EFFECT OF THE ACUTE TOTAL GAMMA RADIATION IN A SUBLETHAL DOSE ON THE BIOPHYSICAL PROPERTIES OF RED BLOOD CELLS, LIPID PEROXIDATION, ANTIOXIDANT SUPPLY AND HEMOCOAGULATING PROPERTIES OF ERYTHROCYTES

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ABSTRACT

The aim of the investigation was to study the effect of acute, total gamma-irradiation in a sublethal dose on the biophysical properties of erythrocytes, the intensity of lipid peroxidation, antioxidant supply and hemocoagulating properties of erythrocytes. The experiments were carried out on 11-12-week-old age guinea pigs, males and females in equal numbers. The animals were exposed to a single total radiation at a dose of 4.5 Gy (sublethal dose, LD 50/30). The studies were carried out on the 7th day after exposure to radiation (at the height of radiation sickness). The development of radiation damage was accompanied by intense erythropoiesis and the appearance of erythrocytes with a high resistance to hemolysis and an increased sedimentation rate. After acute gamma irradiation, depletion of the antioxidant system was noted. It manifested in a decrease in the activity of superoxide dismutase of erythrocytes by 19.7% (p<0.01) and the concentration of serum ceruloplasmin by 21.5% (p<0.01). The content of thiobarbituric acid reactive substances (TBARS) and their accumulation while the incubation of erythrocytes remained within the normal range. The erythrocytes of the irradiated animals exhibited increased procoagulant and decreased antiheparin activity, which reflects conformational changes in highly radiosensitive fatty acid chains of phospholipids in their membranes. A decrease in the fibrinolytic activity of erythrocytes in irradiated animals was found.

Keywords: Gamma-irradiation, erythrocytes, lipid peroxidation, antioxidants, hemocoagulation.
INTRODUCTION

The hematopoietic system is very sensitive to the action of ionizing radiation, and the wide representation of hematopoietic tissue in the body determines its obligatory damage at any type of radiation exposure (1). Under the influence of ionizing radiation, there is a violation of both the structural organization of cells and an increase in the oxidation of lipids of biomembranes (2, 3). At the same time, the erythrocyte is a radio-resistant cell, as it does not have DNA and is not capable of RNA synthesis, but this feature decreases their ability to post-irradiative repair. The main radiation target is plasma membrane of an erythrocyte. Under ionizing irradiation, reactive oxygen and nitrogen forms, which activate enzymes (nicotinamide adenine dinucleotide phosphate oxidase, lipoygenases, nitric oxide synthase, cyclooxygenases) involved in damage to cell membranes are formed (4). All physiological mechanisms of damages of hemostasis erythrocyte link are not clear now, that is important to take into account the presence of post-irradiative hemopoiesis depletion.

One of the manifestations of acute radiation sickness is a violation of hemostasis, which is manifested by bleeding. Modern research shows that disseminated intravascular coagulation plays a very important role in the death of people and animals from radiation damage in relatively low doses. The mechanisms of such development should be investigated (5). However, to date and the role of damaged erythrocytes in impaired hemostasis remain almost unexplored (6, 7).

The aim of this investigation was to study the effect of acute, total gamma radiation in a sublethal dose on the bio-physical properties of erythrocytes, the intensity of lipid peroxidation, antioxidant supply and hemocoagulating properties of erythrocytes.

MATERIALS AND METHODS

Animals

The experiments were performed on 11-12-week-age guinea pigs, 441.7±9.8 g weigh, males and females in equal numbers, kept separately. These animals are one of the best models, including for the study of radiation damage, since they do not synthesize ascorbic acid, similar to humans (8). Laboratory animals were kept in a vivarium of the Ukrainian Medical Stomatological Academy (UMSA is Poltava State Medical University after 05.05.2021) in a room that did not contain specific pathogens, with a natural lighting cycle at a constant temperature (21±1°C) and humidity (50%±10%), on a standard diet.

Compliance with Ethical Standards

This study was carried out in accordance with the national “General Ethical Principles of Animal Experiments”, which is consistent with the provisions of the European Convention for the Protection of Vertebrate Animals used for experiments or other scientific purposes (Strasbourg, March 18, 1986). This experiment was approved by the UMSA Ethics and Bioethics Commission.

