Avosentan Reduces Albumin Excretion in Diabetics with Macroalbuminuria

René R. Wenzel,* Thomas Littke,[†] Susan Kuranoff,[†] Christiane Jürgens,[‡] Heike Bruck,[‡] Eberhard Ritz,[§] Thomas Philipp,[‡] and Anna Mitchell,[‡] for the SPP301 (Avosentan) Endothelin Antagonist Evaluation in Diabetic Nephropathy Study Investigators

*Department of Internal Medicine, A.Ö. Krankenhaus Zell am See, Academic Teaching Hospital of the Paracelsus University Salzburg, Zell am See, Austria; [†]SPEEDEL Pharma AG, Basel, Switzerland; and [‡]Department of Nephrology and Hypertension, University Hospital of Essen, Essen, and [§]Department of Nephrology, University of Heidelberg, Heidelberg, Germany

ABSTRACT

Despite the first-line use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), there is still a large need to improve the prevention and progression of diabetic nephropathy and its associated cardiovascular events. Endothelin antagonists have shown anti-inflammatory, antifibrotic, and antiproteinuric effects in experimental studies. This study was a randomized, placebo-controlled, double-blind, parallel-design, dosage-range study of the effect of the endothelin-A antagonist avosentan (SPP301) on urinary albumin excretion rate (UAER) in patients with diabetic nephropathy. We randomly assigned 286 patients with diabetic nephropathy, macroalbuminuria (UAER 0.2 to 5.6 mg/min), and BP <180/110 mmHg to 12 wk of avosentan (5, 10, 25, and 50 mg) or placebo, in addition to standard ACEI/ARB therapy. Relative to baseline, all avosentan dosages decreased mean relative UAER (−16.3 to −29.9%) compared with placebo (35.5%). Median relative UAER decreased with all avosentan dosages (−28.7 to −44.8%) compared with placebo (12.1%). Creatinine clearance and BP were unchanged at 12 wk. The main adverse events were peripheral edema (12%), mainly with high (≥25 mg) dosages of avosentan; significant increases in liver enzymes did not occur. Twenty-one (7.3%) patients experienced adverse events that led to withdrawal from study medication. In summary, the endothelin-A antagonist avosentan given in addition to standard ACEI/ARB treatment decreases UAER in patients with diabetic nephropathy and macroalbuminuria.

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In the United States and Europe, diabetic nephropathy (DN) is the leading cause of ESRD,[1] and the incidence of DN continues to rise. Persistent proteinuria is the hallmark of DN, a condition that is characterized by rise in BP, a deterioration of GFR, and a dramatic increase in cardiovascular events. The degree of albuminuria is closely related with the incidence of these events.[2,3] The initial stages of the disease involve subtle morphologic changes in the renal glomeruli, with progression to microalbuminuria, macroalbuminuria, and ultimately ESRD.[4] The awareness of the important role of proteinuria has improved, leading to a more aggressive therapy of hypertension and blockade of the renin-angiotensin system (RAS). Current first-line therapies include blockade of the RAS with angiotensin-converting enzyme inhibitors (ACEIs) and/or

Correspondence: Dr. René R. Wenzel, Department of Internal Medicine, Cardiology–Nephrology–Cardiovascular Research, A.Ö. Krankenhaus Zell am See, Teaching Hospital of the Paracelsus University Salzburg (Austria), Paracelsusstrasse 8, A-5700 Zell am See, Austria. Phone: +43-6542-777-2312; Fax: +43-6542-777-66; E-mail: rrwenzel@me.com

angiotensin receptor blockers (ARBs); these treatments reduce proteinuria and delay time to ESRD in type 1 and type 2 DN; importantly, reduction in proteinuria is associated with an improved cardiovascular outcome in patients with DN.[2,3,5–9] Recently, a newly developed renin inhibitor (aliskiren) was shown to have an additional effect on proteinuria in DN[10]; however, there is still a large need to improve prevention of DN and reduce its progression to ESRD and associated cardiovascular events.[9,10]

The endothelin system regulates a number of renal functions and can induce proteinuria by various mechanisms.[11–15] Plasma and urinary endothelin-1 (ET-1) levels are elevated in patients with diabetes and correlate with reduced renal function, increased BP and albuminuria,[16] and severity and duration of diabetes.[17] Endothelin receptor antagonists (ERAs) have demonstrated renoprotective effects in experimental models of diabetic and nondiabetic nephropathy,[18–20] independent of their effects on BP, as well as in a preliminary clinical trial.[21] Antifibrotic effects of ERAs in experimental disease that reduce proteinuria, renal fibrosis, and survival are mainly ET_A receptor mediated.[22,23] Macrophage infiltration in renal tissue and urinary TGF-β and prostaglandin E2 metabolites can be reduced using an ET_A-selective antagonist, an effect that is associated with a reduction in albuminuria in rats with streptozotocin-induced diabetes. This indicates that the activation of renal ET_A receptors mediates renal inflammation and TGF-β production in diabetes.[24,25]

Avosentan (SPP301) is a new, once-daily, orally available ET_A antagonist in clinical development for the treatment of DN.[26] In this study, we investigated the effects of 12 wk of treatment with avosentan on urinary albumin excretion rate (UAER) as an indicator of progression of DN.

