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ORIGINAL PAPER

Effect of lisinopril on renal tissue damage in unilateral ureteral obstruction in rats

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Abstract In this study, it was aimed to investigate apoptosis in renal injury and the effect of lisinopril in rat model, which constitute unilateral ureteral obstruction. The retroperitoneal ureter was ligated with a 4.0 silk for the experimental model of ureteral obstruction in Wistar albino rats. Untreated group ($n = 20$) received no treatment. For the lisinopril-treated group ($n = 20$), 20 mg/kg/day of drug was given orally. Ultrastructural differences were analyzed using electron microscopic technique; apoptotic distribution was analyzed using the TUNEL method. After electron microscopic evaluation, on the 4th and 14th day in the untreated group, edema in the glomeruli, loss of microvillus and apoptotic cells in proximal tubule cells and sclerosis in the glomeruli were detected. On the 4th day in the lisinopril-treated group, the kidney was ultrastructurally normal and a less number of apoptotic cells were only observed on the 14th day. On light microscopic examination on the 4th and 14th day in the untreated group, while the glomeruli were normal in structure, the boundary of the proximal tubule was disrupted and some picnotic cells in both the proximal and collecting tubules were observed. In both 4th and 14th day of the lisinopril-treated group, kidney showed normal structure, although in some places picnotic cells in the collecting tubules were observed. In conclusion, lisinopril was effective and it may prevent early renal damage in the direct obstruction model.

Keywords Lisinopril · Apoptosis · Renal damage · Ureteral obstruction · Rat

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Introduction

Urinary obstruction is the most important reasons that cause renal failure in infants and children [1]. Unilateral ureteral obstruction (UUO) initiates renal injury [2] characterized by tubular dilatation and/or atrophy, infiltration of leucocytes (macrophages), fibroblast proliferation and an increase in matrix proteins, concluding in progressive interstitial fibrosis [3] and end-stage renal disease [4]. A number of recent studies have shown an association between the renin–angiotensin system (RAS) and apoptotic alterations in the kidney [5–7].

After ureteral obstruction, there is a positive correlation between histological tubular differences and a decreased glomerular filtration rate. In the kidney, some ultrastructural differences can be observed in the proximal and collecting tubules after ureteral obstruction. Chronic ureteral obstruction can also cause collagen deposition and tubulointerstitial fibrosis. In addition, tubular cell apoptosis may be related to renal tissue loss and dysfunction in UUO.

Angiotensin (AT)-2 induce renal apoptosis by promoting oxidative stress and vasoconstriction, and enhancing the expression of adhesion molecules and cytokine synthesis such as transforming growth factor β1, tumor necrosis factor α, platelet-derived growth factor, insulin-like growth factor, osteopontin, vascular cell adhesion molecule-1, monocyte chemotactic protein-1, intercellular adhesion molecule-1 and nuclear factor kappa-β [7, 8].

Angiotensin-converting enzyme (ACE) inhibitors could improve the effects of UUO; so in our study, we aimed to investigate the degree of apoptosis in renal injury and the effect of lisinopril, an ACE inhibitor, in a rat model with unilateral ureteral obstruction.

Apoptosis is a process by which single cells undergo programmed cell death and are removed by elimination of abnormal cells from the tissue [9]. It is a genetically determined that biologically activate process plays a role opposite to mitosis in tissue-size regulation, shaping organs during mammalian morphogenesis and removing cells that are immunologically reactive against the organism, when infected or genetically damaged [10]. While chromatin condesation is clearly visible in apoptotic cells, the cell membrane is stable in contrast to necrosis. The regulation of apoptosis is very complex and involves the expression of various genes, which can display either positive or negative regulatory effects. Controlling of apoptosis is regulated by extrinsic or intrinsic pathways. The extrinsic pathway is activated after stimulation of the Fas/Fas ligand, TRAIL and tumor necrosis factor (TNF)/TNF receptor [11, 12]. The intrinsic pathway is controlled by the secretion of cytochrome-*c* from mitochondria [13]. After extrinsic or intrinsic pathways, different caspase such as caspase 8, 9 activate and trigger of other caspases (3/6/7). Recent evidence has strongly suggested that apoptosis is an important regulator of glomerular cell numbers in renal injury [14].

Materials and methods

All animal procedures were approved by the Committee on Human Care of Laboratory Animals at the Ege University, Faculty of Medicine, Izmir, Turkey.

Animals

Male Wistar albino rats (220–260 g each; $n = 60$) were used for this study. All were housed in a standard controlled, 12-h light–dark environment for a minimum of 3 days before being used in experiments.

