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The influence of a single dose of adriamycin on fetal rat liver; histological and histochemical evaluation

Adriamycin, an antineoplastic antibiotic has already proved hepatotoxic activity (5). When it is given to adult individuals in therapeutical doses it causes changes typical of apoptosis, not only in neoplastic cells, but also in liver, where it is metabolized (1, 9).

The researches performed on animals show that Adriamycin (ADR) administered during pregnancy had strong teratogenic activity and caused plenty of congenital defects in the experimental animals. Especially prone to disorders is anterior gut, which includes the liver bud (7, 10). The influence of ADR given to female long time before planned pregnancy on her fetuses is not known.

In pregnant females there are noticed a lot of physiological differences, which affect pharmacodynamics of drug given during pregnancy: increased plasma volume, increased heart and kidney load, decreased blood pressure, the influence of changes in hormonal status. Metabolism and drug pharmacodynamics in pregnancy are also conditioned by fetal or mother blood circulation, and hormonal and enzymatic influence of placenta. Then the effect of fetus on drugs metabolism, and that of drugs on fetus – enzymatic insufficiencies, excretion of drug to amniotic fluid, secondary absorption of drug etc. That big complexity of this issue makes that in the end it is difficult to describe how drug acts on fetal or pregnant female organism.

Another problem is with drugs given before planned pregnancy. Part of these drugs is eliminated and their activity is stopped very quickly, others act and their side-effects are visible even very late. Adriamycin given intravenously is quickly eliminated from plasma and slowly discharged with urine and bile (14): 40–50% of the administered dose is excreted within 7 days with bile, and 5–15% of the administered dose – within 5 days with urine (1, 3).

The purpose of the present study is histological evaluation in light microscopy of changes in fetal liver of female rat individuals, which 4 weeks before fertilization were administered Adriamycin in the dose 5 mg/kg of body weight intraperitoneally.

MATERIAL AND METHODS

In the experimental group there were 16 rat's fetuses chosen randomly, two fetuses taken from each eight rat pregnant females, which 4 weeks before fertilization had been administered ADR in the dose 5 mg/kg of body weight intraperitoneally.

Analogically, for the control group there were randomly chosen 16 rat's fetuses, two fetuses from each eight rat pregnant females, which 4 weeks before fertilization had been administered 0.5 ml of 0.9% NaCl intraperitoneally.

After mothers decapitation (on the 20th day of pregnancy) fetuses were observed and counted. Fetuses chosen for further investigations were weighted, and after their decapitation liver sections were collected from them. Taken for histopathologic examinations liver sections were fixed in 10% formaline buffered to 7.4 pH (with phosphate buffer) and after dehydration in increasing concentrations of ethanol (40%, 50%, 60%, 70%, 80%, 90% "pure" alcohol) were lighted in xylene and embedded in paraffin.

Then preparations were cut into 5 μm slides, which were then stained with hematoxylin and eosin, according to Masson's method visualizing connective tissue and with Mc Manus' method of detection of neutral polysaccharides (PAS). Semithin slides were stained with 1% methylen blue and Azur II in 1% aqua solution of sodium tetraborate. The stained slides were observed in light microscopy. Preparations documentation was performed with Jenaval Contrast Carl Zeiss microscope. The body mass and the number of fetuses were subjected to a statistical analysis. The results were presented as mean values with standard deviation and were assessed statistically with t-student test. Five per cent risk of conclusion error and statistical significance of differences with $p<0.05$ was admitted. Evaluation of histopathological parameters was presented as a description.

RESULTS

Fetuses from the experimental group weighted on the 20th day of fetal life had comparatively to fetuses from the control group lower mass with statistical significance (mean–2.80 g and 3.57 g) (8). The mean number of fetuses in the control group was 10.00 and in the experimental group 6.12, the difference was statistically significant (8). Liver of fetuses from the experimental group observed macroscopically, fulfilled most of the abdominal cavity and did not vary significantly from liver of fetuses from the control group.

Preparations of liver of fetuses from the control group show a difference comparatively to liver of adult rats, which was due to liver embryogenesis. Not very frequent hematopoietic focuses with maternal blood cells were present. The liver was hyperemic, but it architecture was proper. In sinusal vessels lumen there were present blood cells, and among them some immatured forms (blasts). General architecture of parenchyma of rat's fetal liver from the experimental group was preserved. Indistinctness of liver parenchyma drowning and focal disintegration of liver trabeculas was observed (Fig. 1). Focal damage of hepatocytes was visible (lysis, "naked nuclei") and also pyknotic nuclei (Fig. 1).

Decreasing number of hematopoetic cells, and changes of their appearance – deformations, features of cell damages, "naked nuclei" were observed (Figs. 1, 3). Within parenchyma cell division figures were visible, which were the evidence of regeneration of liver parenchymatous cells. Comparatively to the control group swollen parenchyma and steatosis could be noticed.

