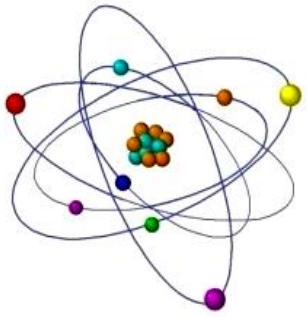
IONIZING RADIATION-INDUCED CHANGES IN THE ABSORPTION SPECTRUM OF ERYTHROCYTE MEMBRANE PROTEINS



***Kalmakhelidze S.L[1,2]., Shekiladze E.R[1]., Ormotsadze G.L[1,2],Gvilava I.V[1]., Tsimakuridze M.P[1]., Sanikidze T.V[1,2]., Kipiani N.V[1].**

[1]Tbilisi State Medical University, Georgia
[2] I.Beritashvili Center for Experimental Biomedicine, Laboratory of Radiation Safety Problems, Georgia

*Corresponding author: sofokalmakhelidze@gmail.com

ABSTRACT*: Under influence of ionizing radiation different types of oxidative modifications of the protein occur, including carbonylation, direct amino-acid oxidation, oxidative cleavage of the protein backbone, or amino acid side chains. Aromatic amino acids are significantly more reactive with the dominant reaction pathway, including the connection of •OH to the aromatic ring. In the case of Tyr, the connection of •OH and subsequent hydrogen extraction lead to the formation of peroxyl radicals, which in the absence of reductants, form Tyr dimers that are implicated in the formation of intra- and inter-protein linkages [7, 8]. Erythrocyte membrane contains numerous integral membrane proteins, usually, they show absorption maximum between 275 and 280 nm, which are caused by the absorbance of the aromatic amino acids tryptophan (Trp) and tyrosine (Tyr) and, to a small extent, by the absorbance of cystine (i.e., of disulfide bonds). Our study aimed to determine Ionizing radiation-induced changes in the absorption spectrum of erythrocyte membrane proteins.*

Mice whole-body irradiation with ^{137}Cs was performed at a dose rate of 1,1Gy/min for the total dose of 5 Gy with a "Gamma-capsule-2". The Erythrocyte membrane was separated according to Hasts Method and absorbance spectra were measured with a spectrophotometer.

Results show that absorption for proteins of erythrocytes' membrane at 280 nm wavelength time-dependent decreased after irradiation and after one month reaches 75% of the control level. This decrease may be related to Tyr-phosphorylation of B3p in radiation-induced oxidative stress conditions, which markedly reduces its affinity for ankyrin, leading to the release of band 3 from the spectrin/actin membrane skeleton, enhancement of the lateral mobility of band 3 protein in the bilayer, progressive vesiculation and loss from the plasma membrane of radiated cells, triggering a cascade of events inducing alteration of deformability, the resistance of erythrocytes membrane, its destabilization.

Key words: γ-radiation, erythrocyte membrane proteins, absorption spectra

INTRODUCTION

Post radiation damages cascade is divided into early, within 90 days after onset of the radiation exposure, which is characterized by massive cell death, tissue dysfunction, and late phase, which occurred some months or years after irradiation, where tissue damage is progressive and irreversible. The total effect of ionizing radiation (IR) exposure on the whole body and isolated cells is dependent on radiation dose, as well as individual radiosensitivity of tissues [1].

In living body tissues, IR exposure is carried out mainly by non-specific mechanisms that are also occurred at various damages (physical and chemical influence) [2, 3]. Cell damage can occur by direct bombarding the biological important macromolecules (proteins, lipids, and DNA) with high energetical photons or indirect action with highly reactive free oxygen radicals (ROS)produced in the fluid phases of tissues as a result of radiolysis of water [4,5]. Different types of oxidative protein modifications are known, including carbonylation, direct amino-acid oxidation, oxidative cleavage of the protein backbone, or amino acid side chains [6]. Aromatic amino acids are significantly more reactive with the dominant reaction pathway, including the connection of •OH to the aromatic ring. In the case of Tyr, the connection of •OH and subsequent hydrogen extraction lead to the formation of peroxyl radicals, which in the absence of reductants, form Tyr dimers that are implicated in the formation of intra- and inter-protein linkages [7, 8].

Erythrocyte membrane contains numerous integral membrane proteins including the glycophorins, the Rh proteins, and transport proteins such as band 3 (AE1, anion exchanger 1, SLC4A1), Na^+, K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase, etc.

