РАДІОБІОЛОГІЯ ТА РАДІОЕКОЛОГІЯ

УДК 577.352.315

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DOSE DEPENDENT REARRANGEMENT OF CELLULAR MEMBRANES INDUCED BY IONIZING RADIATION

The radiation-induced effects at dose rate of 0.35 Gy/min (in vivo) and of ultra-low doses (in vitro) on the cell membranes structural state were shown. The modifications of the membrane protein and lipid components and their dynamic state were revealed at experimental irradiation conditions by fluorescent probe analysis. The principal component analysis of the research data indicates the dose-dependent decrease of plasma membrane structural orderliness of the small intestine enterocytes with the increase of the ionizing irradiation acute dose of 0.5, 1.0, 2.0, 3.0 Gy at dose rate of 0.35 Gy/min. The complex response of the biological structure – the erythrocytes plasma membrane, on the ionizing radiation action at ultra-low doses that occurred through macromolecular structural rearrangements was also demonstrated. The features of the structural rearrangement of the cellular membranes depending on the ionizing radiation dose (dose rate) are found out.

Keywords: ionizing irradiation, dose rate, ultra-low doses, factor statistical analysis, cell membranes, structure.

Introduction

The investigation of biological action of ionizing radiation in low doses and dose rates, determination of mechanisms of origin of the induced effects are still actual nowadays [1 - 3]. The values of low doses are not equal for living systems of different levels of biological organization and depend on specific biological effect manifestation caused by acute and (or) chronic irradiation.

Today radiobiological researches have clearly proved that even low doses of radiation lead to numerous changes in the cells. And if the radiation intensity decreases, the maximal effect is observed at lower doses. The dose-effect dependence is not linear. Probably it is connected with great differences between the dose values which cause lesions in the biological objects or activate their recovery. While these systems don't function completely, the damaging effect increases in dose-dependent manner but then decreases when the recovering systems are fully activated. Besides, an effect again can be intensified with the dose increase, when damages prevail above renewal. In general the organism reaction to the irradiation depends on a dose, dose rates and exposure time. Even at lowest doses of low intensity the changes in the DNA structure and cellular membranes occur [1, 4] that are steady for a long time after irradiation.

There are no specific structures which could play a role of receptors for ionizing radiation in living systems. The influence of the radiation on different cells and tissues begins with ionization and excitation at atom level that triggers the processes that can be manifested on higher levels of biological organization: molecular (radiolysis of water and simple organic compounds), supramolecular (DNA breaks, lipid oxidation, enzyme inactivation), cellular (nucleus and membrane destruction), tissue (elimination of cell populations, morphological damage) and at the organism level [5, 6]. Thus the primary mechanisms of the ionizing radiation influence on biological objects consist in consecutive physical and chemical transformations: excitation, primary and secondary ionization, and as a result of it the free radicals production which can react with each other and with biomolecules [6, 7]. Various chemical disorders in the cell caused by ionizing radiation lead to the biological effect development.

Namely the biological membranes which directly participate in cell function maintenance and interaction with the environment initiate the cell response on the ionizing radiation influence, including low doses and dose rates [1, 8 - 10]. Physical and chemical radiation processes which occur in different cellular membranes are similar by their nature: the molecular transformation to free radicals, molecule destruction, molecule chemical modification etc. The final membrane response on the ionizing radiation influence depends on its structural peculiarities and cell environment. Besides, it shouldn't be forgotten that biomacromolecules, receptor areas of the biomembranes play a main role in the majority of biochemical and

biophysical processes. Local irradiation influence on these biomacromolecules and receptors areas is decisive for these effects [11].

Within the universally recognized fluid-mosaic model the membrane structure is being studied from the positions of dynamic properties and interconnections with membrane functions. The created physical model of the membrane shows not only heterogeneous membrane structure, but also protein and lipid dynamics and interactions in the membranes [12]. Unlike the lipid molecules which have the substantial lateral mobility the protein molecules are not so mobile. It can be explained by the protein association with other membrane proteins or cytoskeleton. Some proteins are able to rotate in the membrane plane; however, this process can be slowed down by the formation of protein aggregates. The lateral protein mobility is determined by both protein properties and microfluidity of the lipid environment. Thus, in the membrane the proteins have strong defined orientation which is named as the absolute asymmetry. Unlike the proteins the lipids migrate from one side of the membrane to another with high frequency (flip-flop mechanism) [12].

Scientists have given considerable attention to the investigations of biophysical properties of cellular membranes under the influence of lethal and sublethal doses of ionizing radiation. The results of these researches reviewed partly in several monographs [13 - 15].

The aim of this work consists in research of structural response of cellular membranes *in vivo* and *in vitro* at the single action of ionizing radiation in the low and ultra-low doses of different dose rates.

