Original Article

Haemodynamic effects of valsartan in acute renal ischaemia/reperfusion injury

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Abstract

Background. Acute deterioration of renal function is an important side-effect of angiotensin-converting enzyme (ACE) inhibitors, especially if accompanied by other nephrotoxic events. Angiotensin II receptor1 blockers (ARB) are thought to have fewer side-effects on renal perfusion and function. We examined the effects of valsartan (VAL) on kidney function as well as the contribution of the nitric oxide (NO) system in a rat model of ischaemic acute renal failure (ARF).

Methods. ARF was induced by 40 min of clamping of both renal arteries in female Sprague–Dawley rats. Renal haemodynamic and tubular parameters were determined during post-ischaemic infusion of vehicle, VAL, VAL and the NO-synthase substrate l-arginine, and VAL together with inhibition of NO synthases (NOS) by L-NAME.

Results. Clamping induced acute renal failure with marked decreases in glomerular filtration rate (GFR) and renal plasma flow (RPF) accompanied by a rise in renal vascular resistance (RVR) and fractional sodium excretion. Valsartan caused a slight but significant improvement of GFR and RPF without full recovery of renal function and caused a lowering of RVR and tubular sodium loss. l-arginine-co-administration had no additive beneficial effect. Valsartan-induced changes were not significantly depressed by unspecific inhibition of NOS.

Conclusions. Inhibition of the angiotensin II-receptor1 diminishes the deleterious effects of ischaemia and reperfusion on glomerular function and on the renal microcirculation. An involvement of the NO system could not be demonstrated.

Keywords: acute renal failure; angiotensin II receptor antagonist; l-arginine; NO system; valsartan

Introduction

Drugs that influence the renin–angiotensin system are used with increasing frequency in the treatment of chronic renal, heart, and vascular disease. A well-known side-effect of angiotensin-converting enzyme (ACE) inhibition is acute worsening of renal function due to the predominant dilatation of the efferent arterioles and consequent decrease in glomerular filtration [1]. The effects of angiotensin II-receptor1 blockers (ARB) on renal function and perfusion parameters are less well described. In bilateral renal-artery stenosis [2] and after kidney transplantation [3], ARB administration caused renal functional impairment. However, in anaesthetized rats, two different selective ARB caused increases in both glomerular filtration rate (GFR) and cortical plasma flow [4]. In volume-depleted rats, ARB caused lesser reductions in vascular resistance in efferent arterioles than ACE inhibitors while preserving single-nephron GFR [5]. Heller et al. [6] observed a beneficial effect of pre-ischaemic ARB administration in a rat model of acute renal failure. In healthy humans a single dose of candesartan caused an increase in effective renal plasma flow (RPF) without changes in GFR [7], whereas losartan reduced GFR up to 50% after volume depletion [8]. Also, after uninephrectomy and subsequent artificial stenosis of the remaining renal artery of dogs, reductions in systemic blood pressure due to losartan as well as captopril were accompanied by acute decreases in renal blood flow and glomerular filtration [9]. Taken together, ARB may have different effects on renal haemodynamics depending on the state of hydration and the kind of pre-existing renal damage.

Over the last ten years, tight interactions between angiotensin II (Ang) and nitric oxide (NO) in the regulation of renal perfusion and function have been described. In isolated perfused afferent arterioles of the rabbit, Ang effects depended on the presence or absence of NO [10]. Administration of ARB reduced the decrease in renal blood flow following NO-synthase inhibition in rats [11], while inhibition of NOS reduced
the rise in GFR induced by infusion of losartan, at least in spontaneously hypertensive rats [12].

At present it is not known how kidney perfusion and function change both during and after acute ischaemic renal failure, especially when ARBs are given during this vulnerable phase. We therefore examined whether acute angiotensin II-receptor blockade by valsartan causes improvement of renal perfusion in rats with acute renal failure (ARF). Results from these studies may help to determine when to continue or begin ARB in human ARF. Furthermore, we examined the involvement of the NO-system in the effects caused by ARB infusion.

