The angiotensin converting enzyme inhibitor enalapril in acute ischemic renal failure in rats

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Summary. The influence of the renin-angiotensin system on renal hemodynamics, tubular pressure and tubulo-glomerular feedback was investigated with the angiotensin converting enzyme inhibitor MK 421 (enalapril), in uninephrectomized rats with and without ischemia-induced acute renal failure. In animals with normal renal function proximal tubular pressure and tubulo-glomerular feedback response were lowered by enalapril long-term treatment, whereas glomerular filtration rate and renal blood flow were not influenced by the drug.
After 45 and 70 minutes ischemia there was no difference between treated and untreated animals in the severely impaired glomerular filtration rate. Renal blood flow remained unaffected by the treatment. The histological damage due to ischemia (tubular casts, tubular necrosis and medullary capillary congestion) was not influenced by enalapril. As tubulo-glomerular feedback had been significantly inhibited during renin-angiotensin inhibition, its importance in mediating acute renal failure remains doubtful; other factors such as tubular obstruction and medullary congestion may be crucial.
Key words. Acute ischemic renal failure; angiotensin converting enzyme inhibition; enalapril; tubulo-glomerular feedback/proximal tubular pressure; renal histology; medullary capillary congestion.

Research work devoted to the mechanisms of acute renal failure has implicated the renin-angiotensin system and the tubulo-glomerular feedback as pathogenetic mediators. The presence of the intrarenal renin angiotensin system[2], the juxtaglomerular and macula densa cells favor this concept. In acute renal failure the elevated plasma renin activity and the enlarged juxtaglomerular apparatus indicate an activation of the renin-angiotensin system. This may in fact play a crucial role, for example by maintaining blood pressure, adjusting renal blood flow[1], modulating effective glomerular filtration pressure[4, 5, 15] and regulating tubulo-glomerular feedback[12, 16]. According to Thurau[18], the tubulo-glomerular feedback regulates the glomerular filtration rate. In renal failure, diminished tubular reabsorption leads to increased flow at the macula densa which decreases the glomerular filtration rate by mediation of the renin-angiotensin system. Accordingly, reduction of renin release by administration of NaCl or deoxy-corticosterone acetate protects against renal failure[3]. If renin is essential in the pathogenesis of renal failure, inhibition of renin release, inhibition of angiotensin conversion or action of angiotensin II would be expected to improve renal function. This hypothesis has been tested with negative results using angiotensin II immunization[9] and angiotensin antagonists[13, 17]. However, both methods are directed mainly against circulating angiotensin II, and the agents may not have reached the juxtaglomerular cells. Using angiotensin converting enzyme inhibitors (ACEI), the results were conflicting[6–8, 10, 11, 14]. In two studies[11, 14], the ACEI SQ 20'881 i.v. reduced feedback response, while in another one[8], captopril given i.a. after renal ischemia did not inhibit tubulo-glomerular feedback.
In the present study, the effect of a more potent ACEI, MK 421 (enalapril maleate), on tubulo-glomerular feedback, renal blood flow and glomerular filtration rate was investigated in animals with and without ischemic renal damage.
Material and methods. Animals and diet. Female Wistar rats weighing 150–250 g were used for most experiments. Male rats of similar size were used for micropuncture studies. Animals were fed ad libitum either standard rat chow (Nafag 185, Gossau, Switzerland) containing 5% of NaCl or an otherwise similar low NaCl diet containing less than 0.17% of NaCl. The rats had free access to water.
Drug treatment. Enalapril maleate (MK 421) was obtained from Merck, Sharpe & Dohme, Zurich, Switzerland. In short-term studies, the drug was given by gavage at a dose of 5 mg/kg, starting 1 h prior to initiation of renal ischemia. This was repeated every 8 h. In long-term studies, enalapril treatment was started at least 14 days before the experiments. The drug was added to the drinking water (150 mg/l); the resulting daily dose was 15–30 mg/kg.
Measurement of tubulo-glomerular feedback and proximal tubular pressure. This was done according to a previously described method[9]. Under thiobarbital anesthesia (Inactin®, 110 mg/kg i.p.), the left kidney was fixed and embedded. Tubules were punctured with 2–14 µm glass capillaries and pressure recorded with a servo-null recording device. For stop flow pressure (SFP) measurements, an oil block was filled into the proximal tubule, and the loop of Henlé was perfused with Ringer solution at a rate of 0, 20 or 40 nl/min. The tubulo-glomerular feedback (TGF), expressed as △% TGF, was calculated from the SFP using the equation △% TGF = $(SFP_{40} - SFP_0) \times 100/SFP\ 0$, where SFP_0 is the SFP_0 without perfusion and SFP_{40} is the SFP during perfusion at a rate of 40 nl/min.
Ischemia-induced acute renal failure. A right unilateral nephrectomy was performed under ether anesthesia. Not earlier than 14 days later, the animals were anesthetized with pentobarbital (Nembutal®, 35 mg/kg i.p.). The body temperature was kept constant at 38 °C. After i.v. injection of heparin (100 U/kg) into treated and untreated animals the left renal artery was occluded for 45 or 70 min.
Animals with 45-min ischemia time were kept in metabolic cages. Creatinine clearance was determined on days 1 and 2 after renal ischemia. Creatinine was measured with a Beckman Auto Analyzer. In animals with 70-min ischemia time, preparation was started 3 or 24 h later for hemodynamic, clearance and micropuncture studies. Under thiobarbital anesthesia, tracheostomy was performed, and two jungular vein catheters were inserted. In addition, the femoral artery and the left ureter were cannulated. An electromagnetic square wave flowmeter (Carolina Medical Electronics) was placed around the left renal artery. Fluid loss was replaced by injection of 0.5 ml of normal saline, followed by infusion of 9 ml/kg/h. Measurements were started 1 h after completion of surgery.
Creatinine clearance was determined during 120 min in 20-min collecting periods.
Control animals were sham-operated using the same procedure except for renal arterial clamping.
Assessment of ACEI. Measurement of arterial blood pressure after i.v. bolus injection (96 ng/kg) of angiotensin I was used to assess the systemic action of enalapril. The dose of angiotensin I was chosen on the basis of a dose-response curve carried out in untreated animals (fig. 1).
Histologic examination. The kidneys were rapidly removed and fixed in 4% buffered (pH 7,4) formalin, and the tissue was embedded in paraplast. Sections (4 µm) were stained with HE, CAB and PAS.
Statistics. All results are given as mean ± SEM. Student's t-test was used to assess statistical significance.