Materials and Methods

Radiation-induced structural modifications of cellular membranes under the influence of ionizing radiation of a wide dose range were evaluated by the fluorescent probes method (described in detail earlier in our numerous articles [16 - 18]). In particular, the parameters which described physical properties of the membrane surface areas, structural orderliness of lipid phase, and the spatial organization of protein-lipid complexes were used. In the performed researches it was taken into account that the most probable regions of the protein tryptophan residues localization were protein hydrophobic areas which could be both in the lipid and protein surrounding in the membrane [19, 20]. Also, it was assumed that ANS fluorescent probe (1-anilinenaphtalene-8-sulfonate) was localized at the lipid – water interface [19], and pyrene was localized in the region of phospholipid fatty acid chains [21] (Fig. 1).

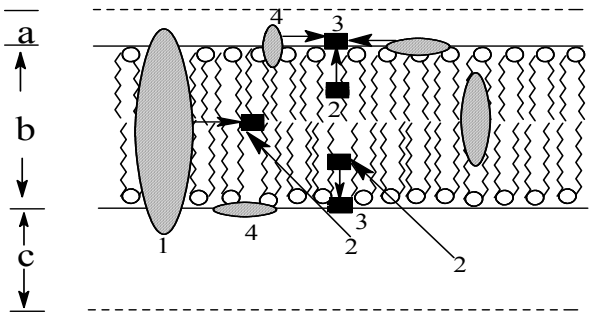

Fig. 1. Schematic representation of fluorophores preferential localization in the membrane (a, c - protein membrane phase, b - lipid membrane phase): *1*, *4* – protein tryptophan residues; *2* – pyrene molecules; *3* – ANS (1-anilinenaphtalene-8-sulfonate) molecules.

In the researches on acute X-irradiation white outbred rats were irradiated at doses of 0.5, 1.0, 2.0, 3.0 Gy (dose rate 0.35 Gy/min, 0,5 mm Cu and 1 mm A1 filter, strength of current 10 mA, voltage 200 kV, focal distance 50 cm). The enterocytes plasma membranes of rat small intestine [14] were used in the experiments.

The investigation of the irradiation ultra-low doses of low dose rate was carried out *in vitro* in the incubation medium which contained the suspension of erythrocyte membranes and β-emitter – ^{14}C-leucine [22, 23]. The incubation was performed for hour. The absorbed dose values of radiation were calculated according to Loevinger R. et al. [24]:

$$D_i = 21{,}3 \cdot E_\beta \cdot C_t \cdot t,$$

where D_i – the absorbed dose for time t, Gy; E_β – the mean value of the radionuclide radiation energy, MeV; C_t – specific radionuclide concentration (C_i/l); t – cell exposition time to radionuclide, hours; 21,3 – the experimental conversion constant of specific radioactivity (C_i/l) into absorbed dose units, Gy. The preliminary studies showed no effect of nonradioactive leucine on the investigated parameters. The radiation absorbed doses were from 10^{-8} to 10^{-5} Gy, and dose rates were from 10^{-8} to 10^{-5} Gy/ hr. The erythrocyte membranes were obtained from the blood of the conditionally healthy donors [23].

Results and discussion

In 1 day of acute X-irradiation the dose-dependent changes of studied structural parameters of the intestinal enterocyte plasma membrane were seen (Fig. 2). The more detailed analysis of the surface membrane area with the membrane bound fluorescent probe ANS have shown a reduction of the ANS fluorescence intensity (F_{ANS}) with the decrease of quantum yield (QY) (see Fig. 2, *a*). This fact can be explained by the changes of physical properties of the probe microenvironment in the

