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Ultrastructural and biochemical changes of renal cortex, liver and blood under the multiple influence of small-doses irradiation in experimental conditions

Zmiany ultrastrukturalne i biochemiczne w korze nerek, wątrobie i komórkach krwi pod wpływem wielokrotnego napromieniowania niskimi dawkami w warunkach doświadczalnych

INTRODUCTION

The organism injury under ionizing radiation may be regarded as stress model with famous originality of irradiation effects on the different levels of the spatial and time organization of cells, cellular and noncellular structures. The main attention is concentrated on liver and kidney under irradiation, which are responsible for detoxication and extracting processes, respectively. To the present day there have been no reports on the effect of x-ray and gamma-irradiation in the structural and functional changes of nuclei and cytoplasmic components of eukaryotic cells, which determine their death [3].

The aim of this study was to investigate morphologic characteristics and oxidative stress parameters of white rats blood, liver and kidney under small-doses irradiation.

MATERIAL AND METHODS

The investigations were carried out on white outbred male rats. The animals were exposed to ionizing radiation in a dose of 0.25 Gy 4 times using "Agat" (irradiation origin – ^{60}Co). The resulting dose was 1 Gy. The liver and kidney tissues specimens underwent electron microscopy examination. Ultrastructural studies of the liver and kidney were carried out on 1 mm^3 slices. The samples were fixed for transmission electron microscopy in 2% osmium tetroxide in 0.1M phosphatic buffer (pH 7.36) at 4 ^{0}C for 3h and were postfixed in 2% osmium tetroxide prepared in the same buffer. The fixed samples were dehydrated in ascending grades of ethanol and were embedded in araldite and epone [2]. Semi-thin sections were prepared using an ultramicrotome (УМТП-3М, Ukraine) [7, 8]. Sections were stained with uranyl acetate and lead citrate to

examination under an electron microscope (YEMB-100K, Ukraine). Oxidative stress was assessed by measuring the activities of catalase, superoxide dismutase (SOD) and the level of thiobarbituric acid reactive substances (TBARS) production. Lactat and pyruvat contents were also determined. Determination of statistical significance of differences between groups was done by Student's t-test.

RESULTS AND DISCUSSION

Considerable ultrastructural changes were found of cells, cellular and noncellular elements of renal cortex connective tissue and liver tissue exposed to irradiation that are manifested in the shape of local separate sites of necrosis, precipitation, coagulation, masses of mucoid and fibrinoid, mitochondria fragmentation, proapoptotic and apoptotic bodies. The structural alterations of cells, cellular and noncellular elements of nephron and renal cortex connective tissue are to be accounted for by the presence of radiation-induced necrosis and by modified apoptosis. The mosaic changes of blood plasma and others major biological media of renal cortex and liver tissues under our experimental conditions indicate the phase character of activation thrombin- or plasmingenesis with the subsequent assembling of the local or disseminated coagulation-peptization dystrophy in conformity with the effecting factors [4]. Besides, kidney and liver tissues showed tissue sites and cells analogous to the same in the control group, and the presence of a more considerable amount of lipoprotein drops with light electronic density, peroxisomes, glyoxysomes, mitochondria, endoplasmatic reticulum channels extended, ribosomes, polysomes. The cytoplasm of nephron epithelial cells contained the cavities, which connected with nucleus and occupied the disorganizing formations (Fig. 1). Kovalyshyn V.I. [5] interpretes these formations as nephron seeds and fulfilling the role of proliferation, and Bhanu P.J. [1] interpretes as porosomas. The radiation-induced process of the assembling of the cavities occupied seeds which we observed and also their desorganization state may be a proof of the harmful influence on predicted structural and functional chromosome unit. Knigavko V.G. et al. [3] showed that this unit is a likely membrane associated superstucture unit (MASU) or something else. In support of this hypothesis, we showed previously that this unit may be cytoplasm flowers of eukaryotic cells which are derivates of telomere vital activity like chromosom formations with high electron density [5].

The data presented indicate that irradiation causes the same effects in kidney and liver parameters that are related to oxidative stress and tissue morphological characteristics. The level of TBARS increases significantly in all investigated tissues in response to induced irradiation (Fig. 2).

