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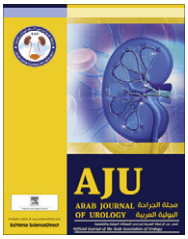

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ORIGINAL ARTICLE

Renoprotection against complete unilateral ureteric obstruction: Is there an ultimate choice?

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Antioxidants

ABBREVIATIONS

ESRD, end-stage renal disease; ERPF, effective renal plasma flow; (U)UO, (unilateral) ureteric obstruction; AT, angiotensin II; ACE, angiotensin-converting enzyme; NO,

Abstract ***Objectives:*** To evaluate and compare the relative contribution of different therapeutic agents for renoprotection against complete unilateral ureteric obstruction (UUO), using a rabbit model sampled at different times.

Materials and methods: Eighty-four male New Zealand White rabbits were divided into seven groups of 12 rabbits; a sham group, a control (left UUO + no medication) or left UUO and treated with either enalapril, losartan, verapamil, L-arginine or antioxidant (vitamin E and selenium mixture). Rabbits in the control and treated groups were subjected to 3, 10 and 21 days of complete ureteric ligation and then killed humanely. The control and treated groups were evaluated at baseline and at the end of the experiment, by measuring split effective renal plasma flow (ERPF) using diuretic renography, and the split glomerular filtration rate (GFR) using selective creatinine clearance. Renal histopathology was evaluated using a tubulo-interstitial damage score.

Results: In the sham group there was no significant effect on any of the evaluated variables. For split ERPF, losartan showed the highest renoprotective effect, saving 44% and 77% of ERPF at 3 and 21 days after UUO, respectively. Losartan was also the best renoprotective agent for GFR. For renal histopathology, enalapril showed the earliest and greatest improvement as assessed by the damage score, reaching 60% at 21 days after UUO. L-Arginine was the next best effect to blockade the renin-angiotensin system for renoprotection.

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nitric oxide; NF, nuclear factor; RB, receptor blocker; C_{cr}, creatinine clearance; RAS, renin-angiotensin system

Conclusion: We suggest that blockade of the renin-angiotensin system provides the best renoprotection against the effects of complete UUO.

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Introduction

The global burden of end-stage renal disease (ESRD) is increasing [1]. It has long been recognized that, left untreated, chronic kidney disease of diverse causes inevitably progresses to ESRD once ≈75% of the GFR is lost, even when the initiating pathological processes are no longer active [2]. At present there are no definitive cures for most acquired kidney diseases, and there is no reasonable expectation that gene therapy will be available soon to treat genetic forms of kidney diseases. Renal transplantation is limited by organ shortage [3], and therefore the best management at present is to prevent the progression of renal diseases, termed 'renoprotection'.

Obstructive uropathy is a common cause of renal impairment [4]. A substantial vasoconstriction of the renal vascular bed is the predominant change observed after ureteric obstruction (UO) for ⩾24 h. An imbalance between vasoconstrictor and vasodilator substances might explain the haemodynamic changes apparent in this setting.

Several vasoconstrictor substances are known to have a role during UO, of which angiotensin II (AT) was extensively reviewed. Besides its powerful vasoconstrictor effect, studies using angiotensin-converting enzyme (ACE) inhibition or AT type 1a (AT_{1a}) receptor knockout mice indicated that AT generation and action result in at least half of the eventual fibrosis of obstructive nephropathy [5]. Oxidative stress, mediated partially by AT, potentiates the vasoconstrictor role of AT because of increased catabolism of nitric oxide (NO). Also, it up-regulates the expression of adhesion molecules, chemoattractant compounds and cytokines [6].

The decreased activity of vasodilators like NO was also implicated. In unilateral UO (UUO), the renal vasculature remains responsive to the vasodilator actions of NO and blood-flow changes associated with UUO involve impairment of the NO synthetic pathways in the kidney [7].

