Time course of the antiproteinuric and antihypertensive effects of direct renin inhibition in type 2 diabetes

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Inhibition of renin with an active site inhibitor, aliskiren, lowers blood pressure (BP) in diabetic patients. Here, we studied the time course of the antihypertensive and antiproteinuric effect of renin inhibition in 15 patients with type 2 diabetes and elevated urinary albumin/creatinine ratios (UACRs) to check whether aliskiren can decrease proteinuria. After a 4-week washout of previous medications, patients received aliskiren and furosemide daily for 28 days followed by a 4-week withdrawal period. Twenty-four-hour BPs were measured at baseline throughout treatment and withdrawal periods. The UACR was significantly reduced after 2–4 days of treatment with another significant reduction after 28 days. Systolic blood pressure (SBP) was significantly lower after 7 days with no further reduction after 28 days. The BP returned toward baseline 3 days after withdrawal, whereas the UACR was still significantly reduced compared with baseline 12 days after withdrawal. Our study shows that aliskiren reduced 24 h SBP, and this was associated with a reduction in albuminuria in type 2 diabetic patients.

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Treatment of diabetic nephropathy relies on blockade of the renin–angiotensin–aldosterone system (RAAS).[1] Angiotensin II receptor blockers (ARBs) like irbesartan, losartan, and valsartan are standard therapy in patients with type 2 diabetes and micro- or macroalbuminuria after results of landmark trials.[2–5] In smaller studies, improved ways of blocking the RAAS has been investigated, with albuminuria reduction as the main end point. Blockade of the aldosterone receptor,[6] dual-blockade using both angiotensin-converting enzyme (ACE) inhibitors and ARBs,[7,8] and ultrahigh doses of ARBs[9] have shown additional effect on albuminuria but has not yet demonstrated prevention of end-stage renal disease or death in diabetes.

Large trials have shown that albuminuria is a marker for renal and cardiovascular risk in patients with type 2 diabetes and diabetic nephropathy.[10] Lowering albuminuria is associated with a reduced risk for renal and cardiovascular end points.[11] Thus any residual albuminuria in these patients calls for more effective RAAS blockade.

Renin inhibition is a new option to block the RAAS at the first rate-limiting step. Renin inhibition with aliskiren lowers blood pressure (BP) in hypertensive patients with or without type 2 diabetes,[12,13] but it is not known whether this treatment can decrease albuminuria. Renin inhibition with remikiren leads to albuminuria reduction in nondiabetic hypertensive patients.[14]

Preliminary data suggest a more complete suppression of the intra-renal RAAS with direct renin inhibition as compared with ARBs and ACE inhibitors.[15] In contrast to ACE inhibitors/ARBs, all components of the RAAS are suppressed except plasma renin concentration.[16]

The aim of this study was to investigate the time course of the antihypertensive and the antiproteinuric effects of direct renin inhibition by aliskiren in patients with type 2 diabetes and micro- or macroalbuminuria. We also investigated the effect on RAAS components and on biomarkers of cardiovascular risk.

RESULTS

A total of 37 patients gave informed consent, 16 of these were screen failures mainly due to BP above the safety limit or low urinary albumin/creatinine ratio (UACR) levels. After 4 weeks washout (Figure 1), 21 patients were considered eligible for dosing at baseline. Six of these were early dropouts (one withdrew consent, two had normoalbuminuria at baseline and three were excluded following rise in BP shortly after first dose (days 3, 3, and 7). Two patients who were excluded during the post-treatment washout were included in the analysis, as they went through the treatment period with all measurements. In accordance with this, BP at baseline was higher in the 8 patients who dropped out of the study compared with the 13 patients who completed the study (Table 1). As the dropouts came at an early point in the treatment phase, no relation to study drug was suspected.

Thirteen patients completed all phases of the study. Clinical data of the 21 eligible patients, the 15 included in the analysis, and the 13 patients who completed all study periods are given in Table 1.

24 h BP

At baseline, mean (s.d.) 24 h BP was 143/75 (12.8/9.6) mm Hg, daytime BP was 147/77 (12.3/10.0) mm Hg, and nighttime BP was 130/68 (14.3/10.0) mm Hg. The mean 24 h systolic BP (SBP) was significantly reduced with 6–8 mm Hg on days 7 ($P=0.037$), 14 ($P=0.006$), and 28 ($P=0.035$) compared with baseline (Figure 2). Three days after the end of treatment, SBP was still significantly lower compared with baseline ($P=0.016$), but returned toward baseline at day 35. Daytime SBP changes were similar; nighttime SBP did not change significantly.

Twenty-four-hour mean diastolic BP (DBP), daytime DBP, and nighttime DBP were not significantly different from baseline during treatment and post-treatment, but baseline levels were low (75 mm Hg) (Figure 2).

