ANNALES
UNIVERSITATIS MARIE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LVIII, N 2, 108 SECTIO D 2003

Department and Institute of Histology and Embryology with the Laboratory
for Experimental Cytology, Medical University of Lublin

AGNIESZKA PEDRYCZ, MARCIN WIECZORSKI,
KRYSTYNA CZERNY

Comparison of methods of inducing experimental nephrotic syndrome in lab animals

Nephrotic syndrome is the group of clinical-biochemical signs due to proteinuria crossing compensative capacity of the organism. Constant big loss of proteins with urine is the cause of hipoproteinemia (hipoalbuminemia) and dysproteinemia, increase of cholesterol and lipids concentration in blood serum, oedemas. The degree of these changes depends on increasing the permeability of glomerular capillary vessel wall (10). Nephrotic syndrome could appear without evident aetiology, it could develop with existing glomerulopathia or could overlap the symptoms of other diseases, rarely could it be congenital or familiar (10).

The studies performed before the Second World War stated that morphological changes typical of diseases with big proteinuria were placed in kidney glomerulus as a thickening of the glomerular capillaries basal membrane. Introducing new methods of staining, electron microscopy and a broad use of intravital kidney biopsy made it possible to assess more exactly all elements of glomelural filter in the mechanism of proteinuria. The studies show that there is a characteristic syndrome of morphological changes for glomerular filter damages. This is thickening and changing of the basal membrane structure; changes in building and connecting of podocyte processes. Farquhar described basal membrane (a study with intravenous ferritine infusion) as a basic filtration barrier. Changes in epithelial cells are probably secondary due to a bigger amount protein permeability (3). It was confirmed in the light of microscopy picture: proteins and lipids passing to urinary space and then to the main tubule were absorbed by epithelial cells giving the picture of droplet-hyaline degeneration and steatosis (8).

MATERIAL AND METHODS

This paper compares different methods of attempting to induce experimental nephrotic syndrome by different factors described in literature by numerous researchers with an experimental model of nephrotic syndrome obtained by authors after a single dose of adriamycin given to rats intraperitoneally. The study performed on 32 female Wistar rats was divided into 4 groups (2 experimental groups and 2 control groups). At the beginning of the experiment female rats from the experimental group were given adriamycin in a dose 5 mg/kg of body weight intraperitoneally and female rats from the control groups were given 0.5 ml 0.9% NaCl intraperitoneally. The animals had 24 hours urine collection performed, total protein, albumin, cholesterol, lipids, urea and creatynine concentration assessed in blood. The blood was taken from the heart after decapitation performed after 4 weeks (experimental group

I) and after 7 weeks (experimental group II). The left kidney was always taken for histological examination, from which histological slides were performed. The slides were stained with hematoxyline and eosine and then watched in light microscopy.

RESULTS

Full nephrotic syndrome appered already 4 weeks after adriamycin administration. The proteinuria, hipoproteinemia with dysproteinemia, hipoalbuminemia, hiperlipidemia, hipercholesterolemia deepen during the experiment (after 7 weeks). The histological picture of rat kidney from all experimental groups was similar and disclosed in glomerules: dilatation of urine space, damaged glomerular capillary vessels loops and glomerulosclerosis; in convoluted tubules: cast in tubular light and focal damages in tubular epithelium (11) – Fig. 2.

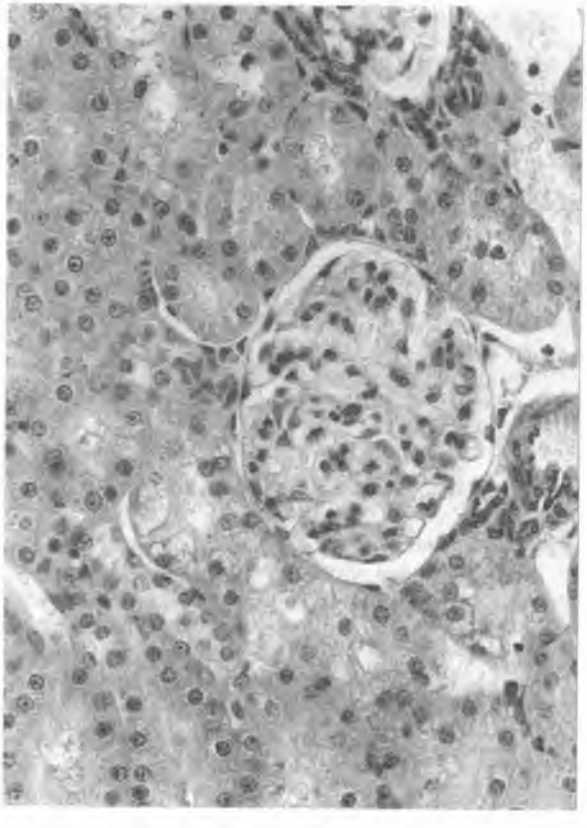

Fig. 1. Control group. The picture of a kidney part of a female rat in light microscopy. Stained H & E. Magn. 320x

