Nephrology Dialysis Transplantation

Original Article

Relative roles of endothelin-1 and angiotensin II in experimental post-ischaemic acute renal failure

Mirjana Jerkić[1,2], Zoran Miloradović[2], Đurđica Jovović[2], Nevena Mihailović-Stanojević[2], Juan Vicente Rivas Elena[1], Danica Nastić-Mirić[3], Gordana Grujić-Adanja[3], Alicia Rodríguez-Barbero[1], Jasmina Marković-Lipkovski[4], Srećko B. Vojvodić[5], Marta Vicens Manero[6], Marta Pérez Prieto[6] and José Miguel López-Novoa[1]

[1]Instituto Reina Sofía de Investigación Nefrológica, Departamento de Fisiología y Farmacología, Universidad de Salamanca, [6]Departamento de Bioquímica Clinica, Hospital Universitario de Salamanca, 37007 Salamanca, Spain, [2]Laboratory of Cardiovascular Physiology, Institute for Medical Research, [4]Institute of Pathology and [5]Institute of Biophysics, Medical School, 11001 Belgrade and [3]Institute of Nuclear Medicine–Clinical Center, Belgrade, Yugoslavia

Abstract

Background. The relative roles of endothelin (ET)-1 and angiotensin (ANG) II in post-ischaemic acute renal failure (ARF) have not been fully established so far. With the aim of contributing to this goal, we assessed in this study the effect of ANG II and ET-1 blockade on the course of post-ischaemic-ARF.

Methods. Anaesthetized Wistar rats received i.v. either bosentan (a dual ET receptor antagonist; 10 mg/kg body weight) or losartan [ANG II type 1 (AT_1) receptor antagonist; 5 or 10 mg/kg body weight] or both, 20 min before, during and 20 min after ischaemia. Rats in the control group received the vehicle via the same route. Survival and renal function were monitored up to 8 days after the ischaemic challenge, while haemodynamic parameters were measured 24 h after ARF.

Results. Our results demonstrate that bosentan treatment has a more beneficial effect on experimental ARF than losartan. The survival rate was remarkably higher in bosentan-treated rats than in both rat groups treated with losartan. In the ARF group treated with bosentan, renal blood flow (RBF) was increased by 129% in comparison with the untreated ARF group, whereas in the losartan-treated ARF groups, RBF was only ∼35 or 38% higher than in control ARF rats. The glomerular filtration rate was markedly higher in bosentan-treated rats than in all other ARF groups on the first and second day after ischaemia. Tubular cell injury was less severe in bosentan-treated rats than in the control ARF rats, but in losartan-treated groups it was similar to that in the ARF group. Concurrent blockade of both ET and AT_1 receptors did not improve ARF because this treatment induced a marked decrease in blood pressure.

Conclusions. These results suggest that ET-1 blockade is more efficient in improving the early course of post-ischaemic renal injury than ANG II inhibition, and that blockade of ET-1 might be effective in prophylaxis of ischaemic ARF.

Keywords: acute renal failure; bosentan; ischaemia–reperfusion injury; losartan; rats

Introduction

Ischaemic acute renal failure (ARF) occurs frequently in clinical practice after major cardiovascular surgery or severe trauma, haemorrhage, hypotension (e.g. cardiac arrest), sepsis and/or volume depletion. The pathophysiological mechanisms involved in the initiation and maintenance phase of ischaemic ARF are quite complex and incompletely understood.

In spite of the fact that total renal blood flow (RBF) is reduced in post-ischaemic ARF to some 40 or 50% of its normal value, it is insufficient to account for the subsequent large fall in the glomerular filtration rate (GFR). This also explains, at least partly, why all attempts over the last 30 years to improve renal function in ARF by increasing RBF have more or less failed. It has become clear that factors other than a decrease of total RBF play an important role in functional renal disturbances caused by ischaemic

Correspondence and offprint requests to: José Miguel López Novoa, MD, PhD, Instituto Reina Sofía de Investigación Nefrológica, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Avenida Campo Charro s/n, 37007 Salamanca, Spain. Email: jmlnovoa@usal.es

insult. Both intrarenal vasoconstriction and physical congestion of the medullary vasculature play important roles in persistent regional disturbances of the RBF. These alterations maintain the tubular segments within the outer medulla in a persistent state of severe hypoxia and both intensify and prolong initial ischaemic insult [1]. Recent work has also shown that injury of the vascular endothelium, in addition to renal tubular damage, contributes to the intrarenal vasoconstriction associated with ARF. Lesions of endothelial cells in ischaemia–reperfusion injury shift the balance between vasoconstrictors and vasodilators, favouring the former, and exacerbating the degree of vasoconstriction. The involvement of several vasoconstrictors, including endothelin 1 (ET-1) [1] and angiotensin II (ANG II) [2], in these haemodynamic changes has been identified. Namely, it has been reported that renal injury induces synthesis of endogenous ET-1, which is then able to ensure continuation of its own production, after the initial injury ends. In addition, ET-1 has greater vasoconstrictor activity in the renal vasculature than in any other vascular bed and it has direct glomerular and tubular effects [3]. At high concentrations, ET-1 induces severe reduction of RBF and GFR, a fall in the urinary flow rate and a decrease of urinary sodium excretion. ET-1 is also able to contract mesangial cells. Renal effects and mesangial cell contraction induced by ET [4] are mediated, at least in part, by platelet-activating factor (PAF). ANG II is also a potent intrarenal vasoconstrictor. It modulates the GFR by directly affecting the tone of both efferent and afferent arterioles, but it also affects mesangial cell function [2]. Additionally, ANG II has multiple tubular effects, which are well known. There is also evidence that ischaemic renal injury increases plasma renin activity (PRA) in the initial stages of both clinical and experimental forms of ARF [5]. Also, it has been suggested that the interaction between the renin–angiotensin system (RAS) and ET pathways accelerates renal injury. Moreover, gene expression of ET-1 is in part upregulated by ANG II in renal failure [6].

Results obtained so far from animal studies indicate that ischaemia-induced ARF is attenuated by the infusion of the ET-converting enzyme inhibitor phosphoramidon [7], and ET receptor antagonists [8,9]. However, it is interesting to note that there is a significant difference between the rat and the dog models. While both selective ET_A and mixed ET receptor antagonists were effective in the rat model, only mixed antagonists were effective in the dog model. Although the first generation of ET receptor antagonists entered clinical trials by the early 1990s, these agents have not yet reached the clinic. However, in a double-blind, placebo-controlled crossover study involving 10 healthy volunteers, bosentan, when administered with cyclosporin A (CsA) for 7 days, had the potential to attenuate CsA renal toxicity by markedly blunting the renal hypoperfusion effect of CsA [10].

Recently, it has been demonstrated that ANG II type 1 (AT_1) receptor blockade accelerates renal function recovery in the post-ischaemic kidney [11]. However, there are, to our knowledge, no reports about coherent comparisons of the roles of ET and RAS in ARF, i.e. published records of simultaneous observations of their effects under the same conditions. Trying to contribute to the insight into these complex phenomena, we have compared the effects of blocking both ET receptor subtypes with the effects of AT_1 blockade on post-ichaemic ARF. For this purpose, we have studied renal and cardiovascular function 24 h after reperfusion and the survival rate up to the eighth day after ARF induction. Measurements in the acute (24 h) study included RBF and GFR, excretory values (as indicators of tubular damage) and renal histological scores. Moreover, we also measured systemic haemodynamic parameters [cardiac output (CO) and total vascular resistance] in order to elucidate whether changes of renal haemodynamics are of local origin or stem from changes of systemic haemodynamic parameters. Finally, we also explored how simultaneous blockade of both ET and AT_1 receptors affects the course of post-ischaemic ARF. In the follow-up study, we have assessed survival rate of animals, evaluated tubular damage and measured renal functional parameters.

