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Changes in rat liver after single dose of Adriamycin. Histological, histochemical and biochemical evaluation

Adriamycin is an anthracycline antibiotic used in antineoplastic treatment (1, 2, 4, 5, 6, 11, 12, 15). For cytotoxic activity of this antibiotic are probably responsible free radicals (semichinon) which are created in that drug transformation processes. (1, 2, 18). Adriamycin given in intravenous injection is quickly eliminated from plasma and secreted with bile and urine (6, 17). Liver is a main way of drug elimination. Mean 40–50% of a given dose of adriamycin is eliminated with bile during 7 days from drug administration (1, 2, 4, 15). Drug molecules degeneration runs in stages, the last of which is demethilation, sulfuration and conjugation with glucurenic acid (1, 2, 16). The liver is an organ which main function in detoxication, and because of that, is the most prone to toxic activity of drugs.

MATERIAL AND METHODS

In experiment 16 rats were used. The animals were randomly chosen from group of female Wistar rats aged 2.5–3 months with body mass from 200 to 250g. Females from experimental group had administered adriamycin in the dose of 5 mg/kg of body weight, intraperitoneally (8 individuals) and females from control group had administered also intraperitoneally 0.5 ml 0.9% NaCl.

Liver sections were collected 4 weeks after drug administration and after proper preparation (they were dehydrated in increasing concentrations of alcohols, in xylene, and embedded in the paraffin blocks) were stained with hematoxylin and eosin, with method according Masson, McMannus (PAS) and semithin slides stained with methylen blue and Azur II.

From heart was collected blood for determination of AlAT, AspAT, bilirubin. Preparations were observed in light microscopy. Photo documentation was performed with Jenaval Contrast Carl Zeiss camera.

RESULTS

After decapitation of rats from experimental group and after opening the abdominal cavity and the thoracic cavity, there was observed swollen subcutanous tissue, retroperitoneal tissue, swollen liver and kidneys, transudates to body cavities, enlarged liver with rounded lower edge and a little tuberous surface.

In histological preparation of liver of female rats from experimental group there were observed indistinctness of parenchymatous cells contours and focal disintegration of hepatocyte architectonics (Fig. 1, 4) different size of their nuclei, nuclear chromatin with features of

dispersion, focally increased number of nucleoli. Hepatocyte cytoplasm in hematoxylin and eosin staining was bright, without cytoplasmic granules or microglanular acidophils (Fig. 1, 3). There was also observed a narrowed sinusal lumen an increased number and size of Browicz-Kupffer cells, focuses of necrosis, defragmentation of liver trabecules and "naked nuclei" which were the evidence of cell degeneration, fat droplets which were the evidence of significant steatosis, vessel damage and connected with that parenchymal hyperaemia (Fig. 1). In the region of central vein and in portochongial spaces appeared widen and long cholangial ductules irregularly shaped (Fig. 2, 3). If the ductule was more mature, then surrounding granulocytes infiltration was smaller, but the amount of connective tissue was then increased, which was especially visible in staining according to Masson (Fig. 4).

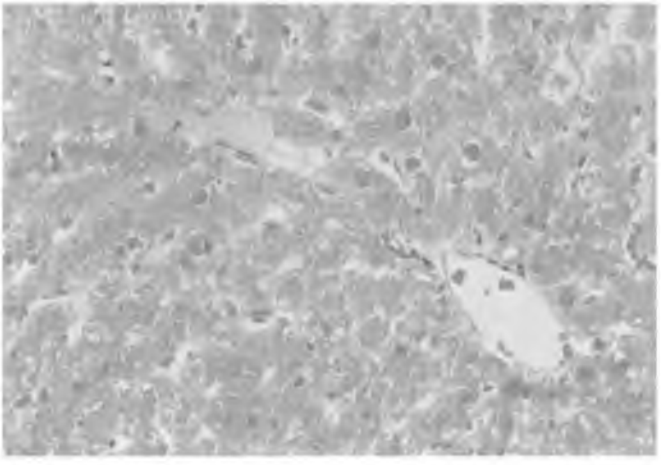

Fig. 1. Section of liver of rat from experimental group (female rat 4 week after administration adriamycin). Hematoxilin and eosin staining. Magn. 400x

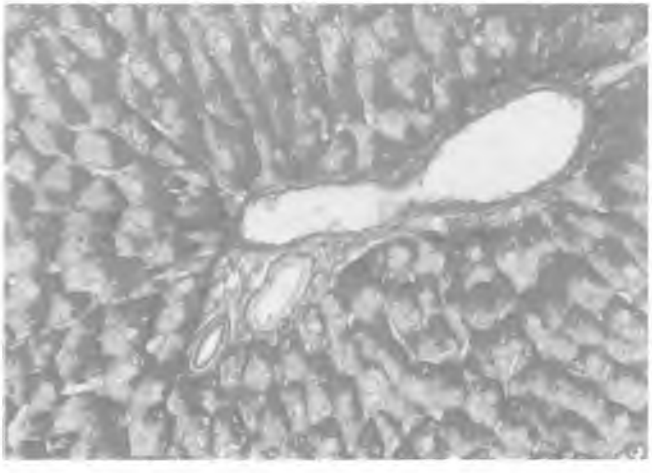

Fig. 2. Section of liver of rat from experimental group (female rat 4 week after administration adriamycin). Staining with PAS method. Magn 400x