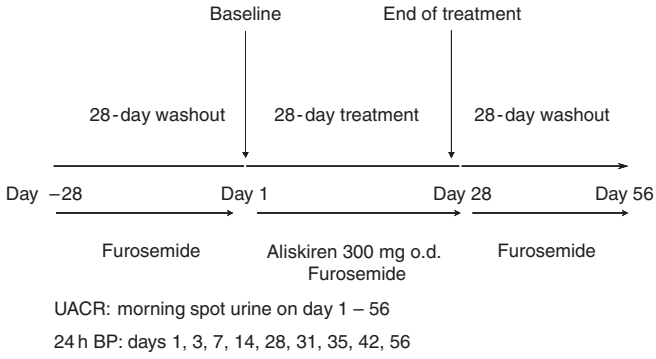

Figure 1 | Study timeline. Four weeks washout, 4 weeks of treatment, followed by 4 weeks withdrawal.

Urinary albumin/creatinine ratio

Baseline UACR was 173 (39–1725) mg g^{-1} (median and range). Aliskiren treatment was associated with a significant decrease in albuminuria throughout the treatment period (Figure 2a). UACR values decreased progressively compared with baseline with a 17% reduction on days 2–4 ($P=0.04$), a 31% reduction on days 8–10 ($P<0.001$), and a maximum reduction of 44% at end of the treatment (days 26–28) ($P<0.001$). Five of the 15 patients had >50% reduction in albuminuria at the end of treatment compared with baseline, 11 of the 15 had >25% reduction, and 13 had >10% reduction (data not shown). During post-treatment washout, UACR remained significantly below baseline for 12 days (days 29–40). Urinary sodium excretion did not differ significantly during the study (data not shown). There was no significant correlation between the relative change from baseline in UACR and in 24 h SBP ($r=0.298$, $P=0.347$).

The time course of the changes in UACR and 24 h BP were not concordant, as significant changes in UACR happened earlier (days 2–4) than changes in 24 h BP (day 7). UACR was further reduced during the treatment period, whereas the 24 h BP did not change further after day 7 (Figure 1).

Estimated glomerular filtration rate

Estimated glomerular filtration rate at baseline was 75.5 ml per min per 1.73 m^2. At the end of treatment, this had decreased by 6 ml per min per 1.73 m^2 and returned toward baseline during post-treatment washout. None of these changes were statistically significant.

Table 1 | Demographics of patients with type 2 diabetes, hypertension, and albuminuria

Demographics	All randomized patients (*n*=21)	Patients completing active treatment (*n*=15)	Patients completing study (*n*=13)
Age	64.6 (7.3)	63.5 (10.3)	65.8 (5.9)
Height (cm)	173.1 (5.5)	174.3 (5.1)	173.7 (5.0)
Weight (kg)	97.1 (17.7)	100.0 (16.1)	98.8 (16.1)
Male gender, *n* (%)	18 (86%)	13 (87%)	12 (92%)
Caucasian, *n* (%)	21 (100%)	15 (100%)	13 (100%)
Hb_{A1c}	8.0 (1.3)	7.9 (1.2)	8.0 (1.2)
Plasma creatinine (μmol l^{-1})	97 (32)	97 (34)	94 (31)
UACR (mg g^{-1})[a]	158 (18–5060)	173 (39–1725)	158 (39–1069)
24 hour BP (mm Hg)	146/76 (15/10)	143/75 (13/10)	139/72 (9/6)
Mean daytime BP (mm Hg)	144/77 (19/13)	147/77 (12/10)	141/75 (17/10)
Mean nighttime BP (mm Hg)	133/71 (18/13)	130/68 (14/10)	128/67 (14/10)

BP, blood pressure; UACR, urinary albumin/creatinine ratio.
All values are mean and (s.d.) except UACR values.
[a]Median (range).

