Physiol. Res. 48: 429-435, 1999

Enalapril in Subantihypertensive Dosage Attenuates Kidney Proliferation and Functional Recovery in Normotensive Ablation Nephropathy of the Rat

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Received December 16, 1998
Accepted May 11, 1999

Summary

Most studies on the antiproliferative action of angiotensin converting enzyme inhibitors (ACEI) were performed in a rat hypertensive remnant kidney model with 5/6 kidney ablation which raised objections about the antihypertensive effect of ACEI and the influence of other antihypertensive drugs administered to remnant kidney control rats. To prevent these objections, a normotensive 4/6 remnant kidney model was elaborated and a subantihypertensive dosage of enalapril was used to evaluate its antiproliferative action. Subtotally nephrectomized rats (Nx) markedly increased the remnant kidney weight during a 4-week period and this rise was prevented by the treatment with enalapril (NxE) (Nx +297±35 mg vs. sham-operated +145±32 mg, $p<0.001$; NxE +154±35 mg vs. Nx $p<0.001$). While collagen concentration in the kidney cortex was not increased in sham-operated rats (Sham) in comparison with the control group (Ctrl) at the beginning of the study, the subsequent increase was significant in the Nx group and enalapril did not attenuate this increase (Sham 148±5 mg/100 g w.w. vs. Nx 164±2 mg/100 g w.w., $p<0.01$; NxE 161±4 mg/100 g w.w. vs. Sham $p<0.05$). The tubular protein/DNA ratio increase, which was significant in the Nx group, was inhibited by enalapril (Nx 26.2±10.5 vs. NxE 15.3±2.6, $p<0.05$). The protein/DNA ratio was much lower in glomeruli, with no significant changes in either the Nx or NxE groups. Serum urea concentrations were slightly higher in the Nx group than in the sham-operated group, but markedly elevated in the NxE group (Nx 10.71±0.76 mmol/l vs. Sham 6.10±0.33 mmol/l, $p<0.001$; NxE 28.9±2.6 mmol/l vs. Sham $p<0.001$). Creatinine concentrations in the Nx group were increased in comparison with the sham-operated group and markedly increased in the NxE group (Nx 63.7±3.56 μmol/l vs. Sham 37.2±2.84 μmol/l, $p<0.001$; NxE 107.0±5.2 μmol/l vs. Sham $p<0.001$). The clearance of creatinine was lower in the Nx group than in the sham-operated group and was markedly reduced in the NxE group (Nx 0.89±0.06 ml/min.g kidney wt. vs. Sham 1.05±0.16 ml/min.g kidney wt., $p<0.01$; NxE 0.58±0.029 ml/min.g kidney wt. vs. Sham, $p<0.001$). Enalapril improved proteinuria in comparison with the Nx group (NxE 5.6±0.6 mg/24 h vs. Nx 16.1±3.4 mg/24 h, $p<0.05$). Thus remnant kidney proliferation is substantial even in normotensive rats. It includes both proliferation and collagen accumulation with partial recovery of kidney weight and function, but is accompanied by enhanced proteinuria. Enalapril attenuates the proliferation and decreases proteinuria but prolongs kidney function recovery.

Key words

Remnant kidney model • Ablation nephropathy • Enalapril • Collagen • Proteins • DNA • Proteinuria • Creatinine clearance

Introduction

Angiotensin II (ANG II) exerts two effects in the kidney: a) hemodynamic action (Anderson *et al.* 1985, Zoja *et al.* 1989, Neurigner and Brenner 1993, Rosenberg *et al.* 1994, Harris and Martinez-Maldonado 1995, Junaid *et al.* 1997), influencing especially glomerular hemodynamics. b) proliferation and hypertrophy of mesangial cells and vascular smooth muscle cells with accumulation of mesangial matrix and interstitium (Zoja *et al.* 1989, Wolf and Neilson 1993, Dzau and Re 1994, Harris and Martinez-Maldonado 1995, Junaid *et al.* 1997). Both ACEI and AT_1 receptor antagonists contribute to the antihypertensive and antiproliferative effects as has been shown in clinical as well as in experimental studies (Remuzzi *et al.* 1990, Dubey *et al.* 1992, Kakinuma *et al.* 1992, Lafayette *et al.* 1992, Wolf and Neilson 1993, Dzau and Re 1994, Harris and Martinez-Maldonado 1995).