Materials and methods

Experiments were performed on 179 male Wistar rats, weighing ∼300 g, bred at the Institute for Medical Research, Belgrade and fed on a standard chow for laboratory rats (Veterinarski zavod, Subotica, Yugoslavia). All procedures were conducted in conformity with guidelines of international and national institutions for the care and use of laboratory animals: Conseil de l'Europe (published in the Official Daily N. L358/1-358/6, 18 December 1986), Spanish Government (published in Boletín Oficial del Estado N. 67, pp. 8509–8512, 18 March 1988) and the US National Institutes of Health (Guide for the Care and Use of Laboratory Animals, NIH publication no. 85-23). They were also approved by our institutional ethics committees. We used a non-peptide, potent and mixed ET_A and ET_B receptor antagonist, bosentan (Ro 47-0203/001; 4-*tert*-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-metoxy-phenoxy)-2,2'-bispyrimidine-4-yl]-benzenesulfonamide sodium salt, gift of Dr Martine Clozel, Actelion Ltd, Allschwil, Switzerland). Bosentan sodium salt was always freshly prepared as 10 μM solution in double-distilled water. The solution was heated gently thereafter in a waterbath at 50–60°C until bosentan completely dissolved, and then cooled down slowly to room temperature. The AT_1 receptor blocker, losartan (DUP 153), was obtained from Du Pont, (Wilmington, DE) and dissolved in saline. ET-1 (Sigma, E-7764) and ANG II (Sigma, A-9525) were dissolved in saline and prepared as 0.5 and 1 μM solutions, respectively.

Pilot study

The effectiveness of both the ET and AT_1 receptor blockade was tested on five rats in each group. The animals were anaesthetized with an intraperitoneal injection of sodium

pentobarbital (35 mg/kg body weight), and placed on a heated table to maintain their body temperature at 37°C. The femoral artery (used for blood pressure measurement) and femoral vein were cannulated. After completion of the surgical procedure, a 60–90 min equilibration period was allowed before the experimental protocol was accomplished. The blood pressure response toward an i.v. bolus injection of 100 ng/kg body weight of ET-1 was analysed with and without previous ET receptor blockade with bosentan (10 mg/kg body weight, infused during 85 min). The same was done for bolus injection of 300 ng/kg body weight of ANG II with and without previous infusion of losartan (10 or 5 mg/kg body weight). Mean arterial pressure (MAP) was measured with a pressure transducer and recorded on a direct-writing recorder. The pressure response to ET-1 or ANG II was calculated for each animal as the percentage of MAP change from its baseline value.

Acute renal failure experiments

The animals were divided into six groups: (i) sham-operated rats (sham); (ii) control animals with acute renal failure (ARF); (iii) animals with ARF and bosentan infusion (ARF + B); (iv) animals with ARF and losartan infusion (10 mg/kg body weight; ARF + L-10 mg); (iv) animals with ARF and losartan infused in a lower dose (5 mg/kg body weight; ARF + L-5 mg); and (vi) animals with ARF and simultaneous infusion of both bosentan and losartan, through two separate femoral veins (ARF + B + L).

The rats were surgically prepared as described above. In addition, right kidneys were removed, the left renal artery was carefully separated from the renal vein and rats were subjected to renal ischaemia by clamping the renal artery for 45 min. In the sham-operated rats (group i, $n = 17$), a sham operation of the renal ischaemia was performed. In these experiments, rats received the vehicle (isotonic saline, at a rate of 1.44 ml/kg body weight) and their arterial pressure was determined during an 85 min control period. Bosentan and/or losartan infusion was administered in the femoral vein 20 min before, during and 20 min after renal ischaemia, while the control ARF group (group ii, $n = 24$), received saline (at a rate of 1.44 ml/kg body weight) via the same route. The bosentan group (group iii, $n = 20$) received 10 mg/kg body weight of bosentan (at a rate of 118 μg/kg/min), the losartan group (group iv, $n = 20$) received 10 mg/kg body weight (at a rate of 118 μg/kg/min), group v ($n = 19$) received a lower dose of losartan (5 mg/kg body weight; at a rate of 59 μg/kg/min) and group vi ($n = 19$) received bosentan plus losartan (each one in dose of 10 mg/kg body weight and at a rate of 118 μg/kg/min), and changes in arterial pressure were recorded.

After 20 min of reperfusion, infusions were stopped, femoral catheters were removed, surgical wounds were sutured and rats were allowed to recover and fully wake up. Rats were then placed in individual metabolic cages and urine was collected.

Studies 24 h after reperfusion

Haemodynamic studies. Rats were anaesthetized again (sodium pentobarbital, 35 mg/kg body weight), and surgically prepared as described before. In addition, their left carotid arteries and right jugular veins were prepared for CO measurement using a previously described [12] modification of Coleman's application of the dye dilution technique [12]. Indocyanine green was used as the indicator, and a recording densitometer (Beckman Instruments, Columbia, MD) was utilized for the determination of dye in the blood and registration of the dilution curve. A non-cannulating ultrasonic flowprobe (1RB, internal diameter = 1 mm) was placed around the renal artery and connected to a Transonic T106 Small Animal Flowmeter (Transonic System Inc., Ithaca, NY) for RBF measurement.

At the end of each experiment, blood samples were taken from all animals and the left kidneys extracted from eight animals of each group for histological examination.

Histological examination. Preparation of kidneys for histological examination has been described previously [13]. Briefly, renal tissue was fixed in 10% buffered formalin solution. Later, the kidney was dehydrated in alcohol, blocked in paraffin wax, and 5 μm thick sections were cut and stained by haematoxylin–eosin and periodic acid–Schiff. Glomeruli, tubulointerstitium and blood vessels were assessed separately by light microscopy examination. Acute tubulointerstitial lesions were graded with respect to the following manifestations: swelling and vacuolization of tubular cells; tubular dilatation; loss of brush borders; cast formation; interstitial oedema and interstitial mononuclear leukocyte infiltration; tubular cell necrosis; or focal infarctions. The level of each manifestation was graded with 1 for low, 2 for moderate, 3 for high, and 0 for the lack of manifestation. The sum of these changes was taken as the histopathological score for comparison between groups.

Analytical methods. Urinary volume was measured using a graduated cylinder with an accuracy of 0.1 ml. Blood samples were centrifuged immediately at 3000 r.p.m. during 20 min, and plasma was frozen to −20°C. Urinary and plasma concentrations of creatinine were determined by the alkaline pycrate method using a Beckman 42 spectrophotometer. Concentrations of sodium (Na^+) in plasma and urine were measured by an IL 943-flame photometer (Instrumentation Laboratory, Milan, Italy).

A standard formula was used to calculate creatinine clearance, as an estimate of GFR. Fractional excretion of Na^+ was calculated as a percentage of creatinine clearance. Total vascular resistance (TVR) was calculated as a quotient of MAP and CO (assuming that mean right atrial pressure is zero), while renal vascular resistance (RVR) was calculated as a quotient of MAP and RBF.

We also measured 24 h urinary excretion of three tubular enzymes [γ-glutamyl transpeptidase (γGT) alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)]. All measurements were made using a centrifugal automatic analyser (Hitachi 917, Tokyo, Japan). γGT transforms the γ-glutamyl residue of L-γ-glutamyl-3-carboxy-4-nitroanilide into glycylglycine. In this reaction, the 5-amino-2-nitrobenzoate is released in proportion to the activity of γGT. Its concentration was measured using the Randox commercial kit (Crumlin, UK). ALP activity was analysed by the p-nitrophenyl phosphate method using a commercial kit (Roche diagnostics, Mannheim, Germany). LDH activity was analysed by the pyruvate NADH method using a commercial kit (Roche diagnostics). The excretion of all three enzymes was expressed in mU/mg of creatinine.

ET measurement and PRA. In the next eight rats from the five groups, plasma ET and PRA were measured. These animals underwent the same treatment during the first day but were not subjected to haemodynamic measurements during the second day. As in previously described measurements, we evaluated plasma ET and PRA 24 h after treatment (sham operation or induction of ARF, etc.). For this purpose, animals were guillotined and blood samples were collected into chilled, siliconized plastic centrifuge tubes containing both Na_2EDTA (1 mg/ml) and aprotinin (1000 kIU/ml) for plasma ET measurement and only Na_2EDTA (2 mg/ml) for PRA measurement. We used an RPA 535 ET-1, 2 Biotrack Assay System with Amprep C_2 RPN 1903 columns (Amersham, Little Chalfont, UK) and a REN-CT2 ANG I radioimmunoassay kit (CIS bio international, Saclay, France) respectively, according to the manufacturers' instructions.