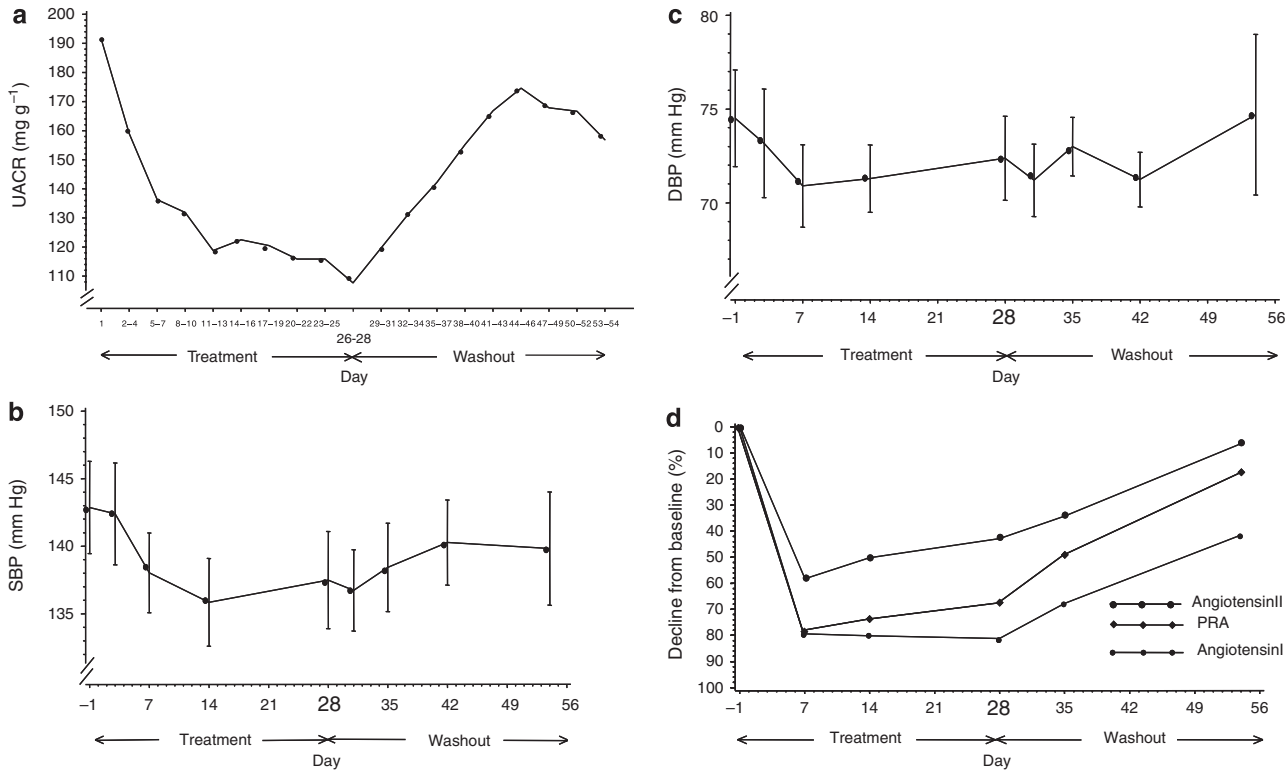

Figure 2 | Effect of renin inhibition on UACR, 24 h BP and RAAS components. (**a**) UACR (mg g^{-1}) (geometric means) in 15 type 2 diabetic patients with micro- or macroalbuminuria treated with aliskiren for 28 days followed by 28 days washout. (**b**) 24 h SBP (s.e.m) in 15 type 2 diabetic patients with micro- or macroalbuminuria treated with aliskiren for 28 days followed by 28 days washout. (**c**) 24 h DBP (s.e.m) in 15 type 2 diabetic patients with micro- or macroalbuminuria treated with aliskiren for 28 days followed by 28 days washout. (**d**) Plasma renin activity, Ang I and Ang II (ratio of geometric means relative to baseline) in 15 type 2 diabetic patients with micro- or macroalbuminuria treated with aliskiren for 28 days followed by 28 days washout.

RAAS components

Levels of RAAS components are given in Table 2. Aliskiren treatment was associated with significant increases in prorenin (30% on day 28, $P<0.001$ vs baseline) and renin concentration (404% from baseline on day 7, $P<0.001$) and decreases in plasma renin activity (PRA) (67–77%, $P<0.001$ vs baseline), angiotensin I (Ang I), and angiotensin II (Ang II) (Figure 1d). One week after withdrawal, prorenin levels were not significantly different from baseline. Comparable reductions in Ang I levels were observed on days 7 (80% reduction), 14 (80% reduction), and 28 (81% reduction) (Figure 1d). The reduction in Ang II levels on day 7 (58%) were slightly greater than on day 28 (43%) ($P=0.044$ for the comparison of the 2 days).

During post-treatment washout, renin concentrations decreased, but remained elevated compared with baseline by 73% ($P<0.001$) on day 54. There was a significant correlation between change in UACR and change in renin concentration (Spearman's ρ, $P=0.033$). After withdrawal, PRA remained significantly reduced from baseline on day 35 (49% reduction, $P=0.010$). PRA on day 54 was not significantly different from baseline.

After withdrawal, Ang I and Ang II levels remained significantly suppressed. Ang I levels were still reduced 42% at day 54 ($P<0.001$), and Ang II levels were reduced by 34% ($P=0.010$) at day 35 but returned to baseline level at day 54. Angiotensinogen was measured in 13 of the 15 patients, and ACE activity was measured in 14 of the 15 patients. No significant changes were found (Table 2).