RESULTS

Of 501 patients screened, 286 underwent randomization to receive treatment (Figure 1). Demographic and baseline characteristics were similar across treatment groups (Table 1). Six of 252 patients were not pretreated with an ACEI or an ARB. Concomitant antihypertensive and antidiabetic therapy was similar in all treatment groups (Table 2). Changes in co-medication (antihypertensives, diuretics, statins) during the trial where performed only in a minority of patients; there were no statistically significant differences regarding the changes in comedication (see Supplemental Appendix 5).

Primary Efficacy Parameter
Mean UAER levels at baseline ranged from 0.79 ± 0.79 mg/min in the avosentan 10-mg group to 1.21 ± 1.43 mg/min in the avosentan 50-mg group (Table 3). Median UAER levels at baseline were 0.49 to 0.78 mg/min and were similar across all treatment groups (Table 2). *Versus* placebo, the mean absolute change in UAER from baseline to week 12 was significant in each
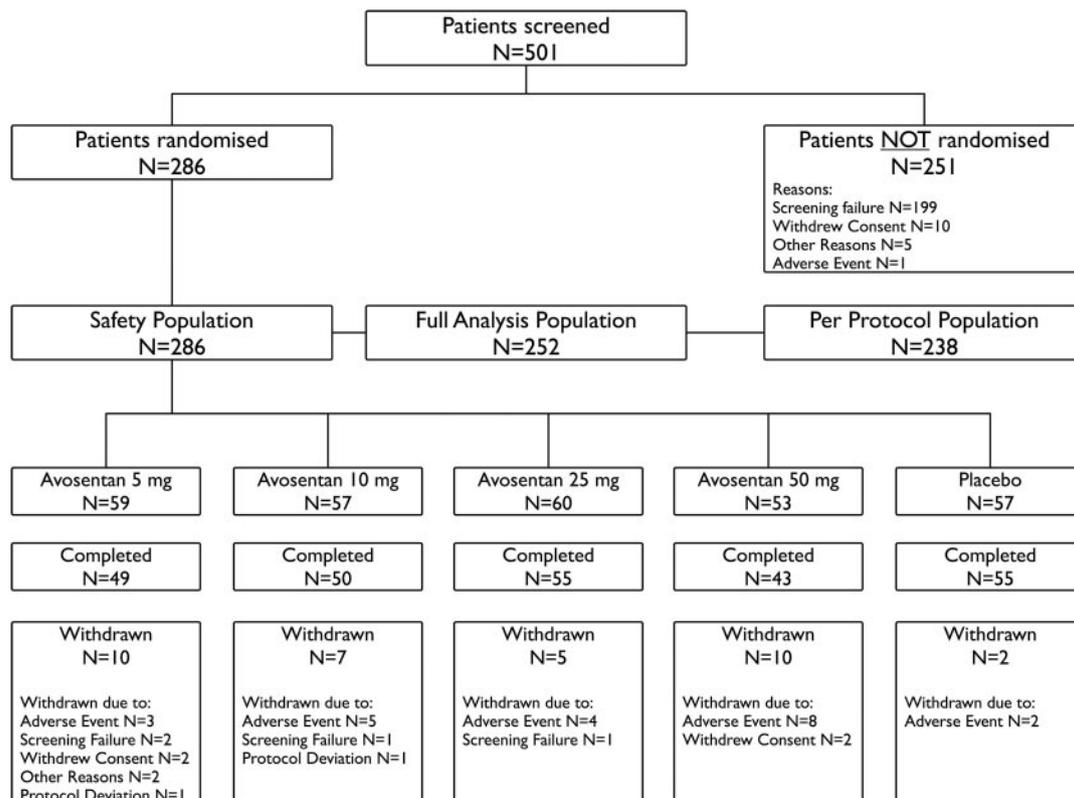

Figure 1. Flow of participants through each stage.

Table 1. Demographics and baseline characteristics of the full analysis population (n = 252)

Parameter	Avosentan (mg)				Placebo (n = 55)	P^b
	5 (n = 49)	10 (n = 50)	25 (n = 55)	50 (n = 43)		
Age (yr; mean ± SD)	60.0 ± 11.0	58.7 ± 9.0	60.8 ± 10.0	58.4 ± 10.0	58.4 ± 10.0	0.66
Weight (kg; mean ± SD)	92.4 ± 18.0	93.5 ± 15.0	88.6 ± 16.0	92.8 ± 16.0	91.2 ± 17.0	0.59
BMI (kg/m²; mean ± SD)^a	31.8 ± 6.0	32.2 ± 5.0	31.3 ± 7.0	32.2 ± 5.0	30.5 ± 5.0	0.54
SBP (mmHg; mean ± SD)	146.0 ± 13.0	147.0 ± 14.0	140.0 ± 19.0	146.0 ± 17.0	144.0 ± 16.0	0.60
DBP (mmHg; mean ± SD)	84.0 ± 7.0	83.0 ± 10.0	83.0 ± 9.0	82.0 ± 8.0	81.0 ± 8.0	0.49
Creatinine clearance (ml/min; mean ± SD)	82.0 ± 30.0	81.0 ± 29.0	75.0 ± 30.0	84.0 ± 26.0	79.0 ± 29.0	0.52
Men (n [%])	36 (74.0)	37 (74.0)	36 (66.0)	30 (70.0)	48 (87.0)	0.08
Age subgroup ≥65 yr (n [%])	21 (43.0)	16 (32.0)	24 (44.0)	15 (35.0)	13 (24.0)	0.16
Type 2 diabetes (n [%])	44 (90.0)	46 (92.0)	46 (84.0)	36 (84.0)	49 (89.0)	0.62
ACEI only (n [%])^c	44 (90.0)	42 (84.0)	50 (91.0)	40 (93.0)	47 (86.0)	0.59
ARB only (n [%])^c	2 (4.1)	5 (10.0)	3 (5.5)	2 (4.7)	5 (9.1)	0.68
ACEI plus ARB (n [%])	1 (2.0)	3 (6.0)	1 (1.8)	0 (0.0)	1 (1.8)	0.37
Neither ACEI nor ARB (n [%])	2 (4.1)	0 (0.0)	1 (1.8)	1 (2.3)	2 (3.6)	0.50
Statins (n [%])	14 (29.0)	12 (24.0)	14 (26.0)	10 (23.0)	24 (44.0)	0.13
Oral antihyperglycemic therapy (n [%])	26 (53.0)	27 (54.0)	25 (46.0)	23 (54.0)	25 (46.0)	0.80
Insulin therapy (n [%])	34 (69.0)	39 (78.0)	41 (75.0)	33 (77.0)	36 (66.0)	0.58