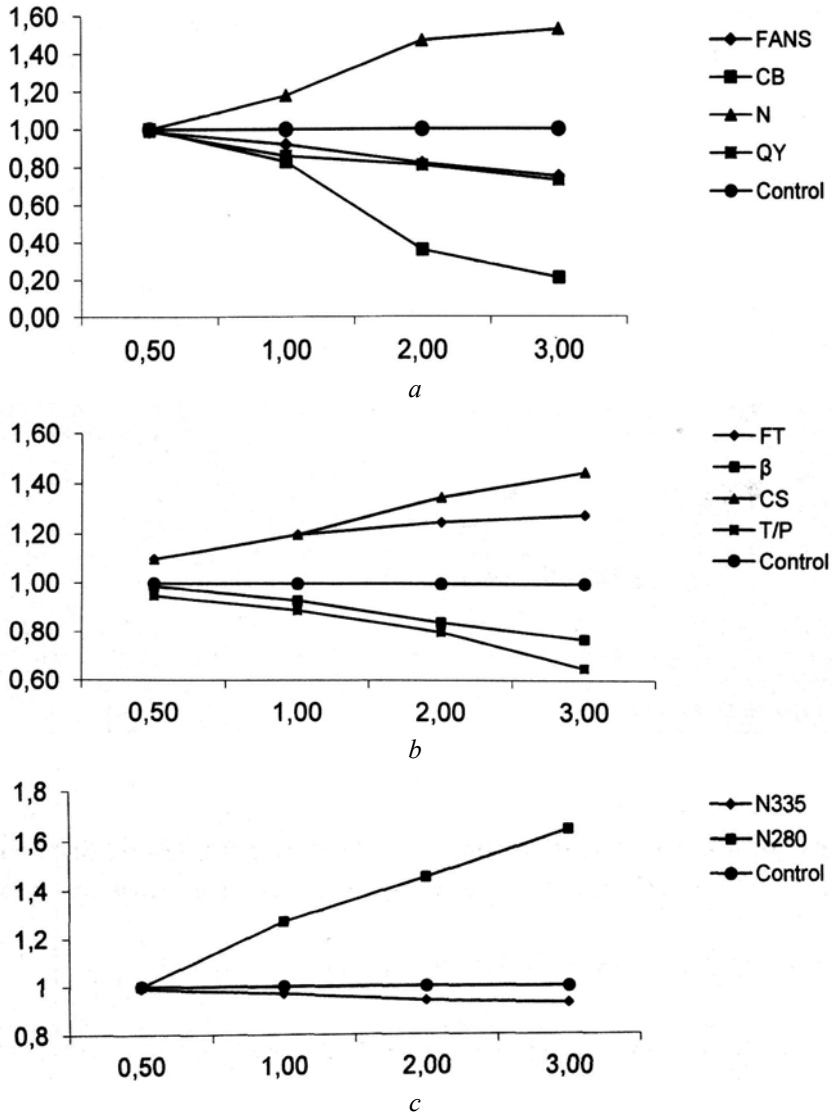

Fig. 2. The structural state parameters of the intestinal enterocyte plasma membrane under the ionizing radiation influence in doses of 0.5, 1.0, 2.0, 3.0 Gy and dose rate of 0.35 Gy/min. The fluorescence parameters of the membrane-bond ANS probe (*a*); the tryptophan fluorescence parameters (*b*); the parameters of the lipid component microfluidity (*c*). The values (relative units) are plotted on the ordinate axis. Radiation doses (Gy) are plotted on the abscissa axis. Notice: the intensity of the ANS fluorescence (FANS); the fluorescence intensity of tryptophan residues (FT); CB – the ANS binding constant (K_{ANS}) and N – the number of ANS binding sites; QY – quantum yield of the ANS fluorescence. N335 and N280 – the pyrene excimerization degree; CS – the Stern-Volmer constant (K_{SV}); β – the ratio of tryptophan residues; T/P – the $(F_o - F)/F_o$ value, which describes the IRET in tryptophan – pyrene pair.

membrane. The established oppositely directed changes of the number ANS binding sites and ANS binding constant (K_{ANS}) with a separate adsorption center (see Fig. 2, *a*) can be related to the modification of the membrane structure, which is defined both by changes of lipid components in the area of polar "heads" and glycerol residues of phospholipids and the changes of membrane proteins [19, 20]. On this basis conformational changes of membrane protein and lipid components can lead to the appearance of additional ANS binding sites considering that the fluorescence of mixed bilayer in a complicated fashion depends on its composition [19, 20].

The fluorescence intensity of protein tryptophan (Trp) residues of plasma membrane of intestinal enterocytes (see Fig. 2, *b*) shows dose-dependent increase up irradiation dose of 3.0 Gy. It can be caused by conformational changes of protein molecules as well as Trp residues transition in more hydrophobic area, since other spectral characteristics of tryptophan fluorescence (spectrum maximum and width) remain unchanged.

In control about 90 % of plasma membrane Trp residues are available for the quenching by the neutral polar acrylamide quencher. After irradiation a part of available residues (β) somewhat diminishes. Perhaps it happens because of Trp

screening in consequence of conformation changes of the protein matrix [25]. According to the diffusion mechanism the Trp fluorescence quenching by acrylamide indicates the possibility of quencher diffusion through the protein globule matrix or the fluctuations of the protein matrix which provides acrylamide penetration. Thus, the increase of effective quenching constant of the Trp fluorescence (K_{SV} – the Stern-Volmer constant), which changes reflect the protein intra-molecular dynamics, is probably caused by the decrease of protein molecule rigidity in the post-radiation period.

The revealed changes of the tryptophan fluorescence, such as the florescence intensity, the decrease of the Trp residue availability for the acrylamide quenching and K_{SV} increase, depend on conformational reorganization of protein molecules in the membrane, the increase of the protein intramolecular dynamics and the interaction nature of Trp residues with surrounding groups, because the Trp fluorescence is sensitive to the neighbor groups mobility [25, 26].

The microfluidity of the enterocytes plasma membrane is estimated by the reciprocal value to the degree of pyrene excimerization N ($N = F_{ex}/F_m$, where F_{ex} – the fluorescence intensity of pyrene excimers and F_m – the monomer fluorescence intensity) [21, 27]. The increase of the pyrene excimerization degree at $\lambda_{ex} = 280$ nm (N_{280}) indicates the decrease of the lipid phase microfluidity in the field near the protein phase (annular lipids), i.e. disturbance of hydrophobic interactions between lipid molecules and protein α-helix at the used irradiation conditions (see Fig. 2, *c*).