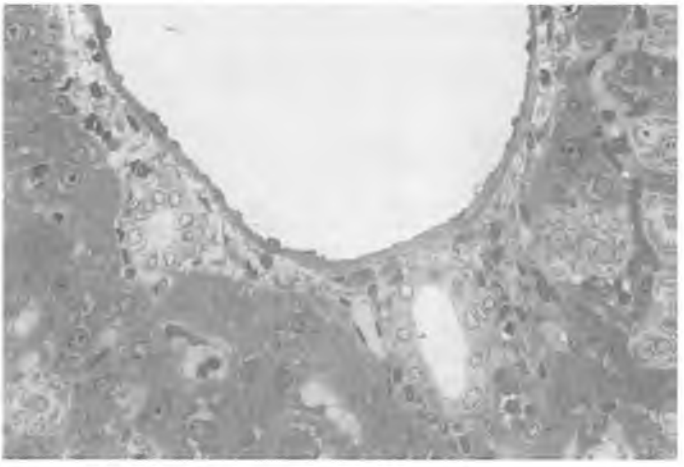

Fig. 3. Section of liver of rat from experimental group (female rat 4 week after adriamycin administration). Semithin slide. Methyled blue and Azur II staining. Magn 400x

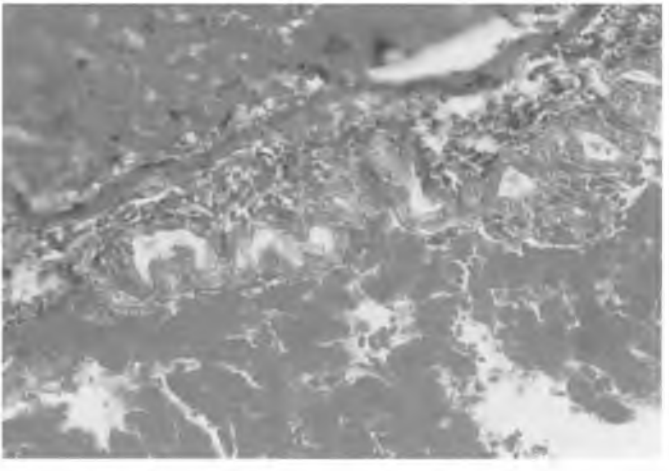

Fig. 4. Section of liver of rat from experimental group (female rat 4 week after administration adriamycin). Staining according Masson. Magn 400x

In preparation with PAS staining there was observed an evident decrease of glicogen granules, bright PAS(-)negative ductular cells with PAS(+) positive substances secreted into ductular lumen (Fig. 2). Mean bilirubin, Alat, Aspat concentrations increased with statistical significance in experimental group as compared to control group (Tab. 1).

Tab. 1 Bilirubin, Alat, Aspat concentrations in blood serum

	Bilirubin (MG%)		AlAT (u)		AspAT (u)	
Group	exp.	contr.	exp.	contr.	exp.	contr.
Mean	0.36	0.15	93.25	79.00	236.63	152.13
Standard deviation	+\-0.09	+\-0.04	+\-10.39	+\-7.23	+\-100.19	+\-27.34
Statistical significance	0.004		0.008		0.055	
T-student test	4.999		3.184		2.247	

DISCUSSION

In liver there are metabolised different chemical compounds and among others – drugs. Drug can cause impairment of liver function and morphological damage. Adriamycin – an antibiotic used in anticancer treatment is metabolised mainly in liver. In literature there could be found reports about liver damaged after administration of adriamycin in lab animals, described by investigators using big doses of a drug or small doses given several times (6–10). There are significantly less reports about liver damage after adriamycin in human (3, 13, 14). A dose given to rats in the present study (5 mg/kg of body weight) is described in literature as the one which causes organs damage in experimental animals but is not the cause of their insufficiency (16). It is similar to the dose administered to human in antineoplasm treatment. It enables observation of the earliest, small changes in liver caused by that drug. The description of those changes could be helpful in answering another question: how to prevent liver damage during adriamycin treatment.

Observed in the present study liver steatosis had microdroplet character. That kind of steatosis was described in case of acute steatosis, acute yellow atrophia of liver in pregnancy and in case of children with encephalopathy. Mostly that steatosis affected most of hepatocytes causing liver insufficiency. The presence of erythrocytes in hepatocytes, cytoplasm visible in the present study is the evidence of cell membrane damaged, and vessel damage and liver parenchymal hyperaemia are the evidence of disintegration of architectonics similar to that which appears in cirrhosis of the liver after toxical damage and in portal hypertension typical of cirrhosis.

Ductular proliferation described in the present study is possible in hepatitis, in cirrhosis (especially after necrosis), in cholestasis and in chronic toxic liver damage. In the present study could be found also changes which are the evidence of apoptosis in hepatocytes. They included: pyknotic cell nuclei with chromatin condensed circumpherentially, endoplasmic membranes damage without inflammatory reaction.

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SUMMARY

The purpose of the present study was histological evaluation of mature liver of rat after exposition to a single dose of antineoplastic antibiotic. Changes visible in hepatocytes had features of apoptotic changes. Ductular proliferation, liver small droplets steatosis, liver vessels damage, transudation red blood cells into liver parenchyma and into hepatocytes which had cell membrane focally damaged was also observed.

Histologiczne, histochemiczne i biochemiczne zmiany w wątrobie szczura po adriamycynie

Celem pracy była ocena histologiczna wątroby szczura poddanego działaniu pojedynczej dawki antybiotyku przeciwnowotworowego. Zmiany widoczne w hepatocytach miały charakter zmian apoptotycznych. Obserwowano również rozrost kanalików, kropelki tłuszczu, zniszczenie naczyń wątrobowych, obecność krwinek czerwonych w miąższu wątroby i w cytoplazmie hepatocytów, które miały ogniskowo zniszczoną błonę komórkową.