The antiproliferative effect of ACEI seems to be present even in subantihypertensive doses (Kakinuma *et al.* 1992, Harris and Martinez-Maldonado 1995). This attracts the attention to the possibility of ACEI administration even to normotensive kidney disease patients. Unfortunately, the effect of ACEI in normotensive experimental models has not yet been evaluated. The interference of hypertension can be eliminated just by the administration of other antihypertensive therapy to control rats. However, the objections to this approach are evident. Because of both experimental and clinical relevance of this alternative, we have developed and performed a study on the normotensive remnant kidney model with the administration of ACEI in doses with a slight but insignificant antihypertensive effect. The proliferation was determined by direct chemical analysis but not histochemically.

Material and Methods

Male Wistar rats (Velaz, Prague, 185-205 g initial body weight) were pair-fed with standard rat chow containing approximately 25 % protein. They were randomly divided into the following groups: a) Control group (Ctrl, 10 rats) for the determination of initial values at the beginning of the study, b) sham-operated group (Sham, 10 rats), c) remnant kidney group (Nx, 14 rats) subjected to 4/6 kidney ablation by the removal of both poles of the left kidney and a week later by the removal of the right kidney, and d) remnant kidney group treated with enalapril (50 mg/l in the drinking water) after right kidney ablation (NxE, 17 rats).

Sham-operated and both experimental groups were pair-fed for 4 weeks after right kidney ablation, at which time they were sacrificed and all necessary analyses performed. All surgical procedures and sacrifice were performed under thiopental anesthesia. The study was approved by the Ethics Committee of our Institute for the experimental animals.

Body weight and systolic blood pressure measured by tail plethysmography (Williams *et al.* 1939) were examined on the day of randomization, before any manipulations, and on the day before sacrifice. After the blood pressure determination, the animals were kept in metabolic cages for 24 hours and urine was collected for determinations of protein, thromboxane metabolites, i.e. TXB_2 + 2,3-dinor TXB_2.

Blood was obtained from the abdominal aorta. Afterwards, the left remnant kidney was removed, weighed and cortical tissue samples excised for analyses. The glomeruli and tubuli were separated by the sieving method (Spiro 1967).

Collagen (Reddy and Enwemeka 1996), proteins (Smith *et al.* 1985) and DNA (Harris 1987) were determined by standard methodology, TXB_2 + 2,3-dinor TXB_2 production after spontaneous blood coagulation at 37 °C and in the urine immunochemically by commercial kits (Institute. of Isotopes Ltd. Corp., Budapest, Hungary). Standard chemical analyses were performed by Vitros 250 (Johnson and Johnson, Rochester, NY, USA).

Statistical evaluation of the results was performed by Student's t-test for unpaired values and by the Wilcoxon test. Analysis of variance (ANOVA) was used for comparison of the subgroups. The results were expressed as means ± S.E.M.

Results

Because of the pair-fed schedule, the body weight in all groups increased comparably during the 4-week study (Table 1). The blood pressure slightly increased in all groups; but remained in the normotensive range, the BP increase in the NxE group tended to be somewhat lower.

No weight difference was found between the left and right kidney in a control group of rats at the

beginning of the study. Thus, it was possible to estimate the weight of the remnant kidney after weighing of the removed right kidney and ablated left kidney poles. Just 70 % ablation was performed and the remnant kidney weight was found to be 585±19.7 mg at the beginning of the study.

At the end of the experiment, the kidney weight (Table 2) was still moderately lower in the Nx group and markedly lower in the NxE group in comparison with the kidney weight of the sham-operated group. No difference was found in the kidney dry weight in the Nx group in comparison with sham-operated rats, while it was significantly decreased in the NxE group as compared with the sham-operated group.

Table 1. Body weight and blood pressure values before and four weeks after right kidney ablation

	Before	After
Body weight (g)		
Sham-operated rats	197±2.5	270±4.5
Nx rats	200±2.5	274±8.5
NxE rats	194±3.1	260±7.6
Systolic blood pressure (mm Hg)		
Sham-operated rats	86±2.0	107±2.1
Nx rats	85±2.0	105±3.9
NxE rats	85±4.5	97±2.8

Nx – rats with remnant kidney, NxE – rats with remnant kidney treated with enalapril.

