Diabetologia (2010) 53:1576–1580
DOI 10.1007/s00125-010-1789-6

SHORT COMMUNICATION

Optimal antiproteinuric dose of aliskiren in type 2 diabetes mellitus: a randomised crossover trial

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Received: 19 January 2010 / Accepted: 19 April 2010 / Published online: 18 May 2010

Abstract
Aim The optimal antiproteinuric dose of aliskiren is unknown. This study compared the effect of placebo and increasing doses of aliskiren on urinary albumin excretion rate (UAER).
Methods The trial was a double-blind crossover design. Twenty-six patients with type 2 diabetes mellitus, hypertension and albuminuria were randomised to 2-month treatments with placebo or aliskiren 150 mg, 300 mg or 600 mg once daily, in random order. Primary endpoint was change in UAER; secondary endpoints included changes in 24-h BP, GFR, biomarkers and components of the renin–angiotensin–aldosterone system.
Results Placebo geometric mean UAER was 350 mg/day, mean 24-h BP was 137/81 (SD 12/9) mmHg, GFR was 85 (SD 26) ml min^{-1} 1.73 m^{-2}. Aliskiren 150, 300 and 600 mg daily reduced UAER significantly by 36% (95% CI 17–51), 48% (33–60) and 52% (38–63) respectively ($p<0.001$) compared with placebo. UAER reduction during the 600 mg dose was not significantly different from the 300 mg dose. Twenty-four-hour systolic BP was reduced by 4.5, 8.0 and 9.2 mmHg versus placebo, significant for 300 and 600 mg ($p\leq0.001$). Twenty-four-hour diastolic BP was reduced by 3.0, 4.1 and 4.4 mmHg, significant versus placebo ($p=0.019$, $p=0.001$ and $p<0.001$). GFR was reduced by 3.0, 5.1 and 6.5 ml min^{-1} 1.73 m^{-2}. hsPRA was reduced by 63%, 70%, and 82% ($p<0.001$ for all). Adverse events, most frequently dizziness and fatigue, occurred during all doses.
Conclusions In patients with type 2 diabetes mellitus, hypertension and albuminuria there is no improved antiproteinuric effect when using 600 mg aliskiren daily compared with the maximal recommended antihypertensive dose of 300 mg.

Trial registration Clinicaltrials.gov NCT00464776
Funding Novartis Pharma AG

Keywords Albuminuria · Aliskiren · Diabetic nephropathy · Renin–angiotensin system · Type 2 diabetes

Abbreviations

ADMA	Asymmetrical dimethyl arginine
ANG I	Angiotensin I
ANG II	Angiotensin II

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ARB	Angiotensin II receptor blockers
CRP	C-reactive protein
DRI	Direct renin inhibition
ESRD	End-stage renal disease
GCP	Good Clinical Practice
HMW	High molecular weight
PAI-1	Plasminogen activator inhibitor-1
PRA	Plasma renin activity
PRC	Plasma renin concentration
RAAS	Renin–angiotensin–aldosterone system
SAE	Serious adverse event
sICAM-1	Soluble intercellular adhesion molecule-1
sVCAM-1	Soluble vascular adhesion molecule-1
vWF	von Willebrand factor
UAER	Urinary albumin excretion rate

Introduction

Renin–angiotensin–aldosterone system (RAAS) blockade is an established therapy for incipient and overt diabetic nephropathy. Randomised trials have investigated ACE inhibitors, angiotensin II receptor blockers (ARBs) [1] and a direct renin inhibitor (DRI) [2]. The trials used antihypertensive doses not necessarily optimal for renoprotection. Several dosage studies [3, 4] have demonstrated dose-dependent antiproteinuric effects. After the introduction of the renin inhibitor aliskiren and evidence of its antiproteinuric effects [2, 5] we aimed to investigate the antiproteinuric effect of increasing doses of aliskiren, by comparing urinary albumin excretion rate (UAER) during placebo and after 2 months of treatment with aliskiren 150 mg, 300 mg or 600 mg once daily. In addition we measured biochemical data of the RAAS and biomarkers of cardiovascular risk.