Experimental procedure

Animals were divided into two groups, each of 10 animals. The first group was intact animals (healthy animals that were not exposed to any effects). Animals of the second group were subjected to a single total exposure at a dose of 4.5 Gy (sublethal dose, LD 50/30).

It is known that radiosensitivity is quite adequately characterized by a radiation dose that causes 50% death of certain mammal species. The average LD 50/30 for a guinea pig is 4.5 Gy, and the greatest degree of granulocytopenia (maximum first emptying) characterizing the height of acute radiation syndrome occurs 7-8 days after acute exposure (9, 10).

Radiation was carried out in the installation “Agat-R”. $^{60}$Co was used as a source of ionizing radiation. The radiation dosage received by each animal was determined according to the calculation results, based on the dosage rate measurements by a type 27012 clinical dosimeter. The measurement error of the dosage was within the limits of 8-10%.

The studies were carried out on the 7th day after the previous exposure (in the midst of radiation sickness) (11). The biological material was taken under hexenal anesthesia at a dose of 120 mg/kg intraperitoneally.

Tissue study

The objects of the study were whole blood, plasma, serum and whole red blood cells of experimental animals.

Evaluation parameters and biochemical estimations.

The number of red blood cells was determined in an automatic counter PCE 210; hemoglobin was measured by a MiniGem 540 hemoglobinometer.

To study the electrophoretic properties of red blood cells a modified method of fractional erythrocyte sedimentation rate was used (12).

Peroxidation resistance of erythrocytes was determined by the Jager F.C. method. (14). The following blood parameters have been studied: the resistance of erythrocyte membranes to hydrochloric hemolytic (13), the accumulation of thiobarbituric acid reactive substances (TBARS) in red blood cells (15), the activity of superoxide dismutase (16) and catalase in red blood cells (17), the content of ceruloplasmin in blood serum (15), conjugated dienes in blood serum (15), total serum lipids (15), low density lipoprotein and very low density lipoprotein (LDL and VLDL) and serum cholesterol (15).

When studying the hemocoagulating and fibrinolytic properties of red blood cells a standard platelet-free substrate plasma was used. In one sample, whole erythrocytes from
animals after irradiation were added to the substrate plasma. In the second sample, whole erythrocytes of intact animals were added to the substrate plasma. Plasma substrate with physiological saline served as a control in the study. The effect on recalcification time, thrombin time and plasma fibrinolytic activity was determined (18).

All the values were expressed as mean ± standard error of mean (SEM). The data was analyzed by Student’s T-test. The analysis of the normality of the distribution of indicators was carried out using one-sample Kolmogorov-Smirnov Test. The data was analyzed by Student’s T-test. All p value less than 0.05 were considered to be statistically significant. The statistical analysis was performed using SPSS (Version 13.0).

RESULTS

A radiation dose of 4.5 Gy 6 days after exposure did not significantly affect the number of circulating red blood cells (4.37±0.08×10^12 / L in intact animals versus 4.41±0.28×10^12 / L in irradiated animals, p>0.5).

Given the significant role of the surface charge in maintaining the structural and functional integrity of red blood cells, the reaction of erythrocyte sedimentation in irradiated animals was investigated. 15 minutes after, the height of the plasma column during erythrocyte sedimentation in irradiated animals exceeded the same value in the intact group by 45.2 % (1,80±0,12 mm versus 1,27±0,14 mm, p<0.05). At the 30th and 45th minutes, the values in the experimental and the control groups were close to each other, and only at the 60th minute a sharp acceleration of erythrocyte sedimentation with an increase in plasma column height during erythrocyte sedimentation was of 68.4 % in the irradiated animals in comparison with the intact group (8,00±0,82 mm versus 4,75±0,42 mm, p<0.01).

The changes in the process of erythrocyte agglutination, which depends on the electric charge of the cells, by the 60th minute indicates the destruction of the electrostatic system of red blood cells, as a result the transport and exchange function of the entire bloodstream decreases and the risk of erythrocyte blood clots increases.

The development of radiation damage was accompanied by a change in the resistance of red blood cells to acid hemolysis, which may be associated with a qualitative change in the composition of red blood cells. The total duration of the hemolysis process, the onset time of the hemolysis maximum and the destruction time of the most stable forms of red blood cells significantly increased in combination with a decrease in the total number of decaying red blood cells in irradiated animals. The total duration of the hemolysis process increased by 27.3% in irradiated animals (p<0.01), as well as the time of hemolysis maximum increased by 27.1% (p<0.02), the number of destroyed red blood cells in hemolysis decreased by 36.8% in relation to the same number in irradiated animals (p<0.05) (Table 1).

