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Reduced progression of adriamycin nephropathy in spontaneously hypertensive rats treated by losartan

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Abstract

Background. The aim of the study was to investigate the antihypertensive effects of angiotensin II type-1 receptor blocker, losartan, and its potential in slowing down renal disease progression in spontaneously hypertensive rats (SHR) with adriamycin (ADR) nephropathy.

Methods. Six-month-old female SHR were randomly selected in six groups. Two control groups (SH_6, SH_{12}) received vehicle. Groups ADR_6, $ADR+LOS_6$ and ADR_{12}, and $ADR+LOS_{12}$ received ADR (2 mg/kg/b.w. i.v.) twice in a 3-week interval. Group $ADR+LOS_6$ received losartan (10 mg/kg/b.w./day by gavages) for 6 weeks and group $ADR+LOS_{12}$ for 12 weeks after second injection of ADR. Animals were killed after 6 or 12 weeks, respectively. Haemodynamic measurements were performed on anaesthetized animals, blood and urine samples were taken for biochemical analysis and the left kidney was processed for morphological studies.

Results. Short-term losartan treatment, besides antihypertensive effect, improved glomerular filtration rate and ameliorated glomerulosclerosis resulting in decreased proteinuria. Prolonged treatment with losartan showed further reduction of glomerulosclerosis associated with reduced progression of tubular atrophy and interstitial fibrosis, thus preventing heavy proteinuria and chronic renal failure. Losartan reduced uraemia and increased urea clearance in advanced ADR nephropathy in SHR. Histological examination showed that losartan could prevent tubular atrophy, interstitial infiltration and fibrosis in ADR nephropathy.

Conclusion. Losartan reduces the rate of progression of ADR-induced focal segmental glomerulosclerosis to end-stage renal disease in SHR.

Keywords: adriamycin; losartan; renal disease progression; spontaneously hypertensive rats

Introduction

Hypertension manifested with sustained high blood pressure has been known as a major risk factor of many cardiovascular pathophysiological conditions including arteriosclerosis, stroke, heart failure, coronary artery disease and progressive renal damage [1]. Spontaneously hypertensive rats (SHR) develop kidney damage in advanced age [2] similar to nephropathy in patients with essential hypertension [3].

On the other hand, focal segmental glomerulosclerosis (FSGS) is characterized by nephrotic range proteinuria and progressive renal failure in humans, like in experimental models with similar glomerular lesions [4].

Adriamycin (ADR), doxorubicin hydrochloride, is a highly potent antineoplastic agent that induces cell killing by causing a variety of biochemical alterations [5]. Its administration into rats results in heavy proteinuria accompanied by glomerular sclerosis and tubulointerstitial damage similar to the morphological picture of FSGS in humans [6,7]. Intensive proteinuria associated with focal loss of podocyte foot processes, swelling and vacuolization of epithelial and mesangial cells are landmarks of early stage of ADR nephrotoxicity, while focal segmental glomerular sclerosis or even global sclerosis, tubular atrophy and interstitial fibrosis are characteristics of late-stage ADR nephropathy [8]. The degree of renal toxicity is a function of the cumulative dose of ADR [5]. Its metabolism results in reactive oxygen species formation, indicating their role in the course of ADR-induced nephropathy [9].

Angiotensin II (ANG II) plays an important role in the progression of chronic renal failure (CRF) [10]. Studies in various human diseases and animal models have shown that angiotensin I converting enzyme inhibitors (ACEI) are superior to other antihypertensive drugs in protecting kidney against progressive deterioration, even in conditions without systemic hypertension, suggesting that ANG II also has non-haemodynamic effects on progressive renal disease [11] such as growth regulation, immunomodulation, matrix interaction and fibrogenesis [10]. In the previous study from our laboratory, we found that ACEI, captopril, normalized systolic blood pressure (SBP) and preserved

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glomerular and interstitial structure and function, without changes in tubular and renal blood vessel structure, and therefore failed to reduce CRF [12]. Other authors found controversial evidence for the beneficial effects of combined therapy with ACEI and ANG II AT-1 receptor antagonists on the course of nephrotic proteinuria in different experimental models and humans [13]. The observed reduction of proteinuria induced by co-treatment was attributed to the blockade of ANG II synthesized in the pathways including non-ACE conversion. In addition, it is well known that ANG II is synthesized and released locally in the kidney.

Since there was no reduction of renal disease progression in SHR with ADR nephropathy after ACE inhibition, we decided to perform the same experimental design with ANG II AT-1 receptor antagonist, losartan. Thus, the aim of the present study was to examine short-term (6 weeks) and long-term (12 weeks) effects of losartan on blood pressure regulation, renal haemodynamics, kidney function and structure, and the ability of this antihypertensive agent to slow down the progression of FSGS in SHR with ADR nephropathy.

Materials and methods

Materials

The SHR used in this study were bred at the Institute for Medical Research, Belgrade, Serbia, and they were descendants of breeders originally obtained through Taconic Farms, Germantown, NY, USA.

Six-month-old female SHR, weighing about 200 g, were examined for control values of SBP by an indirect method using a tail-cuff, pneumatic pulse detector and the direct recorder (Physiograph Four, Narco Bio-System, Houston, TX, USA). After assuring that all animals were hypertensive, we divided them randomly into six groups. Two control SHR groups (SH_6 and SH_{12}) drank tap water throughout the experiment and they were killed after 6 or 12 weeks, respectively. Four groups of SHR received ADR twice [2 mg/kg of body weight (b.w.) intravenously through the femoral vein during anaesthesia with 35 mg/kg sodium pentobarbital] with an interval of 3 weeks and they were killed after 6 or 12 weeks. The first two of these four groups (ADR_6 and ADR_{12}) received vehicle in addition to ADR. After the second injection of ADR, the two other groups ($ADR+LOS_6$ and $ADR+LOS_{12}$) were treated with losartan (LOS, DUP 153, Du Pont, Wilmington, DE, USA, 10 mg/kg b.w./day by gavages) during the next 6 or 12 weeks of the experiment. Animals were kept under appropriate conditions, fed with standard laboratory chow for experimental animals (Vetrerinarski zavod, Subotica, Serbia) and supplied by water without restriction.