The changes in mean levels of aldosterone were not significant (Table 2).

Biomarkers

We also measured the impact of direct renin inhibition on biomarkers of cardiovascular risk. Levels are shown in Table 2. There were no significant changes in levels of any of the biomarkers during the study compared with baseline.

Safety

A total of four (26.7%) patients experienced eight adverse events during the study. The most common adverse event, occurring in 2 of 15 (13.3%) patients, was mild hypoglycemia, occurring during post-treatment (days 35 and 38). One patient discontinued the study due to hypertension on day 42 (post-treatment washout) following sitting and standing BP measurements of 170/80 and 177/87 mm Hg, respectively. No event of hyperkalemia (plasma potassium >5.0 mmol l^{-1}) was recorded during the study.

Table 2 | RAAS components and biomarker levels at baseline, at the end of treatment (day 28), and after post-treatment washout (day 54), *n*=15

	Baseline	End of treatment	End of study	
Angiotensinogen (nmol l^{-1})	1032 (210)	1072 (211)	995 (157)	NS
Prorenin (ng l^{-1})	393 (282)	499 (320)	407 (265)	$P<0.001$[a]
Renin (ng l^{-1})	26 (20)	193 (287)	89 (152)	$P<0.001$[a]
PRA (pmol AngI per ml per h)	4.1 (3.1)	1.3 (0.92)	4.2 (1.5)	$P<0.001$[a]
Ang I (pmol l^{-1})	39.5 (23.4)	7.4 (3.7)	33.0 (51.4)	$P<0.001$[a]
ACE activity (U)	40.1 (8.1)	41.5 (8.8)	40.2 (11.2)	NS
Ang II (pmol l^{-1})	15.1 (11.1)	8.7 (7.4)	14.4 (8.7)	$P=0.044$[a]
Aldosterone (ng l^{-1})	81.8 (45.8)	72.5 (37.4)	85.5 (37.7)	NS
hs-CRP (mg l^{-1})	3.33 (3.47)	3.69 (3.87)	4.18 (5.29)	NS
vWF (%)	151 (52)	144 (45)	144 (48)	NS
sVCAM-1 (μg l^{-1})	886 (303)	956 (471)	913 (342)	NS
sICAM-1 (μg l^{-1})	637 (294)	619 (263)	618 (253)	NS
PAI-1 (μg l^{-1})	98 (49)	135 (87)	131 (99)	NS
NT-proBNP (pmol l^{-1})	338 (73)	308 (60)	318 (59)	NS
ADMA (μmol l^{-1})	0.49 (0.05)	0.49 (0.06)	0.50 (0.06)	NS

ACE, angiotensin-converting enzyme; ADMA, asymmetrical dimethyl arginine; Ang I, angiotensin I; Ang II, angiotensin II; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal-pro brain natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; PRA, plasma renin activity; sICAM-1, serum-soluble intercellular adhesion molecule-1; sVCAM-1, serum-soluble vascular adhesion molecule-1; vWF, von Willebrand factor.
Values are mean (s.d.).
[a]End of treatment (day 28) compared with baseline.

DISCUSSION

We found that treatment with aliskiren 300 mg once daily was associated with a decrease in UACR by 17% after 2–4 days with a 44% reduction after 28 days of treatment. Twenty-four-hour SBP decreased 6 mm Hg by day 7 and 8 mm Hg by day 14. The time course of changes in UACR and SBP was not concordant. After withdrawal, UACR remained significantly below baseline for 12 days, whereas SBP was significantly below baseline for only 3 days. There was no significant correlation between change in UACR and change in SBP.

In a time-course study with losartan 100 mg daily in type 1 diabetes and diabetic nephropathy, Andersen *et al.*[17] found a 25% reduction in UACR on day 4, from a baseline level higher than that in our study (676 mg g^{-1}) and with a larger reduction in SBP (12 mm Hg). The authors did not find any separation between changes in BP and changes in UACR.

The UACR reduction in our study is in the same order of magnitude as with other RAAS inhibitors in similar populations with type 2 diabetes. In the IRMA 2 study, 300 mg of irbesartan once daily reduced urinary albumin excretion by 38%.[2] In 102 hypertensive Chinese patients with type 2 diabetes, Chan *et al.*[18] demonstrated an overall 56% reduction in proteinuria after 1 year of treatment with enalapril 40 mg day^{-1}. Aliskiren treatment on top of conventional treatment, including losartan 100 mg once daily, in type 2 diabetes and nephropathy has recently shown a 20% albuminuria reduction in patients with more severe disease than in our study.[19] This study also demonstrated that the maximal albuminuria-lowering effect occurred after 1 month of treatment.

In our study, we used the dose of aliskiren developed for optimal treatment of arterial hypertension. The optimal dose for albuminuria reduction is not necessarily the same, as is the case with irbesartan, where higher doses may be required for optimal renoprotection.[9] This will need further investigation.

