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Pseudoductules in the rat liver in experimental adriamycin--induced nephrotic syndrome

Nephrotic syndrome is the group of clinical and biochemical symptoms due to intensive proteinuria. Protein loss with urine is the result of glomerular degeneration. (5). The causes of that condition are heterogeneous (glomerulonephritis, nephrotic syndrome in course of several diseases like: diabetes mellitus, amyloidosis, connective tissue diseases, congenital or familial nephrotic syndrome). In this study nephrotic syndrome was induced by a single dose of adriamycin given intraperitoneally (5 mg/kg of body weight).

Adriamycin is an antibiotic from antracyclines group with antineoplastic activity (3). It is used in mono- and polychemotherapy of neoplasm of: breast, thyroid gland or urinary bladder. In clinic is used in dose 1 to 6 mg/kg of body weight. Given intravenously is fast eliminated from blood and slowly excreted with urine and mostly with bile, 40-50% of used doses during 7 days from administration.

MATERIAL AND METHODS

The studies were performed on 18 white female Wistar rats with initial body weight ranging from 200-250 g aged 4-5 months. The animals were divided into the following groups: I – 6 control rats treated with 0.5 ml 0.9% NaCl i.p. in one dose; II – 6 rats treated i.p. with adriamycin in one dose 5 mg/kg of body weight. The animals were decapitated 4 weeks after drug administration; III – 6 rats treated with adriamycin in the same way as in group II and decapitated 8 weeks after drug administration.

The animals were fed with standard food and they drank *ad libitum*. Once a week they were weighted and their urine was taken for analysis (10).

From decapitated animals there was taken: blood from the heart (for biochemical analysis) and the right lobe of the liver (for histological analysis). The sections were fixed

in buffered 10% formalin and processed routinely to paraffin block. Then the specimens were cut semiserially into 5 μm slides and stained with hematoxylin/eosin (Fig. 2, 3) and periodic acid Schiff (PAS) according to Mc Mannus – Fig. 4 (1, 12). Then the slides were observed in light microscopy. Additionally the specimens were fixed in glutaraldehyde. The samples were cut into semithin sections 0.75mm and stained with methylene blue-Azur II for light microscopy (Fig. 1). Photographs of the samples were taken with Jenaval Contrast Carl Zeiss light microscopy camera.

RESULTS

In experimental group II surroundings of central veins and portacholangial spaces there were present pseudoductules irregular in shape, dilated and long (Fig. 1, 2). They

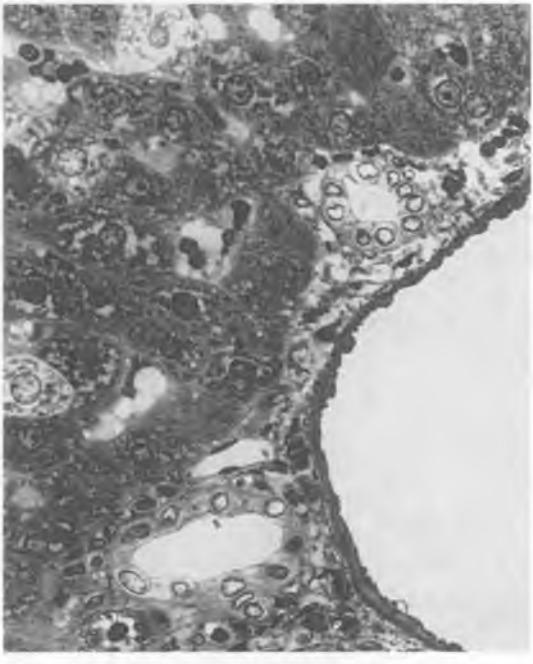

Fig.1. Experimental group II (4 weeks after adriamycin administration). Semithin slide. Methylene blue-Azur II staining. Magn. 400x

had well visible lumen. Their longitudinal cross–sections were parallel or perpendicular to epithelial basal membrane and they were close to it. The pseudoductules consisted of big cells with basophilic PAS (-) negative cytoplasm, clear borders and big nucleus. The

