Peculiarities of the course of acute radiation injury in mice against the background of iodine and selenium preparations

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Abstract. In experiments on mice the study of the radioprotective and radiotherapeutic efficacy of the combined use of the selenium-containing feed additive DAFS-25k and the iodine-containing preparation Monclavit-1 was carried out. It has been shown that intragastric administration of DAFS-25k oil solution 3 hours before the total, external, single exposure to γ-radiation, and the subsequent (in 12 hours) intragastric administration of an aqueous solution of Monclavit-1, increases the survival rate of irradiated animals to 67% versus 17% in the control animals. The mechanisms of radioprotective efficacy are assumed to be the antioxidant activity of selenium compounds in DAFS-25k, normalization of thyroid function and relief of gastrointestinal syndrome due to the pronounced antimicrobial activity of Monclavit-1.

1 Introduction

In the pathogenesis of radiation pathology an important role is assigned to the aspects of the primary indirect and mediated action of ionizing radiation. Notably the severity of the course and the outcome of acute radiation injury can be determined by the antioxidant status and functional state of the thyroid gland [1, 2].

Today the provision of the human and animal body with iodine and selenium is an urgent problem not only for Russia, whose vast territories are endemic due to deficiency of these elements in the diet, but also for other countries as well. [3].

The microelement pair "iodine and selenium" is essential for the functioning of the thyroid gland, primarily for the metabolism of thyroid hormones. While iodine is part of thyroid hormones, selenium is essential for the biosynthesis of selenium proteins in thyroid metabolism. Selenium-dependent iodothyronine deiodinases control the processing of excess thyroid hormones, and intracellular, secreted selenium-dependent glutathione peroxidases are involved in the antioxidant defence of the thyroid gland [4].

Among the syndromes of acute radiation injury, the gastrointestinal tract requires special attention. Direct and indirect effects on the epithelium of the stomach and intestine lead to the development of inflammatory processes in organs, damage to the mucous membrane,

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disruption of the microbial composition of flora, the development of toxemia, bacteremia. All this largely determines the clinical picture and outcome of acute radiation injury [5].

The practical application of inorganic selenium compounds is difficult due to their high toxicity. Organic selenium compounds turned out to be much less toxic and therefore more promising. A similar trend is noted in relation to iodine preparations: organic forms come to replace inorganic forms, as well as iodophores, in which iodine is enclosed in a high polymer molecule. We have previously found that iodophor Monclavit-1, administered before and after exposure to general, external, single γ-radiation contributes to a more favourable course of acute radiation injury in rats and guinea pigs used as experimental models by normalizing the function of the thyroid gland. However, the effectiveness of this drug decreased significantly with an increase in the severity of radiation pathology [2].

When studying the radioprotective efficacy of the organoselenium compound selentetracysteine, it was found that when administered 1 hour before irradiation, the survival rate increased by 17%, and when administered 24 hours before, by 58% in mice with a single, general external exposure to γ-radiation at a dose of 7.3 Gy. The radioprotective effect of selentetracysteine was achieved by researchers due to the positive effect of the substance on hematopoiesis, the increase in the number of endogenous colonies grown on the spleen detected 10 days after irradiation, and an increase in the total antioxidant activity of blood plasma [6].

At the same time, the question of the radioprotective and radiotherapeutic effect of formulations for the combined use of iodine and selenium compounds has remained practically unexplored so far, this laid the ground for the present experimental work.

Thus, the purpose of our work was to evaluate the radioprotective and radiotherapeutic efficacy of the combined use of DAFS-25k and Monclavit-1 in mice exposed to a general, external, single γ-radiation.

2 Materials and methods

The experiment was performed on 126 white, outbred laboratory male mice, live weight 22.0±1.2 grams. The experimental work was carried out in accordance with the requirements of: Order of the Ministry of Health of the Russian Federation dated 01.04.2016 No. 199n "On Approval of the Rules of Good Laboratory Practice", "Code of Ethics" (1985), including the section "International Recommendations for Biomedical Research Using Animals ", Declaration of Helsinki by the World Medical Association (2000), requirements of the Federation of European Scientific Associations on the Care and Use of Laboratory Animals in Scientific Research (FELASA).