Rat model of ureteral obstruction

This study utilized a previously described rat model of ureteral obstruction [15]. In this model, rats anesthetized with ketamine (80–100 mg/kg, Ketalar, Parke Davis, USA) underwent a standard midline laparotomy to expose the left ureter. The left ureter was ligated with a 4/0 silk ligature. The skin was sutured and closed, after a 2-h operation. For the lisinopril-treated group ($n = 20$), 20 mg/kg/day of lisinopril (Merck & Co., Inc., USA, Canada) was dissolved in distilled water and given by orogastric lavage to rats. In the untreated (control) group, the left ureteral obstruction was performed as previously described, but rats were not given any medicine. In the unobstructed group (sham group), treatment was not performed. Ten of the animals from each group on the 4th, and the rest of the ten animals from all groups on the 14th day after treatment were killed and bilateral nephrectomy was performed.

Tissue specimen preparation

All left kidneys were divided into two groups; one part of the samples was fixed in a 10% formalin solution for 24 h, embedded in paraffin, and sliced into 5 μm-thick sections. Sections were then processed for both histochemical and TUNEL assay as described below. The rest of the samples was fixed with 2.5% gluteraldehyde for 4 h at 4°C for electron microscopic analyses.

Histomorphological analysis

Sections were routinely stained with either hematoxylin–eosin (H–E) or Masson Trichrome and then examined under a light microscope (Olympus BX40, Tokyo, Japan).

Electron microscopic analysis

The samples were postfixed with 1% osmium tetroxide for 1.5 h and, after routine processing, embedded in epon (R1080, Agar Scientific, Essex, UK). The thin sections were contrasted with uranyl acetate (U007, TAAB, Berks, England) and lead citrate (L003, TAAB, Berks, England). They were then examined using Zeiss, LIBRA 120 electron microscope.

TUNEL assay

Deparaffinized and rehydrated sections, prepared as described above, were stained using a commercial kit (Apoptag, S7101, Chemicon, Billerica, MA, USA) according to the manufacturer's instructions. They were incubated 30 min with 20 µg/mL proteinase K, rinsed again three times for 5 min with PBS, and then incubated in TdT-enzyme solution at 37°C for 1 h. Subsequently, the sections were rinsed three times with stop/wash buffer (supplied from Chemicon) at room temperature for 15 min each. The sections were then incubated with anti-digoxigenin conjugate (ready to use, supplied from Chemicon) for 30 min. After that, they were washed with PBS for 10 min and covered with mounting medium (Scytek Laboratories, Logan, UT, USA). As a negative staining control, TdT was omitted during the tailing reactions.

Evaluation of sections

The slides stained with TUNEL technique were evaluated using a light microscope (Olympus BX40, Tokyo, Japan). The average number of apoptotic cells was determined by counting TUNEL-positive cells in five neighboring medium-power fields, where the maximum number of stained cells was observed and the total was divided by five. Cells in areas with necrosis, poor morphology or margins of sections were not included.

Statistical analysis

Results were expressed as mean ± SE. The software SPSS 10.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical evaluation. Differences among groups were statistically analyzed with one-way ANOVA where appropriate. A $p < 0.05$ value was assumed to indicate statistically significant differences.

Results

Histological results

On histological evaluation of the left kidney tissue of all groups, stained with hematoxylin–eosin, the cortex and medulla were easily distinguished. In the lisinopril-treated group, the glomerulus, proximal and distal tubules and Henle were normal in structure, while in the collecting tubules a few picnotic cells were observed. In the untreated group, polymorphonuclear leucocytes infiltration was observed and, in addition, picnotic cells were more detectable in the proximal and distal tubules and collecting tubules when compared with the lisinopril-treated group (Fig. 1).

Masson Trichrome staining were detected in both the glomerulus and tubules, but there were no differences between groups (Fig. 2).

TUNEL results

TUNEL (TDT-mediated dUTP-biotin nick end-Labeling) staining was done in the study to determine apoptotic cells. TUNEL-positive cells were not detected in the unobstructed group. On sections of the untreated group at 4 and 14 days, TUNEL-positive cells were not detected in the glomerulus cells, whereas in the proximal and distal tubules and collecting tubules cells, TUNEL-positive cells were observed on both 4th and 14th days: 21.1 ± 0.57 and 15.8 ± 0.25%, respectively. The renal medulla was examined on the same day and the number of positive cells on the 4th and 14th days were 25.6 ± 0.37 and 30.9 ± 0.73%, respectively. When compared with the cortex, more TUNEL-positive cells were observed in the medulla (Fig. 3a, b), which was significant ($p < 0.001$) (Table 1).

