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ACE inhibition prevents renal failure and death in uninephrectomized MWF/Ztm rats

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ACE inhibition prevents renal failure and death in uninephrectomized MWF/Ztm rats. Many studies have consistently documented that angiotensin converting enzyme (ACE) inhibitors prevent proteinuria and glomerulosclerosis in progressive renal disease, but very few data are available on whether they also prevent renal failure and death. The mechanisms of the beneficial effect of ACE inhibition are only partially understood. Recent data suggest that angiotensin II modulates renal synthesis of endothelin-1, a vasoactive peptide implicated in the process of renal injury. Here we investigated in a long-term study whether ACE inhibition ameliorated renal function in uninephrectomized (UNx) male MWF/Ztm rats. Three groups of rats at nine weeks of age underwent UNx or sham-operation. Nephrectomized animals were left untreated or treated with the ACE inhibitor lisinopril in drinking water. In untreated UNx animals systolic blood pressure, serum creatinine, urinary protein and renal synthesis of endothelin-1, evaluated by its urinary excretion, were significantly increased, as compared with control animals with two kidneys. End-stage renal failure developed in all untreated UNx rats that died within 9 to 14 months from UNx. ACE inhibitor significantly reduced systolic blood pressure, completely prevented proteinuria and renal function deterioration, and reduced endothelin-1 excretion. All UNx rats treated with lisinopril were alive 14 months after UNx. These results show that ACE inhibition prevents end-stage renal failure induced by UNx in male MWF/Ztm, and that the beneficial effects of angiotensin II inhibition in this model are related to modulation of renal synthesis of endothelin-1.

In 1985 Anderson and coworkers [1] published the first observation that angiotensin converting enzyme (ACE) inhibitors reduced urinary protein and prevented glomerulosclerosis in a rat model of progressive nephropathy. Since then an impressive number of further studies confirmed the reno-protective properties of this class of compounds in many different experimental settings, as well as in humans with progressive renal insufficiency [reviewed in 2, 3]. Of interest, despite the enormous efforts devoted to confirm the properties of ACE inhibitors to reduce urinary proteins and glomerular structural abnormalities, there are virtually no studies addressing whether or not the treatment prevented or retarded renal insufficiency in animal models.

The mechanisms of the beneficial effect of ACE inhibitors in retarding renal disease progression has been extensively investigated in the last 10 years, but is not fully clarified yet despite considerable advances in knowledge. A plausible explanation is that ACE inhibitors protect against proteinuria and glomerular structural abnormalities by selectively reducing efferent arteriolar resistance, thereby reducing glomerular hypertension [4]. Others have suggested that ACE inhibitors prevent progressive renal disease in animal models due to their properties of limiting angiotensin II-induced mesangial cell proliferation [5, 6]. Since angiotensin II also induces extracellular matrix protein genes in mesangial cells [7], the latter has been considered a possible additional pathway by which ACE inhibitors, by blocking angiotensin II activity, may reduce glomerulosclerosis. We provided experimental evidence that, beside reducing glomerular capillary pressure, ACE inhibitors have a direct effect on glomerular membrane function in spontaneous glomerular injury in male MWF/Ztm rats [8, 9]. Thus, ACE inhibition enhanced hydraulic permeability and prevented the size-selective defect in glomerular barrier function by reducing the fraction of large unselective pores as well as peak pore radius.

A number of other studies have also addressed the effect of angiotensin II receptor blockade on glomerular barrier function. In rats with renal ablation that have established proteinuria four weeks after surgery, angiotensin II blockade significantly reduced proteinuria by restoring remnant glomerular size-selectivity to normal [10]. Angiotensin II blockade also protected against renal injury in experimental diabetes, as documented by amelioration of glomerular size-selective function in this model [11]. In humans, converting enzyme inhibitors improved glomerular size-selectivity in diabetic nephropathy [12, 13] and in IgA nephropathy [14].

So far the favorable effects of ACE inhibitors on renal disease progression have largely been attributed to their effect of inhibiting the biological activity of angiotensin II, and this is supported by studies that specific inhibitors of angiotensin II receptors share identical properties. However, there are data that infusion of angiotensin II sufficient to increase blood pressure and filtration fraction does not alter membrane pore structure [15]. Recent evidence is available indicating that angiotensin II modulates the renal synthesis of endothelin-1 (ET-1), a vasoactive peptide [16] recently implicated in the process of renal injury [17]. Endothelin-1 secretion by cultured rat mesangial cells was stimulated by angiotensin II in a concentration-dependent manner [18]. Furthermore, infusion of low and high doses of angiotensin II in the rat resulted in an enhanced urinary excretion of ET-1 [19], which likely reflects the renal synthesis of the peptide [20]. Additional data would suggest that ACE inhibitors interfere with ET-1. Of

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interest, rats with chronic infusion of ET-1, which develop hypertension [21] in the absence of changes in angiotensin II levels, normalized their blood pressure when given an ACE inhibitor [22].