It was confirmed that renal damage progresses despite an improvement of renal function after relief of UUO in adult rats [8]. AT stimulates nuclear factor (NF)-κB activation, leading to increased TNFα synthesis, which, in turn, can further activate NF-κB. This creates an autocrine-reinforcing loop [9]. Binding of TNFα to its receptors activates several signal-transduction pathways that result in the expression of a variety of transcription factors, cytokines, growth factors, receptors, cell-adhesion molecules, mediators of inflammatory processes, acute-phase proteins and major histocompatibility complex proteins [10]. This necessitates renoprotection against renal damage during UUO to prevent its progress to ESRD even after the release of obstruction.

Extensive studies using several renoprotective approaches were conducted to provide renoprotection against UUO, using available data about the pathophysiology of obstruction. Although extensive, few studies compared the magnitude of the renoprotective effect of these therapies [11]. These studies either used different animal models or variable intervals after obstruction. Even studies that avoided these defects did not include renal histopathological changes as a point of comparison.

The present study used a controlled experimental approach that aimed to evaluate and compare the relative contribution of different therapeutic agents in renoprotection against complete UUO, using a rabbit model at different sampling intervals. We aimed to identify, if any, an ultimate agent for renoprotection in such conditions.

Materials and methods

In all, 84 male New Zealand White rabbits (2.5–3 kg, aged 7–11 months) were used; they were housed in a room with controlled environmental conditions (20–22 C, relative humidity 50–55% and 12-h light/dark cycles). The rabbits were fed standard pelleted rabbit food and had free access to tap water. To maintain hydration, rabbits were injected subcutaneously with 10 mL Ringer-lactate daily for 1 week before the experiment.

The rabbits were divided into seven groups of 12 each, i.e. (1) a sham-operated (abdominal incision with no ureteric ligation and receiving no treatment), then six groups with left ureteric ligation and receiving; (2) no treatment (serving as the control); (3) the ACE inhibitor enalapril at 0.5 mg/kg once daily; (4) the AT receptor blocker (RB) losartan at 10 mg/kg once daily; (5) the calcium-channel blocker verapamil at 3 mg/kg once daily; (6) L-arginine at 1 g/100 mL drinking water, a total dose of 1.25–1.5 g/day; and (7) antioxidant, as vitamin E and selenium at 1 mL once daily, which provided the rabbit with vitamin E (100 mg/kg) plus selenium (1 mg/kg).

The doses used were obtained by the conversion of doses used for rats and dogs in previous studies, using the surface area table obtained from Surface Area Ratios of Some Common Laboratory Species and Man [12] and revised using Plumb's Veterinary Drug Handbook [13]. All drugs were introduced into the rabbit's oesophagus

to be swallowed, using a special rabbit gavage, except L-arginine that was ingested with drinking water.

Each group was then subdivided into three equal subgroups that were killed humanely at 3, 10 and 21 days, respectively, after UUO. These intervals were based on our previous pilot study using 12 rabbits carried out 1 month before the start of the present study (unpublished data). Changes occurring at 3, 10 and 21 days were found to be the most frequent and significant.

All experimental procedures were conducted between 09.00h and 14.00 h. The rabbits were anaesthetized by intramuscular injection with a mixture of ketamine (35 mg/kg), xylazine (5 mg/kg), and midazolam (1 mg/kg). After anaesthesia, a longitudinal para-median abdominal incision was made (≈6 cm from the middle line) on the left side. The abdominal muscles were cut and the left ureter was freed gently along its course. The ureter was left intact in the sham group, whereas it was cut between 4/0 silk ligatures in the control and study groups [14].

A cannula (22 G) was introduced into the most proximal part of the ureter and urine was collected for 2 h thereafter. Another intravenous cannula (24 G) was introduced to collect a blood sample (3 mL) from the left renal vein. Finally, the incision was closed; the rabbit was transferred to a clean cage and treated using routine postoperative care.

After 3, 10 and 21 days, according to the designated subgroup, the rabbits were re-opened, urine was collected again for 2 h using the same procedure as before and another blood sample was withdrawn. Then the two kidneys were harvested for histopathological examination and the rabbits killed humanely. The right kidney served as a control for the left. The study was approved by the local ethical committee.

