EVALUATION OF ANTI-RADIATION EFFICACY OF THE *STAPHYLOCOCCUS AUREUS*-DERIVED THERAPEUTIC AGENT

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The study is relevant due to the fact that the decrease in microbial toxicity observed during the radio-inactivation of microorganisms is accompanied by synthesis of radioprotective substances and exertion of the radioprotective effects associated with administration of such microbial agents to exposed animals. The study was aimed to assess radioprotective efficacy of the exposed *Staphylococcus aureus* variants. The study showed that the *Staphylococcus aureus* culture treated with a single dose of gamma radiation (30–40 kGy) ensured protection of 55–66% of the lethally irradiated animals. Multiple exposures of the test microorganism to the gradually increasing doses of gamma radiation induced an even larger increase in radioresistance resulting from the synthesis of endogenic radioprotectors, particularly peroxidase, the antioxidant enzyme, and IL1β cytokine, ensuring interception of the radiation-induced toxic radicals and thereby preventing post-exposure pancytopenia in the bone marrow. The experiments involving white mice exposed to the absolutely lethal gamma radiation doses (7.9 Gy, $LD_{100/30}$) showed that a single subcutaneous administration of the *St. aureus* radioresistant variant (strain $209R_{70}$) in a dose of 2×10^8 bacterial cells per animal 3 days after the exposure ensured the 77.7% survival rate, while 100% of untreated animals died. Based on the findings it was concluded that inclusion of the exposed agents of microbial origin would make it possible to increase the efficacy of the combination radioprotectors.

Keywords: *Staphylococcus aureus*, gamma rays, radio inactivation, radio modification, laboratory animals, anti-radiation effectiveness

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Compliance with ethical standards: all the procedures involving model animals were conducted in accordance with the Good Laboratory Practice and the Directive 2010/63/EU of the European Parliament and of the Council (2010) on the protection of animals used for scientific purposes.

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ОЦЕНКА ПРОТИВОРАДИАЦИОННОЙ ЭФФЕКТИВНОСТИ ЛЕЧЕБНОГО СРЕДСТВА НА ОСНОВЕ *STAPHYLOCOCCUS AUREUS*

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Актуальность проведенных исследований заключается в том, что снижение токсичности микроорганизмов в процессе их радиоинактивации сопровождается синтезом радиопротекторных субстанций и проявлением радиозащитного действия при введении этих микробных препаратов в организм облученных животных. Целью исследования было изучить радиозащитную эффективность облученных вариантов золотистого стафилококка. В работе установлено, что культура *Staphylococcus aureus*, подвергнутая однократному гамма-облучению в диапазоне доз от 30 до 40 кГр, обеспечивает защиту от 55 до 66% летально облученных животных. Многократное облучение тест-микроба постепенно возрастающими дозами гамма-лучей индуцировало еще большее возрастание радиорезистентности, обусловленное синтезом эндогенных радиопротекторов, в частности антиоксидантного фермента пероксидазы и цитокина IL1β, обеспечивающих перехват радиоиндуцированных токсических радикалов, предотвращая тем самым пострадиационную панцитопению в костном мозге. В опытах на белых мышах, облученных гамма-лучами в абсолютно летальных дозах (7,9 Гр, $ЛД_{100/30}$), показано, что однократное подкожное введение радиорезистентного варианта *St. aureus* штамм $209R_{70}$ в дозе 2×10^8 микробных клеток на особь через 3 суток после облучения обеспечивало 77,7% выживаемость при 100% гибели нелеченых животных. На основании полученных результатов сделано предположение, что включение облученных препаратов микробного происхождения позволит повысить эффективность комплексных радиозащитных средств.

Ключевые слова: золотистый стафилококк, гамма-лучи, радиоинактивация, радиомодификация, лабораторные животные, противорадиационная эффективность

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The principal bases of today's radiation microbiology are widely used in medicine and veterinary medicine (sterilization of the substances of microbial origin, antibiotics, blood, serums, vaccines, culture media, estimation of the irradiated feed and food biosafety) [1, 2]. The radio-inactivated bacteria and viruses, as well as antigens (radio-vaccines, radio-antigens) are widely used in infectious diseases as preventive and diagnostic options. The determined antibacterial effects of ionizing radiation have made it possible to postulate the provisions most important for radiation microbiology and virology that provide the basis for modern radiation biology and radiation genetics of microorganisms and are used to obtain or construct radio-vaccines and radio-antigens [3–6]. Furthermore, the important role is played by the information that radio-inactivation of microorganisms is accompanied by the rapid decrease in microbial toxicity and the changes in microbial metabolism with induction of the synthesis of substances having radioprotective properties [7–10].

