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Dose-Dependent Acute and Sustained Renal Effects of the Endothelin Receptor Antagonist Avosentan in Healthy Subjects

THESE

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chef du Service de Néphrologie

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par

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Dose-dependent acute and sustained renal effects of the endothelin receptor antagonist avosentan in healthy subjects

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Rapport de synthèse

Enjeu :

L'incidence d'insuffisance rénale terminale augmente d'environ 5-6% par année dans nos régions. L'une des causes majeures d'insuffisance rénale est la néphropathie diabétique qui représente selon les pays entre 25 et 40% des néphropathies terminales. La progression de la néphropathie diabétique peut être ralentie de manière efficace par un bon contrôle du diabète et de l'hypertension artérielle et par le blocage du système rénine-angiotensine. Néanmoins, malgré l'application stricte de ces thérapies préventives, la néphropathie de bons nombres de patients diabétiques continue de progresser. Il est donc important de développer de nouvelles stratégies permettant de préserver la fonction rénale des patients diabétiques soit en améliorant le contrôle de la pression artérielle soit en diminuant la protéinurie.

Contexte :

Il existe un certain nombre d'évidences expérimentales que le blocage des récepteurs de l'endothéline pourrait avoir un effet positif sur le devenir de la néphropathie diabétique en diminuant de manière efficace la protéinurie même chez des animaux déjà traités efficacement avec un bloqueur du système rénine-angiotensine. Dans des études de phase 2 impliquant l'avosentan, un antagoniste des récepteurs de l'endothéline actuellement en cours de développement pour le traitement de la néphropathie diabétique, on a pu démontrer que cet antagoniste, prescrit à des doses oscillant entre 5 et 50 mg par jour per os, diminue la protéinurie d'environ 20-40% chez des patients déjà traités avec un IEC ou un antagoniste de l'angiotensine. Toutefois, une grande étude de phase III conduite avec ce médicament chez des patients diabétiques a du être interrompue précocement en raison de l'apparition d'oedèmes et d'une surcharge hydrosodée conduisant dans certains cas à une décompensation cardiaque aiguë. La rétention hydrosodée est un effet secondaire connu des antagonistes de l'endothéline déjà sur le marché. Toutefois, pour l'avosentan, on ne savait pas si des doses plus faibles du médicament avaient aussi un effet négative sur la balance hydrosodée. En outre, les mécanismes rénaux responsables de la rétention hydrosodée sont encore mal connus chez l'homme. C'est pourquoi, nous avons organisé et réalisé cette étude de pharmacologie clinique chez le volontaire sain posant 2 questions : 1) des doses faibles d'avosentan produisent-elles aussi une rétention hydrosodée chez l'homme ? et 2) quels sont les mécanismes rénaux pouvant expliquer la rétention hydrosodée ?

Cette thèse est donc une étude clinique de phase I testant chez 23 volontaires sains les effets rénaux de différentes doses d'avosentan ou d'un placebo pour établir la courbe dose-réponse des effets rénaux de ce médicament. L'idée était également de définir quelle dose est sure et bien tolérée pour être utilisée dans une nouvelle étude de phase II. L'avosentan a été administré par voie orale une fois par jour pendant 8 jours à des doses de 0.5, 1.5, 5 et 50 mg. Les effets rénaux hémodynamiques et tubulaires ont été étudiés chez chaque sujet lors de la première administration (jour 1) et après une semaine de traitement (jour 8). Le médicament a induit une prise de poids dose-dépendante déjà présente à 5 mg et maximale à 50 mg (+ 0.8 kg au jour 8). Nous n'avons pas mesuré d'impact de l'avosentan sur l'hémodynamique rénale ni sur les électrolytes plasmatiques. En revanche, nous avons constaté une diminution dose-dépendante de la fraction d'excrétion de sodium (jusqu'à -8.7% avec avosentan 50 mg). Cette diminution était en rapport avec une augmentation dose-dépendante de la réabsorption

proximale de sodium. Nous avons également constaté une baisse de la pression artérielle aux doses élevées et une hémodilution marquée par une baisse de l'hématocrite suggérant une rétention hydrique à la plus haute dose.
Nos résultats suggèrent donc que l'avosentan induit une rétention sodée rénale dose-dépendante expliquée avant tout par une rétention du sodium au niveau du tubule proximal. Cet effet n'est pas observé à des doses plus basses que 5 mg chez le volontaire sain , suggérant que ce médicament devrait être évalué pour son activité réno-protectrice à des doses inférieures ou égales à 5 mg par jour. La raison pour laquelle les hautes doses produisent plus de rétention sodée est peut être liée à une perte de sélectivité pour les sous-types (A et B) de récepteurs à l'endothéline lorsque l'on administre des doses plus élevées que 5 mg.

