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DIAGNOSTICS OF HIDDEN DAMAGE IN BIOLOGICAL MEMBRANE UNDER EXPOSURE TO LOW-DOSE γ-RADIATION

E. K. Kozlova, A. P. Chernyaev, P. Yu. Alekseeva, A. M. Chernysh, A. A. Dolgopolova, and M. A. Nazarova

Experimental data on the effect of low doses of gamma radiation on the membrane of human erythrocytes are presented. Hidden damage of erythrocyte membrane after exposure to ^{226}Ra has been detected by applying a calibrated electric pulse. This method is compared with the classical method for assessing the state of a membrane by measuring osmotic fragility. A mathematical model for adequate description of experimental results is proposed.

Studying a biological membrane as an object for exposure to radiation is a vital problem of modern physics and biophysics [1, 2]. The objective of this work has been the experimental investigation of hidden changes in biophysical properties of membranes under the effect of low-level gamma radiation. The method of calibrated electroporation of membranes used in the investigations makes it possible to evaluate changes in membrane properties at the early stages of their damage [3, 4]. The data of the experiments are compared with the experimental results obtained by measuring the osmotic fragility.

EXPERIMENTAL METHODS AND RESULTS

One hour after the preparation the human erythrocyte suspension (3 ml) was irradiated for 30 min with gamma-quanta from a ^{226}Ra source. The absorbed radiation dose was 2.5 R. After the irradiation the suspension was exposed to a pulsed electric field in the solution (the field strength $E = 1700$ V/cm, pulse duration 6 ms) which caused electroporation of erythrocyte membranes. The effect of γ-radiation was assessed by the erythrocyte hemolysis rate. The erythrocyte suspension preparation procedure and selection of operating parameters of ionizing radiation, electrical field and temperature are presented in detail in [3, 4].

The kinetics of changes in the state of a membrane during several days after preparation of the erythrocyte suspension is illustrated in Fig. 1. Figure 1*a* shows the rate of decrease in the number of erythrocytes as a function of time, $V(t)$, for previously irradiated and nonirradiated suspensions exposed to pulsed electric field. The rate at which the number of erythrocytes decreases is $V = \Delta n/\Delta t$, where Δn is a change in the number of erythrocytes in a time interval after the pulsed electric field application, and $\Delta t = 20$ min. The rate of hemolysis caused by electroporation increased on the first day after irradiation, decreased on the second and third days, and increased again on the fourth day (curve *1*). For comparison, the figure presents respective results for a nonirradiated control suspension (V_{control}, curve *2*). The trend of the curves for exposed and nonexposed suspensions is identical, but curve *1* is shifted relative to curve *2* for one day along the time axis.

Figure 1*b* shows the dependence of the ratio of the rates, $V_\gamma/V_{\text{control}}$, on time after preparation of the suspension. On the first day $V_\gamma > V_{\text{control}}$. In our experimental conditions $(V_\gamma/V_{\text{control}}) = 1.6 \pm 0.2$ ($\alpha = 0.95$). On the second and third days, the inverse effect was observed: $V_\gamma < V_{\text{control}}$. We note that during the first five days the optical density of the irradiated and nonirradiated suspensions did not change and was equal to the initial density ($D_0 = 1$).