Material and methods

Drugs

L-arginine (L-Arg) and N-monomethyl-L-arginine (L-NMMA) were purchased from Sigma (Deisenhofen, Germany); FITC inulin was ordered from Bioflo (Uppsala, Sweden) and sodium-para-aminohippurate (PAH) from Merck, Sharpe & Dohme (West Point, USA). Valsartan (VAL) was kindly provided by Novartis Pharma (Wehr, Germany).

Four groups of animals consisting of eight rats each were investigated: control animals were treated with Ringer saline as vehicle; the VAL group was treated with valsartan 12.5 μg/kg/h; the VAL-L-Arg group was given valsartan 12.5 μg/kg/h combined with L-Arg 500 mg/kg/h; and the VAL-L-NMMA group was given valsartan 12.5 μg/kg/h in combination with L-NMMA 1.0 mg/kg/h.

Valsartan for intravenous infusion was prepared according to manufacturer’s instructions: the drug was dissolved in 0.1 N potassium hydroxide and titrated to a neutral pH with hydrochloric acid. Valsartan dosage was determined from previous findings in anaesthetized animals (data not shown); the highest dose with no significant effect on systemic blood pressure was chosen for this study.

Dosages of L-Arg and L-NMMA were chosen from previous studies in clamping-induced ARF showing only slight changes on blood pressure following infusion of these substances [13].

Experimental procedure

Female Sprague-Dawley rats (Charles River, Sulzfeld, Germany; n = 32, weight 242 ± 11 g) with free access to water and standard rat chow prior to the experiments were used. Animals were housed with a regular 12 h-light-dark-cycle under steady temperature, pressure and humidity conditions, according to the regulations of the German TierSchutzgesetz as well as the NIH principles of laboratory animal care.

Anaesthesia was induced by thiobutabarbitatal 100 mg/kg i.p., which lasts for the entire duration of the experiment. The animals were placed on a thermoregulated heating table to maintain body temperature at 37.5°C. A tracheostomy with insertion of an endotracheal tube was performed to allow spontaneous breathing. Thereafter, the left femoral vein was cannulated with a PE 50 catheter (Portex, Hythe, UK) for constant infusion of Ringer lactate at 2 ml/h for replacement of fluid and electrolyte losses and for administration of drugs. The infusion rate of Ringer lactate was chosen from previous experiments [13] to avoid dehydration and activation of the renin–angiotensin system. A second PE 50-catheter was placed in the left femoral artery for continuous measurement of arterial blood pressure using a pressure transducer (Hellige, Freiburg, Germany) and for blood collection. The urinary bladder was catheterized with a PE 10-catheter inserted through a suprapubic incision for monitoring urinary flow and for collection of urine samples. A bilateral horizontal flank incision was then performed and both renal pedicles were prepared; the renal arteries were gently divided from the veins renales. After completion of surgery, a bolus of 3 mg fluorescence-marked inulin together with 2 mg para-aminohippurate in 2 ml of isotonic saline was slowly administered intravenously, followed by a continuous infusion of both substances (1.5 ml/h).

After an equilibration period of 30 min the protocol shown in Figure 1 was initiated. Directly after the baseline sampling-period, both renal arteries were clamped for 40 min and venous outflow was not inhibited. A subsequent resting period was then followed by 120 min of drug infusion.

Each urine collection period, indicated by the dark-shaded bar in Figure 1, lasted 20 min, and a corresponding blood sample (300 μl each) was drawn after 10 min and immediately centrifuged. For all collection periods, inulin clearances and PAH clearances were calculated for estimation of GFR and RPF respectively. At the same time, mean arterial blood pressure was recorded. Renal blood flow (RBF) was calculated as: \( RBF = \frac{RPF}{(1 - hemaocrit)} \). Renal vascular resistance (RVR) was computed by the formula: \( RVR = \frac{MAP}{RBF} \). Haemocrit was measured by a Technicon H1-analyser (Bayer Diagnostics, Wiesbaden, Germany).