Spatial relationships between protein and lipid molecules in the membrane can be estimated by the method of inductive resonance energy transfer (IRET) [25] from a donor to acceptor molecules in fluorophore pair. The value $(F_o - F)/F_o$ is estimated as a result of the donor fluorescence quenching by the acceptor that reflects the IRET effectiveness (F_o – the donor fluorescence intensity in the absence, and F – in the presence of acceptor). The IRET reduction from Trp residues (a donor) to the pyrene fluorescent probe (an acceptor) gives evidence of the effectiveness decrease of the fluorescence quenching (see Fig. 2, *b*). Consideration must be given to the conformational changes of protein molecules that can affect this process in the membrane. Taking into account the IRET effectiveness and spectral characteristics of the membrane Trp fluorescence, the penetration of protein molecules into the lipid phase and/or conformational changes which lead to the protein association are typical for the plasma membrane in the post-radiation period.

Thus, the structural membrane order of enterocytes reduces if a single dose of ionizing radiation with dose rate of 0.35 Gy/min increases. The evidence of it is the decrease of the lipid microfluidity, the rise of intramolecular mobility of proteins and their conformational changes. It causes the disorder of protein-lipid interactions.

The structural state of the membrane surface layer of erythrocytes was evaluated using the ANS fluorescent probe as it was described earlier. The revealed changes of membrane-bound ANS fluorescence can be related to the processes of ANS interaction with the membrane and the change of fluorescence quantum yield of the probe. It can indicate the local structural reorganization of the membrane in the probe binding sites that is determined by both physical properties of its microenvironment (polarity and microfluidity) and the modification of membrane proteins or lipids and it occurs mostly at absorbed doses of 10^{-7} and 10^{-5} Gy (Fig. 3, *a*).

The changes of the membrane lipid microfluidity under the ionizing radiation influence have been estimated by the method of the independent microfluidity determination of bulk lipid phase and annular lipids (are located closer than 3 nm from the protein globule). Obtained results indicate the structural order changes of the membrane lipids depending on irradiation dose (see Fig. 3, *b*).

The revealed differently directed changes of the protein Trp fluorescence in the erythrocyte membranes (see Fig. 3, *c*) can be caused by both conformational reorganization of a protein molecule and the intramolecular protein dynamics as well as the nature of the Trp residues interaction with neighbor groups. In order to estimate the protein conformational changes under the influence of studying factors we detected the Trp fluorescence quenching by the outer neutral polar quencher acrylamide. Determined parameters of fluorescence quenching: the quenching constant value (K_{SV}) and the ratio of the available for quenching tryptophan residues (β), also changed depending on irradiation dose (see Fig. 3, *c*).

Researching IRET in fluorophore pairs it was also found out diverse changes of the $(F_0 - F)/F_0$ value. It indicates the changes of the energy transfer efficiency in the experimental conditions (see Fig. 3, *d*).

Relying upon the scheme (see Fig. 1) on IRET in fluorophore pair Trp – pyrene it is necessary to take into consideration that this parameter characterize the transfer of protein molecules in membrane lipid phase. By IRET value in fluorophore pair pyrene – ANS the thickness of membrane lipid component can be evaluated. The decrease of IRET efficiency as a result of the distance increase between these fluorophores reflects structural membrane