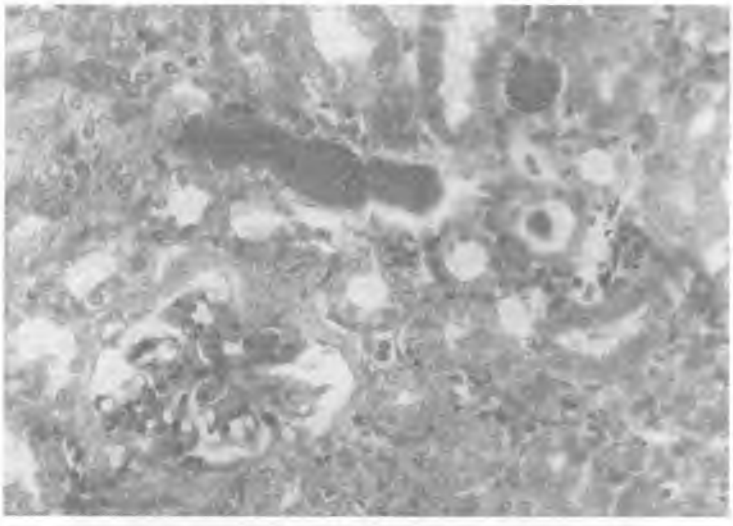

Fig. 2. Experimental group II. The picture of a kidney part of a female rat in light microscopy. Stained H & E. Magn. 320x

DISCUSSION

To explain the pathogenesis of nephrotic syndrome attempts to induce this syndrome in experimental animals were performed with different factors. The following were used: bismuth preparations, salts of chromium, uranium, phosphorus, arsenous oxide, barbiturates derivatives (luminal, veronal) and sublimate. These methods were quite complicated and did not give a full-presented, "pure" nephrotic syndrome, and the mortality of animals during experiments was above 50%.

Another method was autoimmunisation with suspension of homological renal tissue. There were attempts to use heterological antiserums. To cause a decrease of proteins in the organism a big amount of blood was taken from animals, and insufficiency was completed with erythrocyte suspension in Ringer fluid (8). In this way short lasting hipoproteinemia was obtained with evident dysproteinemia (decrease of albumin, gamma globulin and increase of alpha and beta globulin concentration). But it was not a full-presented nephrotic syndrome. It was without oedemas.

An interesting way to induce nephrotic syndrome in rats was using Puromycin – one of the derivatives of aminonucleosids (4). Puromycin was isolated in 1952 by Porter and Hewitt from fungi *Streptomyces alboniger* as an antibiotic with wide spectrum activity (1,4). To induce nephrotic syndrome Sherman and Taylor first used Puromycin in 1954 (1). Frenk et al. (5) administered aminonucleosid to rats in a dose 0.003 ml 0.5% aqual solution on 1g of body weight every 12 days. They noticed oedemas, proteinuria, hipoproteinemia and hiperlipidemia. In a histological examination of kidneys they described hypertrophia of glomerular basal lamina, lipid granules in endothelial cells, casts in tubules and a decreased amount of mitochondria in tubular cells (1,5). In that model of nephrotic syndrome protein in urine could be provided between 6 and 9 days from the beginning of drug administration, and the amount of protein in urine increased in the next days, the maximum being on 10 day (7). The mechanism of nephrotoxic activity of Puromycin (14) was analyzed. Hartmann et al. (6,14) noticed that adenine stopped the nephrotic

syndrome induced by Puromycin. It suggests that Puromycin activity is connected with nucleic acids metabolism. Puromycin also has a similar structure to adenosin. But studies with isotopes did not confirm that hypothesis. Fisher (4) tried to explain the nephrotoxic activity of amino-nucleosid and its influence on some ensymatical systems. He noticed decreased activity of cytochrom oxidase and succinic dehydrogenase in renal cells. Borowsky (1) noticed that Puromycin derivatives given in the same or higher doses than Puromycin did not induce the nephrotic syndrome and Puromycin induced the nephrotic syndrome in rats only (7).

Another method of experimental induction of the nephrotic syndrome was administration of mercury chloride in 80mg% solution in isotonic NaCl in a single dose 0.75 um/100g of body weight intraperitoneally. In that method the biggest concentration of protein in urine was developed on 2 day after drug administration, then its concentration was decreasing. Mercury causes kidneys function disturbance via inactivation of sulfhydryd groups of renal cortex increases permeability of convoluted tubules cells membrane and destroys basal lamina (7).