The aims of the present study were: (1) to investigate whether ACE inhibition prevents the development of renal failure in aging male MWF/Ztm rats subjected to unilateral nephrectomy; and (2) to study whether in this model the ACE inhibitor interfered with the generation of ET-1 at a renal level.

Methods

Twenty-one male MWF/Ztm rats, bred and raised in our facilities, were used in this study. Animals were allowed free access to food (standard rat chow containing 20% protein by weight) and water. At nine weeks of age, animals were divided in three groups, matched for urinary protein excretion, and then subjected to unilateral nephrectomy (UNx) or sham operation under ether anesthesia. Five sham operated animals (group 1) received no treatment during the total observation period. Uninephrectomized animals were divided in two groups: eight rats were left untreated (group 2), and eight rats were treated with the ACE inhibitor, lisinopril (Zeneca Pharmaceuticals, Macclesfield, UK), in the drinking water at the dose of 50 mg/liter throughout the duration of the study starting one week after nephrectomy (group 3). At three month intervals systolic blood pressure (SBP) was recorded by tail plethysmography in awake animals and 24-hour urine collections were obtained using metabolic cages for determination of protein and ET-1 excretion rate. At the same time intervals blood samples were collected from the tail vein for determination of serum creatinine using routine laboratory technique [23]. Proteinuria was determined by the Coomassie blue G-dye binding assay with bovine serum albumin as standard [24].

Endothelin-1 concentration in urinary samples was determined by radioimmunoassay (RIA) as described [20]. Briefly, 10 ml of 24-hour urine samples were applied to a Sep-Pak C_{18} disposable column (Waters, Mildford, MA, USA) previously conditioned by consecutive washing steps with different methanol:water solutions containing increasing amounts of solvent as described [20]. The adsorbed peptide was eluted with methanol:water (85:15 vol/vol). The eluates were liophylized and stored at −20°C until RIA was performed. Extraction recovery, determined by addition of trace amounts of ^{125}I-ET-1 (2,000 Ci/mmol; Amersham International, Buckingamshire, UK) to all samples before extraction, was about 80%. The lowest detectable concentration that could be measured was 0.4 pg/tube. Results are expressed as pg/day. Intra- and inter-assay variability averaged 9% and 12%, respectively. The cross-reactivity of the antibody with ET-1 isoforms and validation of RIA measurement are already reported [20].

Animals were kept in our animal room and followed until spontaneous death to study the survival time. After spontaneous death, or under ether anesthesia for control animals with two kidneys and for treated animals, kidneys were removed and tissue fragments were immersion-fixed in Dubosq-Brazil fluid and embedded in paraffin. Sections, 3 μm in thickness, were stained with Masson's trichrome, hematoxylin and eosin, and by the periodic-acid Schiff techniques. Sections including superficial and juxtamedullary glomeruli were evaluated as previously described [25]. Briefly, at least 100 glomeruli were examined for each animal and the percentage of glomeruli affected by focal or global glomerular sclerosis was determined. Tubular changes (atrophy, casts and dilatation) and interstitial fibrosis and inflammation were graded from 0 to 4+ (0 = no changes; 1+ = changes affecting <25% of sample; 2+ = changes affecting 25 to 50% of sample; 3+ = changes affecting 50 to 75% of sample; 4+ = changes affecting >75% of sample). Renal biopsies were analyzed by the same pathologist blind to the nature of the experimental groups.