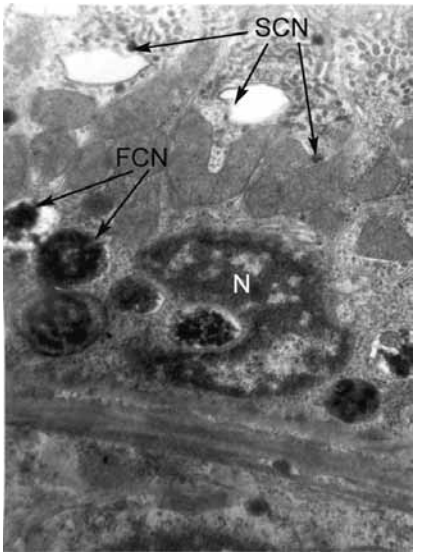

Fig. 1. Ultrastructure of the tubule's epithelial cell of the nephron's proximal part with saved nucleus (N) and cellular structures except disorganized seeds (SCN) incorporate into a cytoplasmic cavities and flowers (FCN) these cells of rats exposured gamma-rays in resulting dose 1 Gy (original ×12000)

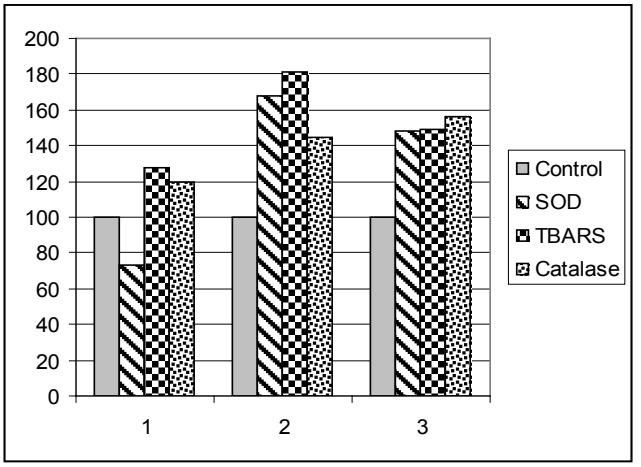

Fig. 2. Changes of biochemical parameters in rats blood (1), liver (2) and kidney (2) under small-doses irradiation; SOD – superoxide dismutase, TBARS – thiobarbituric acid reactive substances

Our present data indicate that ionizing radiation was accompanied by a significant diminution of SOD activity in rats blood and increase SOD activity in liver and kidney tissues (by 27%, 68% and 48,5%, respectively). Besides, catalase activity was significantly increased in all investigated specimens (blood, liver and kidney homogenates). At the same time, the pyruvate and lactate levels in the irradiation group of animals were higher than that in the control group. It is known that ultrastructural alterations could be triggered by changes in the intracellular redox state, which is reflected in an increased number of reactive oxygen species (ROS). In fact, ROS may behave as second messengers, favoring the activation of transcription factors under redox control such as NF-αβ, AP-1, HIF-1α, as reported by Wiener C.M. et al. [9]. The unfavorable consequences of these cellular events include inflammation, fibrosis, or apoptosis. An increase in the levels of lactate and pyruvate were noted in our investigation and the presence of numerous peroxisomes, glyoxisomes, mitochondria, indicates the endogenous intake aqua and ATP in cytoplasm, which according to Gilbert N. Ling [6] are necessary for providing a living state of protoplasm.

CONCLUSIONS

Our studies revealed that gamma-rays damage of the telomeres like chromosomes formations with high electrodensity, their precursors and derivatives determine the death of eukaryotic cells.

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SUMMARY

The studies investigated ultrastructural and biochemical characteristics of cells, cellular and noncellular structures of white rats renal cortex, liver and blood tissues in experimental conditions. It was established that the despite cytoplasm being provided by endogenous aqua and ATP under the multiple influence of small-doses irradiation, the structural and functional elements of eukaryotic cells proliferation process, which is associated with the telomeres activity, are damaged.

Keywords: ionizing radiation, gamma-irradiation, ultrastructure, kidney, liver, antioxidant enzymes, cell, nucleus, cytoplasm, porosoma.

STRESZCZENIE

W warunkach doświadczalnych oceniono właściwości ultrastrukturalne i biochemiczne komórek, struktur komórkowych i niekomórkowych kory nerek, wątroby i komórek krwi szczurów. Stwierdzono, że pod wpływem wielokrotnego napromieniowania dochodzi do uszkodzenia elementów ultrastrukturalnych i biochemicznych procesów proliferacji komórek eukariotycznych, co jest związane z aktywnością telomerów.

Słowa kluczowe: promieniowanie jonizujące, napromieniowanie gamma, ultrastruktury, nerki, wątroba, enzymy antyoksydacyjne, komórka, jądro, cytoplazma, porosom