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Dependence of Erythrocyte Shape Parameter on the Low Dose γ-Irradiation

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Abstract. In this work it is experimentally shown that depending on the dose of irradiation the shape parameters of erythrocytes population of peripheral blood exhibit specific changes in the early post-radiation period. As a result of low dose irradiation (35μSv/h) during 5 minute by ^{226}Ra, the rigidity of erythrocytes membrane was occurred, which was accompanied by the decreasing of cell area. In case of longer irradiation (15 minute) the instability of membrane was occurred in re-swelling manner but membrane rigidity was maintained. Thus, such erythrocyte parameters as area and perimeter cannot characterize the state of erythrocyte. According to that, a new shape parameter (α) was suggested by us to assess the state of erythrocyte. It correlates those two parameters and provides the opportunity to determine more accurately the dose dependent shape changes of erythrocytes.

Keywords: erythrocytes, radiation, rigidity, shape parameter, perimeter, area

Introduction

All living organisms are continuously exposed by background ionizing radiation coming from natural sources such as radioelements in the soil or cosmic radiation, as well as man-made ionizing radiation (medical procedures, consumer products etc.).

Erythrocyte is a suitable candidate for monitoring the radiation effect for many reasons. First of all, it is a representative sample for the whole body exposure, since it circulates all over the body, second, it is accessible and easy in its separation to obtain cells with intact membrane. Additionally, being annucleated, it represents a useful model for measuring the membrane properties without the interference of intracellular membranes. Normal red blood cells (RBCs), at a rest condition have shapes close to a circle, but when they are subjected to certain conditions, they have the ability to undergo strong deformations [1, 2]. Erythrocyte deformability is altered under various pathophysiological conditions such as diabetes, vascular diseases, sepsis etc. It was shown that gamma irradiation also leads to deformation of RBC and rigidity of membrane was recorded [3]. A rigid cell would greatly increase the blood viscosity. Thus, the deformability of the red blood cell membrane is one of the conditions for its viability. This deformability is determined by the molecular and osmotic state of the cell [4].

This work intends to study the radiation effects of low doses of γ-radiation (35μSv/h) on the RBC shape parameters. The dose rate of 35μSv/h is only about 200 times higher than that of the background radiation which is about 0.18μSv/h. Usually the dose rates investigated exceed this value by hundred of orders of magnitude. Much less is known about the cellular response to low doses of ionizing radiation such as those typical for medical diagnostic procedures, normal occupational exposures or cosmic-ray exposures at flight altitudes, and there are only a few reports on their action on RBCs [5]. Basic research data and human epidemiological data show that cellular responses to low absorbed doses of ionizing radiation cannot be predicted by extrapolating from the responses to high doses.

It is well known that gamma-irradiation of erythrocytes induces alterations at the three different functional units of the membrane: 1 – Lipid bilayer, 2 – Protein components, 3 – Cytoskeleton at the membrane surfaces.

Literature data and experimental findings reinforce our understanding that the cell membrane is a significant biological target of radiation. Thus, the role of the biological membrane in the expression and course of cell damage after radiation exposure must be considered.

Thus, the aim of present work is to investigate the low dose-dependent changes of erythrocytes shape parameters in the early post-radiation period, as well as to find out a suitable parameter to assess more accurately the state of erythrocyte after gamma irradiation.

Material and Methods

Adult male Wister rats weighing 200g were used. Rats were kept under standard conditions along the experimental period, 12/12 h light-dark regimen. Food and water were supplied daily *ad libitum*. One milliliter of blood was taken immediately into heparinized vial. Erythrocytes were isolated from blood via centrifugation in phosphate buffer, pH 7.4 using a standard method [6]. The blood was centrifuged (4500 rpm, 4ºC) for 20 min and the pellets were collected. Pelleted cells were suspended in phosphate buffer pH 7.4 (NaH_2PO_4/Na^2HPO_4, 200 mM) and centrifuged again (4500 rpm, 4 ºC). This procedure was repeated 3 times. Washed RBCs were suspended in the physiological solution and investigated under MEIJI (Japan) microscope connected to PC. Experimental data were analyzed by NOVA 3.5.PC program. Areas and perimeters were quantified for control and experimental samples.

^{226}Ra was used as the source of γ rays. Its activity was $2.7x10^4$ Bq and the dose rate was 35μSv/h. Dose rate was measured before and after each treatment by IdentiFINDER R400 dosimeter. A special box was constructed in order to hold inside all alpha particles emitted by the ^{226}Ra during its alpha decay. That is giving the opportunity to subject experimental samples to gamma rays.

