Avosentan for Overt Diabetic Nephropathy

Johannes F.E. Mann,* Damian Green,† Kenneth Jamerson,‡ Luis M. Ruilope,§ Susan J. Kuranoff,‖ Thomas Littke,‖ and Giancarlo Viberti,¶ for the ASCEND Study Group

*Schwabing General Hospital, and Department of Medicine IV, University of Erlangen and KfH Kidney Centre, Munich, Germany; †Quintiles Ltd., Strasbourg, France; ‡University of Michigan, Ann Arbor, Michigan; §Hospital 12 de Octubre, Madrid, Spain; ‖Speedel Pharma Ltd., Basel, Switzerland; and ¶King's College London School of Medicine, Guy's Hospital, London, United Kingdom

ABSTRACT

In the short term, the endothelin antagonist avosentan reduces proteinuria, but whether this translates to protection from progressive loss of renal function is unknown. We examined the effects of avosentan on progression of overt diabetic nephropathy in a multicenter, multinational, double-blind, placebo-controlled trial. We randomly assigned 1392 participants with type 2 diabetes to oral avosentan (25 or 50 mg) or placebo in addition to continued angiotensin-converting enzyme inhibition and/or angiotensin receptor blockade. The composite primary outcome was the time to doubling of serum creatinine, ESRD, or death. Secondary outcomes included changes in albumin-to-creatinine ratio (ACR) and cardiovascular outcomes. We terminated the trial prematurely after a median follow-up of 4 months (maximum 16 months) because of an excess of cardiovascular events with avosentan. We did not detect a difference in the frequency of the primary outcome between groups. Avosentan significantly reduced ACR: In patients who were treated with avosentan 25 mg/d, 50 mg/d, and placebo, the median reduction in ACR was 44.3, 49.3, and 9.7%, respectively. Adverse events led to discontinuation of trial medication significantly more often for avosentan than for placebo (19.6 and 18.2 *versus* 11.5% for placebo), dominated by fluid overload and congestive heart failure; death occurred in 21 (4.6%; $P = 0.225$), 17 (3.6%; $P = 0.194$), and 12 (2.6%), respectively. In conclusion, avosentan reduces albuminuria when added to standard treatment in people with type 2 diabetes and overt nephropathy but induces significant fluid overload and congestive heart failure.

J Am Soc Nephrol 21: 527–535, 2010. doi: 10.1681/ASN.2009060593

Data from animal studies and observational data from humans suggest that proteinuria is not simply a biomarker of renal disease but also contributes to progressive renal damage, ultimately leading to ESRD.[1] Proteinuric nephropathies are a leading cause of ESRD, and, despite current available treatments, most patients still exhibit residual proteinuria and disease progression.[1–4] In controlled trials, 15 to 20% of patients who had type 2 diabetes and overt diabetic nephropathy and had been intensively treated still reached ESRD after only 2.5 to 3.5 years.[2,3] Clearly, there is a need for the development of new strategies to reduce further and perhaps arrest the rate of loss of renal function.

Endothelin 1, *via* the activation of the endothelin type A (ET_A) receptor, seems to have a central role in the pathogenesis of proteinuria.[5,6] In short-term (up to 12 weeks) proof-of-concept clinical studies, avosentan, a predominant ET_A receptor antagonist, reduced proteinuria in people who had

Received June 9, 2009. Accepted December 3, 2009.

Published online ahead of print. Publication date available at www.jasn.org.

This trial has been registered at http://www.clinicaltrials.gov (identifier NCT00120328).

Correspondence: Dr. Giancarlo Viberti, Unit for Metabolic Medicine, Cardiovascular Division, King's College London Medical School, 5th Floor Southwark Wing, Guy's Hospital, London SE1 9RT, UK. Phone: +44-20-7188-1910; Fax: +44-20-7188-0146; E-mail: giancarlo.viberti@kcl.ac.uk

diabetes and were on maximal dosages of inhibitors of the renin-angiotensin system.[7–9] This antiproteinuric effect was achieved without significant changes in BP.

We therefore examined the effect of avosentan on time to doubling of serum creatinine, ESRD, or death (A Randomised, Double Blind, Placebo Controlled, Parallel Group Study to Assess the Effect of the Endothelin Receptor Antagonist Avosentan on Time to Doubling of Serum Creatinine, End Stage Renal Disease or Death in Patients With Type 2 Diabetes Mellitus and Diabetic Nephropathy [ASCEND]) and evaluated, as secondary outcomes, changes in urine albumin excretion and in estimated GFR (eGFR), as well as cardiovascular outcomes in people with type 2 diabetes and overt diabetic nephropathy. Safety aspects were monitored throughout the study.