Reduction of microorganisms' toxicity with simultaneous induction of the radioprotective substance synthesis during attenuation or radio-inactivation provided the basis for assessment of the exposed microorganisms' capability of exerting radioprotective activity in cases of body's exposure to ionizing radiation [11, 12]. Furthermore, it was found that the use of both corpuscular Gram-negative bacteria vaccines (*Salmonella*, *Escherichia*, *Klebsiella*, etc.) and cellular components of microbial metabolits (endo-, exotoxins, polysaccharides, DNA) contributed to the significant increase in the exposed animals' survival rate, when the microbial products (MPs) were prescribed several hours or 1–2 days before, or during the first hours or days after the exposure [13–16].

The study was aimed to assess the effects of gamma rays on *Staphylococcus aureus* and the possibility of using the exposed microbial variants as radioprotective agents.

METHODS

The *Staphylococcus aureus* strain (st.) 209 obtained from the state collection of microorganisms of the Federal Center for Toxicological, Radiation, and Biological Safety, Kazan, Russia, was used as a test strain. Cultures were grown in the Kitt–Tarozzi liquid medium supplemented with 1% normal bovine serum (Federal Center for Toxicological, Radiation, and Biological Safety; Russia), tempered at the temperature of 37 °C for 72 h before the exposure. The 3-day culture grown was poured into glass flasks and pelleted by centrifugation at 3000 rpm for 40 min. Supernatant was drained, and the centrifugation pellet was diluted with sterile distilled water to the concentration of 1×10^9 microbial cells (m. c.) per cm^3. The resulting suspension of the *St. aureus* culture was packed in vials, 10 cm^3 per vial; these were secured with rubber stoppers and rolled in with aluminum caps. After that the vials containing the test culture were irradiated using the Researcher gamma device (Baltiets factory; Estonia) with the ^{60}Co radiation source, exposure dose rate of 2.652×10^{-2} A/kg, absorbed doses of 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 kGy. The degree of the gamma-irradiated St. aureus suspension inactivation was determined by plating suspensions on the Kitt–Tarozzi medium with subsequent 7-day thermostating and daily registration of the presence or absence of microorganism growth.

To select radioresistant mutants, the single subcultures grown were repeatedly plated on the Kitt–Tarozzi medium containing normal bovine serum until confluence was achieved. The resulting subcultures were further exposed to the above steadily growing doses of gamma radiation in case of confluence.

The exposed cultures were subjected to microbiological analysis, for which serial dilutions in the sterile phosphate buffer were prepared and analyzed using the colony forming unit (CFU) assay by conducting standard procedures involving reinoculation of Petri dishes with the meat peptone agar (MPA). The latter were incubated for 24 h at a temperature of 3 °C. CFU were enumerated in three Petri dishes with 30–300 colonies using the New Brunswick Scientific Rietran II R automatic colony counter (New Brunswick Scientific; USA), the arithmetic mean was determined for each sample. Cell viability was expressed as the mean log10 ± SD for three iterations.

Smears of the cultures grown were made, Gram stained, and examined by microscopy using immersion and 90x magnification.

Anti-radiation activity of the exposed *St. aureus* variants and radioresistant *St. aureus* st. 209R variant was tested using the lethally irradiated outbred white mice with the body weight of 18–20 g. Acute radiation syndrome (ARS) was simulated using the stationary Puma gamma device (Isotope JSC; Russia) with the ^{137}Cs radiation source at a dose of 7.9 Gy ($LD_{100/30}$), dose rate of 2.5×10^{-5} A/kg, gamma field non-uniformity not exceeding 10%.

The experiments involved 117 white mice divided into 13 groups, 9 animals per group. Animals of 12 groups were exposed to the lethal gamma radiation dose (7.9 Gy), three days later they were once subcutaneously administered 0.2 cm^3 (2×10^8 m. c./animal) of the original non-exposed *St. aureus* st. 209 culture (group 1), St. aureus st. 209 culture exposed to the dose of 30 kGy (group 2), *St. aureus* st. 209 culture exposed to the dose of 35 kGy (group 3), *St. aureus* st. 209 culture exposed to the dose of 40 kGy (group 4), *St. aureus* st. 209 culture exposed to the dose of 45 kGy (group 5), *St. aureus* st. 209 culture exposed to the dose of 50 kGy (group 6), *St. aureus* st. 209 culture exposed to the dose of 55 kGy (group 7), *St. aureus* st. 209 culture exposed to the dose of 60 kGy (group 8), *St. aureus* st. 209 culture exposed to the dose of 65 kGy (group 9), *St. aureus* st. 209 culture exposed to the dose of 70 kGy (group 10), radioresistant *St. aureus* st. $209R_{70}$ culture (group 11). Exposed animals of the control group (group 12) were administered 0.2 cm^3 of saline under the same conditions. Animals of group 13 remained non-exposed and untreated; they were used as biological controls.