All experiments were done according to the local guidelines for animal research and principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Official Daily N. L 358/1-358/6, 18 December 1986). They were also approved by our institutional ethic committee.

Measurements

Before direct haemodynamic measurements, rats were weighed and placed in individual metabolic cages and urine was collected in the next 24 h for biochemical analysis.

Haemodynamic measurements

Direct haemodynamic measurements were performed in anaesthetized (35 mg/kg sodium pentobarbital, intraperitoneally) rats, after urine collection. Systolic and diastolic arterial pressure (SAP, DAP) were measured through a femoral artery catheter (PE-50, Clay-Adams Parsippany, NY, USA) connected to a low-volume displacement transducer (P23 Db, Statham, Oxnard, CA, USA) and the direct writing recorder (Physiograph Four, Nacro Bio System Inc., Houston, TX, USA), while mean arterial pressure (MAP) was obtained by electronic integration. For renal haemodynamic measurements, the renal artery was gently separated from the surrounding tissue, and the ultrasonic flow probe 1RB (internal diameter 1 mm) was placed around the renal artery. Renal blood flow (RBF) was recorded by the Transonic T106 Small Animal Flowmeter (Transonic System Inc., Ithaca, NY, USA), and renal vascular resistance (RVR) was calculated according to the standard formula, normalized to body weight, and expressed as mmHg $\times$ min $\times$ kg/ml.

Biochemical measurements

At the end of the study, all rats were killed and blood samples were taken for determination of creatinine, urea and protein concentrations. Lithium-heparin (Li-heparin, Sigma, USA) was used as an anticoagulant. Twenty-four-hour urine samples for determination of urinary flow were collected in graduated cylinders with an accuracy of 0.1 ml. These urine samples also were used for measuring creatinine, urea and protein concentrations. Plasma and urine creatinine was measured by the Jaffe reaction and plasma and urine urea by the urease reaction. Protein concentration in plasma and urine were determined by commercial kit Randox (Crumlin, Antrim, UK). We have used spectrophotometer for all analysis (Cobas Mira, Roche, Elitech Diagnostic). A standard formula was used to calculate endogenous creatinine clearance and urea clearance and protein excretion. The water reabsorption rate was calculated according to Kusaka *et al*. [14].

Histological examination

Left kidney was excised, dissected longitudinally and fixed in 10% buffered formalin solution. Renal tissue samples were dehydrated in alcohol, blocked in paraffin wax, cut into 5-μm slices and stained by haematoxilin & eosin (H&E) and periodic acid Schiff (PAS) reaction for light microscopy observation.

The severity of histological changes was estimated according to renal scarring. Sclerotic changes in glomeruli were graded as follows: 0 normal glomeruli, 1+ slight segmental changes in small number of glomeruli, 2+ segmental and global changes in most glomeruli, 3+ general global

sclerosis. Tubular dilatation with luminal PAS positive material and atrophy of tubular epithelium as well as interstitial infiltration and interstitial fibrosis were graded from 0 to 3+, according to extension of changes. Vascular injury was graded as follows: 0 normal vessels, 1+ slight vacuolization of media, 2+ hyperplasia of smooth muscle cells and 3+ PAS positive necrosis. The sum of these changes represents the histopathological (HP) score for comparison between treated and control groups.

Statistical analysis

The data are given as mean ± SEM. One-way analysis of variance (ANOVA) was used for multiple comparisons between experimental groups. Newman–Keuls and Duncan tests were performed as *post hoc* multiple comparisons (Statistica for Windows). *P*-values <0.05 were considered significant.

Results

Body weight

Six weeks after second injection of ADR, no difference in the body weight was found between experimental groups (Table 1). After 12 weeks, control SHR and SHR that received losartan after ADR gained body weight compared to respective groups at Week 6 and compared to ADR_{12}, while rats from the ADR group stagnated in body weight during the experiment (Table 1).

Haemodynamic parameters

Directly measured SAP, DAP and MAP in all experimental animals are shown in Table 2, while renal haemodynamics (RBF and RVR) are presented as clustered column values across the categories obtained after 6 and 12 weeks of treatment in Figure 1. With regard to SAP, DAP and MAP, there were no differences between control SHR and SHR that received ADR. SAP and MAP were significantly decreased in the group with 6 weeks' losartan administration in comparison to the control group and ADR alone.

Haemodynamic parameters obtained from the renal artery pointed out that ADR led to remarkable reduction of RBF at late stage of ADR nephropathy, and thereafter RVR was significantly increased. Long-term losartan treatment reduced SAP only, without effects on DAP and MAP. Six weeks' application of AT-1 receptor antagonist, losartan, resulted in mild augmentation of RBF with a mild decrease of RVR. However, 12 weeks' losartan treatment induced an increase of RBF by 59.56% and a statistically significant decrease of RVR.

