ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 2, 154 SECTIO D 2004

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Increased apoptosis in the adult rat liver after a single dose of adriamycin administration

Apoptosis – ([Gr.] – dropping of the leaves) is the natural death of cells which takes place via activation of genes, which produce kill cells. That process is starting only when cells are not necessary, damaged or they do not obtain external stimuli. During a few hours, when apoptosis process in the nucleus and cytoplasm becomes condensed, the killed cell is defragmented with creating surrounded by membranes apoptotic bodies, which then are phagocytized via necrophages or other cells without induction of inflammation. It is a way of quick removing of dead cells, without exposing on toxins secreting from them. Cellular death via apoptosis takes place mainly in single cells, opposite to death due to toxic or pathogenic factors, where a lot of cells are killed (necrosis).

The evaluation of liver cytomorphology is a difficult issue. It is due to, among others, big capacities of the liver for regeneration (2). Changes due to drugs could appear with the same intensity in all hepatocytes of lobule or in some of its zones. It depends on the type of resorbed substance. Histopathological changes in the liver due to external toxins are expressed in parenchymatous degeneration, vacuolar acidophilic or adipose degeneration, as well as necrosis of hepatocytes, activating of Browicz cells, or infiltrations in portocholangial ducts (3).

In the present study were evaluated features of the natural death of hepatocytes of adult rats, after a toxic factor – adriamycin had been administered in a single dose of 5 mg/kg of body weight four weeks before histological investigation.

MATERIAL AND METHODS

In the experiment 16 female Wistar rats were used. Animals were divided into two groups: experimental and control. Animals were administered adriamycin intraperitoneally in a dose of 5 mg/kg of body weight. In control group animals were administered 0.5 ml of 0.9% NaCl i.p. Animals were decapitated after four weeks. For lab investigation blood from the heart was collected for determination of bilirubin, ALAT and AspAT.

Sections taken from the liver for further investigation were fixed in fixation fluid which consisted of: 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M phosphate Sorensen buffer. Then it was treated with osmium tetraoxide, stained in uranyl acetate and dehydrated in increasing concentrations of ethanol and embedded in Aralchit ACM Fliska resin. Preparations were cut into 0.5–0.7 μm thick sections and 60 nm ultrathin sections with ultramicrotom Reichert Ultracut S.

Ultrathin sections were stained with 8% solution of uranyl acetate and lead citrate according to Raynolds. Electron microscopy documentation was made with Tesla BS-500 microscopy.

RESULTS

Table 1. Results of biochemical investigations

	Bilirubin		AlAT		AspAT	
Group	control	experimental	control	experimental	control	experimental
Mean value	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	
Stud. T test	4.999		3.184		2.247	

A statistically significant increase of bilirubin, AlAt, AspAT in the animal experimental group in comparison with the control group was observed.

The picture of hepatocytes from the experimental group in electron microscopy revealed features of significant focal damage. Numerous fat drops in cytoplasm which were evidence of steatosis were observed (Fig. 1). In some cells "wash out" cytoplasm, without organellas and "empty" cells were observed. Rarely cell lysis and indistinctness in cell structures were observed, which is the feature of necrosis. In cytoplasm small droplet degeneration was visible. Mitochondrias, whose number was decreased were very often swollen with bright matrix and partial destruction of crests (Figs. 2, 3). Some mitochondria had external membrane ruptured, and their contents fill out to cytoplasm. In some damaged mitochondria creation of myeline structures could be observed.

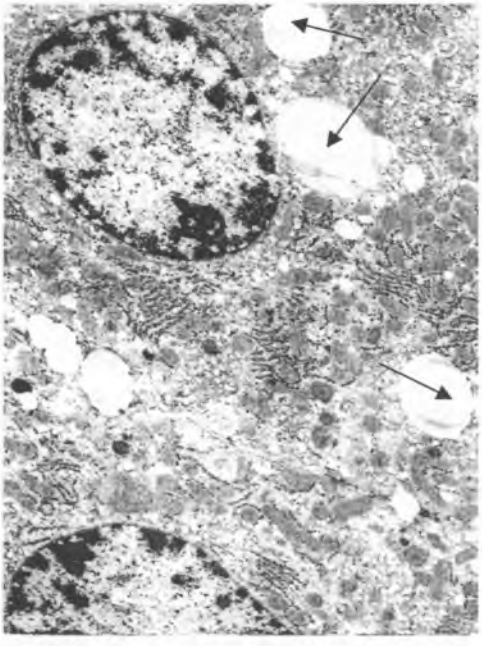

Fig. 1. Electronogram of hepatocytes of a rat from experimental group (4 weeks after adriamycin administration). Features of steatosis present – fat droplets (arrows). Magn. 3500 x