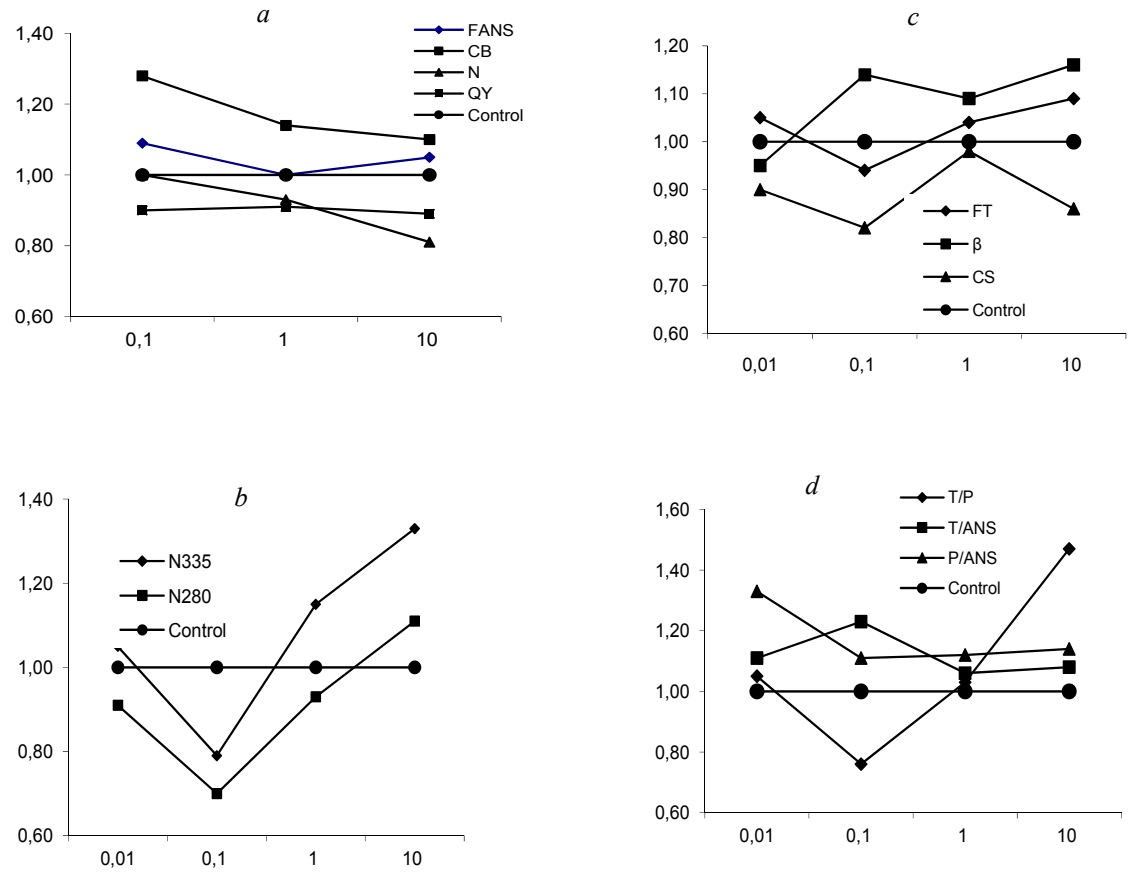

Fig. 3. The parameters of the erythrocyte membrane structural state under the ionizing radiation influence (β-radiation of ^{14}C-leucine) in vitro at absorbed doses of 0.01 to 10 μGy. Notice: T/ANS – the $(F_o - F)/F_o$ value, which describes the IRET in tryptophan – ANS pair; P/ANS – the $(F_o - F)/F_o$ value, which describes the IRET in pyrene – ANS pair and other designations as at the legend of the Fig. 2.

rearrangement leading to the increase of its effective thickness. The determination of IRET efficiency in Trp - ANS pair, that is localized at the interface of lipid – water and in protein phase showed differences of the value (Fo - F)/Fo that characterize IRET efficiency versus control parameter. Taking into account that critical distance in Trp - ANS pair is equal to 2,0 - 3,5 nm to membrane thickness of 4,0 nm it is clearly that more substantial contribution into energy transfer belongs to fluorophores situated on one side from lipid phase in membrane. Thus, changes of this parameter reflect the radiation-induced structural modification of surface membrane area.

In view of obtained results the increase of the degree of protein penetration into the membrane hydrophobic bilayer at the absorbed dose of 10^{-5} Gy can be noted. The decrease of the energy transfer efficiency in Trp – pyrene pair at the absorbed dose of 10^{-7} Gy may be the evidence of the rise of the protein exposure degree into the water phase and/or it may indicate the protein molecules aggregation, which leads to the distance enlargement between a donor and an acceptor. The obtained results correspond to the Trp fluorescence data at these irradiation doses. The represented results on the IRET effectiveness in Trp - ANS pair indicate the structural modification of surface areas of erythrocyte membranes at the absorbed dose of 10^{-7} Gy. Besides, the obtained results of the IRET effectiveness in Trp - ANS pair indicate the efficient thickness decrease of the membrane lipid component in the experimental conditions.

Thus, it should be noted the complex response of the biological structure – the erythrocytes plasma membrane, affected by ultra-low doses of ionizing radiation, that is implemented through the membrane macromolecular structural reorganization. It was discovered the diverse radiation effect on the protein and lipid membrane component at ultra-low doses. The structural order of the membrane lipid component increases at the absorbed dose of 10^{-7} Gy but it decreases at 10^{-5} Gy. The structural reorganization of protein molecules are accompanied by the increase of the tryptophan residues exposure on the membrane surface (at 10^{-7} Gy) or the exposure decrease (at 10^{-5} Gy). The revealed diverse changes of the membrane structural state

characteristics at the dose interval of 10^{-7} - 10^{-5} Gy indicate specificity of the membrane structural response on ultra-low dose radiation.

Taking into account a complex way of the cellular membrane reactions at ionizing radiation influence, the method of factor statistical analysis (the principal component analysis) was applied as it had been described earlier [10, 14].

Using this method primarily the sample of such transformed indexes was updated which determine factors (a_k), that provide the maximally complete description of correlation between studied parameters at their minimal number. Thus, it is assumed that there are a few "significant" parameter changes which really determine the state of the research object among all statistical links between data. To estimate the state of research object it is possible to confine the determination just by few main factors that greatly facilitate classification by their significance.

The factor analysis results are represented in the geometric interpretation [28, 29]. For it the sample correlation matrix and the principal components model are formed on the basis of the initial sample (a set of statistical indicators of the obtained parameters values which are classified by their relationship with definite parameter subset). Among these components a relatively small number p is selected by their own values. Further the p-dimensional space is constructed. The coordinates of this space are the expansion coefficient $a_k^{(i)}$ of vector data for each i-test object by the corresponding factors. Each of researched parameters is represented by a point in this p-dimensional space. Close parameters group in the coordinate space and form the area which belongs to the marked class. The mass center of each group, which is called as the centroid, characterizes the average value of expansion coefficients for objects with similar characteristics.

The analysis of the research results of the structural state of intestinal enterocyte and erythrocyte membranes were carried out by the principal components method. Each of experimental parameters was normalized to appropriate control values. The obtained investigation results were represented in the space of the features of the first four principal components (a_1, a_2, a_3, a_4).