Biochemical parameters

Plasma creatinine (Pcr) and plasma urea (Pu) levels, urine flow (Uf) and water reabsorption (Wr) are given in Table 1. The plasma creatinine level remained unchanged in all SHR after 6 and 12 weeks of experiment, while urinary creatinine dropped significantly in ADR-treated groups (SH_6 versus ADR_6: 7.93 ± 0.52 versus 5.29 ± 0.44 mmol/l, $P < 0.01$; and SH_{12} versus ADR_{12}: 6.36 ± 0.58 versus 3.66 ± 0.6 mmol/l, $P < 0.01$). At the early stage of ADR nephropathy, we found no difference in the glomerular filtration rate (GFR) estimated by clearance of endogenous creatinine (ADR_6 versus SH_6: Figure 2). However, the GFR was markedly elevated after 6 weeks of losartan treatment, as compared to SHR without ADR.

The plasma urea concentration in ADR_6 (Table 1) and the urinary urea concentration in SHR killed 6 and 12 weeks after the second ADR injection were significantly decreased compared to control SHR ($P < 0.001$), without any differences of urea clearance (Figure 2). Twelve weeks after application of ADR, SHR had developed uraemia (Table 1) concomitant with decreased urinary urea concentration (SH_{12} versus ADR_{12}: 939.84 ± 50.60 versus

Table 1. Body weight and biochemical parameters in experimental groups

	SH_6 ($n = 15$)	ADR_6 ($n = 15$)	$ADR+LOS_6$ ($n = 9$)	SH_{12} ($n = 9$)	ADR_{12} ($n = 7$)	$ADR+LOS_{12}$ ($n = 10$)
Body weight (g)	197.33 ± 1.82	190.33 ± 2.86	192.22 ± 4.09	205 ± 2.04$^{\#}$	186.43 ± 3.57§§§	205.5±2.41$^{\#,***}$
Plasma creatinine (μmol/l)	62.4 ± 2.26	61.8 ± 3.92	52.38 ± 5.07	55.78 ± 3.76	60.71 ± 4.87	62.3 ± 2.09
Plasma urea (mmol/l)	9.87 ± 0.45	7.77 ± 0.43§	9.09 ± 0.62	8.86 ± 0.50	10.09 ± 0.94$^{\#}$	7.53 ± 0.42*
Urine flow (μl/min/kg)	28.08 ± 2.24	48.95 ± 6.26§	48.08 ± 7.89§	39.64 ± 2.91$^{\#\#}$	73.08 ± 6.29$^{\S\S\S,\#}$	64.401 ± 5.28§§
Water reabsorption (%)	99.17 ± 0.06	98.74 ± 0.10	98.94 ± 0.24	99.06 ± 0.10	98.15 ± 0.24$^{\S\S,\#}$	98.52 ± 0.14§

$^{\S}P < 0.05$, $^{\S\S}P < 0.01$ and $^{\S\S\S}P < 0.001$ versus the control group; $^{*}P < 0.05$ and $^{***}P < 0.001$ versus the ADR group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, the difference between two examined time points (6 and 12 weeks) in the same group.

Table 2. Arterial blood pressure in experimental groups

	SH_6 ($n = 11$)	ADR_6 ($n = 11$)	$ADR+LOS_6$ ($n = 10$)	SH_{12} ($n = 9$)	ADR_{12} ($n = 8$)	$ADR+LOS_{12}$ ($n = 10$)
SAP (mmHg)	211.09 ± 10.41	207.27 ± 6.67	175.40 ± 8.93§,*	215.33 ± 11.97	193.38 ± 10.65	176.30 ± 5.58§
DAP (mmHg)	151.73 ± 6.65	157.27 ± 3.53	136.90 ± 10.44	156.89 ± 8.65	141.75 ± 10.70	141.30 ± 4.19
MAP (mmHg)	175.82 ± 7.67	177.00 ± 4.53	152.20 ± 9.29§,*	176.78 ± 8.81	161.88 ± 10.18	155.80 ± 3.45

$^{\S}P < 0.05$ versus the control group, $^{*}P < 0.05$ versus the ADR group.
SAP = systolic arterial pressure; DAP = diastolic arterial pressure; MAP = mean arterial pressure.

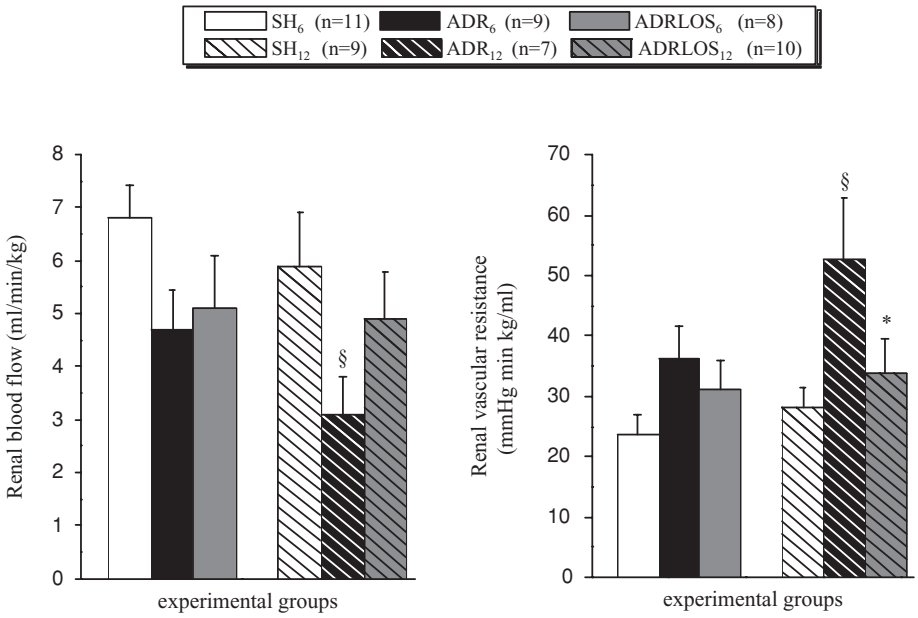

Fig. 1. Renal blood flow and renal vascular resistance in experimental groups. $^{\S}P < 0.05$ versus the control group; $^{*}P < 0.05$ versus the ADR group.