Perspectives :

Les résultats de ce travail de thèse ont donc permis de caractériser les propriétés rénales d'un nouvel antagoniste des récepteurs de l'endothéline chez l'homme. Ces résultats ont aussi permis de guider le développement futur de ce médicament vers des doses plus faibles avec l'espoir de garder les effets bénéfiques sur la protéinurie tout en améliorant le profil de tolérance du médicament par l'utilisation de doses plus faibles.

Dose-Dependent Acute and Sustained Renal Effects of the Endothelin Receptor Antagonist Avosentan in Healthy Subjects

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The endothelin receptor antagonist avosentan may cause fluid overload at doses of 25 and 50 mg, but the actual mechanisms of this effect are unclear. We conducted a placebo-controlled study in 23 healthy subjects to assess the renal effects of avosentan and the dose dependency of these effects. Oral avosentan was administered once daily for 8 days at doses of 0.5, 1.5, 5, and 50 mg. The drug induced a dose-dependent median increase in body weight, most pronounced at 50 mg (0.8 kg on day 8). Avosentan did not affect renal hemodynamics or plasma electrolytes. A dose-dependent median reduction in the fractional renal excretion of sodium was found (up to 8.7% at avosentan 50 mg); this reduction was paralleled by a dose-related increase in proximal sodium reabsorption. It is suggested that avosentan dose-dependently induces sodium retention by the kidney, mainly through proximal tubular effects. The potential clinical benefits of avosentan should therefore be investigated at doses of ≤5 mg.

In humans, the endothelins (ETs) comprise three vasoactive peptides (ET-1, ET-2, and ET-3). The three ET isoforms bind to two cell-surface receptors, ET receptor subtypes A (ET_A) and B (ET_B). ET-1 has the highest affinity for the ET_A receptor, followed by ET-2 and ET-3, with all ETs exhibiting equal affinity for the ET_B receptor.[1] Important functions of ET_A and ET_B receptors include vasoconstriction and vasodilatation, ET clearance, salt balance, cell proliferation, and extracellular matrix production.[2] ET-1 is the major and best-studied isoform in humans, and it plays a significant role in the pathophysiology of a variety of diseases, such as hypertension, heart failure, renal failure, and pulmonary disorders.[3–5] Consequently, several selective and mixed (dual) ET-1 receptor antagonists have been developed or are in development for various pathological conditions.[6]

Avosentan (SPP301) is an orally administered competitive antagonist of ET-1 with a 50- to 600-fold higher selectivity for the ET_A than for the ET_B receptor, depending on the receptor binding assay used (O. Baltatu, personal communication). Following multiple oral administrations of up to 60 mg per day, the pharmacokinetics of avosentan and its hydroxymethyl metabolite (Ro 68-5925) are linear up to a dose of 40 mg. The terminal half-lives of avosentan and its metabolite are in the range of 7–10 h, whether after a single dose or after repeated doses. Urinary excretion rates for avosentan and its metabolite are <0.1 and <5%, respectively.[7] The drug is in clinical development for use in treating diabetic nephropathy.[6]

Overexpression of ET-1 and ET receptors has been demonstrated in diabetic nephropathy in glomeruli and tubular epithelial cells;[4] upregulation of the renal ET system exacerbates proteinuria,[8] a powerful predictor of renal disease progression.[9] Experimental data suggested that ET_A receptor antagonists may preserve renal function in diabetic rats.[10] In fact, in pilot and dosage-finding studies, a reduction in urinary albumin excretion in patients with diabetic nephropathy was demonstrated following chronic preferential selective ET_A receptor blockade with avosentan.[6,11] In a recent placebo-controlled multicenter phase III morbidity and mortality study, avosentan doses of 25 and 50 mg were administered once daily to patients with diabetic kidney disease. The study was stopped prematurely after 18 months for safety reasons. Although avosentan significantly lowered proteinuria, there were noticeably more cardiovascular events (i.e,, fluid overload and congestive heart failure) in the avosentan groups than in the placebo group.[12] It was hypothesized that avosentan at the doses tested could trigger renal retention of sodium and water, leading to fluid overload, edema, and, secondarily, cardiovascular events. In fact, for several ET receptor antagonists (ERAs), fluid retention resulting in edema, weight gain, and, in some cases, worsening of congestive

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heart failure conditions was reported, thereby supporting this hypothesis.[6]

These findings gave impetus to this mechanistic study, which was initiated to further investigate the safety findings in the avosentan phase III trial. A placebo-controlled crossover study was conducted in healthy male subjects to assess the acute and sustained renal effects of avosentan and the dose dependency of these effects. The specific objectives were (i) to determine the effects of avosentan on renal hemodynamics (i.e., glomerular filtration rate and effective renal plasma flow) and sodium excretion, (ii) to evaluate the influence of avosentan on plasma and urinary electrolytes, and (iii) to characterize the systemic effects (blood pressure and heart rate) of avosentan. For this purpose, a wide range of avosentan doses (0.5–50 mg) were administered to the study subjects for 1 week on a once-daily regimen.

