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The influence of a single dose of adriamycin on the pregnant rat female liver – histological and histochemical evaluation

The pregnancy is the physiological period for woman's organism but it predisposes to different diseases, not described in not pregnant women. Apart from the circulatory system, which in pregnant rats takes a part of nutrifying mother and fetus, so the heart muscle has to pomp increased blood volume; other organs like liver and kidney also carry on increased work. For example, if a patient used earlier estrogen preparation, then just liver of a pregnant woman is prone to recurrent cholestasis, which occurred in trimester III of pregnancy. Another danger for pregnant female liver is e.g., idiopathic acute liver steatosis with unexplained till now etiology, probably connected with tetracyclines administration. In microscopic investigation of liver sections, the features of hepatocytes necrosis are rare, but hepatocytes from the central part of hepatic lobule contain small vesicles with fat, mainly fatty acids (in other types of liver steatosis triglycerides are accumulated). The mortality rate of mother and fetus is very high.

In the present paper there was histologically evaluated the liver of pregnant rats which four weeks before fertilization were administered adriamycin in a single dose of 5 mg/kg of body weight. It is known from literature (10) that such doses induce numerous changes in adult rat liver without causing its insufficiency.

MATERIAL AND METHODS

In the experiment there were used 16 white female Wistar rats, 200–250 g in weight; 2.5 to 3 months old. The animals were divided into two groups: experimental and control – 8 females in each. At the very beginning of the experiment females from the experimental group were administered adriamycin in a single dose 5 mg/kg of body weight intraperitoneally, and females from the control group were administered 0.5 ml of 0.9% NaCl as well, in a single dose, intraperitoneally.

After four weeks females were paired with males. Pregnant females were decapitated on 20th day of pregnancy. From all animals were collected sections from the right lobe of the liver, which after macroscopical observation were fixed in 10% buffered formalin. Then after dehydration in increasing concentration of alcohol the sections were brighted in xylene and then embedded in paraffin blocks. Paraffin blocks were cut into 5μm thick sections, and then were stained with hematoxylin and eosin, by the Masson's method imaging connective tissue and by the Mc Mannus method for detecting natural polysaccharides – PAS. The stained sections were observed in light microscopy. Documentation of preparations was made with Jenaval Contrast Carl Zeiss microscope.

Part of liver sections were fixed in fixation fluid containing 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M Sorensen phosphate buffer. Then they were treated with osmium tetraoxide,

stained with uranyl acetate, dehydrated in increasing ethanol concentration and embedded in Aralchit ACM Fliska resin. Preparation were cut into 0.5-0.7 μm thick sections. The performed in that way semi-thin slides were stained with 1% methylene blue with Azur II in 1% water solution of sodium tetraborate. Documentation of preparations was made with Jenaval Contrast optic microscope.

RESULTS

During the experiment the animals from the experimental group showed appetite, thirst and motility similarly to animals from the control group. Body mass of pregnant female rats increased significantly both in the experimental and control groups comparatively to initial body mass, but females from the experimental group weighted on 20th day of pregnancy statistically significantly less (mean 364.5+/-42.1g) than females from the control group (mean 395.5+/-8.2) ($p = 0.049$).

The liver of animals from the control group was pink, smooth, with sharp edges. After decapitation of rats from the experimental group and after opening of the abdominal cavity and thoracic cavity, subcutaneous, retroperitoneal space, liver and kidney oedemas were visible as well as exudates to cavities. The liver was enlarged comparatively to rats' liver from the control group, had rounded lower edge and even a little nodulous surface. In histological preparations females from the control group showed evident indistinctness of contours of parenchymatous cells, focal disintegration architectonics in shape and size of hepatocytes, and different size of nuclei, mainly more marked, than in the control group (Fig. 1). Nuclear chromatin showed the evidence of scattering. Very often an increased number of nucleoli was observed.

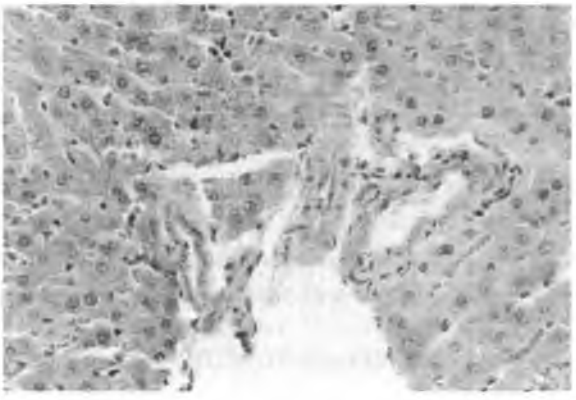

Fig. 1. Section of liver of a rat from the experimental group. Hematoxylin and eosin staining. Magn. 320x

The hepatocytes cytoplasm in hematoxylin and eosin staining was brighter comparatively to the control group. Lack of cytoplasmic granules or small granules and more acidophilic cytoplasm were observed (Fig. 1). It was visible in methylene blue and Azur II staining as well (Fig. 3). Narrowed sinus light was observed, and number of Browicz cells was increased and their size was enlarged.

