ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LXI, N1, 75 SECTIO D 2006

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Late effects of adriamycin action – histological assessment of kidneys of the rat offspring

Adriamycin (ADR) – an antibiotic, is applied in therapy of cancers. It is giving good results while treating cancer of breast, bladder, lungs, bronchi, thyroid gland, ovary, chorioncarcinoma of uterus, non-Hodgkin lymphomas, neuroblastoma, hepatoblastoma, acute lymphoblastic leukaemias, acute myeloblastic leukaemias, Hodgkin disease, sarcomas of soft tissues and the bone, Wilms and Ewing tumours. At intrauretheral applying ADR is giving good results in treating of superficial tumours of the bladder (3).

There are known reports describing injured kidneys in human subjected to ADR therapy. However, these are very thin coincidences, because in human more exposed to toxic action of ADR is heart and that is why cardiotoxical activity of ADR is limited in human.

The use of ADR in antineoplastic therapy in young people and children is common, and the question arises whether ADR can have distant action especially to the offspring of mothers who in the past were treated with ADR. Such potential possibility could not be excluded.

It is known from the literature that ADR given to pregnant females is acting teratogenically. It is inducing a number of congenital defects both in people and in laboratory animals. It was proved that this drug given to rats intraperitoneally even in the dose of 2 mg/kg of body weight in 6–9 day of pregnancy causes the syndrome of the congenital anomalies similar to the human VATER syndrome (spinal disorders, anal atresia, tracheo-oesophageal fistula, oesophageal atresia, and radial bones dysplasia) (8). A foetal rat's model of the oesophageal atresia and tracheal atresia was obtained (11), with ADR given in the early period of pregnancy. Nevertheless, the influence of ADR given to females before planned pregnancy to their offspring is unknown.

MATERIAL AND METHODS

In the experimental group there were used 12 four weeks' rats, which were selected randomly by taking two from the offspring of each of the six pregnant females, which 4 weeks before fertilization were administered ADR intraperitoneally in the dose 5 mg/kg of body weight.

In a similar way in the control group were used 14 four weeks' rats, which were selected randomly by taking two from the offspring of each of the seven pregnant females, which 4 weeks before fertilization were administered 0.9% NaCl intraperitoneally in the dose 0.5 ml. Right after birth and just before decapitation the rats were weighted, the size of litter and percent of still-born foetuses were determined.

Collected for histopathological investigations kidney sections were fixed in 10% formalin buffered to pH 7.4 (with phosphate buffer) and after dehydratation in growing concentrations of the ethanol (40%, 50%, 60%, 70%, 80%, 90%, "absolute" ethanol), they were illuminated in xylene and then embedded in paraffin blocks. Then the specimens were cut into 5 μm thick sections, which next were stained with hematoxylin and eosine and according to the Masson's method depicting the connective tissue. Coloured preparations were examined under the light microscope. Documentation of preparations was performed with the help of the Jenaval Contrast Carl Zeiss microscope.

Subjected to a statistical analysis were body weight differences, amount of alive and dead newborn rats. Results of examinations were compared with figures of averages and standard deviations of average. A risk of 5% conclusion mistake, and significant statistical differences at $P<0.05$ were accepted. The assessment of histopathological parameters was shown in the periphrastic form.

RESULTS

Offspring from the experimental group just after birth were weighted. Weight values were statistically significantly lower than in the control group. Also in the experimental group dead births were noted while in the control group all newborn babies were alive (Table 1). Mean number of litter was significantly higher in the control group than in the experimental group; average increase of the body weight of the animals was significantly smaller in the experimental group after 4 weeks than that of animals in the control group.

