Original Article

Dual blockade of the renin–angiotensin system in type 1 patients with diabetic nephropathy

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Abstract

Background. Albuminuria and hypertension are predictors of poor renal and cardiovascular outcome in patients with diabetes. Approximately 30% of type 1 patients with diabetic nephropathy (DN) have albuminuria >1 g/day, and blood pressure >135 and/or >85 mmHg despite antihypertensive therapy with recommended doses of ACE inhibitor (ACEI) and diuretics. We tested the effect of dual blockade of the renin–angiotensin system (RAS) in these patients.

Methods. We performed a randomised double blind crossover trial with 2 months treatment with Irbesartan 300 mg o.d. and placebo added on top of previous antihypertensive treatment. We included 21 type 1 patients with DN responding insufficiently to ACEI and diuretics, as defined above. At the end of each treatment period, albuminuria, 24-h blood pressure and glomerular filtration rate (GFR) were measured.

Results. Addition of 300 mg Irbesartan to the patients' usual antihypertensive therapy induced a mean reduction in albuminuria of 37% (95% CI 20–49, $P<0.001$); from 1574 mg/24 h (95% CI 1162–2132) to 996 mg/24 h (95% CI 699–1419), a reduction in 24-h blood pressure of 8 mmHg systolic (95% CI -2 to 18) and 5 mmHg diastolic (95% CI 1–9) ($P=0.11$ and 0.01, respectively) (from placebo, mean (SE) 146 (4)/80 (2) mmHg). GFR remained unchanged. Serum potassium increased (mean 4.3 to 4.6 mmol/l, $P=0.02$). Intervention to reduce serum potassium was needed in two patients with GFR <35 ml/min/1.73 m^2. Otherwise the dual blockade with Irbesartan was safe and well tolerated.

Conclusions. Dual blockade of the RAS may offer additional renal and cardiovascular protection in type 1 patients with DN responding insufficiently to conventional antihypertensive therapy, including recommended doses of ACEI and diuretics.

Introduction

Albuminuria and hypertension are predictors of poor renal and cardiovascular outcome in patients with diabetes [1,2]. Antihypertensive treatment, especially with ACE inhibitor (ACEI), has been shown to reduce albuminuria, to diminish loss of kidney function and to improve survival in type 1 patients with diabetic nephropathy (DN) [1–4]. Therefore, ACE inhibitors are recognised to induce renal and cardiovascular protection in these patients. Studies of diabetic and non-diabetic kidney disease suggest the initial degree of reduction in albuminuria after blockade of the renin–angiotensin system (RAS) predicts an attenuated rate of decline in glomerular filtration rate (GFR), as reviewed by Rossing [1].

At the Steno Diabetes Center, $\sim 30\%$ of type 1 patients with DN have albuminuria >1 g/day and blood pressure >135 and/or >85 mmHg despite antihypertensive combination therapy, including recommended doses of ACEI and diuretics [5].

Recently, a superior effect of dual blockade of the RAS compared with single blockade has been reported in type 2 patients with microalbuminuria [6]. Similar data are not available in type 1 patients with elevated urinary albumin excretion.

Therefore, we evaluated the short-term effect of dual blockade of the RAS by adding Irbesartan, an angiotensin II receptor antagonist (ARA), in type 1 patients with DN responding insufficiently

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to conventional antihypertensive treatment, including recommended doses of ACEI and diuretics.