TUNEL-positive cells were almost negative in lisinopril-treated group, on the 4th and 14th days; TUNEL-positive cells were observed in the glomerulus, proximal and distal tubule, (respectively, 0.3 ± 0.01 and 0.1 ± 0.15%), in the medulla and collecting tubule cells on the 4th (0.2 ± 0.13%) and 14th days (3.7 ± 0.26%). The data from the 4th day were not statistically significant compared with the unobstructed group, whereas data from the 14th day showed a statistical significance ($p < 0.001$). In the early stage of the lisinopril-treated group, the appearance of apoptotic cells was fewer, while as the treatment continued, the number of apoptotic cells increased (Fig. 3c, d).

Electron microscopy results

In the untreated group, tubule epithelial cells, as well as deterioration of the apical microvilli and thick large cytoplasmic bleb extending into the lumen, which were similar to apoptotic cells, were detected. Near the portion of the lumen, intercellular connections were tracked. Mitochondria and other organelles were normal, except in some where the cristae of mitochondria were indistinct (Fig. 4a). Sections from glomeruli showed pedicels and podocytes dilated due to edema (Fig. 4b).

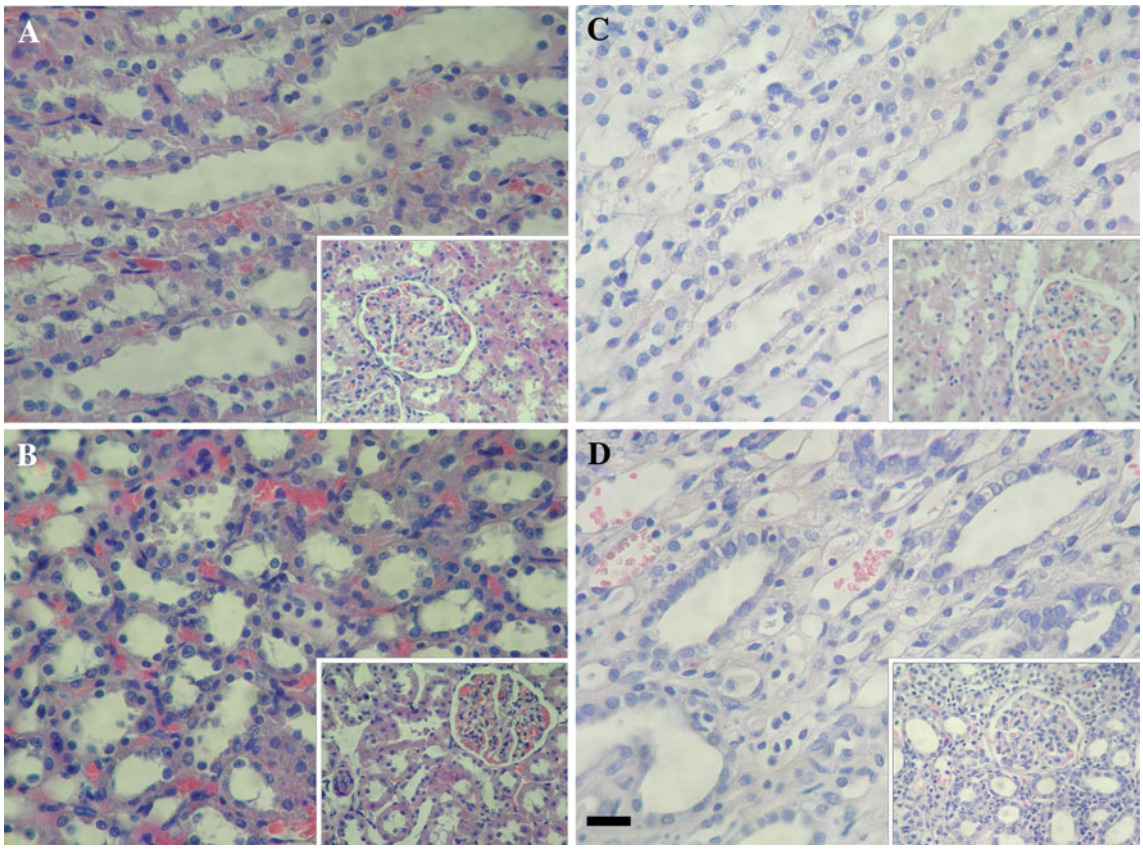

Fig. 1 Histological examination of untreated (**a**, **b**) and lisinopril-treated (**c**, **d**) groups of kidney from the 4th (**a**, **c**) and 14th (**b**, **d**) days after operation. Cortex figures are inserted in medulla figures (H–E). *Scale bar* 25 μm

In the untreated group, tubular epithelial cell degeneration was detected. In addition, some of the cells were ultrastructurally similar with karyolysis. Increase of collagen deposition was also detected in the intertubular space. Distribution of organelles in the cytoplasm was also decreased and these cells showed a degenerative view (Fig. 4c, d).

