SOME AGE ASPECTS OF THE COURSE OF NEPHROTOXIC NEPHRITIS IN RATS

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Experiments on noninbred rats (aged 4 weeks and 4 months, respectively), with induced nephrotoxic glomerulonephritis showed that the course of the disease in younger rats differed from that in the older animals by its less marked proteinuria, which was selective in character, and by the smaller decrease in the concentration index of endogenous creatinine and of diuresis. Acid and ammonia formation in the young and adult rats were depressed equally. The morphological changes in the kidneys of the young rats were less severe than in the adult rats and were localized chiefly at the convoluted tubules. The level of DNA-synthesizing activity in the epithelial nuclei of the glomeruli was higher than the corresponding index for the epithelium of the convoluted tubules. The relative weight of the kidneys in young rats with nephritis showed a smaller increase than in the adult rats. The blood β-lipoprotein level in the young rats was increased eightfold. Restoration of the normal indices for the urine and blood occurred more rapidly in the young rats than in the adult animals.

KEY WORDS: nephrotoxic nephritis in young rats; kidney function; selectivity of proteinuria.

Despite an extensive literature on the subject of glomerulonephritis in children [2-5, 9] and nephrotoxic nephritis in sexually immature animals [1, 8, 10-12, 14], the special features of the pathogenesis of this disease at different ages and, in particular, the degree of involvement of different parts of the renal nephron in the pathological process have been inadequately studied.

The object of this investigation was to compare the course of nephrotoxic glomerulonephritis in young and adult rats with differential analysis of the disturbance of function and morphological changes in different parts of the renal nephron.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male rats of two age groups: 1) 55 rats aged 4 weeks weighing initially 40-50 g; 2) 65 sexually mature rats aged 4 months and weighing initially 170-200 g. In 40 young rats of group 1 and in 45 sexually mature rats of group 2 nephrotoxic glomerulonephritis was produced by Masugy's method [13].

The heterologous nephrotoxic serum used was obtained from rabbits immunized with a homogenate of rat kidney cortex. The nephrotoxic serum (titer 1 : 1000 to 1 : 1200) was injected parenterally into the rats on 3-5 successive days in a dose of 0.8 ml/100 g body weight daily. Intact rats served as the control to each age group. Parallel observations were made on the experimental and control animals.

In rats with experimental nephritis, besides the general condition and degree of edema, quantitative indices of the proteinuria and its selectivity also were studied (the index of selectivity was calculated). The endogenous creatinine concentration index was determined. To assess the functional state of the tubular system of the nephrons, acid production (titratable acids and pH of the urine) and ammonia production were studied. The quantity of titratable acids and ammonia was expressed per gram weight of the kidneys. Diuresis also was determined; the residue of the urine was examined under the microscope. Since the investigation was carried out on animals of two different age groups, differing in body weight, diuresis was calculated per unit of body weight and per unit of body surface, calculated by the equation $V = 0.09\sqrt[3]{p^2}$.

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TABLE 1. Changes in Indices of Function in Young and Adult Rats with Nephrotoxic Nephritis (M ± m)

Index	Healthy		Glomerulonephritic			
	young rats	adult rats	young rats		adult rats	
			10th day	30th day	10th day	30th day
Titratable acids, ml/10 ml	1,98±0,12	2,62±0,13	1,05±0,13	2,06±0,42	1,22±0,13	2,58±0,18
Titratable acids calculated per unit weight of kidney	4,3	3,91	1,9	3,1	1,2	2,5
Ammonia in urine, mg/ml	0,228±0,025	0,368±0,06	0,16±0,022	0,32±0,07	0,238±0,024	0,428±0,1E
Ammonia in urine per unit weight of kidney	4,9	6,4	3,01	4,9	2,4	4,2
Ammonia coefficient, %	10,3	12,3	13,2	13,4	16,3	14,2
Proteinuria, ‰	0,0015±0,06	0,0045±0,029	4,68±1,3	0,4±0,05	23,86±4,5	6,2±0,4
Weight of kidneys, g	0,464±0,025	0,673±0,023	0,535±0,026	0,688±0,051	0,89±0,07	1,016±0,039
Rel. weight of kidneys	0,50	0,27	0,58	0,61	0,49	0,51
Diuresis, ml/h	0,256±0,086	0,634±0,21	0,4±0,049	0,30±0,062	0,195±0,032	0,159±0,07