^{a}BMI, body mass index.
^{b}Global test over all five treatment groups, likelihood ratio χ^2 (G test): H_0, the proportion of patients is equal in each treatment group; H_1, in at least one treatment group, the proportion of patients is different.
^{c}A dose of ACEI or ARB was considered high on the basis of a selection of dosage levels for individual drugs on the basis of large clinical studies and/or official recommendations for their effect on controlling proteinuria guidelines.[33]

Table 2. Concomitant antihypertensive and antidiabetic therapy (n = 252)

Antihypertensive and Antidiabetic Therapy (n [%])^a	Avosentan (mg)				Placebo (n = 55)	P^b
	5 (n = 49)	10 (n = 50)	25 (n = 55)	50 (n = 43)		
ACEI	40 (82.0)	36 (72.0)	43 (78.0)	32 (74.0)	43 (78.0)	NS
ARB	3 (6.1)	4 (8.0)	4 (7.3)	0 (0.0)	5 (9.1)	NS
alpha-1 Blockers	3 (6.1)	6 (12.0)	7 (13.0)	7 (16.0)	11 (20.0)	NS
β Blockers	10 (20.0)	18 (36.0)	18 (33.0)	16 (37.0)	17 (31.0)	NS
Diuretics	17 (35.0)	18 (36.0)	27 (49.0)	22 (51.0)	26 (47.0)	NS
Calcium channel blocker	16 (33.0)	16 (32.0)	21 (38.0)	21 (49.0)	20 (36.0)	NS
Insulin therapy	34 (69.0)	39 (78.0)	41 (75.0)	33 (77.0)	36 (65.0)	NS
Oral antidiabetic therapy	26 (53.0)	27 (54.0)	25 (46.0)	23 (54.0)	25 (46.0)	NS
Biguanides	19 (39.0)	15 (20.0)	20 (36.0)	15 (35.0)	17 (31.0)	NS
Sulfonylureas	13 (27.0)	14 (28.0)	10 (18.0)	8 (19.0)	13 (24.0)	NS
Thiazolidinediones	2 (4.0)	1 (2.0)	0 (0.0)	0 (0.0)	1 (2.0)	NS
α-Glucosidase inhibitors	1 (2.0)	3 (6.0)	5 (9.0)	3 (7.0)	3 (6.0)	NS
Meglitinides	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	1 (2.0)	NS

^{a}The number of patients are not additive because some patients received a combination of more than one antihypertensive or antidiabetic treatment.
^{b}Global test over all five treatment groups, likelihood ratio χ^2 (G test): H_0, the proportion of patients is equal in each treatment group; H_1, in at least one treatment group, the proportion of patients is different.

avosentan dosage group, with an apparent dosage response (Table 2). Data for the mean relative change data were similar: UAER decreased significantly with avosentan 5, 10, 25, and 50 mg, respectively (−20.9, −16.3, −25.0, and −29.9%) but increased with placebo (35.5%; $P < 0.01$ for all dosages; Figure 2A, Table 3).

There were different findings when the UAER data were analyzed using median values. The median absolute decreases in UAER with avosentan were similar for the 5- and 10-mg dosage groups (−0.15 mg/min) and for the 25- and 50-mg dosage groups (−0.21 mg/min). In contrast, there was a median absolute increase of 0.05 mg/min in the placebo group

(Table 3). Avosentan 5, 10, 25, and 50 mg decreased median relative UAER levels by −28.7, −42.2, −44.8, and −40.2%, respectively, *versus* a 12.1% increase with placebo (Figure 2, Table 3). For the median relative changes, a flat dosage-response curve was observed, and all dosages of avosentan, except the 5-mg dose, demonstrated similar efficacy. The proportion of patients who experienced ≥30% relative reduction in median UAER was 46.9, 58.0, 56.4, and 60.5% for 5, 10, 25, and 50 mg of avosentan, respectively, compared with 23.6% for placebo. In a *post hoc* analysis adjusting changes of UAER for systolic (SBP) and diastolic BP (DBP), there was no corre-