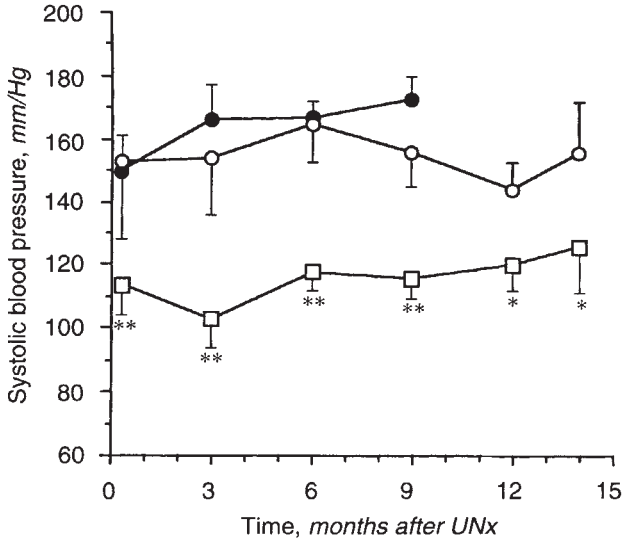

Fig. 1. *Periodic measurements of systolic blood pressure in control and in UNx animals.* Values of untreated UNx animals stop at 9 months after nephrectomy because of spontaneous death. Symbols are: (●) UNx; (○) control; (□) UNx + Lis. *$P < 0.05$ vs. Control group at the same time; **$P < 0.01$ vs. Unx and control group at the same time.

All results are expressed as mean ± SD. Data were analyzed by two-way analysis of variance and differences between individual groups assessed by the Tukey-Cicchetti test for multiple comparisons [26]. Statistical analysis of estimates of renal damage by morphological studies were compared with Mann-Whitney test for non-parametric data. Statistical significance level was defined as $P < 0.05$.

Results

Untreated controls and UNx animals (groups 1 and 2) developed hypertension with age (SBP >140 mm Hg), as reported in Figure 1, while UNx animals given lisinopril (group 3) were maintained normotensive during the study period, with values of SBP never exceeding 130 mm Hg. As shown in Figure 2, untreated control animals developed spontaneous proteinuria with age that averaged about 400 mg/24 hrs after 14 months of observation. Animals of group 2 also developed massive proteinuria with age (on average 552 ± 117 mg/24 hrs 9 months after UNx). At variance, UNx animals treated with lisinopril were significantly protected from proteinuria that averaged only 36 ± 14 mg/24 hrs at nine months after UNx and then slightly increased (averaging 152 ± 63 mg/24 hrs) by the end of the study. Food intake was comparable in the three animal groups and body weight, nine months after nephrectomy, was not significantly different (averaging 477 ± 7, 458 ± 22 and 465 ± 27 g, respectively, in the three groups). Water diuresis was significantly higher in untreated UNx animals as compared to both UNx animals treated with lisinopril

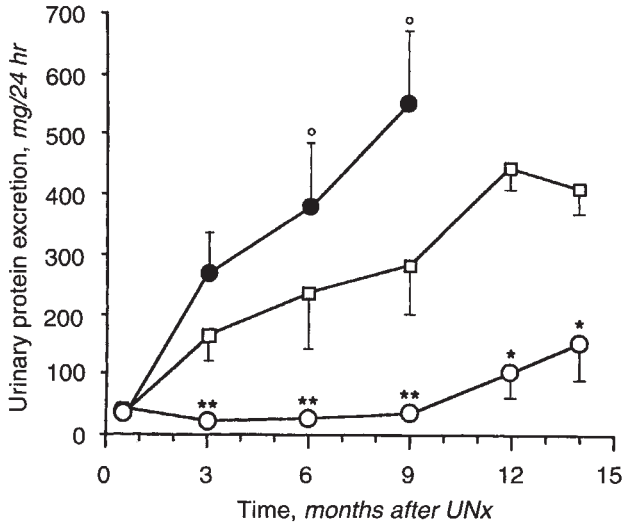

Fig. 2. *Urinary protein excretion rate in control and in UNx animals.* Values of untreated UNx animals stop at 9 months after nephrectomy because of spontaneous death. Symbols are: (●) UNx; (□) control; (○) UNx + Lis. $^{*}P < 0.05$ vs. control group at the same time; $^{**}P < 0.01$ vs. UNx and control group at the same time; $^{\circ}P < 0.01$ vs. Control and UNx + Lis group at the same time.

and to sham operated controls, values recorded at month nine averaged respectively 16 ± 2, 34 ± 9 and 24 ± 3 ml/day in groups 1, 2 and 3 ($P < 0.01$ UNx vs. controls and $P < 0.05$ UNx vs. UNx + lisinopril).