Eight day follow-up studies

Eight or nine rats from each group were followed-up during 8 days after induction of ARF or sham operation. During this time, rats were kept in metabolic cages. Rats' survival and urine flow were continuously monitored and blood samples were taken on the second, fourth, sixth and eighth day after the ischaemic challenge. The minimum, necessary quantity of blood was being taken from their tail vein, collected into heparinized haematocrit tubes and then centrifuged at 11 000 r.p.m. for 5 min. Separated plasma was then frozen to −20°C. At the end of the study, a final blood sample was taken from each animal and it was then sacrificed by injection of an overdose of sodium pentobarbitone. We measured plasma and urinary Na^+ as well as creatinine concentrations in collected samples, as described before. From those values, we calculated creatinine clearance and fractional excretion of Na^+. We also measured urinary excretion of three enzymes (γGT, ALP and LDH) by applying the same methods as detailed above. The values obtained were expressed with respect to the creatinine excreted.

Statistical analyses

Measured values are expressed as mean ± SEM. Statistical significance of interval differences between all groups was analysed by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test for multiple comparisons. Histopathological scores were analysed using the non-parametric Kruskal–Wallis multiple comparison test. In all cases, differences with *P*-values < 0.05 were considered to be statistically significant.

Results

Effectiveness of ET and AT_1 receptor blockade

We used a 'pharmacologically' high dose of ET-1 (100 ng/kg body weight) and ANG II (300 ng/kg body weight) to prove the effectiveness of ET and AT_1 receptor blockade since their respective plasma concentrations 24 h after induction of ischaemic ARF

are ∼30 (our results) and 100 pM [14]. We found that the dose of bosentan used (118 μg/kg body weight/min) fully blocked hypotensive and significantly reduced hypertensive effects of ET-1, 100 ng/kg body weight (from 36.6 ± 4.2% to 19.6 ± 3.4%). The same dose of losartan completely abolished the hypertensive effects of ANG II, 300 ng/kg body weight (from 79 ± 14% to 5.1 ± 4.0%), while the lower losartan dose (5 mg/kg body weight) significantly reduced the hypertensive effects of ANG II (from 79 ± 14% to 9.0 ± 5.2%; $P < 0.01$).

Results obtained 24 h after renal ischaemic injury

Mean arterial pressure and heart rate. No change of MAP was observed during the first 10 minutes of infusion of either bosentan or losartan. At the same time, MAP dropped significantly in the group treated with both bosentan and losartan (Figure 1A). MAP decreased after renal artery clamping in all groups with ARF, being lowest in the ARF + B + L group. After clamp removal, MAP rapidly increased in both the ARF + B group and the control ARF group, but underwent only a slight increase in both groups treated with losartan. MAP in the group treated with the higher dose of losartan became significantly lower than that in the control ARF and ARF + B groups. However, MAP in the group treated with the lower dose of losartan was always very similar to that in the control ARF and ARF + B groups. On the other hand, in the ARF + B + L group, MAP did not recover after renal ischaemia, thus remaining significantly lower than in all other groups. At 24 h after induction of ARF or nephrectomy, MAP values were similar in the sham, ARF, ARF + B and ARF + L-5 mg groups, while in the ARF + L-10 mg group these values were lower than in the sham group. Finally, MAP in the ARF + B + L group remained the lowest of all the groups, being significantly less than in the sham and ARF + B groups. It is also important to note that, contrary to all the other groups, MAP in the ARF + B + L group decreased significantly during treatment with respect to its basal value. This did not occur in any other group, i.e. MAP was not significantly altered during treatment with respect to its own basal value.

Heart rate (HR) dropped significantly in all groups after clamping or nephrectomy (Figure 1B). Once the clamp was removed, HR increased in all groups, but it remained significantly lower in the ARF + L-10 mg rats than in ARF rats and sham-operated animals. On the other hand, HR in the ARF + B, ARF + L-5 mg and ARF + B + L groups was significantly lower than in the sham group only. At 24 h after ischaemia–reperfusion injury or sham operation, HR was not different between groups.

Cardiac output (CO) and total peripheral resistance (TPR). Values of systemic haemodynamic parameters 24 h after induction of ARF or sham operation are shown in Table 1. We have observed that CO and TPR did not change 24 h after reperfusion in the ARF
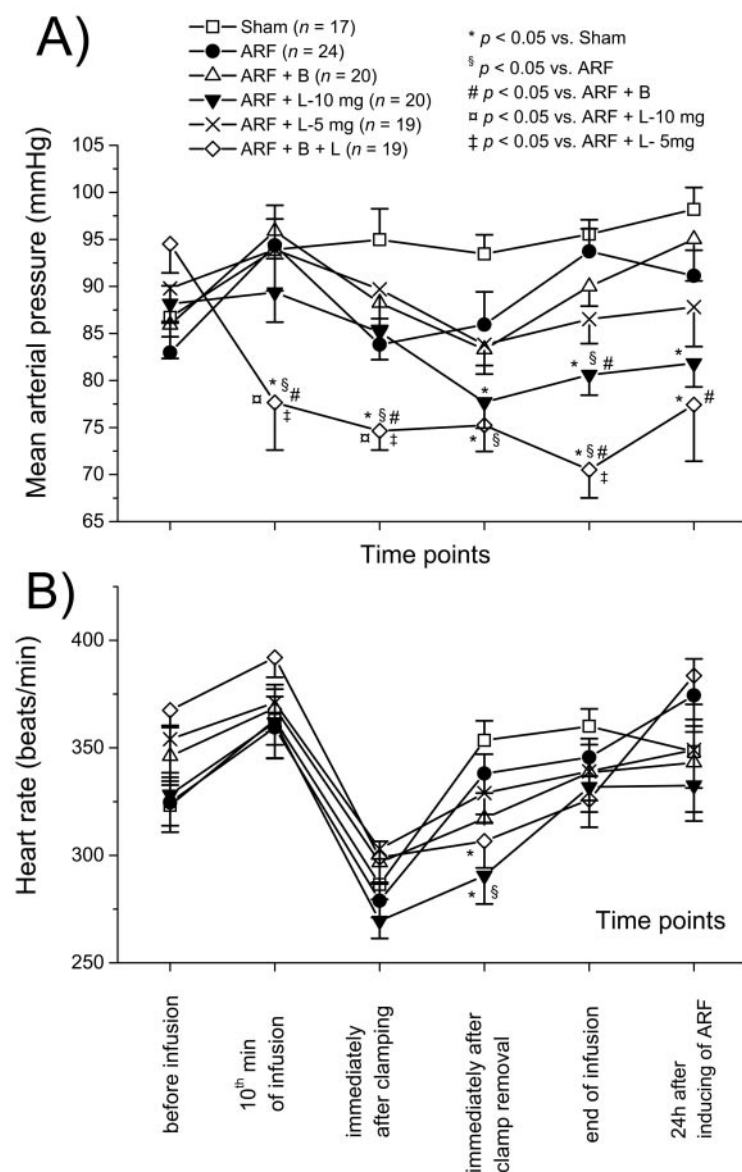
Fig. 1. (**A**) Mean blood pressure and (**B**) heart rate in: sham-operated rats; control rats with acute renal failure (ARF); bosentan-treated ARF rats (ARF + B); ARF rats treated with losartan (ARF + L-10 mg and ARF + L-5 mg); and ARF rats treated with both bosentan and losartan (ARF + B + L).

