MORPHOLOGY AND PATHMORPHOLOGY

Delayed Effects of Hypotensive Drugs on Structural Characteristics of the Renal Glomerular System in Hypertensive NISAG Rats

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Comparative morphometrical study of the renal glomerular system was carried out in hypertensive NISAG rats treated with hypotensive drugs during the prepubertal period. Blockade of the renin-angiotensin system with enalapril or losartan during the critical period of ontogeny (the 2nd month of life) produced a long-term hypotensive and renoprotective effect. Treatment with α-adrenoblocker terazosin during this period of ontogeny produced a less pronounced hypotensive effect, though with renoprotection. Corinfar (Ca^{2+} channel blocker) was least effective.

Key Words: *arterial hypertension; hypotensive drugs; kidney; morphometry*

The search for drugs preventing or at least inhibiting the development of hereditary arterial hypertension is now in progress. These studies are mainly carried out on experimental models of arterial hypertension allowing long-term ontogenetic studies with the use of a wide spectrum of methods, including the morphological ones [1,4,6,9-14]. In the majority of studies, the efficiency of the test drugs is evaluated by the degree of systemic blood pressure (BP) reduction and duration of the hypotensive effect. The renoprotective effect of antihypertensive agents receives less attention.

We considered it important to characterize the structural and functional status of the renal glomerular system, the most important functional system of the organ, within the framework of evaluation of the preventive effects of drugs.

We carried out a comparative morphometric evaluation of the renal glomerular system in hypertensive NISAG rats treated with hypotensive drugs during the prepubertal period.

MATERIALS AND METHODS

The study was carried out on hypertensive NISAG rats. On day 28 of life, the rats were separated from mothers; 5 groups consisting of 5 males each were formed. On days 28-58 of life the rats daily received *per os* a suspension of finely powdered hypotensive drugs in 0.25-0.50 ml water. Group 1 animals received enalapril (angiotensin-converting enzyme blocker) in a daily dose of 25 mg/kg; group 2 animals received losartan (type 1 angiotensin receptor blocker) in a daily dose of 10 mg/kg; group 3 rats received terazosin (α_1-adrenoreceptor blocker) in a daily dose of 2 mg/kg; group 4 animals

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received corinfar (Ca^{2+} channel blocker) in a daily dose of 15 mg/kg; and group 5 received placebo (the same volume of water).

Basal BP was monthly measured in the experimental and control animals under light ether narcosis by an indirect method on the tail. The experiments were carried out with consideration for humanitarian regulations presented in Directions of the European Community (86/609/EC) and "Regulations for Manipulations on Experimental Animals".

The animals were sacrificed at the age of 6 months under ether narcosis and material for electron microscopy was collected. Kidney specimens were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% OsO_4, dehydrated, and embedded in eponaraldite. Diameter, volume, and numerical density of renal glomeruli (RG) were measured in semithin sections of the kidneys stained with toluidine blue at ×640. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined under a JEM-100SX electron microscope (JEOL). Ultrastructural stereomorphometrical analysis of cellular and non-cellular components of RG was carried out on negative images (×8000) using a square test grid (72 points) with an 8-μ step and a ruler with 0.2-μ graduation mark.

The data were statistically processed using Statistica 6.0 software. The significance of differences was evaluated using Student's *t* test.

RESULTS

In control rats a significant elevation of BP was noted during the early prepubertal period and persisted until the end of the experiment (Table 1).

After administration of antihypertensive drugs to rats during the 2nd month of life, a direct hypotensive effect after treatment was noted in groups treated with renin-angiotensin blockers (enalapril and losartan) and adrenoreceptor blocker (terazosin). Blockade of Ca^{2+} channels with corinfar was ineffective. Until the age of 6 months, the most pronounced delayed hypotensive effect was observed in rats treated with enalapril; the effect of losartan was somewhat less pronounced. The dynamics of BP in animals treated with terazosin and corinfar was unstable throughout the experiment, but to the end of observation BP decreased in comparison with the control.