The exposed, control and experimental animals were monitored for 30 days, dead animals and survivors were registered. The effects of the test substances of microbial origin were assessed based on survival rate and lifespan (ALS), blood morphology and biochemistry by the methods widely accepted in radiation hematology, as well as based on the antioxidant defense status (based on the malondialdehyde synthesis level).

Considering the fact that irradiation of animals, plants and microorganisms is associated with formation of toxic radiolytic products (radiotoxins), the experiments were conducted based on indication of these metabolites in the original and exposed staphylococcal cultures. The indirect hemagglutination assay (IHA) was used for indication of radiotoxins in the studied samples. For that the indirect hemagglutination reaction (IHR) was conducted using the anti-radiation antibody erythrocyte diagnosticum (ARAED) represented by the formalinized and tanned sheep erythrocytes sensitized with the anti-radiotoxic hyperimmune serum we had developed.

Immunochemical analysis of the disintegrants of the exposed *St. aureus* st. 209 variants was performed by conducting IHR with ARAED. For that serial two-fold (1:2, 1:4, 1:8, etc.) dilutions of the antigen in saline were prepared, each dilution was added one drop (330 μL) of the formalinized and tanned

sheep erythrocytes sensitized with the anti-radiotoxic serum (ARAED). The mixture of the test antigen and the diagnosticum was mixed thoroughly to obtain a homogenous suspension and left in the thermostat for 2–2.5 h at a temperature of 37 °C.

The reaction results were assessed using the method widely accepted in immunology. Quantitative estimates of the reaction were expressed as the radiotoxin titers (1:2, 1:4, 1:8, etc.) or logarithms to the base 2 (1:2 = $1\log_2$; 1:4 = $2\log_2$, etc.).

The reaction was supported by appropriate controls. The standard quinoid radiotoxin obtained from the lethally irradiated *St. aureus* st. 209 was used as a positive control in IHR, while the non-exposed variant of the culture was used as a negative control.

In the next phase of the study, peroxidase activity was determined in the cell suspension and the culture fluid of the radioresistant *St. aureus* st. $209R_{70}$ variant [17]. Furthermore, pyrogallol, which was oxidized to purpurogallin with maximum absorbance, was used as the oxidizable substrate. Optical density was measured in the SF-46 spectrophotometer (LOMO LLC; Russia). The cell suspension culture fluid of the original and radioresistant *St. aureus* variants were obtained by the generally accepted method through centrifugation of broth culture; centrifugation pellet was diluted to the concentration of 1×10^3 m. c./cm^3, and supernatant was used as the culture fluid.

The test solution contained 0.8 mg of the 0.006 M sodium phosphate buffer, pH 6.8; 0.12 cm^3 of the enzyme extract (suspension, centrifuge liquid); 0.5 cm^3 of the 0.15% H_2O_2; 1.1 cm^3 of H_2O and 0.5 cm^3 of the 0.003 M pyrogallol. In controls, 0.5 cm^3 of H_2O were added instead of H_2O_2.

The enzyme activity was determined using the following formula:

$$A = D_{t2}–D_{t1} / (t2–t1) \times c,$$

where A — enzyme activity, *D* — optical density, *t* — time, *c* — concentration.

Measurement was performed for 2.5–3 min.

In the next series of experiments, we assessed the mechanism underlying the anti-radiation effects of *St. aureus* st. 209R70 on the irradiated body. For that, the experiments were performed involving 30 white mice with the body weight of 15–20 g, divided into three groups, 10 animals per group. Animals of groups 1 and 2 were exposed to the lethal dose of gamma radiation (7.9 Gy, $LD_{100/30}$) using the Puma device. Animals of group 1 received a single subcutaneous injection of 0.2 cm^3 of the radioresistant *St. aureus* st. $209R_{70}$ variant with a titer of 1×10^8 m. c./animal 3 days after the exposure. Animals of group 2 exposed to the specified gamma radiation dose were administered 0.2 cm^3 of sterile injection solution (exposure control). The non-exposed animals of group 3 received nothing and were used as biological controls.

The exposed animals were monitored throughout 30 days; clinical features and the ARS course were assessed. Anti-radiation activity of the agent was estimated based on survival rate, ALS, as well as blood morphology, antioxidant defense system status, cytokine synthesis, and response of the cell renewal system (depletion and restoration of hematopoietic cells in the bone marrow).

