PHYSIOLOGY

Delayed Effects of Enalapril on Structural Characteristics of the Glomerular Apparatus in the Kidneys of NISAG Rats

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Structural characteristics of renal glomeruli were studied in adult hypertensive NISAG rats (hereditary stress-induced hypertension) receiving antihypertensive drug enalapril, an inhibitor of angiotensin-converting enzyme, on days 28-58 of life. Treatment with enalapril (25 mg/kg perorally) in the early period of postnatal ontogeny produced delayed hypotensive and nephroprotective effects.

Key Words: *arterial hypertension; kidney; enalapril; morphometry*

The use of antihypertensive drugs in the early ontogeny to produce a stable hypotensive effect is now intensively discussed. Studies on animals with experimental [3,4,8,15] and inherited arterial hypertension [10,14] yielded ambiguous results. It was concluded that the presence and degree of the delayed hypotensive effect depend not only on the type of drugs, but also on the terms and duration of therapy.

Hypertensive nephrosclerosis is the major cause of kidney diseases. There is a wide spectrum of drugs not only reducing blood pressure (BP), but also alleviating symptoms of kidney pathology. Special attention is paid to drugs modulating activity of the renin-angiotensin system (RAS), e.g. antagonists of angiotensin I receptors and inhibitors of angiotensin-converting enzyme (ACE).

Here we studied whether administration of an ACE inhibitor enalapril in the early ontogeny produces a protective effect on the glomerular apparatus of the kidneys in rats with inherited stress-induced arterial hypertension (NISAG rats).

MATERIALS AND METHODS

Experiments were performed on NISAG rats. The animals were kept in a vivarium under standard conditions and had free access to water and food. NISAG rats were bred from outbred Wistar rats by BP rise under stress conditions (Institute of Cytology and Genetics) [1].

The female was placed in an individual cage on the 3rd week of pregnancy. Rat pups stayed with their mother for 28 days and then male and female pups were kept separately. Rat pups received enalapril in a daily dose of 25 mg/kg on days 28-58 of life. A finely dispersed suspension of the drug in 0.2 ml water was administered *per os* using a syringe and a metal cannula. Controls received an equivalent volume of water.

Basal BP was measured in animals aging 2, 3, 4, 5, and 6 months by the tail-cuff method. The rats were killed by the end of the study. Kidney samples were fixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate

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buffered saline and then with 1% osmium tetroxide, dehydrated, and embedded into Epon-araldite. The diameter, volume density, and numerical density of renal glomeruli were estimated on semithin sections of the kidneys stained with toluidine blue (×640). Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-100SX microscope. Ultrastructural stereomorphometric study of cells and noncellular components of the renal glomeruli was performed using negative images at ×5000. We used a square test grid (72 points, increment 8 μ) and a ruler (graduation mark 0.2 μ). The results were analyzed by means of Statgraphics 4.0 software. The significance of differences was evaluated by Student's test.

RESULTS

BP in control rats increased significantly (180 mm Hg) in the early prepubertal period and remained unchanged until the end of the study (Table 1). Hypertension development was accompanied by significant structural changes in the kidneys. Hypertrophy of the renal glomeruli was found in 6-month-old rats. The mean diameter of glomeruli in these animals was much greater than in Wistar rats. Some capillaries in most glomeruli were sharply narrowed. Other capillaries were widened; sludged erythrocytes were revealed in the lumen of these capillaries. The relative volume of endotheliocytes decreased, which reflects flattening of the endothelial layer. Podocytes were characterized by functional strain. Foot processes were thickened and shortened. Morphometry showed that the length of the contact between these processes and the basal membrane in experimental animals was 1.5 times longer than in Wistar rats. These changes reflect reduced effectiveness of filtration barrier in glomerular capillaries. The basal membrane was thickened. The relative mesangial volume increased. These signs reflect impaired blood circulation in the renal glomeruli, increased functional strain of podocytes, and progression of the initial stage of glomerulosclerosis.

Treatment with enalapril over the 2nd month of life decreased BP, which remained below the control level in the follow-up period (Table 1). The structure of the renal glomeruli in 6-month-old experimental animals differed from that in control rats. The diameter of glomeruli was intermediate between the values observed in control NISAG and Wistar rats. The length of contact between podocyte processes and basal membrane, as well as the width of the basal membrane, was of intermediate value. The relative volume of the basal membrane in a glomerulus in experimental animals was much lower compared to the control, but practically did not differ from that observed in normotensive Wistar rats. These specific features indicate a favorable prognosis and reflect a nephroprotective effect of enalapril.