Table 1. An average size of litter, body weight of newborn babies

Group	Experimental	Control	Statistical significance
Average number of dead births	0.83+/–0.75	0.00	P=0.04
% of dead births	14.7	0.0	
Average number of living births	4.8+/–0.16	8.86+/–0.89	P<0.0001
Medium size of litter	5.63+/–1.36	8.86+/–0.89	P=0.0012
Average body weight of newborn rats in grams	3.23+/–0.70	4.46+/–0.34	P=0.006
Average body weight after 4 weeks	30.01+/–1.23	35.44+/–0.95	P<0.0001
Average increase of the body weight after 4 weeks	26.76	30.98	

Table 2. The structure of the sex of the offspring

Groups	Number of mothers	Number of living births	Including	
			females	males
Experimental	6	29	16	11
Control	7	62	39	23

Kidneys of the offspring from the experimental group differed from kidneys of the offspring from the control group. These changes were focal. In renal tubules destroyed cell membrane of tubular epithelium was found. Epithelial cells were focally destroyed. Tubular lumen was widened and naked nuclei and fragments of homogenous secretions were visible in it. Widening of the urinary space and destroyed loops of capillaries were visible in glomerules. In preparations stained by the Masson's method it was possible to notice the increase of the amount of the connective tissue between tubules and in glomerules – glomerulosclerosis.

DISCUSSION

ADR is administered intravenously in the process of treating cancer disease after dissolving in 100–250 ml 5% glucose or 0.9% NaCl, in infusion for 15–30 min. It is applied in poly- and monochemotherapy. In monotherapy it is administered in the dose 60–75 mg/m^2 every 3 weeks. The total cumulative dose should not exceed 550 mg/m^2. The acceptable curative dose to 1–10 mg/kg of body weight (11).

The highest level of ADR in the blood serum takes place one hour after the application and the accumulation in tissues depends on the dose. ADR penetrates the cells through passive diffusion, whose size depends on the concentration of the drug in blood. It accumulates in the cells in the nucleus and lysosomes.

ADR given in intravenous injection is eliminated from plasma fast. It is discharged slowly unchanged in 5%–15% with urine and about the 50% dose with bile (1, 3), in c 23% as adriamycinol (14). Drug does not permeate to the cerebrospinal fluid. However, it permeates through the placenta to the mother's milk. The demotion of the drug molecules is running in stages (1), from which the last is demethylation, sulfuration or combining with the glucuronic acid. Side-effects during taking ADR are typical of cytostatics: myelosuppression, loosing of hair, nausea, vomiting, inflammation of the oral cavity, the erosion in the region of the tongue, diarrhoea, after intrauretheral administration – haematuria, disorders of giving urine back, painful and frequent giving urine back.

In the literature it is possible to find descriptions of cases of pregnant women treated with ADR.

Paskulin observed a woman, who during having polychemotherapy with ADR became pregnant and still took the drug in trimester I. The child was born on time but at birth numerous anomalies were observed, including: high-arched palate, microcephaly, flat nasal bridge, bilateral syndactyly in the first and second fingers, hand cleft between the second and third fingers and hypoplasia of the fifth fingers, and dystrophic nail of the fourth finger of the left hand. The patient's growth and development were deficient (10).

Kerr observed the mother treated with doxorubicin in trimester 2 and 3. The child, apart from the fact that it was born prematurely, had anaemia and neutropenia, however, it had no physical anomalies and developed correctly through the first year of his extrauteral life (6). Liu and Hutson carried out investigations of embryogenesis of the bladder and proved that day 7 of pregnancy is critical in the development of the bladder in rats and ADR given on that day is inhibiting the development of the bladder (7). Kavlock et al. noticed that application of ADR in the pregnant rat causes changes in the development of the renal wart in the foetus which are also present after 2 weeks of the extrauteral life (5).

