EFFECT OF ETHACRYNIC ACID ON THE COURSE OF CYTOTOXIC GLOMERULONEPHRITIS

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The writers previously showed that several modern diuretics have an anti-inflammatory action, and that ethacrynic acid is the most active preparation in this respect [2]. The pharmacotherapeutic efficacy of ethacrynic acid was subsequently demonstrated on several models of inflammatory diseases [1]. The aim of this investigation was to study the effect of this diuretic on the course of experimental Masugi cytotoxic glomerulonephritis, one of the most adequate models of clinical pathology [5].

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred male rats weighing 140-180 g, kept in individual cages with unrestricted access to food and water. Nephritis was produced by injection of nephrotoxic serum (NTS), obtained by the method of Nikiforova and co-workers [4]. Combined injection of NTS, recommended by several research workers [5] was used: 0.8 mg/100 g body weight intravenously and the same dose subcutaneously next day. Half of the animals received injections of ethacrynic acid in a dose of 10 mg/kg subcutaneously daily for 15 days, whereas the other half served as the control.

Throughout the experiment (20 days) the 24-hourly diuresis and excretion of sodium, potassium, and creatinine of the rats were recorded. Blood levels of urea and total protein also were determined. Twice in the course of the experiment a histological study of the kidneys was carried out: 5 days after injection of NTS (10 rats) and at the end of the investigation (12 rats). For this purpose the kidneys were fixed in 10% neutral formalin solution and embedded in paraffin wax in the usual way. Sections stained with hematoxylin and eosin were examined under the microscope. Histological investigation was concentrated on the state of all parts of the nephron.

The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

In the animals of the control group, starting with the first days after injection of NTS, the rats developed the typical picture of acute glomerulonephritis. Massive proteinuria was observed with an increase in the daily excretion of protein with the urine from 0.9 ± 0.11 to 26 ± 9.1 mg as early as on the 2nd day ($p < 0.05$). This parameter later stabilized until the 11th day of the experiment, after which the proteinuria increased progressively to 73.6 ± 12.8 mg toward the end of the period of observation. The proteinuria led to a fall in the total plasma protein level: by 16% on the 5th day and by 52% at the end of the experiment. During this time interval the urea concentration in the blood was significantly increased (from 7.8 ± 0.5 to 10.5 ± 0.8 mA, $p < 0.05$). Table 1 gives data on the excretory function of the kidneys after injection of NTS. Clearly the 24-hourly diuresis remained unchanged until the 11th day of the experiment, after which a tendency was observed for it to increase, with a maximum after 15-17 days. More or less the same trend was observed with changes in

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TABLE 1. Effect of Ethactynic Acid (10 mg/kg daily, subcutaneously) on Parameters of Excretory Function of the Kidneys in Rats with Cytotoxic Glomerulonephritis (M ± m)

Parameter	Group of animals	Control	Experimental				
			3	7	11	15	19
Diuresis, ml/day	Control	4,8±0,8	5,1±0,9	4,2±0,8	6,3±0,9	9,2±2,0	8,9±1,9
	Experimental	4,1±0,6	3,3±0,5	2,9±0,6	3,0±0,3**	3,6±0,8**	3,4±0,8**
Sodium excretion, μ moles/day	Control	6,6±0,5	8,2±0,8	9,0±1,4	7,6±0,7	7,6±0,9	7,4±0,9
	Experimental	8,2±0,7	9,9±1,2	11,6±1,8	9,0±2,0	10,1±1,3	9,2±1,3
Potassium excretion, μmoles/day	Control	430,0±50,9	832,0± ±101,3*	765,0±104,1*	754,0±46,3*	752,0±28,9*	777,0±50,4*
	Experimental	525,0±59,5	805,0±30,7*	823,0±67,7*	739,0±46,7*	843,0±53,8*	696,0±67,9
Creatinine excretion, μmoles/day	Control	18,4±1,8	21,7±2,1	20,4±1,7	30,3±4,4*	31,1±3,3*	31,5±3,3*
	Experimental	15,1±1,5	19,0±2,7	14,1±1,3**	19,3±1,1*,**	20,5±3,4**	20,0±2,7**

Legend. *) Changes compared with initial level are significant ($p < 0.05$); **) changes in experimental group compared with control are significant.

24-hourly excretion of creatinine, which was significantly increased from the 11th day until the end of the experiment. Sodium excretion was unchanged throughout the experiment but potassium excretion with the urine increased significantly starting with the 3rd day, and it remained at that level throughout the rest of the investigation.