In recent years researches have paid attention to adriamycin – antibiotic from antracycline group, which induces the nephrotic syndrome. First antibiotic from antracycline group-Rubidomycin (daunomycin) was isolated from fungi *Streptomycies species s. coeruleorubidus* in 1962 in France (9). Adriamycin (doxorubicin) is a dose derivative of rubidomycin (14-hydroxy daunomycin) produced mutant of *Streptomyces peuceticus s. caesius* (9). Sternberg and Philips (12) described the first experimental damage of kidney by antracycline in 1967 in rats which obtained intravenously daunomycin. Since that time some studies about the nephrotoxic activity of adriamycin in rats (12) and in rabbits (13) have been performed. In human nephrotoxic the activity of adriamycin on kidney parenchyma was rarely described. Experimental nephrotic syndrome in rats appeared even after a single dose of adriamycin and it was increasing with time even one year after adriamycin administration (12,13). There are plenty of papers to explain the cause of antracycline antibiotic nephrotoxicity. The immunological cause was excluded, because in kidney glomelural structures the immunoglobulin and component deposits were not found and the thesis that antibiotics had an independent toxic effect on kidney was suggested. The cause of proteinuria induced by antracyclines was the influence of these drugs on glomerular and tubular cells, a change of endothelial wall function and a loss of negative change of glomerulus (2). Another concept of antracyclines nephrotoxity mechanism was by Bristow et al. (2), who suggested histamine and catecholamine action because after antibiotics administration their level in the organism was increased. They pointed that histamine and adrenergic blocker administration protects kidneys from changes. Zima et al. and many others authors (15) stated that the most probable hypothesis is the action of free radicals, which are produced due to antracycline biotransformation.

REFERENCES

1. Borowsky B. A. et al.: Structural analogues of puromycin in production of experimental nephrosis in rats. Proc. Soc. Exp. Biol. Med., 97, 857, 1958.
2. Bristow M. R. et al.: Histamine and catecholamines mediate chronic cardiac and renal damage associated with adriamycin administration. Clin. Res., 27, 156, 1979.
3. Farquhar M. G.: An electron microscopic study of glomerular permeability. The Anat. Rec., 136, 191, 1960.
4. Fisher E. R. et al.: Aminonucleoside nephrosis in rats. AMA Arch. Pathol., 65, 545, 1958.
5. Frenk S. et al.: Experimental nephrotic syndrome induced in rats by Aminonucleoside. Renal lesions and body electrolyte composition. Proc. Soc. Exp. Biol. Med., 89, 424, 1955.
6. Hartman J. D. et al.: The effect of adenine in aminonucleoside nephrosis. J. Lab. Clin. Med., 59, 158, 1962.

7. Kowalska-Pyłka H.: Badania nad aminoacydurią u szczurów w nerczycy doświadczalnej wywołanej aminonukleozydem-Puromycyną i chlorkiem rtęciowym. Praca doktorska, AR Lublin 1963.
8. Kruś S.: Diagnostyka mikroskopowa. In: W. Gluzińska (eds.): Patomorfologia nerek. PZWL, Warszawa 1986.
9. Matusewicz W. et al.: Leki antracyklinowe. Pol. Arch. Med. Wewn., 73, 242, 1985.
10. Papierkowski A.: Zespół nerczycowy u dzieci. Cz. 1, 2. Medicus, 3, 4, 8, 1992.
11. Pedrycz A.: Ocena wpływu ciąży na nerkę szczura w przebiegu modelowego, doświadczalnego zespołu nerczycowego wywołanego Adriamycyną. Praca doktorska, AM Lublin 2002.
12. Sternberg S. S. et al.: Biphasic intoxication and nephrotic syndrome in rats given daunomycin. Proc. Am. Assoc. Cancer Res., 8, 64, 1967.
13. Suzuki Y. et al.: Nephrotic effects of adriamycin in rabbits. Fed. Proc., 37, 320, 1978.
14. Wilson S. G. F. et al.: Studies on the mechanism of production of a nephrotic syndrome in rats by a nucleoside. Paediatrics, 25, 228, 1960.
15. Zima T. et al.: ICRF-187 (dextrazoxan) protects from adriamycin-induced nephrotic syndrome in rats. Nephrol. Dial. Transplant., 12, 1975, 1998.

SUMMARY

The purpose of this study was an attempt to compare different methods of inducing the experimental nephrotic syndrome by different factors performed by numerous researchers, with the experimental model of the nephrotic syndrome obtained by the present authors after a single dose of adriamycin.

Porównanie metod wywoływania zespołu nerczycowego u zwierząt doświadczalnych

Celem pracy była próba porównania różnych metod wywoływania zespołu nerczycowego u zwierząt doświadczalnych, opisanych przez licznych autorów, oraz modelu zespołu nerczycowego uzyskanego przez autorów pracy podaniem jednorazowym adriamycyny.