RESULTS

Avosentan induced a dose-dependent increase in body weight over the course of the study, the effect being most pronounced at a dose of 50 mg (Figure 1), with a median increase on day 8 of 0.8 kg (mean ± SD, 1.0 ± 1.0 kg). At that time point, the changes in body weight were significantly different from 0 for those on 5- and 50-mg doses ($P < 0.05$). Avosentan doses of 0.5 and 1.5 mg had no effect on body weight. No acute (day 1) changes in hemoglobin or hematocrit were observed after administration of avosentan, despite the protocol-defined acute water load. However, on day 8, a dose-dependent decrease in hemoglobin and, to a lesser degree, hematocrit was found (for both, $P < 0.01$ for trend), with a marked hemodilution induced by the 50-mg dose (Figure 2); the changes from baseline were virtually identical if analyzed on the basis of mean values (data not shown).

Avosentan caused a dose-dependent decrease in diastolic blood pressure as compared to baseline, both on day 1 and day 8 ($P < 0.01$ for trend) (Table 1). For those on a dose of 50 mg avosentan, the maximum median absolute decrease amounted to 11.5 mm Hg (at 2 h after the dose on day 8). This was also reflected in mean and median diastolic blood pressure areas under the curve (AUCs), which decreased over the course of the study and (on the basis of least-squares means from the analysis of variance) were significantly lower on day 8 as compared to placebo for both the 5 mg (difference in least-squares means, 36.7 h·mm Hg; $P = 0.018$) and the 50-mg doses (difference, 49.2 h·mm Hg; $P = 0.004$). Avosentan doses <5 mg had no sustained effect on diastolic blood pressure AUC. There were no clear or consistent effects of avosentan on heart rate or systolic blood pressure (data not shown).

In terms of renal hemodynamic parameters, no relevant changes or clear trends were observed over the course of the study (Table 2). Likewise, plasma electrolytes were not affected by avosentan treatment. In particular, plasma sodium concentrations remained essentially stable over the duration of the study (data not shown).

On day 1, dose-dependent changes in urinary sodium excretion and fractional excretion (FE) of sodium (FE_{Na}) were found. In the placebo group, the ingestion of fluids before and after intake of study medication increased sodium excretion, as reflected by the increase in FE_{Na} (mean ± SD, 0.8 ± 0.9% at 6 h). Avosentan showed a dose-dependent effect on sodium excretion. Specifically, mean ± SD of absolute changes from baseline in FE_{Na} for the 0.5-, 1.5-, 5-, and 50-mg doses at 6 h after dosing were 0.9 ± 0.8, 0.6 ± 0.4, 0.3 ± 0.2, and −0.2 ± 1.0%, respectively ($P < 0.01$ for trend). On day 8, this effect was still present but was less pronounced because a new sodium balance had been reached (Figure 3). The reduction in FE_{Na} was associated with a dose-dependent increase in proximal reabsorption of sodium on days 1 and 8, as reflected by decreases in the FE of lithium at most avosentan dosages (Figure 4). On day 1, mean ± SD of absolute changes from baseline in FE_{Li} for the 0.5-, 1.5-, 5-, and

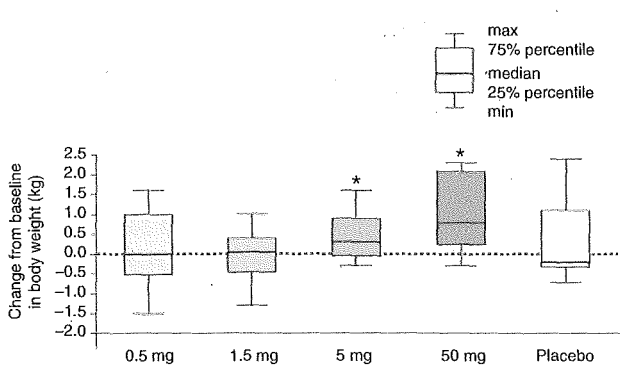

Figure 1 Change from baseline in body weight after multiple oral administrations of avosentan or placebo. Values were determined on day 8. $n = 8$–9 per group. *Mean and median significantly different from 0 ($P < 0.05$, one sample t-test and Wilcoxon signed-rank test, respectively).