Table 1. Systemic and renal haemodynamic parameters, urine flow and histopathological score 24 h after ARF or sham operation

Experimental groups	CO (ml/min/kg)	TPR (mmHg min kg/ml)	RBF (ml/min/kg)	RVR (mmHg min kg/ml)	Urine flow (µl/min/kg)	Histopathological score
Sham ($n=9$)	307 ± 32	0.340 ± 0.038	16.1 ± 2.6	6.8 ± 1.1	15.9 ± 2.6	0.71 ± 0.18
ARF ($n=16$)	262 ± 30	0.437 ± 0.069	5.58 ± 0.78*	20.7 ± 3.0*	20.9 ± 1.6	10.56 ± 0.59*
ARF + B ($n=11$)	275 ± 41	0.414 ± 0.075	12.8 ± 2.8§	10.95 ± 2.0§	20.7 ± 2.1	5.56 ± 0.31§,†
ARF + L-10 mg ($n=11$)	358 ± 26	0.233 ± 0.02§	8.5 ± 1.3*	12.4 ± 1.9§	19.1 ± 2.5	9.50 ± 0.55*,#
ARF + L-5 mg ($n=11$)	384 ± 38	0.233 ± 0.023§	9.02 ± 0.34*	9.73 ± 0.47§	23.2 ± 5.0	8.29 ± 0.29*
ARF + B + L ($n=11$)	382 ± 42	0.214 ± 0.017§	6.6 ± 1.4*	11.5 ± 1.8§	9.1 ± 2.4§,#,†,‡	10.29 ± 0.5*,#

Sham-operated rats; control rats with acute renal failure (ARF); bosentan-treated ARF rats (ARF + B); ARF rats treated with losartan (ARF + L-10 mg and ARF + L-5 mg); and ARF rats treated with both bosentan and losartan (ARF + B + L). CO = cardiac output; TPR = total peripheral resistance; RBF = renal blood flow; RVR = renal vascular resistance; *$P < 0.05$ *vs* sham, §$P < 0.05$ *vs* ARF, #$P < 0.05$ *vs* ARF + B, †$P < 0.05$ *vs* ARF + L-10 mg, ‡$P < 0.05$ *vs* ARF + L-5 mg.

group, as compared with the sham-operated rats. No significant difference in CO was observed between the groups with ARF. However, TPR in both groups treated with losartan as well as in the ARF + B + L group was lower than in the ARF group.

Renal haemodynamic parameters. Changes in renal haemodynamic parameters are shown in Table 1. At 24 h after induction of ARF, RBF was significantly reduced, while RVR was significantly higher than in the sham-operated animals. In the ARF + B group, RBF was significantly higher than in ARF rats. In the two losartan-treated ARF groups as well as in the ARF + B + L group, RBF was not different from the values in the control ARF group. RVR was lower in all treated groups that in the untreated, ARF group (Table 1).

Renal function. Data for renal function 24 h after induction of ARF are shown in Table 1 and Figures 3 and 4. Urinary flow remained unchanged in all ARF groups except in the ARF + B + L group. In this group, urine flow was significantly lower than in all other groups.

Creatinine clearance was used as an estimation of GFR; we did not use a more appropriate method, e.g. determination of inulin clearance, because that would have put an unbearable burden on our animals, already undergoing a very difficult procedure.

The GFR was found to be drastically decreased 24 h after induction of ARF (Figure 4A). In the bosentan-treated group with ARF, the GFR was markedly higher than in the group with ARF alone. In the other groups, no improvement of the GFR was observed.

Plasma creatinine level was markedly elevated 24 h after ischaemic challenge. The creatinine concentration was found to be same in both the ARF + L-5 mg group and the ARF + B + L group as in the control ARF group (Figure 4B). In ARF groups treated with bosentan and the higher losartan dose, the level of plasma creatinine was significantly decreased in comparison with all others ARF groups.

The fractional excretion of sodium (FE_{Na+}), a marker of tubular function, was > 11 times greater in the ARF group than in the Sham group (Figure 3B), thus suggesting an altered tubular function in rats with post-ischaemic ARF. No significant difference in FE_{Na+} was observed among groups with ARF, with the exception of the ARF + B group, in which the increase in FE_{Na+} was significantly lower than in all other groups with ARF. This indicates a protective effect of bosentan on tubular function.

Histological analysis. The morphological examination of renal tissue revealed significant differences between experimental groups of animals.

In kidneys that underwent ARF only, the most prominent lesions were: a widespread tubular necrosis (most manifested in the cortico-medullary zone) and a huge number of PAS-positive casts in the medulla

(Figure 2B). The intensity of interstitial oedema varied from specimen to specimen in this group. Dilatation of some segments of tubuli was observed mainly in proximal tubuli and, to some extent, in distal tubuli. In some cases, the dilatation was associated with loss of brush border of proximal tubular epithelium. In most cases, swelling and vacuolization of proximal tubular epithelial cells was found in some segments only. Glomeruli and blood vessels were similar to those in the sham-operated rats.

In the ARF + B group, less intense lesions were noticed in comparison with the ARF group. Tubular dilatation was less or even absent in some kidney specimens of this group. Interstitial oedema was rare (Figure 2C), while tubular necrosis was decreased in the cortico-medullary region. In addition, fewer intraluminal casts were present. In contrast to the ARF + B group, most kidney specimens from the ARF + L-10 mg group were quite similar to those from the ARF group. In only a few kidney specimens could a slight decrease of tissue lesions be observed, such as a less widespread tubular necrosis and fewer casts (Figure 2D). In the ARF group treated with the lower dose of losartan, histological findings were almost the same as in the ARF + L-10 mg group (Figure 2E).

Most of the kidney specimens from the ARF + B + L group were also similar to those from the control ARF group. Some of the histopathological changes were even a bit worse than in the ARF group. Swelling and vacuolization of proximal tubular epithelial cells (Figure 2F) were found in a slightly greater number of cases than within the group with ARF only.

The sum of the histopathological grades, marking the changes described above, is shown in Table 1. The histopathological score is drastically higher in rats with ARF than in sham-operated animals. In the ARF + B group, the histopathological score was markedly lower than in all other groups with ARF. No other treatment had protective effect on the histological damage induced by ischaemia–reperfusion.

Excretion of urinary enzymes. To evaluate tubular damage in rats subjected to renal ischaemic injury, we measured 24 h urinary excretion of three enzymes: γGT, ALP and LDH. γGT is an enzyme whose maximal activity is located in the brush border of proximal renal tubular epithelium, ALP has been localized to the S3 segment of the renal tubule, whereas LDH is mainly located in the distal tubular cells. We chose these three enzymes because it is known that renal ischaemia provokes severe injury to the proximal brush border, that the S3 segment of the renal tubule is a site believed to be particularly vulnerable to ischaemic and toxic damage, while cast formation is predominantly localized in distal nephron segments. The results are shown in Table 2. Excretion of all enzymes was drastically elevated 24 h after ischaemic challenge.