Subjects and methods

Subjects

From the Steno Diabetes Center, we included 21 type 1 patients with DN responding insufficiently to conventional antihypertensive treatment, as defined below. DN was diagnosed if the following criteria were fulfilled: persistent albuminuria > 300 mg/24 h in two out of three consecutive determinations, presence of diabetic retinopathy, and no clinical or laboratory evidence of other kidney or renal tract disease [7,8]. DN responding insufficiently to conventional antihypertensive treatment was defined as office arterial blood pressure > 135 and/or 85 mmHg and albuminuria > 1000 mg/ 24 h, despite treatment with the recommended doses of ACEI (captopril 100 mg (14 patients) or enalapril (five patients)/ lisinopril (two patients) 20 mg daily) and diuretics (18 patients were treated with a median of 80 mg furosemide (range 40–1000 mg), four patients with thiazide as only diuretic (2.5 mg), and three patients received two types of diuretic). Twelve patients received additional antihypertensive medication. Ten patients were treated with a calcium channel antagonist (amlodipine 10 mg, $n = 5$; diltiazem 120–240 mg, $n = 4$; verapamil 120 mg, $n = 1$). Three patients received a β-blocker (metoprolol 200 mg, $n = 2$; atenolol 50 mg, $n = 1$), one patient received prazosin 1 mg, and one patient received methyldopa 250 mg.

All patients had been dependent on insulin treatment from the time of diagnosis and received at least two daily injections of insulin. Patients were on a diabetic diet (45–55% carbohydrates, 30–35% fat and 15–20% protein) without restriction of sodium or protein intake.

Exclusion criteria at the start of the study were: serum potassium > 4.8 mmol/l, pregnancy, no use of contraceptives, age < 18 years, alcohol or medicine abuse, inability to understand the patient information, contra-indication to treatment with ARAs, systolic blood pressure < 100 mmHg, GFR < 20 ml/min.

Twenty-four patients were screened, and 21 fulfilled all inclusion criteria, did not meet any exclusion criteria and were included in the study. Nineteen patients completed the study (see below).

Design

We performed a randomised, double-blind crossover trial. The randomisation was done in blocks of four by the manufacturing company (Sanofi-Synthelabo, Paris, France). This was kept in a concealed envelope by a third person and not broken until all data were entered in a database after the last patient visit (the code for a patient found dead in bed was broken earlier by a third person). Each patient received 2 months of treatment with Irbesartan 300 mg o.d. and 2 months of placebo. The study medication was added on top of the patient's usual antihypertensive treatment. This was left unchanged throughout the study except in two patients, who had their dose of diuretics increased because of fluid retention (one after the placebo period and one after Irbesartan treatment).

Patients attended the clinic for a total of five study visits: one screening visit and subsequent visits 2 and 8 weeks after the start of each treatment period. At the screening visit

albuminuria was determined in three 24-h urine samples, office arterial blood pressure was measured twice after 10 min rest with 2 min intervals, and serum potassium, sodium, creatinine and glycated haemoglobin A_{1c} were determined.

Blood pressure, serum potassium and serum creatinine were measured 2 weeks after the beginning of each treatment period for safety reasons.

At the end of each treatment period we assessed clinical end points, including the primary end point albuminuria, and the secondary end points 24-h arterial blood pressure and GFR.

Drug compliance was assessed by tablet counts. The study protocol was in accordance with the Declaration of Helsinki and was approved by the local ethical committee. All patients gave their informed consent to participate in the study.

Methods

Albuminuria was determined in three consecutive 24-h urine collections, completed immediately before each visit at the end of each treatment period, by turbidimetry (Cobas Mira Plus; Roche, Montclair, NJ, USA). During determination of GFR, urine was collected quantitatively to determine albuminuria, IgG and IgG_4 (ELISA) [9]. In addition, sodium, urea, creatinine and carbamid excretion in the urine were determined (Cobas Mira Plus, Roche).