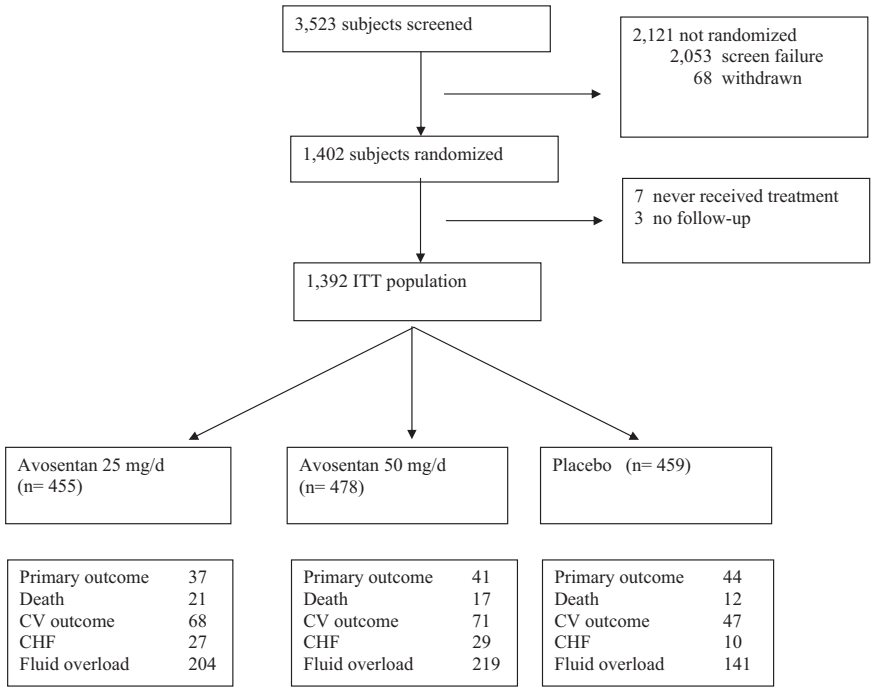

Figure 1. Screen failure was almost exclusively due to an ACR below the inclusion criterion of 35 mg/mmol or a serum creatinine outside the inclusion criteria. For definitions and details of primary and secondary outcomes, see Table 2 and the Concise Methods section. CV, cardiovascular; CHF, a component of the CV outcome (typical signs and/or symptoms of heart failure and having received new therapy for CHF and being admitted to hospital for at least 24 hours). Fluid overload was not defined by the trial protocol but taken from the adverse event reports of the local investigators.

RESULTS

The Steering Committee terminated the trial prematurely on the recommendation of the Data Safety and Monitoring Board (DSMB) because of an excess of cardiovascular events with avosentan, mainly congestive heart failure (CHF) and fluid overload. The median treatment period was 4 months with avosentan 25 and 50 mg and 5 months with placebo. At study termination, 3523 patients had been screened and 1402 randomly assigned. Of the latter, seven did not receive any trial medication, and no follow-up information was available for three, leaving 1392 patients for the intention-to-treat analysis (Figure 1). Treatment exposure was 183, 192, and 224 patient-years for avosentan 25 and 50 mg and placebo, respectively. Baseline characteristics were similar between groups (Table 1) and typical for stages 3 to 4 chronic kidney disease (CKD) and overt diabetic nephropathy.

Primary and Secondary Outcomes

The proportion of patients who met the primary composite end point of doubling of serum creatinine, ESRD, or death was not significantly different among the three groups (Table 2, Figure 2). Fewer patients on avosentan experienced ESRD, but more died compared with placebo. These differences were NS. The reason for death could be ascertained in 29 of the 46 adjudicated cases; death was due to cardiovascular causes in 74%. After the trial had ended and participants stopped trial medication, four additional deaths were reported with avosentan 25 mg/d (see Table 2). Cardiovascular outcomes were more frequent with avosentan, specifically CHF (Table 2).

The eGFR declined during the study in all three groups by 2.5 to 4 ml/min per 1.73 m^2 during 6 months (Table 3). The decrease in eGFR was slightly greater in the avosentan 50-mg group compared with placebo at both 3 months ($P = 0.030$) and 6 months ($P = 0.018$) with no significant difference for avosentan 25 mg. The median albumin-to-creatinine ratio (ACR) significantly declined similarly by 40 to 50% in both avosentan groups (NS between the two avosentan groups) and by 8 to 10% with placebo ($P < 0.0001$ *versus* both avosentan groups, adjusted for changes of BP; Figure 3, Table 3). When the changes in ACR were corrected for the changes in eGFR, these differences persisted (see Supplemental Appendix 1).

Changes of Body Weight and BP

Mean ± SD body weight increased by 0.4 ± 3.0, 0.3 ± 2.9, and 0.0 ± 2.7 kg, respectively, at 3 months and then remained stable up to 6 months. BP declined by 0.0 to −0.5 mmHg systolic and diastolic with placebo and by −4.1 to −6.1 mmHg systolic and by −3.0 to −4.4 mmHg diastolic in both avosentan groups ($P = 0.003$ to 0.118 for placebo *versus* avosentan; range of mean values for systolic and diastolic BP and of P values at 3 and 6 months, for details see Supplemental Appendix 2). After 3 months, the proportion of patients who