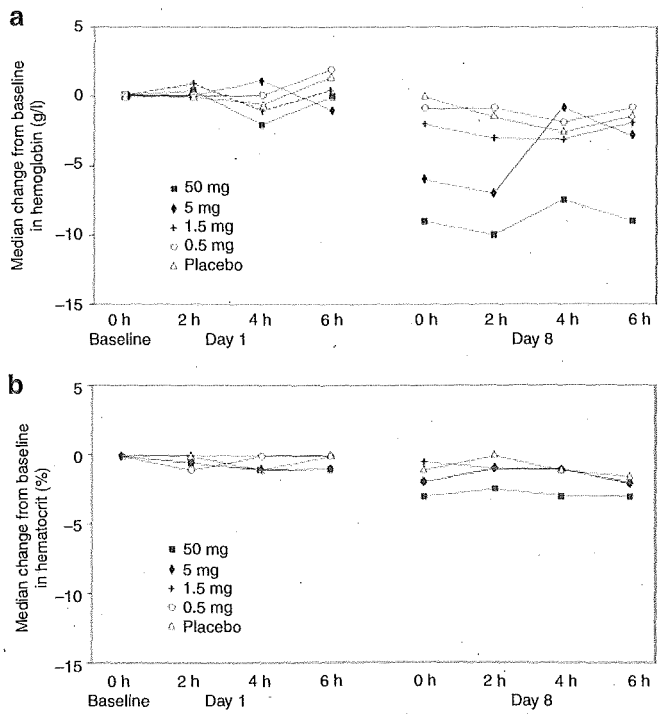

Figure 2 Median changes from baseline in (**a**) hemoglobin and (**b**) hematocrit after single and repeated oral administrations of avosentan or placebo. Baseline was defined as the hemoglobin/hematocrit level measured at time 0 h on day 1 when the first dose of study medication was administered. $n = 8$–9 per group.

Table 1 Change from baseline in diastolic blood pressure following single and multiple oral administrations of avosentan or placebo

	0.5 mg ($n = 9$)	1.5 mg ($n = 8$)	5 mg ($n = 9$)	50 mg ($n = 8$)	Placebo ($n = 9$)
Day 1, 4 h aa	0.22 ± 14.8 (−3.0)	−0.13 ± 7.0 (0)	−4.00 ± 9.3 (−5.0)	−6.63 ± 4.6** (−8.0)	−0.67 ± 6.2 (−1.0)
Day 1, 6 h aa	3.33 ± 9.8 (6.0)	3.13 ± 5.2 (3.5)	−3.33 ± 8.1 (−5.0)	−3.13 ± 5.8 (−2.5)	5.44 ± 6.1 (3.0)
Day 8, predose	−3.67 ± 9.5 (−2.0)	−5.13 ± 11.2 (−4.0)	−7.22 ± 8.0* (−8.0)	−9.50 ± 4.2** (−10.0)	0.11 ± 9.4 (−2.0)
Day 8, 4 h aa	−3.22 ± 13.3 (−1.0)	−2.88 ± 8.4 (−3.5)	−3.33 ± 6.8 (−3.0)	−10.5 ± 5.3** (−9.5)	2.11 ± 4.5 (1.0)
Day 8, 6 h aa	−0.56 ± 10.3 (0)	−3.63 ± 8.3 (−3.5)	−4.56 ± 6.2 (−8.0)	−11.8 ± 5.4** (−11.0)	4.22 ± 7.4 (3.0)

Data are given in mm Hg. All values are presented as mean ± SD (median). Baseline was assessed before first dosing on day 1.
aa, after administration.
*$P < 0.05$, **$P < 0.01$: one sample t-test vs. hypothetical 0 value representing no change in blood pressure.

Table 2 Renal hemodynamic responses to single and multiple oral administrations of avosentan or placebo

	0.5 mg ($n = 9$)	1.5 mg ($n = 8$)	5 mg ($n = 9$)	50 mg ($n = 8$)	Placebo ($n = 9$)
GFR					
Day 1, predose	114.6 ± 18.1 (114.0)	127.3 ± 12.3 (128.5.0)	108.3 ± 12.9 (107.0)	116.5 ± 9.1 (119.5)	106.9 ± 45.5 (110.0)
Day 1, 6 h aa	122.1 ± 33.7 (115.0)	130.8 ± 26.6 (131.5)	102.0 ± 25.1 (104.0)	123.5 ± 40.8 (112.5)	99.9 ± 39.9 (97.0)
Day 8, predose	130.0 ± 38.3 (121.0)	130.8 ± 51.5 (117.5)	113.4 ± 21.7 (116.0)	100.5 ± 36.7 (112.0)	121.7 ± 28.3 (119.0)
Day 8, 6 h aa	110.2 ± 16.2 (105.0)	113.0 ± 16.8 (113.0)	115.7 ± 32.0 (109.0)	99.0 ± 14.9 (95.5)	110.7 ± 17.6 (107.0)
ERPF					
Day 1, predose	625.8 ± 158.8 (616.0)	728.0 ± 192.3 (734.5)	580.3 ± 82.5 (555.0)	649.8 ± 149.7 (583.5)	596.7 ± 233.3 (636.0)
Day 1, 6 h aa	666.7 ± 230.1 (599.0)	754.3 ± 227.7 (733.0)	599.2 ± 112.8 (650.0)	720.8 ± 207.6 (701.0)	653.2 ± 283.0 (639.0)
Day 8, predose	691.8 ± 244.3 (653.0)	747.5 ± 290.5 (765.0)	706.9 ± 177.9 (698.0)	643.0 ± 309.9 (680.0)	750.0 ± 173.3 (799.0)
Day 8, 6 h aa	608.2 ± 120.3 (560.0)	678.6 ± 195.7 (665.0)	767.9 ± 201.3 (685.0)	724.0 ± 220.7 (705.5)	753.9 ± 176.3 (665.0)