In the lisinopril-treated group on the 4th day, epithelial cells of tubule were detected to be normal and the apical microvilli extending into the lumen were rather preserved. In addition, glomerulus podocyte and pedicel were very protected (Fig. 4e, f).

In the lisinopril-treated group at 14th days, tubule epithelial cells and distribution of cell organelles, were detected normally, but rather the loss of microvilli was detected (Fig. 4g, h). In Fig. 4, two adjacent tubule cells which in organelles were protected have normal collagen fibers. In addition, pedicel in glomerulus was also much protected.

Discussion

There are increasing numbers of antenatal or adult models which show the effect of UUO on renal tissue. To date, UUO is mainly produced by surgical methods [15] as we did in this study. Several animals such as rabbit, sheep, opossum and chick embryo have been used antenatal and adult models [16–20], and most of them produced by ligating the ureter. It seems the changes in renal morphology after UUO depend on the time of onset, duration and degree of obstruction [21]. In adult models, the gradual destruction or atrophy of the renal parenchyma was associated with increase in the size of the hydronephrosis. Hydronephrotic atrophy may be associated with destruction of all the renal parenchymal tissue, and a thin-walled sac of watery fluid remains [21]. Hydronephrotic atrophy takes about 4 months in rats [22]; however, the acutely obstructed kidney may increase its weight within hours after UUO due to parenchymal edema [23]. In our study as we observed the nephrectomy materials, the right kidney of all groups and both kidneys of the unobstructed group were morphologically normal, but in the obstructed groups (lisinopril and untreated) we observed especially on the 14th day growth in size of kidney, and serious dilatation of the renal pelvis and proximal portion of the ligated ureter, as expected.

We demonstrated the effect of lisinopril, an angiotensin-2 inhibitor, on renal tissue damage in UUO in rats histologically by observing renal tissue with light and electron