The cytokine-inducing activity of the radioresistant *St. aureus* st. $209R_{70}$ variant was assessed by determining interleukin 1β (IL1β) concentrations in blood serum and bone marrow suspension by enzyme-linked immunoassay (ELISA) 24, 48, 72 h after the exposure and administration of the therapeutic agent. The Mouse IL1β ELISA kit (Biosourse, R&D, and Eudogen; USA) had an IL1β detection limit of 50 ng/cm^3 [18].

To estimate the levels of IL1β secretion by the red bone marrow cells, the femur was dissected in aseptic environment, crushed thoroughly in 0.5 cm^3 of saline supplemented with heparin. The resulting suspension was incubated for 5 h at 37 °C, and then centrifuged for 10 min at 800 g. The levels of cytokines in supernatant were determined per million bone marrow cells and the total number of myelokaryocytes in the femur.

The 30-day survival was used as an integral indicator of the test agent anti-radiation effect. Hemoprotective effect of the agent was estimated by enumeration of peripheral blood cells in the MINOS STO automatic analyzer (Horiba ABX Diagnostics; France). The antioxidant defense system functional competence was determined by measuring serum concentrations of the lipid peroxidation (LPO) stable aldehyde products reacting with thiobarbituric acid [19].

Statistical data processing was performed by parametric methods. Significance of differences between the indicators compared was determined using the Bonferroni adjusted Student's t-test.

RESULTS

Assessment of the *St. aureus* 209 test strain sensitivity to gamma radiation showed that the microorganism was highly radioresistant. Our findings showed that the lack of growth was observed in the sample exposed to the dose of 70 kGy only, while slow growth was reported in the dose range of 45–65 kGy [20].

After being exposed to the doses of 40–70 kGy, no culture growth was observed throughout 4 days after inoculation; sporadic colonies grew up in cases of prolonged cultivation (120 h). That is why we continued the experiments aimed to study the development of resistance to gamma radiation by the original culture during sequential exposure of the surviving colonies to the increasing radiation doses. Furthermore, sporadic colonies that grew up after the lethal exposure (40 kGy) were subjected to the prolonged passage on the Kitt–Tarozzi, MPA and MPB media until confluence was reached in order to obtain the radioresistant *St. aureus* variant. Such procedures were repeated many times using the increasing gamma radiation doses of 45, 50, 55, 60, 65, and 70 kGy.

When performing microscopic examination of the smears obtained from the exposed and Gram stained culture, Gram-positive single and paired cocci in the form of asymmetric grape bunches typical for this culture were clearly visible in the field of view.

The results of the study showed that the radioresistant *St. aureus* st. $209R_{70}$ variant, the resistance of which to gamma radiation twice exceeded that of the original strain, was obtained from the original *St. aureus* st. 209 culture by selection of single surviving colonies after the exposure to each radiation dose and prolonged passage on the appropriate culture media during passage 10 following the exposure to the dose of 70 kGy.

In the next phase of the study, St. aureus 209 exposed to the specified doses of 30–70 kGy (30, 35, 40, 45, 50, 55, 60, 65, 70) and the radioresistant variant (*St. aureus* st. $209R_{70}$) were tested for radioprotective properties in the lethally exposed white mice.

A slight swelling at the injection site that resolved within 24 h was reported in some animals after subcutaneous administration of the exposed *St. aureus* st. 209 cultures. Swelling, local hyperemia, pain at the injection site were observed in the control group in animals administered *St. aureus* st. 209 (original non-exposed culture).

Anti-radiation activity of the tested non-exposed and exposed *St. aureus* 209 variants assessed in the lethally exposed white mice is provided in Table 1.

Table 1. Survival of the lethally exposed white mice against the background of using the test *St. aureus* st. 209 variants 3 days after the single subcutaneous injection of the therapeutic agent, *n* = 9

Group number	*Staphylococcus aureus* st. 209 variant native and exp. to various doses	Agent administration method and volume, cm^3	ALS, days	Survival rate, %
1	Original st. *St. aureus* 209	subcutaneous, 0.2	8	22.2
2	*St. aureus* st. 209, exp. to the dose of 30 kGy	subcutaneous, 0.2	12.7	66.6
3	*St. aureus* st. 209, exp. to the dose of 35 kGy	subcutaneous, 0.2	13.7	66.6
4	*St. aureus* st. 209, exp. to the dose of 40 kGy	subcutaneous, 0.2	11.5	55.5
5	*St. aureus* st. 209, exp. to the dose of 45 kGy	subcutaneous, 0.2	11.2	44.4
6	*St. aureus* st. 209, exp. to the dose of 50 kGy	subcutaneous, 0.2	11	44.4
7	*St. aureus* st. 209, exp. to the dose of 55 kGy	subcutaneous, 0.2	9	44.4
8	*St. aureus* st. 209, exp. to the dose of 60 kGy	subcutaneous, 0.2	8.7	22.2
9	*St. aureus* st. 209, exp. to the dose of 65 kGy	subcutaneous, 0.2	8.4	22.2
10	*St. aureus* st. 209, exp. to the dose of 70 kGy	subcutaneous, 0.2	7.8	22.2
11	*St. aureus* st. $209R_{70}$ (radioresistant variant)	subcutaneous, 0.2	17.5	77.7
12	Exposure control	–	6.8	0
13	Biological control	–	–	–

Note: m. c. — microbial cells; st. — strain; exp. — exposed.

