 P.M. to 7:00 A.M.). Values were averaged for each hour before calculating the mean 24-h, day, and night BPs.

GFR was measured after a single intravenous injection of 3.7 MBq ^{51}Cr-labeled EDTA at 8:00 A.M. by determining the radioactivity in venous blood samples taken 180, 200, 220, and 240 min after the injection (19,20). Extra renal loss was corrected for by subtracting 3.7 ml/min (21). The small underestimation (10%) of ^{51}Cr-EDTA renal clearance versus renal clearance of insulin was corrected for by multiplying the EDTA clearance by 1.10 (21). The results were standardized for 1.73 m^2 body surface area. The mean day-to-day coefficient of variation in GFR is 4% in our laboratory.

From venous samples, serum potassium, sodium, creatinine, and cholesterol concentrations were determined (Cobas Mira Plus; Roche), and HbA_{1c} was measured by high-performance liquid chromatography (normal range 4.1–6.1%) (Variant; Bio-Rad, Richmond, CA). Blood samples for AngII concentrations were collected in prechilled tubes after 30 min supine rest and immediately centrifuged at 4°C, and plasma concentrations were measured radioimmunologically (22). Blood samples for renin concentration were also collected after 30 min supine rest, and plasma renin concentration was determined using the principle of antibody trapping (23), as modified by Millar et al. (24). Aldosterone was measured using a commercial kit (DSL-8600; Diagnostic Systems Laboratories, Webster, TX).

Statistical analysis

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 diabetic nephropathy. On the basis of these data, a sample-size calculation showed a necessary minimum of 16 patients to detect a 25% change in urinary albumin excretion rate between any two treatment periods in a cross-over design with four treatment periods ($\alpha = 0.05$ and $\beta = 0.8$).

Normally distributed variables are expressed as mean ± SE. Values for albuminuria, θ_{Alb}, θ_{IgG}, θ_{IgG4}, AngII, renin, and aldosterone were logarithmically transformed and expressed as geometric

Table 1—*Effects on kidney function and arterial blood pressure of increasing doses of candesartan in 23 type 2 diabetic patients with nephropathy*

Parameter	Placebo	Candesarian dose		
		8 mg	16 mg	32 mg
Albuminuria (mg/24-h)*	700 (486–1,007)	470 (270–778)†	288 (272–462)†‡	332 (176–559)†‡
GFR (ml/min/1.73 m^2)	84 ± 6	79 ± 6†	78 ± 6†	77 ± 6†
θ Albumin (10^{-6})*	163 (101–262)	96 (56–166)†	85 (48–151)†	104 (59–210)†
θ IgG (10^{-6})*	58 (35–95)	36 (22–57)†	36 (21–63)†	39 (22–71)†
θ IgG_4 (10^{-9})*	42 (27–64)	30 (18–49)†	22 (12–39)†	34 (19–64)†
Systolic BP (mmHg)				
24-h	147 ± 4	138 ± 4†	138 ± 4†	134 ± 4†
Day	153 ± 4	143 ± 4†	144 ± 4†	140 ± 4†
Night	135 ± 4	126 ± 4†	124 ± 4†	122 ± 4†
Diastolic BP (mmHg)				
24-h	78 ± 2	73 ± 2†	73 ± 2†	71 ± 2†
Day	81 ± 2	76 ± 2†	76 ± 2†	74 ± 2†
Night	70 ± 2	66 ± 2†	67 ± 2†	65 ± 2†

Data means ± SEM. *Geometric mean (95% CI); †$P < 0.05$ versus placebo; ‡$P < 0.01$ versus 8 mg.

mean (95% CI) because of their positively skewed distribution. Changes in these variables during treatment with candesartan as compared with placebo are expressed in percent.

Comparisons of all clinical end points, including albuminuria, BP, and GFR between each treatment period, were performed using linear mixed models (25). The software used was R version 1.5.1 (http://www.r-project.org) (26). The adapted model was one with fixed effects of treatment level (placebo and candesartan 8, 16, and 32 mg daily), visit (1, 2, 3, and 4), and carryover (i.e., treatment level in the previous period), and a random effect of person was included to account for the person-dependencies in data. For the simplest models, the *P* value and effects correspond to results obtained from paired *t* test and two-way ANOVA, but these models allow for more elaborate exploration of the material. Tests for presence of effects were performed as likelihood-ratio tests, and final estimates were reported as restricted maximum likelihood estimates (25).

