PATHOMORPHOLOGY OF HEPATOCYTES OF RATS TREATED WITH ADRIAMYCIN

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Abstract

The aim of this study was ultrastructural and light microscopical assessment of the adriamycin-induced apoptosis in rats’ hepatocytes. The studied material consisted of Wistar rats divided into four equal groups: two experimental - rats treated with a single intraperitoneal dose of adriamycin (5 mg/kg b.w.) and decapitated after 4 and 7 weeks; and two control - rats treated with a single intraperitoneal dose of 0.9% NaCl (0.5 ml) and decapitated after 4 and 7 weeks. Specimens of the liver were observed under light and electron microscopes (semi thin slides). Typical apoptotic lesions were observed. The applied dose of adriamycin seems to be suitable to induce the cell death through apoptosis and too small to cause necrosis.

Key words: rats, hepatocytes, adriamycin, apoptosis, pathomorphology.

The metabolism of adriamycin or its metabolites occurs in the liver where it is degraded and finally combined with glucuronic acid. Adriamycin is mainly excreted with the bile (40%–50% of the dose administered) partly in the form of metabolites and partly unchanged (5, 11).

The hepatic cells are therefore most exposed to the effects of free radicals formed in the process of biodegradation of adriamycin and responsible for the development of oxidative shock in the cells, which leads to apoptosis. Apoptosis always occurs in these cells and is observed just after adriamycin administration as it was reported in previous investigations (1, 4, 6, 12) and in the authors’ studies on the effects of adriamycin on the kidneys and liver of mature rats, pregnant rats, and rats’ foetuses (2, 10).

The aim of this study, which is a continuation of the series of investigations concerning adriamycin-induced apoptosis (8, 9), was ultrastructural and light microscopical assessment of the adriamycin-induced apoptosis in rats’ hepatocytes.

Material and Methods

The studied material consisted of 32 white Wistar female rats of the baseline body weight – 200-250 g aged 2.5-3 months. The rats were randomly selected according to the simultaneity principle of control and experimental groups. They were kept in 0.2 m² metal cages, four animals in each cage, at the temperature of 20 +/-2ºC and humidity- about 60%. The cage lining was suitably changed. The air temperature, lightening, and noise slightly changed during the day. The rats received standard feed and water ad libitum.

The animals were divided into four equal groups: group I – rats treated with a single intraperitoneal dose of adriamycin (5 mg/kg b.w.) and decapitated after 4 weeks; group II - control rats treated with a single intraperitoneal dose of 0.9% NaCl (0.5 ml) and decapitated after 4 weeks; group III - rats treated with a single intraperitoneal dose of adriamycin (5 mg/kg b.w.) and decapitated after 7 weeks and group IV - control rats treated with a single intraperitoneal dose of 0.9% NaCl (0.5 ml) and decapitated after 7 weeks.

After decapitation, the specimens from the right hepatic lobe were collected and fixed in the solution consisting of 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M phosphatic buffer according to Sorensen (3), and in osmium tetroxide (OsO4). The specimens were contrasted in uranyl acetate, dehydrated in the alcohol-acetone series, and embedded in the Araldit AMC Fluka resin. Semi-thin (0.5-0.7 µm) and ultrathin (60 nm) sections were prepared.

The semi-thin sections were stained with 1% methylene blue with azure II in 1% water solution of
sodium tetraborate and evaluated under light microscope and photographed using the Jenaval Contrast Carl Zeiss camera. The ultrathin sections were stained with 8% solution of uranyl acetate in 0.5% acetic acid and lead citrate according to Raynolds. The material was evaluated under the electron microscope Tesla BS-500.

Results

During necropsy, numerous transudates to the pleural and peritoneal cavity and subcutaneous, retroperitoneal, hepatic, and renal tissues oedema were observed in rats of groups I and III.

The light microscopic structure of the liver in control groups II and IV was normal and was characterised by typical architectonics with clearly visible contoured lobules. The cytoplasm of hepatocytes was stained blue with visible basophilic evenly distributed granules. The nuclei were stained brightly blue and one or two nucleoli were clearly visible in their inside.

The histological structure of the liver in experimental groups I (Fig. 1) and III (Fig. 2) was similar. The effacement of the membrane of parenchymal hepatic cells and focal disintegration of architectonics concerning the shape and size of hepatocytes were visible. Hepatic trabeculae were focally fragmented (Fig. 2). The characteristic different sizes, shapes, and colours of nuclei were observed. Chromatin showed the features of dispersion. Some nuclei revealed peripheral chromatin condensation. An increased number of nucleoli in the nuclei were often observed. Some cells had no cytoplasm, especially around the nuclei (Fig. 2). Other cells had fine-granular, completely dark blue cytoplasm without the nucleus or with nucleus of reduced perimeter, changed shape, and colour (Fig. 1). The cytoplasm of some cells also showed numerous vacuoles (vacuolar degeneration) (Fig. 2). The lumen of sinuses was focally reduced (Fig. 1).

Fig. 1. Group I. The semi-thin liver section of the rat treated with adriamycin and decapitated 4 weeks later. The photomicrograph visualises normal hepatocytes accompanied by residual cells with dark cytoplasm, without the nucleous or with the nucleous of changed shape and colour. Hepatocytes with bright cytoplasm and with the nucleus of various shape and dispersed chromatin are also visible. Methylene blue+azure II, 500x.