Table 1 shows that the Staphylococcus exposure to the gamma radiation doses of 30–40 kGy leads to modification of the test microorganisms associated with the increase in anti-radiation activity, ensuring 55–66.6% of the lethally exposed animals' survival rate, which 2–3 times exceeds the baseline anti-radiation activity level in group 1. The further increase in the exposure dose of the original culture has a negative effect on the microorganisms: the anti-radiation activity level of animals exposed to the doses of 45–70 kGy drops from 44.4 to 22.2%, respectively.

In contrast to the original culture single exposure to the doses of 30–70 kGy, multiple exposure of the original culture obtained during the experiments from the *Staphylococcus* subcultures using the gradually increasing doses had a modifying effect on the test microorganisms. Anti-radiation activity of the radioresistant *St. aureus* st. $209R_{70}$ variant 3.5 times exceeded that of the original strain and accounted for 77.7%. Furthermore, it was noted that the increase in another important radioprotection indicator, the average lifespan of dead animals (ALS), was reported along with the increase in survival rate of the lethally exposed animals when using the exposed *St. aureus* variants as radioprotective agents.

According to the data provided, ALS of the animals suffering from ARS after using the non-exposed *St. aureus* st. 209 variant was 8.0. The use of cultures exposed to the doses of 30–50 kGy resulted in the increase of this indicator to 11.0–13.7 days (variants 6, 5, 4, 3, 2). The use of the radioresistant *St. aureus* st. $209R_{70}$ variant as a radioprotective agent ensured the increase in the average lifespan of dead animals to 17.5 days vs. 8.0 days reported for the original *Staphylococcus* strain, which 2.19 times exceeded the value of the original strain.

The above experiments repeated using the other laboratory animal species, white rats exposed to the lethal doses (9.5 Gy, $LD_{100/30}$) and treated with the radio-modified *St. aureus* st. 209 variants (30, 35, 40, 45, 50, 55, 60, 65, 70) and the radioresistant variant (St. aureus st. $209R_{70}$), yielded similar results.

Analysis of the data provided in Table 1 shows that the exposure of the original *St. aureus* st. 209 culture to the gamma radiation doses of 30–70 kGy has a multidirectional effect on the exposed cultures' capability of inducing various degrees of body's radioresistance to lethal irradiation. Furthermore, it was found that the *St. aureus* st. 209 cultures exposed to the doses of 30–40 kGy increased the anti-radiation activity level to 66.6%, while the exposure of the original culture to the

Table 2. Results of the radiotoxin indication in the *Staphylococcus aureus* st. 209 variants exposed to various gamma radiation doses

Staphylococcus aureus culture and its exp. variants	Radiation dose, kGy	Radiotoxin concentration, log_2	Survival rate of the lethally exp. animals against the background of using the exp. *St. aureus* variants
Original *St. aureus* st. 209 (non-exposed)	–	0.7 ± 0.01	22.2
St. aureus st. 209 (30)	30	2.0 ± 0.3	66.6
St. aureus st. 209 (35)	35	3.0 ± 0.5	66.6
St. aureus st. 209 (40)	40	4.0 ± 0.7	55.5
St. aureus st. 209 (45)	45	6.0 ± 0.9	44.4
St. aureus st. 209 (50)	50	7.0 ± 1.2	44.4
St. aureus st. 209 (55)	55	7.5 ± 0.9	44.4
St. aureus st. 209 (60)	60	8.0 ± 1.5	22.2
St. aureus st. 209 (65)	65	9.0 ± 1.7	22.2
St. aureus st. 209 (70)	70	10.0 ± 2.1	22.2
St. aureus st. $209R_{70}$ (radioresistant variant)	70	2.4 ± 1.6	77.7

Note: st. — strain; exp. — exposed.

