Ultrastructural evaluation of renal tubular epithelial cells of the rats' kidneys after anthracyclines therapy

Ultrastrukturalna ocena komórek nabłonka kanalików nerkowych po terapii antracyklinami

Agnieszka Pedrycz[1], Piotr Siermontowski[2], Dorota Polz[3], Janusz Kocki[4], Agnieszka Puchalska-Kosiecz[5]

[1]Department of Histology and Embryology with Laboratory of Experimental Cytology, Medical University of Lublin, 20-080 Lublin, Poland
[2]Military Institute of Medicine, Maritime & Hyperbaric Medicine Department, Gdynia Poland..
[3]Departament of Virology, Medical University of Lublin, Lublin, Poland.
[4]Laboratory of Clinical Genetics, Medical University of Lublin, 20-080 Lublin, Poland
[5]Isth Department Oncologic Gynecology et Gynecology Medical University of Lublin, Poland

Abstract

Anthracyclines are antineooplastic antibiotics. In the present study Adriamycin (ADR) induced apoptosis of renal tubular epithelial cells.

The study material consisted of Wistar female rats, which were divided into 2 control and 2 experimental groups. Experimental groups: rats treated with a single intraperitoneal dose of Adriamycin- 5mg/kg body weight and decapitated after 4 (gr. I) and 7 (gr. III) weeks. Control groups: rats treated with a single intraperitoneal dose of 0.9% NaCl- 0.5ml and decapitated after 4 (gr. II) and 7 (gr. IV) weeks.

Semi-thin - 0.5-0.7μm and ultrathin - 60nm slideswere prepared from left kidneys segments taken from the rats.

Under electron and light microscope using semi-thin slides we observed apoptosis of kidney' cells. In experimental groups a large number of cells showed damaged pyknotic nuclei of reduced perimeter, changed shape and dark blue-black in semi-thin specimens. Naked pyknotic nuclei were visible in the renal tubular lumen. Those changes increased in the time of the experiment.

Keywords: ultrastructure of renal tubular epithelial cells, semi thin slides, apoptosis, Adriamycin

Streszczenie

Antracykliny są antybiotykami przeciwnowotworowymi. W niniejszym doświadczeniu adriamycyna (ADR) wywołała apoptozę komórek nabłonka kanalików nerkowych.

Materiał do badań składał się z samic szczura szczepu Wistar, które zostały podzielone na 2 kontrolne i 2 doświadczalne grupy. Grupy doświadczalne: szczury, którym podano jednorazową dootrzewnową dawkę Adriamycyny - 5mg/kg masy ciała i dekapitowano po 4 (gr. I) i 7 (gr. III) tygodniach. Grupy kontrolne: szczury, którym podano jednorazową dootrzewnową dawkę 0,9% NaCl- 0,5ml i dekapitowano po 4 (gr. II) i 7 (gr. IV) tygodniach.

Z wycinków pobranych nerek wykonano preparaty półcienkie 0.5-0.7μm i ultracienkie - 60nm oglądane następnie pod mikroskopem świetlnym i elektronowym.

Obserwowano nasiloną apotozę komórek nabłonka kanalików nerkowych. W grupach doświadczalnych znaczna część komórek zawierała pyknotyczne jądra o zmniejszonej średnicy, zmienionym kształcie i ciemnym, niebiesko-czarnym kolorze, obserwowanym na preparatach półcienkich. Nagie, pyknotycze jądra komórkowe widoczne były również w świetle kanalików nerkowych. Opisywane zmiany nasiliły się, w czasie trwania doświadczenia.

Słowa kluczowe: ultrastruktura komórek nabłonka kanalików nerkowych, preparaty półcienkie, apoptoza, adriamycyna

Introduction

The term apoptosis was used for the first time by Kerr and colleagues in 1972, who described it as a basic biological phenomenon considerably affecting the tissue kinetics [1]. They distinguished necrosis from apoptosis demonstrating that the morphological and biochemical processes involved in these two phenomena were different. However, the terms "apoptosis" and "programmed cell death' were used by them interchangeably.

At present, some authors use the term "programmed cell death" in relation to the physiological cell death during the development and "apoptosis" to cell death caused by external factors [2].

Apoptosis is essential throughout the life of organisms [3].

In embryogenesis it determines the normal development of tissues and organs (atrophy of the web between fingers, elimination of T lymphocytes recognizing

their own antigens). In foetal life apoptosis also controls the development of the placenta. Inadequately increased apoptosis of the trophoblast may lead to pathology of pregnancy, e.g. pre-eclampsia [4].