Table 1. Baseline demographic, clinical, and biochemical characteristics

Characteristic	Avosentan 25 mg (n = 455)	Avosentan 50 mg (n = 478)	Placebo (n = 459)	P	
				Avosentan 25 mg *versus* Placebo	Avosentan 50 mg *versus* Placebo
Age (years; mean ± SD)	61.2 ± 8.8	61.0 ± 9.1	60.8 ± 8.9	0.788	0.976
Female (n [%])	140 (30.8)	157 (32.8)	155 (33.8)	0.512	0.819
Disease history (n [%])					
coronary artery disease	143 (31.4)	135 (28.2)	149 (32.5)	0.816	0.128
CHF	66 (14.5)	69 (14.4)	62 (13.5)	0.189	0.432
stroke or transient ischemic attack	39 (8.6)	33 (6.9)	40 (8.7)	0.763	0.140
peripheral vascular disease	37 (8.1)	31 (6.5)	26 (5.7)	0.064	0.890
hypertension	409 (89.9)	427 (89.3)	412 (89.9)	0.742	0.984
current smoking	2 (0.4)	3 (0.6)	4 (0.9)	0.395	0.459
diabetic retinopathy	183 (40.0)	191 (40.2)	167 (36.4)	0.230	0.329
Physical examination					
SBP (mmHg; mean ± SD)	137.1 ± 13.8	137.0 ± 14.3	135.4 ± 15.1	0.162	0.299
DBP (mmHg; mean ± SD)	77.9 ± 9.2	77.5 ± 8.6	77.2 ± 9.5	0.358	0.573
BMI (kg/m^2; mean ± SD)	29.9 ± 6.2	30.4 ± 6.5	30.1 ± 6.2	0.975	0.193
body weight (kg; mean ± SD)	84.5 ± 21.0	85.0 ± 21.0	84.0 ± 19.9	0.960	0.347
edema (n [%])	70 (15.4)	58 (12.1)	68 (14.8)	0.446	0.938
Laboratory results					
HbA_{1c} (%; mean ± SD)	8.0 ± 1.5	8.1 ± 1.6	8.0 ± 1.5	0.927	0.989
creatinine (μmol/L; mean ± SD)	185.1 ± 50.2	186.9 ± 50.8	187.7 ± 50.9	0.990	0.999
eGFR (ml/min per 1.73 m^2; mean ± SD)	33.8 ± 11.2	33.2 ± 10.9	33.0 ± 10.6	0.875	0.992
ACR (mg/mmol)				0.979	0.408
median	160.9	166.5	173.2		
IQR	82.45–274.35	85.80–284.50	89.85–319.45		
hemoglobin (g/L; mean ± SD)	122.6 ± 17.7	121.3 ± 17.4	121.0 ± 16.6	0.502	0.944
Medication (n [%])					
insulin	315 (69.2)	315 (65.9)	291 (63.4)	0.057	0.943
glitazones	54 (11.9)	50 (10.4)	57 (12.4)	0.633	0.804
ACEIs	308 (67.7)	293 (61.3)	273 (59.5)	0.167	0.269
ARBs	195 (42.9)	207 (43.3)	182 (39.7)	0.340	0.980
dihydropyridine calcium channel blockers	204 (44.8)	204 (42.7)	207 (45.1)	0.529	0.206
β blockers	178 (39.1)	156 (32.6)	176 (38.3)	0.803	0.138
diuretics	293 (64.4)	309 (64.6)	297 (64.7)	0.935	0.555
loop diuretics	200 (44.0)	231 (48.3)	208 (45.3)	0.874	0.741
statins	257 (56.5)	264 (55.2)	275 (59.9)	0.176	0.187

BP was measured in the sitting position; first morning urine was provided on 3 consecutive days and the mean value is represented. Heart failure New York Heart Association stage III or IV CHF was an exclusion criterion. BMI, body mass index; DBP, diastolic BP; HbA_{1c}, glycosylated hemoglobin; IQR, interquartile range; SBP, systolic BP.

achieved target BP was 66.9% with avosentan 25 mg, 62.6% with avosentan 50 mg, and 50.0% with placebo.

Adverse Events

Serious adverse events occurred more often with avosentan as did adverse events leading to premature discontinuation of trial medication (Tables 4 and 5). Signs and symptoms of fluid overload were significantly more frequent with avosentan (Figure 4). In particular, there were more reports of pulmonary edema and of CHF with avosentan (Tables 2 and 5). Of participants who permanently discontinued trial medication as a result of adverse events, the most common reason was symptoms of fluid overload, reported by 44 of 89, 38 of 87, and eight of 53 participants with avosentan 25 mg, 50 mg, and placebo, respectively, followed by worsening of renal function in 18 of 89, 20 of 87, and 18 of 53, respectively.

Mean ± SD hemoglobin levels decreased in patients who were taking avosentan 25 mg by 11.4 ± 11.7 g/L, avosentan 50 mg by 11.0 ± 12.6 g/L, and placebo by 0.1 ± 9.0 g/L from baseline to 3 months ($P < 0.001$ for both avosentan groups *versus* placebo) and remained stable thereafter (data not shown). Anemia was reported as an adverse event more frequently with avosentan than with placebo (Table 4). The incidence of an increase in any liver function test above the upper limit of normal was not different between avosentan and placebo groups (65 [14.2%], 80 [16.7%], and 96 [20.9%] with avosentan 25 and 50 mg and placebo, respectively). A small proportion of patients who exhibited an increase in one or

Table 2. Occurrence of the adjudicated primary composite outcome of doubling of serum creatinine, ESRD, or death and its individual components and of secondary outcomes

Parameter	Avosentan 25 mg (n = 455)	Avosentan 50 mg (n = 478)	Placebo (n = 459)	*P*	
				Avosentan 25 mg *versus* placebo	Avosentan 50 mg *versus* placebo
Primary composite outcome	37 (8.1)	41 (8.6)	44 (9.6)	0.557	0.791
death	21 (4.6)[a]	17 (3.6)	12 (2.6)	0.225	0.194
ESRD	20 (4.4)	24 (5.0)	30 (6.5)	0.136	0.405
doubling of serum creatinine	2 (0.4)	4 (0.8)	9 (2.0)	0.405	0.060
Cardiovascular outcome	68 (14.9)	71 (14.9)	47 (10.2)	0.049	0.089
CHF	27 (5.9)	29 (6.1)	10 (2.2)	0.008	0.050

All events were adjudicated and are n (%). The cardiovascular outcome was defined as the composite of coronary or peripheral vascular revascularization, amputations (except from trauma), nonfatal acute myocardial infarction, stroke, and CHF. Of 66 patients with CHF (typical signs and/or symptoms of CHF and having received new therapy for CHF and being admitted to hospital for at least 24 hours), 49 occurred in those with baseline eGFR below the median of 33 ml/min per 1.73 m^2.

[a]For death in the avosentan 25-mg group, we added four deaths that were reported after closure of the trial and discontinuation of trial medication and could not be adjudicated because of insufficient information; there were 17 adjudicated deaths in that group. Excluding those four deaths did not materially alter the difference to placebo (P = 0.313).