Table 3. Peroxidase activity of the cells of the original and radioresistant *St. aureus* st. 209 cultures

Bacterial strain	Peroxidase activity (c^{-1} mg^{-1})	
	Cell suspension	Culture fluid
St. aureus st. 209 (original culture)	$0.123 \times 10^{-3} \pm 0.01$	$0.031 \times 10^{-3} \pm 0.01$
St. aureus st. 209 R_{70} (radioresistant culture)	$0.267 \times 10^{-3} \pm 0.03^{××}$	$0.09 \times 10^{-3} \pm 0.009^{×××}$

Note: ×× — $p < 0.01$; ××× — $p < 0.001$; st. — strain.

doses of 45 kGy and above had an opposite effect, reducing radioprotective activity of the exposed *Staphylococcus* variants to 22.2%.

The results of the quinoid radiotoxin indication in the disintegrators of the *St. aureus* exposed to various gamma radiation doses in IHA with the antibody erythrocyte diagnosticum are provided in Table 2.

Table 2 shows that the *Staphylococcus* exposure to gamma radiation induces the increased synthesis of toxic radiolytic products (radiotoxins), the low doses of which (2.0–4.0 log_2) have a stimulant effect on the body. The survival rate of the lethally irradiated animals increases to 66.6%, while excess production of toxic products (6.0–10.0 log_2) decreases the exposed animals' survival rate; the anti-radiation level is between 44.4 and 22.2%. Therefore, the optimal gamma radiation exposure for the Staphylococcus culture is 30–35 kGy, and the exposure dose increase results in the increased synthesis of radiotoxin by the microorganism, i.e. is associated with reduction of the exposed *St. aureus* variants' anti-radiation factors.

In this regard, the increase in the anti-radiation effect of the radioresistant *St. aureus* st. $209R_{70}$ achieved by the repeated exposure of the original culture and its subcultures to the gradually increasing doses of 30–70 kGy is of interest. It is well known that the radioresistance development is accompanied by synthesis of antioxidant enzymes (catalase, superoxide dismutase). We conducted experiments on determining peroxidase, the antioxidant enzyme. This enzyme was selected for the study due to the fact that peroxidase represents the antioxidant enzyme, the major function of which is to disrupt peroxides, toxic radiolytic products that are dangerous for cell functioning.

The peroxidase activity measurement results for the *St. aureus* st. 209 and *St. aureus* st. $209R_{70}$ variants are provided in Table 3.

Table 3 shows that both test *St. aureus* variants exert peroxidase activity. However, that of radioresistant variant is 2.17 times higher ($p < 0.01$) compared to the original variant of microorganism. Similar upward trend of peroxidase activity was observed in the culture fluid, where the test microorganisms were grown. Furthermore, peroxidase concentration in the culture fluid obtained when growing *St. aureus* st. $209R_{70}$ was 3 times higher ($p < 0.001$), than that in the original culture.

The data on the peroxidase antioxidant enzyme concentration increase in the culture fluid during incubation of *St. aureus* st. $209R_{70}$ obtained in this experiment show that the test culture can synthesize and express this enzyme *in vivo*, i.e. in the bodies of intact and exposed animals, exerting the antioxidant and, as a result, anti-radiation effect.

Considering the above, we conducted a series of experiments on exploring the mechanism underlying body's radioprotection in the context of using the radioresistant *St. aureus* st. $209R_{70}$ variants as potential anti-radiation agents.

The dynamic monitoring of the experimental animals showed that the single subcutaneous injection of the radioresistant *St. aureus* st. $209R_{70}$ variant had a radio-modifying effect, modifying both the ARS course and the exposed animals' survival rate and increasing the lifespan of dead animals. Furthermore, it was found that the control (exposed) animals had severe ARS and ALS of 6.8 days. In contrast to the controls, animals of the experimental group receiving *St. aureus* st. $209R_{70}$ as an anti-radiation agent had milder ARS. The animals' survival rate was 77.7%, and ALS of dead animals was 17.5 vs. 6.8 days in the control (exposed) group.

The increase in the lethally exposed animals' survival rate associated with using the test agent was accompanied by adjustment of radiation-induced pancytopenia (Table 4).

Table 4 shows that lethal irradiation of white mice causes a hemotoxic effect associated with the bone marrow depletion (myelokaryocyte death) and hematopoiesis suppression (significant leucopenia and lymphopenia). The use of the test agent had hemoprotective and myeloprotective effects, preventing severe pancytopenia, preserving the myelocyte and granulocyte pool in the bone marrow and peripheral blood.

Hemoprotective effect of the test agent was realized via inhibition of the toxic radiolytic product (malondialdehyde) synthesis and the more intense synthesis of cytokines, the immunohematopoiesis mediators (Table 5).

Table 5 shows that lethal irradiation of animals with gamma rays is associated with the rapid increase in serum MDA levels (5.85-fold, $p < 0.001$), along with the decrease in the IL1β immunoregulatory cytokine synthesis.

