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Abstract

The presented study describes renal tubular epithelial cells morphology in rats during adriamycin therapy. It was demonstrated that one dose of the compound can induce increasing-with time-apoptosis in the cells. A statistically-significant increase in the number of apoptotic cells was observed in experimental groups compared to controls. The highest percentage of apoptotic cells was found in rats 7 weeks after adriamycin administration. The number of apoptotic cells in these animals was statistically significantly higher than that in the rats 4 weeks after adriamycin administration.

Key words: rats, adriamycin, renal tubular epithelial cells, morphology, apoptotic index.

Already in the mid-XIX Century it was demonstrated that cell death played an important role in the physiological processes of multicellular organisms, particularly during embryogenesis (8). Until the end of the 60’s of the Century, the cell death was believed to result exclusively from pathological processes in the organism. Apoptosis was not distinguished from the other ways of cell death, which are known today (7).

In 1964, the term “programmed cell death” was introduced. It was observed that during the development of this way of cell death was not an incidental but a multi-stage, planned, and controlled process (7).

The studies on apoptosis have demonstrated that the factors contributing to the development of neoplasms also include accelerated proliferation of cells and loss of cell ability to die at a defined time.

The effectiveness of antineoplastic drugs is measured by their capacity to inhibit proliferation and induce the process of apoptosis of the neoplastic cells (6, 13). Better understanding the mechanisms regulating apoptosis could improve the effectiveness of anti-oncological drugs and reduce their toxicity. The destruction of proteins and cytoplasmic organelles is an important element in life and death (1).

Anthracyclines, which include adriamycin (ADR), are antibiotics used in oncology. In the cellular nucleus adriamycin binds DNA (3). In the process of its transformation semiquinone is formed. It is a free radical form responsible for the cytotoxic effects of the drug (14). There are a number of reports describing adriamycin–induced apoptosis in various human and animal organs.

Five to fifteen percent of the administered dose of adriamycin is excreted in its unchanged form in the urine within 5-7 d after administration (3). The kidneys’ cells are therefore exposed to the effects of free radicals formed in the process of biodegradation of adriamycin.

The presented study describes the morphological and apoptotic changes in renal tubular epithelial cells during anthracycline therapy in rats.

Material and Methods

The study material consisted of 32 white Wistar female rats of baseline body weight of 200-250 g and 2.5-3 months of age. The rats were randomly selected according to the simultaneity principle of control and experimental groups. The animals received standard feed and water ad libitum. The rats 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; group IV - control rats treated with a single intraperitoneal dose of 0.9% NaCl – 0.5 ml and decapitated after 7 weeks.

After the decapitation of the animals, the specimens from the left kidney were collected for further examination. The specimens were fixed in 10% buffered formalin (pH 7.4), dehydrated in a graded ethanol, cleared in xylene, and then embedded in paraffin. Five micrometre sections were stained with haematoxylin and eosin.

The epithelial cells of renal convoluted tubules were analysed. Special attention was paid to the features suggesting apoptosis. The photographic documentation was prepared using the Jenaval Contrast Carl Zeiss camera. The results were presented in descriptive form.

The degree of apoptosis in the renal sections was determined quantitatively using the apoptotic index (AI). Three sections from each individual were examined. The nuclei stained dark with haematoxylin with reduced perimeter and diameter were accepted as the pyknotic ones, i.e. being one of the possible manifestations of apoptosis. In the haematoxylin and eosin stained sections magnified about 1,000x (linear areas: 985x, square spaces: about 1,000,000x), the number of pyknotic nuclei was counted in 100 cells in a particular experimental group and compared with the respective control group. The results were presented as percentages. The projection microscope (MP3, PZO, Poland) was used. The examinations included only the epithelial cells of renal tubules.

The results were presented as means and standard deviation of the mean using the ONE WAY ANOVA test. Five-percent-error risk and statistical significance at P≤0.05 were accepted.

The study was approved by the Local Ethics Committee in Lublin.

**Results**

In all control groups of the rats, the kidneys were not macroscopically different. They were bean-shaped, surrounded by an easy-to-remove capsule. Their surface was smooth and red-brown in colour. The cross-sections showed the cortex and medulla, which were different in shade. The blue-red medulla was indented towards the yellowish-red cortex dividing it into renal columns. In the cortex, dots corresponding to renal glomeruli were visible.

The kidneys in experimental groups I and III were bigger than those in the corresponding control groups. They showed similar macroscopic morphology. Their surface was smooth and pale pink in colour. The section revealed the parenchyma everted from beneath the capsule and a clearly-marked border between the medulla and cortex.

In all control groups (II, IV), the microscopic picture of the kidneys was normal. The renal tubules were characterised by clearly-visible empty lumen without pathological deposits (Fig. 1). The proximal convoluted tubules were quite regularly arranged. Their wall was formed by the one-layer cubic epithelium consisting of cells whose walls were not distinct. The cytoplasm was pink and acidophilic (Fig. 1). The cell nuclei, 4-5 on cross-section, were round and arranged regularly in the centre.