Renin inhibition has shown renoprotective potential in animal studies. In diabetic rats with renal injury, aliskiren treatment attenuated SBP, albuminuria, and glomerulosclerotic index compared with diabetic controls. Furthermore, tubular interstitial fibrosis was more reduced compared with diabetic rats treated with the ACE inhibitor, perindopril.[20]

The mechanism by which RAAS blockade alters the filtration of proteins in diabetic nephropathy is not fully understood. Trevisan and others[21] have proposed that reductions in intraglomerular pressure with RAAS blockade lowers albuminuria. Previous studies of the permeability of the glomerular membrane indicate an improved size selectivity with RAAS blockade.[22,23] Treatment with enalapril improved charge selectivity by attenuating loss of heparin sulfate from the glomerular basement membrane in diabetic rats.[24] In recent studies, the role of nephrin, a protein present in the slit diaphragm of the podocyte, has been investigated. It appears from human studies[25] that nephrin levels are inversely associated with the degree of proteinuria, and that RAAS blockade improves nephrin expression to control levels. The effect of aliskiren treatment on the mentioned pathways remains to be elucidated.

The effect of aliskiren treatment on ambulatory BP has been assessed in a few randomized studies. In nondiabetic patients with mild-to-moderate hypertension, a reduction in daytime ambulatory SBP was found. The authors reported a placebo-controlled reduction, compared with baseline, of 11 mm Hg after 4 weeks with 300 mg aliskiren once daily as the only antihypertensive treatment.[12] Other aliskiren studies have found similar reductions in office SBP.[13] In our study, 24 h BP reductions are smaller than in the above-mentioned

studies, probably because baseline 24 h SBP was only slightly elevated.

Aliskiren inhibits renin from converting angiotensinogen to Ang I by binding to the active site; however, it can also bind to prorenin. Recently, a (pro)renin receptor has been discovered,[26] through which prorenin may exert effects in an angiotensin-independent manner.[27] Whether prorenin bound to aliskiren also exerts an effect via this receptor is unknown, but obviously this could have implications for the angiotensin-independent (pro)renin receptor pathways in the kidney that are potentially deleterious.[28,29] If receptor binding of prorenin/renin contributes to the pathophysiology of diabetic nephropathy, and if renin inhibition interrupts this sequence, this could be a new pathway of action in RAAS blockade.

The reduction in PRA is accompanied by a reactive increase in renin and prorenin, which is of interest in relationship with the potential activation of the (pro)renin receptor under these conditions. On the one hand, the receptor may be down-regulated by such high renin/prorenin levels,[30] whereas on the other hand, the activation of the receptor may be disturbed due to aliskiren-induced conformational changes in the renin/prorenin molecule. Further research is needed to establish the percentage of plasma renin and prorenin that is bound to aliskiren. Such research should also take into consideration the fact that high prorenin levels are associated with the development of diabetic microangiopathy.[31] Aliskiren treatment had no effect on the markers of endothelial dysfunction and inflammation, that is, biomarkers of cardiovascular risk. This is in contrast to irbesartan treatment, which lowers markers of inflammation.[32] An effect cannot be excluded due to profiles of patients with relatively low cardiovascular risk, short-term treatment, or lack of power. In general, aliskiren was well tolerated, and the adverse events reported were mild and similar to other aliskiren studies.

The study design was primarily intended to investigate the time course of aliskiren treatment, and the results imply that direct renin inhibition may be a new treatment of diabetic kidney disease. As this was an open trial, these results will need confirmation, also given the small number of participants and the number of dropouts (although not considered related to study drug). The results are thus preliminary, but support the concept that RAAS blockade with direct renin inhibition can decrease albuminuria. Further double-blind, randomized trials of efficacy and safety of aliskiren in diabetic nephropathy are needed[19] before aliskiren can be added to currently recommended renoprotective treatments. All patients in this study were Caucasians, which limits interpretation of the results concerning race differences. The lasting effect on the clinical variables and the RAAS components after withdrawal could be of clinical value, as drug holidays will have less impact on UACR and BP levels.

In conclusion, aliskiren 300 mg once daily was associated with a reduction in UACR and 24 h SBP in patients with type 2 diabetes and albuminuria. This is a small exploratory study that needs confirmation in a larger trial.

MATERIALS AND METHODS

This open single-centre trial enrolled patients with type 2 diabetes (WHO criteria), hypertension ($\geqslant$135 or $\geqslant$85 mm Hg) and micro- or macroalbuminuria (UACR $>30\,mg\,g^{-1}$ $<2000\,mg\,g^{-1}$). UACR was measured at baseline after 4 weeks of washout, with no RAAS blocking treatments allowed. Patients were between 30 and 80 years with an estimated glomerular filtration rate $\geqslant$40 ml per min per $1.73\,m^2$. Exclusion criteria included a safety limit for BP at any time in the study ($>$170/105 mm Hg), heart failure (NYHA Class II–IV), Hb_{A1c} $>$11%, and nondiabetic renal disease.

