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Extensive proteinuria in rats after single dose of adriamycin – etiology

The explanation the mechanism of proteinuria has been the subject of studies for several years. Evident progress took place using of protein electrophoresis in body fluids and then introducing the kidney biopsy and improving the microscope techniques. Wurmann and Wunderly used the term proteinuria first in place of the term albuminuria widely used. They noticed the presence of globulin in urine apart from albumin (4).

At the beginning of 20th century the kidney diseases were divided into diseases of vessels, glomerules and tubules (6). Tubules diseases were called nephrosis because it was stated that changes in epithelium were the basis of disease with clinical and biochemical features due to excessive proteinuria. With time it was stated that proteinuria appear as well in evident glomerular changes for example in amyloidosis. According to up-to-date knowledge, proteinuria with kidney aetiology is due to the damage of proper glomerular filter, which lets more amounts of blood serum proteins than physiologically go to primary urine. Insufficient recurrent absorption of tubular cells seem does not seem to play such an important role. It was noticed in the studies of Chinard et al., who described higher concentrations of proteins in primary urine of patients with nephrotic syndrome than in physiological glomerular filtrate (3).

MATERIAL AND METHODS

The studies were performed on 24 female Wistar rats aged 2.5 to 3 months with initial body mass 200–250g. The animals were divided into 4 groups: two controls and two experimental ones. Females from the experimental group (I, II) were given at the beginning of the experiment adriamycin in a single dose 5 mg/kg of body weight intraperitoneally. Females from the control group (III, IV) the beginning of the experiment were given 0.5 ml 0,9% NaCl intraperitoneally. During the experiment the rats were weighed and urine protein concentration was examined by stripe test every week. 24 hours before decapitation the animals were prepared for 24 hours urine collection. In the collected urine protein concentration was measured. The rats were decapitated: 4 weeks (experimental group I and control group III) and 7 weeks (experimental group II and control group IV) after adriamycin administration. The left kidney from decapitated animals was taken, which was assessed microscopically and weighed. The kidney mass was estimated in percent of the rat body mass. Slides for electron microscopy were performed from kidney (14). Then the slides were observed with electron microscopy TESLA

BS-500 and photographs of the samples were taken for documentation. The differences in urine parameters, body mass and kidney mass were statistically estimated.

RESULTS

Initial body mass of all female rats from the experimental group did not vary with statistical significance from the initial body mass from the control group and it ranged from 212.00 g to 225.33 g (Tab. 1). 4 weeks after the beginning of the experiment the animal body mass from the experimental group increased similarly. 7 weeks after drug administration the body mass of animals from experimental group II decreased with statistical significance (an average – 36.10 g) comparatively to the body mass of animals from control groups, which increased their body mass in the same period with an average 1.34 g. One female from the experimental group II died in 7th week of the experiment.

Kidneys from all control groups animals did not differ microscopically. Kidney taken to investigation was bean-shaped with a capsule which could be easily taken off. The organ surface was red-brown smooth. Kidneys taken from female rats from experimental groups did not differ macroscopically. They differed from control groups kidneys. The picture shows the features 'big, white kidney". Comparing to control kidney it had bigger dimensions, and pale-pink-grey colour. The kidney surface was smooth. After dissection of the kidney parenchyma everted from the capsule was visible. The mass of the kidneys is displayed in Table (Tab. 2).

Table 1. Body mass

Groups	No.	Average initial body mass (g)	Average body mass after 4 weeks (g)	Increase of body mass after 4 weeks (g)	Average body mass after 7 weeks (g)	Increase of body mass after 7 weeks (g)
Experimental	I	220.36+/-1.59	270.04+/-1.98	49.68		
	II	225.33+/-0.65	276.1+/-7.11	50.77	240+/-8.01	-36.1
Control	III	212+/-15.27	266.44+/-16.15	54.44		
	IV	224.62+/-0.77	272.66+/-7.59	48.04	274+/-4.72	1.34
Significance	gr.I to gr.III gr. II to gr. IV	P.=0.14 P.=0.051	P.=0.525 p.=0.337		p.<0.0001	

Table 2. Kidney's mass

	Exp.	Groups	Contr. groups	
	I after 4 weeks	II after 7 weeks	III after 4 weeks	IV after 7 weeks
Average kidney mass at the end of the experiment	1.67 (+/-0.49)	2.38 (+/-0.24)	0.95 (+/-0.95)	1.03 (+/-0.058)
Significance	p.=0.02	p.=0.0002		
Kidney's mass in% of the body mass	0.06%	0.10%	0.04%	0.04%