As shown in Figure 3, all control animals with two kidneys (group 1) were alive 14 months after the sham operation, while all untreated UNx animals (group 2) died between 9 and 14 months after nephrectomy. Treatment of UNx animals with the ACE inhibitor lisinopril (group 3) completely prevented spontaneous death, so that all animals were alive 14 months after nephrectomy. At this age animals of groups 1 and 3 were sacrificed to obtain samples of kidney tissue for morphological evaluation. Untreated UNx animals (group 2) died because of uremia, as documented by values of serum creatinine represented in Figure 4. Last values of serum creatinine measured in this group of animals, as determined monthly after the ninth month from UNx, averaged 3.46 ± 1.32 g/dl. At variance, animals with two kidneys (group 1) showed values of serum creatinine always normal, up to 12 months from sham operation, with a slight tendency to increase at 14 months, at which time serum creatinine averaged 1.21 ± 0.30 g/dl. UNx animals treated with lisinopril (group 3) did not develop renal failure and showed stable values of serum creatinine during the entire duration of the study (14 months after UNx serum creatinine averaged 1.02 ± 0.07 g/dl). Kidney weight was significantly higher in UNx animals than in sham operated controls (1.84 ± 0.22 vs. 1.43 ± 0.06 g, $P < 0.01$). In UNx animals treated with lisinopril kidney weight averaged 1.65 ± 0.13 g, a value not significantly different from both groups 1 and 2.

The results of the morphological evaluation are reported in Figure 5 and 6. As previously reported [8, 25], control male MWF/Ztm rats with two kidneys (group 1) developed important glomerular lesions spontaneously with age, affecting on average 69% (range 46 to 87%) of glomeruli. Also interstitial nephrosis (mean score 2.6) and tubular damage (mean score 2.6) were present in these animals. Untreated UNx animals developed more severe renal damage. Glomerulosclerosis incidence averaged 94% (range 89 to 98%) with 13.5% of glomeruli on average affected by global sclerosis. Both interstitial nephrosis and tubular damage had a mean score of 3.7 in these animals. At variance, treatment with lisinopril importantly prevented damage of glomerular and tubular structure. In animals of group 3 glomeruli affected by sclerosis were only 9.1% (range 4 to 18%) with none of them showing global sclerosis. Interstitial nephrosis and tubular damage were minor in these kidneys (score average 1.0 for both). Thus, glomerular and tubular structure were significantly protected, not only in comparison with UNx animals left untreated, but also with control animals with two kidneys.

Urinary ET-1 excretion values for the three groups of animals are reported in Figure 7. In sham-operated animals ET-1 excretion was almost constant during the observation period, with average values ranging from 46 to 70 pg/24 hrs. Three months after nephrectomy, ET-1 excretion increased in animals of group 2 (116 ± 47 pg/24 hrs) in respect to sham-operated controls, although statistical significance was not reached. Urinary ET-1 excretion values became significantly ($P < 0.01$) different from sham operated controls starting from six months after the surgical procedure. At this time, ET-1 excretion averaged 210 ± 56 pg/24 hrs. Urinary ET-1 values remained constantly elevated (185 ± 75 pg/24 hrs, $P < 0.01$) over sham-operated animals at nine months after nephrectomy. The last value of urinary ET-1 excretion, measured in animals of group 2 between 10 and 12 months after nephrectomy, further increased with time averaging 430 ± 102 pg/24 hrs (data not shown in Fig. 7). Lisinopril treatment significantly ($P < 0.01$) reduced the increase in ET-1 excretion induced by UNx at all time points evaluated. As compared to control animals with two kidneys urinary ET-1 excretion in lisinopril treated animals was significantly reduced at three months after nephrectomy (44 ± 15 vs. 70 ± 10 pg/24 hrs, $P < 0.05$). In contrast, in animals given lisinopril urinary ET-1 was significantly higher than controls at 6 and 9 months after the surgical procedure (6 months, 110 ± 13 vs. 46 ± 5 pg/24 hrs; 9 months, 88 ± 13 vs. 52 ± 5 pg/24 hrs, $P < 0.05$). By the end of the observation period (14 months from UNx) animals treated with lisinopril showed ET-1 excretion values comparable to those measured in control rats with two kidneys (47 ± 10 vs. 61 ± 6 pg/24 hrs).