TABLE 2. Changes in Blood Biochemistry in Young and Adult Rats with Nephrotoxic Nephritis (M ± m)

Index	Healthy		Glomerulonephritic			
	young rats	adult rats	young rats		adult rats	
			10th day	30th day	10th day	30th day
Total serum protein, g%	5,17±0,19	6,83±0,16	4,56±0,14	5.73±0,1	5,75±0,12	6,63±0,11
Serum β-lipoproteins, mg%	99,1±24,8	188,2±17,5	853,9±12,7	160,1±38	1176,1±110	237,8±42,4
Nonprotein nitrogen, mg%	27,8±0,53	29,8±0,4	35±0,35	33±0,7	34±0,4	31±3,1

TABLE 3. Changes in [^{3}H]-Thymidine Labeling Index of Glomeruli, Tubular Epithelium, and Connective-Tissue Fibroblasts of Kidneys of Young and Adult Rats with Nephrotoxic Nephritis

Group of animals	Adult rats					Young rats				
	number of labels per 100 glomeruli	cortex		medulla		number of label per 100 glomeruli	cortex		medulla	
		epithelial cells	connective tissue cells	epithelial cells	connective tissue cells		epithelial cells	connective tissue cells	epithelial cells	connective tissue cells
Healthy	13,9	0,230±0,049	0,345±0,045	0,073±0,013	0,230±0,035	7,6	0,172±0,029	0,130±0,02	0,035±0,009	0,120±0,023
With glomerulonephritis	32,5	1,810±0,460	2,860±0,780	1,030±0,201	2,630±0,547	23	0,341±0,060	0,407±0,054	0,103±0,005	0,190±0,017
P	<0,05	<0,05	<0,05	<0,01	<0,01	<0,05	<0,05	<0,05	<0,01	>0,05
Comparative intensity of label, % of control	231	787	817	1471	1143	302	198,3	315,4	294,3	141,7

As an additional parameter of the functional state of different parts of the nephron the number of DNA-synthesizing nuclei was counted in the epithelial cells of the glomeruli and convoluted tubules (in the cortex and medulla of the kidneys), by means of Merkulov's histoautoradiographic method [6]. [^{3}H]-Thymidine was used as the radioactive label.

The biochemical indices determined included total protein and its fractions in the blood and urine, and γ-globulin, nonprotein nitrogen, creatinine, and β-lipoproteins in the blood serum. The kidneys of some animals were investigated morphologically. After fixation in 10% formalin and embedding in paraffin wax, sections were stained with hematoxylin-eosin and with picrofuschin by Van Gieson's method.

EXPERIMENTAL RESULTS

The following differences were found in the course of nephrotoxic nephritis in the young and adult rats. The proteinuria in the young rats was significantly less than in the adults. On the 10th day of the disease the protein concentration in the urine of the young rats was 4.68 ± 1.30 ‰ and in the adult rats 23.86 ± 4.5 ‰. By the 30th day it had fallen in the young rats to 10% of the level on the 10th day (at the height of the disease), and in the adult rats to 25%, i.e., the proteinuria fell more rapidly in the course of the disease in the young rats.

Glomerular filtration of proteins in the acute period of nephritis was selective in type in all the rats. After more than 30 days the selectivity of glomerular protein filtration was disturbed in some of the experimental adult rats. The endogenous creatinine concentration index in the young rats was reduced by a lesser degree than in the adult animals. The results are evidence of a less severe disturbance of glomerular function in the young rats than in the adults.

Tubular functions – acid and ammonia production – were depressed equally in the young and adult rats with nephritis. Acid production was disturbed first and to a greater degree: Acid production was reduced to 47% below normal in the young rats and 54% in the adult rats; ammonia production was reduced to 30 and 36%, respectively, below normal; the increase in the ammonia coefficient confirmed the above picture (Table 1). As regards nephritic edema, most rats developed abdominal ascites, although edema on the hind and forelimbs and snout, characteristic of the adult rats, was less common. The relative weight of the kidneys in the young rats with nephritis was increased by a lesser degree than that of the adult rats.