In the present study ADR given to rats intraperitoneally in the amount of 5 mg/kg of body weight 4 weeks before planned pregnancy develops changes in kidneys of the offspring observed after 4 weeks of the extrauteral life. The dose of 5 mg/kg of body weight given intravenously or intraperitoneally is located in borders of acceptable curative doses given to people during monochemotherapy of cancers. In experimental animals there develops nephrotic syndrome (NS) 4 weeks after ADR administration manifesting itself with: proteinuria, hipoproteinemia with dysproteinemia, hipercholesterolemia, hipertriglicerydemia and with swellings (2, 15). Histological changes in kidneys include: dilatation of the urinary space in glomerules, destroyed loops of capillaries, increased amount of fibres of the connective tissue – glomerulosclerosis (4), destroyed epithelial cells in tubules, the widened light of tubules filled up with secretion (12). The presence of protein casts in the tubular light confirms the proteinuria. The filling of some tubular lights, observed in the present study is in the majority fragments of removed cells of the epithelium, however focally homogenous secretion was also visible.

We made similar observations in our own examinations in foetuses of mothers, which 4 weeks before planned pregnancy were given ADR intraperitoneally in the dose 5 mg/kg of body weight. Changes proving the proteinuria in foetal kidneys seemed to be more intensified than in the offspring where prevailed changes of glomerulosclerosis character. It seems probable that NS after ADR in rat's mothers was also observed in their offspring, as the distant effect of ADR action. Changes observed in kidneys, especially a damaged filtration barrier due to glomerulosclerosis, destroyed endothelial cells and destroyed visceral layer of glomerular capsule cells suggest such a possibility. In order to confirm this thesis the examining of urine and blood of the offspring in terms of NS exponents (proteinuria, proteinemia, cholesterolemia, etc.) seems to be correct.

ADR is acting by inhibition of the synthesis of nucleic acids. It also suppresses the activity of enzymes of the respiratory chain (succinate dehydrogenase and NADH oxidases) (9). In the proc-

esses of ADR transformation there arise free radicals, which are responsible for cytotoxic and also nephrotoxic action of this drug (1, 13, 15). Apart from that, ADR suppresses the enzymatic system removing free radicals, which additionally increases damage in the cell. Perhaps it is exactly free radicals that are responsible for late histological changes in kidneys of the offspring of female rats which got the ADR dose long time before planned pregnancy.

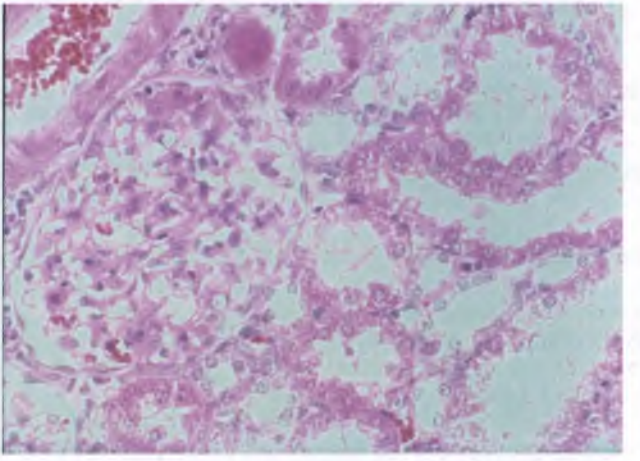

Fig. 1. Experimental group. Focally damaged tubular epithelial cells. Homogeneous secretion in tubular lumen. Magn. ca 320x. H+E staining

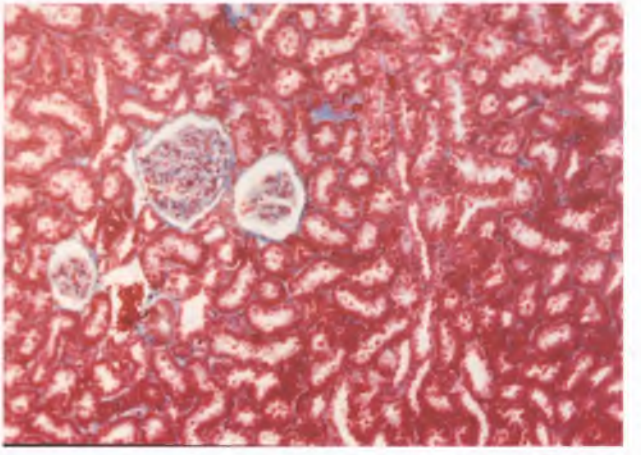

Fig. 2. Experimental group. Dilatation of urinary space in glomerules, glomerulosclerosis (grey colour in glomerulus). In the tubular light nuclei of damaged epithelial cells. Magn. 160x. Staining according to Masson