Discussion

We have documented that the ACE inhibitor, lisinopril, effectively reduced systemic blood pressure in aging male MWF/Ztm rats. Actually at variance with untreated control and UNx animals, values of SBP were normal in UNx rats on lisinopril during the whole study period. Urinary protein excretion values were also significantly lower at all observation times in UNx animals on lisinopril as compared to untreated UNx and normal controls. The effect of lisinopril of restoring glomerular permeability properties in this model was associated with a remarkable effect of limiting glomerular structural injury as reflected by significantly less glomerulosclerosis and tubulointerstitial injury in animals treated with lisinopril as compared to untreated nephrectomized rats and normal controls. Renal pathology data were totally unexpected and quite remarkable. Actually the effect of lisinopril on signs of renal injury was such that UNx animals had also fewer lesions than age-matched controls with two kidneys and the differences were very impressive (Fig. 5). The major finding of the current

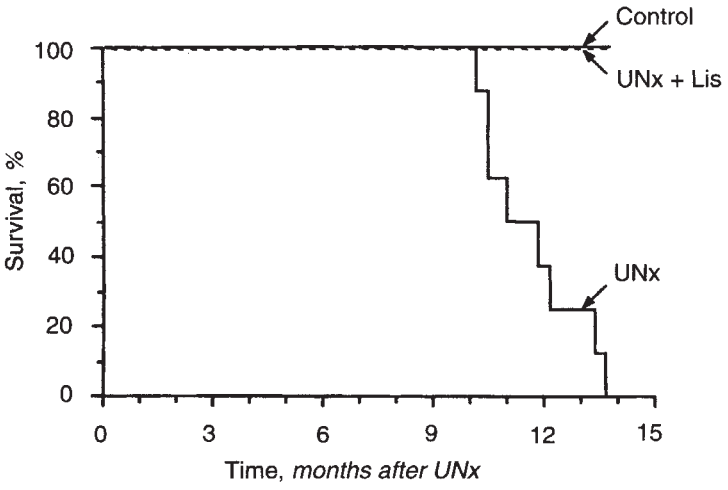

Fig. 3. *Survival curve of control, untreated UNx and lisinopril-treated UNx animals.* All control rats (group 1) and lisinopril-treated UNx rats (group 3) were alive at the end of the observation period. All untreated UNx animals (group 2) died before 14 months after nephrectomy.

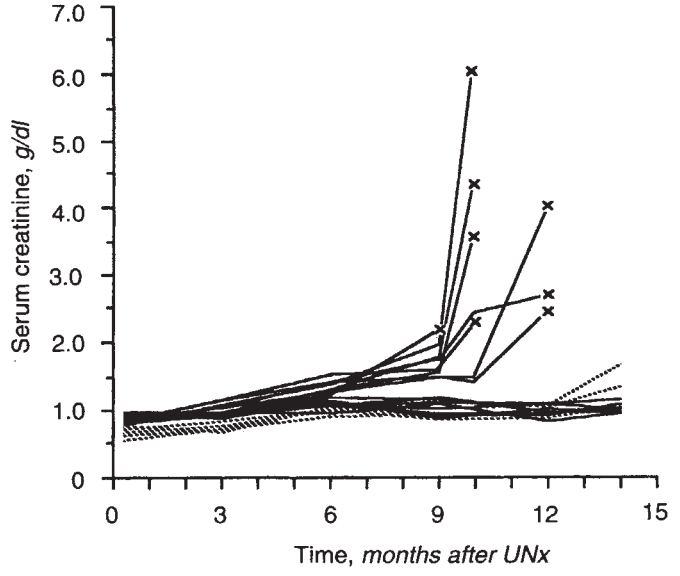

Fig. 4. *Periodic measurements of serum creatinine in control and UNx animals.* Lines represent time course of serum creatinine in individual animals. In untreated UNx animals crosses represent last values measured before animal death (group 1). Symbols are: (--------) control; (————x) Unx; (————) UNx + Lis.