Data are given in ml/min. All values are presented as mean ± SD (median). There were no significant differences between groups.
aa, after administration; ERPF, effective renal plasma flow; GFR, glomerular filtration rate.

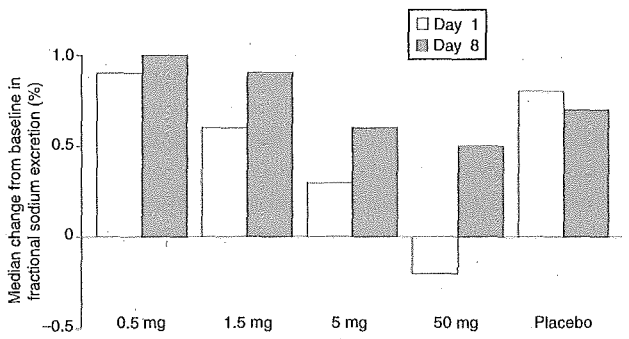

Figure 3 Median absolute change from baseline in fractional excretion of sodium after single and multiple oral administrations of avosentan or placebo. Values were determined at 6 h after the dose; baseline was assessed before first dosing on day 1. $n = 7$–9 per group.

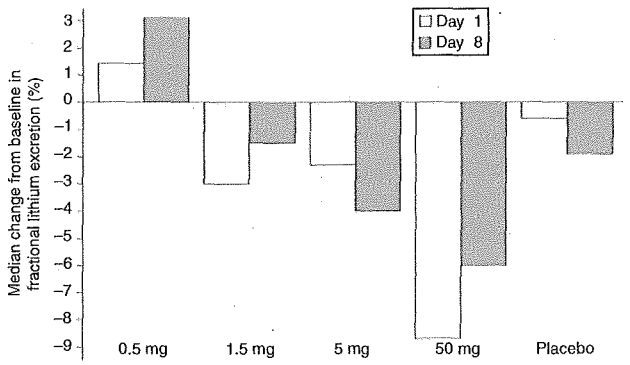

Figure 4 Median absolute change from baseline in fractional excretion of lithium after single and multiple oral administrations of avosentan or placebo. Values were determined at 6 h after the dose; baseline was assessed before first dosing on day 1. $n = 8$–9 per group.

50-mg doses and placebo at 6 h after dosing amounted to 1.4 ± 8.2, −3.0 ± 7.4, −2.3 ± 4.7, −8.7 ± 7.4, and −0.6 ± 9.8%, respectively ($P < 0.05$ for trend). Avosentan had no significant effect on urinary excretion of other electrolytes, phosphate, uric acid, or creatinine over the course of the study.

Pharmacokinetics

The main pharmacokinetic parameters of avosentan are summarized in Table 3. After single-dose administration of 0.5, 1.5, 5, or 50 mg, there was a dose-proportional increase in rate (peak plasma concentration) and extent (AUC_{0-t}) of avosentan absorption. Following multiple-dose administration, dose proportionality was observed up to a dose of 5 mg. There was no significant accumulation of avosentan after repeated administrations.

Tolerability

No unexpected or serious adverse events were observed. Twenty subjects reported a total of 92 adverse events (AEs) of mostly mild to moderate severity, of which headaches and hot flushes were the most common. For these AEs, it was apparent that

Table 3 Main pharmacokinetic parameters of avosentan following single and multiple oral administrations of different doses in healthy volunteers

	0.5 mg ($n = 9$)	1.5 mg ($n = 8$)	5 mg ($n = 9$)	50 mg ($n = 8$)
Day 1				
t_{max} (h)	2.0 (1.9–5.9)	4.0 (1.9–5.9)	2.0 (1.9–5.9)	2.0 (1.9–4.1)
C_{max} (ng/ml)	15.4 ± 1.2 (14.7)	48.2 ± 1.3 (53.8)	174.5 ± 1.5 (184)	2,253 ± 1.4 (2,369)
AUC_{0-t} (h·ng/ml)	204 ± 1.3 (199)	672 ± 1.4 (710)	2,065 ± 1.6 (2,216)	24,091 ± 1.3 (24,835)
Day 8				
t_{max} (h)	2.0 (2.0–4.0)	4.0 (1.9–4.1)	3.9 (1.9–5.9)	1.9 (1.8–4.0)
C_{max} (ng/ml)	19.6 ± 1.2 (17.4)	54.3 ± 1.2 (56.2)	228.9 ± 1.6 (206)	1,883.7 ± 1.3 (1,938)
AUC_{0-t} (h·ng/ml)	247 ± 1.3 (223)	641 ± 1.4 (656)	2,106 ± 1.4 (2,121)	15,646 ± 1.3 (14,985)

Data are median (range) for t_{max} and geometric mean ± geometric SD (median) for the other parameters.