Analytical procedures

Inulin concentrations were determined by fluorescence spectrometry using an LS-50 luminescence-spectrometer (Perkin–Elmer, Überlingen, Germany), and PAH concentrations by spectrometry at 550 nm using a microplate reader (Molecular Devices, Crawley, UK). Sodium concentrations in serum and urine were measured by flame photometry (Autoanalyzer FCM 6341, Eppendorf, Hamburg, Germany).

<table>
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<th>Duration</th>
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<th>60</th>
<th>90</th>
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<th>240</th>
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<th>300 min</th>
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<tr>
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<td>Baseline period</td>
<td>Clamping period</td>
<td>Resting period</td>
<td>Infusion period</td>
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Fig. 1. The experimental protocol.
**Statistical analyses**

Results are presented as means ± SEM. The normal distribution of all mean values was checked by the Kolgomorov–Smirnov test. Two-way ranked ANOVAs with repeated measurements and two-sided Student’s t-tests, as appropriate, were used for statistical analysis. A P value ≤ 0.05 was considered statistically significant. Analyses were done using SPSS 6.1 for Windows (SPSS GmbH, München, Germany).

**Results**

Results from the entire study are summarized in Table 1. Mean arterial blood pressure was not different between the four experimental groups before the infusion period. VAL significantly decreased MAP compared with control during the infusion period (77 ± 8 mmHg vs 96 ± 11 mmHg; P = 0.02) and compared with VAL/1-NMMA from infusion (P = 0.002) until the end of the experiment. From clamping onwards, MAP declined in all groups except the VAL/1-NMMA group, in which the NO-synthase antagonist stabilized MAP over time.

Urine volume (Figure 2) rose in all groups following the clamping procedure and corresponded to the induction of acute polyuric renal failure. Immediately after clamping, increases of up to eight times baseline urinary volumes were observed, followed by a slow decrement in urine excretion in the control group. A similar decrease was found after stabilization in the VAL group and further increases of urinary volumes in VAL/1-Arg and VAL/1-NMMA during drug infusion.

The increase during 1-Arg co-administration was statistically significant compared with control and VAL during drug infusion and thereafter. In the control group, urine volume returned almost to baseline levels at about 17 μl/min by the end of the experiment.

Glomerular function was directly determined by FITC-inulin clearance. As shown in Figure 3, baseline GFR during the 50-min equilibration period was about 1.4 ml/min in all groups. Clamping induced a steep decline in GFR, resulting in values that were 15–15% of baseline, with no differences between experimental groups. Valsartan with or without co-administration of 1-Arg or 1-NMMA caused a small but significant increase in MAP compared with control during the infusion period (P = 0.02) and compared with VAL/1-NMMA from infusion (P = 0.002) until the end of the experiment.

![Figure 2. Urine volume in microliters per minute, means ± SEM; c. significant differences of control and Val compared to the VAL/1-Arg group using t-test and ANOVA.](image)

<table>
<thead>
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<th>Table 1. Results from the entire study</th>
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<tr>
<td><strong>Groups</strong></td>
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<td>Urinary volume (μl/min)</td>
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<td>VAL</td>
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<td>VAL/1-Arg</td>
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<td>MAP (mmHg)</td>
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<td>RVR (kPa/min/ml)</td>
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All results expressed as means ± SEM. Statistical significance with P ≤ 0.05 using ANOVA; a compared with control; b compared with VAL; c compared with VAL/1-Arg; d compared with VAL/1-NMMA.
rise in GFR during the infusion period, which was also detectable after cessation of drug infusion. However, GFR never returned to baseline. No significant differences were observed between the three groups.

RPF was measured by PAH clearance. Similarly to GFR, RPF rapidly decreased after induction of renal ischaemia. RPF decreased from baseline values of 5.5 ml/min to very low levels of 0.22 ml/min (control) and 0.47 ml/min (VAL and VAL+L-Arg). At this time, no significant differences could be detected between the groups. Infusion of valsartan alone led to an increase in RPF, which, at the end of the experiment was significantly higher than in control \((P = 0.03)\). Co-administration of L-Arg with VAL caused a distinct increase in RPF especially during the infusion period, resulting in statistically significant differences between control and VAL+L-NMMA groups. Compared with the control groups, Valsartan caused increases in RPF that were independent of pharmacological interventions on the NO system (Figure 4).