Table 3. Effects of avosentan and placebo on mean and median absolute and relative UAER levels in the full analysis population ($n = 252$)

Parameter	Avosentan 5 mg		Avosentan 10 mg		Avosentan 25 mg		Avosentan 50 mg		Placebo	
	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12
UAER (mg/d)[d]	1598.00 ± 1495.00	1278.00 ± 1474.00[c]	1135.00 ± 1132.00	983.00 ± 1312.00[c]	1354.00 ± 985.00	912.00 ± 925.00[d]	1743.00 ± 2055.00	1006.00 ± 1098.00[d]	1560.00 ± 1726.00	1797.00 ± 2028.00
UAER (mg/min)[d]	1.11 ± 1.04	0.89 ± 1.02[c]	0.79 ± 0.79	0.68 ± 0.91[c]	0.94 ± 0.68	0.63 ± 0.64[e]	1.21 ± 1.43	0.70 ± 0.76[d]	1.08 ± 1.20	1.25 ± 1.41
Absolute UAER (mg/min)[d,e]		−0.22 ± 0.61		−0.11 ± 0.55		−0.31 ± 0.62		−0.51 ± 1.24		0.16 ± 0.72
Relative UAER (%)[d,e]		−20.90 ± 49.70		−16.30 ± 65.30		−25.00 ± 62.30		−29.90 ± 45.70		35.50 ± 107.28
UAER (mg/min; median [IQR])[a]	0.64 (0.36 to 1.48)		0.49 (0.28 to 0.90)		0.69 (0.41 to 1.37)		0.78 (0.31 to 1.50)		0.64 (0.34 to 1.41)	
Absolute UAER (mg/min; median [IQR])[b,e]		−0.15 (−0.36 to −0.03)[e]		−0.15 (−0.33 to 0.01)[e]		−0.21 (−0.59 to −0.02)[d]		−0.21 (−0.82 to −0.02)[e]		0.05 (−0.17 to −0.46)
Relative UAER (%; median [IQR])[a,e]		−28.70 (−50.80 to −7.60)		−42.20 (−55.30 to 1.20)		−44.80 (−69.90 to −6.60)		−40.20 (−61.90 to −7.80)		12.10 (−21.90 to −49.60)

[a]IQR, interquartile range.
[b]P values for the median relative change from baseline are interpreted from the log-transformed mean changes (analysis of covariance [ANCOVA]).
[c]$P < 0.01$, [d]$P < 0.0001$, [e]$P < 0.001$ for the mean absolute change from baseline to week 12 versus placebo (ANCOVA).
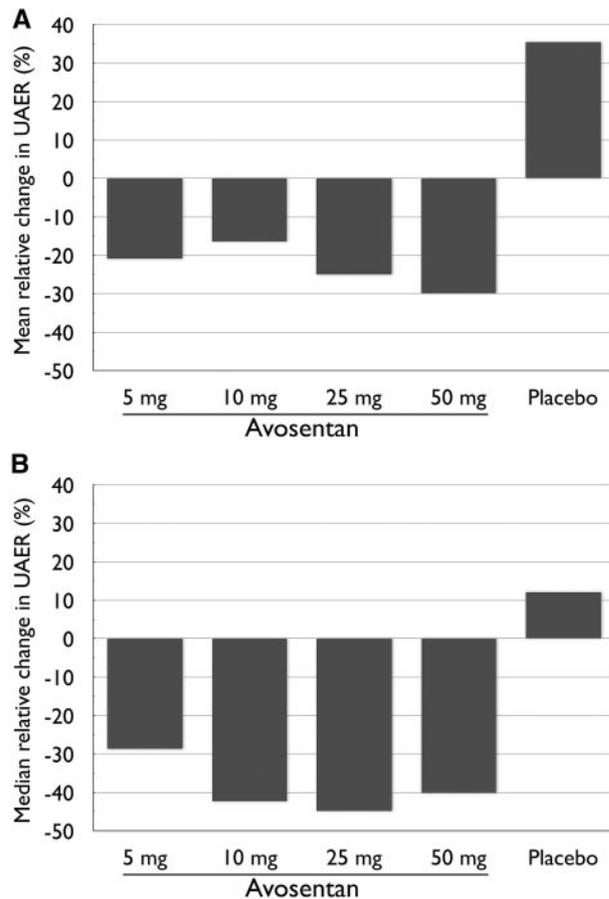[d]Mean ± SD.
[e]Change from baseline.

Figure 2. (A and B) Effect of avosentan (5, 10, 25, or 50 mg) and placebo on mean (A) and median (B) relative change in UAER from baseline to week 12 of treatment given in addition to standard renin angiotensin aldosterone system blockade (with an ACEI and/or an ARB) in the full analysis population (n = 252).

lation of BP with the avosentan-induced reduction in macroalbuminuria (NS). Analysis of the median relative change in UAER from baseline to week 12 in the safety population and per protocol population showed similar results to those reported for the full analysis population (see Supplemental Appendix 4).