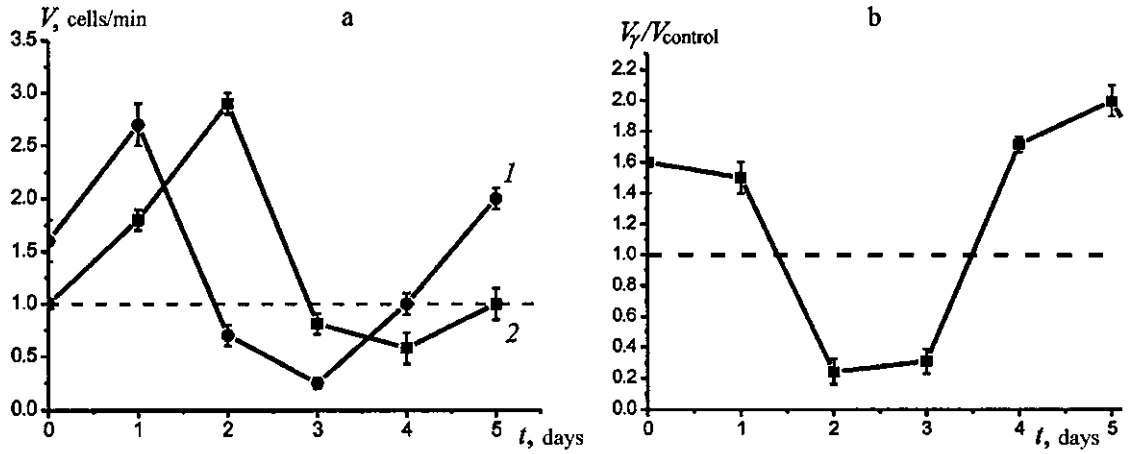

Fig. 1

Rate of change of erythrocyte number caused by electroporation as a function of time interval after suspension preparation, $V(t)$: (a) irradiated suspension (1), nonirradiated suspension (2) (the rate of change for control suspension on the day of preparation is taken as unity rate); (b) ratio of rates, $V_\gamma/V_{\text{control}}$. ^{226}Ra source activity $A = 9.25$ mCi, radiation dose 5 R, temperature $t = 20\,^\circ$C. In all experiments optical density of suspensions before electroporation was equal to unity.

The method of electroporation revealed hidden effects of γ-radiation on membranes as early as on the first day after exposure. Our method of detecting hidden damages was compared with the classical technique of assessing the state of a membrane by measuring osmotic fragility which is determined by the concentration $C_{50\%}$ of NaCl when 50% of initial erythrocytes are hemolyzed [5]. However, this method has not shown any significant difference between the osmotic fragility of irradiated and nonirradiated suspensions on the first or subsequent days (Fig. 2). Osmotic fragility decreased in the same way for both suspensions on the first and fifth days when the optical density of suspension dropped to below unity.

MODEL

The number and radius of pores that are formed in a membrane exposed to a pulsed electric field depend on an induced transmembrane potential and the threshold electrical-breakdown potential φ_p. Gamma radiation changes local properties of biological membranes and, hence, φ_p. Let us isolate a unit area on the membrane surface and divide this area into N equal regions. A pore may be formed in each of these regions. The number of regions N is large. Formation of a pore in each region depends on the properties of this region. The membrane of an erythrocyte is initially heterogeneous in its electrochemical properties. The reason for this heterogeneity is proteins, various lipid species, interfaces between molecules, ionic channels, water pores, asymmetry of lipids at the external and internal sides of a cell, and defects in the membranes. Therefore, potentials φ_p are different in different regions of a membrane. The properties of each of N regions are determined by two electroporation parameters: φ_p and a pore radius. The assortment of macroscopic unit areas of a membrane, N, can be considered to be a statistical ensemble. We select volume $d\varphi_p$ near point φ_p in the phase space of states. At the given time instant this volume contains points characterizing the states of dN ensemble systems out of their total number N. Then the limit of their ratio $\lim\limits_{N\to\infty}\left(\frac{dN}{N}\right) = f_N(\varphi_p, t)\,d\varphi_p$ determines the distribution density (statistical distribution function) of microscopic states of systems in the ensemble at the time instant t. To calculate the total area of the pores formed we should find the integral

$$S = \frac{N}{\sigma\sqrt{2\pi}} \int\limits_{\varphi_{\min}}^{\varphi} \exp\left(-\frac{(\varphi_p - \varphi_{p\,\text{av}})^2}{2\sigma^2}\right)\left(\frac{k}{\varphi_p}\right)^2 d\varphi_p,$$

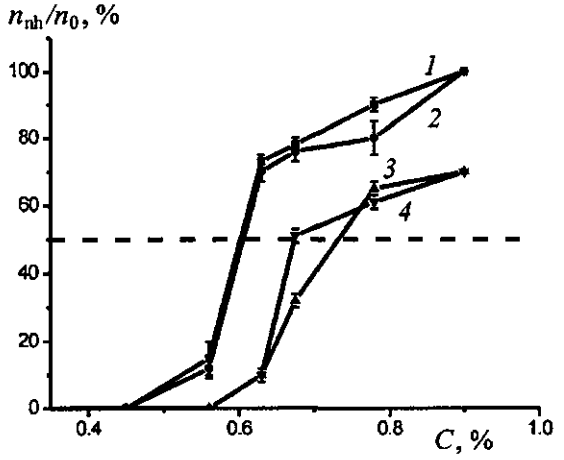

Fig. 2

Percentage of nonhemolyzed erythrocytes in suspension (n_{nh}/n_0) as a function of NaCl concentration (C). First day: (*1*) control suspension, (*2*) suspension after irradiation. Fifth day: (*3*) control suspension, (*4*) suspension after irradiation.