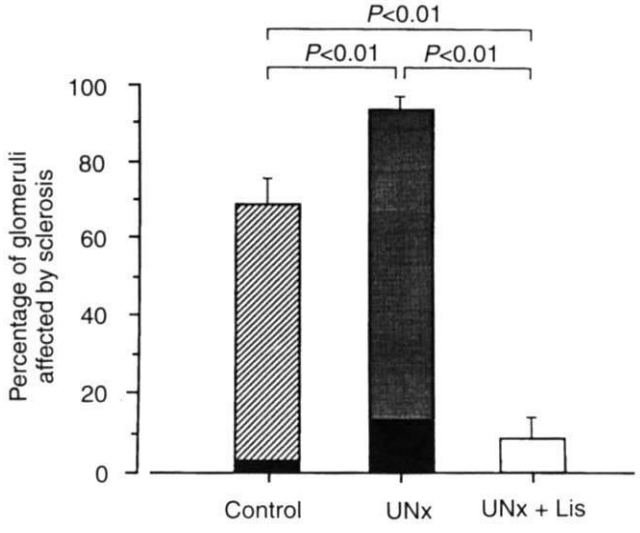

Fig. 5. *Percentage of glomeruli affected by sclerosis in control and UNx animals by the end of the observation period.* Bars represent the total percentage of glomeruli affected by sclerosis while black areas represent the percentage of glomeruli affected by global sclerosis. In UNx animals treated with lisinopril glomeruli affected by global sclerosis were not observed.

study, however, is that end-stage renal failure and death, that invariably developed in UNx animals 10 to 14 months after surgery, were completely prevented by lisinopril. Finally, we found that lisinopril also significantly reduced the elevated values of urinary ET-1 that followed UNx.

To our knowledge this is the first demonstration that ACE inhibition, besides ameliorating glomerular membrane selectivity and reducing glomerular injury, protects animals from progressive renal failure and death. Thus, the current results rise the question of whether ACE inhibition represents the best possible approach to limit the evolution of human nephropathies to renal failure. So far the most convincing human study of this kind is the one of Lewis and coworkers [27] that indeed shows that captopril retarded the rate of loss of renal function in the progressive nephropathy associated with type I diabetes. Quite consistent with the present animal data the above study in diabetic patients also documented that captopril did significantly decrease the combined risk of renal failure and death. Data on the protective effect of the ACE inhibition on renal function deterioration in diabetic humans of the above trial were in line with the results of small, short-term studies published previously [28, 29].

Whether the renal effect of drugs that reduce systemic blood pressure in animal and human nephropathies is simply the consequence of blood pressure lowering effect or is due to some local effect not necessarily related to systemic blood pressure is one of the most debated issue in the renal literature these days. In the Lewis's study the effects of captopril of limiting diabetic renal disease progression appeared independent of its antihypertensive effect. In a recent study from our group the calcium channel blocker nitrendipine, despite an effect on systemic blood pressure

