THE IMMUNE STATUS OF THE SMALL INTESTINE IN THE BIOINFORMATICS-ASSISTED CHARACTERIZATION OF THE LATE POSTRADIATIONAL PERIOD FROM THE PERSPECTIVE OF ENHANCING THE FUNDAMENTAL NATURE OF RESEARCH

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Abstract. The present research is aimed at studying the immune component of the mucous membrane of the jejunum – the most active segment of the small bowel where one can most clearly observe the processes of parietal, membrane, and intracellular digestion, ensuring the homeostasis in the whole body; whereas any changes occurring after a low-dose total γ-exposure led to a decrease in the barrier and protection functions. It must be noted that a prolonged postradiational period is not rehabilitational for structural component recovery, despite the fact that some of them returned to their normal state. The morphofunctional changes were characterized by a barrier imbalance of sialomucin gel and alkaline phosphatase light-optical density indices, as well as a mast cell population against the background of strong correlation relationships, involving mitotic cells and intraepithelial lymphocytes into the interaction. Thus, functional interaction could be witnessed and the mast cells displayed a radioprotective effect. Lymphoid tissue demonstrated homeostasis problems

through the formation of lymphocytic infiltrate in the epithelium, and of M-cells, as well as of subepithelial lymphoplasmacytic infiltrate, and lymphoid nodules, which point to an increase in the antibody response.

Key words: jejunum mucous membrane, low doses, lymphoid tissue, γ-radiation.

Introduction

Nowadays, the issue of low-dose radiation exposure has already been addressed in the documents by leading international organizations conducting research into radiation effects and protection. With the development of the nuclear power industry, the need for integrating the existing experimental and epidemiological evidence in the sphere of low-dose radiation has definitely become obvious.

Currently, one of the essential areas of research is studying the immune system components that, at all the stages of body development until the old age, participate in the body's defense reactions to various environmental exposure, including radiation, and maintain the body's structural and functional integrity. Immunological responses may induce regeneration, protection, and radioresistance processes. It is the lymphocytes that have the status of the chief cells involved in the immune process and ensuring the immune reaction specificity. There is demonstrative evidence to prove the lymphocytes' ability to

stimulate proliferation, which accompanies every morphogenetic process and regulates cell differentiation.

Regenerative processes and the patterns in their manifestation are of the utmost importance; they require new bioinformatics solutions at the immune reaction level and are going to facilitate the development of new approaches to cell therapy.

This research is aimed at assessing the immune status of the mucous membrane of the jejunum after single whole-body and fractionated ionizing radiation exposure with a wide range of low dose parameters and long-term follow-up periods.

Research materials and methods

The experiment was done on outbred albino eugamic male rats, 4 months of age. The experimental rats were subjected to single homogeneous whole-body and fractionated (fivefold) γ-radiation MeV with the spectrum of 1,2 MeV on a "Khizotron" unit (60Co).

The total dose of fractionated radiation was distributed over 5 days in a growing range of exposure doses from 10 to 100 cGy, and 50 cGy/hr for isolated radiation. The postradiation follow-up period was 180; 365; 545 and 730 days. The assessment of long-term radiation effects involved a calculation of the absorbed dose, equivalent to the exposure dose, measured in Sieverts (Sv), with low-level radiation doses in the long term.

In accordance with the plan of the experiment, 53 groups were formed, with the age control. In total, there were 477 rats.

The material for the research were proximal jejunum fragments, 1.3-1.5 cm in size, extracted 12-15 cm from the stomach. In order to conduct morphological and immunohistochemical examinations, the jejunum was immersed in Becker's solution and 10% neutral formaldehyde solution, followed by the standard histological preparation and embedding in a homogenized paraffin coating. Paraffin blocks were formed and marked, and 6 μm thick microtome cuts were made. For visualization purposes, the preparations were stained with haematoxylin and Bismarck brown, which enabled the description of the pattern and the characterization of the epithelium and the subepithelial stroma with the vascular component and the mast cells.