The development of arterial hypertension in control rats (group 5) was paralleled by pronounced structural changes in the kidneys (Table 1). At the age of 6 months, these animals were characterized by hypertrophy (compared to normotensive Wistar rats) and significant structural heterogeneity RG. Glomerular capillaries were sharply narrowed (Fig. 1, *a*) or, vice versa, significantly dilated, the epithelium was flattened. Hyperplasia of podocyte membrane structures, flat cytopodias, and longer areas of their contact with the basal membrane were noted (Fig. 1, *b*). The mesangium was enlarged,

TABLE 1. Morphometric Parameters of RG in NISAG Rat at the Age of 6 Months after Treatment with Hypotensive Drugs during Early Ontogeny (*M*±*m*)

Parameter	Wistar	NISAG				
		placebo	losartan	enalapril	terazosin	corinfar
Basal BP, mm Hg	118.0±3.7	184.00±5.56	168.3±3.0	164.00±2.38*	169.40±2.67	172.0±4.3
Density of glomeruli per mm^2	7.6±0.4	7.16±0.94	6.83±0.76	6.65±0.56	7.40±0.86	6.86±0.46
Relative volume of glomeruli, %	6.15±0.38	6.76±0.95	6.44±0.84	5.86±0.60	7.51±0.70	6.48±0.52
Diameter of glomeruli, μ	102.5±1.7*	111.90±3.96	102.90±2.68*	106.40±2.91	106.40±3.28	107.20±2.36
Relative volume of capillaries, %	6.24±0.25	5.66±0.29	5.06±0.28	5.04±0.22	5.56±0.33	6.60±0.27*
Relative volume of podocytes, %	33.52±1.38	28.08±1.18	35.12±1.78	28.72±1.40	25.72±1.31	31.04±1.28
Relative volume of endotheliocytes, %	14.46±1.00	13.20±1.10	12.56±1.13	13.33±1.02	12.66±1.11	10.41±0.74*
Relative volume of urinary space, %	11.07±0.51	10.23±0.54	8.3±0.6	9.35±0.57	11.39±0.75	10.11±0.54
Relative volume of capillary lumen, %	18.19±1.20	17.67±1.28	15.53±1.58	16.58±1.43	19.98±1.71	20.76±1.27
Relative volume of mesangium, %	6.80±0.73*	9.68±0.97	8.10±1.28	9.63±0.89	7.91±1.12	8.98±0.91
Relative volume of basal membranes, %	8.48±0.39*	12.48±0.47	10.63±0.44*	10.97±0.43*	10.40±0.44*	11.57±0.35
Width of basal membrane, nm	198.30±5.64*	280.99±12.00	255.9±10.2	268.05±8.52	257.10±11.31	295.15±9.70
Length of cytopodia contact with basal membrane, nm	366.6±31.4*	639.55±56.33	518.90±34.93	555.08±36.12	595.80±45.63	610.00±39.45

Note. *$p<0.05$ compared to placebo.