The study was approved by the local ethics committee and was conducted according to the Declaration of Helsinki Principles and Good Clinical Practice. The study was conducted at the Steno Diabetes Center, Gentofte, Denmark.

Washout. After giving informed consent, patients were screened and underwent a 4 week washout, with all antihypertensive medications paused (Figure 1). During washout and throughout the study, patients were given long-acting furosemide in a stable dose to prevent BP elevation and fluid retention and to minimize the interference from changes in dietary sodium intake. To detect important changes in BP, patients were handed automatic BP devices (U&A 779, A&D Instruments Ltd., Abingdon, UK) for measurements twice daily, to ensure that BP did not exceed 170/105 mm Hg. These measurements were not used in any efficacy evaluation. Estimated glomerular filtration rate was calculated using the revised MDRD formula[33] at baseline, at the end of treatment and 7 and 26 days after the end of treatment.

Treatment. After washout, patients attended a baseline visit for ECG, blood sampling, physical examination, and 24 h ambulatory BP. Patients then received 300 mg aliskiren once daily and furosemide in a stable dose for 28 days.

Post-treatment. To assess a possible rebound effect, the treatment was followed by a 28-day washout. All patients continued furosemide in the same dose as during treatment.

Outcome measurements

Twenty-four-hour BP was measured at baseline, during treatment (days 3, 7, 14, and 28) and during post-treatment washout (days 31, 35, 42, and 54) using a 24 h auscultatory BP device (Takeda TM2421, version 7) (A&D Medical, Tokyo, Japan). BP was measured every 15 min during the day (0700 to 2300 hours) and every 30 min during the night (2300 to 0700 hours). Values were averaged for each hour before calculating mean day, night, and 24 h values.

Urinary albumin/creatinine ratio

Morning urine samples were collected to measure UACR at screening, at baseline, during the treatment period (daily from days 1 to 28), and during post-treatment washout (days 29–54).

Twenty-four-hour urine collections were performed at baseline, during treatment (day 3), and during post-treatment washout (day 35) to measure albumin, creatinine, sodium, and urea. This was to detect fluctuations in sodium excretion, which could influence the effect of RAAS blockade.

Laboratory methods

Urinary albumin and creatinine concentrations were determined in morning urine samples on a turbidimetric Hitachi 912 System (Roche Diagnostics, Mannheim, Germany).

Blood samples for prorenin, PRA, renin concentration, angiotensinogen, Ang I, ACE activity, Ang II, and aldosterone levels were

taken after 30 min of supine rest, and after centrifugation the plasma was frozen (−80°C). These parameters were measured at baseline, during treatment (days 7, 14, and 28) and during post-treatment washout (days 35 and 54).

Renin concentration was measured with an immunoradiometric kit (Renin III, CisBio, Gif-sur-Yvette, France). Total renin concentration was determined simultaneously with the same kit, after previous changing of the prorenin conformation into a form recognizable by the IRMA antibodies by incubating for 48 h at 4°C with aliskiren.[34] Subtraction of renin concentration from total renin concentration gave the estimated prorenin concentration. PRA was measured by in-house radioimmunoassay of Ang I formed during incubation of plasma for 1 h at 37°C. No inhibitors were present during the incubation, but instead the antibody-trapping method was used.[35]

Plasma angiotensinogen concentration was determined based on conversion of angiotensinogen to Ang I by exogenous human renin using antibody trapping[36] as modified by Millar *et al.*[37]

Plasma Ang I and Ang II were measured using in-house radioimmunoassays and ethanol extraction of plasma samples. Antibodies were raised in rabbits and calibrators were purchased from NIBSC (Hertfordshire, UK). ACE activity was determined using a commercial radioenzymatic assay (ACE direct, Bühlmann-Laboratories AG, Schönenbuch, Switzerland).

Aldosterone was measured with a radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA).

Biomarkers of inflammation, endothelial dysfunction, and cardiovascular risk were measured: high-sensitivity C-reactive protein (EIA, DAKO A/S, Glostrup, Denmark); plasma von Willebrand factor (RIA, Precision BioLogic Inc., Dartmouth, Canada); serum-soluble vascular adhesion molecule-1, and serum-soluble intercellular adhesion molecule-1 (EIA, Diaclone, Besançon, France); plasma plasminogen activator inhibitor-1 (HYPHEN BioMed kit, Andresy, France); serum N-terminal-pro brain natriuretic peptide (EIA, Biomedica kit, Wien, Austria); and plasma asymmetrical dimethyl arginine (high-performance liquid chromatography).[38] Biomarkers were measured at the same study days as the RAAS components.