In irradiated animals, the erythrogram maximum is shifted to the right, which, apparently, is associated with a sharp rejuvenation of the erythrocyte pool and indicates an abnormally highly stable erythrocyte entering the vascular bed, and the flattening of the erythrogram reflects the dysregulation of erythropoiesis.

Thus, intense erythropoiesis and the appearance of red blood cells with high resistance were noted in irradiated animals already on the 7th day.

After acute gamma radiation, depletion of the antioxidant system was observed, which manifested itself in a decrease in the activity of erythrocyte superoxide dismutase (SOD) by 19.7 % (p<0.01) and serum ceruloplasmin concentration by 21.5 % (p<0.01). The content of TBARS and their accumulation during the incubation of erythrocytes remained at the level of the intact group (Table 2).

An increase in the number of total serum lipids of the irradiated animals was noted by 38.7 % (p<0.05), while the content of low and very low density lipoproteins under the influence of radiation did not change.

It can be assumed that the changes obtained are caused by the release of phospholipids of cell membranes, including erythrocyte membranes, which underwent structural modification during lipid peroxidation (LPO). In turn, the release of phospholipids with pronounced thromboplastic properties affects the state of red blood cell hemostasis (Table 3).

Red blood cells of irradiated animals reduced plasma recalcification time more significantly than red blood cells of intact guinea pigs. Moreover, the thromboplastic activity of red blood cells of irradiated animals decreased during washing, whereas it did not change in intact guinea pigs. The erythrocyte supernatant shortened the recalcification time in the same way in both groups of animals. The erythrocytes of animals exposed to acute gamma radiation had less antihemarin activity than the erythrocytes of the intact group. When washing, the antihemarin activity of erythrocytes in intact and irradiated animals was unchanged.

The red blood cells of animals of both studied groups had a pronounced fibrinolytic effect. However, the fibrinolytic activity of the erythrocytes of the irradiated animals was 15.0 % (p<0.02) less than in intact animals, which may be due to the increased activity of antiplasmin and inhibitors of plasminogen activation in the erythrocyte stroma. The fibrinolytic activity of the washed red blood cells of both groups of animals did not differ significantly. The supernatant did not have a pronounced effect on the rate of the euglobulin clot lysis.
### Table 1. Effect of sublethal gamma radiation on the resistance of red blood cells to acid hemolysis in guinea pigs

<table>
<thead>
<tr>
<th>The studied indicators</th>
<th>Intact animals, n= 10</th>
<th>Animals after radiation, n= 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.23±0.12</td>
<td>6.66±0.26 p&lt;0.01</td>
</tr>
<tr>
<td>Total duration of the hemolysis process (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.25±0.08</td>
<td>4.13±0.14 p&lt;0.02</td>
</tr>
<tr>
<td>Time of hemolysis maximum (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.50±2.24</td>
<td>12.31±2.37 p&lt;0.05</td>
</tr>
<tr>
<td>Number of destroyed red blood cells in hemolysis maximum (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.61±0.28</td>
<td>5.36±0.12 p&lt;0.05</td>
</tr>
<tr>
<td>Destruction time of the most stable forms of red blood cells (min)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: p is the significance indicator of differences between indicators of intact and irradiated animals.

### Table 2. Effect of sublethal gamma radiation on peroxidation and blood lipid metabolism in guinea pigs

<table>
<thead>
<tr>
<th>The studied indicators</th>
<th>Intact animals, n= 10</th>
<th>Animals after radiation, n= 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.04±0.17</td>
<td>3.03±0.29 p&gt;0.05</td>
</tr>
<tr>
<td>Spontaneous erythrocyte hemolysis (% hemolysis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.22±1.65</td>
<td>38.78±1.67 p&gt;0.05</td>
</tr>
<tr>
<td>Diene conjugates (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The level of TBARS before the incubation of red blood cells, (μmol/L erythrocyte)</td>
<td>10.65±1.13 10.97±1.36 p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>The level of TBARS after 1.5 hours of incubation of erythrocytes (μmol/L erythrocyte)</td>
<td>12.32±0.81 12.21±0.25 p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>The increase in TBARS during the incubation of red blood cells (μmol/L erythrocyte)</td>
<td>3.08±0.78 3.61±0.73 p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase (U)</td>
<td>0.76±0.03 0.61±0.04 p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Catalase index</td>
<td>1.65±0.16 1.61±0.16 p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Ceruloplasmin (mg/L)</td>
<td>44.70±2.97 33.50±2.89 p&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>1.27±0.15 1.19±0.11 p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Total lipids (g/L)</td>
<td>1.68±0.25 2.33±0.11 p&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>LDL and VLDL (g/L)</td>
<td>1.33±0.36 1.17±0.08 p&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Note: p is the significance indicator of differences between indicators of intact and irradiated animals.
Table 3. Effect of gamma radiation at a sublethal dose on the hemocoagulating and fibrinolytic properties of red blood cells in guinea pigs