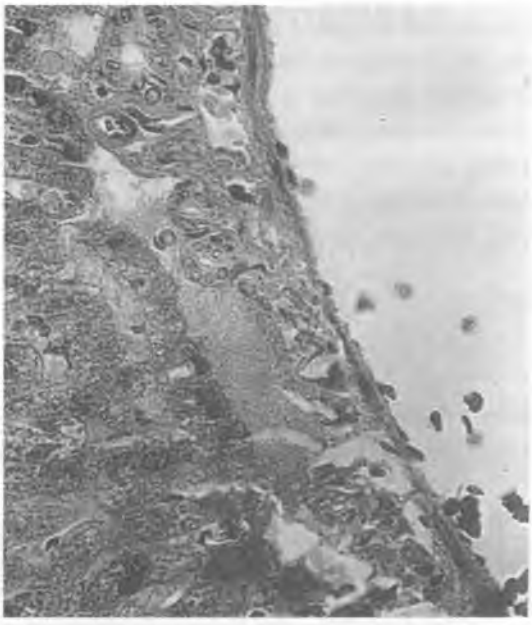

Fig. 2. Experimental group II. H/E staining.
Magn. 400x

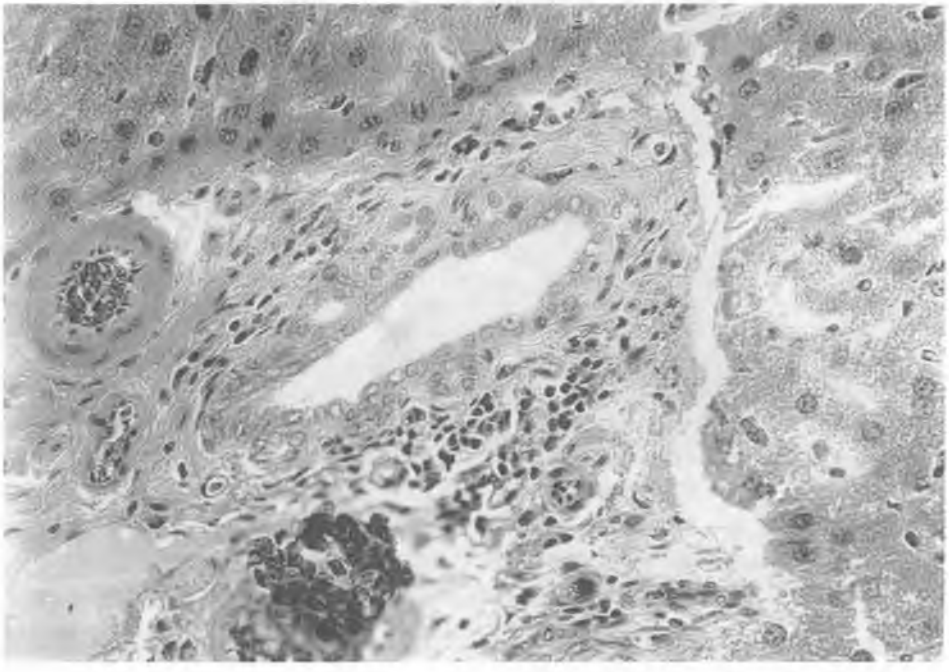

Fig. 3. Experimental group III (8 weeks after adriamycin administration). H/E staining.
Magn. 400x