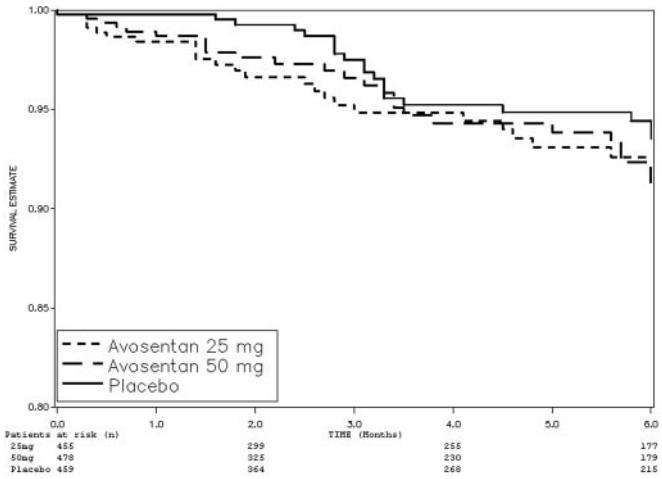

Figure 2. Kaplan-Meier plot shows time to doubling of serum creatinine, ESRD, or death in patients who had type 2 diabetes and diabetic nephropathy and were treated with avosentan 25 mg/d, avosentan 50 mg/d, or placebo (n = 1392). There were no significant differences among groups. The plots were truncated at 6 months because of premature termination of the trial.

more liver function tests had increases up to twice the upper limit of normal of these tests.

DISCUSSION

Although ASCEND was terminated prematurely because of safety concerns, it provides new information about the effects of a predominant ET_A receptor antagonist in patients with type 2 diabetes and stages 3 to 4 CKD. At the dosages used, avosentan induced a major decrease in proteinuria but also symptoms of fluid overload with serious consequences in some patients that led to the discontinuation of the trial. Of particular concern was a trend to a higher mortality with avosentan. eGFR fell to a greater extent with avosentan 50 mg/d, and anemia, hypoglycemia, and hypotension were reported more frequently by the investigators in patients who received avosentan.

Avosentan reduced albuminuria by 40 to 50% in patients who had an average baseline albuminuria close to 1.5 g/g creatinine; exhibited reasonable BP control[8,9]; and received extensive treatment with angiotensin-converting enzyme inhibitors (ACEIs) and/or angiotensin receptor blockers (ARBs), other antihypertensive drugs, diuretics, and statins. In another study on diabetic nephropathy, a 50% reduction in albuminuria was associated with a relative risk reduction for ESRD of approximately 50%.[10] In people with less albuminuria, the association of albuminuria reduction and reduction of ESRD risk may be less pronounced.[11] Our findings on proteinuria confirm previous observations in shorter small-scale studies over several weeks with avosentan in patients with overt diabetic nephropathy but with less advanced renal insufficiency[9] and in acute studies with infusion of BQ-123, another ET_A receptor antagonist.[12] The effect on albuminuria is likely due to inhibition of the renal ET_A receptor, because other researchers have found that the mixed type $ET_{A/B}$ receptor antagonists have a weaker or no effect on proteinuria.[12–14] The slightly greater decrease in eGFR that was observed with avosentan does not seem to explain the substantial difference in albuminuria between placebo and avosentan,[15] because this persisted after correction for the changes in eGFR.

It is generally thought that additional lowering of albuminuria in diabetic nephropathy may protect the kidney from progressive loss of function; however, because of the early termination of ASCEND, the number of primary outcomes was insufficient to test the latter hypothesis with avosentan. Primary outcomes occurred at approximately the same rate with avosentan and placebo, and the decrease in eGFR, a secondary outcome, seemed to be slightly faster with avosentan. Whether the faster fall in eGFR was the result of avosentan-induced lower intraglomerular pressure, an effect that could translate into long-term benefit,[1,15] remains a moot point. ESRD seemed to occur less frequently with avosentan, and we cannot

Table 3. Change in eGFR (ml/min per 1.73 m^2) and in ACR (mg/mmol)

Time	Avosentan 25 mg (n = 455)	Avosentan 50 mg (n = 478)	Placebo (n = 459)	*P*	
				Avosentan 25 mg *versus* Placebo	Avosentan 50 mg *versus* Placebo
eGFR ml/min					
baseline					
n	455	478	459		
mean ± SD	33.8 ± 11.2	33.2 ± 10.9	33.0 ± 10.6		
3 months					
n	303	330	345		
mean ± SD	31.7 ± 11.5	30.4 ± 11.6	30.9 ± 10.5		
change from baseline (mean ± SD)	−1.66 ± 6.01	−2.71 ± 7.03	−1.70 ± 5.87	0.932[a]	0.030[a]
6 months					
n	192	200	236		
mean ± SD	29.5 ± 10.0	29.3 ± 11.4	30.0 ± 11.7		
change from baseline (mean ± SD)	−3.35 ± 6.18	−4.08 ± 6.94	−2.50 ± 6.87	0.184[a]	0.018[a]
ACR (mg/mmol)					
baseline					
n	455	478	459		
median	160.9	166.5	173.2		
IQR	82.5 to 274.4	85.8 to 284.5	89.9 to 319.5		
3 months					
n	294	308	334		
median	100.3	105.3	166.7		
IQR	40.7 to 198.3	42.8 to 219.8	68.0 to 328.6		
% median change	−40.50	−38.30	−7.66	<0.001[b]	<0.001[b]
IQR	−60.70 to −10.40	−62.50 to −1.38	−32.60 to 21.30		
6 months					
n	194	199	234		
median	89.2	89.4	164.8		
IQR	28.9 to 200.2	32.2 to 182.3	65.5 to 283.5		
% median change	−44.30	−49.30	−9.69	<0.001[b]	<0.001[b]
IQR	−71.4 to −6.5	−72.8 to −17.5	−41.4 to 31.0		

All parameters were measured in a central laboratory, eGFR was calculated with the six-item MDRD formula. For each ACR value, the geometric mean of three consecutive first morning urine values was entered into the database. With repeated measures ANOVA, changes of ACR were significant at $P < 0.001$ for both dosages; changes of eGFR were significant for the 50-mg dosage *versus* placebo ($P = 0.0238$) but not for the 25-mg dosage ($P = 0.5160$).