The grouping results of research parameters of the plasma membrane structural state of the small intestine enterocytes under the acute ionizing radiation influence with dose rate of 0,35 Gy/min indicate the quite expressed grouping depending on radiation dose (Fig. 4).

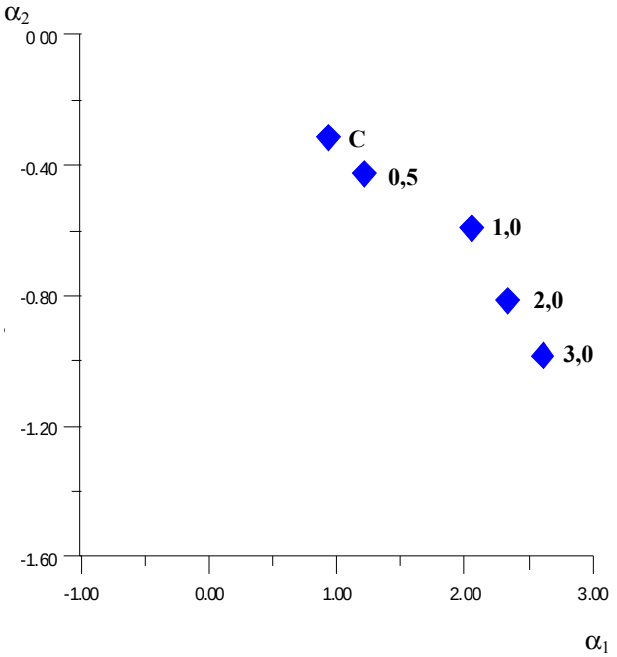

Fig. 4. The space grouping of principal components a_1 - a_2 of the structural state parameters of the intestinal enterocyte membrane under the acute ionizing radiation influence. Notice: C – control; the radiation dose of 0.5, 1.0, 2.0 and 3.0 Gy.

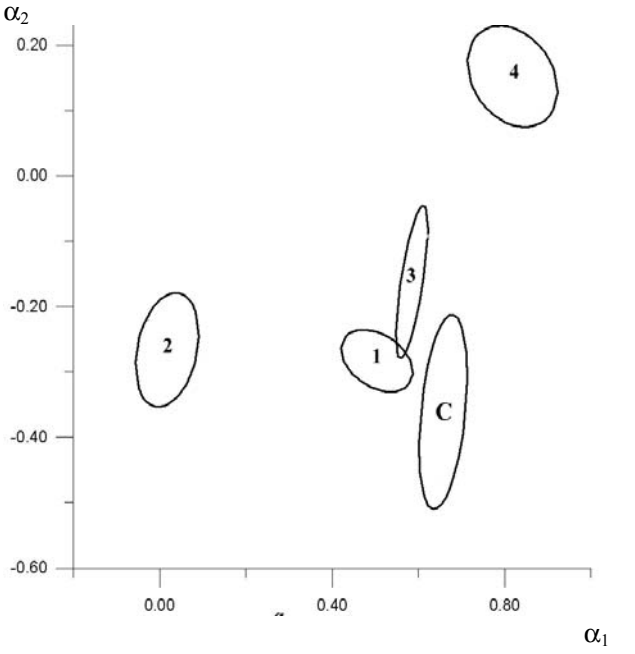

Fig. 5. The space grouping of principal components a_1 - a_2 of the structural state parameters of the erythrocyte membrane under the acute ionizing radiation influence. Notice: C – control; the radiation dose of 10^{-8} Gy (*1*); 10^{-7} Gy (*2*); 10^{-6} Gy (*3*) and 10^{-5} Gy (*4*).

Also the analysis of the grouping results of structural parameters of the erythrocyte membrane under the ionizing radiation influence indicates their evident grouping in the experimental conditions (Fig. 5). The grouping of the membrane state projections mostly occurs by the a_1 component if the

dose rate increases from 10^{-8} to 10^{-7} Gy/hr, but if the dose rate increases from 10^{-6} to 10^{-5} Gy/hr it occurs by the a_2 component. The main contribution in determination of the research objects state by the a_1 and a_2 components is introduced by the different parameters which variety causes the differences in the membrane structural modification depending on irradiation dose and dose rate.

Thus, there is no monotony in the dependence of the membrane structural state on irradiation dose (and dose rate) in conditions of acute irradiation at the dose rate of 10^{-8} to 10^{-5} Gy/hr. Moreover, in such conditions the radiation-induced effect is observed only under the ultra-low dose influence. The using of the mathematical approach has allowed us to reveal the peculiarities of the ionizing radiation influence on the cellular membrane structure.

Conclusion

The specificity of the ionizing radiation biological effect consists in ionization and excitation of atoms and molecules with subsequent formation of highly reactive peroxides and free radicals. Naturally, quantitatively such changes depend on irradiation dose, i.e. absorbed energy, but they appears at any dose. Thus, the membrane structural modification can occur as a result of direct action on membrane protein and lipid components as well as can be mediated by oxidative processes.