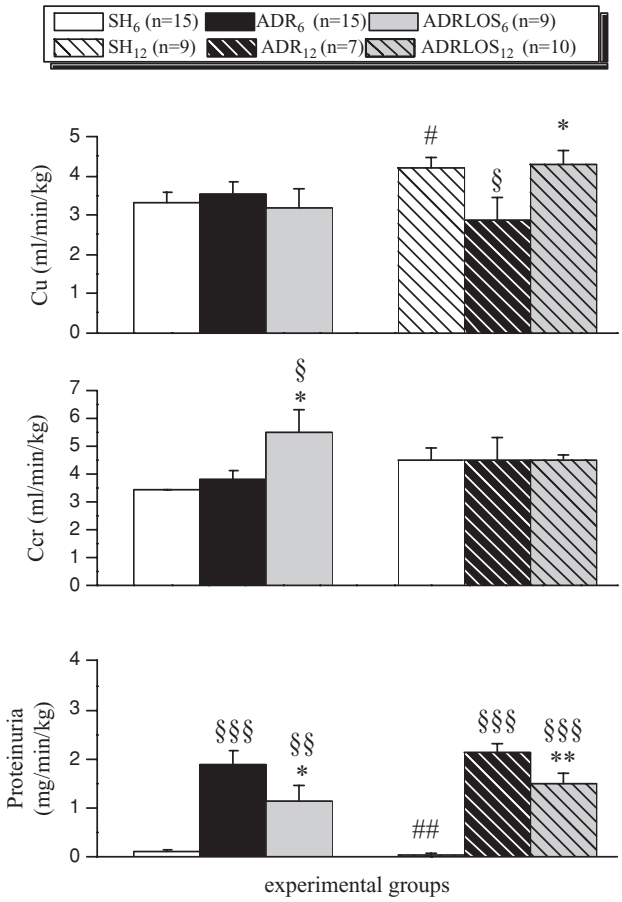

Fig. 2. Urea clearance (Cu), creatinine clearance (Ccr) and proteinuria in experimental groups. $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{\S\S\S}P < 0.001$ versus the control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the ADR group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, the difference between two examined time points (6 and 12 weeks) in the same group.

395.86 ± 79.81 mmol/l, $P < 0.001$), and therefore they reduced urea clearance drastically (Figure 2). However, losartan completely prevented the development of uraemia in long-term study and improved urea clearance (Table 1, Figure 2). In accordance with pronounced elevation in the urine flow in SH_{12}, urea clearance was significantly increased compared to values obtained in same group after 6 weeks.

ADR in a cumulative dosage of 4 mg/kg induced massive proteinuria, as shown in Figure 2 (SH_6: 0.13 ± 0.02 versus ADR_6: 1.88 ± 0.28 mg/min/kg, $P < 0.001$ and SH_6: 0.13 ± 0.02 versus $ADRLOS_6$: 1.13 ± 0.22 mg/min/kg, $P < 0.01$; SH_{12}: 0.05 ± 0.01 versus ADR_{12}: 2.14 ± 0.18 and SH_{12}: 0.05 ± 0.01 versus $ADRLOS_{12}$: 1.50 ± 0.20 mg/min/kg, $P < 0.001$). Both short- and long-term losartan applications successfully reduced ADR-induced proteinuria ($ADRLOS_6$ versus ADR_6 $P < 0.05$, and $ADRLOS_{12}$ versus ADR_{12} $P < 0.01$).

Water reabsorption, as a marker of functional state of tubules, declined significantly in advanced ADR nephropathy (Table 1).

Histological studies

Morphological changes in the kidney of all examined SHR are shown in Table 3 and Figures 3 and 4. Histological examinations of kidney specimens obtained 6 and 12 weeks after the second injection of ADR demonstrated significant increase in the HP score compared to respective controls (SH_6, SH_{12}). Short treatment with the AT-1 receptor antagonist losartan ($ADR+LOS_6$) shows tendency to decrease the HP score in relation to the ADR_6 group, while long-term losartan treatment results in significant reduction of morphological changes compared to the ADR_{12} group. After 12 weeks of experiment, however, the score of

