ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 2, 30 SECTIO DDD 2010

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Structural biochemical assessment of the status of the nuclear apparatus of the rat spleen lymphoid cells under radiation treatment

Strukturalne badania biochemiczne stanu aparatu jądrowego komórek limfoidalnych śledziony szczurów w warunkach napromieniania

INTRODUCTION

Studies on the influence of different radiation doses are of important practical value, as they make it possible to detect the early signs of radiation injury, assess the organism's compensatory reactions, and find out the specific effects of different types of ionizing radiation (IR) by comparing their impact with that of X rays [2]. As a result of the wide range of applications of IR sources in different fields of science and practical activity, the problem of the influence of IR on different types on the organism and the radiosensitivity of organs and tissues has acquired a particular theoretical and practical importance [4].

One of the most profound effects of ionizing radiation on the organism is alteration of the immune system, being the most radiosensitive one [6]. Multiple studies *in vitro* have revealed that low doses of ionizing radiation are able to induce apoptosis-type lymphocyte death. This form of radiative cell inactivation – the interphase death – occurs before the onset of mitosis. Under very heavy radiation doses, it occurs directly "under the ray" or immediately after exposure. In the dose range up to 10 Gy, death occurs within the first few hours past exposure and can be registered in the form of various degenerative alterations of the cell; most often cells with a sharp nuclear pyknosis and chromatin fragmentation appear under a microscope 2 to 6 hour after IR exposure. In the cell cytoplasm, hydrolytic enzymes leading to DNA degradation get activated and accumulate, and the inherent lymphocyte genetic program of the interfase death – apoptosis – is realized [5].

Various types of apoptosis lauched by the action of different agents may have significantly different initial stages; however, the key stages of the process development have a common underlying mechanism and identical morphological manifestation.

One of the most informative methods of monitoring the immune system during apoptotic events is two-wave photometry of local regions of luminescent images of lymphocytes after fluorescent staining.

MATERIAL AND METHODS

We studied non-strain white rats weighing 150–170 g with vivarium maintenance on an adequate nutrient-balanced diet. The animals were exposed to IR under RUM-17 apparatus with doses between 1.0 and 1.78 Gy under the following conditions: Cu 0.5 mm and Al 1.0 mm filters, voltage 200 kV, ray focal distance 50 cm, dosage power 0.17 Gy/min and 0.34 Gy/min, respectively. The animals were decapitated three hours after exposure. Spleen lymphocytes were extracted employing the commonly used method in a phycol–verografin density gradient (viscosity – 1.077 g/cm^3). To assess biochemically the status of the nuclear fluorescently stained DNA of fixed cells we used microscopy, digital imaging, and computer-assisted two-color photometry of digital color images using adapted Photoshop software that is able to automatically calculate the luminescence intensity ratio of the assessed regions in red (640 nm) and green (540 mn) spectral bands [$\alpha=I_{640}/I_{530}$] (precision: $\alpha\pm0.05$). This approach is thought to represent an integral index of the degree of nuclear chromatin spiralization and the status of the synthetic machinery of the cell.

RESULTS

To assess biochemically the status of the nuclear DNA of fixed cells we used microscopy, digital imaging, and computer-assisted two-color photometry of digital color images using the Photometry software [1], which allows assessment of the degree of the chromatin structural status in the rat spleen lymphoid cells.

Morphological traits of apoptotic cells include condensation of nuclear material, its fragmentation with resulting compact apoptotic bodies confined within the plasma membrane and containing well-preserved organelles (Fig. 1). These features distinguish apoptotic cells from necrotic ones in which the integrity of chromatin, organelles, and the plasma membrane is significantly mutilated.

According to the obtained results presented in Fig. 2, an increase of the parameter α by 32% compared to control after IR exposure with the dose of 1.0 Gy is observed, which may be linked to the structural changes in DNA within chromatin.