At screening, office blood pressure was measured twice after 10 min of rest using a standard clinical mercury sphygmomanometer. Arterial blood pressure after each treatment period was assessed by taking 24-h ambulatory blood pressure measurements using the Takeda TM2420 device, model 6 and 7 (A&D, Medical Inc., Tokyo, Japan). Blood pressure measurements were taken every 15 min during the daytime (from 07:00 to 23:00) and every 30 min during the night-time (from 23:00 to 07:00). Values were averaged for each hour before calculating the mean 24-h day- and night-time arterial blood pressures. GFR was measured after a single intravenous injection of 3.7 MBq EDTA at 08:00 by determining the radioactivity in venous blood samples taken 180, 200, 220 and 240 min after the injection [10,11]. Extrarenal loss was corrected for by subtracting 3.7 ml/min [12]. The small underestimation (10%) of [^{51}Cr]EDTA renal clearance *vs* renal clearance of inulin was corrected for by multiplying the EDTA clearance by 1.10 [12]. 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, haemoglobin and cholesterol concentrations were determined. Serum potassium was measured by an indirect ion-selective method (normal range 3.5–5.0 mmol/l) (BM/HITACHI system; Boehringer Mannheim GmbH, Roche Laboratory Systems, Mannheim, Germany). Our haemoglobin method has a normal range of 7–10 mmol/l in females and 8–11 mmol/l in males (Sysmex SF 3000; Sysmex Corp., Kobe, Japan). Glycated haemoglobin A_{1c} (HbA_{1c}) was measured by high-performance liquid chromatography (normal range 4.1–6.1%) (Variant; Bio-Rad Laboratories, Hercules, CA, USA). Blood samples for angiotensin II levels were drawn in prechilled tubes after 30 min supine rest and immediately centrifuged at 4°C. Plasma concentrations were measured radioimmunologically [13]. Blood samples for renin concentration were taken after 30 min supine rest. Plasma renin concentration was determined using the principle of antibody trapping [14] as modified by Millar *et al.* [15].

Statistics

At screening, normally distributed variables are expressed as mean (SD), otherwise as median (range). During Irbesartan and placebo treatment, normally distributed values are expressed as mean (SE). Albuminuria, serum creatinine and renin concentration were logarithmically transformed and are expressed as geometric mean (95% CI) due to their positively skewed distribution. When evaluating the effect of Irbesartan, all comparisons of normally or log-normally distributed parameters (albuminuria, serum creatinine and renin concentration) were done with a paired student's t-test. Changes in variables between visits are expressed as means (95% CI). 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 standard deviation (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 type 1 patients with DN. On the basis of these data, a sample-size calculation revealed a necessary minimum of 16 patients to detect a 25% difference in change in urinary albumin excretion rate ($\alpha = 0.05$ and $\beta = 0.8$).

A linear regression analysis was made between the change in arterial blood pressure and the change in albuminuria (ln (albuminuria during Irbesartan treatment/albuminuria during placebo)). A P value of < 0.05 was considered significant (two-tailed). Data were evaluated using SPSS 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics at screening of the 21 patients included in this study are shown in Table 1. Two patients did not complete follow-up. One patient was found dead in bed during the placebo treatment, 2 days before completing the study. The cause of death was unknown and autopsy was not carried out. One patient was excluded from the study because of elevated serum potassium (see below). The following results are based on the 19 patients completing the study, except for the treatment effect on serum potassium, which is based on 20 patients.

Table 1. Baseline clinical data in 21 type 1 patients with diabetic nephropathies

Age (years)	45 (10)
Sex (male/female)	17/4
Duration of diabetes (years)	29 (8)
Duration of diabetic nephropathy (years)	10 (5)
Retinopathy (background/proliferative)	4/17
Office arterial blood pressure (mmHg)	156 (15)/87 (10)
Albuminuria (mg/24 h)[a]	1866 (1493–2333)
No. of antihypertensive agents, including ACEI and diuretics[b]	3 (2–5)
No. of patients receiving calcium channel antagonist/beta-blocker/others	10/3/2[c]

Values represented are mean (SD).
[a]Geometric mean (95% CI).
[b]Median (range).
[c]One α-blocker and one methyldopa.

The median duration of each treatment period was 63 days (range 56–72 days) and compliance as assessed by tablet count was 100% (95–100%) during placebo treatment and 100% (80–100%) during Irbesartan (one patient 80% and another 85%, all others $> 95\%$).

The patients suffered both systolic and diastolic hypertension, and had highly elevated albuminuria despite conventional antihypertensive treatment with three different agents, including recommended doses of ACEI and diuretics (Table 1).

Albuminuria, and 24-h diastolic and mean blood pressure were significantly lower during dual blockade of the RAS when Irbesartan 300 mg was added to conventional antihypertensive treatment as compared with placebo (Table 2).