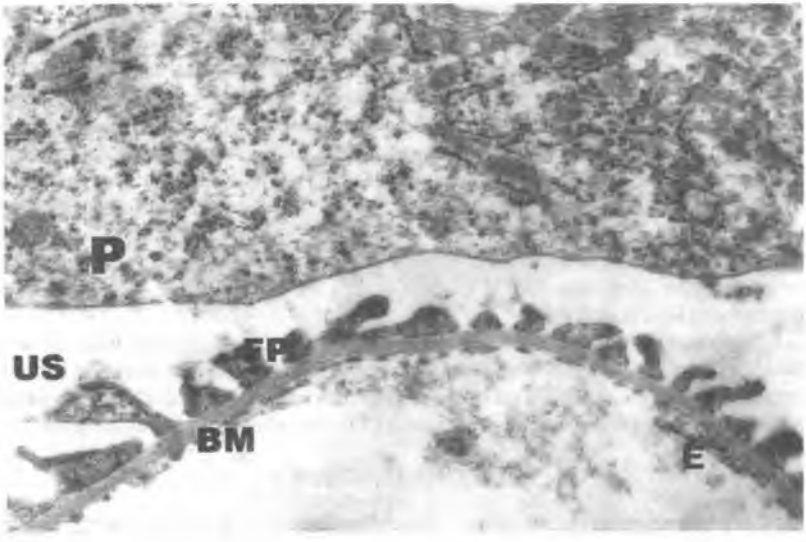

Fig. 1. Control group. The picture of a part of a rat female kidney normal glomerulus in electron microscopy. The barrier blood - primary filtrate. P–podocyte, V–vacuole, BM–basement membrane, FP–foot processes, N–nucleus, US–uriniferous space, RBC–erythrocyte, E–endotheliocyte. Magn. 11000x

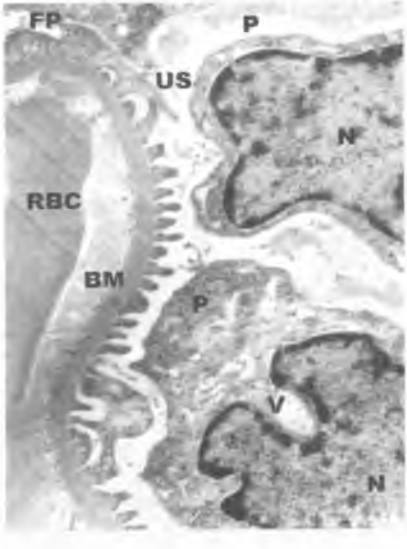

Fig. 2. Experimental group I. A part of a rat female kidney glomerulus in electron microscopy. In podocyte's (P) cytoplasm some vacuolas (V). Dilatation of reticulum endoplasmic tubules (arrow). Food processes of podocytes are connected. Magn. 3 000x

The picture of rat kidney from the control group in electron microscopy did not vary from normal description (Fig. 1). Changes of glomerules in electron microscopy in all experimental groups were similar. In podocyte cytoplasm optically empty vacuoles were observed or ones with not homogenous material, some of them with the diameter bigger than the nucleus diameter. Similar vacuoles were also seen in some of the endothelial cells. In some of podocyte perinuclear vacuoles were observed (the evidence of oedema), seen as a light "hallo" around the nucleus. The reticulum endoplasmatic tubules were focally dilated. In podocyte cytoplasm some mitochondria were swollen. Focal connecting of podocytes processes and thickening of endothelial basal membrane of glomerural vessels were observed (Fig.2,3). Protein concentration in female rat urine investigated with stripe test was higher than 10g/l in all experimental groups 4 week after adriamycin administration. In control groups it did not change essentially during the experiment and was less than 0.3g/l.

In average, protein concentration in urine from 24 hours collection was: a) in control group 7.96+/-0.37mg/24hours and not differ with statistical significance between groups; b) in experimental groups (Tab. 3, Fig. 2) extensive proteinuria was noticed already 4 weeks after drug administration. It was more evident with time (7 weeks after drug administration).

Table 3. Protein in urine

Study	Group	No.	Average	Standard deviation	To group		To control	
					significance	test t of stud.	significa-nce	test t of stud.
Protein in urea (mg/24h)	control	III	7.96	(+/-0.27)				
		IV	7.90	(+/-0.22)	p=0.74	0.34 III		
	experimental	I	84.38	(+/-5.40)			p<0.0001	31.57 III
		II	127.02	(+/-9.11)	p<0.0001	9.00 I	p<0.0001	29.23 IV

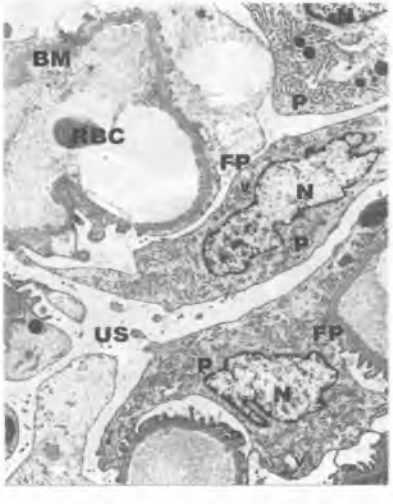

Fig. 3. Experimental group II. A part of rat female kidney glomerulus in electron microscopy. In podocyte's (P) cytoplasm some vacuoles (V). Dilatation of reticulum endoplasmic tubules (arrow). Food processes of podocytes are connected (FP). Focal thickenning of basement membrane(BM). Magn. 8 000x