Focal necrosis, defragmentation of hepatic trabecules and "naked nuclei" were observed, which was the evidence of cell degradation. Visible fat droplets were the evidence of significant steatosis of the liver. Blood vessel damage causing parenchymal hyperaemia (there was observed erythrocytes penetration into liver parenchyma) (Fig. 1). Around the central vein and portocholangial space appeared widened and long tubules with irregular shape (Figs. 1, 4) which had well visible lumen. Tubules accummulated around lamina border, its cells had light basophile PAS(-) negative cytoplasm and a big nucleus. Tubules produce PAS(+) positive substance. Tubules were often surrounded by inflammative infiltrations with granulocytes prevalence, especially accumulated near PAS(+) positive masses. If the tubule was more mature, then the granulocytes infiltrations were smaller, but the amount of connective

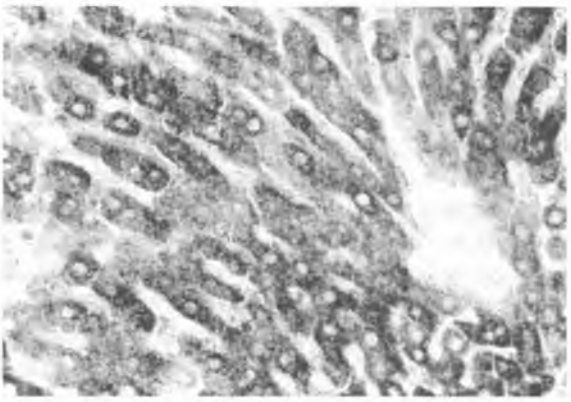

Fig. 2. Section of liver of a rat from the experimental group. PAS staining. Magn. 320x

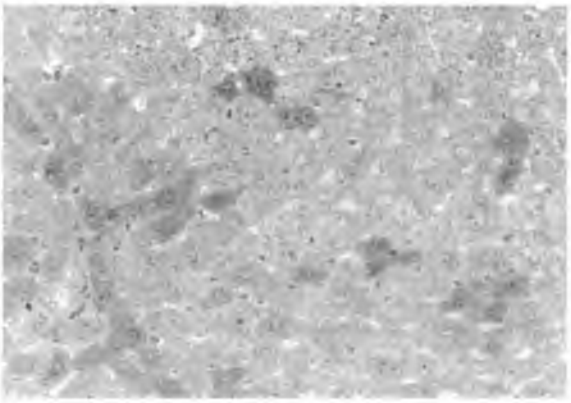

Fig. 3. Section of liver of rat from the experimental group. Semi-thin slides. Methylene blue with Azur II staining. Magn. 320x

tissue increased (which was especially visible in staining according to Masson) – Fig. 4. In PAS-stained preparations there was observed a decrease of glycogen granules comparatively to the control group, and bright PAS(-) negative tubular cells with PAS(+) positive substance secreted into tubular lumen (Fig. 2).

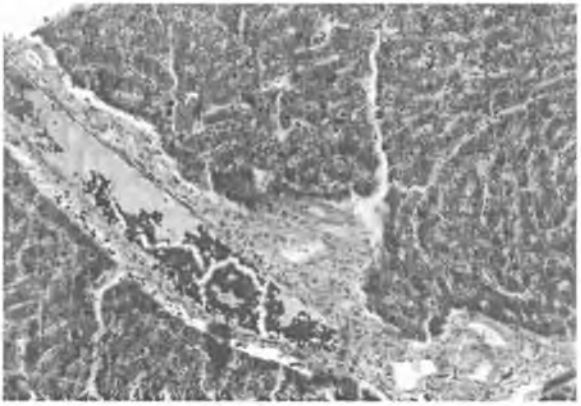

Fig. 4. Section of liver of a rat from the experimental group. Staining according to Masson. Magn. 320x

DISCUSSION

Because of numerous metabolic pathways the liver is especially exposed to toxic activity of exogenous substances including drugs.

As a result of their activity all cells or different parts of cells could be damaged (5, 8). Histopathological changes arising in the liver after using exogenous toxic compounds are most often expressed in parenchymatous degeneration, vacuolar degeneration, and hepatocytes necrosis, which was visible in the present study, as well as inducing of Browicz cells or infiltrations in portocholangial spaces (2). Different cell organelles show different sensitivities to toxic compounds activities. Detoxication in the liver takes place mainly in hepatocytes microsomes. Biotransformation process of toxic substances is placed in hepatocyte endoplasmic reticulum whose vacuolisation is observed in light microscopy as a vacuolar degeneration (3, 6, 9). With the development of smooth endoplasmic reticulum in hepatocytes the amount of glycogen granules in the liver decreased, which was observed in the present study. Changes caused by the drug could appear with the same intensity in all lobules or in some lobular zones. It depends on the kind of substance absorbed. It should be added that the cytomorphological evaluation of the liver is a difficult issue. It is due to big regeneration capacities of the liver (1).