Table 4. Blood indicators of white mice on day 10 after the exposure and single subcutaneous injection of *St. aureus* st. $209R_{70}$, $n = 10$

Indicators	Group of animals		
	Control	Exposure	Exposure + treatment *St. aureus* st. 209 R_{70}
Peripheral blood leukocyte counts $×10^9$/L	5.4 ± 0.7	1.95 ± 0.1×××	4.98 ± 0.5
Peripheral blood neutrophil counts $×10^9$/L	2.17 ± 0.3	1.03 ± 0.5××	1.95 ± 0.2
Peripheral blood lymphocyte counts $×10^9$/L	4.39 ± 0.4	1.63 ± 0.3×××	3.98 ± 0.9
Myelokaryocyte counts in the hip bone marrow $×10^6$/L	27.1 ± 1.3	15.7 ± 0.5××	26.3 ± 0.7

Note: ×× — $p < 0.01$; ××× — $p < 0.001$; st. — strain.

Table 5. Concentrations of malondialdehyde (MDA) and interleukin 1β in blood serum and bone marrow of the lethally irradiated white mice treated with *St. aureus* $209R_{70}$ 8 days after the exposure and treatment, $n = 10$

Indicator	Group of animals		
	Control	Exposure	Exposure + treatment *St. aureus* $209R_{70}$
MDA concentration in blood serum, μmol/g of protein	0.87 ± 0.05	5.09 ± 0.37***	1.05 ± 0.15
IL1β concentration in blood serum, ng/mL	55.1 ± 3.7	41.1 ± 5.9	87.1 ± 2.5
IL1β concentration in bone marrow of the femur, ng per 1 million cells	1.93 ± 0.31	1.05 ± 0.17**	1.78 ± 0.25

Note: ** — $p < 0.01$; *** — $p < 0.001$.

In this context, the use of substances of microbial origin (radioresistant *St. aureus* st. $209R_{70}$ variant) has an antioxidant effect, inhibiting free radical oxidation and peroxidation of lipids and decreasing synthesis of the LPO aldehyde product, malondialdehyde.

At the same time, the agent of microbial origin used showed itself as the enhancer of the interleukin IL1β synthesis in peripheral blood and bone marrow of the exposed animals.

The reported biochemical alterations in the immunohematopoietic organs associated with the effects of the agent of microbial origin ensure the 70% survival rate in the lethally irradiated animals.

DISCUSSION

In recent years, domestic and foreign researchers have accumulated experimental data suggesting that substances of microbial origin (endotoxins, polysaccharides, toxoids, etc.) can increase body's resistance to ionizing radiation when studying various aspects of the mechanism underlying the anti-radiation effect [4, 11, 13–16]. Considering the above, we have carried out this study focused on assessing radioprotective properties of the radio-modified *St. aureus* variant.

The experiments conducted have shown conclusively that the Staphylococcus exposure to the gamma radiation doses of 30–40 kGy has a modifying effect on the microorganisms, it increases their anti-radiation activity by 22.2% compared to the original culture. Such results are well consistent with all the literature data on the issue. Many authors have shown that corpuscular bacteria, microbial polysaccharides, exo-, endotoxins, and toxoids increase survival rate of the experimental animals exposed to the ionizing radiation doses of about $LD_{80\text{-}90/30}$ by 20–30% [21]. There is no doubt that staphylococcal lipopolysaccharides contained in vaccines and other bacterial formulations have the same properties [22].

Staphylococcus was selected as a model to construct the microbial radioprotector due to the following facts: first, these microorganisms produce potent exo- and endotoxins, which convert to toxoids having radioprotective properties under exposure to physical and chemical factors (UV, ionizing radiation, formaldehyde, etc.) [23]; second, *St. aureus* has a powerful antioxidant system and is capable of inducing antioxidant enzymes [24] and cytokines [25]; third, highly effective medicines with the broad-spectrum biological activity are derived from phagolysates of pathogenic Staphylococcus strains.

When conducting this study, we proceeded from the premise that the role of microorganisms in protection against the effects of ionizing radiation is well understood. The animal body's defense mechanisms in the form of enhanced proliferation of the granular and lymphoid hematopoietic cells directly involved in the immune response are activated under exposure to the substances of microbial origin; platelet, granulocyte counts and hemoglobin levels are increased, along with the activity of the endogenous and exogenous cells of the lymphoid system of the spleen, lymph nodes [26]. Furthermore, it was also considered that irradiation of microorganisms with the doses, insufficient to disrupt their DNA molecules, but quite enough for DNA rearrangement involving alteration of the DNA chain fragments, can lead to formation of the mutant bacteria with different cultural and morphological properties and some acquired useful qualities, specifically the ability to produce certain substances that are useful in terms of human practice [27].