For all groups, the split GFR was measured by estimating the split endogenous creatinine clearance (C_{cr}), using blood and urine samples collected from the corresponding kidney during the experiment just after inducing obstruction, and just before death, according to the assigned subgroup. The concentrations of creatinine in serum and urine were measured using an auto-analyser apparatus (CX7; Beckman, USA) which was adjusted to measure the rabbit samples. C_{cr} was calculated from the equation of Edelstein and Cronin [15] as (urinary creatinine in mg/dL × urine volume in mL/24 h)/(serum creatinine as mg/dL × 1440 min).

The split effective renal plasma flow (ERPF) was measured using diuretic renography [16], before the onset of obstruction to measure basal levels, and then repeated at 3, 10 and 21 days after UUO, according to the assigned subgroup. The rabbits were sedated using intramuscular injection with ketamine 50 mg/kg and xylazine 10 mg/kg. The ^{99m}Tc-MAG3 was injected into an ear vein of the sedated rabbit at 0.25 MBq/kg, and the procedure continued for 20 min. After the eighth frame of renogram, frusemide was injected at 2.2 mg/kg. The curves of the renogram were interpreted by an expert radiologist, and the split GFR and ERPF were calculated.

For histopathology, the harvested kidneys were perfused briefly with PBS to rinse out the blood. The kidney was rapidly placed in a container containing 10% neutral buffered formalin for histopathological examination. The kidney specimens were processed for paraffin blocks and sections 3 μm thick were cut and stained with haematoxylin and eosin, and with periodic acid-Schiff. Light-microscopic sections of the stained films were examined (10 random fields with a 40× objective) for tubular vacuolization, tubular dilatation, tubular necrosis, intratubular detachment, tubular cell brush border integrity and interstitial oedema. The tubulo-interstitial damage score was calculated by averaging the mean score of each of the following pathological characteristics: 1, no abnormality; 2, mild lesions affecting 10–25% of the kidney samples; 3, lesions affecting 25–50%; 4, lesions affecting 50–75% and 5, lesions affecting ⩾75% of the kidney samples [17].

The data for renal function and histopathology were analysed to ascertain whether changes that occurred with time and within one kidney were significant, using a one-way ANOVA. If there was a significant difference, multiple comparisons vs. a control group (Tukey's method) were done. A *t*-test was used to determine whether mean values at selected sample times differed between groups. The results are expressed as the mean (SEM) and considered significant when $P < 0.05$.

Results

All rabbits survived the treatments and there were no surgical complications in any of them. Renal function was stable in the sham-operated rabbits (Table 1). All the measured variables of renal function showed a significant deterioration ($P = 0.05$) in the control group when compared with the sham-operated group at all sample times (Table 1).

Compared with basal values, left UUO caused a progressive decrease in split ERPF at the three sample times in the control and study groups. In the control group, the percentage decrease of split ERPF was 65.9%, 84.3% and 91.3% of the basal value at 3, 10 and 21 days after inducing UUO, respectively (Table 1).

All five renoprotective agents reduced the deterioration in split ERPF, by different degrees, compared with the control group. This effect was significant as early as 3 days after UUO for losartan and L-arginine, whereas the effect of enalapril and verapamil started to be significant at 10 days and beyond. Although it reduced the deterioration in split ERPF, the effect of antioxidant was insignificant compared with the control group at all sample times (Table 1).

The effect of the five renoprotective agents on saving the split ERPF after UUO compared with the control group is shown in Table 2. Of all study groups, losartan

Table 1 Summary of the effects of the used therapeutic agents on renal functions.