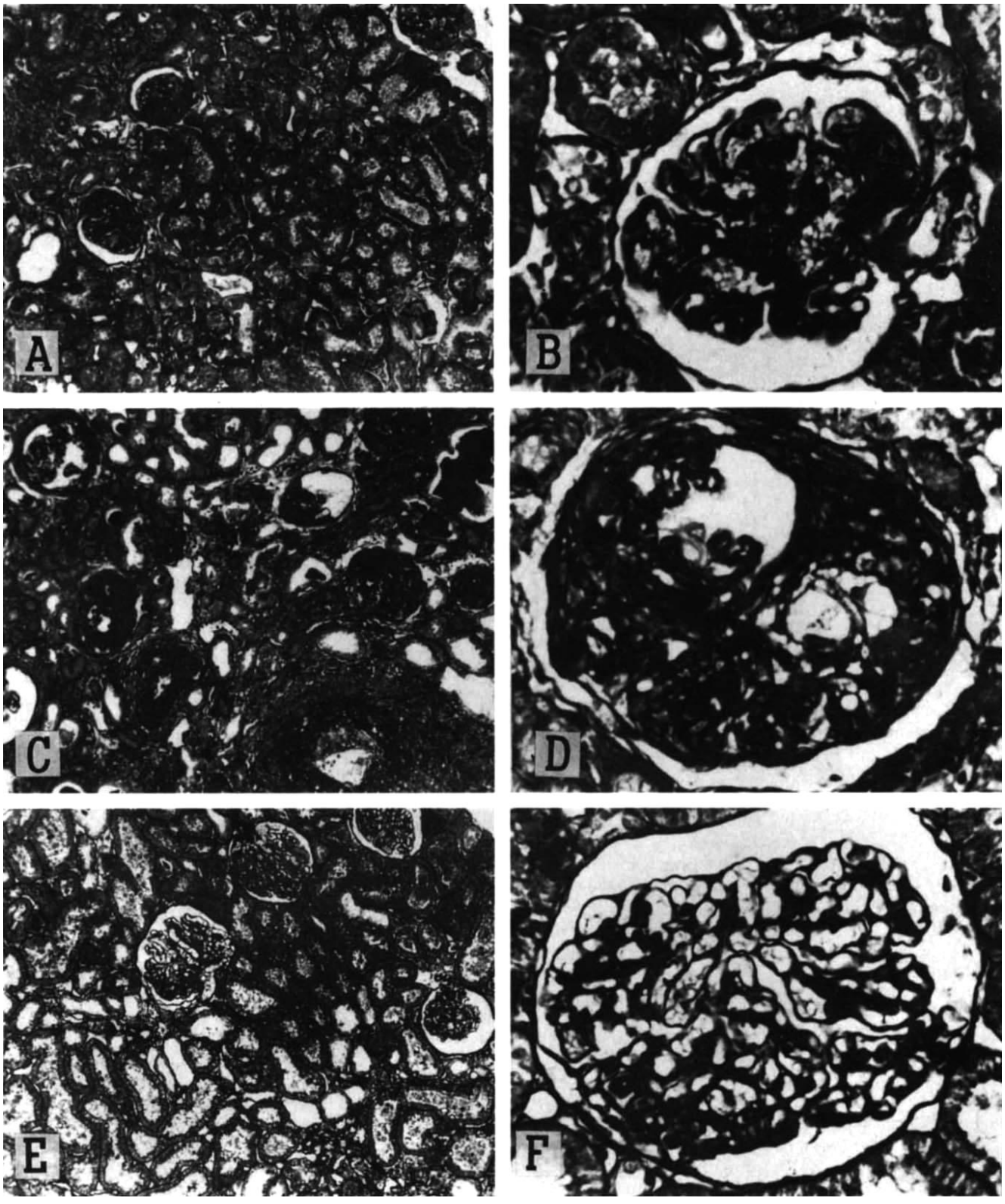

Fig. 6. *Representative light micrographs at low (×100) and medium (×400) power magnification of glomeruli from control (group 1,* **A** *and* **B***), untreated UNx (group 2,* **C** *and* **D***) and lisinopril-treated UNx animals (group 3,* **E** *and* **F**). Paraffin sections, 3 μm thick were stained with Masson's thrichrome.

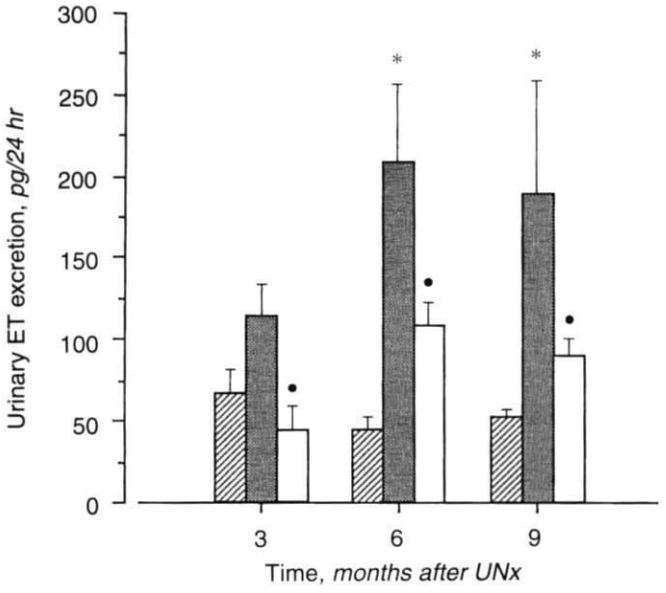

Fig. 7. *Time course of urinary excretion rate of ET-1 in unilateral nephrectomized rats given lisinopril (□, UNx + lis) or no treatment (▨, UNx) and in sham operated animals (▨, control).* •$P < 0.01$ vs. UNx and $P < 0.05$ vs. control at the same time; *$P < 0.01$ vs. control at the same time.

control comparable to that of the ACE inhibitor lisinopril, did not protect animals against proteinuria and glomerular lesions [9]. This, however, remains a controversial issue given the fact that other animal and human studies [30, 31] did indeed find a protective effect of calcium channel blockers which is generally less effective than ACE inhibition.