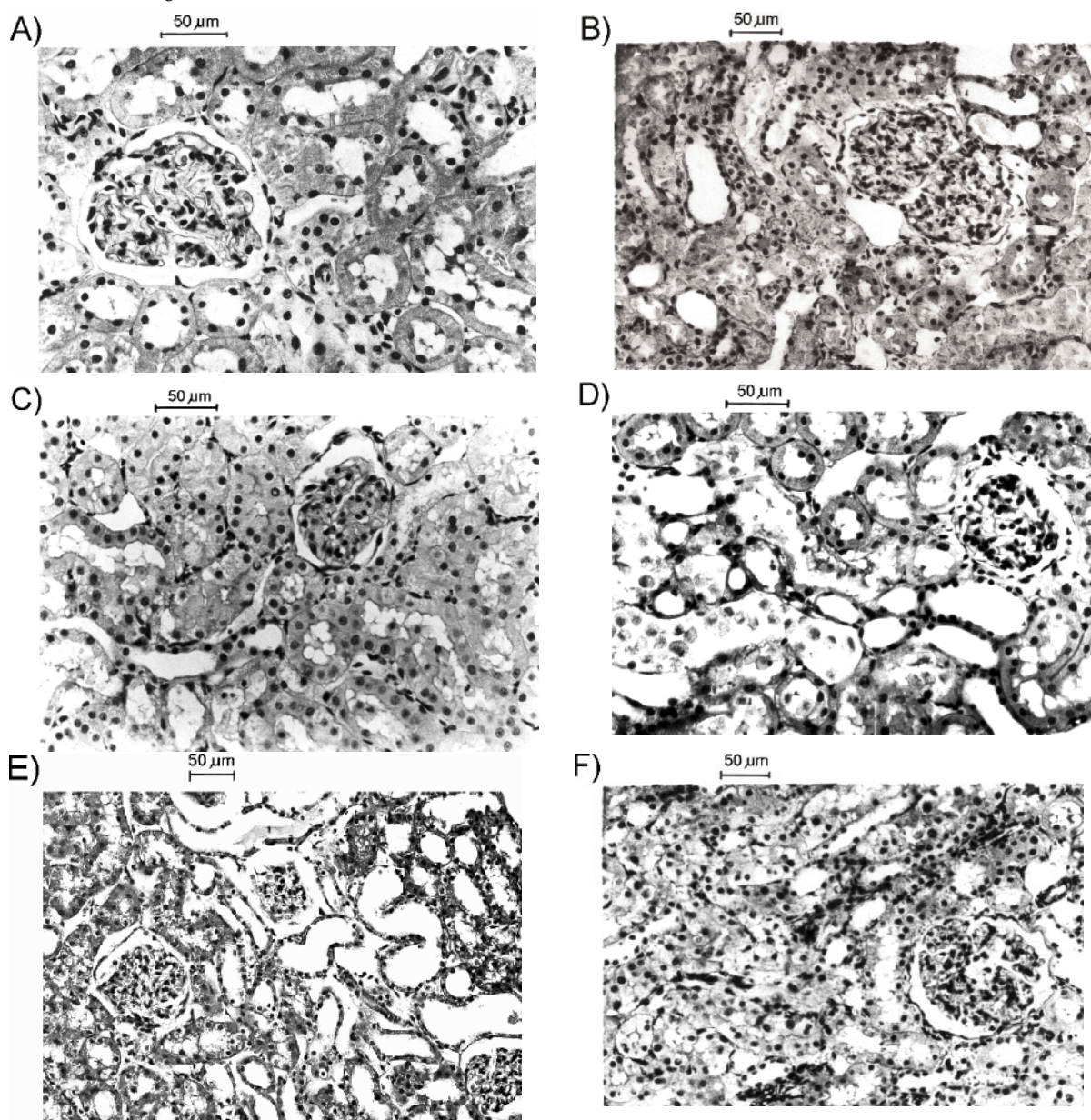When we compared γGT excretion among ARF groups, we found that only its value in the ARF + B

Fig. 2. (**A**) Sham: normal glomerulus and tubuli (×320). (**B**) ARF: dilatation of tubuli, intensive interstitial oedema and widespread areas of tubular necrosis in the cortico-medullary zone (×250). (**C**) ARF + bosentan: no tubular dilatation and no interstitial oedema (×320). (**D**) ARF + losartan (10 mg/kg body weight): dilatation of tubuli, slight interstitial oedema, and areas of tubular necrosis in the cortico-medullary zone (×320). (**E**) ARF + losartan (5 mg/kg body weight): tubular dilatation, interstitial oedema, areas of tubular necrosis in the cortico-medullary zone and hyalin intratubular casts (×200). (**F**) ARF + bosentan + losartan: oedema and vacuolization of proximal tubular epithelial cells, slight dilatation of tubuli and focal interstitial oedema with a small amount of mononuclear leukocytic infiltration (×250).

group was significantly less than in the control ARF group. In this (ARF + B) group, the enzyme value was not significantly different from that in the sham-operated group, and was significantly lower than that in the ARF + B + L group.

Excretion of ALP was significantly less in both ARF + B and ARF + L-10 mg groups than in the control ARF group. However, the enzyme value in the ARF + L-5 mg group was not different from that in the ARF group. The enzyme excretion in the ARF + B + L group was higher than that in all other groups.

Excretion of LDH was also lower in the ARF + B group, but in this case the difference did not reach statistical significance. Urinary LDH excretion was significantly higher in the ARF + B + L group than in all other groups.

ET and PRA measurements. We found that 24 h after induction of ARF, plasma ET level and PRA increased by 85 and 76%, respectively. In the ARF + B group, we noticed a further increase of the plasma level of ET, as a consequence of ET receptor blockade (Table 3).

Table 2. Urinary enzymes during 8 days after ARF or sham operation

Days	Sham	ARF	ARF + B	ARF + L-10 mg	ARF + L-5 mg	ARF + B + L
γGT in urine (mU/mg of creatinine)						
1	155 ± 13	776 ± 128*,#	337 ± 51§	575 ± 95*	662 ± 168*	791 ± 130*,#
2	32.8 ± 3.8	103 ± 18*,#,‡	14.4 ± 5.3§,‡	55.3 ± 9.7#	62 ± 14#	96 ± 14*,#,‡
4	30.8 ± 4.3	76.4 ± 6.4*,#,‡	14.3 ± 5.0§	29.3 ± 2.2	23.3 ± 6.6§,†	49 ± 5.1*,§,#,‡
6	52 ± 13	114 ± 15	39.1 ± 7.7§	61 ± 16	60 ± 27	59 ± 14
8	63 ± 18	126 ± 28	28 ± 13§	66 ± 20	91 ± 25	86 ± 11
LDH (EC 1.1.1.27) in urine (mU/mg of creatinine)						
1	9.2 ± 1.7	72 ± 12†	29.8 ± 2.7†	91.5 ± 9.9†	159 ± 19†	881 ± 204*,§,#,‡
2	9.5 ± 1.7	37.5 ± 3.7*,#,‡	9.8 ± 1.4§,‡	22.5 ± 1.3*,#	14.9 ± 1.6§,‡,†	43 ± 4.2*,#,‡
4	7.57 ± 0.87	23.6 ± 2.3*,#,‡	3.23 ± 0.21§,†	6.09 ± 0.71§,†	4.38 ± 0.62§,†	10.3 ± 1.6§
6	6.2 ± 1.2	29.1 ± 2.0*,#,‡	4.63 ± 0.72§,†	8.4 ± 1.6§,†	4.56 ± 0.86§,†	37 ± 13*,#,‡
8	5.8 ± 1.0	4.5 ± 1.1	4.40 ± 0.53	5.12 ± 0.62	9.1 ± 1.6*,#,‡	7.9 ± 1.1
ALP (EC 3.1.3.1) in urine (mU/mg of creatinine)						
1	6.9 ± 1.5	34.1 ± 2.4*,#,‡,†	8.3 ± 1.0§,†	14 ± 1.1§,†	30 ± 3.8†	93 ± 6.0*,§,#,‡
2	1.64 ± 0.24	11.20 ± 0.81*,#,‡,†	4.69 ± 0.92*,§,‡	7.60 ± 0.70*,#	7.1 ± 1.1*,§,†	24 ± 1.1*,§,#,‡
4	1.30 ± 0.37	4.72 ± 0.68	4.7 ± 1.3	5.71 ± 0.32	4.4 ± 1.3	18 ± 8.9*
6	1.77 ± 0.42	3.3 ± 1.0	4.9 ± 1.5	5.4 ± 1.2	3.37 ± 0.56	8.6 ± 5.1
8	2.61 ± 0.42	5.9 ± 2.5	4.7 ± 1.4	5.23 ± 0.57	7.8 ± 2.2	13.9 ± 6.2

Sham-operated rats; control rats with ARF; bosentan-treated ARF rats (ARF + B); ARF rats treated with losartan (ARF + L-10 mg and ARF + L-5 mg); and ARF rats treated with both bosentan and losartan (ARF + B + L). *$P < 0.05$ *vs* sham; §$P < 0.05$ *vs* ARF; #$P < 0.05$ *vs* ARF + B; ‡$P < 0.05$ *vs* ARF + L-10 mg; †$P < 0.05$ *vs* ARF + B + L.