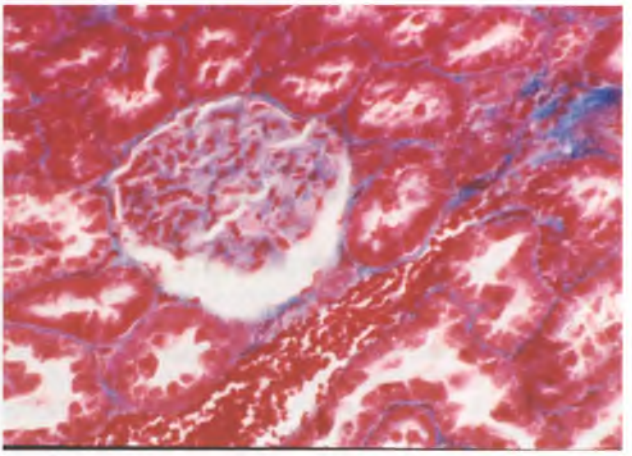

Fig. 3. Experimental group. Dilatation of urinary space in glomerulus, glomerulosclerosis (grey colour in glomerulus), in the tubular light small amount of homogeneous secretion, or epithelial cells parts. Magn. 320x. Staining according to Masson

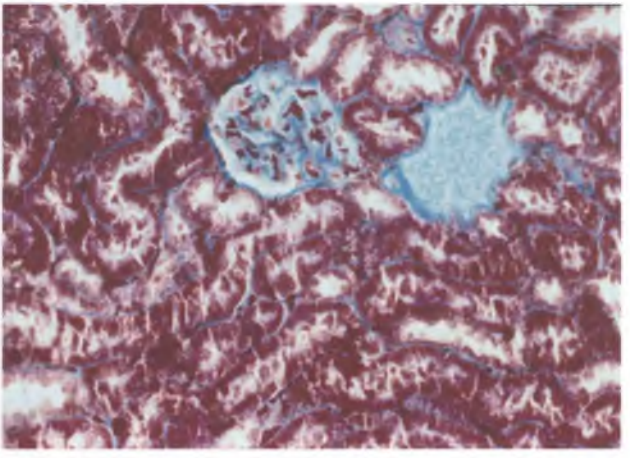

Fig. 4. Experimental group. In glomeruli visible increase of amount of connective tissue (grey colour), and damaged capillaries loops. In kidney parenchyma visible increase of connective tissue and focally damaged epithelial cells in tubules. In the light of some tubules small amount of homogeneous secretion. Magn. 320x. Staining according to Masson

REFERENCES

1. Bachur N.R.: Daunorubicinol, a major metabolite of daunorubicin: isolation from human urine and enzymatic reactions. J. Pharm. Exp. Ther., 177, 573, 1971.
2. Bertani T. et al.: Adriamycin-induced glomerulosclerosis in the rats. Am. J. Kidney Dis., 7, 12, 1986.
3. Blum R.H., Carter S.K.: Adriamycin, a new anticancer drug with significant clinical activity. Ann. Int. Med., 80, 249, 1974.
4. Hall R.L., Wilke W.L.: The progression of adriamycin-induced nephrotic syndrome in rats and the effect of captopril. Toxicol. Appl. Pharmacol., 82, 164, 1986.