During the individual life apoptosis is involved in continuous maintenance of the functions of organisms (maintenance of constant number of cells in individual organs, removal of dangerous cells, i.e. infected with viruses, with damaged DNA, neoplastic) and the ageing process of tissues.

The changes in apoptosis are connected with many diseases, including neoplastic diseases [5]. Therefore apoptosis is the aim of numerous studies [6]. During neoplastic transformation of the tissues one observes increased apoptosis, which is seen in colon carcinoma, melanoma, retinoblastoma. Suppressed apoptosis is observed in neoplasms with mutated p-53, leukemias, breast, ovary and prostate cancer.

Apoptosis is crucial in viral infections. The use of drugs inhibiting apoptosis is likely, among other things, to delay the development of immunity deficiency in AIDS.

In present study Adriamycin (ADR) induced apoptosis. We observed this phenomenon in electron and light microscope using semi-thin slides.

Material and Methods

The study was approved by the Local Ethics Committee in Lublin at the the Medical University of Lublin, no. 32/2000, 551/2005

The study material consisted of 32 white Wistar female rats of the baseline body weight – 200-250g aged 2.5-3 months. 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 4 equal groups in which the effects of Adriamycin were evaluated:- 8 females each. Group I – rats treated with a single intraperitoneal dose of Adriamycin-5mg/kg body weight and decapitated after 4 weeks – 8 individuals. Group II- control rats treated with a single intraperitoneal dose of 0.9% NaCl- 0.5ml and decapitated after 4 weeks - 8 individuals. Group III- rats treated with a single intraperitoneal dose of Adriamycin - 5mg/kg body weight and decapitated after 7 weeks – 8 individuals. Group IV- control rats treated with a single intraperitoneal dose of 0.9% NaCl – 0.5ml and decapitated after 7 weeks - 8 individuals.

After decapitation the specimens from the left kidney were collected for further examinations. Specimens were fixed in the agent consisting of 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1M phosphatic buffer according to Sorensen (2) and in osmium tetroxide (OsO_4).

After that specimens were contrasted in uranyl acetate, dehydrated in the alcohol-acetone series, embedded in the Araldit AMC Fluka resin.

The following sections were prepared semi-thin - 0.5-0.7μm and ultrathin - 60nm.

The semi-thin sections were stained with 1% methylene blue with Azure II in 1% water solution of sodium tetraborate, evaluated under the 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

In control groups the semi-thin specimens showed bright, round, big nuclei with a clearly marked nucleolus in renal tubular epithelial cells

The renal tubular cells revealed focal darker nuclei with slightly reduced perimeter, which were likely to correspond to the pyknotic nuclei characteristic of apoptotic cells. The naked nuclei were visible in the lumen of some tubules. Those phenomena, however, were incidental, visible only in some individuals and statistically within the limit of error.

In experimental groups a large number of cells showed damaged pyknotic nuclei of reduced perimeter, changed shape dark blue-black in semi-thin specimens (Fig.1). Naked pyknotic nuclei were visible in the tubular lumen

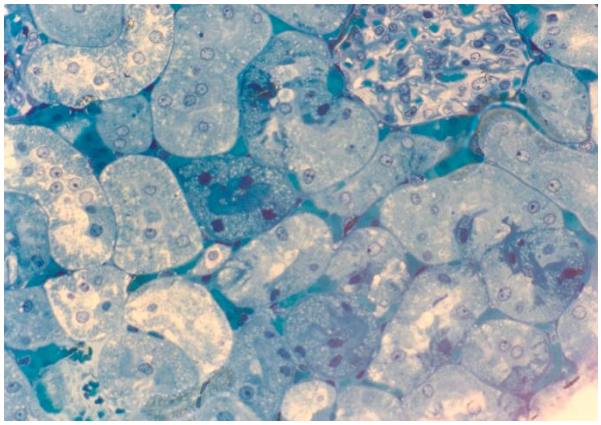

Figure 1. EXPERIMENTAL GROUP I. The kidney section of the rat decapitated 4 weeks after administration of a single Adriamycin dose. The photomicrograph shows focally destroyed renal tubules, a fragment of renal glomerulus and irregular tubules. Naked nuclei are visible in the lumen. Epithelial cells with brightened cytoplasm and dark distorted pyknotic nuclei are seen. Congestion is visible between tubules. The semi-thin specimen. Methylene blue+Azure II staining. Magnification x 400.