Secondary Efficacy Parameters

Mean urinary protein excretion rate (UPER) decreased by 0.2 mg/min (10 mg of avosentan; Table 4) to 0.8 mg/min (50 mg of avosentan; Table 4), corresponding to a decrease of 0.3 to 1.2 g/d compared with an increase of 0.2 mg/min (0.3 g/d) after placebo ($P < 0.01$ to 0.001 *versus* placebo). Total cholesterol was similar at baseline in all groups (no significant differences) and decreased significantly by 5 to 17 mg/dl with avosentan, irrespective of statin use. Total cholesterol was increased in the placebo group (Table 4). Plasma triglycerides decreased with active treatment, whereas the placebo group displayed a NS increase (Table 4). For all other secondary parameters, including SBP and DBP, glycosylated hemoglobin (HbA_{1c}), and body weight, mean values were similar across all treatment groups and remained unchanged with avosentan or placebo (Table 4).

Safety Parameters

A mild decrease in erythrocytes was observed from baseline to week 12 (active treatment −0.2/picoliter; placebo ± 0/pl) and in hemoglobin (−0.6 *versus* 0 g/dl; Table 4). In 86% of patients receiving avosentan (*versus* 95% placebo), the decrease in hemoglobin was <2 g/dl. These changes were not deemed clinically relevant by the treating physicians and did not necessitate any hospitalizations or blood transfusions.

Overall, liver enzymes remained stable during avosentan treatment (Table 4). With the exception of one patient, all increases in aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were less than three times the upper limit of normal (ULN), were asymptomatic, were not accompanied by increases in bilirubin, and did not differ in frequency from those observed in the placebo group. The proportion of patients with mild increases in ASAT (less than three times the ULN) was highest in the placebo group and lowest in the 50-mg group. The proportion of patients with mild increases in ALAT (less than three times the ULN) was similar in the total active treatment groups compared with the placebo group, with the 50-mg group again displaying the smallest number of patients with increases. One patient reported a transient and asymptomatic increase in ALAT of more than eight times the ULN in the 10-mg group.

Adverse Events

In the safety population (n = 286), 161 (56.3%) patients reported adverse events (AEs) during the study (Table 5), most (87%) of which were mild or moderate in severity. Severe AEs were reported in 8 and 5% of patients taking avosentan and placebo, respectively; most were considered unrelated to treatment. With the exception of 50 mg of avosentan, the proportion of patients who experienced AEs was higher in the placebo group than in all other avosentan dosage groups (Table 5). The most common AEs were edema, abnormal electrocardiogram, anemia, and headache (Table 5).

Five deaths occurred during the study period: One in each of the four avosentan groups and one in the placebo group. Causes of death were subdural hematoma, myocardial infarction (n = 2), congestive heart failure, and gastric bleeding. One death (myocardial infarction) was considered possibly related to the study treatment (the rest were considered unlikely related to the study treatment).

Post hoc analysis of fluid retention episodes showed a dos-

Table 4. Effects of avosentan and placebo on secondary parameters and selected safety end points in the full analysis population (n = 252) or safety population (n = 286)[a]

Parameter	Placebo		Avosentan 5 mg		Avosentan 10 mg		Avosentan 25 mg		Avosentan 50 mg	
	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12
UPER (mg/min)	1.55 ± 1.60	1.74 ± 1.40	1.69 ± 1.40	1.27 ± 1.40	1.25 ± 1.40	1.05 ± 1.30[c]	1.42 ± 1.10	0.90 ± 0.80[d]	1.74 ± 2.00	0.93 ± 0.80[d]
Heart rate (bpm)[b]	75.4 ± 12.0	73.0 ± 12.0	75.0 ± 14.0	75.1 ± 11.0	71.8 ± 12.0	73.2 ± 14.0	76.1 ± 13.0	76.0 ± 12.0	74.5 ± 11.0	76.7 ± 13.0
Systolic BP (mmHg)	144 ± 16	147 ± 22	146 ± 13	142 ± 17	147 ± 14	144 ± 17	140 ± 19	141 ± 18	146 ± 17	144 ± 18
Diastolic BP (mmHg)	81 ± 8	80 ± 10	84 ± 7	82 ± 6	83 ± 10	83 ± 9	83 ± 9	80 ± 8	82 ± 8	80 ± 10
Cholesterol (mg/dl)	221 ± 43	230 ± 52	227 ± 58	210 ± 56[e]	217 ± 47	212 ± 49[e]	230 ± 45	213 ± 46[e]	223 ± 49	209 ± 36[e]
Triglyceride (mg/dl)[b]	199 ± 103	230 ± 147	214 ± 153	178 ± 152	225 ± 137	226 ± 183	231 ± 127	197 ± 124	245 ± 235	235 ± 188
HbA$_{1c}$ (%)	7.3 ± 1.4	7.5 ± 1.5	7.7 ± 1.2	7.6 ± 1.1	7.5 ± 1.3	7.5 ± 1.4	7.8 ± 1.3	7.4 ± 1.2	7.9 ± 1.8	7.8 ± 1.6
Creatinine (mg/dl)	1.2 ± 0.5	1.3 ± 0.5	1.1 ± 0.4	1.2 ± 0.5	1.2 ± 0.6	1.2 ± 0.5	1.2 ± 0.5	1.3 ± 0.6	1.1 ± 0.5	1.2 ± 0.5
Creatinine clearance (ml/min)	79 ± 29	78 ± 31	82 ± 30	81 ± 31	81 ± 29	80 ± 28	75 ± 30	75 ± 31	84 ± 26	80 ± 25
Hemoglobin (g/dl)[b]	13.8 ± 1.6	13.7 ± 1.5	13.9 ± 1.4	13.2 ± 1.5	13.6 ± 1.5	13.3 ± 1.5	13.5 ± 1.4	12.7 ± 1.3	13.4 ± 1.5	13.0 ± 1.7
Body weight (kg)[b]	92.2 ± 18.1	91.8 ± 17.0	91.4 ± 17.2	93.2 ± 18.3	93.3 ± 14.7	94.2 ± 14.5	89.3 ± 17.1	89.1 ± 16.7	91.0 ± 17.4	92.8 ± 18.6
ALAT (ULN)[b]	22 ± 10	23 ± 11	24 ± 11	25 ± 13	22 ± 10	22 ± 8	21 ± 10	21 ± 7	21 ± 8	20 ± 10
ASAT (ULN)[b]	22 ± 8	22 ± 8	22 ± 8	22 ± 9	21 ± 7	20 ± 6	20 ± 6	19 ± 5	20 ± 5	20 ± 5