AUC_{0-t}, area under the curve from 0 to the last quantifiable concentration; C_{max}, peak plasma concentration; t_{max}, time to peak plasma concentration.

the frequency of occurrence was dose dependent. Headaches and hot flushes were most frequently recorded in the avosentan 50-mg group (headache, 46; hot flushes, 10), followed by the 5-mg group (headache, 8). No headaches or hot flushes were reported with avosentan 1.5 mg or placebo. Two episodes of periorbital edema were reported in two subjects receiving avosentan 50 mg. There were no clinically relevant changes in vital signs, electrocardiograms, or clinical laboratory parameters over the course of the study.

DISCUSSION

This study, the first to explore the dose-dependent effects of the ERA avosentan on renal and systemic hemodynamics, examined plasma and urinary electrolytes, with a particular emphasis on renal sodium handling. The study was triggered by the premature termination of a phase III trial as a result of an increased incidence of fluid overload and congestive heart failure at avosentan doses of 25 and 50 mg.[12] In our mechanistic study in healthy subjects, we found that avosentan induced an increase in body weight suggestive of fluid retention, the effect being most pronounced at high doses. Hemodilution was seen, particularly at the 50-mg dose. This was apparently due to isotonic fluid retention, given that no changes in plasma sodium were noted. Avosentan doses of 5 and 50 mg induced dose-dependent decreases in diastolic blood pressure, suggesting peripheral vasodilation. No reflex tachycardia was observed. Avosentan had no influence on renal hemodynamics, but a dose-dependent decrease in fractional urinary sodium excretion was found, suggesting that avosentan has effects on sodium retention at higher dosages. This decrease was less pronounced after multiple avosentan dosing, suggesting that a new sodium balance was achieved over time, most likely resulting from an adaptation in sodium excretion by the distal convoluted tubules of the nephron. An examination of the effects of avosentan on segmental renal sodium handling showed a dose-dependent increase in proximal sodium reabsorption. Finally, avosentan was generally well tolerated at low doses; cerebral vasodilation may have caused the moderate headaches reported by subjects at higher avosentan doses.

The results observed in our mechanistic study are consistent with published data on other ERAs. Both ET_A and dual ET-1 receptor antagonists can cause fluid retention. For instance, bosentan therapy was associated with worsening congestive heart failure, which was felt to be a consequence of fluid retention.[13,14] In addition, dose-dependent fluid retention has been reported for selective ET_A receptor antagonists such as ambrisentan, sitaxsentan, and atrasentan.[6,15] In one study with atrasentan, two patients on a very high dosage (75 mg/day) developed severe hyponatremia.[16] Patients given lower doses had a 33% incidence of peripheral edema, which was associated with a 1-kg gain in body weight.[17] In the same study, a mild hemodilution effect was observed as a decrease in hemoglobin concentrations.

In the human kidney, ET_A receptors are expressed in large quantities in cortical vessels, in pericytes of descending vasa recta, within glomeruli, and in interstitial cells from the inner and outer medulla. ET_B receptors are most abundant in cells from the inner medullary collecting duct and in glomeruli.[4] In the renal medulla, activation of ET_B receptors abates sodium and water absorption and causes natriuresis and diuresis, whereas ET_B blockade appears to result in sodium and water retention.[4,18] Consequently, the fluid retention observed with selective ET_A receptor antagonists may be caused by some remaining activity on the ET_B receptor. For avosentan, it has been suggested that it should be classified as a dual ERA rather than as a selective ET_A receptor antagonist, given its weaker selectivity for the ET_A receptor as compared to other selective ET_A receptor antagonists.[6] Therefore, avosentan's effect of promoting sodium retention when administered at a dose of 50 mg may originate from ET_B blockade in the kidney caused by a loss of receptor selectivity at high doses. It is known that ET_B receptors are also located on the proximal tubule, and this is in agreement with our study results using endogenous lithium clearance. This suggests that the observed water retention was promoted mainly by the proximal tubular effects of avosentan on sodium handling. Yet an effect on distal tubular sodium reabsorption cannot be entirely ruled out. Of note, proximal tubules carry no ET_A receptors.[18–20] However, given that ETA receptors may exert a natriuretic effect in female subjects, as has recently been described,[21] there may

be gender-related differences in the effect of ERAs on sodium reabsorption. Moreover, fluid retention has been reported to occur even with a highly selective ET_A receptor antagonist such as sitaxsentan.[22] Therefore, one cannot exclude the possibility that ET_A receptor blockade *per se* promotes sodium retention.