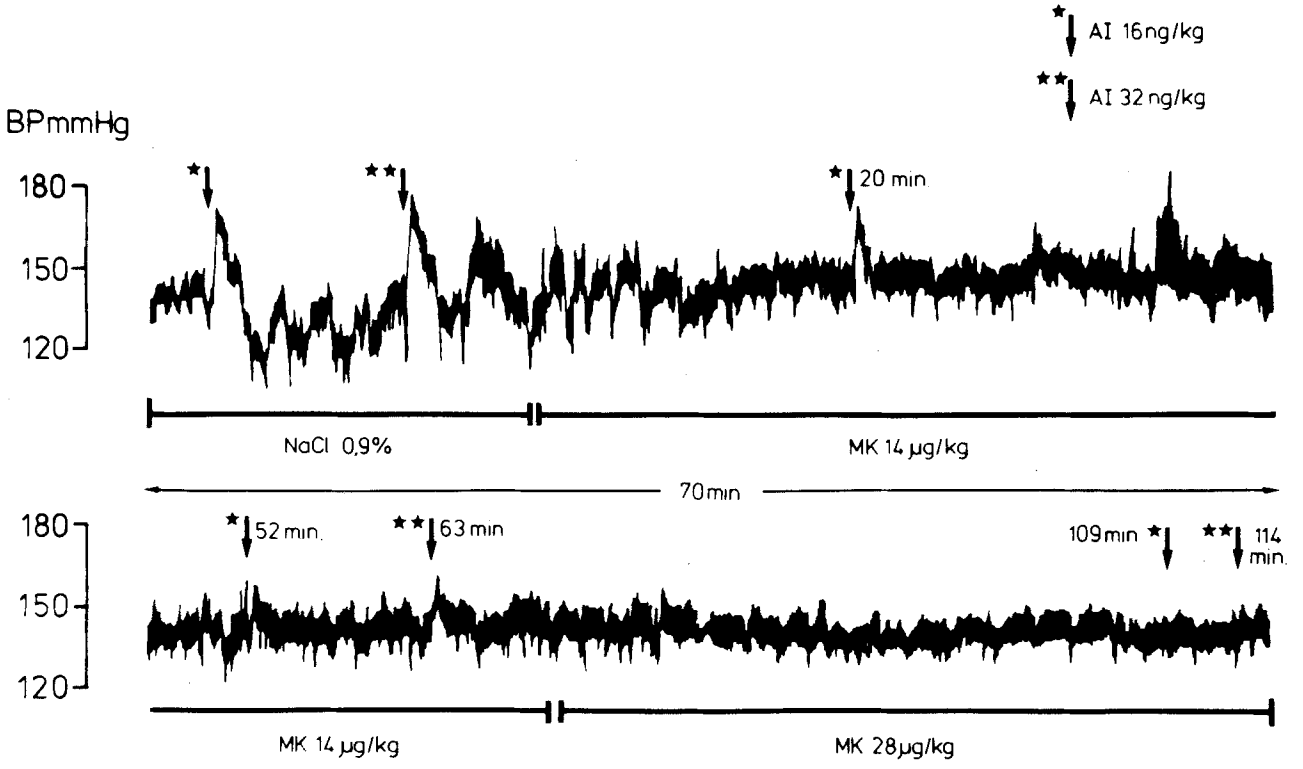

Figure 1. BP response to angiotensin I bolus injection during infusion with NaCl and NaCl/MK 421.