[a] One-way ANOVA from summary data.

[b] Wilcoxon test.

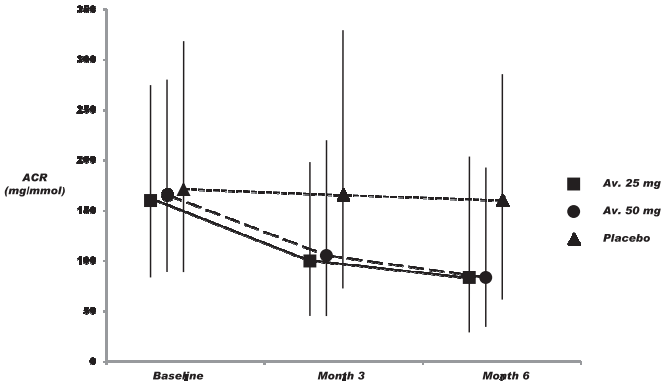

Figure 3. Urine ACR changed significantly ($P < 0.0001$; see Table 3) in the avosentan (av)-treated groups during the first 6 months of the trial. Medians and interquartile ranges are given. Similar differences were found for fractional excretion of urine albumin (see Supplemental Appendix 2).

exclude that a beneficial effect on the kidney was outweighed by increased early mortality. Avosentan caused a moderate decrease in systolic BP by 3 to 6 mmHg, compared with <0.5 mmHg with placebo, that does not fully explain a change of proteinuria by approximately 40 to 50%.[15,16] ET_A receptor antagonists can lower BP[17] and more so in combination with ACEIs[18] and in those with CKD.[13]

The administration of avosentan at the dosages of 25 and 50 mg/d was associated with symptoms of fluid overload that led to life-threatening complications in some patients. The modest weight gain in ASCEND of approximately 0.5 kg during 6 months with avosentan may be explained by fluid retention but also fluid redistribution. Edema formation has been observed before with ET_A and mixed type $ET_{A/B}$ receptor antagonists.[17–19] For avosentan, a predominant type A antagonist, these effects were seen mainly at dosages of ≥5 mg/d, but they were not found to be life threatening in shorter term studies of patients with less advanced renal disease.[8,9] It may be that at

Table 4. Occurrence of adverse events

Adverse Event (*n* [%])	Avosentan 25 mg (*n* = 455)	Avosentan 50 mg (*n* = 478)	Placebo (*n* = 459)	*P*	
				Avosentan 25 mg *versus* Placebo	Avosentan 50 mg *versus* Placebo
Patients with ≥1 adverse event	322 (70.8)	346 (72.2)	309 (67.0)	0.164	0.325
Patients with ≥1 serious adverse event	149 (32.7)	145 (30.3)	112 (24.3)	0.001	0.122
Withdrew because of adverse events	89 (19.6)	87 (18.2)	53 (11.5)	0.001	0.020
Patients with symptoms of fluid overload	204 (44.8)	219 (45.8)	141 (30.7)	0.0001	<0.0001
Anemia	49 (10.8)	64 (13.4)	16 (3.5)	0.0002	<0.0001
hypoglycemia	20 (4.4)	23 (4.8)	13 (2.8)	0.139	0.052
hyperkalemia	16 (3.5)	19 (4.0)	14 (3.0)	0.407	0.120
hypertension	11 (2.4)	12 (2.5)	17 (3.7)	0.280	0.171
hypotension	6 (1.3)	14 (2.9)	4 (0.9)	0.322	0.047

Symptoms of fluid overload (see also Table 5) were taken from the reports of adverse and serious adverse events of the investigative centers and included reports of heart failure, edema, fluid overload, fluid retention, hypervolemia, dyspnea, effusions, weight increase, and rales (see Concise Methods section). Anemia, hypertension, hypotension, hyperkalemia, and hypoglycemia were not defined but were also taken from the reports of the clinical investigators. Statistics: Cochran-Mantel-Haenszel test controlling for investigative center.

Table 5. Frequency of adverse events relating to fluid overload as reported by the clinical investigators on adverse event forms (not adjudicated)

Signs of Fluid Overload (*n* [%])	Avosentan 25 mg (*n* = 455)	Avosentan 50 mg (*n* = 478)	Placebo (*n* = 459)	*P*	
				Avosentan 25 mg *versus* Placebo	Avosentan 50 mg *versus* Placebo
Peripheral edema	78 (17.1)	80 (16.7)	77 (16.7)	0.706	0.822
Other edema	42 (9.2)	55 (11.5)	25 (5.4)	0.053	0.006
Fluid overload	28 (6.2)	26 (5.4)	5 (1.1)	<0.001	0.001
Dyspnea	31 (6.8)	34 (7.1)	15 (3.3)	0.052	0.197
Acute pulmonary edema	9 (2.0)	8 (1.7)	4 (0.9)	0.286	0.184
CHF	27 (5.9)	18 (3.8)	10 (2.2)	0.003	0.107

See also Table 2 for CHF as adjudicated secondary outcome, not included here. Symptoms of fluid overload were taken from the reports of adverse and serious adverse events of the investigative centers. The frequency of those symptoms was not different between participants below/above the median of baseline eGFR.