At single action of ionizing radiation at doses of 0.5, 1.0, 2.0, 3.0 Gy with dose rate of 0.35 Gy/min the structural order of the enterocytes plasma membrane changes in dose-dependent way. Thus taking into account, that complete renewal of indexes which characterize the structural state of membranes of enterocytes is observed in 14 days [14 - 16], the activation of protective and recovering processes in cells takes place in the certain interval of time after irradiation. At ultra-low doses of ionizing radiation at the dose rates of 10^{-8} to 10^{-5} Gy/hr the monotony in dependence of the structural state of membranes on the dose rate of irradiation is absent which testifies to complex character of dose-effect, and the removal of this field results in recovering of the tested indexes. Such trends indicate the contribution of the membrane disorders into cellular damage at ultra-low doses of ionizing irradiation.

Nowadays the following features of physical factors effect at low and ultra-low doses are distinguished. Nonmonotonic polymodal dose-effect dependence. The maximal activity is observed only at the definite dose interval: at ultra-low doses the effect increases, if the dose raises the effect decreases and then it rises again. The effect enhancement occurs with intensity decrease within the certain dose and dose-rate intervals. Many similar features of cellular metabolism response upon the action both of the biology active substances in low doses and of the physical factors of low intensities were found out. Such phenomenon can be explained by the high susceptibility of targets including cellular membranes, and also by peculiarities of the reaction kinetics where the main role belongs to the weak interactions.

REFERENCES

1. *Eidus L.Kh.* Membrane mechanism of biological influence of low doses. - M.: Inst. Theor. & Exp. Physics Publishers, 2001. - 81 p.
2. *Burlakova E.B.* New aspect of regularites in the action of low doses of low-level irradiation. In Low doses of radiation: are they dangerous? / Ed. E. B. Burlakova. - N.Y.: Nova Science Publishers, Inc. Hungtington, 2000. - Ch. 1. P. 1 - 14.
3. *Baverstok K.* Radiation- induced genomic instability: a paradigm-breaking phenomenon and its relevance to environmentally induced cancer // Mutat. Res. - 2000. - Vol. 454, No. 1 - 2. - P. 89 - 109.
4. *Eidus L.Kh.* About the induction mechanism of DNA destruction reparation at the ionizing radiation influence on the cells // Radiation Biology and Ecology. - 2000. - Vol. 40, No. 6. - P. 674 - 677.
5. *Kudryashov Yu.B.* Main principles in radiobiology // Radiation Biology and Ecology. - 2001. - Vol. 41, No. 5. - P. 531 - 547.
6. *Kudryashov Yu.B.* Radiation biophysics (ionizing radiation). - M.: Phys. Mat. Lit., 2004. - 448 p.
7. *Kutlakhmedov Yu.O., Voitsitsky V.M., Khyzhnyak S.V.* Radiation Biology. - K.: Kyiv University, 2011. - 543 p.
8. *Benderitter M., Vincent-Genod L., Berroud A. et al.* Radio-induced structural membrane modifications: a potential bioindicator of ionizing radiation exprosure // Int. J. Radiat. Biol. - 1999. - Vol. 75. - P. 1043 - 1053.
9. *Choudhary D., Srivastava M., Sarma A., Kale R.K.* Effect of higt linear energy transfer radiation in biological membranes // Radiat. Environ. Biophys. - 1998. - Vol. 73. - P. 177 - 185.
10. *Khyzhnyak S.V., Voitsitsky V.M.* Radiation-induced structural metabolic modification of enterocytes and lymphoid tissue / Ed. by Ukraine NSA academician M. E. Kucherenko. - K.: Phytosociocenter, 2006. - 245 p.
11. *Schmidt-Ullrich R.K., Dent P., Gramt S. et al.* Signal transduction and cellular radiation responses // Radiat. Res. - 2000. - Vol. 153. - P. 245 - 257.
12. *Gennis R.B.* Biomembranes. Molecular structure and function. - M.: Mir, 1997. - 624 p.
13. *Ryskulova S.T.* Radiation biology of plasmatic membranes. - M.: Energoatomizdat, 1986. - 126 p.
14. *Kycherenko M.E., Khyzhnyak S.V., Vekslyarsky R.Z., Voitsitsky V.M.* Small intestine enterocytes and