Mean (SEM) variable/days after UUO	Group						
	Sham	Control	Enalapril	Losartan	Verapamil	L-arginine	Antioxidant
ERPF (mL/min)							
0 (pre)	86.22 (1.08)	83.98 (2.23)	85.68 (3.50)	84.59 (13.83)	91.26 (7.2)	91.26 (7.2)	87.64 (3.31)
3	84.34 (2.6)	28.64 (2.82)†	44.44 (0.78)	65.68 (3.17)*	52.34 (1.79)	62.34 (1.79)*	34.61 (3.63)
10	85.29 (1.67)	13.15 (0.55)†	51.03 (4.12)*	70.94 (4.27)*	54.28 (1.92)*	64.94 (1.53)*	24.50 (1.89)
21	85.44 (2.02)	8.13 (1.07)†	53.69 (1.89)*	73.02 (5.41)*	56.36 (3.23)*	69.69 (6.42)*	11.88 (0.45)
GFR (mL/min)							
0 (pre)	25.89 (0.88)	23.44 (0.64)	25.95 (0.87)	25.58 (0.84)	25.58 (0.84)	26.25 (1.42)	25.5 (1.65)
3	24.66 (1.05)	13.54 (0.84)†	17.73 (0.68)	20.74 (0.87)	16.74 (0.49)	18.41 (0.45)	14.18 (0.69)
10	25.07 (0.21)	9.73 (0.52)†	16.25 (0.84)	21.58 (0.45)*	16.58 (0.84)	19.58 (0.42)*	9.43 (0.94)
21	25.71 (0.5)	7.47 (0.53)†	16.04 (0.94)*	21.52 (0.25)*	14.86 (1.15)*	19.19 (0.68)*	8.08 (1.39)
Serum creatinine (mg/dL)							
0 (pre)	0.88 (0.05)	0.9 (0.06)	0.78 (0.09)	0.9 (0.07)	0.85 (0.07)	0.70 (0.20)	0.93 (0.48)
3	0.97 (0.04)	1.6 (0.21)†	1.7 (0.15)	1.28 (0.06)	1.25 (0.07)	1.25 (0.05)	1.41 (0.05)
10	0.89 (0.06)	1.87 (0.47)†	1.47 (0.18)	1.18 (0.06)	1.13 (0.07)	1.07 (0.03)	1.34 (0.11)
21	0.88 (0.32)	2.48 (0.08)†	1.26 (0.10)*	1.1 (0.06)*	1.075 (0.05)*	0.97 (0.09)*	1.25 (0.05)*

* Significant vs. control group at the same sample time.
† Significant vs. the sham group at the same sample time.

Table 2 The renoprotective effects of the therapeutic agents on renal function.

Days after UUO/variable		% Saved				
		Losartan	L-Arginine	Verapamil	Enalapril	Antioxidants
3	ERPF	43.6	34.2	$23.3^{L,A}$	$17.8^{L,A,V}$	$5.39^{L,A,V,E}$
	GFR	23.3	12.4^{L}	$7.68^{L,A}$	$10.6^{L,A}$	-2.15^{X}
10	ERPF	68.2	55.5	$43.8^{L,A}$	$43.9^{L,A}$	$12.3^{L,A,V,E}$
	GFR	42.9	33.1^{L}	$23.3^{L,A}$	$21.1^{L,A}$	-4.53^{X}
21	ERPF	76.6	66.7	52.1^{L}	$53.0^{L,A}$	$3.88^{L,A,V,E}$
	GFR	52.3	41.2^{L}	$26.2^{L,A}$	$29.9^{L,A}$	-0.18^{X}

L, significant vs. losartan; A, significant vs. L-arginine; V, significant vs. verapamil; E, significant vs. enalapril; X, not suitable for comparison. All comparisons were made at the same time point.

showed the greatest renoprotective effect, saving 43.6% of ERPF at 3 days after UUO. This increased to 76.6% at 21 days after UUO. The renoprotective effect of losartan was significant compared with all other agents, except for L-arginine, at all sample times. L-arginine ingestion was second to losartan as a potent renoprotective agent, saving up to 66.7% of ERPF at 21 days after UUO. Although the percentage of ERPF saved by losartan was higher than that saved by L-arginine at the three sample times, the difference was not statistically significant (Table 2).

The renoprotective effect of verapamil and enalapril, vs. the control group, appeared to be time-dependent. The effect of verapamil was apparent as early as 3 days, saving 23.3% of ERPF, which increased to 52.1% at 21 days. However, the effect of enalapril increased with time from 17.8% at 3 days to 53.0% at 21 days. The renoprotective effect of verapamil was significant vs. enalapril only at 3 days after UUO. There was no significant difference in their renoprotective effect at 10 and 21 days (Table 2).