Table 3. Plasma levels of endothelin and ANG I 24 h after ARF or sham operation

	ET (fmol/ml)	ANG I (ng/ml/h)
Sham ($n = 8$)	16.0 ± 4.4	3.6 ± 1.3
ARF ($n = 8$)	29.5 ± 4.1	6.34 ± 0.38†
ARF + B ($n = 8$)	47 ± 16	3.40 ± 0.50†
ARF + L-10 mg ($n = 8$)	9.0 ± 2.8#	17.5 ± 1.8*,§,#
ARF + B + L ($n = 8$)	33.4 ± 11.2	17.9 ± 2.7*,§,#

*$P < 0.05$ *vs* sham; §$P < 0.05$ *vs* ARF; #$P < 0.05$ *vs* ARF + B; †$P < 0.05$ *vs* ARF + L-10 mg.

However, PRA in rats of this group was not different from that in sham-operated rats. In the ARF + L-10 mg rats, PRA was significantly higher than in the ARF group, due to blockade of AT_1 receptors. However, the plasma level of ET was found to be the lowest in rats of this group, thus suggesting that ET-1 can be released by ANG II [14]. Finally, the plasma level of ET in the ARF + B + L group was practically the same as in the ARF group, whereas PRA was similar in ARF + B + L and ARF + L-10 mg groups (Table 3).

Results from the follow-up study

Survival rate. We followed-up the survival rate of animals from all groups during 8 days after ischaemic injury (Figure 3A). Almost all rats from the sham-operated and ARF + B groups were alive at the end of experiment. Animals from both control ARF and ARF + L-10 mg groups had a survival rate of ∼75%. Rats from the ARF + L-5 mg group had a better survival rate (87.5 %) than rats from the last two groups but only during the first 4 days. In the ARF + B + L group, a few animals survived to the end of the experiment.

Renal function. Urine flow continued to rise on the second day of our study, reaching the respective maximal values in all groups (results not shown) with the exception of the ARF + B + L group. In this last group, the highest urine flow was found on the fourth day after the ischaemic challenge. However, no difference in urine flow was found between the groups until the end of the study except, again, in the case of the ARF + B + L group. In this last group, urine excretion was higher than in the sham and ARF + B group on the sixth day of the study.

Creatinine clearance rose continuously in all ARF groups (with respect to their own values 24 h after ARF), and the highest values were observed in bosentan-treated rats. On the second day of the study, the GFR in the ARF + B group was significantly higher than in all other ARF groups (Figure 4A). No difference in GFR was found between the two losartan-treated groups at any time point.

The plasma creatinine level was found to be lower in the bosentan-treated ARF group on the second day of the study than in all other ARF groups and was very close to the creatinine level in the sham group (Figure 4B). On the fourth day of the study, plasma creatinine concentration decreased in all ARF groups, being highest in the ARF + B + L and ARF control group.

The fractional excretion of sodium (FE_{Na+}) decreased in all ARF groups 2 days after ischaemic challenge, with the exception of the ARF + L-5 mg and ARF + B + L group. On the second day, FE_{Na+} was least in the ARF group treated with bosentan in comparison with all other ARF groups (Figure 3B). FE_{Na+} slowly decreased in all ARF groups towards the end of the study, being the highest all the time in the ARF + B + L group.

Excretion of urinary enzymes. Excretion of all three urinary enzymes drastically decreased on the second
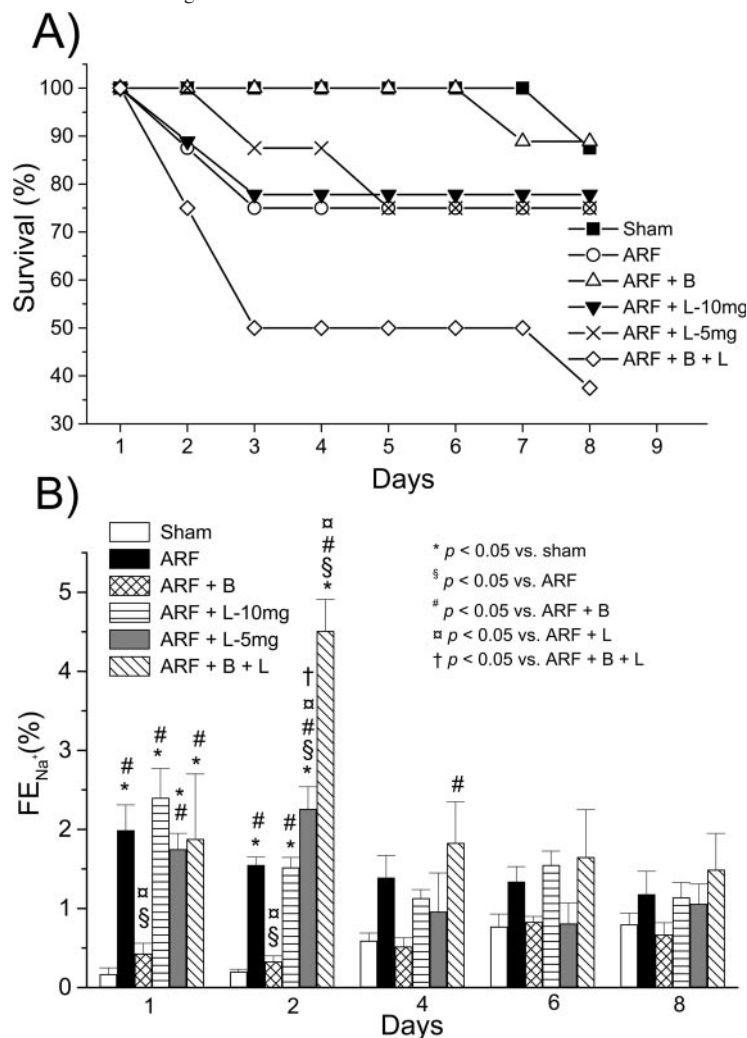
Fig. 3. (**A**) Survival and (**B**) fractional excretion of sodium (FE_{Na+}) in: sham-operated rats; control rats with acute renal failure (ARF); bosentan-treated ARF rats (ARF + B); ARF rats treated with losartan (ARF + L-10 mg and ARF + L-5 mg); and ARF rats treated with both bosentan and losartan (ARF + B + L). As for significance level, $P < 0.05$ *vs* ARF + L refers to the ARF + L-10 mg group.

day of the study in all ARF groups (Table 2). On this day, the excretion of enzymes in the ARF + B group was lower than in all other ARF groups. The observed difference was found to be insignificant only in the case of ALP and LDH values, compared between the ARF + B and ARF + L-5 mg group. During the whole study, urinary excretion of all enzymes was less in the ARF + B group and most of the time it was significantly different from the ARF group. Urinary enzyme excretion was the highest in the ARF + B + L group during all follow-up, while no significant difference in excretion of urinary enzymes was found between the two losartan-treated groups.

Discussion

The results of the present study demonstrate that bosentan, a mixed ET_A and ET_B receptor antagonist, has a more beneficial effect on the early stages of post-ischaemic experimental ARF than the AT_1 receptor blocker losartan. The survival rate is remarkably higher in bosentan-treated rats than in both rat groups treated with losartan. Bosentan improves both renal haemodynamic and functional parameters when administered before and during ischaemia. The protective effects of bosentan in ARF are also confirmed by our findings that renal tubular lesions in animals treated with bosentan were less severe than those in the ARF group.