Erythrocytes' proteins usually show absorption maximum between 275 and 280 nm, which are caused by the absorbance of the two aromatic amino acids tryptophan (Trp) and tyrosine (Tyr) and, to a small extent, by the absorbance of cystine (i.e., of disulfide bonds) [9, 10]. Our study aimed to determine Ionizing radiation-induced changes in the absorption spectrum of erythrocyte membrane proteins.

MATERIALS AND METHODS

The experimental protocol was in accordance with the guidelines for the care and use of laboratory animals as adopted by the Ethics Committee of the Tbilisi State Medical University (TSMU).

Animal care and maintenance

Three-month-old male mice (Mus musculus), were obtained from the Vivarium of Tbilisi State Medical University. They were housed in animal cages, with room temperature maintained at 200-220C, relative humidity of 50-70%, and an airflow rate of 15 exchange/h. Also, a time-controlled system provided 08:00-20:00 h light and 20:00-08:00 h dark cycles. All mice were given a standard rodent chow diet and water ad libitum from sanitized bottle fitted with stopper and sipper tubes.

After acclimatization to laboratory conditions for a week, the mice were divided into two different groups: I - control group (non-irradiated animals), II group - experimental group (gamma-irradiated animals). The blood samples were obtained from animals of the I group - non-irradiated mice, and II-d experimental group of irradiated mice after 48 hours (IIa subgroup) and one month (IIb subgroup) of irradiation.

Mice whole-body irradiation with 137Cs was performed at a dose rate of 1,1Gy/min for the total dose of 5 Gy with a “Gamma-capsule-2”.

The Erythrocyte membrane was separated according to Hasts Method and absorbance spectra were measured with a spectrophotometer.

Erythrocyte membrane isolation by the Hast method

Blood samples, collected in tubes containing anticoagulants were centrifuged at 3000g for 15 min. The obtained erythrocyte sediment was washed 3 times with a 1: 4 volume of solution A, containing 130 μM KCl, and 20 μM Tris-HCl (pH-7.4). For hemolysis of the obtained erythrocyte sediment, the 1:10 volume of solution B, containing 5 μM Tris-HCl, and 1 mm EDTA, was added and the resulting mixture was left all night (for about 15 hours). The next day the suspension was centrifuged at 12,000 g for 20 min. The obtained precipitate was washed again with solution "B" 2-3 times before bleaching. The precipitate was washed again with a 1:10 volume of "A" solution. Absorbance spectra were

measured with a spectrophotometer (SPECTRO UV-UIS DUAL BEAM 8 AUTO CELL (UVS-2800) and (Lambda 38, PerkinElmer, Rodgau, Germani).

RESULTS AND DISCUSSION

Fig.1 shows the absorbance spectrum at the wavelength of 280 nm. The intensity of absorbance spectrum of erythrocytes' membrane proteins from a control group of mice was 2,392, in irradiated mice, 48 hours after radiation (subgroup IIa) the intensity of absorption slightly decreased (2,06) and after one month of irradiation (subgroup IIb) absorption spectrum decreased and was equal to 1,81 (Figure 1).