Experimental and human data are available which consistently document that the protective effect of ACE inhibition on renal function deterioration is always associated with a remarkably improved glomerular barrier permeability to proteins [8, 12, 14]. We and others [10, 11] have previously demonstrated that blocking renin-angiotensin system by converting enzyme inhibitors or angiotensin II antagonists reduces to normal the number and dimension of large unselective pores that form within the glomerular barrier in proteinuric renal diseases. This could be taken as an indication that reducing angiotensin II serves to repair the selectivity of the barrier. Of interest, remarkably similar results have been obtained both in animal and human studies, suggesting that this effect is not confined to a given model but is likely a generalized phenomenon. Our present data further stress the possibility that, on the long term, restoring size-selective and hydraulic permeability functions limit the renal evolution of the disease to uremia. Within the limit of the assumption that ACE inhibitors are better reno-protective than other antihypertensives by virtue of their unique property of improving the permeability of the glomerular membrane to macromolecules and water, it is logical to think to a direct effect on the structural/functional properties of the membrane. This is consistent with very recent findings that the ACE inhibitor enalapril modulates extracellular matrix protein composition [32].

Another interesting finding of the current study is that, despite the fact that male MWF/Ztm rats do develop glomerular damage, this was not associated with renal failure unless uninephrectomy was also performed. Thus the current study is a major support to the view that fewer nephrons, particularly in the presence of a pre-existing renal disease, initiates a program of glomerular and tubulointerstitial events that eventually trigger progression of the disease to renal failure [33]. This is exemplified by the extreme condition of oligomeganephronia—a disease of congenital reduction in the number of glomeruli—that after a period of compensatory hyperfiltration evolves toward overt proteinuria and renal insufficiency [34, 35].

We have previously reported that in rats with progressive renal disease triggered by extensive renal mass ablation urinary ET-1 increases progressively with time and parallels the severity of renal injury [20]. Moreover, renal prepro-ET-1 gene expression increases in these animals [36]. Exaggerated formation of ET-1 in the kidney in a more general way characterizes the development of progressive nephropathies, as documented by subsequent data in other models of disease progression including remnant kidney, immunological nephritis and streptozotocin diabetes [20, 37, 38]. *In vitro* ET-1 mRNA levels in glomeruli of diabetic rats increased with the progression of diabetic nephropathy [38]. These latter findings are consistent with data from our group [20] and from others [39] that enhanced ET-1 urinary excretion values reflect an enhanced renal synthesis of the peptide rather than events in the systemic circulation. In the current study we found that chronic renal failure in UNx male MWF/Ztm rats was also associated with an increased urinary ET-1. Endothelin-1, beside its potent effect of reducing renal blood flow, induces mesangial cell proliferation [40] and stimulates extracellular matrix synthesis [41]. That ET-1 may be a potential mediator of damage in progressive renal disease rests on data that a specific endothelin subtype A receptor antagonist, given to rats with reduction of renal mass, ameliorated proteinuria, limited glomerular injury and prevented renal function deterioration [42]. In the current study we found that lisinopril significantly reduced urinary excretion of ET-1 as compared to untreated nephrectomized animals. However, the reduction in injury seen with in the ACE inhibitor group far exceeded the observed reduction in ET-1 excretion. Specifically, in animals treated with lisinopril proteinuria and sclerosis were almost completely prevented and showed ET-1 excretion values higher than in normal controls, and, on the other hand, despite low ET-1 excretion sham operated controls developed massive proteinuria and glomerular sclerosis. Altogether the above observations would indicate that in this setting, the effect of lisinopril in preventing renal failure and death may not simply derive from its property of lowering ET-1, but other mediators must be involved [43].

The mechanism of the specific suppressing effect of the ACE inhibitor on renal ET-1 formation must remain speculative at the moment. Since in another study [44] captopril-induced suppression of ET-1 formation did not correlate with the fall in blood pressure, there are reasons to believe that factors other than angiotensin II inhibition explain the effect of ACE inhibitors on ET-1. In cultured endothelial cells bradykinin is a potent inhibitor of ET-1 synthesis, and ACE inhibitor inhibits ET-1 secretion by the accumulation of endogenous bradykinin [45]. These data would suggest that drug-induced potentiation of bradykinin might account for the effect of lisinopril on urinary ET-1 observed in the present study. Alternatively, ACE inhibitors may normalize renal ET-1 by enhancing endothelial cGMP, a phenomenon that likely

depends on endothelial nitric oxide synthesis, also promoted by the accumulation of bradykinin [46].

In conclusion, our present data indicate that ACE inhibition effectively prevents kidney structural and functional damage which develops in male MWF/Ztm rats after unilateral nephrectomy leading to end-stage renal failure. The impressive effect of the ACE inhibition in this model of renal failure should stimulate extensive investigation on the possibility that this treatment prevents the progression of proteinuric renal diseases to end-stage renal failure in humans.

Acknowledgments

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