RESULTS — A total of 24 patients were randomized in the trial, 23 of whom were eligible for the final analysis. One patient was excluded from the study after randomization because stomach cancer was diagnosed during the first treatment period, during which the patient had received candesartan 32 mg daily. The incidence was not related to the study medication because symptoms had occurred before enrollment in the study and because the cancer was in an advanced stage at the time of diagnosis.

At entry to the study, the mean ± SD age of the remaining 23 patients who completed the trial was 59 ± 7 years. The known duration of diabetes was 12 ± 7 years, the mean BMI was 31 ± 5 kg/m^2, and most patients were men (21 of 23 patients). A total of 18 patients had diabetic retinopathy, whereas the remaining five patients had no signs of retinopathy. All patients received long-acting furosemide in a median dose of 40 mg (range 30–160) daily.

Albuminuria and 24-h BP were significantly reduced by all three doses of candesartan (Table 1). The statistical analysis showed no evidence of a carryover or a time-sequence effect on end points evaluated in the study, including albuminuria, 24-h BP, and GFR.

Albuminuria was reduced by a mean difference of 33% (95% CI 21–43) during treatment with candesartan 8 mg daily as compared with placebo, whereas candesartan 16 and 32 mg lowered albuminuria by 59% (52–65) and 52% (44–59), respectively. The two highest doses were significantly more effective in reducing albuminuria as compared with 8 mg; no difference was shown between candesartan 16 and 32 mg daily. The reduction in albuminuria was similar in patients with diabetic retinopathy as compared with patients without retinopathy.

Fractional clearances of albumin, IgG, and IgG_4 decreased significantly during treatment with all three doses of candesartan but without significant differences between the three doses (Table 1).

Compared with placebo, 24-h systolic BP was reduced by 9 (2–16), 9 (2–16), and 13 mmHg (6–20) and 24-h diastolic BP was reduced by 5 (2–8), 4 (1–7), and 6 mmHg (3–9) by candesartan 8, 16, and 32 mg daily, respectively (Table 1). The reductions in BP were sustained during the night (Table 1). There were no statistical differences in the reductions of 24-h systolic or diastolic BP between the three doses. The differences in systolic and diastolic 24-h BP reductions were 4 (−4 to 11) and 2 mmHg (−1 to 4) with candesartan 32 vs. 16 mg daily, respectively.

GFR was reduced by 5 (2–9) ml/ min/ 1.73 m^2 by candesartan 8 mg compared with placebo; no further statistical significant reduction resulted from the two higher doses (Table 1).

Plasma renin and AngII concentrations were increased by all three doses compared with placebo; no statistically significant differences existed between doses (Table 2). Plasma concentration of aldosterone tended to decrease during treatment with all three doses of candesartan. However, the decrease was only significantly different from placebo during treatment with 32 mg daily (Table 2).

Protein and salt intake and total cholesterol and HbA_{1c} levels were unchanged throughout the study (Table 2). Hemoglobin concentrations decreased by ~0.4 mmol/l during treatment with candesar-

Table 2—*Effects on laboratory parameters of increasing doses of candesartan in 23 type 2 diabetic patients with nephropathy*