The renal tubules of animals in control groups II, IV, observed under electron microscope had normal structure.

Their wall was built of cubic epithelial cells located on the basilar membrane of normal thickness. The boundaries between cells were clearly visible.

The cells forming the wall of primary convoluted tubule at the pole directed towards the lumen had the brush border composed of microvillaes .

The cellular membrane of the base of epithelial cells of proximal and distal tubules was indented into the cytoplasm forming the peribasal labyrinth (striation) with parallel rows of mitochondria inside (Fig.2).

The mitochondria had normal shape, membranes and mitochondrial crests.

The nucleus in the epithelial cells of tubules was located above the striation, medially and eccentrically (Fig.2). It was round or oval, large and surrounded by the nuclear membrane. Moreover, it had a nucleolus.

Above the nucleus, there was a well developed endoplasmic reticulum – rough and smooth, and lysosomes as well as dark peroxysomes with crystalline medulla.

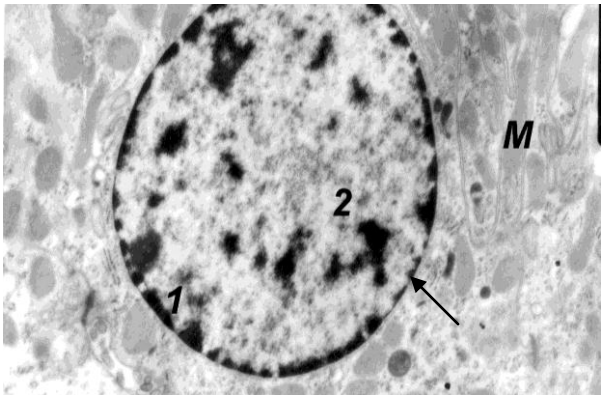

Fig.2. CONTROL GROUP II. The epithelial cell of the wall of the secondary convoluted tubule. The photomicrograph shows a large, round nucleus with smooth surface filled with heterochromatin(1) (electron-dense layer under the internal lamina of the nuclear capsule) and euchromatin (2) (bright areas of the nucleus), nuclear pores (arrow), peribasal striation (the cellular membrane indented to the interior of the cytoplasm) with numerous elongated mitochondria inside (M). TEM Magnification x 7000

The renal lesions observed under electron microscope were focal and concerned all experimental groups (Fig.3, 4). In addition to normal tubules or single normal epithelial cells of the tubular wall, the tubules with dead cells were observed (Fig.4).

The boundaries between epithelial cells of the tubule wall were blurred. Some cells were flattened, with reduced volume, partially or completely destroyed. The tubular lumen was widened with homogenous deposits inside (Fig.3) (experimental group I, III) as well as nuclei, mitochondria, single epithelial cells (experimental group I, III). The brush border in the proximal tubules was focally destroyed.

The partially destroyed cells had numerous vacuoles surrounded by the smooth or rough membrane in the cytoplasm. The rough endoplasmic reticulum had widened channels and was focally completely destroyed. The mitochondria showed abnormal structure. They were oedematous with brightened matrix and destroyed crests. Moreover, the architectonics of peribasal striation was damaged. Numerous lysosomes and peroxysomes with dark, homogenous content were observed (Fig.4). Some cells had only a nucleus and a few only organelles- brightened structure of the cytoplasm.

The nucleus of damaged cells was most commonly located in one of the cell poles, its shape was changed and markedly smaller than the nuclei of normal cells. Condensation and peripherally located chromatin were observed (Fig.3, 4).

Moreover, a prominent cell membrane (best visible in the lumen) and formation of apoptotic alveoli ("cell boiling") were observed (Fig.3). The alveoli contained complete or fragmented nucleus and other organelles (Fig.3, 4).

The lesions observed were characteristic of apoptotic cells.