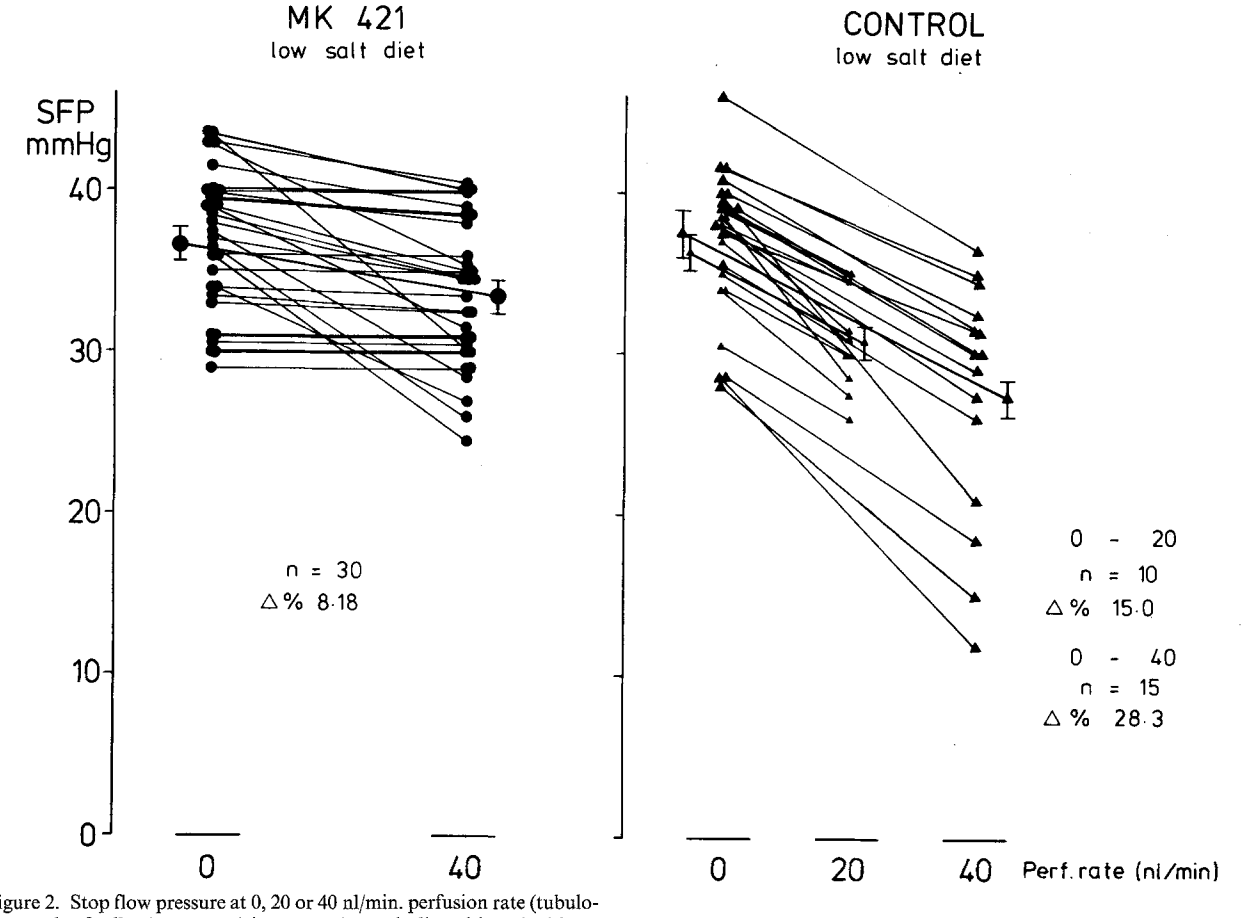

Figure 2. Stop flow pressure at 0, 20 or 40 nl/min. perfusion rate (tubuloglomerular feedback response) in rats on low-salt diet with and without (control) MK 421 treatment.

Results. Effect of angiotensin I during enalapril treatment. Bolus injection of angiotensin I was followed by a marked rise of arterial blood pressure in untreated rats. A typical example is shown in figure 1. The angiotensin I-induced increase of blood pressure was abolished in all animals after enalapril treatment.

Animals with normal renal function. Stop flow pressure, tubulo-glomerular feedback and proximal tubular pressure. Enalapril did not significantly affect stop flow pressure on zero tubular perfusion rate (fig. 2). Tubulo-glomerular feedback, however, was decreased by enalapril in animals receiving normal chow (table 1) and it was dramatically depressed after both short-term and long-term treatment and controls averaged 10.8 ± 0.3 mm Hg (measured in 88 tubules of 4 animals) and 13.3 ± 0.3 mm Hg (71 tubules of 3 animals), respectively. This difference was highly significant ($p < 0.001$).

Blood pressure, renal blood flow (RBF) and glomerular filtration rate (GFR). The effect of enalapril on hemodynamics and renal function of unilaterally nephrectomised animals is shown in table 2. Short-term treatment clearly decreased mean arterial blood pressure and glomerular filtration rate, but did not influence renal blood flow. As expected, long-term treatment also decreased blood pressure. It did not, however, lower glomerular filtration rate and renal blood flow (table 2).