Antioxidants had no significant renoprotective effect on ERPF vs. the control group. Antioxidants saved 5.4% of ERPF at 3 days, and although this increased to 12.3% at 10 days, it decreased again to 3.9% on 21 days. The renoprotection offered by antioxidants was significantly lower than for all other agents (Table 2).

Compared with basal values, left UUO caused a progressive decrease in split GFR at the three sample times in the control and study groups. In the control group, the percentage decrease of split GFR was 42.2%, 58.5% and 68.1% of the basal value at 3, 10 and 21 days, respectively (Table 1).

Only four renoprotective agents reduced the deterioration in split GFR, by different degrees, compared with the control group. Antioxidants failed to save the split GFR of the obstructed kidney when compared with the control group at all sample times (Table 1). Saving the GFR appears to take longer than saving ERPF. Hence, compared with the control group, the effect of losartan and L-arginine was significant at 10 days and after, whereas the effect of enalapril and verapamil started to be significant only at 21 days (Table 1).

The effect of the different renoprotective agents on saving the split GFR compared with the control group is shown in Table 2. As for ERPF, losartan had the

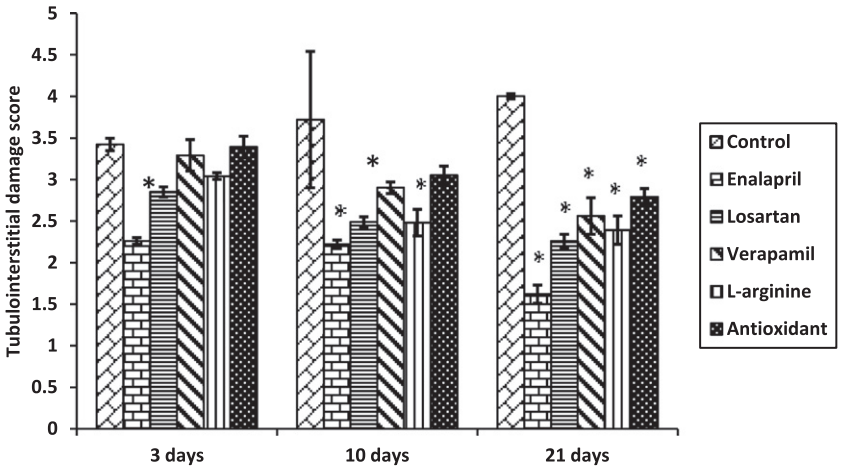

Figure 1 The effect of different therapeutic agents used on renal damage (tubulo-interstitial score) produced by left UUO. All values are expressed as mean ± SE, and * = significant when compared with control group.

greatest renoprotective effect on GFR, saving 23.3% of GFR at 3 days; this increased to 52.3% at 21 days. The renoprotective effect of losartan was significant vs. all other agents at the three sample times. L-arginine had the next greatest effect, saving up to 41.2% of GFR at 21 days. Compared with enalapril and verapamil, the effect of L-arginine was insignificant at 3 days, but its renoprotective effect became significant at 10 and 21 days.

At all sample times the percentage of GFR saved by enalapril and verapamil was very similar, with a slight preference for enalapril, but with no statistically significant difference. Antioxidants failed to save the GFR, which deteriorated further when compared with basal levels before UUO (Table 2).

Compared with basal values, left UUO caused a progressive increase in serum creatinine levels in the left renal vein at the three sample times in the control and study groups (Table 1). Although all the five renoprotective agents improved serum creatinine levels, their effect started to be significant only at 21 days, vs. the control group (Table 1).

Compared with basal values, left UUO caused progressive tubulo-interstitial damage, shown as a progressive increase in the tubulo-interstitial damage score at the three sample times in the control and study groups. In control group, the damage score increased significantly from 3.42 (0.075) at 3 days to 4.00 (0.03) at 21 days (Fig. 1).