In the region of central vein and in portocholangial spaces there were observed ductular cells with basophilic, PAS(-)negative cytoplasm and large nucleus. In preparations with PAS staining brighter cytoplasm with less intensive colour of glycogen granules comparatively to the control group were observed (Fig. 2).

In slides with staining according to Masson, a little amount of connective tissue in the region of central vein and portocholangial space was observed (Fig. 4).

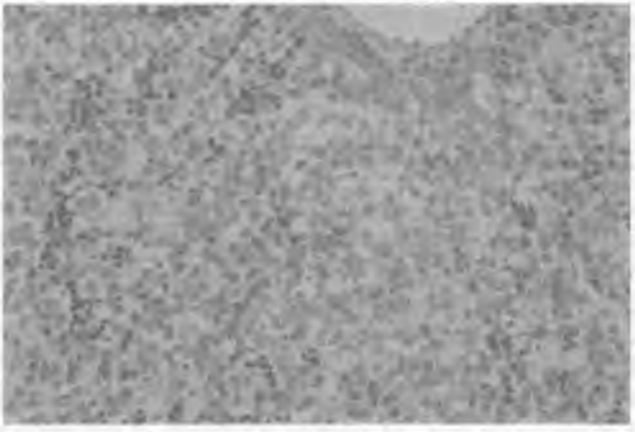

Fig. 1. Fetal liver section of rat from experimental group. H+E staining. Magn. 320x.

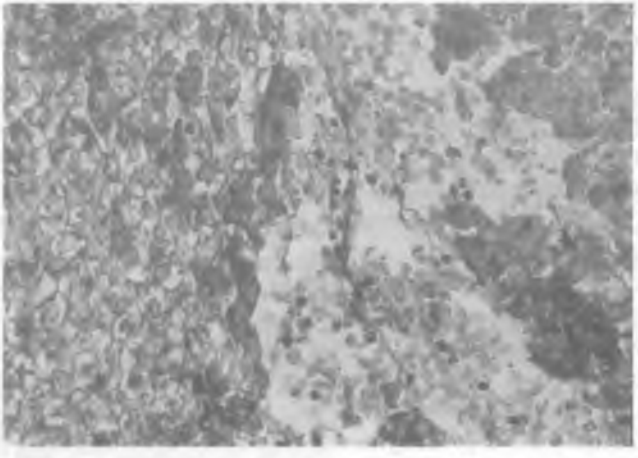

Fig. 2. Fetal liver section of rat from experimental group. Staining with PAS methods. Magn. 320x.

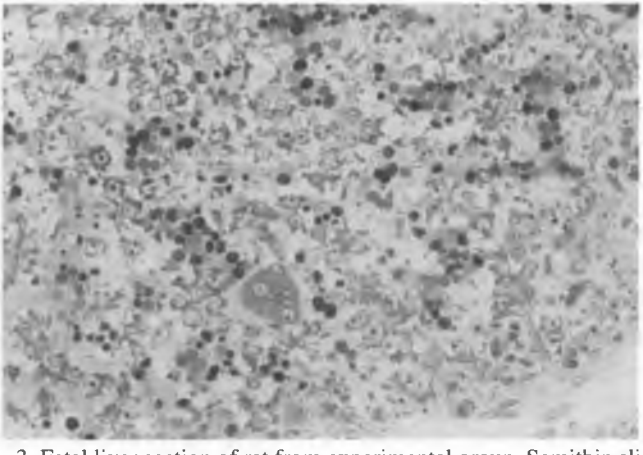

Fig. 3. Fetal liver section of rat from experimental group. Semithin slides.
Methylene blue and Azur II staining. Magn. 320x

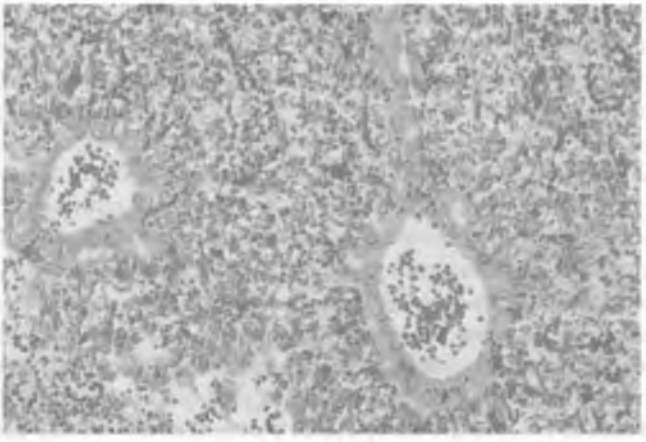

Fig. 4. Fetal liver section of rat from experimental group.
Staining according to Masson. Magn. 160x

DISCUSSION

Different chemical compounds administered the same way as drugs, and as other substances are metabolized in liver and could cause its morphological damage and also impairment of function. The kind and degree of hepatocyte's damage depends on (among others): the kind of drug, its metabolic pathway, way of action and way of administration.