In selecting blockers of ET receptors, we chose to block both ET_A and ET_B receptors because both of them participate in the pathogenesis of ARF [9]. Bird *et al.* [7] compared the effects of the ET-converting enzyme inhibitor (phosphoramidon) and the ET_A receptor antagonist (BMS-182874) on the rat kidney that underwent a 30 min ischaemia. They found that infusion of phosphoramidon protected the function and structure of the kidney more than treatment with BMS-182874. They concluded that both ET_A and non-ET_A receptors mediate ET-induced changes in ischaemic renal failure. More recently, Nishida *et al.* [15] evaluated the role of ET_B receptors in the
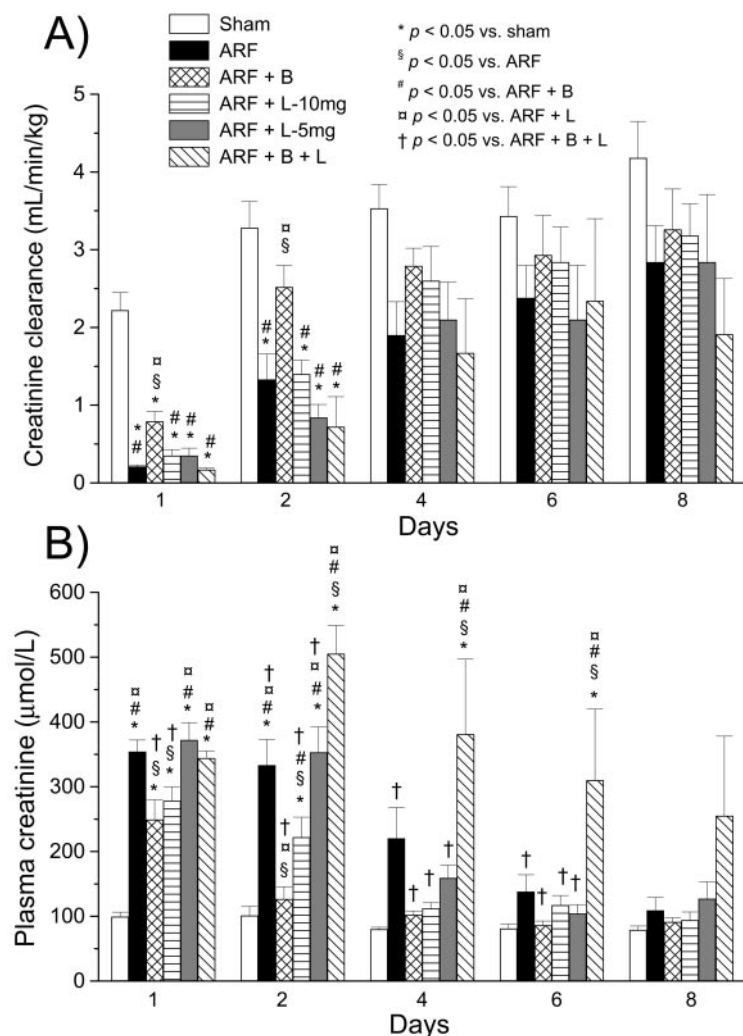
Fig. 4. (**A**) Creatinine clearance and (**B**) plasma creatinine in: sham-operated rats; control rats with acute renal failure (ARF); bosentan-treated ARF rats (ARF + B); ARF rats treated with losartan (ARF + L-10 mg and ARF + L-5 mg); and ARF rats treated with both bosentan and losartan (ARF + B + L). As for significance level, $P < 0.05$ vs ARF + L refers to the ARF + L-10 mg group.

pathogenesis of ARF using 'rescued' ET_B receptor-deficient rats. The authors suggest that ET_B receptors are involved in recovery from ischaemia–reperfusion renal injury though they do not seem to play an important role in the development of ischaemic renal failure.

In the present study, we found that systemic haemodynamic parameters changed neither after infusion of bosentan nor 24 h later in rats that underwent post-ischaemic ARF. However, 24 h after reperfusion, RBF was significantly higher in bosentan-treated animals than in ARF rats. This augmentation of RBF is a consequence of a marked decrease of RVR in the bosentan-treated group, probably because bosentan blocks the renal haemodynamic effects of ET-1. Besides, in relation to this improvement of renal haemodynamics, plasma creatinine became significantly lower, while creatinine clearance (used as an estimation of GFR) became more than three times higher in the ARF + B group than in the group with ARF only. On the second day of the study, the GFR was also higher in the bosentan-treated group than in

all other ARF groups. In addition, FE_{Na+} and urinary excretion of γGT, ALP and LDH was markedly reduced in ARF + B compared with the ARF group throughout the study. These data, together with the histopathological findings, suggest that treatment with bosentan reduces tubular cell injury in ARF. Recent results of Wilhelm et al. [16] may contribute to the understanding of our latter findings. These authors reported a marked increase of ET-1 in the peritubular capillary network of ischaemic kidneys and suggested that ET-induced intrarenal vasoconstriction might have a pathophysiological role in ischaemic acute tubular necrosis.

To summarize, bosentan reduces tubular and haemodynamic changes observed in the early phase of acute post-ischaemic renal failure. The results that we obtained using bosentan are in agreement with findings of Kusumoto et al. [9]. These authors studied the effects of another mixed ET receptor antagonist, TAK-044, on the course of ARF induced by clamping the left renal pedicle for 45 min. They showed that i.v.

administration of TAK-044 (1–10 mg/kg body weight) prior to renal occlusion attenuated the increase of creatinine in plasma and the severity of the morphological damage to the kidney 48 h after ischaemia. Herrero *et al.* [17] investigated the potential protective effect of bosentan against cold ischaemia–reperfusion injury in Lewis rats. They concluded that bosentan is effective in protecting kidneys from both renal function deterioration and tubular necrosis in cold ischaemia–reperfusion damage and that it might be useful in clinical renal transplantation. Nevertheless, Gellai *et al.* [18] found that SB 209670 (another mixed ET receptor antagonist) had no effect in Sprague–Dawley rats when given before, during and after 45 min of renal ischaemia. However, when infused at 30 µg/kg/min for 3 h on the day after ischaemia, it markedly increased the survival rate (by 75%) in rats with severe ARF. The authors concluded that ET receptor antagonists might be more effective in reversing than in preventing renal impairment after ischaemia. We surmise that the discrepancy between their findings and ours most probably stems from differences between rat strains used and the choice of a different double ET receptor antagonist. However, Huang *et al.* [19] recently showed that both non-selective (SB209670) and ET_A-selective (UK-350,926) ET antagonists ameliorated 30 min ischaemia injury in Wistar rats when given in the peri-ischaemic period but not when given 1 h after the ischaemic period. The authors suggest that these compounds might be effective in prevention but not in reversion of ARF, which is in agreement with our findings.

All these data suggest a major role for ET in the early phase of the damage induced in the kidney by ischaemia and the subsequent reperfusion.

The role of the RAS in renal ischaemic injury also remains incompletely understood. In our experiments, we blocked AT_1 receptors with losartan because most effects of ANG II are mediated by those receptors. The AT_1 receptors are responsible for virtually all known 'classical' effects of the RAS such as vasoconstriction, renal salt and water retention, central osmo-control and stimulation of cell growth [20]. Our data reveal that losartan failed to reduce most of the damage to the kidney structure associated with post-ischaemic ARF. Kontogiannis and Burns [11] demonstrated that renal ischaemia–reperfusion injury caused an early increase of intrarenal ANG II levels, associated with a reduction of mRNA for angiotensinogen and proximal tubular AT_1 receptors. They found that blockade of AT_1 receptors with losartan accelerated recovery of renal function after bilateral renal pedicle occlusion for 60 min, i.e. caused a significant decrease in serum creatinine 72 h after reperfusion. The authors suggested that AT_1 receptor blockade increases the GFR by improving renal haemodynamics. Our results only show a small, non-significant rise in RBF in the group treated with losartan 24 h after a 45 min renal ischaemia. We think that the reasons that explain the different findings between our study and theirs are that they used a different model of ARF (bilateral renal pedicle occlusion for 60 min), a different rat strain (Sprague–Dawley), as well as a different dose and time course of losartan application (25 mg/kg s.c. starting at the time of reperfusion).

Losartan had a greater effect on systemic haemodynamics in ARF than bosentan. Since the blood pressure reduction was different between the groups of rats treated with 10 mg/kg body weight of losartan and bosentan-treated rats only at the end of infusion, we added a new group of animals (ARF + L-5 mg group) in which MAP reduction was similar between losartan- and bosentan-treated rats. However, our data show that no losartan dose used had considerable beneficial effects on intrarenal haemodynamic and functional parameters in ARF, and had almost no effect on tubular necrosis induced by ischaemia–reperfusion. In agreement with our results, Kim *et al.* [21] recently reported that pre-treatment with enalapril and losartan did not prevent the reduction of GFR 24 h after ischaemic ARF, suggesting that ANG II was not involved in this reduction.