Many structural characteristics of renal glomeruli in experimental rats did not differ from those in control animals, which attested to high degree of the hypertensive state. Volume characteristics of

TABLE 1. Morphometric Indexes of Renal Glomeruli in Adult NISAG Rats Receiving Enalapril during Early Ontogeny (*M*±*m*)

Index	Wistar	NISAG	
		water	enalapril
Blood pressure, mm Hg	118±4	184±6*	164±2*+
Numerical density of glomeruli, per mm^2	7.6±0.4	7.16±0.94	6.65±0.56
Relative volume of glomeruli, %	6.15±0.38	6.76±0.95	5.86±0.60
Diameter of glomeruli, μ	102.5±1.7	111.29±2.26*	106.40±2.91
Relative volume of capillaries, %	6.24±0.25	5.66±0.29	5.04±0.22
Relative volume of podocytes, %	33.52±1.38	28.08±1.18	28.72±1.40
Relative volume of endotheliocytes, %	14.46±1.00	13.20±1.10	13.33±1.02
Relative volume of the urinary space, %	11.07±0.51	10.23±0.54	9.35±0.57
Relative volume of the capillary lumen, %	18.19±1.20	17.67±1.28	16.58±1.43
Relative volume of the mesangium, %	6.80±0.73	9.68±0.97*	9.63±0.89*
Relative volume of basal membranes, %	8.48±0.39	12.48±0.47*	10.97±0.43*+
Width of the basal membrane, nm	198.30±5.64	281.90±12.08*	268.00±8.52*
Length of contact between cytopodia and basal membrane, nm	366.6±31.4	639.60±56.33*	555.10±36.12

Note. $p<0.05$: *compared to Wistar rats; +compared to water-receiving NISAG rats.

podocytes, endotheliocytes, urinary space, and lumen of capillaries remained practically unchanged. Similarly to control rats, the relative mesangial volume in experimental animals was much higher than in Wistar rats. Visual examination showed that renal glomeruli are heterogeneous by the state of blood capillaries and initial signs of sclerosis.

Structural characteristics of the glomerular apparatus in the kidneys of experimental rats indicate that treatment with enalapril in the early period of life reduces symptoms of stable arterial hypertension in the kidneys of adult animals.

Published data show that enalapril is effective in the therapy of glomerulosclerosis accompanying a variety of kidney diseases [4,12,15]. There are ambiguous data on the delayed effect of enalapril administered during the early ontogeny. The effect of enalapril depends strongly on the period of ontogeny when it is administered. Previous studies showed that neonatal blockade of RAS with enalapril over the first 2-3 weeks of life led to irreversible dysfunction and morphological changes in the kidneys [7,9,11]. This period of life is critical for the regulatory effect of RAS on the development of the kidneys.

The positive therapeutic effect of enalapril is manifested when treatment starts at the age of not less than 3 weeks. Our experiments and previous studies [6,8] revealed a positive effect of enalapril in animals aging 3-4 weeks.

Glomerular hypertrophy was less pronounced in adult NISAG rats receiving enalapril in the prepubertal period. The relative volume of basal membranes in renal glomeruli decreased in experimental rats, which attested to a positive effect of enalapril. The width of the basal membrane tended to decrease in these animals. Published data show that enalapril modulates selectivity of the glomerular membrane by decreasing pore radius [12]. The length of contact of podocyte processes in experimental animals was intermediate between the values observed in control NISAG rats and normotensive Wistar rats. These data indicate that enalapril improves function of the filtration barrier in renal glomeruli.

Mesangial volume in experimental rats was as high as in control animals. Glomerulosclerosis is usually accompanied by thickening of the basal membrane and increase in the mesangial volume. However, these signs do not necessary develop simultaneously [5]. It can be hypothesized that high index of the mesangial compartment in NISAG rats is a genetically determined sign. Published data provide support for this hypothesis. The mesangial volume increased, while BP was not elevated in 3-week-old NISAG rats [2].

Structural study of arteries in the small intestine of SHR rats yielded similar results [14]. ACE inhibitor peridopril produced a delayed hypotensive effect, but did not normalize the structure of arteries in the small intestine. Changes in arterial smooth muscle cells typical of these rats and concerned the synthesis of proteoglycan-containing granules preceded the development of hypertension-associated vascular dysfunction [13]. The data prove support for the hypothesis on hereditary nature of these changes.

The decrease in BP after treatment with the drug probably decelerates nephrosclerosis. It can be hypothesized that the nephroprotective effect of the drug is followed by a decrease in BP, which delays hypertension. Previous studies showed that ACE inhibitors and angiotensin I receptor antagonists are most potent in producing the delayed hypotensive effect [3,4,15]. Our results are consistent with this point of view.

Our study showed that enalapril administered to NISAG rats during the early prepubertal period produces delayed hypotensive and nephroprotective effects.

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