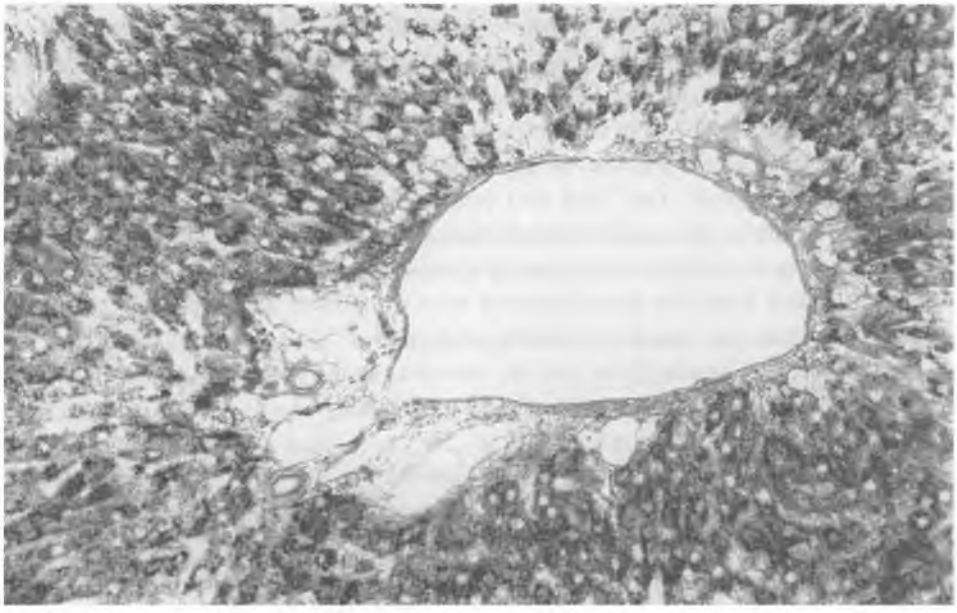

Fig. 4. Experimental group III. PAS staining. Magn. 400x

Tab. 1 Analysis of biochemical results

Material	Investigation	Group	Mean value	Standard deviation	Significance
Blood serum	total protein (g/dl)	K	7.05	+/- 0.32	
		II	6.12	+/- 0.09	p.<0.0001
		III	5.37	+/- 0.26	p.<0.0001
	albumin (g/dl)	K	3.82	+/- 0.44	
		II	2.93	+/- 0.26	p.=0.0003
		III	2.35	+/- 0.36	p.<0.0001
	lipids (mg/dl)	K	65.4	+/- 19.4	
		II	137.8	+/- 33.3	p.=0.006
		III	247.0	+/- 40.5	p.=0.0002
	cholesterol (mg/dl)	K	89.8	+/- 12.5	
		II	143.0	+/- 18.2	p.=0.001
		III	296.0	+/- 82.3	p.=0.005
	creatinine (mg/dl)	K	0.75	+/- 0.21	
		II	0.56	+/- 0.17	p.=0.08
		III	0.67	+/- 0.34	p.=0.64
Urea	protein (mg/24 h)	K	7.9	+/- 0.6	
		II	85.4	+/- 4.1	p.<0.0001
		III	127.1	+/- 6.2	p.<0.0001

p compared to control group.

pseudoductules produced PAS (+) positive substance (Fig. 4). In the surrounding connective tissue there were cumulated also PAS (+) positive substances (4, 8).

In the specimens taken from experimental group III (Fig. 3, 4) the ductules were more numerous. Close to them accumulated inflammatory infiltration with prevalence of granulocytes. Aggregated granulocytes especially surround PAS (+) positive substances close to pseudoductules. Thin PAS (+) positive membrane covered endothelium of pseudoductules. Near more mature pseudoductules infiltrations were much smaller and some of them were surrounded by connective tissue layer.

In the control group the pseudoductules were not present and the picture of specimens did not show any changes or features of damage.

The biochemical results (total protein, albumin, lipids, cholesterol, creatinine from blood serum and protein from urea) are presented in Table 1.

DISCUSSION

Adriamycin nephrotoxicity was described in the literature (2, 6, 7, 9). Most commonly proteinuria was induced by the repeated intravenous doses of the drug. Sano et al. (9) had given adriamycin 4-6 weeks in the dose 2mg/kg of body weight once a week to the total dose 8-12mg/kg of body weight. The inducing intensive proteinuria after a single dose of adriamycin (given i.v.) was described in the literature from the dose 3 mg/kg (11) to 16 mg/kg (6) The intraperitoneal dose – 5mg/kg of body weight used in that study seems to cause fast and full-presented nephrotic syndrome and at the same time did not develop lethal pathology of organs. Full-presented nephrotic syndrome in that study revealed 4 weeks after a single dose of adriamycin (see Tab. 1). First signs – intensive proteinuria appeared already after 3 weeks.

Histological and histochemical kidney examinations as well as biochemical blood and urine results (Tab.1) showed that adriamycin induced an experimental model of nephrotic syndrome in rats, which had not tendency to decrease but even increase in time (4 weeks after drug administration proteinuria was lower than 4 weeks after that time).

Pseudoductules proliferation (French: *formation des neocanaux*) observed in that study is present as well in many liver diseases in human and experimental animals. Spurious ductules, pseudoductules, Hering's bile ductules proliferation, cholangioles proliferation, these are other names of ductules proliferation used in scientific papers. Pseudoductules proliferation in winding form make difficult the outflow of bile from the liver. The picture of such proliferation was observed in extrahepatic cholestasis (4, 8). It was described in viral and alcoholic hepatitis, in hepatic cirrhosis especially after necrosis, and toxical liver damage.

Kruś described the Hering's choleic ductules in the liver between parenchyma and portacholangial space which connect bile canaliculus and bile interlobular ductules (4). In

the liver without any disease they are very difficult to find but they are proliferated in pathological state. These ductules proliferate very fast and live 2 weeks.

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SUMMARY

Pseudoductules proliferation in the liver is the condition observed in some liver diseases in human and experimental animals. It is observed in viral and alcoholic hepatitis, in hepatic cirrhosis and toxical liver damage.

In that study pseudoductules were observed after i. p. single dose of adriamycin (5 mg/kg of body weight) administered to rats. Adriamycin given in this way is used to induce experimental nephrotic syndrome.

Kanaliki rzekome w wątrobie szczura w doświadczalnym zespole nerczycowym wywołanym adriamycyną

Rozplem kanalików rzekomych w wątrobie jest stanem obserwowanym w niektórych chorobach wątroby u ludzi i u zwierząt doświadczalnych. Jest notowany w wirusowym lub alkoholowym zapaleniu wątroby, w marskości wątroby i w toksycznym uszkodzeniu wątroby.

W przedstawionej pracy rozplem ten występował po jednorazowej dootrzewnowej dawce adriamycyny (5 mg/kg wagi ciała) podanej szczurom. Adriamycyna podana w ten sposób jest używana do wywołania doświadczalnego zespołu nerczycowego.