Methods

This was a double-blind, randomised, crossover trial. The design of this exploratory study was placebo-controlled, in order to be able to investigate the direct effects of different doses of aliskiren. Careful safety measures were taken; slow-release furosemide was prescribed to prevent fluid retention and BP elevation. Patients used an electronic blood pressure device (UA 779; A&D Instruments Ltd, Abingdon, UK) to measure home BP daily throughout the study. If home BP at any time exceeded 170/105 mmHg, the patient was excluded.

The protocol was approved by the local ethics committee and the Danish Medicine Agency. Patients with type 2 diabetes mellitus were recruited from Steno Diabetes Center, Denmark. After giving informed consent, patients underwent a 1-month run-in period, in which all antihypertensive treatment was stopped prior to randomisation. A total of 26 patients were randomised and 19 patients completed the study.

Patients attended a randomisation visit before starting treatment periods of 2 months with placebo or aliskiren 150, 300 and 600 mg once daily, in random order (Fig. 1).

Patients were considered eligible for randomisation if they had baseline UAER >100 mg/24 h and <2,000 mg/24 h, baseline office BP above 135/85 mmHg and baseline GFR >40 ml min^{-1} 1.73 m^{-2}. Exclusion criteria included recent cardiovascular disease, heart failure (New York Heart Association class II–IV), HbA_{1c} >11% and malignancy.

After each treatment period, patients collected three consecutive 24-h urine samples for assessment of geometric mean UAER. We performed assessment of GFR (measured as plasma clearance of ^{51}Cr-EDTA) [6] and mounted standard Takeda 24-h BP devices (TM2421; A&D Medical, Tokyo, Japan). BP measurements were performed every 15 min from 7:00 to 23:00 and every 30 min from 23:00 to 7:00 for 24 h.

Laboratory methods UAER was determined on a turbidimetric Hitachi 912 System (Roche Diagnostics, Mannheim, Germany) and creatinine using the enzymatic Jaffé method. Cardiovascular biomarkers, PRC, prorenin, angiotensinogen, PRA, Ang I, ACE activity and Ang II were measured using previously described methodology [7]. Prior to the study, it was anticipated that conventional PRA assays would not have the required sensitivity to detect very low levels of plasma renin activity during the 600 mg treatment. Consequently, we developed a trapping high sensitivity PRA assay with increased sensitivity. Trapping hsPRA (ThsPRA) was carried out like hsPRA with the modification that Ang I antiserum was added at the start of the enzymatic reaction at a quarter of the concentration employed in hsPRA.

Investigators were blinded to randomisation using a validated computer system. Blinded study medications were packed and labelled prior to delivery to the site.

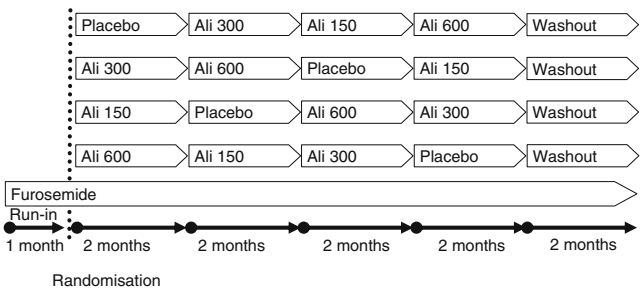

Fig. 1 Study design. Ali, aliskiren

Statistical analysis It was estimated that 20 patients who completed the study could provide 80% power to demonstrate a significant difference between two treatments in antiproteinuric effect (measured as UAER) if the true difference was 15%. This was based on the assumption that intra-individual CV for UAER was 13%. The log-transformed values of UAER were analysed by a PROC MIXED model with sequence, treatment, and period as fixed factors and patient (nested in sequence) as a random factor. Twenty-four-hour average values for systolic blood pressure (SBP) and diastolic blood pressure (DBP) were analysed using a PROC MIXED model with sequence, treatment, and period as fixed factors and patient (nested in sequence) as a random factor. GFR results and all other laboratory assessment data were analysed in a similar way to BP data**.** A two-sided p value <0.05 was considered significant.

Statistical analyses were performed using SAS version 8.2 or higher (SAS Institute Inc., Cary, North Carolina, USA).

Results

Clinical and biochemical results are shown in Table 1. Treatment with aliskiren 150, 300 and 600 mg daily reduced UAER significantly by 36% (95% CI 17–51), 48% (33–60) and 52% (38–63) respectively compared with placebo ($p<0.001$ for all). The 600 mg dose had a significantly larger reduction compared with 150 mg, but not compared with 300 mg.