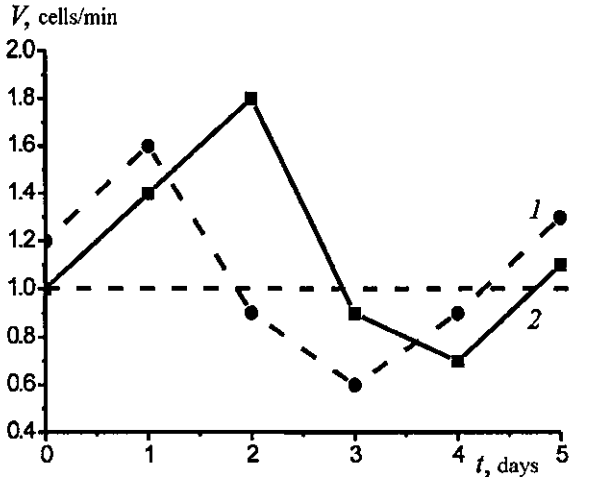

Fig. 3

Theoretical curves of rate of change of erythrocyte number caused by electroporation versus time after suspension preparation, $V(t)$, for (*1*) irradiated and (*2*) nonirradiated suspensions. The rate for control suspension on the day of preparation is taken as unity ($\lambda = 0.013$ day^{-1}, $t_q = 3$ days, $t_{q\gamma} = 2$ days, $\sigma_q = 1$ day).

where $\varphi_{p\,\mathrm{av}}$ is the membrane-averaged threshold potential, $\varphi_{\min}$ is the minimal potential at which one pore is formed in a membrane, and k is the coefficient characterizing the relation of a pore radius to the threshold potential in the given membrane region.

The development of lipid peroxidation processes after irradiation of lipids results in their structural changes that become obvious several days later [1]. Besides, the surface charge of erythrocytes decreases [6]. These processes are also observed in nonirradiated blood, although their rates are lower. It is known that the surface charge of erythrocytes starts dropping quickly after several days of storage of whole blood even if it is kept at low temperatures [7].

Exposure of a membrane to γ-radiation gives rise to the second statistical ensemble N_γ that causes damage of the area S_γ. Peroxidation reduces the average threshold potential which becomes $\varphi_{p\,\mathrm{av}}(t) = \varphi_{p\,\mathrm{av}\,0}\exp(-\lambda t)$, where $\varphi_{p\,\mathrm{av}\,0} = \varphi_{p\,\mathrm{av}}(0)$, and λ indicates a decrease in the average potential. After the exposure, the surface charge and a respective surface potential start decreasing with time, and this decrease proceeds at a higher rate than that in nonexposed suspension: $\psi_s = \psi_{s0}\left(1 - \mathrm{erf}\left(\frac{t-t_q}{\sigma_q}\right)\right)$, where ψ_{s0} is the surface potential for the just-prepared suspension, and t_q, σ_q are constants. In this case the transmembrane potentials from the cathode and anode sides change as follows:

$$\varphi_c = -|\varphi_{\mathrm{cell}}/2| - |\psi_s|, \quad \varphi_a = -|\varphi_{\mathrm{cell}}/2| - |\psi_s|.$$

Then the total area of the pores formed will be $S_\gamma + S = \frac{N+N_{\gamma 0}}{\sigma\sqrt{2\pi}}(A_1 + A_2)$, where

$$A_1 = \int\limits_{\varphi_{\min}}^{\varphi_c} \exp\left(-\frac{(\varphi_p - \varphi_{p\,\mathrm{av}})^2}{2\sigma^2}\right)\left(\frac{k}{\varphi_p}\right)^2 d\varphi_p, \quad A_2 = \int\limits_{\varphi_{\min}}^{\varphi_a} \exp\left(-\frac{(\varphi_p - \varphi_{p\,\mathrm{av}})^2}{2\sigma^2}\right)\left(\frac{k}{\varphi_p}\right)^2 d\varphi_p.$$

The ratio of rates at which the number of erythrocytes decreases depends on the ratio of respective areas:

$$V_\gamma/V_{\mathrm{control}} = (S_\gamma + S)/S.$$

This ratio will grow with a radiation-induced increase of the number of active electroporation sites and will decrease with the surface charge decrease. It is due to the difference in the kinetics of the growth of active sites and change in the surface charge that the ratio $V_\gamma/V_{\text{control}}(t)$ changes in time (Fig. 3). So, the relationship $V_\gamma/V_{\text{control}}(t)$ can be used as an operating characteristic of a γ-radiation biosensor.

CONCLUSIONS

The electroporation method makes it possible to study the kinetics of biophysical changes in erythrocyte membranes that are caused by the exposure of the membranes to γ-radiation in a 2.5 R dose. A mathematical model has been developed that describes adequately the experimental results.

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Department of High-Energy Accelerator Physics