Statistical analysis

The log-transformed values of UACR (days 1–54) were analyzed using a mixed model where the term 'patient' was fitted as a random effect and the term 'day' was fitted as a fixed effect.

The estimated daily average with its standard error was reported. Days 2–52 were divided into intervals of three consecutive days (that is, days 2–4, days 5–7, …, days 50–52), and days 53–54 were divided into an interval of 2 consecutive days. Each 2- or 3-day interval average was compared with the baseline (day 1). Point estimates and corresponding 95% confidence intervals were reported.

For BP data, the average values of the 24 h SBP or DBP were analyzed using the same mixed model. Trends of UACR over time and BP over time were explored using graphical methods. All tests were two sided and used the 5% level of significance.

DISCLOSURE

Hans-Henrik Parving has been an advisor for Merck, Pfizer, Novartis, and Sanofi-Aventis, has received research grant support from Sanofi-Aventis, and owns stock in Merck. All other authors do not report any financial interests.

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REFERENCES

1. Parving H-H, Mauer M, Ritz E. Diabetic Nephropathy, Chap. 36. In: Brenner BM (ed). *Brenner and Rector's The Kidney*, 8th edn, Elsevier: Philadelphia, USA, 2008, pp 1265–1298.
2. Parving H-H, Lehnert H, Bröchner-Mortensen J *et al.* The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 2001; **345**: 870–878.
3. Viberti G, Wheeldon NM. Microalbuminuria reduction with valsartan in patients with type 2 diabetes mellitus: a blood pressure-independent effect. *Circulation* 2002; **106**: 672–678.
4. Brenner BM, Cooper ME, de Zeeuw D *et al.* Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; **345**: 861–869.
5. Lewis EJ, Hunsicker LG, Clarke WR *et al.* Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001; **345**: 851–860.
6. Rossing K, Schjoedt KJ, Smidt UM *et al.* Beneficial effects of adding spironolactone to recommended antihypertensive treatment in diabetic nephropathy: a randomized, double-masked, cross-over study. *Diabetes Care* 2005; **28**: 2106–2112.
7. Rossing K, Jacobsen P, Pietraszek L *et al.* Renoprotective effects of adding angiotensin II receptor blocker to maximal recommended doses of ACE inhibitor in diabetic nephropathy: a randomized double-blind crossover trial. *Diabetes Care* 2003; **26**: 2268–2274.
8. Rossing K, Christensen PK, Jensen BR *et al.* Dual blockade of the renin–angiotensin system in diabetic nephropathy: a randomized double-blind crossover study. *Diabetes Care* 2002; **25**: 95–100.
9. Rossing K, Schjoedt KJ, Jensen BR *et al.* Enhanced renoprotective effects of ultrahigh doses of irbesartan in patients with type 2 diabetes and microalbuminuria. *Kidney Int* 2005; **68**: 1190–1198.
10. de Zeeuw D, Remuzzi G, Parving H-H *et al.* Albuminuria, a therapeutic target for cardiovascular protection in type 2 diabetic patients with nephropathy. *Circulation* 2004; **110**: 921–927.
11. Rossing P, Hommel E, Smidt UM *et al.* Reduction in albuminuria predicts a beneficial effect on diminishing the progression of human diabetic nephropathy during antihypertensive treatment. *Diabetologia* 1994; **37**: 511–516.
12. Stanton A, Jensen C, Nussberger J *et al.* Blood pressure lowering in essential hypertension with an oral renin inhibitor, aliskiren. *Hypertension* 2003; **42**: 1137–1143.
13. Gradman AH, Schmieder RE, Lins RL *et al.* Aliskiren, a novel orally effective renin inhibitor, provides dose-dependent antihypertensive efficacy and placebo-like tolerability in hypertensive patients. *Circulation* 2005; **111**: 1012–1018.
14. van Paassen P, de Zeeuw D, Navis G *et al.* Renal and systemic effects of continued treatment with renin inhibitor remikiren in hypertensive patients with normal and impaired renal function. *Nephrol Dial Transplant* 2000; **15**: 637–643.
15. Fisher NDL, Hollenberg NK. Renin inhibition: what are the therapeutic opportunities? *J Am Soc Nephrol* 2005; **16**: 592–599.
16. Nussberger J, Wuerzner G, Jensen C *et al.* Angiotensin II suppression in humans by the orally active renin inhibitor Aliskiren (SPP100): comparison with enalapril. *Hypertension* 2002; **39**: E1–E8.
17. Andersen S, Jacobsen P, Tarnow L *et al.* Time course of the antiproteinuric and antihypertensive effect of losartan in diabetic nephropathy. *Nephrol Dial Transplant* 2003; **18**: 293–297.
18. Chan JCN, Cockram CS, Nicholls MG *et al.* Comparison of enalapril and nifedipine in treating non-insulin dependent diabetics associated with hypertension: one year analysis. *BMJ* 1992; **305**: 981–985.
19. Parving H-H, Lewis JB, Lewis E *et al.* Aliskiren in the evaluation of proteinuria in diabetes (AVOID). *J Am Soc Nephrol* 2007; **18**: 574A.
20. Kelly D, Zhang Y, Moe G *et al.* Aliskiren, a novel renin inhibitor, is renoprotective in a model of advanced diabetic nephropathy in rats. *Diabetologia* 2007; **50**: 2398–2404.
21. Trevisan R, Tiengo A. Effect of low-dose ramipril on microalbuminuria in normotensive or mild hypertensive non-insulin-dependent diabetic patients. North-East Italy Microalbuminuria Study Group. *Am J Hypertens* 1995; **8**: 876–883.