Animals with ischemia-induced acute renal failure glomerular filtration rate after 45-min ischemia. Prior to ischemia, control and experimental groups had similar filtration rates (table 3). One day after ischemia, glomerular filtration rate was reduced to almost 25%, with no difference between treatment groups. There was some improvement in filtration one day later, but again independent of treatment.

Blood pressure, renal blood flow, glomerular filtration rate and proximal tubular pressure after 70-min ischemia. As compared to sham-operated animals (table 2), blood pressure and renal blood flow were not significantly altered in animals with renal ischemia (table 4). This was true for animals with and without enalapril treatment. Glomerular filtration was almost abolished by extended ischemia, and there was no evidence of improvement between the early (4–6 h) and late 24 h) phase of ischemic damage. Furthermore, there was no difference between untreated animals and those on short-term or long-term enalapril treatment (table 4). Proximal tubular pressure is shown in figure 3. Late after ischemia, normal and dilated-looking tubules were observed in control animals. Enalapril treatment was followed by arterial hypotension and increase of pressure in dilated tubules and the appearance of many collapsed tubular structures with low pressure values.

Renal artery thrombosis. In initial studies, six out of seven enalapril-treated animals had persistent renal artery thrombosis after clamping for 70 min. This was not observed in untreated rats. After installation of prophylactic heparin treatment, thrombosis did not occur any longer.