Table 2. Left/remnant kidney weight and collagen concentrations at the end of the experiment.

	Wet weight (mg)	Dry weight (%)	Collagen (mg/100 g w.w.)
Controls	–	–	143±10
Sham-operated rats	1111±36	24.3±1.30	148± 5
Nx rats	912±31	22.0±1.95	164± 2[a,c]
NxE rats	719±32[b]	20.9±0.71[a]	161± 4[a]

[a] *Nx vs Sham; Nx vsCtrl* $p<0.05$, [b] *NxE vs Nx* $p<0.002$, [c] *Nx vs Sham* $p<0.01$.

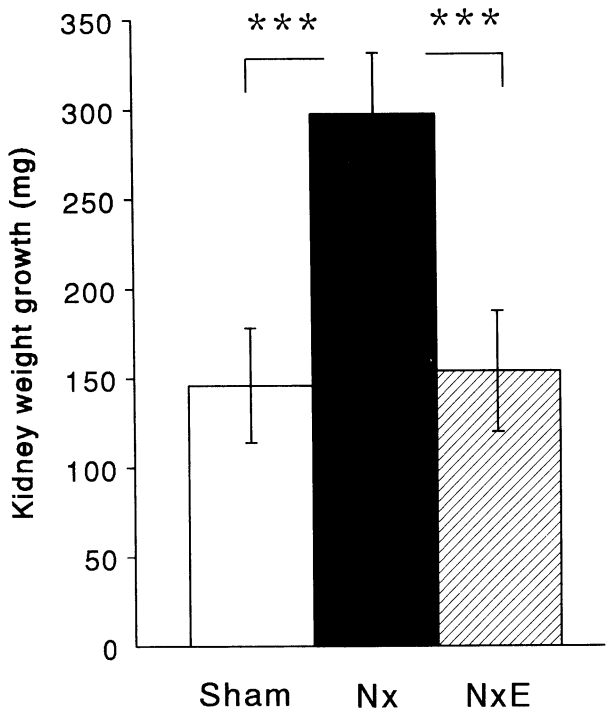

Fig. 1. *Kidney weight growth after a one-month period.* *** $p<0.001$; *data are given as means ± S.E.M.*

The differences were even more illustrative, if the increase of remnant kidney weight was calculated (Fig. 1). During the 4-week period, the remnant kidney weight growth increased markedly (Nx 297±35 mg vs. Sham 145±32 mg, $p<0.001$), and enalapril completely prevented this growth (NxE 154±35 mg vs. Nx, $p<0.001$).

The kidney cortex collagen concentration was 143±10 mg/100 g kidney weight at the beginning of the study which was similar to that in Sham rats (Table 2). The collagen concentration increased slightly in Nx rats and enalapril did not attenuate collagen accumulation (NxE). This may be taken a sign of fibrosis already present in the first month.

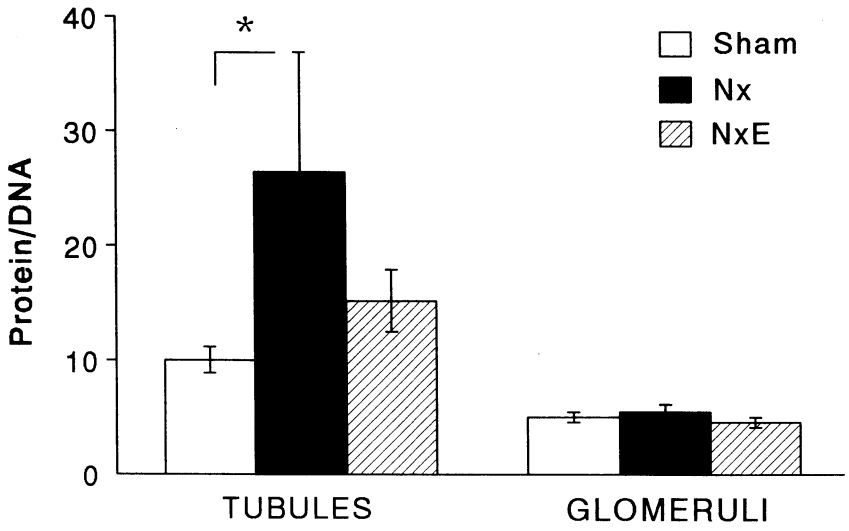

Fig. 2. *Protein/DNA ratio in glomeruli and tubuli of sham-operated rats, rats with remnant kidneys and rats treated with enalapril. * $p<0.05$; data are given as means ± S.E.M.*

