The effect of hydrogen peroxide on the zeta potential of human neutrophils under low-dose rate β-radiation

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Hydrogen peroxide (H_2O_2) is a relatively stable molecule which can penetrate membranes without being inactivated [1]. Local concentrations of H_2O_2, at sites of inflammation may increase through an upregulation of intracellular H_2O_2 production in activated neutrophils [2]. Incubation of neutrophils with the addition of 10^{-4}–10^{-7} M H_2O_2 was accompanied by a change in the ability of cells to generate reactive oxygen [3]. It has been established that H_2O_2 violates the barrier properties of neutrophil membranes only at concentrations greater than 10^{-3} M. The obtained data indicate a high blood neutrophil resistance to the H_2O_2 destructive action. H_2O_2 is the major agent signalling through specific protein targets to support cellular adaptation to a changing environment [4]. The negative surface electrostatic charge on biomembranes plays an essential role in cell signaling, modifying the activity of signaling proteins and structuring of signalosomes [5,6]. β-Radiation in the microgray range increases the surface charge of human blood cells [7], apparently due to changes in the structural state of the plasma membrane [8]. The purpose of this study was to study the response of the neutrophil zeta potential (ZP) to H_2O_2 under low-dose rate β-radiation.

To assess the charge on the plasma membrane of cells was determined by the method of microelectrophoresis. The incubation time of the cell suspension with the radionuclide (^{14}C-Leucine) was 1 hour The radiation doses absorbed by the cell suspension were calculated according to [9].

Data on the effect of H_2O_2 on the neutrophil ZP s are given in the Table.

Table. The effect of β-radiation (100 μGy/h) on the response of human neutrophils to H_2O_2

Experimental conditions	Zeta potential, mV						
	Concentration of hydrogen peroxide, M						
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
H_2O_2	-10.16 ±0,51*	-10.67 ±0,34*	-8.47 ±0,34*	-8.47 ±0,68*	-8.20 ±0,53*	-7.30 ±0,51	-6.44 ±0,41*
H_2O_2+^{14}C	16.43 ±0.50	15.24 ±0.61	16.09 ±0.51	20.00 ±0.36	18.12 ±0.33	17.63 ±0.84	14.74 ±0.51

ZP (control) = -12.87 ± 0.45; ZP (radiation) = 16.60 ± 0.34; * - Statistically significant difference from control, $p < 0.05$.

According to the obtained data, H_2O_2 in the entire range of concentrations reduces the negative surface charge of erythrocytes. The maximum decrease in the absolute value of zeta potential (ZP_{abs}) is observed at a concentration of H_2O_2 - 0.1-1.0 nM. Ionizing radiation not only eliminates this effect, but even increases ZP_{abs} to values exceeding the control. It should be noted that the average concentration of H_2O_2 in human plasma is 3.00 ± 0.09 μM ($p < 0.027$) [10]. The obtained results testify to a very high sensitivity of the ZP to H_2O_2. This indicates a high homeostatic stability of the cellular response to the real concentration of H_2O_2 in the latter. Thus, low dose rate ionizing radiation can not only neutralize the effect of H_2O_2 on the ZP of neutrophil membranes in the metabolic range, but also change its response to in the area where H_2O_2 functions as a secondary messenger of cell signaling systems.

References

1. Winterbourn CC, Kettle AJ, Hampton MB. Reactive oxygen species and neutrophil function. Annu Rev Biochem. 2016;85:765-92.

2. Simms HH, D'Amico R. Polymorphonuclear leukocyte dysregulation during the systemic inflammatory response syndrome. Blood. 1994;83:1398.

3. Коваленко Е.И., Семенкова Г.Н., Черенкевич С.Н. Влияние перекиси водорода на способность нейтрофилов генерировать активные формы кислорода и хлора и секретировать миелопероксидазу in vitro. Цитология. 2007;49(10):839-847.
4. Lorenzen I, Eble JA, Hanschmann EM. Thiol switches in membrane proteins - Extracellular redox regulation in cell biology. Biol Chem. 2020;28;402(3):253-269.
5. Goldenberg NM, Steinberg BE. Surface charge: a key determinant of protein localization and function. Cancer Res. 2010;70 (4):1277-1280.
6. Nishino M, Matsuzaki I, Musangile FY, Takahashi Y, Iwahashi Y, Warigaya K, Kinoshita Y, Kojima F, Murata SI. Measurement and visualization of cell membrane surface charge in fixed cultured cells related with cell morphology. PLoS One. 2020;15 (7):e0236373.
7. Zhirnov VV, Iakovenko IN. The osmotic resistance and zeta potential responses of human erythrocytes to transmembrane modification of Ca2+ fluxes in the presence of the imposed low rate radiation field of 90Sr. Int J Radiat Biol. 2015;91(1):117-126.
8. Zhirnov VV, Khyzhnyak SV, Voitsitskiy VM. The effects of ultra-low dose β-radiation on the physical properties of human erythrocyte membranes. Int. J. Radiat. Biol. 2010;86(6):499–506.
9. Loevinger R, Berman MA. Formalism for calculation of absorbed dose from radionuclides. Phys Med Biol. 1968;13(2):205-217.
10. Lacy F, Kailasam MT, O'Connor DT, Schmid-Schönbein GW, Parmer RJ. Plasma H_2O_2 production in human essential hypertension: role of heredity, gender, and ethnicity. Hypertension. 2000;36(5):878-884.