5. Kavlock R.J. et al.: The significance of the dilated renal pelvis in the nitrofen-exposed rat fetus: effects on morphology and function. Toxicol. Appl. Pharmacol., 94, 287, 1988.
6. Kerr J.R.: Neonatal effects of breast cancer chemotherapy administered during pregnancy. Pharmacotherapy, 25, 438, 2005.
7. Liu-Mi P., Hutson J.M.: Cloacal and urogenital malformations in adriamycin-exposed rat fetuses. BJU-Int., 86, 107, 2000.
8. Merei J.M.: Single umbilical artery and the VATER-animal model. J. Pediatr. Surg., 38, 1756, 2003.
9. Meriwether W.D., Bachur N.R.: Inhibition of DNA and RNA metabolism by daunorubicin and adriamycin in L1210 mouse leukemia. Cancer Res., 32, 1137, 1972.
10. Paskulin G.A. et al.: Combined chemotherapy and teratogenicity. Birth Defects Res. A. Clin. Mol. Teratol., 7, 634, 2005.
11. Qi B.Q., Beasley S.W.: Communicating bronchopulmonary foregut malformations in the adriamycin-induced rat model of oesophageal atresia. ANZJ Surg., 69, 56, 1999.
12. Soares V.A., Vivero R.M.: Reduction of urine volume ameliorates adriamycin-induced nephropathy. Braz. J. Med. Biol. Res., 26, 943, 1993.
13. Speth P.A. et al.: Clinical pharmacokinetics of doxorubicin. Clin. Pharmacokinet., 15, 15, 1988.
14. Young D.M.: Pathologic effects of adriamycin (NSC-123127) in experimental systems. Cancer Chemother. Rep., 6, 159, 1975.
15. Zima T. et al.: ICRF-187 (dexrazoxan) protects from adriamycin-induced nephrotic syndrome in rats. Nephrol. Dial. Transplant., 13, 1975, 1998.

SUMMARY

Adriamycin (ADR), the antineoplastic antibiotic given to rats in the dose 5 mg/kg of body weight, induces already after 4 weeks full sign nephrotic syndrome (NS). In earlier examinations we observed that histological changes in kidneys of the rat's females with the ADR induced NS present before pregnancy were visible also in the fetuses's kidneys of these mothers at the end of pregnancy. In the current study there were histologically examined kidneys of offspring (4 weeks after delivery) deriving from female rats, which 4 weeks before planned pregnancy were administered ADR in the dose 5 mg/kg of body weight intraperitoneally. Histological changes observed in kidneys under the light microscope were similar to the ones described in the foetuses and mothers, however less intensified. In the foreground there were visible damaged renal glomerules – glomerulosclerosis. Walls of kidney's ducts were also damaged, in whose light there were observed the nuclei of damaged cells and little amounts of homogeneous secretion. To confirm the presence of NS in offspring features of NS in urine and blood should be investigated.

Odległe skutki działania adriamycyny – histologiczna ocena nerek noworodków szczura

Adriamycyna (ADR), antybiotyk p-nowotworowy, podana szczurom w dawce 5 mg/kgm.c. wywołuje już po czterech tygodniach pełnoobjawowy zespół nerczycowy (ZN). W poprzednich badaniach zwróciliśmy uwagę, iż zmiany histologiczne w nerkach samic szczura w przebiegu ZN po ADR obecne przed ciążą widoczne są również w nerkach płodów tych matek pod koniec ciąży. W obecnej pracy przebadane zostały histologicznie nerki dzieci (4 tyg. po urodzeniu) pochodzących od matek, które na 4 tygodnie przed planowaną ciążą otrzymały 5mg/kg m.c. adriamycyny dootrzewnowo. Zmiany histologiczne w nerkach obserwowane pod mikroskopem świetlnym były zbliżone do tych opisywanych u płodów i matek, jednakże mniej nasilone. Na pierwszy plan wysuwało się uszkodzenie kłębków nerkowych pod postacią glomerulosklerozy. Uszkodzone były również ściany kanalików, w których świetle obserwowano jądra uszkodzonych komórek i niewielkie ilości homogennej wydzieliny. W celu potwierdzenia obecności ZN u noworodków szczura należałoby ocenić wykładniki ZN w ich krwi i moczu.