All five renoprotective agents reduced the renal damage, as shown by a reduction in the tubulo-interstitial damage score vs. the control group. The magnitude of this tissue sparing varied according to agent used and the duration of use. Of all agents, the improvement in the damage score produced by enalapril was significant as early as 3 days and after, vs. the control group (Fig. 2). The effect provided by losartan and L-arginine started to be significant at 10 days and after. The protection provided by verapamil and antioxidants required 21 days to be statistically significant vs. the control group (Fig. 1).

The effect of the five renoprotective agents on renal tissue sparing after UUO is shown in Table 3. Enalapril showed the greatest renal-tissue sparing, improving the damage score by 34% at 3 days, and increasing to 60% at 21 days. Except for L-arginine, this sparing effect was significant compared with all other agents at all sample times. Losartan showed a slightly higher percentage of tissue sparing than L-arginine; compared with L-arginine, the improvement in the damage score with losartan was significant only at 3 days, with no statistically significant difference at later times.

The renal tissue protection provided by verapamil and antioxidants was the lowest compared with the other agents. Although verapamil gave a greater improvement in the damage score at the three sample times there was no statistically significant difference between the agents.

Discussion

The evidence from both experimental studies and clinical trials suggests that the current practice of renoprotection can, at best, only postpone ESRD and avoid dialysis for a few years. However, nephrologists are striving to reduce the number of patients reaching ESRD.

Although extensive work was done to optimize renoprotection strategies against UUO, few studies have investigated the relative magnitude of renoprotection by comparing the effects of different agents under standardized conditions to determine which agent gives the best results. Moreover, the link between the effect of different renoprotective agents on the histopathological changes of the corresponding kidney and deterioration in kidney function is still lacking. The present study was done to overcome the shortcomings of previous ones.

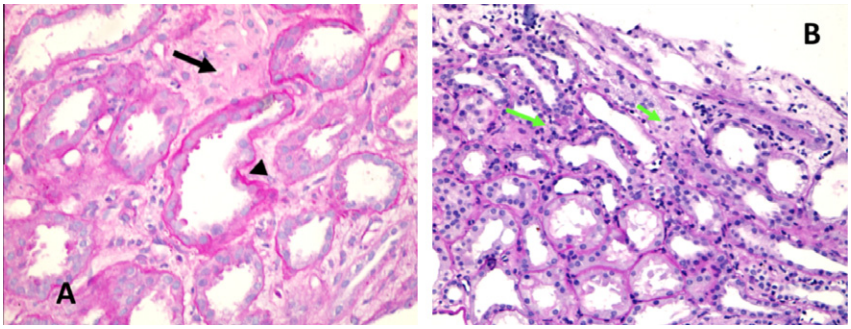

Figure 2 Histopathological changes in renal tissue. (A) Complete UUO with no treatment (showing severe tubulo-interstitial damage). (B) Complete UUO after 10 days of treatment with enalapril (improvement in tissue damage).

Table 3 The percentage improvement in the tubulo-interstitial damage score.

Days after UUO	Damage score				
	Enalapril	Losartan	L-Arginine	Verapamil	Antioxidants
3	34	17^{E}	$11^{E,L}$	3^{E}	$<1^{E,L,A}$
10	40	33^{E}	33	$22^{E,L,A}$	$18^{E,L,A}$
21	60	44^{E}	40^{E}	36^{E}	$30^{E,L}$

L, significant vs. losartan; A, significant vs. L-arginine; E, significant vs. enalapril. All comparisons were made at the same time point.

In agreement with all previous studies, our experiment confirmed that UUO produced a deterioration in renal function, as shown by a significant reduction in split ERPF and split GFR, and a progressive increase in serum creatinine in the renal vein of the obstructed kidney, when compared with the basal values. This deterioration in function was progressive over time, reaching a maximum at 21 days after UUO. The changes in function were also associated with renal tissue damage detected by a significant and progressive increase in the tubulo-interstitial damage score.

Our results supported the role of renin-angiotensin system (RAS) blockade in the progression of UUO into ESRD. We showed that losartan (an AT RB) was the best renoprotective agent for renal function. Losartan saved split ERPF (up to 76.6% by 21 days) and split GFR (up to 52.3% by 21 days) compared with the control obstructed untreated kidney. For renal tissue fibrosis, we showed that enalapril (an ACE inhibitor) had the best renoprotective effect. Enalapril reduced tubulo-interstitial damage by up to 60% at 21 days after UUO compared with the control obstructed untreated kidney.