Twenty-four-hour SBP was reduced by 4.5 (−0.2, 9.2), 8.0 (3.2–12.6) and 9.2 (4.5–13.9) mmHg compared with placebo, significant reductions for the 300 and 600 mg group vs placebo ($p=0.001$ and $p<0.001$), but with no significant difference between 300 and 600 mg ($p=0.598$). Twenty-four-hour DBP was reduced by 3.0 (0.5–5.4), 4.1 (1.7–6.6) and 4.4 (2.0–6.9) mmHg respectively; all doses were significantly different compared with placebo ($p=0.019$, $p=0.001$ and $p<0.001$), and there was no significant difference between 300 and 600 mg ($p=0.814$).

GFR was reduced by 3.0 (−0.6, 6.6), 5.1(1.5–8.8) and 6.5 (2.8–10.1) ml min^{-1} 1.73 m^{-2}, significant reductions compared with placebo for the 300 and 600 mg doses ($p=0.007$ and $p<0.001$), and no difference between doses ($p=0.469$).

During the washout period the UAER increased towards placebo levels. After 6 weeks the UAER was reduced by 10% compared with placebo (NS) and after 8 weeks it was similar to placebo (332 mg/day vs 330 mg/day). After the 8-week washout mean 24-h BP was 136/77 mmHg, as compared with 137/81 mmHg at baseline.

We found a clear dose-dependent effect on the RAAS components as shown in Table 1.

The biomarkers of cardiovascular risk displayed only minor and insignificant changes during the different aliskiren treatments compared with placebo levels, except for aldosterone, which was significantly reduced during treatment with 150 mg and 600 mg, and total adiponectin, which showed a significant increase during 600 mg treatment.

All doses of aliskiren were well tolerated. During the study four patients had a total of five serious adverse events (SAEs). One patient was diagnosed with colon cancer, one patient was hospitalised twice for syncope, one patient developed macroscopic haematuria and one patient was hospitalised due to lower limb wound infection requiring i.v. antibiotic therapy. None of the SAEs were suspected to be related to or caused by the aliskiren treatment. No events of hyperkalaemia were observed. The most frequent side effects were dizziness (four patients, one in each treatment group) and fatigue (two patients, one in the 300 mg and one in the 600 mg group). There were no gastrointestinal adverse events during treatment with 600 mg aliskiren.

Discussion

In this study we investigate the antiproteinuric effect of increasing doses of aliskiren. Although there is no further clinical benefit from increasing the aliskiren dose above the maximal recommended antihypertensive dose, 300 mg (indicating a plateau of the dose–response curve), there are signs of a higher degree of RAAS blockade, reflected by the larger increase in plasma prorenin and plasma renin concentration, and the further decrease in plasma renin activity.

The matter of dosing is important, since most antiproteinuric treatments are applied using antihypertensive doses. Dosing of antiproteinuric RAAS blockade was recently discussed [8] and has so far been investigated in a fraction of the treatments used today [3, 9].

When comparing 300 mg and 600 mg, we found no significant differences in the clinical measures (UAER, 24-h BP), but the changes in RAAS markers imply that 600 mg aliskiren treatment achieved a higher degree of RAAS blockade compared with treatment with 300 mg aliskiren. The increase in PRC is of interest, as it is regarded as a response to the feedback from the Ang II receptors and is thus a sign of increased intrarenal inhibition of the RAAS. Also prorenin levels were significantly higher during 600 mg treatment. Long-term beneficial effects of renin inhibition may in part work by mitigating the negative effects of this (pro)renin receptor pathway. Blocking this (pro)renin receptor using a decoy peptide has so far shown diverging results [10]. Levels of biomarkers of cardiovascular risk measured in our study

Table 1 Clinical results and changes in RAAS components and cardiovascular biomarkers