Histology. Histological examination after long-term treatment with enalapril revealed dilated glomerular and intertubular capillaries as well as dilated tubules with hyaline drops in the proximal segments. Enalapril and a low NaCl diet combined induced nephrocalcinosis in 3 of 4 animals examined, but this was not observed after drug treatment or diet alone. There was no difference whatsoever in histological findings between control animals and those treated with

Table 1. Effect of MK 421 on tubulo-glomerular feedback in uninephrectomized rats (tubules/animals)

Experimental group	Δ % stop flow pressure with 0–40 nl/min perfusion rate	
	Rats on normal food	Rats on low salt diet [a]
Untreated control rats	−29.7 ± 14.3 (26/12)	−28.3 ± 12.9 (15/3)
MK 421 short treatment	–	−4.5 ± 3.3** (18/3)
MK 421 long treatment	−16.0 ± 9.9** (38/4)	−8.2 ± 10.1** (30/4)

[a]) Salt depletion for 2–4 weeks. ** $p < 0.001$ when compared with untreated control rats.

Table 2. Effect of MK 421 on BP, RBF and GFR in uninephrectomized rats

Experimental group	BP mm Hg	RBF ml·min^{-1}·100 g^{-1}	GFR ml·min^{-1}·100 g^{-1}
Untreated controls (n = 20)	124 ± 11	5.9 ± 2.3 (n = 3)	0.44 ± 0.11
MK 421 short treatment (n = 8)	87 ± 14**	4.4 ± 1.3	0.30 ± 0.14*
MK 421 long treatment (n = 9)	94 ± 12**	4.7 ± 1.1	0.41 ± 0.11

* $p < 0.01$ when compared with untreated control rats; ** $p < 0.001$ when compared with untreated control rats.

Table 3. Effect of 45′ RAO in uninephrectomized rats on GFR

Experimental group	Before ischemia GFR ml·min^{-1}·100 g^{-1}	After ischemia GFR ml·min^{-1}·100 g^{-1}	
		Day 1	Day 2
Controls (n = 6)	0.48 ± 0.03	0.13 ± 0.05	0.20 ± 0.05
MK 421 (n = 6) short- or long-term treatment	0.46 ± 0.03	0.16 ± 0.04	0.22 ± 0.04

Table 4. Effect of 70′ RAO in uninephrectomized rats on BP, RBF and GFR

Experimental group	4–6 h after ischemia			24 h after ischemia		
	BP mm Hg	RBF ml·min^{-1}·100 g^{-1}	GFR ml·min^{-1}·100 g^{-1}	BP mm Hg	RBF ml·min^{-1}·100 g^{-1}	GFR ml·min^{-1}·100 g^{-1}
Untreated controls (n = 10)	115 ± 28	2.9 ± 1.8	0.01 ± 0.02	128 ± 21	3.8 ± 1.1	0.02 ± 0.03
MK 421 short treatment (n = 10)	101 ± 20	3.4 ± 1.6	0.02 ± 0.03	107 ± 20*	4.1 ± 1.1	0.01 ± 0.01
Mk 421 long treatment (n = 10)	–	–	–	89 ± 11**	3.1 ± 1.0	0.02 ± 0.04

* $p < 0.05$ when compared with untreated control rats; ** $p < 0.005$ when compared with untreated control rats.