Another morphological change observed in the present study is tubular proliferation. It is typical of several liver pathologies in humans and in experimental animals (for example, after etionine, tioacetamide and others). The most frequent reason of tubular proliferation is inflammation of the liver, cirrhosis hepatis, especially after liver necrosis, and after extra- or intrahepatic cholestasis or in toxic damage of the liver (7).

Tubular epithelial cells proliferated very quickly (frequent mitosis) and longitude of their life is about two weeks, and in hepatocytes – about 300 days. Around proliferated tubules very often accumulated inflammative infiltrations, which were especially gathered around PAS(+) positive substances close to tubules. When tubules (*ductules spuriae*) were more mature, inflammative reaction decreased but increased fibrosis, which was especially clearly visible in preparations stained according to the Masson method.

Observed in the present study liver steatosis had features of small droplet one (accumulation of small droplets of fat into cytoplasm). Such changes were described in cases of acute steatosis of the liver in pregnancy and in children with encephalopathy. Such steatosis very often causes liver insufficiency, because it touches most of hepatocytes. It seems that the main reason of steatosis observed in the present study is ischaemic shock induced by adriamycin, which suppresses oxidation of enzymes (succinate dehydrogenase and NADH oxidase) (4), which in effect reduces lipids oxidation and their accumulation in the liver.

Disorder in protein translation by adriamycin, especially proteins which connect with lipids forming lipoproteins – because only in such form lipids can be eliminated from hepatocytes – can as well be a reason of lipids acumulation in the liver.

Liver vessel damage and penetration of erythrocytes to liver parenchyme visible in preparations from the experimental group are the evidence of disintegration of liver architectonics similar to that which appears in cirrhosis hepatic after toxic damage, including portal hypertension typical in cirrhosis. Erythrocytes present in hepatocytes cytoplasm are the evidence of cell membrane damage.

REFERENCES

1. Bartel H. et al.: Ultrastructure of hepatocyte in regenerating rat liver. Folia Morph., 4, 367, 1977.
2. Brzozowski R.: Toksyczne uszkodzenie wątroby. Pol. Arch. Med. Wew., 61, 331, 1979.

3. Danilewicz M.: Zmiany patomorfologiczne w wątrobach szczurów zatrutych siarczanem talowym w różnych dawkach. Pat. Pol., 1, 89, 1981.
4. Drzewoski J., Matusewicz W.: Farmakologia kliniczna Doksorubicyny (adriamycyny). Pol. Tyg. Lek., 37, 1071, 1982.
5. Grzybek H.: Badania ultrastrukturalne i histoenzymatyczne wątroby i nerek w doświadczalnym zatruciu octanem ołowiu. Pat. Pol., 1-2, 51, 1982.
6. Hagel-Lewicka E., Lewicki Z.: Ultrastructural investigations of the liver in young (suckling) rats receiving tetracycline hydrochloride. Mat. Med. Pol., 12, 259, 1980.
7. Pedrycz A. et al.: Pseudoductules in rat liver in experimental adriamycin induced nephrotic syndrome. Annales UMCS, sect. D, 57 N 1, 2002.
8. Simon K.: Badania nad wpływem kolchicyny na przebieg doświadczalnego, przewlekłego uszkodzenia wątroby u szczurów rasy Buffalo. Praca doktorska. Akademia Medyczna we Wrocławiu, 1993.
9. Vainio H.: On the topology and synthesis of drug-metabolizing enzymes in hepatic endoplasmic reticulum. Academic Dissertation, 5, Turku 1973.
10. Wieczorski M.: Skutki jednorazowego podania adriamycyny na wątrobę płodową i dojrzałą szczura – ocena zmian cytomorfologicznych. Praca doktorska, Akademia Medyczna w Lublinie, 2003.

SUMMARY

The purpose of the study was histological evaluation of the liver of a pregnant rat, which 4 weeks before planned pregnancy was administered adriamycin intraperitoneally in a dose of 5 mg/kg of body weight. Focal damage of hepatocytes ("naked nuclei" which were the evidence of cell damage) were observed in histological preparations. Significant steatosis and vessel damage were visible as well. Around the central vein and in portocholangial space numerous *ductules spuriae* appear.

Wpływ jednej dawki ADR na wątrobę ciężarnej samicy szczura – ocena histologiczna i histochemiczna

Celem pracy była ocena histologiczna wątroby samicy ciężarnej szczura, której na cztery tygodnie przed planowaną ciążą podano dootrzewnowo 5 mg/ kg m.c. adriamycyny. W preparatach histologicznych obserwowano ogniskowe zniszczenie hepatocytów (nagie jądra świadczące o rozpadzie komórek), widoczne było znaczne stłuszczenie oraz uszkodzenie naczyń. Wokół żyły środkowej i w przestrzeni bramnożółciowej pojawiły się liczne pseudokanaliki.