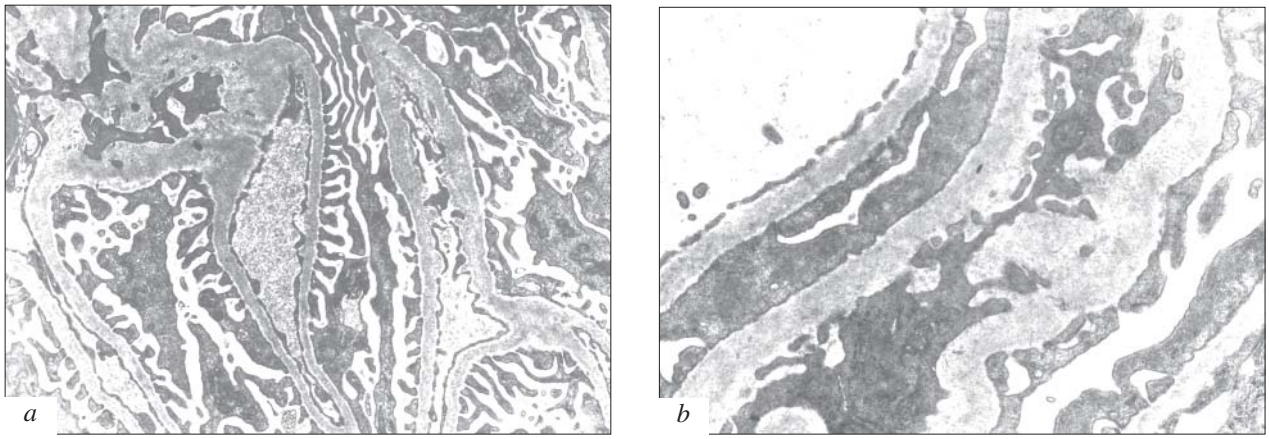

Fig. 1. Electronograms of RG in a control (placebo) NISAG rat. *a*) capillaries with stenosed lumen, growth of mesangial matrix, ×10,000; *b*) podocyte cytopodias are flat and their contact with basal membrane is longer than normally, ×20,000.

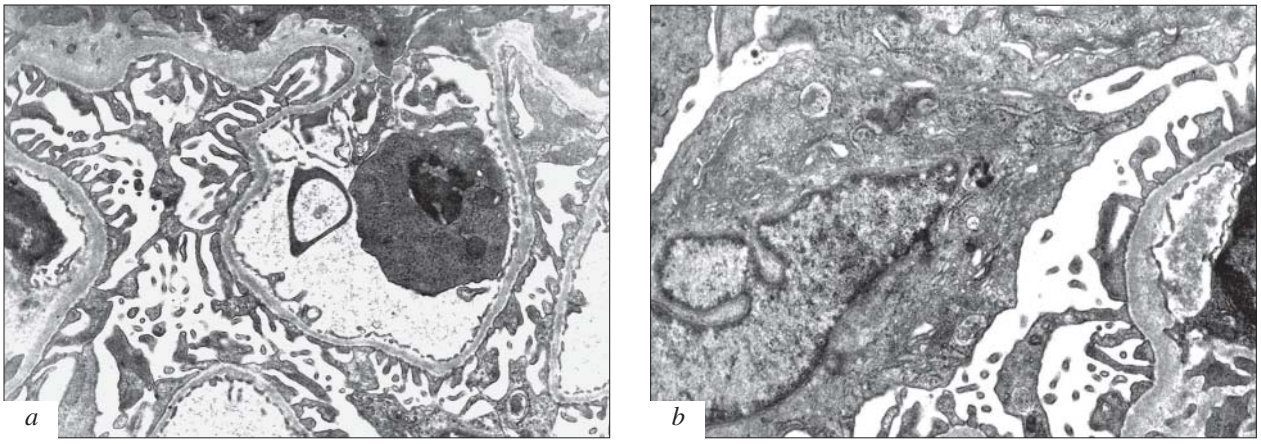

Fig. 2. Electronograms of RG in a NISAG rat treated with losartan during the prepubertal period. *a*) RG structure close to normal (in normotensive Wistar rats), ×10,000; *b*) podocyte with signs of hyperplasia of membrane ultrastructures, ×15,000.

basal membranes were thickened. The complex of these signs indicated hemocirculatory disorders in RG, increased functional strain of podocytes, and initial stages of glomerulosclerosis.

Structural heterogeneity of RG was also detected in group 2 rats. However, morphometrical analysis showed significant differences of their quantitative parameters from the control. RG were virtually not enlarged (Table 1). The relative volume of basal membranes in RG was significantly lower than in control animals and almost did not differ from that in normotensive Wistar rats. The relative volume of the mesangium, the width of the basal membrane, and length of contacts between the podocyte processes and basal membrane were lower. At the same time, structural features characteristic of rats with manifest arterial hypertension persisted in RG: elevated (in comparison with normotensive level) volume parameters of the mesangial matrix and basal membranes, focal heterogeneity of capillaries, and signs of hypertrophic changes in podocytes and endotheliocytes (Fig. 2, *a*, *b*).

A trend to normalization of the majority of morphometric parameters of RG was observed in group 1 rats. The mean RG diameter, relative volume of the mesangium, and width of basal membranes were intermediate between the corresponding values in normo- and hypertensive control, while the relative volume of basal membranes was significantly lower than in group 5. However, initial signs of glomerulosclerosis characteristic of group 5 were detected in RG of group 1 rats.

A similar trend to normalization of the structural parameters of RG was detected in group 3 animals.