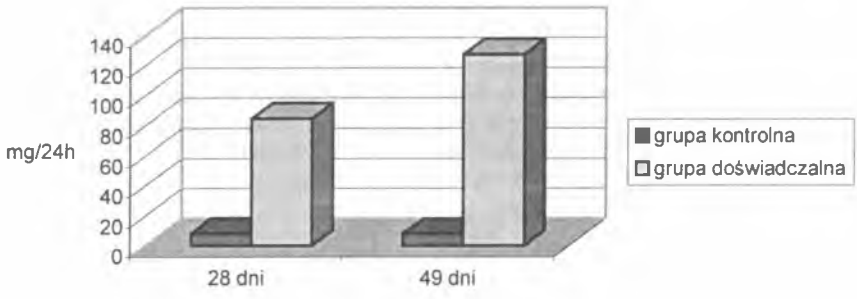

Fig. 4. Urine protein concentration

DISCUSSION

There are many papers on proteinuria after antracycline antibiotics (8,11). Proteinuria assessed in semi-quantitative method in this study surpassed 10g/l 4 weeks after adriamycin administration. Protein in urine in 24 hours collection was on average 84.38 mg/24 hours and after 7 weeks 127.03 mg/24 hours. Similar results were observed by Pomeranz – 115+/-26 mg/24 hours (9). Soares (11) 10 weeks after drug administration in the dose 3 mg/kg of body weight stated an increase of protein in urine even to 250 +/-25.91 mg/ 24 hours; Irwin (5) after 28 weeks 494 mg/24 hours. It is the evidence of proteinuria increasing with time. Many authors considered the mechanism of excessive passing of protein through the glomerular filter to urine, which takes place after adriamycin. Wang (13) observed decreased amounts of negative charges (anions) placed in glomerulus already 3 hours after drug administration in the dose 5 mg/kg of body weight. He noticed as well that this phenomenon got intensified with the disease development. Bertani et al. (2) described the presence of electrostatic disturbances placed in filtration membrane of glomerulus. They did not find morphological damage of basal membrane. In the case of loss of the negative charge, permeability of albumin is much higher than of other proteins (selective proteinuria), 6. In morphological damages the permeability of low and big mass protein is similar, for example albumins and gammaglobulin (non-selective proteinuria), which takes place in proteinuria after adriamycin. Sternberg (12) noticed that 4 weeks after adriamycin administration morphological changes in basal membrane were seen. Wang (13) stated that connecting of podocytes processes – changes seen in electron microscopy were the consequence of a decreased amount of anions in filtration layers. Focal connecting podocytes processes observed in this study was described by Zima et al., Remuzzi et al., Wang (10,13,15). All these authors gave adriamycin in 5 mg/kg of body weight and examined kidneys after 13, 15 and 21 days. Changes in basal membrane touched single glomerules, they were focal and lay in the thickening of basal membranes without deposits. Changes in renal glomerulus structures observed in electron microscopy show the cause of proteinuria – morphological damage of kidney filtration barrier and connected with that excessive permeability of basal membrane for proteins. The present study used adriamycin, which is an antibiotic from antracyclines group with anticancer activity (1). In adriamycin transformation process free radicals are created, which are responsible for cytotoxical and nephrotoxical activity of this drug (15). Free radicals take part in cell membranes peroxidation process. Adriamycin is metabolized in microsoms. As a result of chemical reactions catalysed by microsomal enzymatic system semichinon is produced, which is the form of free radical.

Adriamycin also stops enzymatic system eliminating free radicals, which increases cells damage.

Microscopic study of kidney shows a picture of a big, white kidney (7). A big, white kidney is a microscopic feature of increased permeability of glomerular basal membrane for proteins and lipids. These substances passing in big amounts to primary filtrate create cast in tubular lumen. Protein and lipid deposits give grey-yellow colour of the kidney. A big, white kidney is the most frequent reflection of kidney disease with proteinuria.

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SUMMARY

The purpose of the present studies was to find the cause of proteinuria, which is displayed in rats after one dose of adriamycine. The results show electrostatic (decreased amount of negative charges in glomerulus) and morphological damages of kidney filtration barriers, which causes increased permeability of basal membranes for proteins. An important part in etiology seems to played by free radicals, which arise in the process of adriamycine transformation.

Wzmożony białkomocz u szczurów po jednorazowej dawce adriamycyny – poszukiwanie przyczyn

Przeprowadzone badania miały na celu odnalezienie przyczyny białkomoczu, który pojawił się u szczurów po jednej dawce adriamycyny. Otrzymane wyniki wskazują na elektrostatyczne (zmniejszenie ilości ładunków ujemnych w kłębku) oraz morfologiczne uszkodzenie bariery filtracyjnej nerek i związaną z nim wzmożoną przepuszczalność błony podstawnej dla białka. Dużą rolę zdają się odgrywać wolne rodniki, które powstają w wyniku transformacji adriamycyny.