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Features of proteinuria in rat kidney in experimental nephrotic syndrome

Cechy proteinurii w nerce szczura w doświadczalnym zespole nerczycowym

The group of clinical and biochemical symptoms due to intensive proteinuria is called nephrotic syndrome (10). Proteinuria appears when filtration barrier of glomerulus is damaged. Typical symptoms of nephrotic syndrome include: proteinuria, hypoproteinemia, dysproteinemia, hyperlipidemia, hypercholesterolemia and oedemas (7, 9).

In the present study the experimental nephrotic syndrome was induced by adriamycin, antibiotic from antracyclines group with antineoplastic activity (2, 5). It is used in treatment of bladder tumours, breast tumours and thyroid tumours in mono- and polychemotherapy in dose 1-6 mg/kg of body weight (4, 11).

In this study there were described histological changes, which were the evidence of proteinuria.

MATERIAL AND METHODS

In order to induce nephrotic syndrome adriamycin was used in a single dose of 5 mg/kg of body weight. It was given intraperitoneally. The studies were performed on 18 white female Wistar rats with initial body weight ranging from 200-250 g. The rats aged 4-5 months (14). The animals were fed with standard food and they drunk *ad libitum*. Once a week they were weighted and their urine was taken for analysis (3, 12).

The animals were divided into the following groups: I - experimental group - 6 rats treated i. p. with adriamycin in one dose of 5 mg/kg of body weight.The animals were killed 4 weeks after drug administration, when biochemical features of proteinuria appeared; II - control group for group I - 3 rats treated i. p. with 0.5 ml of 0.9% NaCl in one dose and killed after 4 weeks; III - experimental group - 6 rats treated i. p. with adriamycin in one dose of 5 mg/kg of body weight. The rats were killed 8 weeks after drug administration (time twice longer than in group I); IV - control group for group III - 3 rats treated i. p. with 0.5 ml of 0.9% NaCl in one dose and killed after 8 weeks after drug administration.

From the killed animals there was taken blood from heart (for biochemical analysis - to evidence features of nephrotic syndrome), and always - the left kidney. The sections were fixed in buffered 10% formalin, dehydrated in increasing concentrations of alcohol (from 60% to 100%) and processed routinely to paraffin block (1). The specimens were cut into 3, 5, 7 mm slides and stained with hematoxylin/eosin and periodic acid Schiff (PAS), according to Mc Mannus (15). Additionally the specimens were fixed in glutaraldehyde. The samples were cut into semithin sections 0.75 mm and stained with methylene blue-Azur II for light microscopy (1).

Then all slides were observed in light microscopy. The photographs of samples were taken with Jenaval Contrast Carl Zeiss camera.

RESULTS AND DISCUSSION

Biochemical changes in urine and in blood taken from the rats from experimental groups I and III present the picture of nephrotic syndrome and include: in urine - intensive proteinuria, in blood - hypoproteinemia with dysproteinemia, especially hypoalbuminemia, hyperlipidemia, hypercholesterolemia (8, 13, 14). In the animals from experimental group III (killed after twice longer period following adriamycin administration than the rats from experimental group I) changes in biochemical results were more intensive (5). In the animals from control groups II and IV biochemical results did not change.

The results of histological analysis showing the consequences of proteinuria were the following (6):

In renal glomerules:

In control group II and IV the microscopic picture of glomerules in all rats was homogeneous and did not show any features of damage.

In experimental group I (4 weeks after drug administration) (Fig. 1): 1. Dilatation of urinary space; 2. Irregular shape of visceral lamina of the Bowman's capsule; 3. Thickening of the Bowman's capsule; 4. Damage of vessel loops - they were dilated, deformed, swollen or with partial atrophy.

In experimental group III (8 weeks after drug administration) changes were more intensive and included (Fig. 4, 5, 6): 1. Focal thickening of mural lamina of the Bow-

man's capsule. It was especially evident in sections stained according PAS method. (Fig. 5, 6); 2. Deformation of barrier of mural lamina (Fig. 4, 5); 3. In some glomerules thinning or complete destroying of mural lamina of the Bowman's capsule (Figs 4); 4. Remarkable dilation of urinary space (Fig. 4, 5); 5. Homogeneous deposits in capsular lumen, which sometimes fulfilled all urinary space (Fig. 4); 6. Collecting of fluid in capsular lumen; 7. Thickening of basal membrane of glomerule, especially evident in sections stained according PAS method (Fig. 5, 6); 8. Thickening of capillary vessels wall in glomerules (Fig. 6); 9. Dilatation and deformation of vessel loops (Fig. 5, 6); 10. Complete or partial destruction of vessel loops (Figs 4, 5).

In convoluted tubules (distal and proximal) (6,7):

In control groups II and IV the microscopic picture of convoluted tubules in all rats was homogeneous and did not show any features of damage.