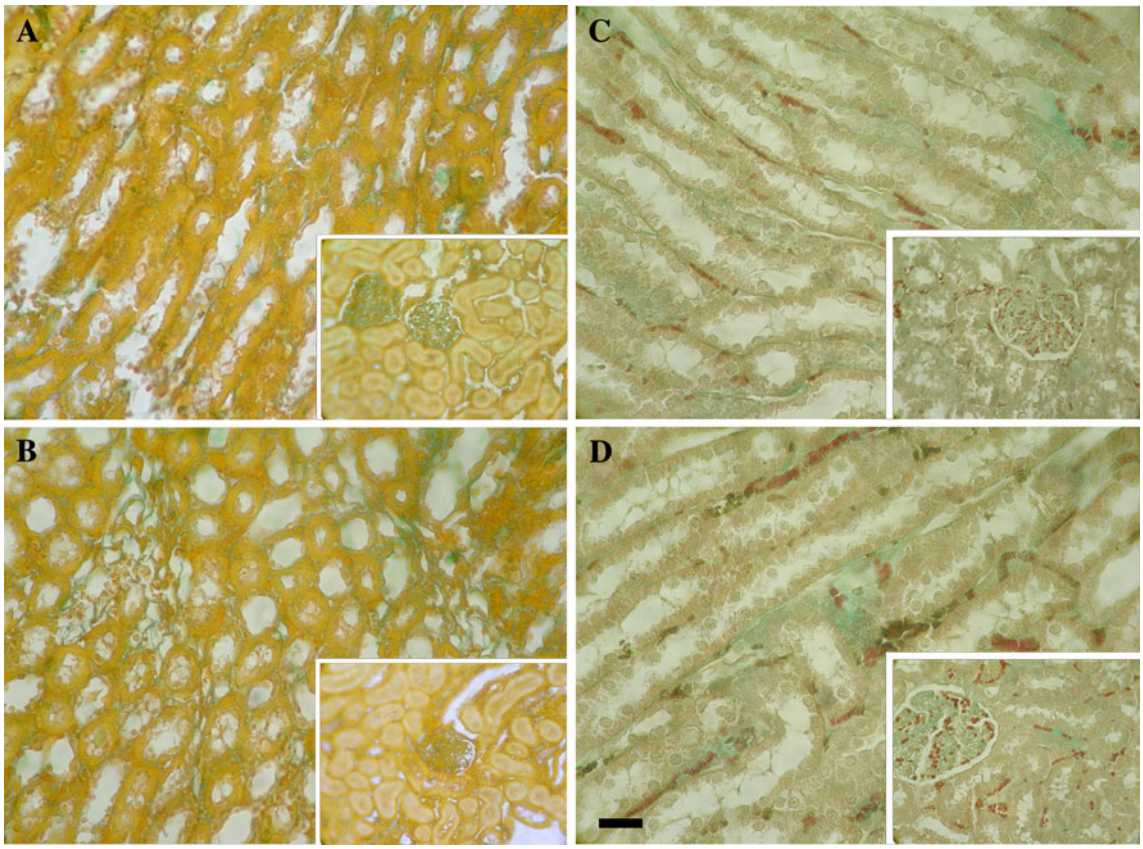

Fig. 2 Masson Trichrom staining of untreated (**a**, **b**) and lisinopril-treated (**c**, **d**) groups of kidney from the 4th (**a**, **c**) and 14th (**b**, **d**) days after operation. Cortex figures are inserted in medulla figures. *Scale bar* 25 μm

microscope and apoptosis using TUNEL method. After electron microscopic evaluation, while edema in glomeruli, loss of microvillus and apoptotic cells were observed in the proximal tubule cells on the 4th day in the untreated group, these differences were more on the 14th day in the untreated group, including sclerosis in the glomeruli, widespread degeneration of the tubule cells, tubule contraction in places and apoptotic–necrotic cells of the proximal tubule cells. In addition, we found that after lisinopril treatment, tubular atrophy, flattened epithelium of tubular cells and tubular deepithelialization, which were very well documented in the ureteral obstruction model (untreated group) in the morphologic analysis, were altered when compared with the untreated group. Therefore, after UUO, the histology of the kidney was disrupted, and after treatment with lisinopril, the histopathological differences improved and only picnotic nuclei were observed in the collecting tubule in the late period. When the sections were stained with Masson Trichrome, there were no differences between the untreated and lisinopril-treated groups. Collagen deposition is a late effect of UUO; therefore, in 2 weeks, there was no collagen deposition pathologically. Light microscopic examinations of all groups supported electron microscopic observation.

In sections on the 4 and 14 days of the untreated group, TUNEL-positive cells were not detected in glomerulus cells, whereas in the proximal and distal tubules and collecting tubules cells, TUNEL-positive cells were observed on both 4th and 14th days: respectively, 21.1 ± 0.57 and $15.8 \pm 0.25\%$. The renal medulla was examined on the same day and the number of positive cells on the 4th and 14th days were 25.6 ± 0.37 and $30.9 \pm 0.73\%$, respectively. When compared with the cortex, there were more TUNEL-positive cells observed and these data were significant ($p < 0.001$). These data show that the medulla was harmed more than the cortex.

We observed apoptosis in the lisinopril-treated group, on the 4th and 14th day, in the glomerulus, and proximal and distal tubule. TUNEL-positive cells were almost negative (respectively, 0.3 ± 0.01 and $0.1 \pm 0.15\%$) in the medulla. TUNEL-positive cells were observed in the collecting tubule cells on the 4th day ($0.2 \pm 0.13\%$) and 14th day ($3.7 \pm 0.26\%$). The data from the 4th day were not statistically significant compared with the unobstructed group, whereas data from the 14th day showed a statistical significance ($p < 0.001$). These data show that in the early stage of the lisinopril-treated group, the appearance of apoptotic cells was fewer, while as the treatment contin-