Of the biochemical indices of the blood, by the 10th day of the disease the serum β-lipoprotein concentration was increased less sharply in the young rats than in the adults, to 853 ± 127 mg %, an eightfold increase above the normal. Changes in the nonprotein nitrogen and total protein of the blood were similar in type in the young and adult rats and were about equally severe (Table 2). The indices of the urine and blood returned to normal more rapidly in the young experimental rats than in the adult rats.

The morphological investigations showed that changes in the glomeruli and tubules characteristic of membranous-proliferative glomerulonephritis were less marked in the kidneys of the young rats than of the adults. In the young rats the epithelium of the convoluted tubules was predominantly affected. These observations agree with the results of Erdman's morphological investigations [11] on young rabbits with experimental nephrotoxic nephritis.

Increased DNA-synthesizing activity of the nuclei of the epithelial cells both of the glomeruli and of the convoluted tubules of the kidneys was discovered autoradiographically in the animals with glomerulonephritis, but the increase was less marked in the young than in the adult rats. The increase in the DNA-synthesizing activity of the epithelial cell nuclei, moreover, was more marked in the glomeruli, whereas in the adult rats it was more marked in the convoluted tubules of the kidneys (Table 3).

The comparative investigation of the course of nephrotoxic glomerulonephritis in adult and young rats thus showed that, despite the common pathogenesis of the disease, a distinguishing feature in the young rats was that changes in the function and morphology of the kidneys were most marked in the tubular system and less marked in the glomeruli.

Some of the features distinguishing the course of nephrotoxic glomerulonephritis in the young rats can be explained from the standpoint of the results of kidney function tests in healthy animals (control group). The kidneys of healthy rats, despite their smaller size, have a greater relative weight; the younger the rat, moreover, the greater the relative weight of its kidneys. These observations suggest that during the experimental production of nephrotoxic nephritis by injection of specific antikidney serum in a dose calculated in terms of body weight, a smaller quantity of antikidney antibodies per unit weight will act on the young rats, with their

relatively larger kidneys, than on the adult rats. Another factor of decisive importance is the different functional and morphological relationships of the kidneys in sexually immature animals. It was noted that in healthy young rats the original urine analyses never revealed protein, whereas in adult rats traces of protein were frequently observed, and sometimes its concentration reached 0.1‰. The values obtained for acid and ammonia production, calculated per unit weight of kidneys, were similar in the adult and young rats (Table 1). This could indicate a relatively high physiological level of function of the system of convoluted tubules in the young rats. Furthermore, irrespective of age, elimination of hydrogen ions in rats under physiological conditions evidently takes place chiefly through association with anions of weak acids, for acid production in young rats amounts to 90% and in adult rats to 87%. The ammonia coefficients in rats is 10-13% irrespective of age (compared with 50-70% in man). These figures are in harmony with statements in the literature to the effect that in rats, unlike in man, acid production in the kidneys is not a function mainly of the distal portions of the convoluted tubules, but takes place throughout the length of the tubular system [7].

The physiological features distinguishing the tubular system of the kidneys in young rats may account for the greater severity of the damage to that system in them than in adult rats in experimental nephrotoxic nephritis. The fact that DNA-synthesizing activity and, consequently, repair processes are predominantly increased in the cells of the renal glomeruli in nephritis in young rats may be the reason for the more rapid recovery of function of the nephron as a whole and the correspondingly more rapid restoration of normal biochemical indices of the urine and blood in young animals compared with the adults. Besides the differences in reactivity of the vascular and nervous systems in sexually immature animals, there are thus also strictly renal factors that determine the milder course of nephritis in young than in adult rats.

The results of the present investigation, in which parallel observations were made on young and adult rats, go some way toward explaining the contradictions in the literature as regards the course of nephritis before the age of puberty [1, 8]. The results of a study of age differences in the course of experimental nephritis could serve as the basis for the development of rational methods of treatment of this disease that depend on age.

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