In the literature there could be found reports about liver disorders after ADR given both to adult persons (6) and to experimental animals (4). The influence of ADR on next generations born from mothers which had been treated with ADR in the past has not been reported.

ADR in the present study was given to rat mothers 4 weeks before planned pregnancy. Because pregnancy in rats lasts about 3 weeks, 4 weeks corresponds to about 1 year in human. One year is long enough after finished ADR therapy, for a woman to decide to have a baby.

From 45 to 65% of the administered ADR dose is eliminated within 7 days, half of that unchanged, and half as metabolites (1, 3). ADR is metabolized mainly in liver. In ADR transformation process arise free radicals, which have cytotoxic and hepatotoxic activities (15).

It was shown that Adriamycin decreased tissue respiration, oxidative phosphorilation and mitochondrial ATP-ase activity. According to some authors these changes are due to disorders of physico-chemical status of mitochondrial membrane (2). Adriamycin induces also decrease of cytochrome P450 and oxidase activity (13), and also decrease of oxidative enzymes in mouse liver, which had administered Adriamycin and lipids peroxidation. It suggests that hepatotoxic activity of Adriamycin is connected with decreasing antioxidative protection (12).

The dose 5 mg/kg of body weight given in this study is known in the literature (9, 15), and it let bear alive offsprings. In antineoplastic therapy in human there is used a dose of about 2 mg/kg of body weight several times to obtain a cumulative dose of about 14 mg/kg of body weight (11).

In experimental group fetal liver slides there were observed changes in the number of hematopoietic cells (decrease) and features of their damage. These changes could be explained with the strongest toxic anthracyclines activity on quick proliferating cells, among which hematopoetic cells were present. On the other hand, numerous cell division figures are the evidence of larger regeneration potential of fetal cells, which is understandable. The loss of cytoplasm basophilia and

the presence of numerous small granules corresponding to enlarged mitochondria, visible in light microscopy, are the evidence of rough endoplasmic reticulum damage.

Toxic liver damage progresses in stages, and according to changes in cell organelles we can conclude about the degree of cell damage. In cells of liver, as a detoxication organ, the longest activity have organelles which have detoxication function. It happens that some organelles convert into those whose function is detoxication – for example endoplasmic reticulum. One of the first signs of cell damage is the loss of ribosomes in rough endoplasmic reticulum. That reticulum is transforming into smooth endoplasmic reticulum, which has detoxication capacity. Changes in these organelles should be observed in electron microscopy. The enlarging of mitochondria and damage of reticulum without doubt are the evidence of liver cells damage.

The results of our study suggest that in the body of mother, ADR or its metabolites could be still present long time after finished treatment. It is also probable that free radicals, which arise during ADR biotransformation act also hepatotoxically in the body of fetus. Fetal liver was examined one day before delivery. Fetuses were already almost prepared to extrauteral life, which suggests that changes observed in the present study could remain also after delivery. If these changes could influence liver function, it would be checked after assessment of ALAT, AspAT, bilirubin and other factors in newborn blood. In the experimental group, the number of fetuses in the litter was significantly lower and their size was smaller than in the control group. It is possible that histological changes arisen in fetal rat liver following ADR administration to rat mothers could appear also in other organs.

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SUMMARY

The purpose of the study was the histological assessment of the liver of fetuses coming from female rats, which 4 weeks before planned pregnancy had been administered Adriamycin in a single dose intraperitoneally. On preparations stained with H+E, with PAS method, and according to Masson and on semi-thin slides there were observed damaged hepatocytes (lysis, "naked nuclei", pycnotic nuclei), decreased number of hematopoietic cells, swollen parenchyma, steatosis. Results showed that Adriamycin given to mother before pregnancy acts cytotoxically also on fetal hepatocytes.

Wpływ jednej dawki adriamycyny na wątrobę płodową szczura – ocena histologiczna i histochemiczna

Celem pracy była histologiczna ocena nerek płodów szczura pochodzących od matek, które cztery tygodnie przed planowaną ciążą dostały jednorazowo dootrzewnowo adriamycynę. Na preparatach barwionych H+E, metodą PAS, metodą wg Massona oraz na preparatach półcienkich obserwowano uszkodzone hepatocyty (lizę, „nagie jądra", piknotyczne jądra), obniżoną ilość komórek hematopoetycznych, stłuszczenie. Wyniki badań wskazują na to, iż adriamycyna podana matce przed ciążą działa cytotoksycznie również na hepatocyty płodowe.