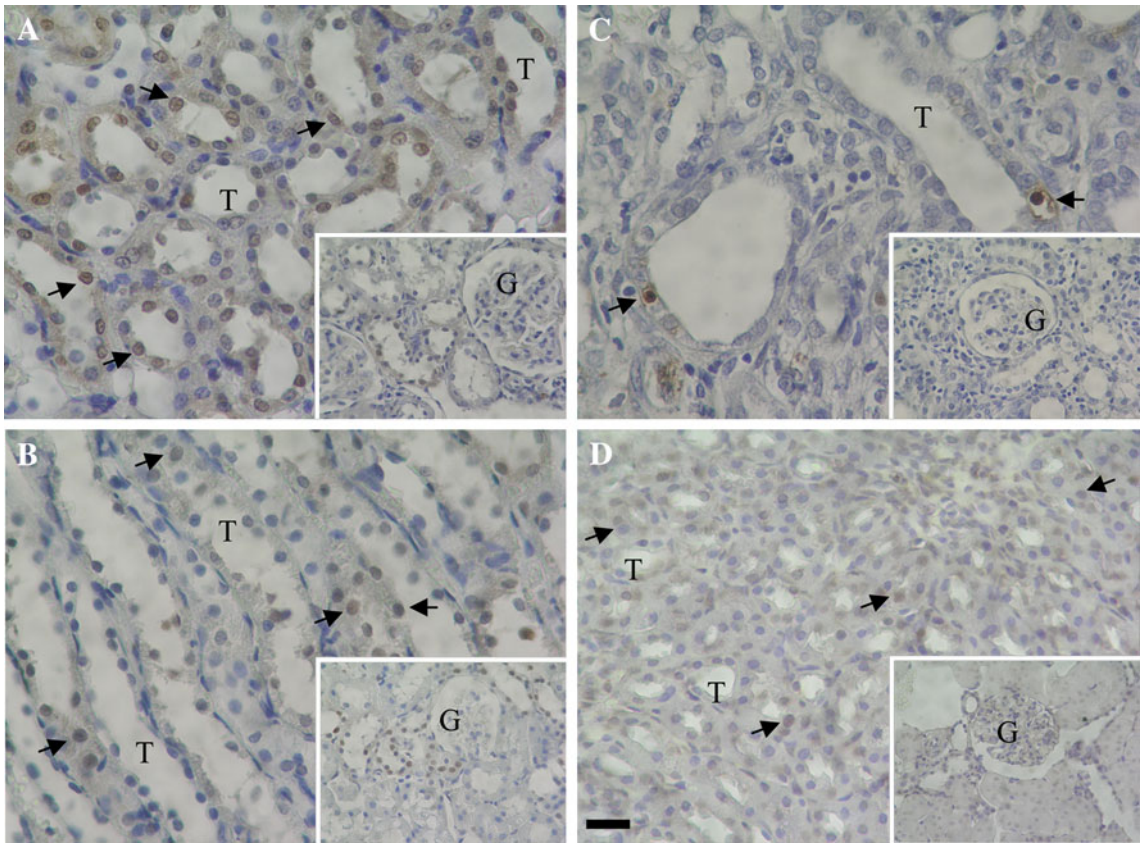

Fig. 3 TUNEL analyses of untreated (**a**, **b**) and lisinopril-treated (**c**, **d**) groups of kidney from the 4th (**a**, **c**) and 14th (**b**, **d**) days after operation. TUNEL-positive cells showed brown nucleus (*arrows*). Cortex figures are inserted in medulla figures. G Glomerul, T Tubul. *Scale bar* 25 μm

Table 1 TUNEL-positive cell numbers from all groups

TUNEL (%)	Sham		Control		Lisinopril	
	4th day	14th day	4th day	14th day	4th day	14th day
Left cortex	–	–	21.1 ± 0.57*	15.8 ± 0.25*	0.1 ± 0.01	0.3 ± 0.15
Left medulla	–	–	25.6 ± 0.37*	30.9 ± 0.73*	0.2 ± 0.13	3.7 ± 0.26
Right cortex	–	–	10.5 ± 0.48	13.6 ± 0.43	–	6 ± 0.26
Right medulla	–	–	28.1 ± 0.62	30.1 ± 0.41	–	11.1 ± 0.40

ued, the number of cells increased in the medulla. Therefore, lisinopril protects the effect of UUO in the early stage, but if the obstruction continues, the effect of lisinopril diminishes. These findings are in conflict with the data of certain studies [4, 8]. Turan et al. [4] investigated the effects of enalapril, another ACE inhibitor, on rat kidneys and found no significantly ameliorating effect when the enalapril-treated group was compared with the untreated group after 2 weeks of UUO. They speculate that enalapril may be effective only in the short term; 2 week of obstruction was too long for drug benefit, as we found similar results in our study: the effect of lisinopril was significant only in the early stage of obstruction. In another study, Radovic et al. [8] also found that neither cilazapril (ACE inh) nor losartan (AT receptor antagonist) had any effect on tubular cell apoptosis, observed on rats after 10 days of UUO. The data can be explained also by the long duration of UUO.

In a recent study, El Chaar et al. [6] have shown the effect of enalapril and 1D11 (monocylonal antibody to TGF-β). They found administration of either 1D11 or enalapril individually significantly decreased all tissue changes, but in combination there was little additive effect and did not provide full protection against damage. In another study, Miyajima et al. [24] have shown similar ameliorating effect of ACE inhibitor on rats. Further investigation is required for the understanding of the mechanisms of UUO and the effects of drugs on obstruction models.