Hypertrophy and hyperplasia were differentiated by determining the protein/DNA ratio separately in glomeruli and tubuli (Fig. 2). This ratio was already higher in isolated tubules as compared to glomeruli in the sham-operated group. However, they also differed in their response to subtotal nephrectomy. The protein/DNA ratio increased markedly in the tubuli, while this ratio was unchanged in the glomeruli of Nx group. Enalapril inhibited the increase of protein/DNA ratio in the tubuli.

Table 3. Serum and urine analyses

		Sham-operated (n = 10)	Nx (n = 10)	NxE (n = 17)
Serum				
Creatinine	(μmol/l)	37.2±2.84	63.7±3.56[a]	107.4±5.22[a]
Urea	(mmo/l)	6.10±0.33	10.71±0.76[a]	28.89±2.57[a]
Glucose	(mmo/l)	8.35±0.40	6.97±0.38	6.68±0.26
Cholesterol	(mmol/l)	1.24±0.02	1.56±0.08	1.96±0.09
Triglycerides	(mmol/l)	0.84±0.10	0.81±0.07	0.76±0.17
TXB_2 products	(ng/ml)	92.2±7.1	99.3±7.2	80.4±8.1
Calcium	(mmol/l)	2.40±0.02	2.55±0.03	2.41±0.03
Magnesium	(mmol/l)	0.90±0.04	1.03±0.07	1.25±0.08
Inorganic phosphate	(mmol/l)	2.22±0.16	2.17±0.16	2.43±0.14
Urine				
Creatinine	(nmol/24 h)	9.01±1.15	5.44±0.70	7.52±0.85
Urea	(μmol/24 h)	628±65	448±44	591±44
TXB_2 excretion	(ng/24 h)	17.4±2.5	17.9±2.5	16.0±2.5
Creatinine clearance	(ml/min/100 g)	483±53	301±26[a]	163±10[a]

[a] *Nx vs Sham, NxE vs Sham, NxE vs Nx $p<0.001$.*

The differences between the groups were also reflected in their blood chemistry (Table 3). Creatinine and urea serum concentrations were moderately elevated in the Nx group and markedly increased in the NxE groups than in sham-operated rats. Their accumulation was caused by the decrease of creatinine clearance. On the other hand, no significant changes were found in TXB_2 serum concentrations which probably excluded the participation of the vascular thromboxane compartment in these proliferative changes. Moreover, glucose, lipids and mineral concentrations were not changed.

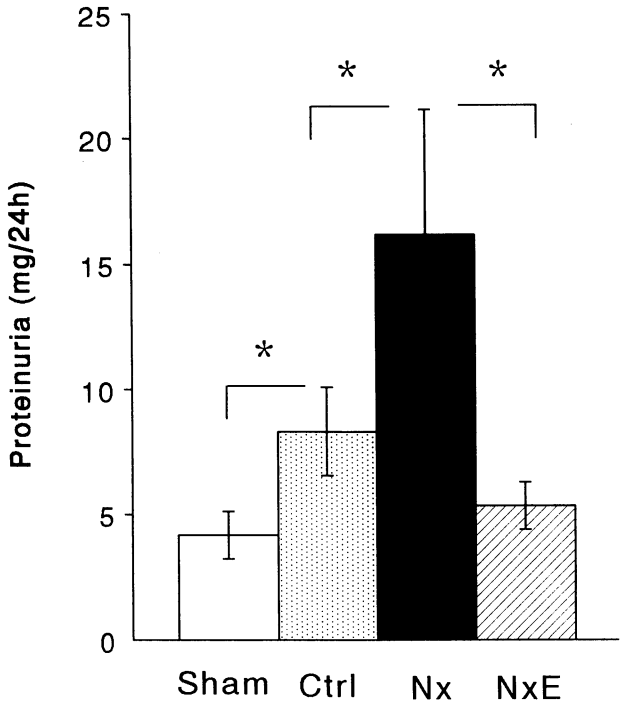

Fig. 3. *The effect of enalapril treatment on proteinuria in rats with remnant kidneys. Ctrl - proteinuria at the beginning of the study; Sham, Nx, NxE - proteinuria after a one-month period. * $p<0.05$; data are given as means ± S.E.M.*