Table 3. Index of morphological changes of the kidney in experimental groups

	SH_6 ($n = 9$)	ADR_6 ($n = 7$)	$ADR+LOS_6$ ($n = 8$)	SH_{12} ($n = 9$)	ADR_{12} ($n = 7$)	$ADR+LOS_{12}$ ($n = 10$)
Presence of scars	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.27	$0.00 \pm 0.00^{*}$
Sclerotic glomeruli	0.44 ± 0.18	$1.86 \pm 0.14^{\S\S\S}$	$1.00 \pm 0.19^{\S,**}$	0.22 ± 0.15	$3.00 \pm 0.00^{\S\S\S,\#\#\#}$	$2.10 \pm 0.28^{\S\S\S,**,\#\#}$
Tubular dilatation	0.00 ± 0.00	$1.86 \pm 0.26^{\S\S\S}$	$1.50 \pm 0.19^{\S\S\S}$	0.00 ± 0.00	$2.57 \pm 0.28^{\S\S\S}$	$1.10 \pm 0.10^{\S\S\S,***}$
Tubular atrophy	0.00 ± 0.00	0.29 ± 0.18	0.38 ± 0.18	0.00 ± 0.00	$2.71 \pm 0.27^{\S\S\S,\#\#\#}$	$1.00 \pm 0.00^{\S\S\S,***,\#\#}$
Vascular changes	1.00 ± 0.29	$1.86 \pm 0.14^{\S}$	1.63 ± 0.18	1.67 ± 0.17	2.14 ± 0.13	1.70 ± 0.15
Interstitial fibrosis	0.00 ± 0.00	0.29 ± 0.18	0.38 ± 0.18	0.00 ± 0.00	$2.57 \pm 0.28^{\S\S\S,\#\#\#}$	$1.00 \pm 0.00^{\S\S\S,***,\#\#}$
Infiltration	0.00 ± 0.00	$1.00 \pm 0.31^{\S\S\S}$	0.50 ± 0.19	0.00 ± 0.00	$3.00 \pm 0.00^{\S\S\S,\#\#}$	$1.40 \pm 0.16^{\S\S\S,***,\#\#}$
HP score	1.44 ± 0.44	$7.14 \pm 0.67^{\S\S\S}$	$5.38 \pm 0.84^{\S\S\S}$	1.89 ± 0.26	$15.86 \pm 1.06^{\S\S\S,\#\#\#}$	$8.30 \pm 0.47^{\S\S\S,***,\#\#}$

$^{\S}P < 0.05$ and $^{\S\S\S}P < 0.001$ versus the control group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the ADR group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, the difference between two examined time points (6 and 12 weeks) in the same group.

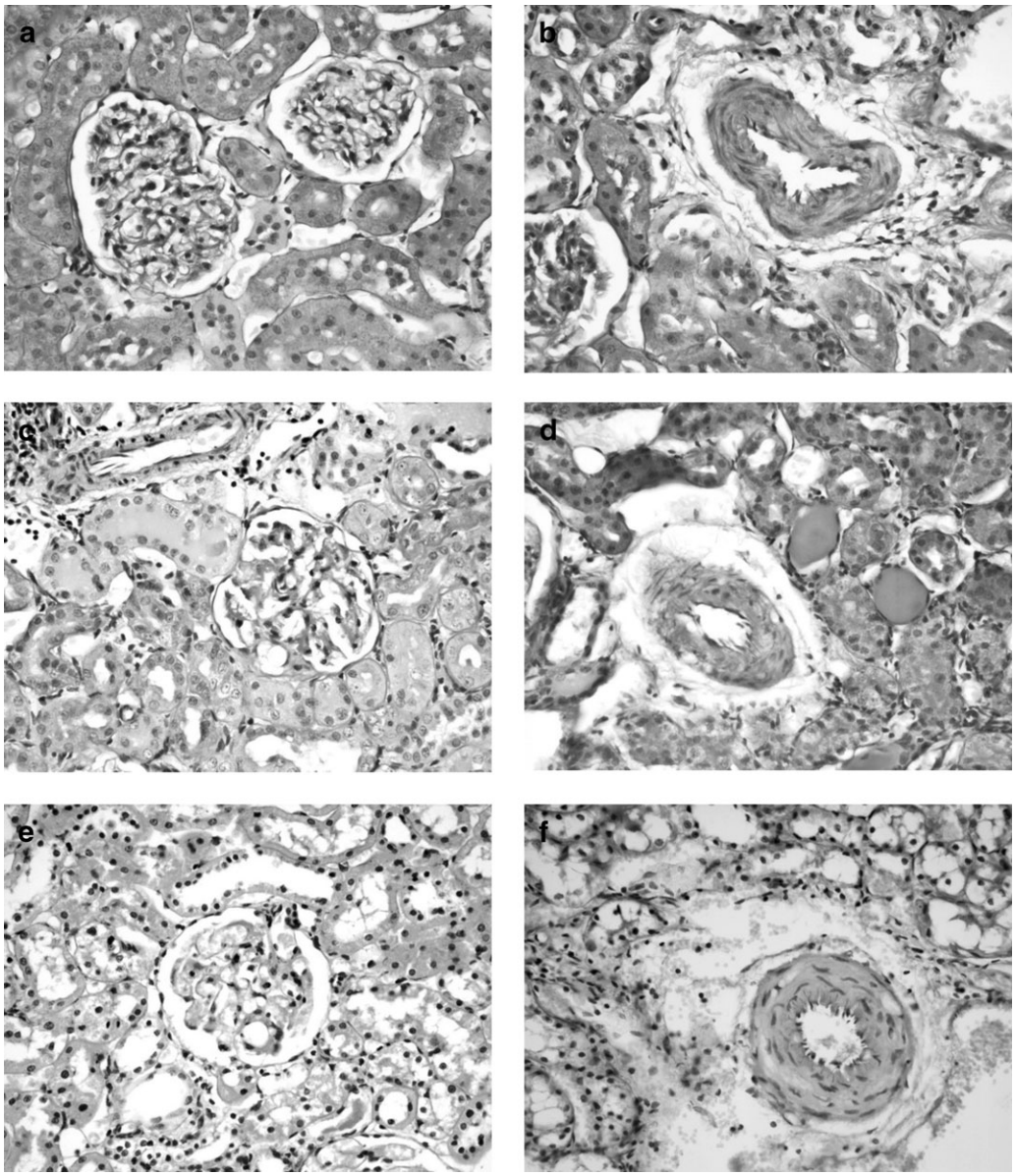

Fig. 3. (**a**) Normal-shaped glomeruli and tubules. (**b**) Blood vessel with slight miointimal hyperplasion, control group SH_6 (PAS; ×400). (**c**) Sclerotic glomerulus with capsular adhesion of glomerular tuff and the presence of foamy content, and focal interstitial mononuclear infiltration. (**d**) Blood vessel with slight miointimal proliferation and foamy transformed vacuoles. Mild tubular dilatation with PAS positive casts, ADR_6 group (PAS; ×400). (**e**) Well-preserved glomerulus and tubules. (**f**) Blood vessel with mild mioelastofibrosis, $ADR+LOS_6$ group (PAS; ×400).