Morphological investigation of the kidneys 5 days after injection of NTS revealed changes characteristic of the development of glomerulonephritis. The changes were most marked in the proximal part of the nephron. Its convoluted and straight parts showed a similar pattern of injury. The cytoplasm of the epithelial cells acquired a more granular structure and the brush border was destroyed, and sometimes was completely absent in the epitheliocytes of several neurons. Some cells were completely destroyed and desquamated into the lumen of the tubule, as was confirmed by the presence of remnants of their cytoplasm and nuclei. Eosinophilic granular contents were seen in the lumen of many parts of the subcapsular nephrons, and in the urinary tubules of some rats dense hyalinelike plugs were present. Dilatation of the lumen of the blood vessels, especially capillaries, was observed in the interstitial tissue; the vessels appeared to be distended by blood cells, in agreement with the picture of stasis of the blood.

Toward the end of the period of observation the morphological picture of the kidneys corresponded qualitatively to that described above but with more marked destructive changes in the epithelial lining of the proximal and distal portions, i.e., nephrotic changes affecting the structural units of the metanephros were more severe.

In the group of rats receiving ethacrynic acid the course of glomerulonephritis differed significantly from that in the control group. The proteinuria, as Fig. 1 (missing in the Russian original — Publisher) shows, was much less severe: from 0.9 ± 0.09 to 10.3 ± 3.51 mg/24 h ($p < 0.05$) on the first days of the experiment and to 18.8 ± 5.22 mg when injection of the preparation ceased. Protein excretion then increased toward the end of the period of observation to 46.8 ± 11.5 mg/day, significantly lower than in the group of control animals. Incidentally, in five of the 12 rats proteinuria at the time of ceasing administration of the toxin was negligibly small or absent altogether, and in three animals it did not develop after withholding of the diuretic. Correspondingly, the total plasma protein concentration fell by a lesser degree: by 36% toward the end of the period of observation (by the 5th day no significant changes could be found). The blood urea was unchanged. Throughout the days of the experiment, the volume of urine excreted remained virtually the same as initially (Table 1). Although ethacrynic acid is a diuretic, in rats this drug in therapeutic doses as a rule has no diuretic action [3]. A tendency for the excretion of creatinine to increase was observed, just as in the control, starting with the 11th day, although it was weaker in intensity. The time course of sodium and potassium excretion was similar to that in the control.

Thus in the group of animals receiving ethacrynic acid changes characteristic of the development of renal pathology were much less severe. This is confirmed to some degree also by morphological investigation of the kidneys of the experimental group of rats. Whereas during the first investigation (5 days after injection of NTS) the morphological picture was virtually identical with the control, by the end of the period of observation (20th day) the difference became evident, especially as regards the severity of the changes. For instance, in the proximal parts the general morphology of the epithelial cells (their shape, the structure of their cytoplasm, the state of the brush border) acquired features characteristic of the corresponding part in intact animals. Signs of vacuolation and desquamation of single cells and detachment of an epithelial sheet from the basement membrane had virtually disappeared. The contents of the lumen of the nephrons were less abundant. Homogeneous hyalinelike plugs were found in the kidneys of only two animals. In most rats these plugs were

completely absent or their volume was small, in which case, wide free zones were visible in the urinary tubules between the somewhat flattened epithelium and the homogeneous contents. Stasis of blood in the vessels of the connective tissue, which was most marked in the control animals, was extremely slight in the rats of this group, showing that the circulation in the kidneys was restored. The morphological picture of the kidneys of rats receiving ethacrynic acid, described above, reflected an improvement compared with the control.

Analysis of the results shows that the development of renal pathology took place through two stages: 1) from the time of injection of NTS until the 11th day, and 2) from the 11th day and until the end of the experiment. This is in agreement with the classical picture of Masugi nephritis, which goes through heterologous and autologous phases [6]. Phase 1 is characterized by rapid binding of NTS antibodies with antigen of the basement membrane of the glomerular capillaries, phase 2 by antibody production to γ-globulin of the heterologous antikidney serum. Ethacrynic acid considerably alleviated the course of nephritis throughout the two stages, although morphologically this was confirmed for phase 2. This action of the diuretic may be linked either with its nonspecific anti-inflammatory effect, described by the writers previously, and with the ability of ethacrynic acid to block histamine release from basophils and mast cells [7, 8], and this may influence the development of the autologous phase of the process.

The functional and morphological features revealed by this investigation thus demonstrate alleviation of the course of experimental cytotoxic nephritis by ethacrynic acid.

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