Considering the need to develop new safe and effective anti-radiation agents for treatment of ARS, we conducted this study focused on assessing anti-radiation effects of the substances of microbial origin represented by medicines derived from *Staphylococcus*. When conducting the study, we used the data on the fact that physical effects on microorganisms induced the increase in the exposed organism's radioresistance through induction of Toll-like receptors (TLR) by microorganisms as a working hypothesis [4].

To test this hypothesis for eligibility, we exposed *Staphylococcus* to gamma radiation in the dose range of 30–70 kGy. The experiments showed that the *Staphylococcus* gamma exposure had a multidirectional effect on the microorganism, it increased or decreased the *St. aureus* radioprotecitive efficacy, depending on the dose. It was found that the *Staphylococcus* cultures exposed to gamma radiation doses of 30, 35 and 40 kGy exerted radioprotective activity, protecting 55.5–66.6% of the lethally exposed white mice from radiation-induced death. However, further increase in radiation dose (45–70 kGy) had an opposite effect, it decreased the level of animal's protection against ARS. The decrease in radioprotective activity of the *St. aureus* variants exposed to high doses is explained by the increased production of radiotoxins in the irradiated cultures, which results in the increased mortality among exposed animals due to summation (potentiation) of toxic effects of the irradiated bacteria and the macroorganism [20].

The repeated exposure of microorganisms to the gradually increasing ionizing radiation doses results in the stepwise radioresistance increase [27] accompanied by changes in cell metabolism together with induction of endogenous radioprotectors [21]; we conducted the study involving obtaining the radioprotective *St. aureus* variant. We obtained the radioresistant *St. aureus* $209R_{70}$ variant that survived under exposure to the supraletal radiation dose (70 kGy) through repeated exposure of the test microorganism to the gradually increasing gamma radiation doses in the range of 30–70 kGy. When studying the mechanism of targeted acquisition of extreme radioresistance by *St. aureus*, it was found that the process of adaptation to the supraletal gamma radiation dose was accompanied by changes in bacterial cell metabolism with the increase in the levels of peroxidase, the antioxidant enzyme

playing one of the key roles in the context of body's exposure to stress factors, including ionizing radiation [17]. Our findings are consistent with the data of other authors, who revealed increased production of antioxidant enzymes (superoxide dismutase, hydroperoxidase), ubiquinone, enhanced recombination DNA repair in the radio-thermo-resistant *St. aureus* mutants [7, 28].

The ability of the radioresistant *St. aureus* variant to synthesize antioxidant enzymes during radio-adaptation ensured the increase in its anti-radiation properties in case of being used as an anti-radiation agent *in vivo*. The experiments involving white mice exposed to the absolutely lethal gamma radiation doses (7.9 Gy, $LD_{100/30}$) showed that the single subcutaneous injection of the radioresistant *St. aures* $209R_{70}$ variant in a dose of 2×10^8 m. c./animal 3 days after the exposure ensured the 77% survival rate, against 100% mortality among untreated animals. The increase in survival rate of the animals exposed and treated with *St. aureus* $209R_{70}$ was accompanied by transition from acute ARS to mild ARS, which resulted from prevention of pancytopenia and depopulation of the bone marrow. The mechanism underlying the hemo- and myeloprotective effect of the gamma-exposed microorganism (*St. aureus* $209R_{70}$) was realized as follows: first, through interception and neutralization by anti-radical enzymes (peroxidase and superoxide dismutase) of the radio-induced toxic radiolytic products ((malondialdehyde), the main target of which is represented by the immunohematopoietic system cells (lymphocytes, monocytes, bone marrow stem cells); second, through induction of the cytokines (IL1β in our study) initiating post-radiation restoration of hematopoiesis via induction by the test microorganism, which is in line with the data reported by other researchers [29, 30].

CONCLUSIONS

The findings suggest that the therapeutic agents derived from *St. aureus* that have been exposed to radiation in the dose range of 30–40 kGy, demonstrate anti-radiation efficacy ensuring 66.6% survival of the lethally irradiated animals, while the radioresistant *St. aureus* st. $209R_{70}$ variant is superior to these agents, since it increases survival of animals exposed to $LD_{100/30}$ to 77.7% and prevents radiation-induced death. Substances of microbial origin are promising and feasible, since agents of this class are harmless and, which is more important, have polyfunctional (immunotropic, antioxidant, hemo- and myeloprotective) properties. Given the above, we believe that it is important to continue the search for measures increasing the efficacy of substances of microbial origin, since inclusion of agents of this class in the range of medications for radiation-induced injury might increase the efficacy of combined anti-radiation treatment.

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