<table>
<thead>
<tr>
<th>The studied indicators</th>
<th>C</th>
<th>Intact animals, n= 10</th>
<th>Animals after radiation, n= 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>The plasma recalcification time with the addition of red blood cells (s)</td>
<td>146.03±2.08</td>
<td>76.03±3.27 p&lt;0.001</td>
<td>65.87±2.67 p&lt;0.001 p1&lt;0.02</td>
</tr>
<tr>
<td>Plasma recalcification time with the addition of washed red blood cells (s)</td>
<td>146.03±2.08</td>
<td>74.37±2.42 p&lt;0.001</td>
<td>80.75±2.65 p&lt;0.001 p1&lt;0.05</td>
</tr>
<tr>
<td>Plasma recalcification time when supernatant is added (s)</td>
<td>146.03±2.08</td>
<td>78.13±2.29 p&lt;0.001</td>
<td>76.62±3.59 p&lt;0.001 p1&gt;0.05</td>
</tr>
<tr>
<td>Thrombin time of plasma with the addition of red blood cells (s)</td>
<td>44.01±1.19</td>
<td>21.01±2.27 p&lt;0.001</td>
<td>32.02±2.53 p&lt;0.001 p1&lt;0.05</td>
</tr>
<tr>
<td>Thrombin time of plasma with the addition of washed red blood cells (s)</td>
<td>44.01±1.19</td>
<td>24.13±1.23 p&lt;0.001</td>
<td>30.13±2.29 p&lt;0.001 p1&lt;0.05</td>
</tr>
<tr>
<td>Thrombin time of plasma with the addition of supernatant (s)</td>
<td>44.01±1.19</td>
<td>28.63±1.34 p&lt;0.001</td>
<td>30.80±1.25 p&lt;0.001 p1&gt;0.05</td>
</tr>
<tr>
<td>Euglobulin plasma clot lysis time with the addition of red blood cells (min)</td>
<td>298.24±6.07</td>
<td>227.41±9.68 p&lt;0.001</td>
<td>261.52±6.10 p&lt;0.001 p1&lt;0.05</td>
</tr>
<tr>
<td>Euglobulin plasma clot lysis time with the addition of red blood cells (min)</td>
<td>298.24±6.07</td>
<td>225.00±8.94 p&lt;0.001</td>
<td>217.40±4.72 p&lt;0.001 p1&gt;0.05</td>
</tr>
<tr>
<td>The time of lysis of the euglobulin plasma clot with the addition of supernatant (min)</td>
<td>298.24±6.07</td>
<td>294.02±0.29 p&gt;0.05</td>
<td>281.10±9.47 p&gt;0.05 p1&gt;0.05</td>
</tr>
</tbody>
</table>

Note: C is plasma control (substrate plasma + physiological saline); p is the significance indicator of differences between the indicators of substrate plasma and plasma with the addition of red blood cells; p1 is the significance indicator of differences between indicators of intact and irradiated animals.

DISCUSSION

According to our data, after acute sublethal irradiation of animals, on the 7th day, the appearance of cells with high resistance to hydrochloric acid hemolytic and an increase in the erythrocyte sedimentation rate associated with a decrease in the charge of their membranes were revealed. Most researchers consider the decrease in the surface charge of erythrocytes after exposure to ionizing radiation as a result of structural rearrangement of the membrane (19-21).

The consequence of irradiation is the activation of LPO and profound changes in the conformation of membrane proteins, including the aggregation of membrane proteins with the formation of -S-S-bridges, which affects the mechanical properties of membranes and their resistance to chemical hemolysis (22).