In experimental group I (Fig. 1, 2, 3): 1. In most of nephrons in proximal convoluted tubules the changed epithelial cells had weakly stained, foamed cytoplasm - the evidence of microvacuolar degeneration (Fig. 3); 2. Homogeneous, PAS(+)positive casts in tubular lumen (both proximal and distal) were present. Tubular epithelial cells were flattened. Irregular shape of tubular lumen, thick basal membranes (Fig. 2); 3. Focal flattening of tubular epithelium and focal dilatation of tubular lumen (Fig. 3).

In experimental group III (Fig. 4, 5, 6): 1. In the lumen most of tubules homogeneous casts were present. They were more numerous than in experimental group I. Indistinctness of tubular epithelium building (Fig. 4, 6); 2. Macro- and microvacuolar degeneration. In proximal tubules big bright vacuolas were present, whose diameter was bigger than nuclear diameter. Sometimes they filled all cytoplasm. In the epithelium numerous granules were present which could be phagosomes (Fig. 4); 3. Droplet - hyaline degeneration: in proximal tubules were present acidophilic dropled; cells were swollen and tubular lumen was contracted or irregular (Fig. 4); 4. Parenchymal steatosis, partial necrosis and inflammatory infiltration was observed.

All those observed changes in nephron structure were the evidence of protein presence in the primary filtrate and in urine (2).

An evident feature of nephrotic syndrome is the increased permeability of capillary vessels wall of the glomerule. Proteins and lipids that passed through to the capsular lumen and then to the tubular lumen were captured by epithelium cells, which gave a histological picture of droplet-hyaline degeneration and steatosis observed in the present study (6, 7, 15).

The way of damaging basal membrane determines the size of penetrating protein particles. It could be organic damages or loss of negative charge (both elements could coexist). In electrostatic disturbances the albumin is permeable (small diameter protein; - selective proteinuria). In organic disturbances also proteins with a bigger diameter, for example gammaglobulin, are permeable (non-selective proteinuria). In this study blood concentration of gammaglobulin was in physiological range (7).

Collecting of fluid in the capsular lumen is present in proteinuria of kidney origin. That picture is also typical of kidney removed on operation as a consequence of vessels ligature (7).

Thickening of basal membrane of capillary vessels observed in this study is observed quite often in light microscopy according to Kruś (7). It is due to protein deposition as well as the adherence of flattened processes of podocytes. So it is rather the thickening of vessels wall. Electron microscopy let us get to know the real thickening of membrane more exactly.

Both biochemical, histological and histochemical changes described in the study were more intensive in experimental group III than in experimental group I (2, 13). It is the proof that nephrotic syndrome induced by a single dose of adriamycin increases in time without any additional activity of other drug (5).

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STRESZCZENIE

Celem pracy było opisanie zmian w nerce szczura, świadczących o białkomoczu w przebiegu modelowego, doświadczalnego zespołu nerczycowego, biorąc pod uwagę czynnik czasu. Zespół został wywołany pojedynczą dawką adriamycyny podaną dootrzewnowo (5 mg/kg). Zwierzęta były uśpione po 4 (I grupa) i 8 (III grupa) tygodniach od podania leku.

Nasze obserwacje pozwalają na stwierdzenie, że: w grupie doświadczalnej III morfologiczne, histologiczne i biochemiczne zmiany były bardziej nasilone niż w grupie I. Składały się na nie: w kłębkach nerkowych zniszczenie blaszki ściennej torebki (jej pogrubienie, zcieńczenie lub przerwanie), gromadzenie się płynu i złogów białkowych w przestrzeni moczowej, poszerzone, zniekształcone, całkowicie lub częściowo zniszczone pętle naczyniowe. W kanalikach krętych nefronu wałeczki białkowe w świetle kanalików, zwyrodnienie drobno- i wielkowodniczkowe, zwyrodnienie kropelkowo-szkliste, stłuszczenie. Zmiany te składają się na obraz intensywnej proteinurii.

EXPLANATION TO FIGURES

Fig. 1. The experimental group I (4 weeks after adriamycin administration). PAS staining. Mag. 400x.