The renoprotective action of enalapril is exerted mainly through decreased formation of AT [18]. Wang et al. [19] found that enalapril significantly reduced the renal interstitial damage index. This was significant at 3, 7 and 14 days after UUO. Enalapril also stimulates the production of NO [20]. Signalling through the AT_1 receptor results in vasoconstriction, stimulation of growth and activation of fibroblasts and myocytes. Enalapril also decreased the deterioration in renal function in partial UUO, and enhanced the recoverability of renal function after relief of obstruction [21].

Although both ACE inhibitors and AT RBs blocked RAS activity, our results showed that the magnitude of their renoprotective effect varied significantly, with apparently different roles on renal function and pathology. This was in agreement with Laverman et al. [22], who reported that the trials with ACE inhibitors and AT RBs show some striking differences.

The differences in the renoprotection provided by ACE inhibitors and AT RBs in relation to their mechanism depend on interference with AT-mediated actions. Beside the reduction in synthesis of AT, ACE inhibitors increase kinin concentrations, as ACE has a kininase-like action. Bradykinin has some renoprotective properties, e.g. decreasing proteinuria and fibrosis [23]. However, AT RBs block the action of AT on the AT_1 receptor, allowing an increased concentration of AT acting on AT_2 receptors. AT_2 receptors show some renoprotective action, like decreased cell proliferation, increased production of NO and vasodilatation [24]. Also, the efficiency of ACE inhibitor blockade of intrarenal AT formation is still uncertain, whereas losartan directly blocks the action of AT both intra- and extrarenally.

We also showed that L-arginine as an exogenous source of NO provides the next greatest effect after RAS blockade for renoprotection against UUO. L-arginine spared renal function, saving split ERPF (up to 66.7%) and split GFR (up to 41.2%), and was better than enalapril, by 21 days after UUO. L-Arginine also decreased the renal tissue damage score (up to 40% at

21 day) compared with the control obstructed untreated kidney.

Felsen et al. [25] showed that arginine infusion 18 h after UUO led to increases in renal blood flow and ureteric pressure that were not detected in control animals. It was suggested that NO can produce resistance to obstruction-induced apoptosis, through the induction of heat-shock protein 70 expression, in neonatal UUO [26].

Although verapamil and antioxidants were effective, the present results did not support their potency as possible renoprotective agents compared with the other agents. This was not in line with previous studies. Loutzenhiser et al. [27] reported that calcium antagonists markedly augment GFR but produce only a modest improvement in RPF in a model of unilateral hydronephrosis induced by unilateral ureteric ligation. Topcu et al. [28] showed that verapamil significantly prevented the impairment of renal function and prevented the upregulation of p53, Fas and proliferating cell nuclear antigen during UUO. Chade et al. [29] showed that reducing oxidative stress by using antioxidants ameliorates renal injury, especially renal fibrosis, in renovascular diseases.

To our knowledge, the present study is the first to compare all agents with a suggested renoprotective effect in UUO using the same experimental conditions in one animal model at different sample times. The histopathological renoprotection provided by these agents was also investigated. Our study can be criticized because we did not assess combinations of the agents that might be assumed to provide better renoprotection. As to the antioxidants used in the study, different combinations of antioxidants and dosages might provide better renal protection.

Although the degree of renoprotection with these agents was estimated, future work investigating the molecular mechanisms for the difference in degree of renoprotection is necessary. This might throw more light on the relative contribution of the pathways to the pathogenesis of UUO.

In conclusion, blockade of the RAS is considered the best choice for renoprotection in UUO. There was no clear difference between ACE inhibitors and AT RBs. This suggests a greater advantage in using a combination of these agents. The use of an exogenous source of NO provides a similar effect but the applicability of this therapy is not imminent. Calcium-channel blockers and antioxidants are useful but not as a single therapy. This suggests that a better role for them might be as adjuvant agents or in cases where RAS blockade is inappropriate.

Conflict of interest statement

None declared.

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