On the standard median paraffin sections stained in haematoxylin, we then counted the number of intraepithelial lymphocytes (IL) on 20 longitudinally sectioned villi, i.e. the calculations were made over an area restricted by the same number of epithelial cells (x900). Taking into account the IL's heterogeneity, we calculated their numeric distribution according to the topographic features in the lower, middle, and upper third of the villi, for the purposes of characterizing and evaluating the migration process. In order to characterize the immune response, we viewed the subepithelial lymphoplasmacytic infiltrate stained by toluidine blue and Romanowsky-Giemsa stain. Using the alcian blue method of Steedman (1950) and the Image J

program, we determined the light-optical density of the sialomucin layer – a morphological substrate of goblet cells covering the epithelial surface of the mucous membrane and having a protective effect. We also considered light-optical density rates of the distribution of the alkaline phosphatase enzyme of enterocyte striated border, equivalent to the processes determining the absorption and transmembrane transport. Its activity dynamics is closely connected with the intestinal epithelium's migration, degeneration, and destruction rates. On the 4- μm thick paraffin sections, using the nuclear antigen-based enzyme-linked immunosorbent assay detection method (PCNA, clone PC10), we were able to observe cambial zone cells in the S-phase, or synthesis phase, during which DNA synthesis and replication take place. It is the S-phase cells that determine the morphogenetic activity of the lymphocytes, i.e., their constructive function, before the cells' preparation for division. The more damage is done to an organ, the higher is the lymphocytes' stimulating activity in cell division processes, which testifies to their ability to transfer the information on regeneration. They may not only stimulate it, but also suppress it, thus maintaining cell balance. The qualitative and quantitative characteristic of the microscopic objects for each animal was done using a binocular microscope equipped with an OPTIKA digital video-photo camera and a computer program modified specifically for this research. The volume of the material necessary for the research was determined using the method of accumulated average. The data obtained was processed using a statistical

method with the help of a standard “Excel” – 2007, “Statistika” – 8.0 for Windows, “SPSS” 13.0. for Windows statistical function pack, employing variation statistics, correlation and adaptometric analysis, and computer simulation methods.

Results and discussion.

The results of the research have demonstrated that, in the long-term chronodynamics, there were age-related changes detected in the control rats’ jejunum mucous membrane, which confirmed their dependence on age with firm indicators in all the criteria by the final day. Furthermore, the criteria under consideration were differently affected by the time parameter.

The results of the descriptive statistics for IL topographical dynamics in the experiment have demonstrated a dependence on all γ-radiation parameters in the low-dose range, as well as determined their functional direction. Following single and fractionated γ-radiation, as exemplified by 180 days after the exposure, there was only a rise in IL in the low-dose range in the lower third of the villi, with the exception of the 1 Sv dose, where there was a decline. In the upper and middle third of the villi, the changes were heterogeneous. We can assume that IL topography determined the lymphocytes’ functional direction, which was of varying nature, determining the differentiation of the newly formed cells and the protection in the area of desquamating enterocytes.

Computer simulation enabled to demonstrate the spline effect with varying degrees of topographic evidence of IL distribution depending on the γ-radiation parameters. The curve of the graphic representation of interpolation defined the spline function and its deformation was caused by the reactions to the emerging shifts in the specified criteria. Experimentally, the spline model was individual and approximated by the γ-radiation parameters.

Taking into account the lymphocytes' key role in ensuring the immune regulation of the body's structural and functional integrity, we determined the contrastive dynamics in terms of the lymphocyte migration index, mitotic index, and the synthetic period cell size in the experiment. The postradiational period chronodynamics modified the γ-radiation dose rage. We revealed a regularity in that, as the number of S-phase cells increases against a rise in the lymphocyte migration index, with the dose of 0.1 Sv after 365 and 545 days; 0.2 Sv – after 180 days; 0.5 Sv – after 180 and 545 days, 1 Sv – after 545 days, the mitotic index reduced, and it definitely increased as the number of S-phase cells and the lymphocyte migration index decreased with the dose of 0.2 Sv after 365 days; 0.5 Sv and 1 Sv – 730 days after a single exposure, which defines the lymphocytes' regulative role.