The lumen of the tubules was starlike, shaded with a brush border. The distal renal tubules had regular round or oval lumen. They were lined with the one-layer cubic epithelium. The epithelial cells, 5-6 on cross-section, had poorly-marked margins.

The microscopic picture of renal tubules in experimental groups I and III was similar. The visible lesions were focal, segmental, and involved single tubules or even epithelial cells of the tubular walls. The markedly widened lumen of some tubules was filled with homogenous secretion (Fig. 2). The epithelial cells of the tubular walls were flattened, damaged, or completely destroyed. Groups of degenerated tubules were visible (Fig. 3). In other tubules, the cells were filled with brightened cytoplasm with numerous dark granules and vacuoles (vacular degeneration). A large number of cells showed damaged pyknotic nuclei of reduced perimeter, changed shape, and dark violet (Figs 2, 3)

The examinations of the apoptotic index included 100 cells from each of three specimens collected from every animal. There were no statistically-significant differences in the number of apoptotic cells between control groups (P=0.43). Statistically-significant differences, however, were observed between experimental groups (P=0.001) as well as experimental vs control groups (P<0.001) (Table 1). A statistically-significant increase in the number of apoptotic cells was observed in all experimental groups compared to controls (P<0.001). The highest percentage of apoptotic cells was found in the rats 7 weeks after adriamycin administration (27.00 ±6.78). This number was statistically-significantly higher than that in the rats 4 weeks after adriamycin administration.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean number of apoptotic cells per 100 cells examined</th>
<th>Standard deviation</th>
<th>ONE WAY ANOVA</th>
<th>ONE WAY ANOVA</th>
</tr>
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<tbody>
<tr>
<td>Controls</td>
<td>I</td>
<td>15.10</td>
<td>±3.03</td>
<td>P=0.001</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>4.10</td>
<td>±2.96</td>
<td>P=0.43</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>4.90</td>
<td>±4.24</td>
<td></td>
</tr>
<tr>
<td>Experimental groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>15.10</td>
<td>±3.03</td>
<td>P=0.001</td>
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</tbody>
</table>
Fig. 1. Control group II. The rat kidney showing normal renal tubules with empty lumen. The cytoplasm of epithelial cells is pink and round violet nuclei are regularly arranged in the centre. A few pyknotic nuclei are visible. The photomicrograph also shows a renal glomerulus with slightly-enlarged urinary space. H+E staining. 350x.

Fig. 2. Experimental group I. The rat kidney section 4 weeks after administration of a single adriamycin dose. The photomicrograph shows numerous apoptotic epithelial cells of the tubular wall characterised by dark-stained distorted pyknotic nuclei and brightened cytoplasm. The renal tubules are irregular and destroyed. Pyknotic nuclei and homogenous secretion are visible in their lumen (arrow). Congestion of the kidney. H+E staining. 500x.

Fig. 3. Experimental group III. The rat kidney section 7 weeks after administration of a single adriamycin dose. Groups of destroyed tubules, pyknotic nuclei, and congestion are visible. H+E staining. 200x.
Discussion

Cell apoptosis, suicidal death determining the life of a whole multicellular organism, is the mechanism naturally balancing cell proliferation. The normal cell constantly receives life signals, which are transmitted by other cells through hormones and growth factors (cytokines). Nutrients are also involved. Moreover, cell survival is determined by its contact with other cells and proper anchoring in the extracellular matrix proteins.

The factors which commit the cell to apoptosis include (12) direct DNA damage due to various stress-inducing factors like hypoxia, UV and \( \gamma \)-radiation, free radicals, abnormal amount of growth factors (10), and, like in this study, antineoplastic drug – adriamycin. Adriamycin in the nucleus of the cell incorporates between two nitrogen bases of the DNA double spiral, which blocks the synthesis of DNA and RNA (3).

Other stimulating factors for the cell to die are: activation of some membranous receptors, external signals (9), activation of some intracellular receptors, deprivation of the cell of contact with the matrix, attack of T lymphocytes (activation of granulosomes - granules of cytoplasmic lymphocytes), and damage to some cell organelles - mitochondria (2, 5) or endoplasmic reticulum (11). If the cell does not receive life signals or receives more death signals then life signals, it stops serving its function and activates its internal programme of suicidal death. Sometimes such a cell is recognised by the immune system as foreign and the immune cells stimulate it from outside to start the process of its death.

Adriamycin-induced apoptosis in the specimens observed under light microscope in this study involved the renal tubular epithelial cells (proximal and distal tubules). Apoptotic index increased with the time of the experiment. In this study, the oedema of some mitochondria (parenchymal dimness ) in the apoptotic cells was noticed. It was observed that low-amplitude swelling of mitochondria was reversible, which shows the adaptation of mitochondria to higher energy demand. If the mitochondria are swollen by more than 20% of their physiological volume, the change is irreversible and denotes cell death. In the presented study the oedema was very highly intensified and irreversibly destroyed the mitochondria.

A relatively significant lesion observed under light microscope was vacuolar degeneration (4). The researchers examining the adriamycin-induced cell damage demonstrated that such vacuoles were formed from the widened tubules of the rough endoplasmic reticulum (14). This was also visible in the presented study in the form of the loss of basophilia of the cytoplasm.

References