Finally, ET_A receptor antagonism is associated with peripheral vasodilation,[23] which may be a contributory factor for the fluid retention observed after high doses of avosentan. Vasodilation may be associated with fluid shift into the extravascular space, thereby causing or worsening sodium and water retention. In the clinical setting, this might be even more pronounced, as indicated by investigations in binephrectomized rats receiving increasing doses of avosentan. The rats showed a concentration-dependent extravasation of fluid, as measured by changes in hematocrit, most likely caused by increased vascular permeability or a precapillary vasodilation as observed with peripheral vasodilators.[24]

Most studies in healthy humans have not demonstrated an effect of selective ET_A or dual ET-1 receptor antagonists on renal hemodynamics, thereby suggesting that ET-1, acting through the ET_A receptor, does not contribute to the maintenance of renal vascular tone in health.[8] This is in accord with the results of our study.

With respect to systemic hemodynamics, the observed reduction in diastolic blood pressure may be explained on the basis of vasoactive changes induced by avosentan. It has previously been reported, in both healthy subjects and patients, that ET_A receptor blockade decreases peripheral vascular resistance, a major determinant of diastolic blood pressure.[25,26] Likewise, the reported AEs of headache and hot flushes are likely attributable to the vasodilating properties of avosentan. Both of these AEs are known to be associated with avosentan and other ERAs (ET_A and dual antagonists).[6,11,15,26] In and of itself, the acute decrease in blood pressure observed in our subjects at high doses of avosentan could also contribute to acute sodium retention by stimulating proximal sodium reabsorption.

In conclusion, our data suggest that avosentan dose-dependently induces sodium retention by the kidney (probably via proximal tubular effects). The maximum effect is observed at the 50-mg dose; some minor effects are apparent even with the 5-mg dose. Doses <5 mg are virtually never associated with such findings, thereby indicating that these low avosentan doses do not affect steady-state sodium handling. From these data, it can be inferred that the incidence of congestive heart failure and fluid overload observed in a phase III morbidity and mortality study at avosentan doses of 25 and 50 mg were most likely of renal origin, caused by the high doses administered. The data support further investigation of the antiproteinuric effect of avosentan at doses of ≤5 mg.

METHODS

Subjects. Twenty-three healthy male subjects were enrolled in this study; all were Caucasian. Their mean age was 25.8 years (range, 18–39 years), and mean body mass index was 23.7 kg/m^2 (range, 20.4–29.0 kg/m^2). All the subjects were considered to be healthy on the basis of medical history, physical examination, vital signs, routine blood and urine analyses, and an electrocardiogram, all assessed at a screening visit. One subject withdrew consent after period 1; the missing period 2 (avosentan 1.5 mg) was completed by a replacement subject. The study protocol was reviewed and approved by an investigational review board (Ethics Committee of the Canton de Vaud, Lausanne, Switzerland), and written consent was obtained from each volunteer after the nature, purpose, and potential risks of the study were explained. The trial was performed in accordance with the Declaration of Helsinki (Somerset West Amendment, 1996).

Study design. This was an open-label, placebo-controlled, two-period crossover study conducted at the Nephrology Research Unit, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland. The subjects were randomized to two groups of six treatment sequences. Within each group, each volunteer received two different doses of avosentan (either 0.5 and 1.5 mg or 5 and 50 mg) or avosentan and placebo in a randomized fashion for 8 days; the two treatments/study periods were separated by a 10-day washout phase. Avosentan and placebo were administered as oral solutions on a once-daily treatment schedule.

The volunteers were studied on a high-salt diet, which commenced 3 days before the first drug administration and was maintained during the course of the study. For this purpose, the subjects received salt tablets to be taken with each meal. This resulted in an additional daily salt load of 6 g sodium chloride (i.e., ~100 mmol sodium). Diet compliance was evaluated by means of 24-h urine collections. The subjects were institutionalized on study days 1 and 8 of each study period to assess the acute and sustained effects, respectively, of avosentan on renal hemodynamics and sodium balance. Consumption of alcohol- and xanthine-containing beverages was forbidden during the study.