Variable	Placebo	Aliskiren 150 mg		Aliskiren 300 mg		Aliskiren 600 mg	
	Geometric mean (range or SD)	Geometric mean (range or SD)	Ratio (95% CI) vs placebo	Geometric mean (range or SD)	Ratio (95% CI) vs placebo	Geometric mean (range or SD)	Ratio (95% CI) vs placebo
UAER (mg/24 h)	350 (42–1,450)	222 (63–829)	0.64 (0.49–0.83)***	182 (36–878)	0.52 (0.40–0.67)***	167 (30–843)	0.48 (0.37–0.62)***
24 h BP (mmHg)	140/80 (14/8)	136/77 (13/9)	−4.5 (−9.2, 0.2)[a]	132/76 (14/8)	−7.9 (−12.6, −3.2)***[a]	131/75 (15/8)	−9.2 (−13.9, −4.5)***[a]
GFR (ml min^{-1} 1.73 m^{-2})	85 (27)	82 (27)	−3.0 (−6.6, 0.6)[b]	80 (27)	−5.1 (−8.8, −1.5)**[b]	79 (30)	−6.5 (−10.1, −2.8)**[b]
Angiotensinogen (nmol/l)	890.1 (640–1,240)	934.9 (563–2,628)	1.05 (0.96–1.15)	914.2 (571–2,628)	1.03 (0.94–1.12)	871.8 (481–1,886)	0.98 (0.90–1.07)
Plasma prorenin concentration (ng/l)	302.31 (41–1,521)	336.18 (43–1,903)	1.14 (1.01–1.29)*	339.32 (41–2,153)	1.15 (1.02–1.30)*	391.36 (62–2,747)	1.33 (1.18–1.50)***
Plasma renin concentration (ng/l)	25.70 (6–123)	70.70 (16–476)	2.95 (2.17–4.00)***	116.99 (10–723)	4.87 (3.59–6.62)***	207.99 (21–1,233)	8.70 (6.40–11.81)***
PRA (ng A1 ml^{-1} h^{-1})	0.81 (0.21–2.43)	0.39 (0.14–1.57)	0.49 (0.35–0.69)***	0.36 (0.07–1.53)	0.44 (0.32–0.62)***	0.23 (0.07–1.05)	0.28 (0.20–0.39)***
hsPRA (ng A1 ml^{-1} h^{-1})	3.15 (0.79–18.0)	1.11 (0.27–7.7)	0.35 (0.22–0.57)***	0.93 (0.27–5.3)	0.29 (0.18–0.46)***	0.56 (0.27–3.9)	0.19 (0.12–0.30)***
ThsPRA (ng A1 ml^{-1} h^{-1})	3.51 (1.27–15.16)	1.62 (0.30–11.45)	0.47 (0.30–0.73)***	1.16 (0.14–7.63)	0.33 (0.22–0.52)***	0.63 (0.03–5.41)	0.18 (0.12–0.28)***
ACE activity (U/l)	31.96 (6.0–60.0)	30.80 (1.0–77.0)	0.95 (0.81–1.10)	30.95 (1.0–63.0)	0.95 (0.82–1.11)	33.99 (7.0–65.0)	1.05 (0.90–1.22)
Angiotensin I (pmol/l)	30.79 (7.8–100.0)	10.73 (4.7–35.0)	0.35 (0.27–0.45)***	9.27 (4.7–24.0)	0.30 (0.23–0.39)***	7.48 (2.8–17.0)	0.24 (0.19–0.31)***
Angiotensin II (pmol/l)	14.50 (2.9–61.0)	10.87 (2.9–91.0)	0.74 (0.50–1.08)	10.64 (1.4–86.0)	0.72 (0.49–1.07)	7.82 (1.2–51.0)	0.53 (0.36–0.78)**
Aldosterone (ng/l)	75.50 (25–205)	49.16 (25–233)	0.67 (0.53–0.84)***	60.81 (25–169)	0.82 (0.65–1.03)	54.38 (25–199)	0.73 (0.58–0.93)**
High sensitivity C-reactive protein (mg/l)	2.26 (0.3–28.8)	2.30 (0.3–17.1)	1.07 (0.71–1.60)	1.59 (0.2–9.5)	0.74 (0.49–1.11)	1.83 (0.2–9.6)	0.84 (0.56–1.27)
Fibrinogen (g/l)	3.65 (2.7–6.4)	3.75 (2.7–6.2)	1.03 (0.95–1.13)	3.51 (2.7–5.7)	0.97 (0.89–1.06)	3.65 (2.6–5.6)	1.01 (0.92–1.10)
vWF (%)	141.8 (70–271)	144.16 (67–281)	0.99 (0.92–1.07)	141.72 (72–304)	0.98 (0.91–1.05)	144.11 (68–273)	0.99 (0.93–1.07)
ICAM-1 (μg/l)	651.3 (426–1,120)	644.2 (398–1,020)	0.99 (0.93–1.06)	678.8 (439–1,230)	1.05 (0.98–1.11)	679.0 (483–1,190)	1.04 (0.98–1.11)
VCAM-1 (μg/l)	1,202.9 (686–1,920)	1,214.6 (700–1,940)	1.02 (0.90–1.15)	1,195.9 (716–1,810)	0.99 (0.88–1.12)	1,267.1 (683–2,390)	1.05 (0.93–1.19)
Adiponectin (total) (μg/ml)	3.26 (1.18–6.92)	3.20 (1.01–7.55)	0.99 (0.94–1.05)	3.37 (1.18–10.62)	1.04 (0.99–1.10)	3.46 (1.35–10.09)	1.07 (1.02–1.13)**
HMW adiponectin (μg/ml)	1.19 (0.24–4.84)	1.21 (0.18–5.67)	1.01 (0.95–1.09)	1.22 (0.27–5.71)	1.02 (0.95–1.10)	1.23 (0.29–5.34)	1.03 (0.96–1.11)
HMW:total adiponectin ratio	0.37 (0.2–0.7)	0.38 (0.18–0.75)	1.02 (0.96–1.09)	0.36 (0.15–0.55)	0.98 (0.92–1.05)	0.36 (0.13–0.58)	0.96 (0.90–1.02)
PAI-1 (μg/l)	80.29 (21–622)	76.86 (18–199)	0.94 (0.70–1.27)	79.70 (9–289)	0.98 (0.73–1.31)	71.40 (12–277)	0.87 (0.65–1.18)
ADMA (μmol/l)	0.47 (0.4–0.5)	0.46 (0.4–0.6)	1.00 (0.96–1.05)	0.47 (0.4–0.6)	1.02 (0.97–1.07)	0.48 (0.4–0.6)	1.04 (0.99–1.08)