radiation. - K: Phytosociocenter, 2003. - 176 p.
15. *Khyzhnyak S.V.* Radiosensitivity of the small intestine epithelium cells. - K.: Kyiv University, 1997. - 148 p.
16. *Khyzhnyak S.V.* The comparative study of physical properties of apical and basolateral membranes of small intestine enterocytes affected by ionizing radiation // Ukr. Biochem. J. - 1998. - Vol. 70, No. 1. - P. 44 - 48.
17. *Khyzhnyak S.V., Kysil O.O., Laposha E.A. et al.* The chronic ionizing radiation influence on the structural properties of the apical and of the mitochondrial membranes of small intestine enterocytes // Radiation Biology. Radioecology. - 2006. - Vol. 46, No. 1. - P. 27 - 33.
18. *Kysil O., Khyzhnyak V., Klepko A., Voitsitskiy V.* The structural and functional state of enterocyte mitochondria under chronic and single ionizing irradiation In book "Current problems of radiation research". - K.: Ukraine NSA, 2007. - P. 134 - 151.
19. *Dobrecov G.E.* Using of fluorescence probes in cell, membrane and lipoprotein study. - M.: Nauka, 1989. - 277 p.
20. *Vladimirov Yu.A., Dobrecov G.E.* Using of fluorescence probes in biological membrane study. - M.: Nauka, 1980. - 320 p.
21. *Dergunov A.D., Kaprelyanc A.S., Kabishev A.A. et al.* The existence of boundary lipids in the reconstructed hydrophobic protein-lipid system // Biochemistry. - 1982. - Vol. 47, No. 2. - P. 296 - 303.
22. *Voitsitskiy V.M., Khyzhnyak S.V., Zhirnov V.V., Laposha O.A.* The structural organization of erythrocyte membranes at the action of ionizing radiation at low doses // Physics of Alive. - 2008. - Vol. 16, No. 2. - P. 58 - 64.
23. *Zhirnov V., Khyzhnyak S., Voitsitskiy V.* The effects of ultra-low dose β-radiation on the physical properties of human erythrocyte membranes // Int. J. of Radiation Biology. - 2010. - Vol. 86, No. 6. - P. 499 - 506.
24. *Loevinger R., Budinger T.F., Watson E.E.* MIRD primer for absorbed dose calculations // New York: Society of Nuclear Medicine. - 1991. - 41 p.
25. *Demchenko A.P.* Luminescence and dynamics of protein structure. - K.: Naukova dumka, 1988. - 280 p.
26. *Lactovich J.* Principles of fluorescence spectroscopy. - M.: Mir, 1986. - 321 p.
27. *Ehutkyn G., Jakubowski S., Hatsko G.G.* The physical and chemical state of plasmatic membranes of rats' adipose tissue and liver in the definite periods after γ-irradiation at the dose of 1 Gy // Radiation Biology. Radioecology. - 1993. - Vol. 33, No. 1. - P. 61 - 65.
28. *Iberla K.* Principal component analysis. - M.: Mir, 1972. - 316 p.
29. *Kramer G.* Mathematical methods of statistics. - M: Mir, 1975. - 648 p.

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ІНІЦІЙОВАНА ІОНІЗУЮЧОЮ РАДІАЦІЄЮ ДОЗОЗАЛЕЖНА РЕОРГАНІЗАЦІЯ КЛІТИННИХ МЕМБРАН

Показано радіаційно-індукований ефект іонізуючого випромінювання потужністю 0,35 Гр/хв (опромінення *in vivo*) та надмалих доз *(*опромінення *in vitro)* на структурний стан клітинних мембран. Методом флуоресцентних зондів установлено модифікацію білкової та ліпідної компонент клітинних мембран та їхнього динамічного стану, що має місце при опроміненні в досліджуваних дозах. Методом факторного аналізу виявлено дозозалежні зміни структурної впорядкованості плазматичної мембрани ентероцитів тонкої кишки за дії іонізуючої радіації в дозах 0,5; 1,0; 2,0; 3,0 Гр потужністю 0,35 Гр/хв. Водночас спостерігається складна відповідь плазматичної мембрани еритроцитів на дію надмалих доз іонізуючої радіації (10^{-8} - 10^{-5} Гр), яка реалізується через макромолекулярні структурні перебудови мембрани. Виявлено особливості структурних перебудов клітинних мембран залежно від дози (потужності) іонізуючої радіації.

Ключові слова: іонізуюча радіація, потужність опромінення, надмалі дози, метод факторного аналізу, клітинні мембрани, структура.

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ИНИЦИИРОВАННАЯ ИОНИЗИРУЮЩЕЙ РАДИАЦИЕЙ ДОЗОЗАВИСИМАЯ РЕОРГАНИЗАЦИЯ КЛЕТОЧНЫХ МЕМБРАН

Показан радиационно-индуцируемый эффект ионизирующего излучения мощностью 0,35 Гр/мин (облучение *in vivo*) и ультрамалых доз (облучение *in vitro*) на структурное состояние клеточных мембран. Методом флуоресцентных зондов установлена модификация белковой и липидной компонент клеточных

мембран, их динамического состояния, которое имеет место при облучении в исследуемых дозах. Методом факторного анализа выявлено дозозависимое изменение структурной упорядоченности плазматической мембраны энтероцитов тонкой кишки при действии ионизирующей радиации в дозах 0,5; 1,0; 2,0; 3,0 Гр мощностью 0,35 Гр/мин. Наблюдается сложный ответ плазматической мембраны эритроцитов в поле действия ультрамалых доз (10^{-8} - 10^{-5} Гр) ионизирующей радиации, реализуемый через макромолекулярные структурные перестройки мембраны. Отмечены особенность структурных перестроек клеточных мембран в зависимости от дозы (мощности дозы) ионизирующей радиации.

Ключевые слова: ионизирующая радиация, мощность дозы, ультрамалые дозы, метод факторного анализа, клеточные мембраны, структура.

Надійшла 24.02.2014
Received 24.02.2014