Proteinuria (Fig. 3) was low at the beginning of the experiment (Ctrl 8.3±1.7 mg/24 h), without an increase during the 4-week period (Sham 4.1±0.87 mg/24 h) but it doubled in the nephrectomized group (Nx 16±3.47 mg/24 h, $p<0.05$. Enalapril inhibited the increase of proteinuria (NxE 5.6±0.6 7 mg/24 h vs. Nx $p<0.05$).

No significant changes were found in urea and creatinine urinary excretion (Table 3). The excretion of TXB_2 + 2,3-dinor TXB_2 was also comparable in all groups, excluding the changes in renal thromboxane production and the significance of prostaglandin mediation of ANG II action under these conditions.

Discussion

The present model of 4/6 ablation was not associated with hypertension development, not only during the 4-week period but even up to 12 weeks, as was found in other experiments in our laboratory. On the other hand, the effect on serum creatinine, urea concentrations and proteinuria as well as on the clearance of endogenous creatinine was remarkable.

The 4-week interval was chosen because glomerulosclerosis developed in our experiments as well as in those of others (Johnson RJ, personal communication) after one month, with maximum changes in the third month. Thus, the 4-week interval reflects the period of intensive proliferation.

The blood pressure-independent effect of ACEI with regard to the development of vascular lesions in chronic renal failure has already been described (Kakinuma *et al.* 1992). The present experiments were focused on the ACEI antiproliferative action. While the kidney weight in the NxE group increased comparably with the sham-operated group, a twofold increase was found in the Nx group (Fig. 1).

Because of the lack of an adequate analytical procedure for the determination of total collagen, only the histomorphological determination of collagen types has been employed in the previous morphological studies. However, a recently developed analytical method (Reddy and Enwemeka 1996) adapted for microsample analysis has been chosen because it enabled direct quantitative determination of total collagen changes.

The slight but significant collagen accumulation excluded marked interstitial and glomerular fibrotic changes. However, the failure of enalapril in the used dosage to prevent the collagen accumulation was probably a consequence of an insufficient increase of proteases.

Due to the primary interest in antiproliferative action of enalapril, the protein/DNA ratio was determined separately in glomerular and tubular fractions. The fact that the glomerular protein/DNA ratio was unchanged made any reasonable interpretation difficult. On the other hand, the increased tubular protein/DNA ratio indicated that the participation of hypertrophy is dominating. The antiproliferative and antihypertrophic effects of enalapril

were remarkable even in the absence of significant antihypertensive action. However, the recovery of kidney function was also attenuated with the exception of proteinuria. This is probably due to the effect of enalapril on permselectivity and not a consequence of its antiproliferative action (Mayer *et al.* 1993). This effect could be of importance in acute kidney failure. Of course, such a finding does not exclude the significance of ACEI in chronic kidney disease development.

The potential significance of additional mechanisms, i.e. TXB_2 and 2,3-dinor TXB_2 excretion, markers of thromboxane mediation in the proliferation, serum lipid accumulation and mineral dysbalance wave been excluded.

In conclusion, the proliferation and weight increase of remnant kidney tissue was extreme even in case of the normotensive remnant kidney model. The collagen accumulation was small but already apparent in this early phase. Enalapril markedly attenuated proliferation and proteinuria. However, it also attenuated the restoration of creatinine clearance, serum urea and creatinine concentrations. It could be suggested that angiotensin II participates even in the progression of normotensive nephropathies and ACEI attenuate its proliferative action with both positive and negative consequences.

Acknowledgements

The authors would like to express their gratitude to Dr. J. Mazánková from Lachema (Brno, Czech Republic) for the gift of the enalapril sample used in these experiments.

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