The observed increase in erythropoiesis at the height of radiation sickness can be explained by the mobilization of reserves of erythrocyte production (23). An increase in the pool of proliferating hematopoietic cells, as well as the acceleration of cell differentiation, bypassing some “normal” stages of their maturation, can be considered as an additional source of enhancing erythropoiesis (24).

In our research, depletion of the antioxidant system was also noted under exposure at a sublethal radiation dosage. It
manifested in a decrease in the activity of superoxide dismutase (SOD) of erythrocytes and the concentration of serum ceruloplasmin without changing the concentration of primary and secondary lipid peroxidation products.

Data on the unchanged amount of conjugated dienes and the concentration of TBARS under total irradiation at a sublethal dose do not contradict the literature.

After radiation, the amount of antioxidant phospholipids in the bloodstream increases and the intensity of free-radical autooxidation of lipids in tissues decreases (25). In turn, it was found that the humoral products of activation of stress-realizing systems - catecholamines and steroid hormones - have antioxidant activity (26, 27). Their hypersecretion can be considered as a response to LPO activation, which develops through a negative feedback mechanism. However, a long-term excess of their normal level in circulation by 5-10 times and more causes the secondary activation of LPO (4).

The absence of an LPO outbreak on the 7th day after sublethal irradiation may be associated with a weakening of the “respiratory activity” of leukocytes. Thus, a number of authors noted the inhibition of the phagocytic activity of neutrophils at the height of radiation sickness, and the release of glucocorticoids at the earliest stages after irradiation inhibits the respiratory burst of neutrophils (28, 29).

In turn, the suppression of the “respiratory explosion” in polymorphonuclear leukocytes is accompanied by a decrease in the power of the pentose phosphate cycle, which can also be observed in erythrocytes (30).

Since the source of the reducing equivalents of the cell antioxidant system is the pentose phosphate pathway, the observed decrease in the SOD activity in erythrocytes can be associated with a possible inhibition of the pentose cycle, a decrease in the level of O2•-, which acts in relation to the enzyme as an inducing and activating factor (31, 32).

The noted decrease in the concentration of the main plasma antioxidant, ceruloplasmin, may be associated with inhibition of the release of the leukocytes endogenous mediator, which is responsible for the synthesis and release of protein reactants of the active phase, including ceruloplasmin (33).

An increase in the concentration of blood serum total lipids with a constant content of low and very low density lipoproteins may be the result of an increase in the amount of high density lipoproteins. A similar antiphase change in the amount of high and low density lipoproteins was observed in works by Serkiz et al. (34).

The studies show that the observed post-radiation changes in erythrocytes affect the structural rearrangement of the cell membrane, accompanied by a change in the conformation of membrane molecules and disruption of the enzyme systems of erythrocytes. Erythrocytes of irradiated animals exhibited increased procoagulant and decreased antiheparin activity, which reflects conformational changes in highly polyunsaturated fatty acid chains of phospholipids in their membranes.

The observed decrease in the fibrinolytic activity of erythrocytes in irradiated animals is possibly explained by the increased activity of antiplasmins and inhibitors of plasminogen activation in the erythrocytes stroma, as well as by the fact that at the height of radiation sickness, the plasminogen proactivators and activators enter the plasma from erythrocytes intensively; the activation of the fibrinolytic chain of hemostasis is observed during this period (35).

CONCLUSIONS
In summary, the evidence of the participation of radiosensitive specialized cells (erythrocytes) in functional disorders in the organism after irradiation was obtained.

It was found that on the 7th day after a single sublethal irradiation of animals, intense erythropoiesis and the appearance of red blood cells with high hemolysis resistance and an increased sedimentation rate were observed. An increase in procoagulant and a decrease in the fibrinolytic activity of erythrocytes, an antioxidant system stress, aimed at maintaining lipid peroxidation, which at this time does not exceed the normal level, despite pronounced signs of impaired function of erythrocyte membranes, were observed.

Further study of the mechanisms of oxidative stress and hemostasis in radiation-induced tissue damages will provide the opportunity to better develop preventive and therapeutic strategies in the future.

ETHICS APPROVAL
This study was carried out in accordance with the national General Ethical Principles of Animal Experiments, which is consistent with the provisions of the European Convention for the Protection of Vertebrate Animals used for experiments or other scientific purposes (Strasbourg, March 18, 1986). This experiment was approved by the UMSA Ethics and Bioethics Commission.

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

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