22. Morelli E, Loon N, Meyer TW *et al.* Effects of converting-enzyme inhibition on barrier function in diabetic glomerulopathy. *Diabetes* 1990; **39**: 76–82.
23. Remuzzi A, Ruggenenti P, Mosconi L *et al.* Effect of low-dose enalapril on glomerular size-selectivity in human diabetic nephropathy. *J Nephrol* 1993; **6**: 36–43.
24. Reddi AS, Ramamurthi R, Miller M *et al.* Enalapril improves albuminuria by preventing glomerular loss of heparan sulfate in diabetic rats. *Biochem Med Metab Biol* 1991; **45**: 119–131.
25. Langham RG, Kelly DJ, Cox AJ *et al.* Proteinuria and the expression of the podocyte slit diaphragm protein, nephrin, in diabetic nephropathy: effects of angiotensin converting enzyme inhibition. *Diabetologia* 2002; **45**: 1572–1576.
26. Nguyen G, Delarue F, Burckle C *et al.* Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest* 2002; **109**: 1417–1427.
27. Saris JJ, 't Hoen PA, Garrelds IM *et al.* Prorenin induces intracellular signaling in cardiomyocytes independently of angiotensin II. *Hypertension* 2006; **48**: 564–571.
28. Jan Danser AH, Batenburg WW, van Esch JHM. Prorenin and the (pro)renin receptor–an update. *Nephrol Dial Transplant* 2007; **22**: 1288–1292.
29. Ichihara A, Suzuki F, Nakagawa T *et al.* Prorenin receptor blockade inhibits development of glomerulosclerosis in diabetic angiotensin II type 1a receptor-deficient mice. *J Am Soc Nephrol* 2006; **17**: 1950–1961.
30. Schefe JH, Menk M, Reinemund J *et al.* A novel signal transduction cascade involving direct physical interaction of the renin/prorenin receptor with the transcription factor promyelocytic zinc finger protein. *Circ Res* 2006; **99**: 1355–1366.
31. Luetscher JA, Kramer FB, Wilson DM *et al.* Increased plasma inactive renin in diabetes mellitus. A marker of microvascular complications. *N Engl J Med* 1985; **312**: 1412–1417.
32. Persson F, Rossing P, Hovind P *et al.* Irbesartan treatment reduces biomarkers of inflammatory activity in patients with type 2 diabetes and microalbuminuria: an IRMA 2 substudy. *Diabetes* 2006; **55**: 3550–3555.
33. Levey AS, Coresh J, Greene T *et al.* Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006; **145**: 247–254.
34. Derkx FH, de Bruin RJ, van Gool JM *et al.* Clinical validation of renin monoclonal antibody-based sandwich assays of renin and prorenin, and use of renin inhibitor to enhance prorenin immunoreactivity. *Clin Chem* 1996; **42**: 1051–1063.
35. Derkx FH, van den Meiracker AH, Fischli W *et al.* Nonparallel effects of renin inhibitor treatment on plasma renin activity and angiotensins I and II in hypertensive subjects: an assay-related artifact. *Am J Hypertens* 1991; **4**: 602–609.
36. Poulsen K, Jorgensen J. An easy radioimmunological microassay of renin activity, concentration and substrate in human and animal plasma and tissues based on angiotensin I trapping by antibody. *J Clin Endocrinol Metab* 1974; **39**: 816–825.
37. Millar JA, Leckie BJ, Morton JJ *et al.* A microassay for active and total renin concentration in human plasma based on antibody trapping. *Clin Chim Acta* 1980; **101**: 5–15.
38. Teerlink T, Nijveldt RJ, de Jong S *et al.* Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. *Anal Biochem* 2002; **303**: 131–137.