[a]Data are means ± SD.
[b]Safety population.
[c]P < 0.01, [d]P < 0.0001, [e]P < 0.001, [f]P < 0.05 for the absolute change from baseline to week 12 versus placebo (ANCOVA).

age-dependent increase in incidence (11.9, 21.1, 15.0, and 32.1% in the 5-, 10-, 25-, and 50-mg groups, respectively) compared with placebo (3.5%). In the *post hoc* analysis, the proportion of patients who withdrew consent or did not complete the study as a result of AEs was dosage dependent (18.9% in the 50-mg group and 5.1% in the 5-mg group *versus* 3.5% in the placebo group).

DISCUSSION

This study is the first to demonstrate that the administration of the ET$_A$ antagonist avosentan with standard treatment for 12 wk significantly reduces UAER in patients with diabetes. A preceding exploratory study revealed that both 20- and 50-mg dosages of avosentan were effective in lowering proteinuria in patients with DN.[21] Because avosentan was considered to be well tolerated at both dosages, 50 mg was chosen as the upper dosage level.

Administration of avosentan in addition to standard therapy at 5, 10, 20, or 50 mg, once daily, significantly reduced mean UAER at week 12 *versus* baseline in a dosage-dependent manner; in contrast, an increase in UAER was observed with placebo. Importantly, macroalbuminuria decreased by up to 0.7 g/d, an effect that is marked and regarded as clinically relevant. Because distribution of albuminuria was not normal, median UAERs were analyzed and showed slightly different results. There was a flat dosage-dependent effect of avosentan with no benefit observed beyond the 25-mg dosage. Of all of the avosentan dosage groups, the 10-mg group had a lower absolute mean and median change in UAER between baseline and 12 wk. The decrease in UAER (mg/min) between baseline and week 12 was ≥30% in all four avosentan treatment groups and considered to be a clinically significant reduction.

This study enrolled patients with significant renal disease (macroalbuminuria). In two large-scale trials of patients with diabetes and microalbuminuria (Renal Insufficiency and Anticancer Medications II [IRMA II] and MicroAlbuminuria Reduction with VALsartan in patients with type 2 diabetes [MARVAL]),[8,27] ARBs demonstrated albuminuria-lowering effects. Thus, in this study, we examined the effects of ET system blockade in addition to standard stable care including ACEIs and/or ARBs in patients with macroalbuminuria. It is not known whether avosentan would have the same antialbuminuric effects in patients with microalbuminuria.

Analysis of secondary efficacy parameters revealed that total cholesterol and UPER were significantly decreased compared with placebo in all avosentan groups. These effects were anticipated because of the correlation of UPER and UAER; however, further investigation is required to determine the mechanism of action. Similar cholesterol-lowering effects have been observed with ACE inhibition.[28]

The beneficial effects of ACEIs and ARBs in the treatment of proteinuria and renal disease are well established, and these agents are regarded as standard treatments for patients with

Table 5. Overview of AEs, serious AEs, and treatment-emergent AEs in the safety population (n = 286)[a]

AE	Avosentan (mg)				Placebo (n = 57)	P[b]
	5 (n = 59)	10 (n = 57)	25 (n = 60)	50 (n = 53)		
Any AE	29 (49.2)	32 (56.1)	33 (55.0)	33 (62.3)	34 (59.6)	0.68
Treatment-emergent AEs	15 (25.4)	13 (22.8)	11 (18.3)	22 (41.5)	12 (35.3)	0.06
Serious AEs	4 (6.8)	7 (12.3)	5 (8.3)	7 (13.2)	3 (5.3)	0.52
Treatment-emergent serious AEs	2 (3.4)	1 (1.8)	1 (1.7)	2 (3.8)	0 (0.0)	0.48
Most commonly reported AEs[c]						
edema[d]	5 (8.5)	8 (14.0)	6 (10.0)	14 (26.4)	2 (3.5)	0.01
abnormal electrocardiogram[e]	6 (10.2)	2 (3.5)	2 (3.3)	3 (5.7)	4 (7.0)	0.52
anemia	2 (3.4)	4 (7.0)	2 (3.3)	4 (7.5)	2 (3.5)	0.71
headache	2 (3.4)	1 (1.8)	3 (5.0)	5 (9.4)	1 (1.8)	0.28