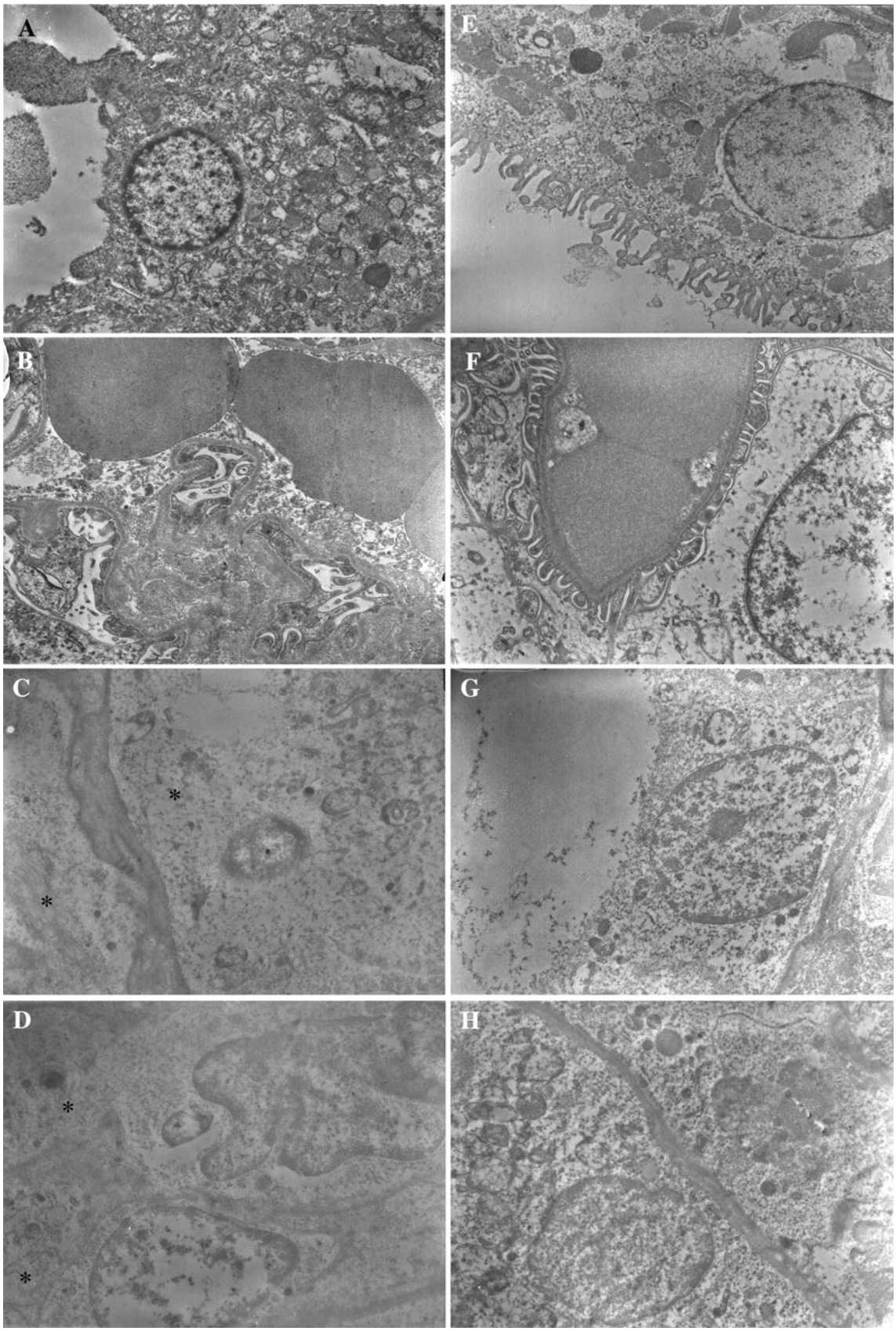

Fig. 4 Electron micrograph of untreated (**a**–**d**) and lisinopril-treated (**e**–**h**) groups of kidney from the 4th (**a**, **b**, **e**, **f**) and 14th (**c**, **d**, **g**, **h**) days after operation. The deposition of collagen fibers in **c** and **d** is labeled (*asterisks*). *Scale bar* 1 μm

In the light of these data, drug administration after complete UUO may not provide full protection against the harmful effects of ongoing obstruction. Ss a clinical aspect, when we experience an obstructive ureteral stone, ACE inhibitors can give the urologist time before surgical treatment.