Twenty-four hour urine collections were available in 18 patients, since one patient developed uterine bleeding disorder. The addition of Irbesartan 300 mg caused a mean reduction in 24-h albuminuria of 37% (95% CI 20–49%) (Table 2). Similarly, we observed a reduction (mean difference) in albuminuria and urinary IgG excretion during GFR measurements of 32% (95% CI 0–54; $P = 0.05$) and 31% (95% CI 4–50%; $P = 0.03$), respectively. No change in the urinary excretion of IgG_4 was found. Albuminuria after placebo treatment did not differ from albuminuria at baseline (Tables 1 and 2; $P = 0.27$).

Twenty-four hour recordings of arterial blood pressure were available from all patients.

Twenty-four hour blood pressure (mean difference) decreased by 8 (95% CI -2 to 18)/5 (95% CI 1–9) mmHg, $P = 0.11/0.01$ during treatment with Irbesartan 300 mg. We found a decline in 24-h mean blood pressure of 6 mmHg (95% CI 1–11) from 102 (SD 10) ($P = 0.02$) when Irbesartan was added. No significant changes were found in the 24-h mean heart rate. The reduction in blood pressure was sustained both during day- and night-time (Table 2).

Linear regression analysis showed a correlation between the change in arterial blood pressure and the change in albuminuria ($r^2 = 0.29$, $P = 0.02$) (Figure 1).

Plasma renin concentration increased by 69% (95% CI 17–145) during the 2 months of Irbesartan 300 mg treatment. We also found a tendency for the serum angiotensin II level to increase (Table 2).

GFR, serum sodium, creatinine, HbA_{1c}, cholesterol and urinary sodium excretion all remained unchanged in response to Irbesartan treatment (Table 2).

Adverse effects

Irbesartan treatment caused an increase in serum potassium to > 5.1 mmol/l in four patients (Table 2). One patient was excluded after 18 days (serum potassium rose from 4.8 to 5.9 mmol/l). One completed the study but was receiving treatment with Resonium 7–30 g daily from day 17 (maximal serum potassium 5.5 mmol/l). In two patients, hyperkaliemia appeared at the end of Irbesartan treatment. Three of seven patients with GFR < 35 ml/min/1.73 m^2 experienced an increase in serum potassium to > 5.1 mmol/l.

Table 2. Response to 2 months treatment with Irbesartan 300 mg o.d. in 19 type 1 patients with diabetic nephropathy receiving conventional antihypertensive treatment, including recommended doses of ACEI and diuretics

	Placebo	Irbesartan 300 mg daily	Mean difference (95% CI)	P value
Albuminuria (mg/24 h)[a]	1574 (1162–2132)	996 (699–1419)	−37% (−49 to −21)	<0.001
24-h blood pressure (mmHg):	146 (4)/80 (2)	138 (4)/75 (2)	−8 (−18 to 2)/−5 (−9 to −1)	0.11/0.01
Day (7–23)	151 (5)/82 (2)	143 (4)/78 (2)	−8 (−18 to 3)/−5 (−9 to 0)	0.14/0.048
Night (23–7)	137 (5)/75 (2)	128 (6)/68 (3)	−9 (−20 to 3)/−6 (−12 to −1)	0.12/0.02
GFR (ml/min/1.73 m²)	52 (5)	50 (6)	−2 (−6 to 2)	0.35
Serum creatinine (μmol/l)[a]	148 (126–174)	155 (127–190)	5% (1–11)	0.12
Serum potassium (mmol/l)	4.3 (0.1)	4.6 (0.1)	0.3 (0.0–0.6)	0.02
Haemoglobin (mmol/l)	7.9 (0.2)	7.6 (0.2)	−0.3 (−0.6 to 0.0)	0.06
HbA$_{1C}$ (%)	9.6 (0.3)	9.9 (0.4)	0.3 (−0.2 to 0.8)	0.26
Serum cholesterol (mmol/l)	5.8 (0.2)	5.6 (0.2)	−0.2 (−0.6 to 0.1)	0.18
Plasma renin concentration (mU/l)[a]	120 (71–202)	203 (103–399)	69% (17–145)	0.008
Serum angiotensin II (pmol/l)	7.1 (0.9)	11.9 (3.1)	4.8 (−0.8 to 10.5)	0.09
Urinary sodium excretion (mmol/24 h)	178 (18)	160 (11)	−19 (−51 to 14)	0.24