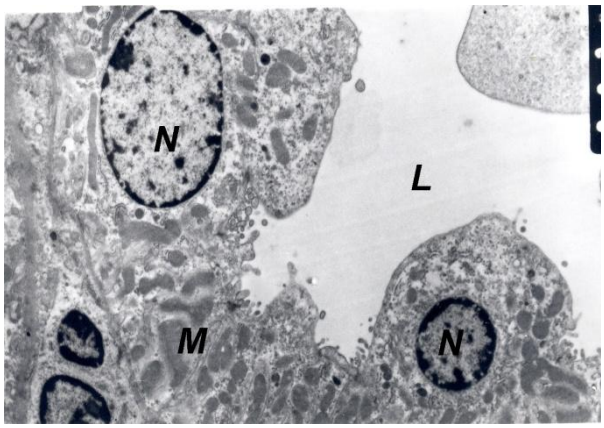

Fig.3.EXPERIMENTAL GROUP I. The renal tubule section of the rat treated with adriamycin and decapitated 4 weeks later. "Cell boiling" is seen. The cell membrane is bulged, apoptotic bodies containing cell elements are formed. Homogenous secretion in the tubular lumen (L). TEM. Magnification x 2000.

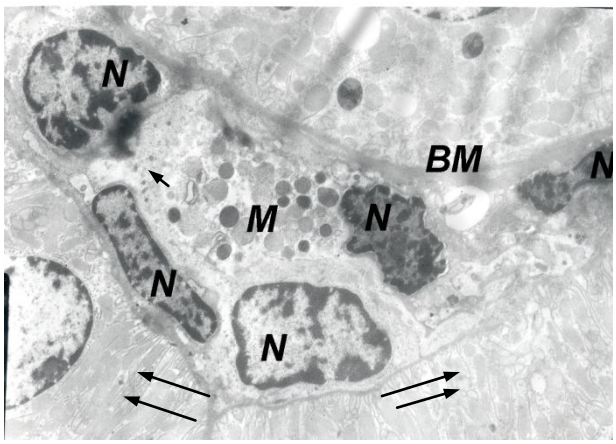

Fig.4.EXPERIMENTAL GROUP III. The kidney section of the rat treated with adriamycin and decapitated 7 weeks later. The damaged tubule with apoptotic cells is present surrounded by the basilar membrane (BM) with normal tubular epithelial cells with normal peribasal striation (double arrows). The apoptotic cells (apoptotic bodies) are visible which contain pyknotic nuclei (N), damaged mitochondria (M) and abundant peroxysomes (arrow). No boundary between dying cells. TEM. Magnification x 3000.

Discussion

In this study we observed several stages in the cell morphology which are characteristic for apoptotic process. On the beginning the cell loosing water shrinked leading to apoptotic volume decrease (AVD) [7]. Next the cell changed its shape and became elongated. The cell surface became folded. It was the result of changes in the structure of the cell membrane (the molecules of phosphatidylserine (lipid) previously directed into the cytoplasm appear on the surface of the cell membrane). Next chromatin undergone condensation and was located marginally - characteristic papules (chromatin is a complex of nuclear DNA and proteins). Condensation of the cytoplasm was observed. In this study we observed widened and undergone disintegration the cisterns of the rough endoplasmic reticulum and Golgi apparatus. Next step described in the literature - DNA undergoes fragmentation thanks to endonucleases – DNA breaks in an orderly manner into the fragments corresponding to distances between the adjacent nucleosomes [8]. Important fact during apoptotic process is, that even the cell skeleton is remodelled, intracellular structures preserve their integrity.

In this study we observed under light and especially under electron microscope, bulging of the cell membrane- "boiling" of the apoptotic alveoli (apoptotic bodies) on the cell surface ("the cell boils"). The vesicles contained fragments of the cell nucleus, other organelles, etc. The cell was fragmented into apoptotic bodies.

The apoptotic cells are recognized by the immune cells and other adjacent cells [9]. Fragmented elements of the cell are absorbed through phagocytosis. The cell residues are phagocytosed without eliciting an inflammatory response.

All these stages was observed in present study in light and electron microscope. Quantitative changes in the kidneys increased in the time of experiment. Both after 4 and after 7 weeks of experiment we observed cells in all stages.

The ultrastructural examination showed an extremely large number of lesions in the mitochondria (mitochondrial pathway of apoptosis). The mitochondria were oedematous, their matrix brightened and crests destroyed, which was also described by other authors in ADR-exposed hepatocytes [10] and other cells [11,12,13].

A quite important lesion observed under electron microscope was vacuolar degeneration [14,15]. Such vacuolas probably originate from destroyed rough endoplasmic reticulum [16]. This was also visible in the present study - in degranulation as well as widening of the endoplasmic reticulum (reticular pathway of apoptosis) observed under electron microscope.

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Correspondence address:

A. Pedrycz
e-mail address: apw4@wp.pl