dosages of 25 to 50 mg, avosentan is less selective for the ET_A receptor and thus caused sodium and water retention and peripheral vasodilation with a potential fluid shift from the intravascular to extravascular space.[20,21] The assumption of ET_B receptor blockade with higher dosages of avosentan is further supported by data that showed a natriuretic effect of selective ET_A receptor blockade in people who were treated with ACEIs.[13] The ET_A receptor mediates vasoconstriction, cellular proliferation, and matrix deposition and also renal sodium retention, whereas the function of ET_B is mainly vasodilatory, depending on its anatomic location, and also mediates action on endothelin clearance and on renal sodium handling. The action of a given endothelin antagonist will depend on its selectivity and on the activation of ET_A *versus* ET_B receptors in a given clinical situation. In healthy individuals, only minor effects on fluid shifts were found at avosentan dosages of ≤5 mg/d.[21]

In long-term trials of patients who had advanced heart failure and received endothelin receptor antagonists (either $ET_{A/B}$ or ET_A), peripheral edema but not pulmonary edema was observed.[17–19] In some heart failure studies, such as Heart Failure ET_A Receptor Blockade Trial (HEAT), ET_A Receptor Antagonism Trial in Heart Failure (EARTH), Research on Endothelin Antagonism in Chronic Heart Failure (REACH-1), and Lowering Cardiac Events in Heart Failure (ENABLE), short-term hemodynamic benefit of endothelin antagonism was noted but no benefit on hard outcomes, and even more adverse events, including death, were reported.[18,19] It is important to note that patients who enrolled in ASCEND were prone to fluid retention by the very nature of their disease, and, indeed, edema was noted in approximately 15% of the participants at baseline physical examination, but participants with New York Heart Association stage III or IV CHF, the predominant population of the heart failure trials mentioned, were excluded.

Some endothelin receptor antagonists have also been associated with hepatic dysfunction and anemia. In our trial, no signal of hepatic toxicity was detected with monthly monitoring, but hemoglobin levels declined by approximately 10 g/L. This decline may be due to hemodilution. A reduction in hemoglobin has been reported with other endothelin receptor antagonists.[17]

The major limitation of this study was the short treatment

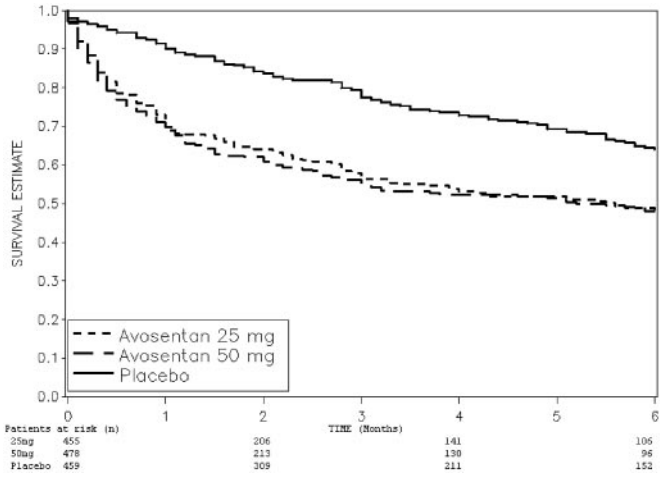

Figure 4. Fluid overload occurred in the avosentan-treated groups. Fluid overload was not defined by the trial protocol but taken from the adverse event reports of the local investigators. All participants were followed at monthly intervals and examined for adverse events. The individual signs and symptoms on the adverse event forms indicating fluid overload are detailed in the Concise Methods section.

exposure time, which precludes conclusions for the primary outcome. Despite its potent albuminuria-lowering effect, it is clear that avosentan at the doses of 25 and 50 mg/d in a population of patients with type 2 diabetes and 3 to 4 CKD stage is not a viable therapeutic option. The clear dosage dependence of the renal and hemodynamic effects of avosentan shown in other studies[7] raises the question as to whether lower dosages of avosentan would maintain the antialbuminuric effect, thereby affording a significantly more favorable risk-benefit ratio, especially at earlier stages of CKD and with more strict control of sodium balance.

CONCISE METHODS

Design Overview

ASCEND was an international, multicenter, randomized, double-blind study of people with type 2 diabetes and overt nephropathy that started in July 2005 and was prematurely discontinued in December 2006 on recommendation of the DSMB because of an excess of cardiovascular events. After a 2-week screening phase to check eligibility, a 42-month treatment phase was scheduled with three randomized treatments, either placebo or avosentan tablets at 25 or 50 mg once daily, as add-on to existing treatment. Tablets were identical in shape, color, and taste. All participants gave written informed consent. Approval from all local and central ethics committees and by regulatory authorities was obtained consistent with the principles of the Declaration of Helsinki.

Setting and Participants

In 551 clinical centers in 36 countries worldwide, men and women who had type 2 diabetes and overt nephropathy and were aged between 21 and 80 years were recruited. Clinical centers were selected, on the basis of their previous experience with clinical trials, by members of the steering committee and by Quintiles, the contract research organization involved. Diabetes had to be known for at least 3 years and had to be treated by oral antidiabetic drugs and/or insulin. Overt nephropathy was defined as urine ACR $\geq$35 mg/mmol ($\geq$309 mg/g) *and* a serum creatinine level between $\geq$115 and 265 mmol/L ($\geq$1.3 to 3.0 mg/dl) in men and between $\geq$106 and 265 mmol/L ($\geq$1.2 to 3.0 mg/dl) in women. Standard treatment for diabetic nephropathy had to include ACEIs or ARBs or their combination for at least 6 months before screening, but participants who were intolerant of ACEIs or ARBs were not excluded. Exclusion criteria were type 1 diabetes, proteinuria of nondiabetic origin, renal transplant, previous nephrectomy, eGFR $\leq$15 ml/min, sitting BP $\geq$160/100 mmHg with or without antihypertensive medication, or New York Heart Association stage III or IV CHF, glycosylated hemoglobin >12%, prolonged QT or QTc >500 ms with normal sinus rhythm; recent (60 days) history of acute myocardial infarction, unstable angina, stroke or transient ischemic attack, percutaneous transluminal coronary angioplasty, percutaneous coronary intervention, coronary artery bypass grafting, or any other major surgical intervention; history of life-threatening arrhythmias including those at high risk for QT/QTc prolongation such as a family history of long QT syndrome, severe hypokalemia, hepatitis B surface antigen or hepatitis C antibody positivity and abnormal liver function (specifically alanine aminotransferase/aspartate aminotransferase >1× upper limit of normal); treatment with spironolactone, eplerenone, or amiodarone; and women who were of child-bearing potential and not using adequate contraception.

