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Protective Effect of Herniarin on the Ionizing Radiation-Induced Alterations of Erythrocyte Membrane Proteins

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ABSTRACT

BACKGROUND.
Exposure to ionizing radiation leads to the intensification of oxidizing processes that directly or indirectly alter target molecules forming reactive oxygen species targeting cell membrane lipids and proteins. There is a need for new antioxidants to reduce the effects of ionizing radiation.

OBJECTIVES
Our study aimed to determine changes in the absorption spectrum of erythrocyte membrane proteins in gamma-irradiated mice and evaluate the potential protective effect of the antioxidant Herniarin.

METHODS
Overall, 18 2-month-old mice were distributed evenly among the control (group I) and two experimental (group II and group III) groups. The whole-body irradiation of experimental mice with ^{137}Cs was performed at a dose rate of 1,1 Gy/min for a total dose of 5 Gy with a "Gamma-capsule-2", and animals of group 3 were treated with Herniarin (20 mg/kg) for five days before and one hour after irradiation. The erythrocyte membrane was separated using Hast Method, and absorbance spectra were measured with a spectrophotometer at 280 nm wavelength (Spectro UV-UIS BEAM 8 AUTO CELL [UVS-2800]) on 2nd, 7th, 14th and 30th post-irradiation days.

RESULTS
The Herniarin-treated mice showed approximately the same results as irradiated mice on the 2nd and 7th post-irradiation days. Furthermore, on days 14 and 30 absorption spectrum of erythrocyte membrane proteins significantly increased ($P<0.05$) in the Herniarin-treated experimental group.

CONCLUSIONS
The ionizing irradiation induces a decrease in the intensity of the erythrocyte membrane absorption spectrum at 280 nm due to intensive tyrosine-phosphorylation of B3p under oxidative stress conditions. Impairments manifested on post-irradiation day 7, were preserved during the whole post-irradiation period. Herniarin, as a potent antioxidant, significantly amplified the absorption spectrum compared to the control group on days 14 and 30 of the post-irradiation period.

KEYWORDS
Erythrocyte; Herniarin; ionizing radiation.

BACKGROUND

Exposure to ionizing radiation results in the formation of reactive oxygen species that directly or indirectly interact with target molecules. The harmful effects of irradiation depend on the dose, exposure time, and involvement of protective mechanisms. The antioxidative defense mechanisms are unable to handle the oxidative stress induced by high-dose ionizing radiation.[1,2]

In physiological conditions, reactive oxygen and nitrogen species play critical roles in cellular functions. The cell irradiation induces water radiolysis and the formation of superoxide and hydroxyl radicals. Therefore, ionizing radiation causes the activation of NOS and the intensive generation of nitric oxide, which reacts with superoxide anion, forming the peroxynitrite anion, targeting cellular molecules, including membrane proteins, lipids, thiols, and DNA.[3,4]

The oxidative stress causes the damage of sulfur-hydril groups of membrane proteins resulting in the lysis of erythrocytes. Alterations of membrane permeability for different ions occur in the post-irradiation period (dose range 2 to 200 Gy),[5] and induce notable changes in the structure of the erythrocyte membrane.[6-8]

Different types of antioxidants scavenge reactive oxygen and nitrogen species. Herniarin is a simple coumarin, which can reduce the concentration of reactive oxygen species (ROS) in lymphocytes and significantly decreases the genotoxic effect of cisplatin in rat bone marrow cells.[9-11]

Our study aimed to evaluate changes in the absorption spectrum of erythrocyte membrane proteins in gamma-irradiated mice and evaluate the potential protective effect of the antioxidant Herniarin.

METHODS

Animal care and maintenance

Experiments were performed on 18 white mice. The experimental protocol was developed under guidance on the care and use of laboratory animals and adopted by the Ethics Committee of Tbilisi State Medical University (TSMU).

2-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 20^0-22^0C and relative humidity of 50-70%. 08:00-20:00 hours of light and 20:00-08:00 hours of dark cycles were provided by a time-controlled system. All mice were fed a standard rodent chow diet and watered from sanitized bottles equipped with stoppers and sipper tubes.

Erythrocyte membrane isolation with Hast method

On the 2nd, 7th, 14th, and 30th days after irradiation, blood samples were collected in anticoagulant-containing tubes and centrifuged at 3000 x g for 15 minutes; obtained precipitate containing red blood cells was subjected to membrane isolation procedure by Hast method. The obtained precipitate was washed three times with a 1:4 volume of solution A containing 130 µM KCl and 20 µM Tris-HCl (pH-7.4). Afterward, a 1:10 solution B containing 5 µM Tris-HCl and 1 mm EDTA was added to the washed precipitate and left overnight (about 15 hours) for hemolysis. The next day, the suspension was centrifuged at 12 000 x g for 20 minutes. The obtained precipitate was washed 2-3 times with solution B and then 1:10 with a volume of solution A before bleaching. Red cell membrane absorption spectra were measured by spectrophotometer (SPECTRO UV-UIS DUAL BEAM 8 AUTO CELL [UVS-2800] and Lambda 38, PerkinElmer, Rodgau, Germany) at 280 nm.