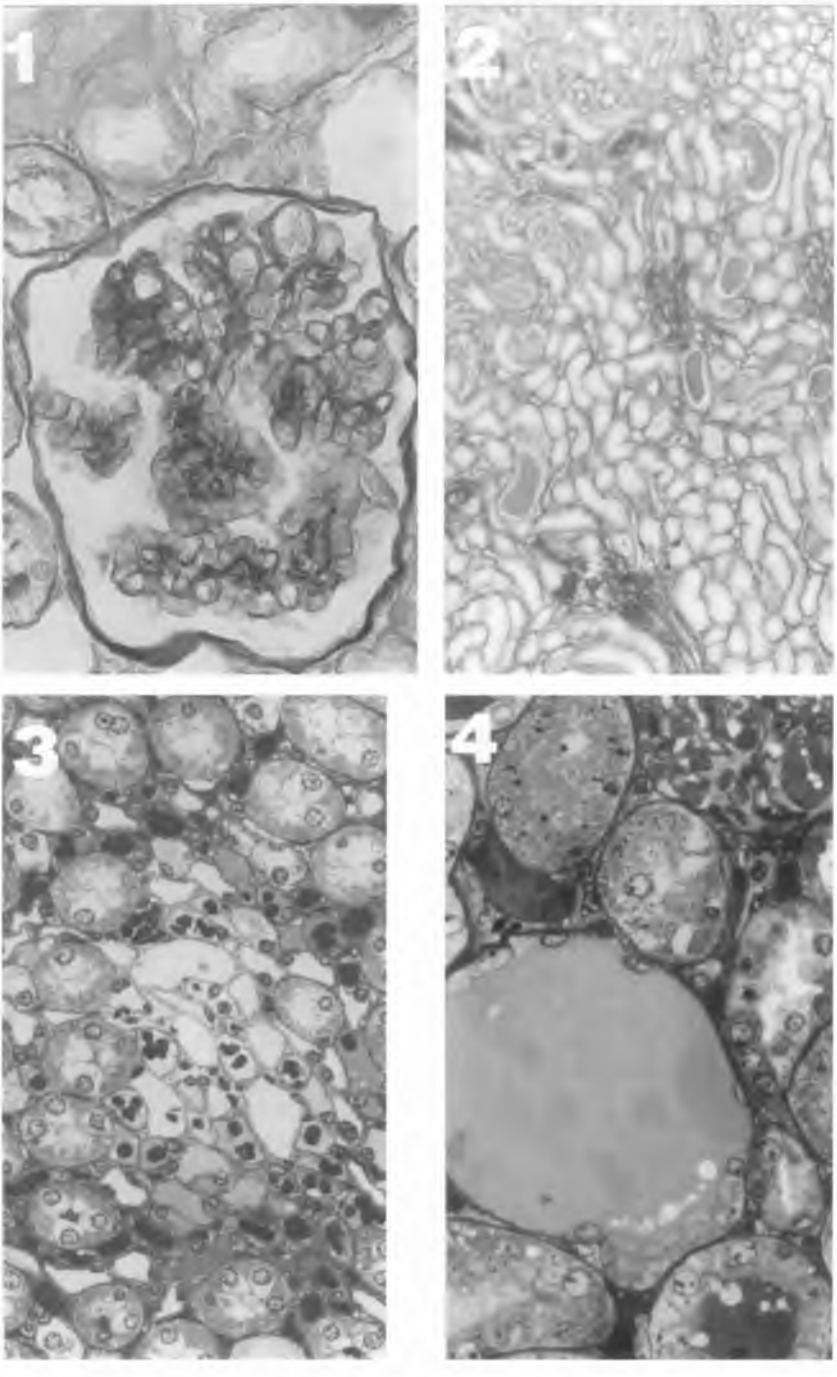
Fig. 2. The experimental group I. PAS staining. Mag. 100x.

Fig. 3. The experimental group I. Semithin slide. Methylene blue-Azur II staining. Mag. 200x.

Fig. 4. The experimental group III (8 weeks after adriamycin administration). Semithin slide. Methylene blue-Azur II staining. Mag. 400x.

Fig. 5. The experimental group III. PAS staining. Mag. 400x.

Fig. 6. The experimental group III. PAS staining. Mag. 400x.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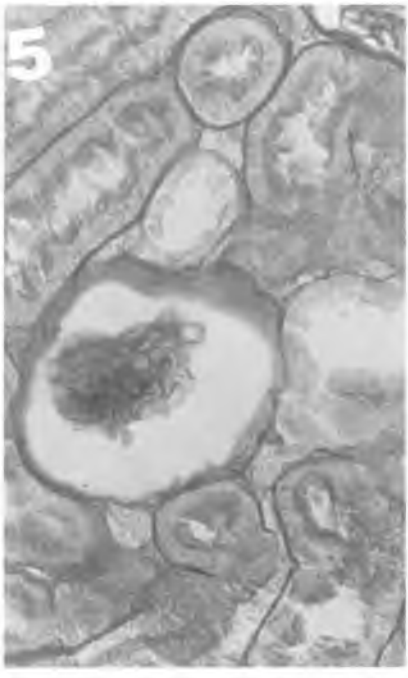
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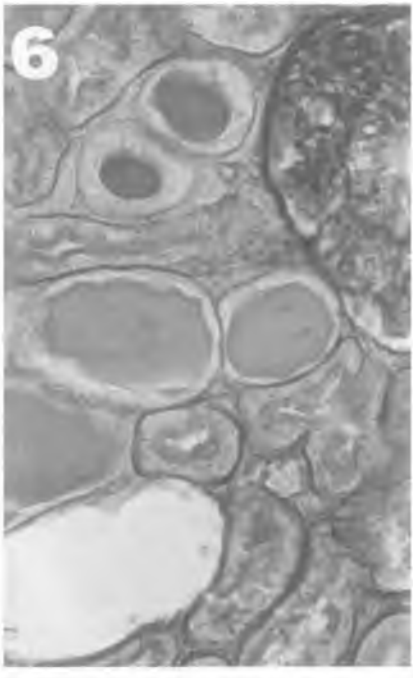
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