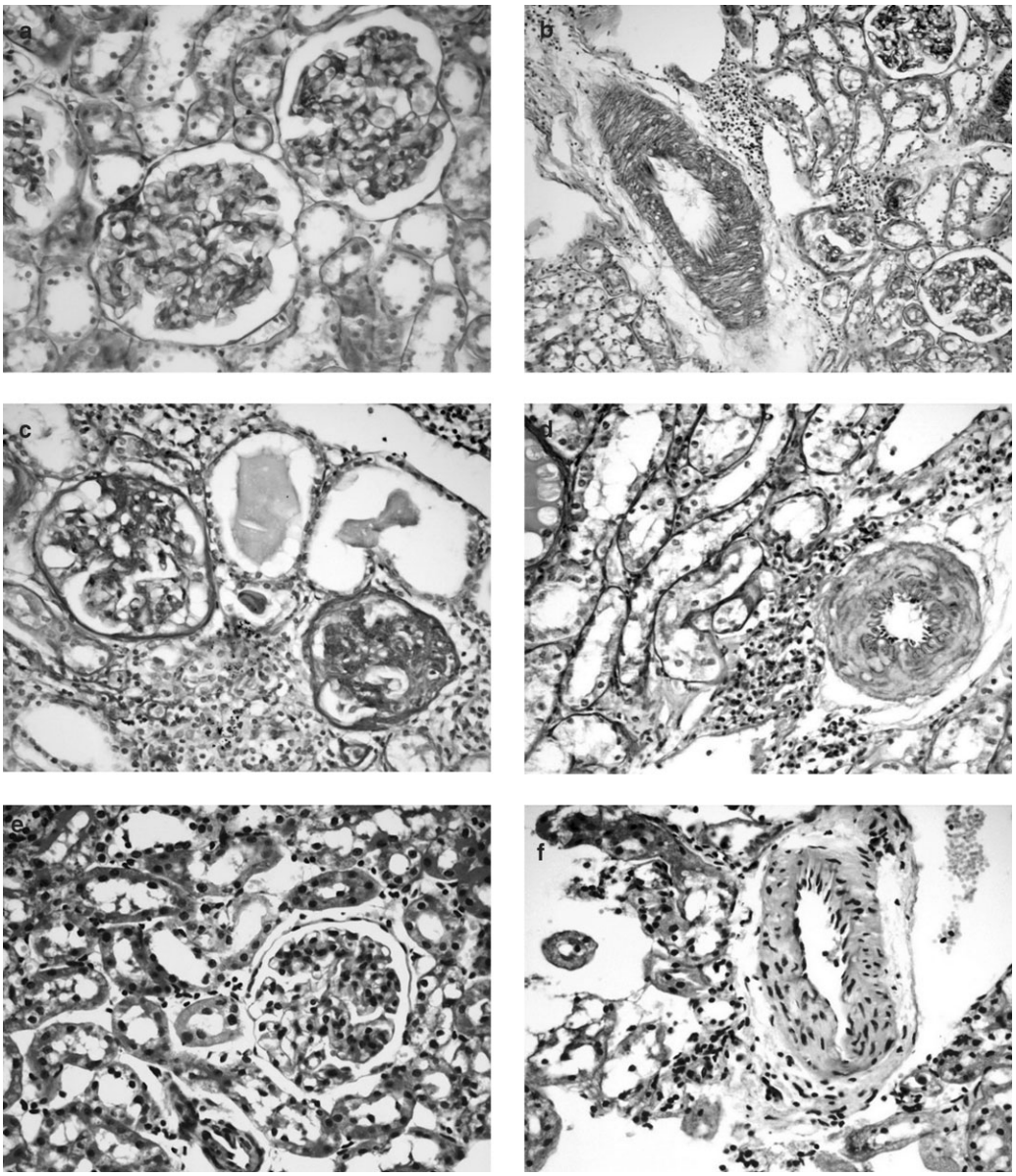

Fig. 4. (**a**) Normal-shaped glomeruli and tubules. (**b**) Blood vessel with mild miointimal proliferation and foamy transformed vacuoles and focal perivascular mononuclear interstitial infiltration, control group SH_{12} (PAS; ×400). (**c**) Left glomeruli with thickening of the GBM, capsular adhesion of glomerular tuff and the presence of foamy content in sclerotic glomerulus, global sclerosis of the right glomeruli, periglomerular interstitial fibrosis and discrete interstitial mononuclear infiltration, tubular atrophy and dilatation with PAS positive casts. (**d**) Narrowing of arteriolar lumen with extensive miointimal proliferation and light foam vacuolization of media, perivascular mononuclear interstitial infiltration, ADR_{12} group (PAS; ×400). (**e**) Well-preserved glomerulus and tubules. (**f**) Blood vessel with slight mioelastofibrosis, $ADR+LOS_{12}$ group (PAS; ×400).

morphological changes in all rats that received ADR was higher than that observed 6 weeks after ADR application.