Fig. 2. Group III. The semi-thin liver section of the rat treated with adriamycin and decapitated 7 weeks later. The central vein and widened sinuses are visible. Effaced architectonics. In hepatocytes, granular brightened cytoplasm, variously shaped nuclei are visible. Methylene blue+azure II staining, 400x.

Fig. 3. Control group II. The liver section of the rat showing distinct boundaries between three hepatocytes (H). In hepatocytes - oval nuclei, mostly low-energy mitochondria (M), and well developed endoplasmic reticulum (RE) are visible. TEM, 2,200x.

Fig. 4. Group I. The liver section of the rat treated with adriamycin and decapitated 4 weeks later. Significantly oedematous mitochondria in the apoptotic hepatocyte with destroyed membrane (M) and disrupted endoplasmic reticulum. TEM, 9,000x.
Fig. 5. Group I. The liver section of the rat treated with adriamycin and decapitated 4 weeks later. Pyknotic nuclei (N) are present in apoptotic hepatocytes. In the centre, the apoptotic cell with the pyknotic nucleus (N), numerous peroxysomes (P), lysosomes (L), and oedematous mitochondria (M) are visible. TEM, 3,700x.

Fig. 6. Group III. The liver section of the rat treated with adriamycin and decapitated 7 weeks later. Pyknotic nuclei (N) of hepatocytes, oedematous mitochondria (M), and quite numerous vacuoles are seen (V). TEM. 3,000x.

The electron microscope image of hepatocytes in all control groups was similar and not significantly different from that described in textbooks (Fig. 3). Distinct boundaries between hepatocytes were visible with intercellular junctions in the form of the zonula occludens, adhesion, or desmosome (Fig. 3). One or two centrally located nuclei, round or slightly oval in shape, large and surrounded by the nuclear membrane were observed in hepatocytes. The nucleus was filled with evenly scattered euchromatin and heterochromatin located under the internal lamina of the nuclear capsule (Fig. 3).

The mitochondria were mainly in the condensed form (low-energy - a large number of crests and highly saturated matrix); however, some high-energy mitochondria were also observed (orthodox) (Fig. 3). The number of elongated mitochondria was higher in the cells located near the portal area. In the vicinity of the central vein, the mitochondria were oval and less abundant, the smooth endoplasmic reticulum was well developed compared to peripheral hepatocytes of lobules where the rough endoplasmic reticulum predominated (Fig. 3).

Moreover, the well developed Golgi apparatus, single lysosomes and peroxysomes with dark crystalline medulla and scattered or accumulated glycogen granules were observed in the hepatocyte cytoplasm (Fig. 3).

The hepatocyte lesions in groups I and III were similar. They concerned single hepatocytes or their groups. A higher number of changed hepatocytes were observed (Figs 4, 5, and 6). The borders between damaged hepatocytes were effaced (Fig. 6). The degenerating hepatocytes were irregularly shaped and smaller than normal ones (Fig. 5). The formation of apoptotic bodies containing pyknotic nucleus and destroyed organelles was observed (Fig. 5).

Some damaged cells had only one nucleus and no organelles. A decreased number of glycogen granules were found in hepatocytes. The membrane of damaged cells was partially or completely disrupted.

The nuclei of damaged cells had various shapes and reduced volume compared to the controls (Figs 4 and 6). Their chromatin was compacted in papules, irregularly scattered, and peripherally condensed (Fig. 6). Vacuoles in the nucleolus were seen in some nuclei. The mitochondria, less numerous compared to controls, were mainly high-energy (Figs 4, 5, and 6). The majority of them were characterised by severe oedema. They had brightened matrix and low rare crests. The internal or/and internal mitochondrial membrane was damaged in some of them (Figs 4 and 6). Moreover, the fragmented endoplasmic reticulum (Figs 4 and 6) as well as increased number of lysosomes (Fig. 5), peroxysomes, and vacuoles (Fig. 6) were observed.

Discussion

Many methods were elaborated to determine the type and course of cell death. The extremely interesting methods allowing identification and observation of single apoptotic cells are based on transmission electron microscopy (TEM). The methods detecting single apoptotic cells as well as their groups (15) include histological staining as the most common and simple way to visualise the morphological changes. The cell elements visualised by stains, particularly cell nuclei, show different morphology in apoptosis and in necrosis (14).

In this study, TEM and light microscopy was used to detect adriamycin-induced apoptosis in rats’ hepatocytes. Quite characteristic lesion seen in this study under electron microscope was adriamycin-increased number of peroxysomes in the cytoplasm of dying cells. An increased number of peroxysomes following the administration of adriamycin (by 200%-400%) was observed by Zipper (16) in the myocardium. He assumed these changes as a cell response to oxidative stress. Similar observations were reported by Mendocha (7) in the rat hepatocytes examined 4 weeks after the administration of adriamycin.

In the presented study, adriamycin was administered in the dose (5 mg/kg b.w) inducing...
apoptosis (13) without causing necrosis. This dose seems to be suitable to induce the cell death through apoptosis and too small to cause necrosis.

References