Values represented are mean (SE).
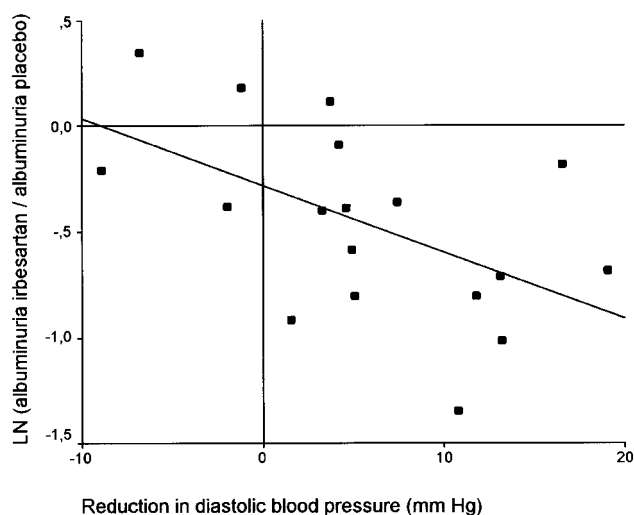[a]Geometric mean (95% CI).

Fig. 1. Linear regression analysis between the change in arterial blood pressure and the change in albuminuria (LN) in type 1 patients with diabetic nephropathy responding insufficiently to conventional antihypertensive treatment, including recommended doses of ACEI and diuretics.

In the 13 patients with GFR >35 ml/min/1.73 m², the increase in serum potassium was not statistically significant (increase of 0.2 mmol/l, 95% CI −0.1 to 0.5, $P = 0.17$).

Haemoglobin decreased from 7.9 to 7.6 mmol/l. In none of the patients was treatment for anaemia initiated during the study. No other adverse effects were reported.

Statistical analysis revealed no evidence of carryover or order effects.

Discussion

The major novel findings in our study are that dual blockade of the renin–angiotensin system by the addition of Irbesartan 300 mg caused a reduction in albuminuria and 24-h blood pressure in type 1 patients with DN responding insufficiently to conventional antihypertensive treatment, including recommended doses of ACEI and diuretics. GFR was preserved.

The rationale for dual blockade of the RAS system is based on the different mechanism of action of the two drug classes. In addition to decreased angiotensin II formation, ACEI decreases the degradation of bradykinin, a powerful vasodilator [16]. However, an insufficient response to ACEI might be explained by incomplete blockade of the ACE enzyme or by the generation of angiotensin II by ACE-independent pathways such as Chymase [17]. The incomplete blockade possibly explains the observation that plasma angiotensin II levels return to normal after chronic ACEI treatment, a phenomenon called 'ACE escape' [18]. Angiotensin II receptor antagonism results in more complete neutralization of the unfavourable actions of angiotensin II by blocking the angiotensin II type 1 receptor. Therefore, treatment with both ACEI and ARAs may offer synergistic blockade of the RAS, not obtainable with either drug alone.

Recently, the CALM-study, which investigated type 2 diabetes with microalbuminuria and hypertension, demonstrated an enhanced reduction in blood pressure by dual blockade (candesartan cilexetil 16 mg and lisinopril 20 mg) compared with therapy with either agent alone [6]. The additional effect of combination therapy was a reduction in systolic blood pressure of 10 mmHg. In the CALM study there was a tendency towards a more pronounced antiproteinuric effect of dual blockade as documented in our study dealing with type 1 patients with overt diabetic nephropathy. Similar findings have been reported in patients with various non-diabetic kidney diseases and creatinine clearance of 20–45 ml/min [19].