Randomization and Interventions

Participants were randomly assigned 2 weeks after the screening visit to avosentan 25 or 50 mg/d or matching placebo on a 1:1:1 basis by an automated interactive voice recognition system in blocks of six. Treatment allocation was not known to patients or their physicians or anybody within or outside the study except a statistician who was not involved in the study and reported exclusively to the DSMB. During the study, a goal BP of <130/80 mmHg was recommended by the trial protocol. When BP was higher at any given visit, investigators were instructed to increase ACEIs or ARBs to maximal tolerated dosages, then to add diuretics followed by calcium antagonists or β blockers. Further recommended drugs were peripheral α blockers, central α agonists, and other agents according to local guidelines of antihypertensive therapy. Renal elimination of avosentan is <1%.

Outcomes and Measurements

The primary outcome was defined as the composite of time to doubling of serum creatinine, ESRD, or death. ESRD was defined as need for dialysis or renal transplantation or an eGFR <15 ml/min per 1.73 m^2. Both doubling of serum creatinine and eGFR $\leq$15 ml/min per 1.73 m^2 had to be confirmed by a second measurement within 4 $\pm$ 1 weeks. Secondary outcomes were changes in eGFR and in urine ACR and cardiovascular outcomes that were defined as the composite of coronary or peripheral vascular revascularization, amputations (except from trauma), nonfatal acute myocardial infarction, stroke, and CHF. For an event to be qualified as CHF, the patient had to have

typical signs and/or symptoms of heart failure *and* receive new therapy for CHF *and* be admitted to hospital for at least 24 hours. For myocardial infarction, two of three criteria had to be met (ischemic symptoms, typical electrocardiogram changes, and cardiac enzymes two-fold above upper limit of normal); for stroke, there had to be a focal neurologic deficit for >24 hours *and* a computed tomography or magnetic resonance scan was strongly recommended. Death was classified as cardiovascular or noncardiovascular. All primary and secondary outcomes, except for eGFR and ACR, were adjudicated by an independent clinical end point committee that was unaware of treatment allocation.

Because the trial was terminated early, all adverse event reports were examined for evidence of fluid overload. The following items in those reports were grouped as indicating fluid overload: Heart failure, edema, fluid overload, fluid retention, hypervolemia, dyspnea, pleural and pericardial effusions, ascites, weight increase, pulmonary rales, and pulmonary edema.

All laboratory parameters were measured centrally. Creatinine in blood and urine was measured by the Jaffe method and urine albumin by the Roche Tinaquant turbidimetric method on Roche Modular analyzers (Roche, Basel, Switzerland). For each urine measurement, first-void morning sample was collected on 3 consecutive days, and the geometric mean albumin concentration value was recorded in the database. From the serum creatinine concentration, eGFR was calculated using the six-variable Modification of Diet in Renal Disease (MDRD) formula that includes age, race, gender, serum albumin, and serum urea.[22,23]

Follow-up Procedures and Monitoring

Participants were followed up at monthly intervals. At each visit, sitting BP after 10 minutes of rest, body weight, and blood for liver function tests (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin) were taken, and adverse events were recorded. Electrocardiogram and serum creatinine and potassium were measured at baseline, at 1 and 3 months after randomization, and every 3 months thereafter. Urine ACR and glycosylated hemoglobin were measured at baseline and every 3 months thereafter.

Statistical Analysis

A sample size of 2364 patients and 747 primary outcomes were calculated to provide a 90% power at the 5% level (two-sided) to detect a 7% (25-mg dose) and 10% (50-mg dose) absolute reduction of the primary outcome compared with the placebo group, assuming a placebo cumulative incidence of 40% at 36 months for the primary outcome. This calculation incorporated two interim analyses of the DSMB, a 1% loss to follow-up, and a constant enrollment over 18 months. Because the study terminated early, most results are presented for the first 3 and 6 months of the trial, for which follow-up data were available for a substantial number of participants.

Continuous data are given as mean ± SD and categorical data as actual frequencies and percentages. The primary analysis used a time-to-event approach using the Kaplan-Meier method and included all randomly assigned participants who received at least one dose of randomized treatment and had at least one postbaseline visit (intention-to-treat population). Treatment comparisons with regard to time-to-event–related data (based on Cox regression of time to occurrence of first event) are displayed as hazard ratio with 95% confidence interval. All P values are two-sided and not adjusted for multiplicity. Treatment group comparisons for categorical data were performed using the Cochran-Mantel-Haenszel χ^2 test controlling for investigator center. Comparisons for continuous variables, eGFR, and ACR between treatment groups were analyzed by a repeated measures ANOVA, with the values at 3 and 6 months being the response variables and treatment group the effect variable, with baseline as a covariate. Urine ACRs were not normally distributed; therefore, median and interquartile range are reported. For statistical comparisons between groups, ACR was log-transformed.

ACKNOWLEDGMENTS

We thank the many trial coordinators, national coordinators, local investigators, and patients for support of ASCEND. Influence Medical Communication, UK, provided support with the tabulation and graphs but had no influence on the scientific content of this report. Three independent committees were appointed before initiation of the study to supervise the conduct of the study and undertake specific functions: The Steering Committee, the Data Safety and Monitoring Board, and the Clinical Endpoint Committee. The composition of these committees is reported in Supplemental Appendix 1.