SHR revealed normal glomerular and tubular structures, demonstrated in Figures 3a and 4a, and indexes of these changes were shown in Table 3. FSGS with capsular adhesion and foamy transformed glomerular cells were present in both ADR groups (Figures 3c and 4c). Similar degree of tubular dilatation was found in both, ADR_6 and ADR_{12} groups, while tubular atrophy with protein casts was mainly seen in advanced ADR nephropathy (ADR_{12}). Focal interstitial mononuclear infiltration was also present in early as well as in the late phase of ADR nephropathy where it was more intensive. The appearance of glomerular basement membrane thickening, extensive tubular atrophy and dilatation, as well as periglomerular interstitial inflammation and fibrosis, were also connected to the advanced stage of ADR-induced injury. Losartan-treated groups showed less prominent glomerulosclerosis ($ADR+LOS_6$ versus ADR_6, $P < 0.01$; $ADR+LOS_{12}$ versus ADR_{12}, $P < 0.01$) independent of treatment duration, since prolonged treatment mostly prevent interstitial fibrosis, tubular atrophy and dilatation, as well as infiltration of mononuclear leukocytes into the interstitial space (Figures 3e and 4e).

Figures 3b and 4b represent the blood vessel with a slight miointimal hyperplasia characteristic for hypertensive states of SHR. Vascular changes, characterized by miointimal proliferation, arterial and arteriolar wall thickening with reduced lumina, and foamy transformations in the intimae and the media were significantly increased in ADR_6 ($P < 0.05$) and ADR_{12} ($P = 0.055$) groups compared to relevant controls (Table 3, Figures 3d and 4d). Losartan could preserve the vascular structure, and therefore the morphological changes were almost similar to the controls (Figures 3f and 4f).

Discussion

Previous studies from our laboratory showed that ACEI captopril ameliorated glomerular and interstitial changes but had insignificant effects on renal disease progression in SHR with ADR nephropathy [12,15]. Our following study with α-, β-blocker, carvedilol and carvedilol–captopril combination (in the same experimental design) pointed out beneficial effects of carvedilol on renal haemodynamics. Carvedilol alone or in combination with captopril was able to reduce tubulointerstitial changes but glomerulosclerosis and progression of renal failure remained unaffected [16]. The treatment also failed to reduce proteinuria significantly [16].

On the other hand, the data from the present study clearly show that losartan reduces proteinuria in both early and late stage of ADR nephropathy associated with hypertension. This resulted in the prevention of glomerular sclerosis in both stages and protection of tubulointerstitial damage in the late phase of ADR nephropathy.

Proteinuria, as an important factor for the progression of FSGS to end-stage renal disease, persisted in our model in both early and late phases of ADR nephropathy. Hall and coauthors [17] found notable renal dysfunction in rats after single injection of ADR followed by 13 weeks' captopril treatment, without slowing down the progression of renal insufficiency. Despite the decrease in the glomerulosclerotic index, Irwin *et al*. [18] were also unable to prevent proteinuria and to improve GFR by an ACEI, enalapril, in Sprague-Dawley rats with ADR nephropathy induced in the same way as in our experiment. However, our results from the present study demonstrate that oral administration of AT-1 receptor antagonist, losartan, in a dose of 10 mg/kg/day resulted in significant reduction of proteinuria at both examined time points. This antiproteinuric effect was in accordance with amelioration of renal lesions, although no difference in MAP was found between examined groups at the late stage of ADR nephropathy. Results obtained from proteinuric patients with IgA nephropathy treated with enalapril or irbesartan indicate that there is no correlation between antihypertensive effects of the drugs and changes in glomerular haemodynamics and urinary protein excretion. In the rat model of diabetic nephropathy, characterized by segmental glomerulosclerosis, increased interstitial volume and progressive proteinuria, Tufescu *et al*. [19] showed that both exercise and losartan could have renal protective effects, directly influencing glomerular cells through the mechanism independent of their haemodynamic effects. This mechanism is probably due to glomerular macrophage infiltration, and glomerular mesangial activation and loss of podocyte foot processes. The results and findings from our study with blockade of AT-1 receptors further support the hypothesis that antiproteinuric effect of the ANG II antagonist is non-haemodynamic. It seems that its activity at the glomerular capillary wall depends on the amelioration of the capillary permselectivity in addition to its beneficial effects on the cell level [20].

The effects of hypertension on renal disease progression were examined in many experimental models as well as in humans. In control SHR and in SHR treated with ADR, in our study, hypertension is a characteristic of the used rat strain [21], in spite of the fact that ADR may induce hypertension in non-hypertensive rats. After the first 6 weeks of the experiment, the beneficial effect of losartan on renal disease progression was due to its antihypertensive action. In the long-term study, both a decrease in blood pressure (with increase in RBF and consecutive reduction in RVR) and losartan-specific effects on preglomerular and postglomerular arterioles could account for the decline of glomerular capillary pressure and therefore increase of membrane permselectivity.

Here, GFR was evaluated by urea and creatinine clearance. At the early stage of ADR nephropathy, 6-week-long losartan treatment completely repaired GFR estimated by creatinine clearance. This renoprotective effect of the treatment was partly related to the blood pressure decrease. However, marked uraemia noticed between 6th and 12th week of the experiment was successfully repressed by long-term losartan treatment as a result of improved GFR measured by urea clearance, irrespective to abundant diuresis induced by ADR in the early and advanced FSGS. In contrast to that, 12 weeks of losartan treatment failed to improve creatinine clearance. There is evidence indicating that measuring GFR by creatinine clearance might be inadequate, especially under conditions associated with uraemia [22]. Namely, the increase of creatinine release in CRF is a consequence of its tubular excretion rather than glomerular filtration [23]. Since the net effect of ANG II on renal function is a combination of AT-1 and AT-2 receptor-mediated events, with generally predominant AT-1 receptor-mediated effects [24], AT-1 blockade induced by losartan results in net renal vasodilatation and increased glomerular filtration. These effects are modulated, however, through the AT-2 receptor by activation of the autocrine cascade that includes bradykinin, nitric oxide and cyclic GMP [24].