The data from the correlation analysis of the criteria under consideration demonstrates that there was a change in the existing relationships and the emergence of new ones over control, which proves and points to a dynamic interdependence of criteria on the new level in the chronodynamics after the γ-

radiation with a wide low-dose range. The quantity of correlation relationships between various parameters was a sign of state dynamics in the experiment.

A correlative adaptometric analysis has demonstrated that, in all follow-up periods after a single gamma exposure except the 1 Sv dose, there was an adaptive state for some of the considered criteria; for example, between the migration index and the mitotic index, there was an adaptive effect after 180 and 365 days with the dose of 0.2 Sv, and a proximity to the control figures was registered at the dose of 0.5 Sv after 730 days. After a fractionated γ-exposure, there was this effect after 365 days with the dose of 0.1 Sv. After 180 days there was no adaptive state recorded between the criteria.

Conclusion.

Based on the results of the computer histophotometry, the reaction of the gut-associated lymphoid tissue depended on the parameters of γ-radiation and the postradiational period, and manifested itself in the formation of lymphocytic infiltrate in the epithelium and of M-cells, as well as of subepithelial lymphoplasmacytic infiltrate, alongside with lymphoid nodules, which point to an increase in the antibody response, informing of homeostasis problems associated with the barrier state disintegration, in terms of the light-optical density of sialomucin distribution and the enterocytes' alkaline phosphatase. Correlation adaptometric analysis defined the adaptive state for several considered criteria in all follow-up periods after a single γ-exposure in the

selective dose range except the 1 Sv dose, and all the doses after fractionated exposure, with the exception of a long-term period of 180 days, which didn't guarantee any outcome of homeostasis. We revealed a certain regularity in that, as the number of S-phase cells increases against a rise in the lymphocyte migration index, with the dose of 0.1 Sv after 365 and 545 days; 0.2 Sv – after 180 days; 0.5 Sv – after 180 and 545 days, 1 Sv – after 545 days ($p<0.05$), after a single exposure; after a fractionated γ-exposure: with the dose of 0.1 Sv after 365, 545, and 730 days; 0.2 Sv – after 545 and 730 days; 0.5 Sv and 1 Sv – after 545 days ($p<0.05$); irrespective of the dosage frequency, the mitotic index reduced, and it increased ($p<0.05$) as the number of S-phase cells and the lymphocyte migration index decreased with the dose of 0.2 Sv after 365 days; 0.5 Sv and 1 Sv – 730 days ($p<0.05$) after a single exposure, and with a lower degree – 365 days after a fractionated dose, which defines the lymphocytes' regulative role in the regeneration processes, along with the signs of their morphogenetic activity.

References:

1. Vorontsova Z.A. An Evaluation of the Immune Status of the Mucous Membrane of the Jejunum in the Model Algorithm of γ-Radiation Effects / Z.A. Vorontsova, V.V. Zyuzina // Allergologia I Immunologia (Allergology and Immunology). – Moscow, 2011. – Vol. 12, №1. – P. 59

2. Grinevich Yu.A. The Immune and Cytogenetic Effects of High- and Low-LET Radiation: monograph / Yu.A. Grinevich, E.A. Demina / ed. by A.A. Yarilin. – Kiev: Zdorovia, 2006. – 200 p.

3. Koterov A.N. Low Doses and Low Ranges of Ionizing Radiation: Regulating the Ranges, the Criteria for their Formation, and the Reality of the XX Century // Meditsinskaya Radiologiya I Radiatsionnaya Bezopasnost (Medical Radiology and Radiation Safety). – 2009. – Vol. 54, № 3. – P. 5-26.

4. Khrupatchev A.G., Khadartsev A.A., Kashintseva L.V., Panova I.V. The Infrastructure of a Universal Computing System for Quantitative Assessment of Hidden Occupational Hazards // Vestnik Novykh Meditsinskikh Tekhnologiy (The Bulletin of New Medical Technologies). – Tula, TulGu, 2012. – № 1. – P. 47 – 49.