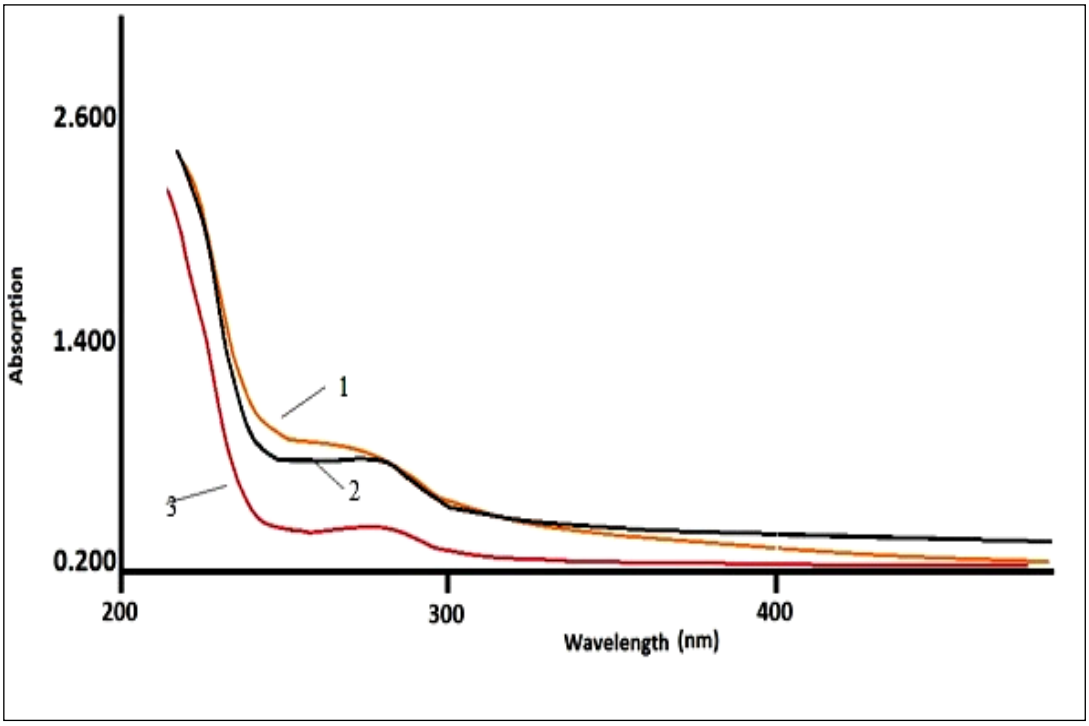


Fig.1 Absorption spectrum of erythrocyte membrane proteins
1 – control group, 2- after 48 hours from irradiation, 3- after one month from irradiation

Since band 3 protein is one of the most common proteins in the erythrocyte membrane and contains a large number of tyrosine residues (at least tyrosine 8, 21, 359, and 904) [11], we assumed that changes in the absorption intensity in the spectra of erythrocyte membrane proteins are to a certain extent associated with changes in tyrosine residues of Band 3 protein.

Band 3 protein is the major integral protein of the erythrocytes' membrane (composes approximately 25% of proteins), which has two primary functions, ion transport (mediates chloride–bicarbonate exchange) and maintenance of protein-protein interactions with cytoskeletal proteins (spectrin, actin, band 4.2) and glycolytic enzymes. The shape, osmotic resistance, and deformability of erythrocytes are critically related to the Band 3 protein (B3p) function [12]. Perhaps because of its many important functions, band 3 is also a prominent substrate of Ser/Thr kinases and is the major substrate of the cell's protein tyrosine kinases. In response to physiologic stimuli such as hypertonic conditions or oxidative stress, phosphorylation of band 3 on tyrosine residues, can increase by several orders of magnitude.

Literature data show, that erythrocytes membrane oxidative damage is leading to B3p dose-dependent clusterization in high-molecular-mass aggregates, through disulfide cross-linking dimerization [13, 14]. Since B3p is normally linked to the cytoskeleton by junctional complexes, the formation of large aggregates of B3p should be restrained by such interactions unless they are weakened by regulatory mechanisms. tyrosine phosphorylation via phosphotyrosine kinases (PTKs) or phosphotyrosine phosphatase (PTP) [15] which appears to be facilitating oxidatively modified B3p clusterization [13]. It was reported, that tyrosine phosphorylation of band 3 markedly reduces its affinity for ankyrin, leading to the release of band 3 from the spectrin/actin membrane skeleton, enhancement of the lateral mobility of band 3 in the bilayer, and progressive vesiculation and loss from the plasma membrane of stimulated cells [11]. It was suggested that B3p as a redox sensor, is regulated by phosphorylation; in oxidative stress conditions, rapid intense Tyr-phosphorylation of B3p affects its interactions with the cytoskeletal proteins triggering a cascade of events inducing alteration of deformability, the resistance of erythrocytes membrane, its destabilization [11] and finally leading to their hemolysis [13]. Irradiation of RBCs by gamma radiation could cause various degrees of damage to RBCs membranes. The damage to the cell membrane is dose-dependent [14].

The results of the study indicate that absorption for proteins at 280 nm wavelength time-dependent decreased after irradiation. This decrease may be related to Tyr-phosphorylation of B3p in radiation-induced oxidative stress conditions [16], which markedly reduces its affinity for ankyrin, leading to the release of band 3 from the spectrin/actin membrane skeleton, enhancement of the lateral mobility of band 3 in the bilayer, progressive vesiculation and loss from the plasma membrane of radiated cells, triggering a cascade of events inducing alteration of deformability, the resistance of erythrocytes membrane, its destabilization.

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