RVR was calculated from MAP and RBF, and the results are shown in Figure 5. At baseline, RVR was about 1.0–1.7 kPa\text{-}min/ml in all groups. Clamping led to a 15- to 20-fold increase of RVR compared with baseline. After clamping, RVR increased further in control animals by up to 100-fold from baseline (with high SEM). VAL administration alone as well as with L-Arg and L-NMMA co-infusion blunted this increase from the beginning of drug infusion and thereafter. During the infusion period, L-Arg co-administered with VAL caused a significant decrease in RVR that was prolonged to the end of the trial. In contrast, competitive NOS inhibition with L-NMMA slightly but significantly increased RVR compared with VAL+L-Arg.

Fractional sodium excretion (FENa) was calculated as a marker for tubular function. Clamping induced a steep rise in FENa in all groups, ranging from 32% in the VAL+L-Arg group up to 46% in the control group. FENa then decreased to about half the maximal values until the end of the experiment. There were no differences between the groups. Infusion of VAL caused reductions in FENa that were independent of changes in the NO system.

**Discussion**

The aim of this study was to describe the haemodynamic effects of intravenous administration of the ARB valsartan after induction of renal ischaemia. Our model of acute ischaemic renal failure was the clamping of both renal arteries for 40 min. Renal dysfunction in this study was characterized by an acute and persistent fall in renal blood flow, a concordant depression of GFR, an increase of filtration fraction indicating the involvement of post-glomerular vasoconstriction in this form of ARF, and a rise in renal
vascular resistance without significant changes in systemic arterial blood pressure. After releasing the clamps, urine volume increases due to ischaemic tubular changes with loss of proximal and distal tubular reabsorption capacity were observed and were characterized by increases in FE\textsubscript{Na} in the control group. This pattern of ARF has been previously described by our group [13].

Valsartan infused for 2 h after induction of warm ischaemia to the kidneys led to a slight but significant improvement in renal function. The dose of valsartan was chosen from preliminary experiments showing no effects on systemic blood pressure following ARB infusion. In the present experiments, a small decline in MAP was observed, making it impossible to rule out systemic effects on renal perfusion and function. However, decreases in perfusion pressure should not cause increases in RPF and GFR, as was seen in the VAL group.

These observations confirm results from other groups. Ang receptor\textsubscript{1} have been observed in both afferent and efferent arterioles of C57BL6 mice, and Ang dose-dependently constricted both vessels in a similar fashion [14]. This vasoconstriction was completely abolished by the selective ARB, CV-11974. In healthy animals under anaesthesia, two different ARB increased cortical blood flow and GFR [4]. In hypertensive human volunteers, Buter et al. [7] demonstrated positive effects of candesartan on effective RPF and filtration fraction. These effects were independent of decreases of systemic blood pressure and renal function before the treatment, which was impaired in some of the 17 patients reported.

In a previous study, clamping of the renal pedicle increased intrarenal Ang levels measured by radioimmunoassay [15]. Losartan treatment at the time of reperfusion resulted in a significant decrease in serum creatinine 72 h after ischaemia, indicating a role for Ang and renal Ang receptor\textsubscript{1} in renal perfusion after ischaemia/reperfusion injury. However, renal haemodynamics and function were not measured directly in this study [15]. In our study during the first 3 h of reperfusion, VAL increased renal blood flow to a greater extent than GFR, resulting in a decreasing filtration fraction, and lowered RVR compared with control. This indicates a protective effect of VAL on ischaemic renal tissue, but without the potential to reverse completely the circulatory changes induced by the clamping procedure. In addition, VAL decreased FE\textsubscript{Na}, which is in contrast to reports of ARB actions on tubular function in healthy animals.

Xie et al. [16] observed an increased natriuresis due to blockade of Ang receptor\textsubscript{1} in the proximal tubules. In the case of reperfusion, improvement of renal blood flow with concurrent increases in medullary perfusion seem to prevail over the direct effects of ARB on the proximal tubular epithelium, resulting in a net decrease in renal sodium loss.