[a]Data are number of AEs (percentage of patients).
[b]Global test over all five treatment groups, likelihoodratio χ^2 (G test): H_0, the proportion of patients is equal in each treatment group; H_1, inat least one treatment group, the proportion of patients is different.
[c]Reported in ≥10 patients across all treatment groups.
[d]Including pulmonary not otherwise specified, eyelid, peripheral, aggravated, anasarca, pitting, fluid retention, and periorbital.
[e]Rhythm disorder, PQ-time prolongation, impaired repolarization, ST-segment elevation, Q-wave.

nephropathy and/or hypertension.[2,3,7,8] In this study, the observation of an additional antiproteinuria effect with avosentan after RAS blockade suggests an additional renoprotective effect in DN, although BP control was not part of the design of this study. The addition of avosentan to standard renoprotective antihypertensive therapy may provide a complementary benefit to treat progressive DN.

In this study, there was no statistically significant difference in SBP or DBP across groups. This is mainly because hypertension was corrected with other antihypertensive agents when necessary. Relatively small sample sizes and short duration of the study make it difficult to evaluate the exact effects of avosentan on BP. Whereas some studies suggested that ET antagonists are renoprotective as a result of antihypertensive properties, others suggested that renoprotection is unrelated to BP. The conflicting findings could be attributed to several variables, including different animal models, selectivity/dosage of ERA, duration of renal disease, time of drug treatment initiation, baseline BP, and the severity of DN. In this study, avosentan's antiproteinuric effect seems to be unrelated to its effects on BP. The precise role of ERAs and BP reduction is uncertain and warrants further investigation; however, the documented anti-inflammatory effects of ETA-selective antagonists in experimental studies hint toward important BP-independent effects.[25]

Avosentan led to mild but significant reductions in total cholesterol levels. Interestingly, it has been demonstrated that reduction in cholesterol improves macroalbuminuria.[29,30] Thus, avosentan may well reduce proteinuria, in part, indirectly *via* its cholesterol-lowering effects. Because proteinuria is positively correlated with severity of renal disease, avosentan may therefore have renoprotective properties.

Overall, all avosentan dosages were well tolerated, and AEs during the 12-wk treatment period were generally minor. Approximately 25% of patients reported AEs that were consid-

ered possibly related to study medication. Five deaths occurred in the safety population, but only one was considered possibly related to the study medication. It should be emphasized, however, that the observed mortality rate is consistent with that generally seen in patients with DN.[31]

Liver abnormalities have been reported with ERAs. This study demonstrated that the frequency of increased liver enzymes with avosentan is very low (0.4%), mild, and not considered clinically relevant. Another undesirable effect associated with ET receptor blockade is anemia, possibly as a result of hemodilution. A decrease in the mean values from baseline to week 12 in hematocrit and hemoglobin was observed with avosentan, which were not dosage dependent. These changes may be attributed to DN-associated decreases in hemoglobin. In addition, ACEIs and ARBs can suppress erythropoiesis and thereby may exacerbate anemia[32]; however, an effect of ERA on worsening of anemia, especially in patients with impaired left ventricular function, has been described.

This study has several limitations. The protocol did not impose BP control beyond that deemed necessary by the treating physician. Although similar across all treatment groups, BP levels were beyond the standard recommended target of 130/80 mmHg,[33] and detailed information about the up-titration of antihypertensive therapy is lacking; however, baseline and final BP values were similar in all treatment groups, and our *post hoc* analysis showed that effects of avosentan on macroalbuminuria did not correlate with BP (see the Results section), indicating that the effects of the compound are likely to be independent from BP reduction. Furthermore, there were only a few changes in concomitant antihypertensive and diuretic medication without any statistically significant differences in the treatment groups (see Supplemental Appendix 5).

Furthermore, we do not have data on urinary sodium and urea excretion as a marker of salt and protein intake, which may have influenced proteinuria. Thus, we may have missed

additional effects on proteinuria independent from the avosentan treatment.[34–37]

Here we report for the first time data demonstrating an albumin-lowering effect of the ET receptor antagonist avosentan when given in addition to renin-angiotensin-aldosterone system blockade in DN. The incidence of AEs, mainly edema, was significantly elevated, especially with high dosages of avosentan (50 mg). As relative median UAER values show, there seems to be no additional antiproteinuric effect with dosages of avosentan above 25 mg; thus, the optimal dosage in terms of risk-benefit ratio may be defined at ≤10 mg.

A large outcome trial is mandatory to confirm these findings and to determine whether avosentan's antiproteinuric effects can be translated into long-term benefits also with lower dosages of avosentan, which are likely to have an optimal tolerability. Nevertheless, the marked and significant reduction in macroalbuminuria after 12 wk of avosentan treatment suggests a clinically valuable nephroprotective effect.