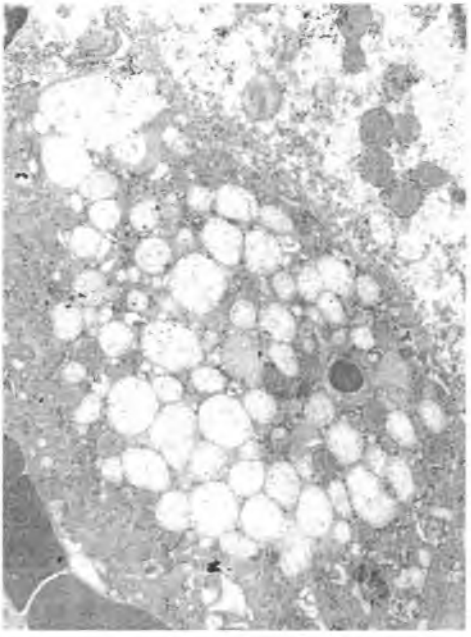

Fig. 2. Electronogram of hepatocytes of a rat from experimental group (4 weeks after adriamycin administration). Swollen mitochondria with brightening of matrix and partial crests destruction. Magn. 3500 x

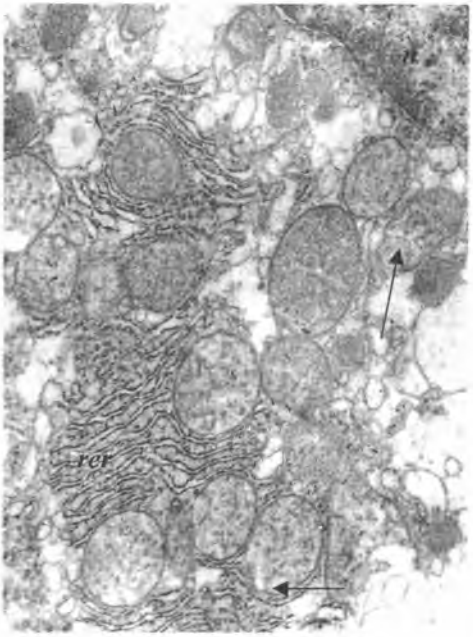

Fig. 3. Electronogram of hepatocytes of a rat from experimental group (4 weeks after adriamycin administration). Swollen mitochondria. Myelin figures visible (arrows). widened perinuclear space. Magn. 8400 x

Hepatocytes nuclei showed different shape and size. In some cells picnotic nuclei with chromatin circurpherentially condensed were observed which were the evidence of apoptosis. In some nuclei evident vacuolisation of nuclei visible. Rough endoplasmic reticulum was often defragmented, especially in the mitochondrial region. The amount of glycogen was decreased. Irregular location of increased numbers of lysosomes was observed. In the region of cholangial tubules numerous autophagosomes were visible.

Comparatively to control group the amount of peroxisomes in hepatocytes was increased (Figs. 1, 4). In vascular endothelium significant focal cell damage was visible. Perisinusal spaces were often widened and swollen, containing cytoplasm of damaged hepatocytes. Connective tissue proliferation was also visible. Erythrocytes present in hepatocyte cytoplasm were another evidence of cell membrane damage (Fig. 4). In the region of central veins and close to portochongial space numerous cholangial ductules were observed. Cells building these ductular wall had bright cytoplasm with a small amount of organellas, big circle nucleus with chromatin condensed circumferentially. In tubular sections, five to eight cells were observed.