On day 1, the renal effects of avosentan were determined after an overnight fast, as previously described.[27,28] The subjects were studied while they were in the supine position (except during voiding). They received a light breakfast followed by a snack 1 h before drug intake; thereafter, they fasted throughout the study procedure. Two intravenous catheters were inserted into antecubital veins, one for the infusion of sinistrine and *para*-aminohippurate (PAH) in a glucose-saline solution and a second one into the contralateral forearm for blood drawing. After the ingestion of 400 ml of water, intravenous infusion of sinistrine and PAH was started; a fixed amount of fluid (150 ml/h) was given orally to sustain urine output. Two baseline urine collections of 1 h each were obtained before drug intake. At the end of these baseline periods (time 0), the volunteers received the study medication. Blood pressure, heart rate, urinary excretion of electrolytes (sodium, potassium, chloride, calcium, endogenous trace lithium), phosphate, uric acid, and clearances of sinistrine and PAH to assess glomerular filtration rate and effective renal plasma flow, respectively, were measured twice before drug administration and at 2-h intervals for 6 h thereafter. Simultaneously, blood samples were drawn for measuring electrolytes, sinistrine, and PAH.

On days 2–7, the subjects attended the study center each morning for ambulatory visits during which the study medication was administered under supervised conditions. Blood pressure and heart rate were recorded while subjects were in the supine position before the drug was given. Body weight and tolerability (AEs) were assessed daily. On day 8, assessments of renal clearances were repeated in the same manner as on day 1. On day 9, safety assessments similar to those performed at the screening visit were repeated.

Calculation of renal parameters. Clearances were calculated according to the traditional formula: $CLx = (Ux \cdot V)/Px$, in which Ux and Px represent urine and plasma concentrations, respectively, of x, and V is the urine flow rate in milliliters per minute. Fractional excretion was calculated as the clearance of x divided by the clearance of sinistrine or glomerular filtration rate, where x denotes lithium or sodium. The segmental renal sodium handling was measured via endogenous lithium clearance as a noninvasive marker of proximal sodium reabsorption.[29] Blood pressure was measured by the conventional auscultatory method.

Pharmacokinetic assessments. Blood samples were collected on days 1 and 8 before drug intake and then at 2, 4, 6, and 24 h thereafter.

On day 8, additional blood samples were collected at 8 and 10 h postdose. From the avosentan plasma concentration–time profile data, the peak plasma concentration, time to peak plasma concentration, and AUC from 0 to the last quantifiable concentration (AUC_{0-t}) were derived.

Analytical methods, drugs, and chemicals. Plasma and urinary sinistrine concentrations were measured by a microadaptation of a diphenylamine procedure on a Technicon AutoAnalyzer (Technicon Instruments, Tarrytown, NY). PAH and electrolyte concentrations were determined as published previously.[30] Calcium, phosphate, and uric acid were quantified photometrically using Technicon RAXT (Technicon Instrument, Tarrytown, NY). Endogenous trace lithium was measured by graphite furnace atomic absorption spectrophotometry.[31]

Avosentan and placebo were provided by Speedel Pharma (Basel, Switzerland). Sinistrine was purchased from Fresenius Kabi Austria (Graz, Austria), and PAH (aminohippurate, sodium salt of *para*-aminohippuric acid) was obtained from Merck (Whitehouse Station, NJ). Plasma concentrations of avosentan were determined using a validated liquid chromatography–tandem mass spectrometry assay[32] slightly modified from the original method.[33] The lower limit of quantification was 0.5 ng/ml.

Data sets and statistical analysis. Two subjects participated in a single period only. In addition, one subject was excluded from statistical analysis because of noncompliance with regular intake of salt tablets during one study period (placebo). Therefore, for statistical analysis of pharmacodynamic data, there were the following numbers of evaluable subjects per treatment: placebo ($n = 9$), avosentan 0.5 mg ($n = 9$), avosentan 1.5 mg ($n = 8$), avosentan 5 mg ($n = 9$), and avosentan 50 mg ($n = 8$); 43 treatments in total. For safety analysis, all 23 subjects were included.

Descriptive statistics were used to summarize the pharmacodynamic end points (and their changes from baseline), AEs, and baseline characteristics. Summary statistics included the mean, SD, and median for continuous variables, as well as frequency and proportion for categorical variables. The main goal of the statistical analysis was to demonstrate the dose dependency of the renal and hemodynamic effects of avosentan. For these parameters, trend analyses for dose response were performed by means of ANOVA, with a value of $P < 0.05$ as the level of significance. In addition, the AUCs from 0 to 6 h for systolic and diastolic blood pressures on days 1 and 8 were calculated and compared by ANOVA between active treatments and placebo ($P < 0.05$). For body-weight changes, explorative statistical analyses were performed using the one sample *t*-test and Wilcoxon signed-rank test as appropriate ($P < 0.05$).

A total of 20 evaluable subjects were planned for this study, but no formal sample size calculation was performed. Based on historical data,[25,26] it was expected that this number would allow detection of a 20% difference in glomerular filtration rate or effective renal plasma flow between the active treatments and placebo with a 95% confidence.

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CONFLICT OF INTEREST

T.L. and T.H. are employees of Speedel Pharma, whose product was studied in this work. The other authors declared no conflict of interest.

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