CONCISE METHODS

Study Design and Protocol

This randomized, double-blind, placebo-controlled, dosage-range, parallel-group study was conducted in 58 European centers (21 in Germany, 13 in Poland, 17 in Hungary, and seven in Slovakia). Recruitment was performed between April 2003 and March 2004. Apart from sites in Germany, where approximately half of the sites were private practices, most of the sites selected were endocrinology or diabetology units located within a hospital setting. From 501 patients screened, 286 were randomly assigned (safety population) and 252 fulfilled the criteria for the full analysis population (Figure 1). Total study duration was 14 wk; during this period, seven visits (weeks −1, 0, 2, 4, 8, 12, and 13) occurred. At each visit, efficacy parameters, vital signs, biochemistry, hematology, body weight, and AEs were obtained according to the schedule (see Supplemental Appendix 3). After a 1-wk screening phase, eligible patients were randomly assigned (Quintiles, Edinburgh, UK), using permuted blocks of five patients to treatment with avosentan 5, 10, 25, or 50 mg or placebo taken as a tablet once daily for 12 wk. A follow-up visit occurred 1 wk later. Patients took their first dose of either placebo or avosentan under the supervision of the investigator, and subsequent doses were taken at the same time each day.

Participants

Male and female patients who had type 1 or type 2 diabetes and DN and were aged 18 to 75 yr were recruited. Eligible patients had a UAER at screening of ≥0.2 and ≤5.6 mg/min and were on standard stable treatment for DN (including ACEI and/or ARB) for at least 4 wk before randomization. Female patients were postmenopausal for >1 yr or surgically sterile.

Exclusion criteria were serum creatinine level >3 mg/dl and/or creatinine clearance <30 ml/min, hemoglobin <10 g/dl, ferritin >50 μg/dl, HbA$_{1c}$ ≤5.8%, BP ≥180/110 mmHg, and ASAT and/or ALAT levels more than three times the ULN. To maintain BP <140/90

mmHg, antihypertensive treatment with diuretics, calcium channel blockers (amlodipine), and α blockers was permitted (Table 2). Glycemia was controlled using oral antidiabetic drugs and/or insulin (Table 2; see Supplemental Appendix 1 for a full list of exclusion criteria).

The study protocol was approved by independent ethics committees and was conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki. All patients gave written informed consent before study initiation.

Efficacy Parameters

The primary efficacy parameter was the absolute change in 12-h UAER between baseline and week 12. Secondary parameters were UPER, serum creatinine concentration, creatinine clearance, HbA$_{1c}$, SBP and DBP, and total cholesterol.

Assessment of UAE

UAE was determined in a central laboratory by immunoturbidimetric measurement, and urinary protein content was assessed by colorimetric (pyrogallol red) testing.[38,39] Efficacy was assessed by the change in 12-h UAER between baseline and week 12. Patients were required to collect two separate 12-h timed overnight urine samples from two consecutive overnight periods before the baseline visit (week 0) and visits at weeks 4 and 12. The mean UAER of the two overnight samples (per patient) was used to calculate the group means and medians. UAERs (mg/d) were extrapolated from the UAER (mg/min) values.

Safety Evaluation

Safety and tolerability end points included effects on plasma lipids (total cholesterol, triglycerides), HbA$_{1c}$, and renal function (creatinine clearance). Safety parameters included vital signs (heart rate, BP), hemoglobin, liver enzymes (ALAT, ASAT) body weight, and incidence of AEs. BP was measured with a conventional sphygmomanometer using Korotkoff phases I and V while the patient was sitting calmly and without being disturbed. Medical history was collected at baseline. Assessment of vital signs, blood and biochemical testing, and AE incidence was carried out at baseline; after 2, 4, 8, and 12 wk of treatment; and at follow-up. Electrocardiograms were performed at baseline and after 12 wk of treatment.

Statistical Analysis

Patient sample size was determined on the basis of the design of a previous explorative study in patients with DN, in which the primary efficacy end point was change from baseline in UPER.[21] The study was powered to assume a 30% reduction of proteinuria between treatment arms and placebo and not intraindividually on the basis of the results of our pilot trial.[21] In this study, the SD of the change of logarithmic values was observed to be 0.4 g/d. Assuming a one-sided test with level of 0.025, a reduction of 30% can be shown *versus* a 10% worsening in the placebo group with 245 patients and a corresponding power of 80% when using a simple *t* test. Because we planned overall five groups, an estimated total of approximately 250 patients needed to be randomly assigned to obtain 225 assessable patients for the final analysis. If any of the dosage groups yielded a 30% change under these assumptions, then the power would be 80% to detect this change if no adjustment for a multiple testing procedure were performed.

The predefined analysis of absolute and relative change in UAER from baseline to week 12 was initially performed using arithmetic means. Because the distribution of all raw proteinuria observations was not normal (Shapiro-Wilk test for normality), median values were also determined. Data from both analyses are represented either as mean ± SD or as median (interquartile range). Secondary efficacy parameters are presented as mean ± SD. An analysis of covariance was used to assess the significance of treatment differences for all efficacy parameters, with baseline UAER and change in DBP as covariates. To maintain the experiment-wise level, we used a hierarchically stepwise testing procedure.[26] This procedure required a complete *a priori* ordering of the four hypotheses, which is naturally provided by the dosage levels and, thus, corrects for multiple comparisons. For missing UAER (mg/min) values, we performed additional analyses using a different imputation method (see Supplemental Appendix 2).

Safety data were analyzed using descriptive statistics and are presented as means ± SD. We performed a *post hoc* analysis of fluid retention episodes.

The safety population comprised all patients who received at least one dose of study medication. The full analysis population comprised all patients who were included in the safety population and were assessable for the primary efficacy parameter (UAER). The per-protocol population analysis comprised all patients in the full analysis population who were treated according to the protocol.

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DISCLOSURES

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