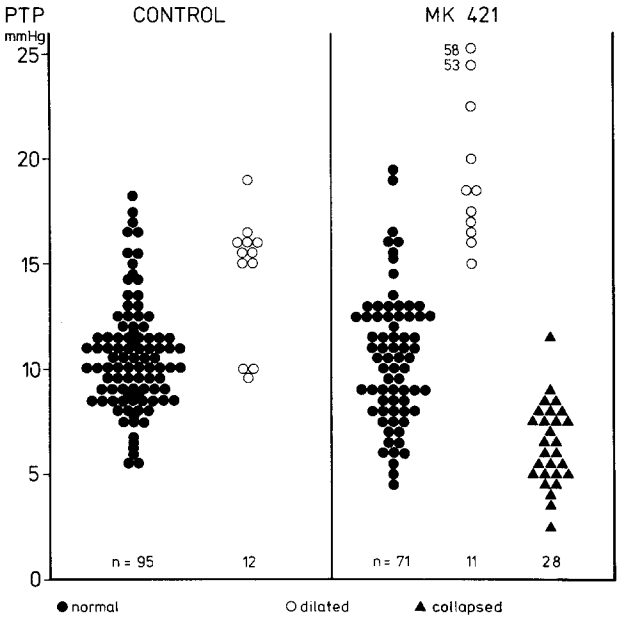

Figure 3. Proximal tubular pressure 24 h after ischemia control (n = 6) and MK-treated (n = 6) animals in normal, dilated and collapsed looking tubules.

enalapril. In the early phase of ischemic renal injury, changes included capillary congestion, which was most marked in the inner stripe of the outer medulla. In the late phase, severe tubular necrosis was seen, localized mainly in the cortex and in the outer stripe.

Discussion. If the renin-angiotensin system and the tubulo-glomerular feedback are in fact the major culprits potentiating ischemic acute renal failure, then inhibition of these factors could be expected to influence the outcome of renal damage. In our experiments, the renin-angiotensin system was fully blocked and the tubulo-glomerular feedback was nearly abolished with a new angiotensin converting enzyme inhibitor, enalapril (MK 421), but no protection by the ACEI could be demonstrated.

First, the administered dose may have been insufficient. We have evidence that this was not the case. The efficacy of our ACEI was tested with an angiotensin I injection at the end of every experiment. The missing rise of blood pressure confirmed the complete blocking of the renin-angiotensin system. Furthermore, the tubulo-glomerular feedback, which is also thought to be mediated by the renin-angiotensin system, was clearly inhibited. Second, the drug may not be specific enough and it may act on targets other than the renin-angiotensin system which could abolish the intended benefit. ACEIs do not only block kininase II, the enzyme cleaving angiotensin I, but they also block the degradation of the vasodilator bradykinin into inactive fragments. Any resulting rise of the kinin level would increase renal blood flow and would therefore potentiate rather than counteract protection by an ACEI. In our experiments, renal blood flow remained constant, but glomerular filtration rate was markedly reduced at the same time. Thus, a major role of the kallikrein-bradykinin system is unlikely.

Third, the ACEI-induced fall in blood pressure may be a factor that in itself worsens renal injury. This may be an explanation for the one group with short-term treatment without ischemia where glomerular filtration rate was slightly reduced parallel with blood pressure, but mean stop flow pressure (reflecting glomerular filtration pressure) was not affected. Furthermore, the situation was different following ischemia where blood pressure was comparable in treated and control animals 4–6 h after reperfusion – yet no protection could be seen. The pressure in dilated, presumably obstructed, tubules was even higher in animals which had received enalapril. Therefore, the weak hypotensive effect as obtained in these studies does not appear to impair glomerular filtration pressure. And finally, too severe ischemic damage may be irreversible and thus resistent to any treatment. This possibility is unlikely on the basis of experiments with a short ischemia time of only 45 min: although the partial recovery of GFR observed in the control group indicated reversibility of the injury within 48 h, treatment did not further improve renal function.

Thus the failure of enalapril to protect from ischemic renal damage suggests that the renin-angiotensin system and the tubulo-glomerular feedback are not the decisive pathogenetic mechanisms[13]. Alternatively, postischemic tubular obstruction may be of prime importance. Evidence for this was obtained both in enalapril-treated and untreated animals from the high proximal tubular pressure in dilated tubules and also in normal looking tubules on the kidney surface. The low pressure in collapsed-looking tubules could be due to leakage from necrotic tubules. Capillary congestion in the inner stripe of the outer medulla may enhance postischemic tubular necrosis; it was unchanged by the ACEI. In summary, enalapril did not afford protection against ischemic renal injury despite significant attenuation of the tubulo-glomerular feedback. The role of tubulo-glomerular feedback as a major factor in ischemic acute renal failure thus appears to be overestimated.

Other mechanisms like tubular obstruction, necrosis and medullary congestion are not influenced by ACEI. They may be more important in the pathogenesis of acute ischemic renal failure.

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