The benefit of VAL on renal perfusion in the current study could be explained simply by the blockade of Ang receptor\textsubscript{1}. However, as another possibility, the effects may be mediated at least partly by the unopposed activation of Ang receptor\textsubscript{2} by excessive Ang. This may lead to a secondary vasodilatation due to the production of vasoactive substances such as NO, bradykinin, or prostaglandins [17]. Our experimental approach cannot rule out these secondary effects of ARBs.

In a previous study using a similar model of acute warm ischaemia we showed a beneficial effect of L-Arg infusion as substrate of NO synthases on renal function and perfusion [13]. Administration of L-Arg for 60 min increased GFR two- to threefold compared to controls. L-Arg also improved renal function in other models of acute renal failure, including contrast-media-induced [18] or cyclosporin-induced ARF [19]. L-Arg in these studies was postulated to enhance production of vasoactive NO by NO synthases in ischaemic tissue thereby improving microperfusion in these areas. Tomé et al. [20] showed beneficial effects of acute NOS activation on GFR and tubular integrity and function 2 days after clamping the renal arteries. However, L-Arg given together with Ang-receptor\textsubscript{1} blockade showed no additive effect on GFR or RPF, whereas a non-significant trend for lower RVR was observed. Together, these data show that simultaneous infusion of the indirect vasodilating agent and ARB after ischaemia and during initial reperfusion showed no additional benefit on renal function compared to VAL alone.

We additionally looked for a possible involvement of the NO system in the effects of VAL on renal function. Therefore, L-NMMA, a non-selective competitive antagonist of NO synthases, was infused simultaneously with ARB. We have previously demonstrated that inhibition of NOS during reperfusion after clamping did not improve GFR and abolished the beneficial effects of L-Arg in this setting [13]. Tomé et al. [20] demonstrated a deleterious effect of L-NAME, an alternative non-selective NOS inhibitor, on GFR, an increase in serum urea, and a distinct rise in renal sodium loss 2 days after ischaemia/reperfusion injury. Madrid et al. [21] administered valsartan after L-NAME-treatment and observed a reversal of the negative effects of NOS inhibition on RBF and GFR in anaesthetized rats. The same was true for cortical renal blood flow reported by the same group [22]. The authors concluded that Ang-receptor\textsubscript{1} blockade reduced renal vasoconstriction induced by NOS-inhibition and that renal cortical perfusion in the anaesthetized rat is modulated by NO interacting with Ang.

Administration of L-NMMA together with VAL during ARF led to non-significant decreases in RPF and GFR when compared with VAL alone. During infusion, RVR increased slightly more than in the VAL group although the dose of L-NMMA used was sufficient to completely reverse the effects of direct NOS-activation with L-Arg [13]. Two explanations are possible in this situation of grossly disturbed renal perfusion and function. Either the beneficial effects of Ang-receptor\textsubscript{1} blockade surpass the described
modulation of renal cortical perfusion by the NO system, or unopposed stimulation of Ang-receptor \(_2\) subtypes accounts for the production of vasodilatory compounds which could not be blocked by the present dose of \(\text{L-NMMA}\).

Urine output was distinctly higher during concomitant \(\text{L-Arg}\) and \(\text{L-NMMA}\) together with VAL compared with control and VAL alone; however, this was significant only during infusion of \(\text{L-Arg}\). We could not demonstrate any significant changes in \(\text{FE}_{\text{Na}}\) amongst the groups. Therefore the increase in urinary volumes could not be attributed to renal sodium loss. A diuretic effect of NOS antagonists has not been described previously and could not explain our data. We observed an increase in urine production that was probably not related to the NO-system. The volume of the substitution solution did not differ between the groups, and pressure diuresis seems an unlikely explanation. One explanation could be an osmotic effect of amino acids; however, this has not been observed before, and urine osmolality was not examined in this series of experiments.

In summary, Ang-receptor \(_2\) blockade with valsartan improved renal haemodynamics after warm ischaemia/reperfusion injury. The contribution of the NO system to this beneficial effect is minor. A secondary involvement of the NO system due to the activation of Ang receptor \(_2\) cannot be ruled out in the present studies.

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