DISCLOSURES

Speedel Pharma Ltd., Switzerland, sponsored the study and appointed the contract research organization, Quintiles Ltd., for study set-up, initiation, management, monitoring, laboratory analyses, data management, and statistical analysis. The contract research organization housed all blinded data during the treatment phase of the study and performed data analyses according to a prespecified statistical plan developed with and approved by the Steering Committee. The Steering Committee members had access to all data analyses, wrote the manuscript, and made the decision to publish, with no restrictions imposed by the sponsor.

All authors contributed to the design, conduct, analysis, and reporting of this study and approved the final draft. S.J.K. and T.L. were employees of the sponsor; all other authors report receiving honoraria for consultancy with the sponsor.

REFERENCES

1. Remuzzi G, Benigni A, Remuzzi A: Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest* 116: 288–296, 2006
2. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345: 861–869, 2001
3. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 345: 851–860, 2001
4. Jafar TH, Schmid CH, Landa M, Giatras I, Toto R, Remuzzi G: Angiotensin-converting enzyme inhibitors and progression of nondiabetic renal disease: A meta-analysis of patient-level data. *Ann Intern Med* 135: 73–87, 2001

5. Hocher B, Schwarz A, Reinbacher D, Jacobi J, Lun A, Priem F: Effects of endothelin receptor antagonists on the progression of diabetic nephropathy. *Nephron* 87: 161–169, 2001
6. Barton M: Reversal of proteinuric renal disease and the emerging role of endothelin. *Nat Clin Pract Nephrol* 4: 490–501, 2008
7. Dieterle W, Mann J, Kutz K: Multiple-dose pharmacokinetics, pharmacodynamics and tolerability of the oral ET(A) endothelin-receptor antagonist SPP301 in man. *Int J Clin Pharmacol Ther* 43: 178–186, 2005
8. Wenzel RR, Czyborra P, Luscher T, Philipp T: Endothelin in cardiovascular control: The role of endothelin antagonists. *Curr Hypertens Rep* 1: 79–87, 1999
9. Wenzel RR, Littke T, Kuranoff S, Juergens C, Bruck H, Ritz E, Philipp T, Mitchell A: The endothelin antagonist avosentan reduces albumin excretion rate in diabetic patients with macroalbuminuria: A randomized, placebo-controlled, double-blind trial. *J Am Soc Nephrol* 20: 655–664, 2009
10. de Zeeuw D, Remuzzi G, Parving HH, Keane WF, Zhang Z, Shahinfar S, Snapinn S, Cooper ME, Mitch WE, Brenner BM: Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: Lessons from RENAAL. *Kidney Int* 65: 2309–2320, 2004
11. Lea J, Greene T, Hebert L, Lipkowitz M, Massry S, Middleton J, Rostand SG, Miller E, Smith W, Bakris GL: The relationship between magnitude of proteinuria reduction and risk of end-stage renal disease: Results of the African American Study of Kidney Disease and Hypertension. *Arch Intern Med* 165: 947–953, 2005
12. Goddard J, Johnston NR, Hand MF, Cumming AD, Rabelink TJ, Rankin AJ, Webb DJ: Endothelin-A receptor antagonism reduces blood pressure and increases renal blood flow in hypertensive patients with chronic renal failure: A comparison of selective and combined endothelin receptor blockade. *Circulation* 109: 1186–1193, 2004
13. Goddard J, Eckhart C, Johnston NR, Cumming AD, Rankin AJ, Webb DJ: Endothelin A receptor antagonism and angiotensin-converting enzyme inhibition are synergistic *via* an endothelin B receptor-mediated and nitric oxide-dependent mechanism. *J Am Soc Nephrol* 15: 2601–2610, 2004
14. Pedersen EB, Thomsen IM, Fjordside LS: Effect of BQ-123, an endothelin antagonist, on renal hemodynamics, tubular function, vasoactive hormones, and blood pressure in healthy humans: A dose response study. *Am J Hypertens* 18: 1578–1585, 2005
15. Sarafidis PA, Khosla N, Bakris GL: Antihypertensive therapy in the presence of proteinuria. *Am J Kidney Dis* 49: 12–26, 2007
16. Weidmann P, Schneider M, Bohlen L: Therapeutic efficacy of different antihypertensive drugs in human diabetic nephropathy: An updated meta-analysis. *Nephrol Dial Transplant* 10[Suppl 9]: 39–45, 1995
17. Epstein BJ: Efficacy and safety of darusentan: A novel endothelin receptor antagonist. *Ann Pharmacother* 42: 1060–1069, 2008
18. Ertl G, Bauersachs J: Endothelin receptor antagonists in heart failure: Current status and future directions. *Drugs* 64: 1029–1040, 2004
19. Kelland NF, Webb DJ: Clinical trials of endothelin antagonists in heart failure: A question of dose? *Exp Biol Med (Maywood)* 231: 696–699, 2006
20. Maillard M, Wang Q, Baltatu O, Burnier M: Do endothelin receptor antagonists induce edema through extravasation of fluids? *J Hypertens* 26[Suppl 1]: 371–372, 2009
21. Smolander J, Vogt B, Maillard M, Littke T, Hengelage T, Burnier M: Dose-dependent acute and sustained renal effects of the endothelin receptor antagonist avosentan in healthy subjects. *Clin Pharmacol Ther* 85: 628–634, 2009
22. Stevens LA, Coresh J, Greene T, Levey AS: Assessing kidney function: Measured and estimated glomerular filtration rate. *N Engl J Med* 354: 2473–2483, 2006
23. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW: Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem* 53: 766–772, 2007

See related editorial, "Endothelin Receptor Antagonists in Proteinuric Renal Disease: Every Rose Has Its Thorn," on pages 392–394.

Supplemental information for this article is available online at http://www.jasn.org/.