1 ml of erythrocyte suspension was filled into specially prepared glass vials (with 24/24/5 mm dimension) and placed under radiation source for 5 and 15 minutes. Control samples were in the same room condition but under natural radiation background (18μRoentgen/h or 0.18μSv/h).

Results and Discussion

The treatment of experimental samples by γ rays during 5 and 15 minutes leads to the deformation of erythrocytes in both cases and the rigidity of cells was mentioned after irradiation (Fig. 1).

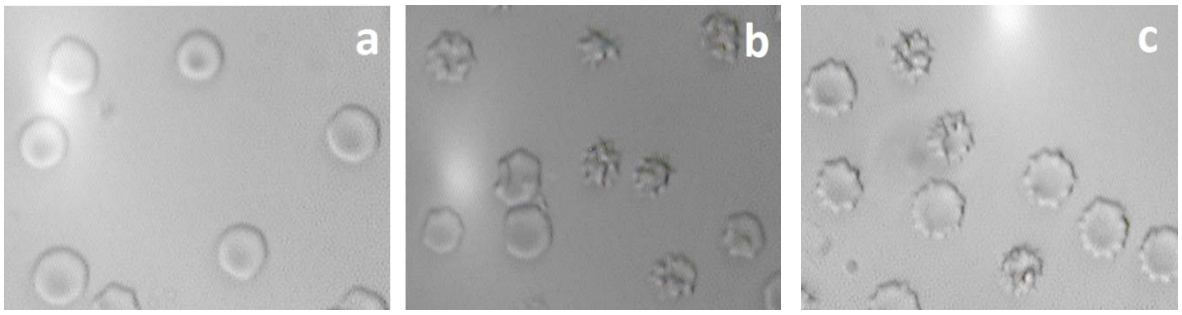

Fig. 1. Gamma radiation-induced rigidity of rat erythrocyte shapes. Typical views of erythrocyte in control (a), 5 minute radiated by ^{226}Ra (b), 15 minute radiated by ^{226}Ra (c).

As a result of 5 minute irradiation the samples by gamma source the instability was occurred in the membrane of erythrocytes which leads to the leakage of intracellular substances from the cell. Similar effect of irradiation by X-ray was recorded by Suzuki et al. [4], according to which RBCs were impaired by irradiation mainly due to dehydration.

Although, in case of 5 minute the increased rigidity is accompanied by the decreasing of their areas by 21.68% (Fig. 2a and 2b), the exposition of samples during 15 minute does not bring to the statistically significant changes of the cell areas as compared to control samples (Fig. 2a and 2c). It means that in this case the instability of membrane was occurred as re-swelling manner of erythrocyte up to its initial area value, which can be explained by the producing the additional charges on the surface of the membrane, leading the increasing of membrane surface potential. As produced charges are similar, so the repulsive forces can modify the tilt of phospholipids' dipole fragment and, as a result, the above mentioned changes take place.

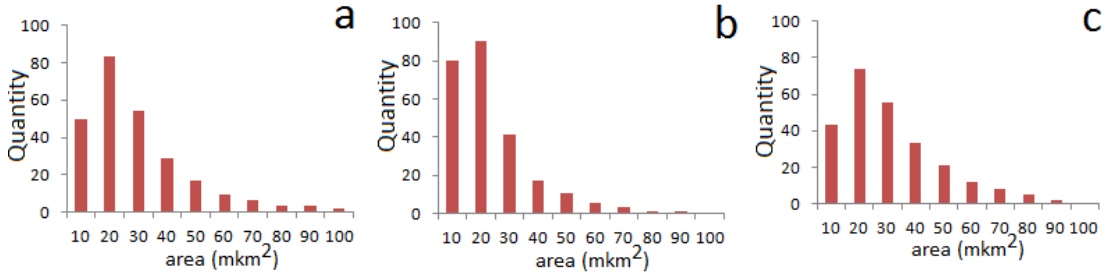

Fig. 2. Variation histograms of gamma radiation-induced changes of rat erythrocytes' areas. (a) control, (b) 5 minute exposed to ^{226}Ra , (c) 15 minute exposed to ^{226}Ra.

Another picture is observed at calculation of cell perimeters. As it can be seen from Fig. 3, the 5 minute treatment of experimental samples decreased the cell perimeter by 14.06%, while 15 minute treatment conversely increased that parameter by 14.69% as compared to control one.