Structural changes in of RG after corinfar treatment (group 4) were different. RG were somewhat smaller than in control animals of this strain, but the majority of analyzed morphometrical values were within the normal range. Significant shifts in the capillary parameters were detected: the volume percentage of capillaries in the renal cortical matter, percentage of glomerular capillaries, and relative volume of capillary lumen increased, while the relative volume of endotheliocytes decreased. In

many RG, capillaries were significantly dilated and contained slugged erythrocytes (Fig. 3). Analysis of these findings showed that corinfar treatment in young age virtually did not reduce manifestations of renal structural shifts characteristic of hypertensive animals, but could induce changes in the microcirculatory bed of the renal cortex.

Hence, enalapril and losartan exhibited the most pronounced delayed renoprotective effect. Numerous experimental studies also showed that drugs inhibiting the renin-angiotensin system used in different doses and different combinations produce significant hypotensive and renoprotective effects. Many scientists explain the delayed hypotensive effect by improvement of renal function [4,11,12]. It was shown not once that enalapril and losartan or their analogs prevented the development of glomerulosclerosis in hypertensive rats [12-14]. Among possible mechanisms of this effect are reduction of mesangial cell hyperplasia and inhibition of enhanced expression of collagen IV and fibronectin in the kidney. Interestingly, that the positive effect of angiotensin-converting enzyme inhibitors and AT1 receptor blockers manifests only if they are used no earlier than at the age of 3-4 weeks [5,12].

Our experiments showed that injection of terazosin (α_1-adrenoblocker) to NISAG rats during the 2nd month of life leads to less pronounced delayed hypotensive effect than treatment with renin-angiotensin inhibitors. Presumably, the choice of this age for the treatment with this drug was erroneous. In light of this it is interesting that terazosin exhibits a stable hypotensive effect in SHR rats only if it is used before the end of nursing period, while the blockade of α-adrenoreceptors during later terms prodcued practically no hypotensive effect [10].

Nevertheless, comparative analysis of morphometrical parameters of RG showed a renoprotective effect of terazosin. The data on adrenoblocker efficiencies are contradictory. Many scientists noted their good therapeutic effect in renal diseases without appreciable effect on BP [7], others claim that the hypotensive effect of adrenoblockers is due to their capacity to reduce manifestations of glomerulosclerosis and normalize filtration rate in RG [3]. These contradictions are presumably caused by the fact that drug efficiency depends on its dose and time of injection, and on the experimental model [2].

The opinions about the delayed effect of adrenoblockers also differ. No lasting effect of these drugs on BP and renal vessels were attained in some studies [8], while others demonstrated a clear-cut delayed hypotensive effect of adrenoreceptor blockade persisting for more than 2 months after drug discontinuation [10].

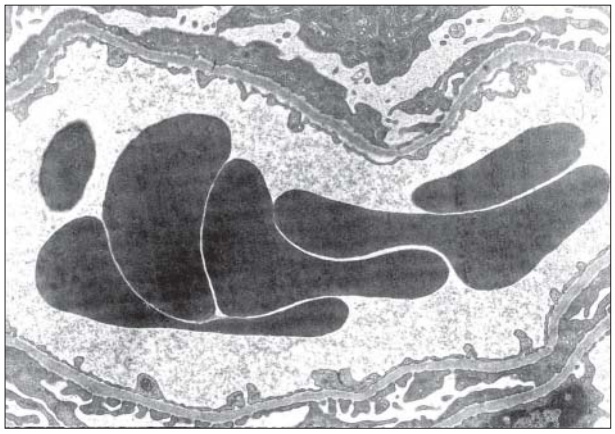

Fig. 3. Electronogram of RG in a NISAG rat treated with corinfar during the prepubertal period. Dilated glomerular capillary with erythrocytes in the lumen, ×10,000.

Corinfar was least effective: it exhibited no delayed hypotensive effect (except after 6 months) or renoprotective effect. Presumably, the dose (15 mg/kg/day) was insufficient for this effect. In experiments on SHR rats, a good therapeutic effect was attained with felodipine (corinfar analog) in a dose of 30 mg/kg/day [6]. It was reported that if BP could not be controlled with nifedipine blockade of Ca^{2+} channels, the use of this drug can lead to elevation of capillary pressure in the renal glomeruli and promote glomerulosclerosis [9].

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