We observed an increase in plasma renin concentration and a tendency towards an increase in the serum angiotensin II level in response to Irbesartan

therapy, indicating incomplete blockade of the RAS on ACEI treatment in recommended doses. A key issue, therefore, is whether the observed beneficial effect of dual blockade of the RAS in our study is obtainable simply by increasing the dose of ACEI. The answer to this dose escalation question is unknown, since the antiproteinuric and antihypertensive effect of captopril >100 mg daily or enalapril/lisinopril >20 mg daily have not been investigated in DN. The patients in the present study received ACEI in doses equal to or higher than those recommended based on studies documenting the renoprotective effect of the drug [3,4]. We have found no evidence from patients with moderate to severe essential hypertension to support an additional antihypertensive effect of doses of captopril up to 600 mg or enalapril up to 80 mg [20–22]. Recently, Agarwal [23] reported that 50 mg of losartan daily in addition to 40 mg of lisinopril daily had no effect on blood pressure and proteinuria in a small heterogeneous group of predominantly severe obese, hypertensive, proteinuric diabetic African–Americans with advanced renal failure. Surprisingly, this study showed a lowered plasma renin activity and enhanced GFR during losartan + ACEI treatment. However, these findings cannot be extrapolated to other ethnic groups since suppressed RAS activity and reduced effect of blocking the action/formation of angiotensin II have been demonstrated in African–Americans suffering from diabetic nephropathy (H.-H. Parving, personal communication) and left ventricular dysfunction [24].

Our patients received Irbesartan and placebo for 2 months. Studies in diabetic [25] and non-diabetic kidney disease [26] have suggested that a maximal antiproteinuric and antihypertensive effect of angiotensin II receptor blockade is present after <1 month of treatment.

The correlation between change in blood pressure and change in albuminuria observed in linear regression analysis in the present study has been demonstrated in several other studies, as reviewed by Parving [27]. This may indicate that the renoprotective effect is partly due to changes in systemic and local haemodynamic factors, causing a reduction in glomerular capillary hydraulic pressure [28]. The reduction in albuminuria might also be due to improved size-selective properties of the glomerular capillary membrane, as demonstrated by ficoll clearance in type 1 patients with diabetic nephropathy treated with an ARA [29], although we could not confirm this finding by using endogenous proteins as markers. Our study did not suggest changes in the charge-selective properties of the glomerular capillary membrane wall. Unfortunately, the present study design cannot distinguish between the specific antiproteinuric effect of dual blockade of the RAS and the effect of lowering blood pressure *per se*. In order to solve this issue, a head-to-head comparison between dual blockade of the RAS and ACE-inhibition in combination with other antihypertensive drugs aiming towards identical blood pressure levels is needed.

The beneficial effect of reducing blood pressure and albuminuria on progression of kidney function in patients with diabetic nephropathy is well established [1,2]. Previous studies in diabetic and non-diabetic kidney disease have indicated that the magnitude of reduction in albuminuria after the start of antihypertensive treatment with or without ACEI is highly predictive for future progression in GFR, i.e. patients with the largest initial reduction in albuminuria have the smallest subsequent rate of decline in GFR [1]. As a consequence, albuminuria may serve as a surrogate end point for the prognosis in diabetic nephropathy in clinical trials and the present short-term study suggests that dual blockade of the renin–angiotensin system has renoprotective effects. However, this has to be confirmed in long-term studies with direct measures of kidney function and survival.

Our beneficial results were obtained despite a relatively high dietary intake of salt [30], a condition lowering the RAS activity and impairing the antihypertensive [31] and antiproteinuric [30] effects of drugs interrupting the RAS.

In conclusion, our short-term study suggest that dual blockade of the RAS offers additional renal and cardiovascular protection in type 1 patients with diabetic nephropathy responding insufficiently to conventional antihypertensive therapy, including recommended doses of ACEI and diuretics. The long-term renoprotective effects of dual blockade need to be evaluated.

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