Parameter	Placebo	Candesartan dose		
		8 mg	16 mg	32 mg
Renin (mU/l)*	12 (9–16)	40 (24–65)†	49 (31–74)†	50 (27–84)†
Ang II (pmol/l)*	8 (6–10)	18 (11–27)†	17 (12–24)†	19 (11–29)†
Aldosterone (pmol/l)*	127 (98–181)	112 (74–165)	108 (73–162)	94 (69–130)†
Serum potassium (mmol/l)	4.1 ± 0.1	4.2 ± 0.1†	4.2 ± 0.1†	4.4 ± 0.1†
Serum cholesterol (mmol/l)	5.6 ± 0.2	5.5 ± 0.2	5.3 ± 0.2	5.6 ± 0.3
HbA_{1c} (%)	8.4 ± 0.3	8.4 ± 0.3	8.5 ± 0.2	8.4 ± 0.2
Hemoglobin (mmol/l)	8.6 ± 0.2	8.3 ± 0.2†	8.2 ± 0.2†	8.2 ± 0.2†
Protein intake (g/kg/24-h)	1.0 ± 0.06	1.0 ± 0.06	0.9 ± 0.05	0.9 ± 0.05
Urinary sodium excretion (mmol/24-h)	219 ± 20	213 ± 12	205 ± 19	211 ± 17

Data are means ± SEM. *Geometric mean (95% CI); †$P < 0.05$ versus placebo.

tan; this decrease was independent of the dose given (Table 2). There was a slight general increase in serum potassium levels during treatment with all three doses of candesartan (Table 2). However, there were no incidences of hyperkalemia.

The therapy was well tolerated without adverse symptoms related to the study medication.

CONCLUSIONS — Our double-blind, randomized, cross-over study suggests that the optimal dose of candesartan is 16 mg daily for renoprotection, as reflected by short-term changes in albuminuria based on three consecutive 24-h collections of urine in hypertensive type 2 diabetic patients with nephropathy. All three doses of the AngII receptor antagonist reduced albuminuria and 24-h BP. However, albuminuria was reduced significantly more by candesartan 16 and 32 mg as compared with 8 mg daily without differences between the two highest doses.

Beneficial effects on albuminuria and BP were obtained despite a very high intake of dietary salt, a condition lowering the activity of the RAS and impairing the effect of agents blocking the synthesis and effects of AngII (27). However, this can be restored by adding diuretic treatment, as was the case in all patients in the present study.

The therapy was generally well tolerated without associated adverse events. We found a slight increase in serum potassium levels, but no incidences of hyperkalemia were observed in these patients, who, in general, had normal or only moderately reduced kidney function. Moreover, there was no incidence of hypotension, even though doses were changed between treatment periods without up-titration, e.g., from placebo to candesartan 32 mg daily. The small decrease in hemoglobin seen in our study might be a direct effect of blocking the actions of AngII, which is known to stimulate erythropoietin (28).

We found a small dose-independent decrease in GFR during treatment with candesartan, which is probably a hemodynamic reversible consequence of the BP reduction as previously suggested (29). The reversibility of the changes in GFR during treatment was also evident from our data, in which we found no carryover or time-sequence effect on the GFR.

In this study, 18 of the patients had clinically established diabetic nephropathy with the coexistence of albuminuria and diabetic retinopathy (30). Approximately one-third of the remaining five patients without diabetic retinopathy may suffer from nondiabetic nephropathies (31). However, the reductions in albuminuria upon treatment were comparable in those with and without diabetic retinopathy.

Several years of observations would be required to evaluate the long-term renoprotective effect of antihypertensive treatment on principal renal end points, i.e., doubling of serum creatinine, development of ESRD, or death. However, short-term changes in albuminuria have previously been shown to predict long-term loss of GFR in both diabetic and nondiabetic nephropathy (6–10), i.e., the greater the initial decline in albuminuria, the slower the subsequent long-term progression in renal disease. Recent data from the Reduction of End Points in Type 2 Diabetes With the Angiotensin II Antagonist Losartan (RENAAL) study in type 2 diabetic patients with overt nephropathy clearly indicated that the initial reduction in proteinuria upon antihypertensive treatment with or without AngII receptor antagonist is highly predictive of long-term renoprotection, i.e., doubling of serum creatinine, development of ESRD, or death (personal communication with Dick de Zeeuw on behalf of the RENAAL study group).