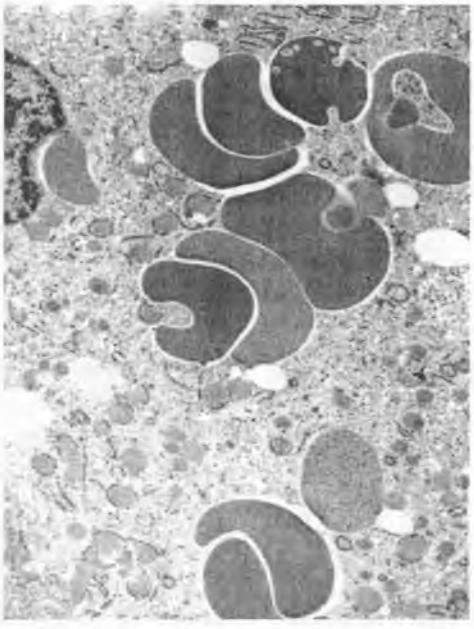

Fig. 4. Electronogram of hepatocytes of a rat from experimental group (4 weeks after adriamycin administration). Erythrocytes in hepatocyte cytoplasm are the evidence of hepatocyte membrane damage, and consequently cell lethal damage. Magn. 2800 x

DISCUSSION

The research on adriamycin influence on individuals in different age showed that older individuals (rats more than 18 months old) had increased oxidative damages (especially lipid peroxidation) comparatively to adult individuals (rats 10 months old) and young ones (rats 2 months old) (1, 11). Lethal dose in young individuals was two times bigger than in old individuals (4). Because of that in the present study were used young individuals – rats at the beginning of experiment were 2.5–3 months old.

The dose of adriamycin in the experiment was chosen in the way that a single dose should induce organ changes, without their insufficiency. According to data from literature in the experiment was used a dose of 5 mg/kg of body weight given peritoneally (9).

In the present study a lot of hepatocytes disclose all lesions including nucleus and cytoplasm condensation. Changes in cell organellas preparing hepatocyte for detoxication function were also observed.

The observed in the present study rough endoplasmic reticulum degranulation and widening of canals were probably due to, according to some authors (10), decreased protein synthesis and secretion in hepatocytes.

Widening of spaces in endoplasmic reticulum could be a sign of vacuolar degeneration (5, 6, 13, 14). These changes could be as well due to glucogenolysis, and it was stated that the numbers of glycogen granules was decreased.

Often the cause of widening smooth endoplasmic reticulum even through decrease in rough endoplasmic reticulum are detoxication processes (7, 12, 14) and hypoxia. In case of adriamycin we could talk about toxic and ischaemic activity ("ischaemic shock").

The increased numbers of lysosomes and the appearance of autophagosomes are probably the result of hepatocytes damage, similar to the influence of other pathogenic factors (8, 12). Peroxisomes,

also called microbodies, are vesicles with diameter 0.15-0.5 μm, surrounded by membrane. Inside they contain homogeneous material of small electron density and crystal structure, the so-called core localised centrally. Peroxisomes play an important part in metabolics and xenobiotics detoxications via oxidation. Peroxisomes are created from smoth endoplasmic reticulum and their enzymes are synthetized in rough reticulum. In the present study a significant increase of peroxisomes numbers was observed in the experimental group after adriamycin administration, which is the evidence of its activity in detoxication processes. The increased number of peroxisomes to 200–400% in the heart after adriamycin administration was described by Zipper (15), as a cell reaction to oxidative stress.

The presented above morphological changes, in tissue, cell and ultrastructural level are the evidence of persistent and irreversible damage of liver tissue via adriamycin. Cells damaged in that way waste away. In the present study cell death touched mainly single cells and necrosis was not observed. It is the evidence of apoptosis – the natural death of cells.

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SUMMARY

The purpose of this study was the evaluation of hepatocytes in the adult rat's liver after adriamycin administration in a single dose. The presented above morphological changes, on tissue, cell and ultrastructural level are the evidence of persistent and irreversible damage of liver tissue via adriamycin. Cells damaged in that way waste away. In the present study cell death touched mainly single cells and necrosis was not observed. It is the evidence of apoptosis – the natural death of cells.

Wzmożona apoptoza w dojrzałej wątrobie szczura po jednorazowej dawce adriamycyny

Celem pracy jest ocena hepatocytów wątroby dorosłego szczura po podaniu w pojedynczej dawce adriamycyny. Prezentowane zmiany morfologiczne na poziomie tkankowym, jak też komórkowym i ultrastrukturalnym są dowodem trwałego i nieodwracalnego uszkodzenia tkanki wątrobowej przez adriamycynę. Tak uszkodzone komórki w konsekwencji obumierają. Śmierć komórki dotyczyła w doświadczeniu pojedynczych komórek i nie obserwowano martwicy. Świadczy to o apoptozie – naturalnej śmierci komórki.