[a] BP changes in mmHg; [b] GFR changes in ml min^{-1} 1.73 m^{-2}

$^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$

were mostly unchanged during aliskiren treatment. This is in agreement with previous short-term studies [5, 7]. Of note, aldosterone levels tended to decrease with all doses, which warrants further investigation, since this is a small study and the connection between RAAS blockade (i.e. aliskiren treatment) and cardiovascular biomarkers has not been well described. The antiproteinuric effect of aliskiren treatment in type 2 diabetes mellitus has been demonstrated as an add-on to standard treatment [2]. The consequences of a high degree of RAAS blockade remain to be further investigated.

This was a small exploratory study of short duration, limiting the potential interpretations. However, the cross-over study design was appropriately powered to detect a clinically meaningful difference in UAER (15%) between doses which can now be excluded, and thus 600 mg should not be used in clinical practice. In conclusion, we found no additional antiproteinuric effect of 600 mg aliskiren once daily as compared with the standard dose of 300 mg daily.

Acknowledgements The authors want to acknowledge the participating patients and the work of lab technicians U. M. Smidt, A. G. Lundgaard and B. R. Jensen. This study was sponsored by Novartis, and we wish to thank C.-M. Yeh for statistical contributions and W. P. Dole, I. Rajman, L. Sandford and M. Prescott for assistance through all study phases.

Duality of interest F. Persson reports having received lecture fees from Novartis and having equity interest in NovoNordisk. P. Rossing reports having received lecture fees from Novartis and Boehringer Ingelheim and a research grant from Novartis, having served as a consultant for Merck, and having equity interest in NovoNordisk. A. H.J. Danser reports having received a research grant from Novartis and having served as a consultant to Novartis. H.-H. Parving reports having served as a consultant for Novartis, Merck, Pfizer and sanofi-aventis, having equity interest in Merck and NovoNordisk and having received lecture fees from Novartis, Merck, Pfizer and sanofi-aventis. H.-H. Parving has received grant support from Novartis, AstraZeneca and sanofi-aventis.

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