In the present study, the effects of each dose level were evaluated after 8 weeks of treatment. Therefore, if the maximal reduction in albuminuria and BP is not reached within 8 weeks, our results would underestimate the effect. However, previous studies of both diabetic and nondiabetic nephropathy have demonstrated that the maximal antiproteinuric and antihypertensive effect by inhibition of the RAS is reached 3–4 weeks after initiation of treatment (32–34). Another potential bias in cross-over studies relates to the possible carryover effect, which would occur in the present study, if the antiproteinuric effect of a given dose persists for more than 8 weeks after discontinuation of treatment. However, we could not detect any significant carryover or time-sequence effect in the statistical analysis of the present study or in our previous studies of RAS inhibition also applying treatment intervals of 2 months (35–37). Furthermore, previous studies in diabetic nephropathy have demonstrated a doubling in albuminuria just 1 month after withdrawal of long-term (>1 year) RAS inhibition (29,38), which corresponds to the maximal reduction of ~50%, which can be obtained by treatment with ACE inhibitors or AngII II receptor blockers, as demonstrated in the present study and previously (37).

The reduction in albuminuria caused by inhibition of the RAS may be due, in part, to reduced systemic BP. Furthermore, in diabetic patients with elevated albuminuria, RAS inhibition has been demonstrated to reduce urinary albumin excretion and intraglomerular BP independently of systemic BP by vasodilatation preferentially of the postglomerular arterioles (39). Interestingly, albuminuria decreased further when the dose of candesartan was increased from 8 to 16 mg daily in our study. This additional reduction in albuminuria could not be explained by an additional reduction in systemic BP or GFR. A similar graded reduction was not detected in the fractional clearance of albumin. The discrepancy may well be explained by the greater variability of urinary albumin excretion in a single 4-h urine collection as compared with our primary end point albuminuria, which was assessed by three consecutive 24-h urine collections.

Charge and size selectivity of the glomerular membrane remained unchanged as measured by the fractional clearance of IgG, IgG_4, and albumin. Applying a more specific and sensitive method of assessing glomerular size-selectivity, using clearance of Ficoll molecules of graded size, has demonstrated improved size-selectivity with AngII receptor antagonists in early diabetic nephropathy (40). Perm-selective changes could not be demonstrated in our study, in which reductions in the fractional clearance of the larger molecule of IgG and its anionic subclass paralleled that of the smaller molecule of albumin.

AngII receptor antagonists may prevent the occurrence of proteinuria by reducing the loss of glomerular nephrin, a protein located at the slit-diaphragm of the glomerular podocyte, which is suggested to play a central role in the function of the glomerular filtration barrier (41). AngII receptor blockade has no impact on tubular protein reabsorption as assessed by changes in urinary excretion of retinol-binding protein (37).

The disassociation between doses needed to inhibit local tissue actions of AngII and circulatory concentrations directly involved in BP regulation may be due to reduced tissue penetration or higher tissue concentration of AngII or its receptor. In our study, renin and AngII concentrations increased expectedly as a compensatory mechanism during treatment with candesartan. However, in accordance with the BP reductions, there were no additional increases in hormone concentrations when doses were increased above 8 mg daily. Therefore, complete inhibition of circulatory RAS was obtained by the lowest dose of candesartan, but higher doses were needed for optimal reduction in albuminuria. This suggests that circulatory concentrations of AngII contributing to the regulation of systemic BP is inhibited at doses of AngII receptor antagonists lower than those needed to block the deleterious effects of AngII locally in the kidney. Data from a nonrandomized open-labeled study of 10 older patients with heavy proteinuria (>1.5 g/day) of different etiology have suggested that additional reduction in proteinuria can be obtained by increasing the dose of candesartan up to 96 mg daily (42).

Within the recommended dose range of candesartan applied in the present study, not all patients reached the recommended BP target, which would therefore have required addition of other types of antihypertensive agents. To further enhance blockade of the RAS, the combination of ACE inhibitors and AngII receptor antagonists may prove beneficial, as suggested by short-term studies of diabetic patients with microalbuminuria (43) and macroalbuminuria (35,36). However, long-term studies are needed to evaluate the effect of dual blockade of the RAS on principal renal end points, such as rate of decline in GFR, doubling of serum creatinine, and ESRD.

In conclusion, our study suggests that the optimal dose of candesartan for renoprotection, as reflected by short-term reduction in albuminuria, in hypertensive type 2 diabetic patients with nephropathy is 16 mg daily.

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