Blockade of AT_1 receptors had a noticeable natriuretic effect in our experimental conditions. FE_{Na+} was markedly higher in losartan-treated rats than in untreated ARF rats during 48 h after ischaemia. Most probably this increase in urinary sodium excretion in losartan-treated groups is not caused by haemodynamic effects but by the blockade of tubular AT_1 receptors. Thus, Navar *et al.* [22] demonstrated the presence of angiotensinogen and angiotensinogen mRNA in proximal tubule cells, which indicates that ANG II or precursors of ANG II are secreted directly into the proximal tubular lumen by the epithelial cells. Micropuncture studies by these authors provided direct evidence that activation of intraluminal AT_1 receptors by ANG II exerts a substantial stimulatory influence on sodium transport by both proximal and distal tubules.

We found PRA to be significantly increased in the ARF groups treated with higher losartan dose in comparison with all other groups, probably as a consequence of the AT_1 receptor blockade. In contrast, the plasma ET level was markedly reduced in ARF rats treated with losartan, being more than three times lower than in control ARF rats (Table 3). It is well known that ANG II stimulates the expression of the ET-1 gene in endothelial and renal cells. Yanagisawa *et al.* [6] demonstrated that in $HgCl_2$-induced ARF, the gene expression of ET-1 is at least partly upregulated at the transcriptional level by endogenous ANG II. Thus, ANG II inhibitors or its receptor antagonists block this stimulatory effect of ANG II on ET-1 synthesis. Our results show that the same effect occurs under conditions of ischaemia-induced ARF.

On the other hand, in the group of ARF rats treated by both bosentan and losartan, ARF was not improved at all 24 h after reperfusion. In our opinion, the main reason underlying this finding is the fact that concurrent administration of bosentan and losartan causes a significant drop in MAP, which was observed not only during the infusion of these substances but also

after 24 h. The hypotensive effect of the combination of bosentan and losartan was not associated with any significant changes of CO or HR, and therefore was probably due to a decrease of total peripheral resistance. Richard *et al.* [23] reported very recently that bosentan did not affect arterial blood pressure in rats in control conditions. In contrast, in losartan-pre-treated rats, bosentan induced a marked, dose-dependent decrease in arterial pressure. The authors concluded that blockade of AT_1 receptors unmasks a vasodilator response to ET antagonists.

In summary, our results clearly indicate that non-selective blockade of ET receptors with bosentan produces a more favourable effect on the early course of ischaemic ARF than AT_1 receptor blockade with losartan. Concomitant blockade of both ET and AT_1 receptors does not improve post-ischaemic ARF because concurrent administration of both receptor antagonists induces a significant decrease of blood pressure. These results strongly suggest that ET-1 has a more important role in the early phases of ischaemia–reperfusion injury than ANG II.

The fact that we applied bosentan before, during and after the ARF induction limits the potential clinical applicability of our results, i.e. in patients with ARF. Further limitations arise from the fact that the rat model of ARF is polyuric whereas in the most frequently observed clinical situation, ARF is oliguric. However, in the search for distinctive relative roles of several mediators in the complicated mechanisms involved in the induction and initial development of ARF, these results can be very useful. In addition, bosentan could be used to prevent ischaemia–reperfusion-associated damage in kidneys obtained for transplantation.

Conflict of interest statement. None declared.

References

1. Lieberthal W. Biology of acute renal failure: therapeutic implications. *Kidney Int* 1997; 52: 1102–1115
2. Ishikawa I, Harris RC. Angiotensin actions in the kidney: renewed insights into the old hormone. *Kidney Int* 1991; 4: 583–596
3. Katoh T, Chang H, Uchida S, Okuda T, Kurokawa K. Direct effects of endothelin in the rat kidney. *Am J Physiol* 1990; 258: F397–F402
4. López-Farré A, Gómez-Garre DN, Bernabeu F, Montañés I, Millás I, López-Novoa JM. Renal effects and mesangial cell contraction induced by endothelin are mediated by PAF. *Kidney Int* 1991; 39: 624–630
5. Stein HJ, Lifschitz DM, Barnes BL. Current concepts on the pathophysiology of acute renal failure. *Am J Physiol* 1978; 234: F171–F181
6. Yanagisawa H, Nodera M, Umemori Y, Shimoguchi Y, Wada O. Role of angiotensin II, endothelin-1, and nitric oxide in $HgCl_2$-induced acute renal failure. *Toxicol Appl Pharmacol* 1998; 152: 315–326
7. Bird JE, Webb ML, Wasserman AJ, Liu ECK, Giancarli MR, Durham SK. Comparison of a novel ETA receptor antagonist and phosphoramidon in renal ischemia. *Pharmacology* 1995; 50: 9–23
8. Gellai M, Jugus M, Fletcher T, De Wolf R, Nambi P. Reversal of postischemic acute renal failure with a selective endothelin A receptor antagonist in the rat. *J Clin Invest* 1994; 93: 900–906
9. Kusumoto K, Kubo K, Kandori H *et al.* Effects of a new endothelin antagonist, TAK-044, on post-ischemic acute renal failure in rats. *Life Sci* 1994; 55: 301–310
10. Binet I, Wallnöfer A, Weber C, Jones R, Thiel G. Renal hemodynamics and pharmacokinetics of bosentan with and without cyclosporine A. *Kidney Int* 2000; 57: 224–231
11. Kontogiannis J, Burns KD. Role of AT1 angiotensin II receptors in renal ischemic injury. *Am J Physiol* 1998; 274: F79–F90
12. Sušić D, Mandal AK, Kentera D. Heparin lowers the blood pressure in hypertensive rats. *Hypertension* 1982; 4: 681–685
13. Jerkic M, Varagic J, Jovovic D *et al.* L-Arginine reduces tubular cell injury in acute postischemic renal failure. *Nephrol Dial Transplant* 1999; 14: 1398–1407
14. Allred AJ, Chappell MC, Ferrario CM, Diz DI. Differential actions of renal ischemic injury on the intrarenal angiotensin system. *Am J Physiol* 2000; 279: F636–F645
15. Nishida M, Ieshima M, Konishi F, Yamashita J, Takaoka M, Matsumura Y. Role of endothelin B receptor in the pathogenesis of ischemic acute renal failure. *J Cardiovasc Pharmacol* 2002; 40: 586–593
16. Wilhelm SM, Simonson MS, Robinson AV, Stowe NT, Schulak JA. Endothelin up-regulation and localization following renal ischemia and reperfusion. *Kidney Int* 1999; 55: 1011–1018
17. Herrero I, Torras J, Riera M *et al.* Prevention of cold ischemia–reperfusion injury by an endothelin receptor antagonist in experimental renal transplantation. *Nephrol Dial Transplant* 1999; 14: 872–880
18. Gellai M, Jugus M, Fletcher T *et al.* Nonpeptide endothelin receptor antagonists. V: prevention and reversal of acute renal failure in the rat by SB 209670. *J Pharmacol Exp Ther* 1995; 275: 200–206
19. Huang C, Huang C, Hestin D *et al.* The effect of endothelin antagonist on renal ischaemia–reperfusion injury and the development of acute renal failure in the rat. *Nephrol Dial Transplant* 2002; 17: 1578–1585
20. Berry C, Touyz R, Dominiczak AF, Webb RC, Johns DG. Angiotensin receptors: signaling, vascular pathophysiology, and interactions with ceramide. *Am J Physiol* 2001; 281: H2337–H2365
21. Kim SJ, Lim YT, Kim BS *et al.* Mechanism of reduced GFR in rabbits with ischemic acute renal failure. *Renal Fail* 2000; 22: 129–141
22. Navar LG, Harrison-Bernard LM, Wang CT, Cervenka L, Mitchell KD. Concentrations and actions of intraluminal angiotensin II. *J Am Soc Nephrol* 1999; 10 [Suppl 11]: S189–S195
23. Richard V, Hogie M, Thuillez C. Angiotensin II receptor blockade unmasks a depressor response to endothelin antagonists in rats. *Fund Clin Pharmacol* 2000; 14: 101–106