Statistical analysis

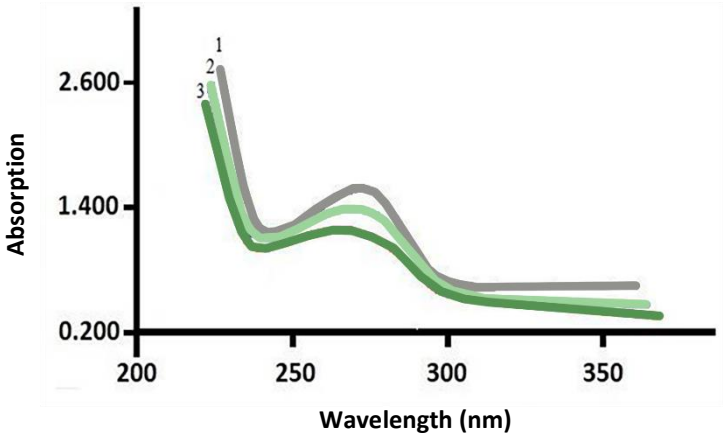
IBM SPSS was used for analyzing data. Analysis of Variance (ANOVA) was used for the assessment of results. A statistical significance was taken $p<0.05$.

RESULTS

In the present study, we investigated the effect of ionizing radiation (5 Gy) on the absorption spectrum of erythrocyte membrane proteins and the possibility of correcting impairments using Herniarin as an antioxidant.

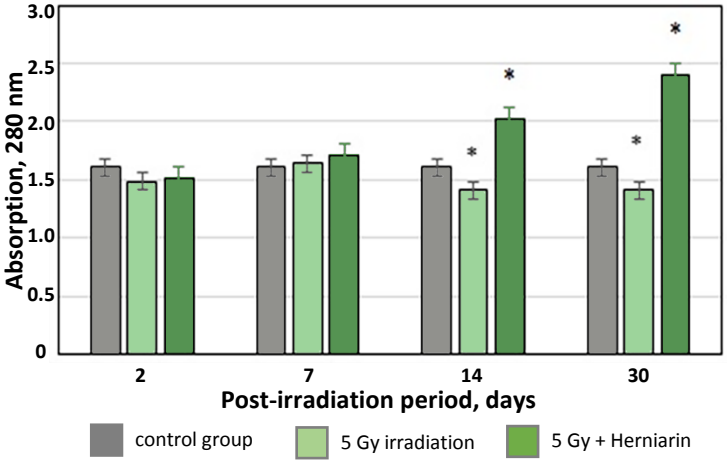
Figures 1 and 2 represent the effect of ionizing radiation on the absorption spectrum of erythrocyte membrane proteins on days 2, 7, 14, and 30 after irradiation. The intensity of the absorption spectrum of erythrocyte membrane proteins of irradiated animals did not significantly differ from the control group results on days 2 and 7 of the irradiation period and significantly decreased on days 14 and 30 of post-irradiation compared to the control group ($p<0.05$). In the Herniarin-treated group (group III), the absorption spectrum of erythrocyte membrane proteins continuously increased on day 7 after irradiation and reached a maximal level on day 30 of the post-irradiation period (increased by 25% on day 14 and by 50% on the day 30.

FIGURE 1. The absorbance spectrum of erythrocytes membrane on the 30th day after irradiation



Explanations: 1- Intensity of absorption in the control group; 2- Intensity of absorption in Irradiated mice treated with Herniarin, 3- Intensity of absorption in Irradiated mice (5Gy).

FIGURE 2. The intensity of absorption at 280 nm of erythrocyte membrane proteins on the 2nd, 7th, 14th, and 30th days after irradiation



DISCUSSION

According to the existing evidence, UV absorption at 270-280 nm occurs due to proteins containing aromatic amino acids (especially tryptophan, tyrosine, and phenylalanine residues).[12] The band 3-protein (B3p), one of the most common proteins in the erythrocyte membrane, contains a large number of tyrosine residues. We assumed that changes in the absorption intensity in the spectra of erythrocyte membrane proteins are associated with the alterations of tyrosine residues of B3p.[13,14]

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B3p is the major integral protein in a human erythrocyte membrane forming mechanical support through the maintenance of interaction with ankyrin and the cytoskeletal network. B3p mediates chloride–bicarbonate exchange, osmotic resistance, and deformability of erythrocytes.[15,16]

P3p is also an essential substrate of phosphotyrosine kinases (PTKs) or phosphotyrosine phosphatases (PTP), which are responsible for the phosphorylation process of band 3-protein tyrosine residues. The intensity of this process increases in response to physiologic stimuli such as hypertonic conditions or oxidative stress.[13,17]

It has been suggested that B3p is a redox sensor of the erythrocyte membrane, which is regulated by phosphorylation. Under oxidative stress, rapid Tyr-phosphorylation of B3p affects its interactions with cytoskeletal proteins and causes changes in membrane cytoskeletal structures,[18] such as the reduction of its affinity for ankyrin and weakening of the interaction between band 3 and the spectrin/actin membrane skeleton; and the enhancement of the lateral mobility of integral protein in the bilayer, which causes the changes in the resistance and deformability of the erythrocyte membrane, its destabilization and finally hemolysis.[15,18]

It was shown that the phosphorylation of tyrosine caused a blue shift and a marked decrease in the 275 nm absorption by tyrosine-containing peptides and free L-tyrosine.[19] Therefore, irradiation of RBCs by gamma radiation causes the formation of reactive oxygen and nitrogen radicals, which induce the intracellular oxidative stress and rapid Tyr-phosphorylation of B3p (that revealed a decrease of the erythrocytes membrane absorption spectra at 280 nm) with dose-dependent damage of RBCs membranes.[20,21]

We can assume that Herniarin reduces ROS levels in the body and prevents tyrosine phosphorylation in B3p due to its antioxidant activity. It also causes an increase in the number of erythrocytes in peripheral blood and, therefore, in the intensity of absorption spectra at 280nm in the late post-irradiation period.[9]

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