ADR induces the decline in body-weight gain by reducing food intake and inhibiting protein synthesis [25], which is in accordance with our findings in SHR with uraemia and proteinuria induced by ADR. Significant decrease of body weight in SHR 12 weeks after ADR application was obtained by Herman [26] and also in the model of bilateral or unilateral ADR-induced proteinuria in Wistar rats [27,28], but not in Lewis rats [27]. Thus, the potential differences regarding body-weight changes in rats treated with ADR might be dose and strain dependent.

Tubulointerstitial injuries are usually present in proteinuric nephropathies, irrespective of their primary cause, because the leakage of proteins into tubular lumina could

directly or indirectly contribute to tubular and interstitial damage [29]. Calculating water reabsorption as an indicator of tubular function, we pointed out that ADR induced heavy proteinuria and ran into enhanced fluid leaking and reduced water reabsorption in the late stage of ADR nephropathy. Losartan has no effects on tubular water reabsorption in the long-term study because of its diuretic action. However, it prevents the development of tubulointerstitial changes and inflammation by diminishing ultrafiltration of proteins into tubulointerstitium, and thereby partly attenuates cellular events responsible for the lesions and decline of tubular function.

Our results from morphological examination also support the assumption that non-haemodynamic mechanisms are more important in slowing down the rate of renal disease progression. In the 6-week study, analysis of renal tissue specimens of SHR that received ADR (cumulative dosage of 4 mg/kg b.w.) confirmed FSGS by the presence of characteristic glomerular lesions and incipient tubulointerstitial changes. However, in the 12-week study, ADR induces more pronounced glomerular sclerotic lesions accompanied with tubular atrophy and interstitial fibrosis. Our results clearly showed that both short- and long-term AT-1 receptor antagonist administration could prevent glomerular segmental and/or global sclerosis. Even more, long-term AT-1 receptor blockade could diminish tubulointerstitial changes such as tubular atrophy, interstitial inflammation and fibrosis.

In conclusion, the short-term study revealed that AT-1 receptor antagonist losartan significantly reduces the progression of ADR nephropathy. We found that losartan, in addition to its antihypertensive effect, could improve GFR and ameliorate glomerulosclerosis resulting in decreased proteinuria and therefore delayed progression of ADR nephropathy. Moreover, prolonged treatment with losartan, besides its beneficial role on renal haemodynamics, could induce further reduction of glomerulosclerosis associated with reduced progression of tubular atrophy and interstitial fibrosis, thus preventing heavy proteinuria and CRF. Taken together, the present results obtained in hypertensive rats with ADR nephropathy confirmed the important role of ANG II AT-1-mediated non-haemodynamic actions in progression of CRF.

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Conflict of interest statement. None declared.

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Anti-proteinase 3 antibodies both stimulate and prime human neutrophils

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Abstract

Background. Anti-neutrophil cytoplasmic antibodies (ANCA) against proteinase 3 (PR3) are postulated to injure vascular endothelium by inducing cytokine-primed neutrophils to release proteolytic enzymes and generate reactive oxygen species. Anti-PR3 induce exocytosis, and since priming is associated with upregulation of plasma membrane proteins by exocytosis of intracellular granules, we tested the hypothesis that anti-PR3 prime neutrophils in the absence of cytokines.

Methods. Isolated human neutrophils were incubated with or without anti-PR3. Superoxide release was determined by measuring the reduction of ferricytochrome C. Exocytosis of secretory vesicles and specific granules was determined by measuring the expression of CD35 and CD66b, respectively, using flow cytometry.

Results. Anti-PR3 (15 μg/mL) directly stimulated superoxide production and enhanced FMLP-stimulated superoxide production. Anti-PR3 (0.5 μg/mL) did not stimulate superoxide production but did enhance FMLP-stimulated superoxide production. Incubation of neutrophils with anti-PR3 resulted in time-dependent exocytosis of secretory vesicles and specific granules. Anti-PR3-induced exocytosis, but not superoxide production, was dependent on p38 mitogen-activated protein kinase.

Conclusions. These data demonstrate that anti-PR3 can directly stimulate production of reactive oxygen species by neutrophils without cytokine priming, and that anti-PR3 prime neutrophils for increased FMLP-stimulated reactive oxygen species production. Anti-PR3 also induce exocytosis via a mechanism separate from their effect on reactive oxygen species production. These findings suggest that anti-PR3 ANCA may activate neutrophils and cause endothelial cell injury by multiple pathways, including some that are independent of priming by a second agent.

Keywords: ANCA; exocytosis; neutrophil; priming; reactive oxygen species

Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA) mediate small vessel injury associated with a group of diseases, including Wegener's granulomatosis, microscopic polyangiitis, renal-limited vasculitis and Churg–Strauss syndrome. ANCA are directed against proteinase 3 (PR3) and myeloperoxidase (MPO), and are postulated to exert their pathogenic effect by binding to those proteins on the surface of neutrophils, interacting with FcγRs and activating the cells. Activated neutrophils are thought to adhere to vascular endothelial cells and induce injury through either production of reactive oxygen species or release of proteolytic enzymes stored in neutrophil granules.

While the role of ANCA in vascular injury is well established, the mechanisms through which ANCA activate neutrophils remain poorly defined. The most commonly invoked mechanism involves interaction of ANCA with target antigens on the surface of neutrophils primed by cytokines, such as tumor necrosis factor α (TNFα). Priming increases plasma membrane expression of ANCA target antigens

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