Conclusion

In the lisinopril-treated group, compared to the untreated group, the number of damaged and apoptotic cells were found to be less. Lisinopril was concluded to be effective in preventing early renal damage in the direct obstruction model. For ongoing obstruction, because of the decreasing effect of lisinopril, surgical treatment is required.

References

1. Chavalier Robert L (2006) Pathogenesis of renal injury in obstructive uropathy. Curr Opin Pediatr 18:153–160
2. Klahr S, Buekert J, Purkerson ML (1977) The kidney in obstructive uropathy. Contrib Nephrol 7:220
3. Jones EA, Shahed A, Shoskes DA (2000) Modulation of apoptotic and inflammatory genes by bioflavonoids and angiotensin II inhibition in ureteral obstruction. Urology 56:346
4. Turan T, Van Harten R, De Water R, Tuncay OL, Kok DJ (2003) Is enalapril adequate for the prevention of renal tissue damage caused by unilateral ureteral obstruction and/or hyperoxaluria? Urol Res 31:212–217
5. Fronkier J (2005) Pharmacologic intervention in urinary tract obstruction—is it possible? Kidney Int 68:894–895
6. El Chaar M, Chen J, Seshan VS, Jha S, Richardson I, Ledbetter SR, Vaughan ED, Poppas DP, Felsen D (2007) Effect of combination therapy with enalapril and the TGF-β antagonist 1D11 in unilateral ureteral obstruction. Am J Physiol Renal Physiol 292:F1291–F1301
7. Klahr S (2001) Urinary tract obstruction. Semin Nephrol 21:133–145
8. Radovic N, Cuzic S, Knotek M (2008) Effect of unilateral ureteral obstruction and anti-angiotensin II treatment on renal tubule and interstitial cell apoptosis in rats. Croat Med J 49:600–607
9. Cummings MC, Winterford CM, Walker NI (1997) Apoptosis. In: Stenberg SS (ed) Histology for pathologists, 2nd edn. Lippincott-Raven Publishers, Philadelphia, pp 3–21
10. Bowen ID (1993) Apoptosis or programmed cell death. Cell Biol Int 17:365–380
11. Stellar H (1995) Mechanisms and genes of cellular suicide. Science 267:1445–1449
12. Nagata S, Golstein P (1995) The Fas death factor. Science 267:1449–1456
13. Borutaite V (2010) Mitochondria as decision-makers in cell death. Environ Mol Mutagen 51(5):406–416
14. Baker AJ, Mooney A, Hughes J et al (1994) Mesangial cell apoptosis: the major mechanism for resolution of glomerular hypercellularity in experimental mesangial proliferative nephritis. J Clin Invest 94:2105–2116
15. Gonçalves GR, Biato AM, Colosimo DGR, Martinusso AC, Pecly ID, Farias KE, Cardoso RL, Takiya MC, Ornellas FRJ, Leite M Jr (2004) Effects of myophenolate moferil and lisinopril on collagen deposition in unilateral ureteral obstruction in rats. Am J Nephrol 24:527–536
16. Beck AD (1971) The effect of intra-uterine urinary obstruction upon the development of the fetal kidney. J Urol 105:784
17. Berman DJ, Maizels M (1982) The role of urinary obstruction in the genesis of renal dysplasia. A model in the chick embryo. J Urol 128:1091
18. Mc Vary KT, Maizels M (1989) Urinary obstruction reduces glomerulogenesis in the developing kidney: a model in the rabbit. J Urol 142:646
19. Steinhardt GF, Salinas-Madrigal L, Farber R, Phillips B, Vogler G (1994) Experimental ureteral obstruction in the fetal opossum: histologic assessment. J Urol 152:2133
20. Wen JG, Frokiaer J, Jorgensen TM, Djurhuus JC (1999) Obstructive nephropathy: an update of the experimental research. Urol Res 27:29–39
21. Ward RM, Starr NT, Snow BW, Bellinger MF, Pysher TJ, Zaino RJ (1989) Serial renal function in an ovine model of unilateral fetal urinary tract obstruction. J Urol 142:652
22. Fink RL, Caridis DT, Chmiel R, Ryan G (1980) Renal impairment and its reversibility following variable periods of complete ureteric obstruction. Aust NZ J Surg 50:77
23. Moody TE, Vaughan ED Jr, Gillenwater JY (1977) Comparison of the renal hemodynamic response to unilateral and bilateral ureteric occlusion. Invest Urol 14:455
24. Miyajima A, Chen J, Poppas DP, Vaughan ED Jr, Felsen D (2001) Role of nitric oxide in renal tubular apoptosis of unilateral obstruction. Kidney Int 59:1290–1303