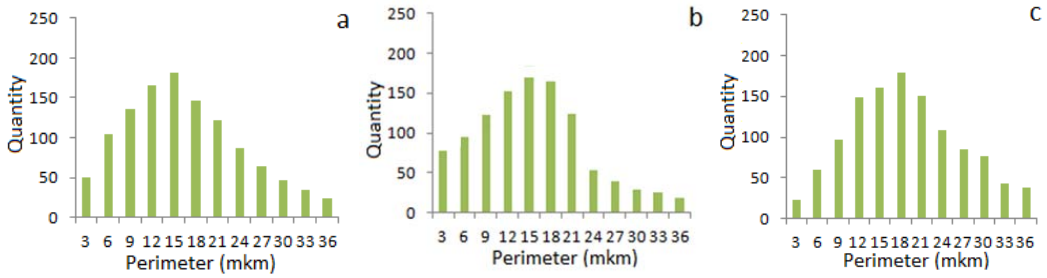

Fig. 3. Variation histograms of gamma radiation-induced changes of rat erythrocytes' perimeters. (a) control, (b) 5 minute radiated by ^{226}Ra, (c) 15 minute radiated by ^{226}Ra.

According to our experimental results, 5 minute treatment-induced dehydration or decreased area of RBC was accompanied by the decreasing of its perimeter (but not proportionally), while 15 minute irradiation-induced re-swelling up to its initial value of area also followed with increased perimeter value (membrane rigidity remains in this case).

Experimental data support the assumption that various parameters of RBC are responsive to radiation in a different rate and manner. According to this statement, it might be concluded that any single parameter of erythrocyte cannot separately describe the state of the cell.

To solve this imbroglio a new shape parameter **(α)** was suggested by us, which can be characterized by the following equation:

$$\alpha = \frac{S}{P^2},$$

where S – is an area of erythrocyte, P – is perimeter.

For erythrocytes at rest condition when they have shapes close to a circle, the shape parameter α will be varied near the value of 0.0796 ±3 %, i.e. 0.077-0.082.

Any deflection from this range will specify the deformation of erythrocyte.

Using that equation in calculation of (α) indexes for experimental findings, the following chart was observed (Fig. 4).

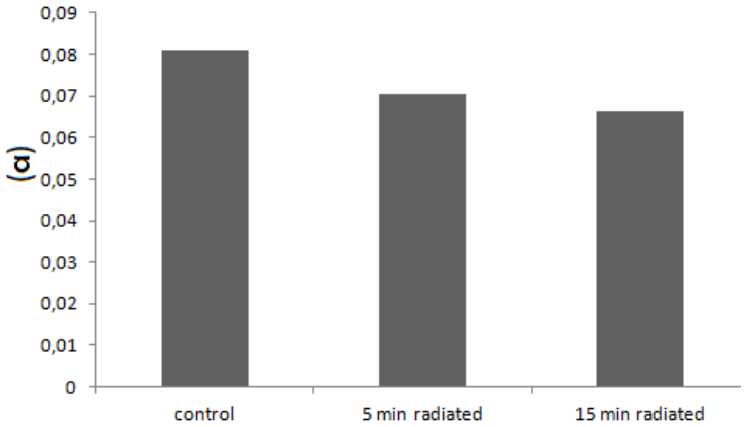

Fig. 4. 5 and 15 min ^{226}Ra gamma radiation-induced changes of rat erythrocytes' shape parameter (α) as compared to the control

As it can be seen from Fig. 4, after 5 and 15 minutes treatment by γ source the shape parameter is decreased in both cases. It is worth to note that 15 minutes treatment decreased α by 18% even though the areas value of this sample didn't exhibit any statistically significant changes as compared to control samples.

Thus, the new shape parameter is more informative and more accurately could describe the cell state after γ irradiation.

Conclusion

As a result of low dose irradiation (5 minute), the instability of membrane was occurred, which leads to the leakage of intracellular substances from cell via trans-membrane osmotic pressure changes.

In case of longer irradiation (15 minute), the instability of membrane was occurred in re-swelling manner, which can be explained by the producing of additional charges on the membrane surface, leading to the increasing of membrane surface potential. If the produced charges are similar, then the repulsive forces are able to modify the phospholipids' dipole fragment tilt and the above mentioned changes take place.

Suggested new shape parameter (α) determines the dose dependent shape changes of erythrocytes.

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