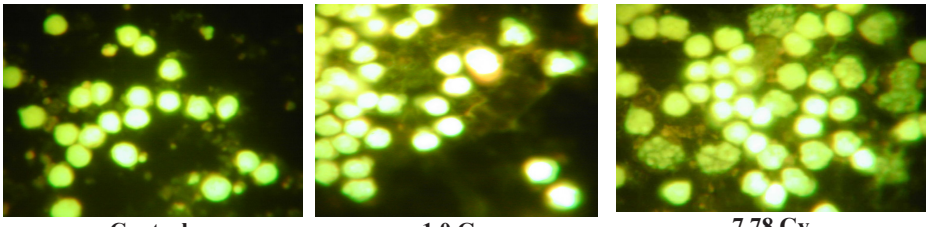

Fig. 1. Microphotographs of rat splenocytes three hours after IR exposure

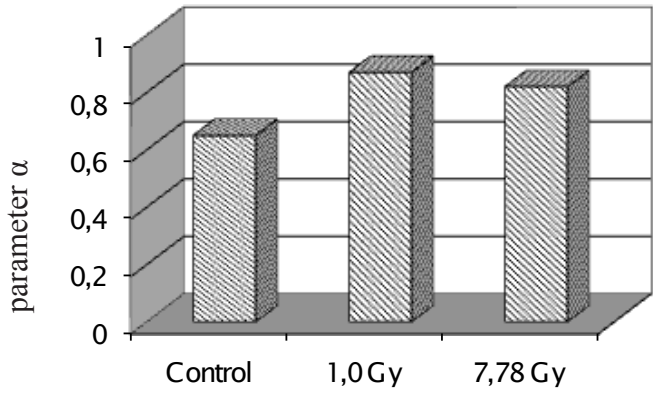

Fig. 2. The value of the parameter α for rat spleen lymphocytes three hours after IR exposure

As a result of the breaks of phosphodiester bonds in DNA molecules induced by the action of ionizing radiation, we used the acridine orange as a stain extensively intercalates into the deoxyribonucleic acid chain, binding with the unblocked phosphate groups [3]. Increasing the dose up to 7.78 Gy leads to an increase in parameter α by 15% compared to control. The significant reduction in the parameter studied compared to the values obtained under the dose of 1.0 Gy may be explained in terms of an increased degree of chromatin compactization, which, in turn, hampers the access of the stain to the phosphate groups.

DISCUSSION

The experimental results we obtained suggest significant changes in the structural characteristics of chromatin under radiation-induced apoptosis.

A number of authors [7] believe that a significant role in the programmed cell death is played by chemical modifications in some histones, which may provide for compactization of the central linker region of chromatin due to an increase charge of nucleosomes and some extra binding of DNA with the histone core.

Therefore, the two-color photometry of the luminescent images of rat spleen lymphoid cells we carried out with the help of a new computer version of data processing allowed us to estimate the changes in the integral index of the degree of structural compactization of chromatin brought about by ionizing radiation.

CONCLUSIONS

Using two-color photometry of local regions of luminescent images of rat spleen lymphocytes and a new computer software for data processing a change in the integral index of the degree of DNA

spiralization (parameter α [$\alpha=_{640}/I_{530}$]) under the action of ionizing radiation was detected, which indicates structural damage of chromatin.

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SUMMARY

Using two-color photometry of local regions of luminescent images of rat spleen lymphocytes and a new computer software for data processing, pyknotic events accompanied by condensation of nuclear material and its fragmentation leading to the formation of apoptotic bodies have been registered. Under IR exposure, a change in the integral index of the degree of DNA spiralization (parameters α [$\alpha=640/I_{530}$]) has been found to occur, which indicates structural damage in chromatin.

Key words: apoptosis, parameter α, fluorescence microscopy, acridine orange, spleen lymphocytes, ionizing radiation

STRESZCZENIE

Stosując dwukolorową fotometrię lokalnych regionów obrazów luminescencyjnych limfocytów śledziony szczurów i nowe oprogramowanie komputerowe do analizy danych, wykazano piknozę jąder i kondensację materiału nuklearnego oraz jego fragmentację prowadzącą do wytworzenia ciałek apoptotycznych. W warunkach napromieniowania wykazano zmiany integralnego wskaźnika stopnia spiralizacji DNA (parametr α [$\alpha=640/I_{530}$]), co wskazuje na strukturalne uszkodzenie chromatyny.

Słowa kluczowe: apoptoza, parametr α, mikroskopia fluorescencyjna, oranż akrydyny, limfocyty śledziony, promieniowanie jonizujące