Three series of experiments were carried out. Each series included the formation of 7 experimental groups of animals, 6 individuals in each. The groups were formed on the basis of analogues. To simulate acute radiation injury, mice were exposed to general, external, single exposure to γ-radiation of ^{137}Cs, at a dose of 6.0 Gy, at a dose rate of 0.99 Gy/min. In accordance with the requirements of the "Methodological guidelines for the preclinical study of the radioprotective properties of pharmacological substances" for the initial selection of effective radioprotective drugs, the of 30-day survival was used.

DAFS-25k (Sulfat LLC, Saratov, Russia) is a feed additive for animals, it includes diacetophenonyl selenide – not less than 95% with a mass fraction of selenium in diacetophenonyl selenide – 25%, sodium sulfite and sodium chloride – not more than 1%, bound water – no more than 4%. DAFS-25k solutions were prepared *ex-tempore*; vegetable oil heated to a temperature of 70°C was used as a solvent. DAFS-25k was administered to mice intragastrically, at a dose of 6.5 mg/kg and 1.6 mg/kg, 3 hours before exposure to γ-radiation, once, the volume of administration was 0.3 ml per animal.

Monclavit-1 (LLC Orgpolymersintez SPb, St. Petersburg, Russia) is a medicinal product for veterinary use containing iodine in the form of a polymer complex. As an active ingredient, it contains a 0.1% solution of crystalline iodine in the form of an aqueous solution of polyvinyl-N-amidecyclosulfoiodide. This drug was used intragastrically in the form of an aqueous solution 12 hours after irradiation, and then with an interval of 48 hours, premixed with water in a 1: 1 ratio, with a total volume of 0.3 ml/animal/administration, 10 times.

Mice of all experimental groups, except for intact animals, from day 5 after exposure to γ-radiation were given probiotic feed additive "Vetom1.1", by the group method with drinking water, dissolving 150 mg in 100 ml of water.

The experimental animals were monitored daily for 30 days, monitoring included an assessment of clinical signs of the disease, thermometry, determination of live weight, registration of death. The absolute content of shaped blood elements of animals was determined using an automatic veterinary hematological analyzer Mindray BC-2800 Vet. Surviving animals were withdrawn from the experiment by means of an overdose (80 mg/kg) of general anesthesia (Zoletil®, Virbac, France) followed by cervical dislocation of the vertebrae.

The data obtained were subjected to statistical processing using the Statistica10 program. The statistical significance of differences in the values of the survival rate and the manifestation of gastrointestinal syndrome between the experimental groups was assessed using Fisher's exact test. Using the Mann-Whitney U-test, the significance of differences between the experimental groups was determined in terms of the absolute content of leukocytes and platelets in peripheral blood. The indices of the total body temperature and live weight in animals were subjected to variational statistical processing using the Tukey test. Differences were considered statistically significant at $p \leq 0.05$.

3 Results

At a single exposure of total, external γ-radiation of ^{137}Cs at a dose of 6.0 Gy, at a dose rate of 0.99 Gy/min, mice developed acute radiation damage. The research of the antiradiation effect of the compounds under study showed a decrease in mortality in animals, which characterizes the of these beneficial effect of these compounds on the course and outcome of acute radiation injury. As can be seen from the data given in table. 1 death of animals was recorded in the period from 6 days to 21 days after irradiation. The mortality rate by 30 days in the "Control of exposure" group was 83%; against On application of Monclavit-1 – 67%; on application of water and DAFS-25k at a dose of 6.5 mg/kg and 1.6 mg/kg – 55% and 50%, respectively; with the combined use of DAFS-25k at a dose of 6.5 mg/kg and 1.6 mg/kg and Monclavit-1 – 44% ($p \leq 0.05$) and 33% ($p \leq 0.05$), respectively.

Table 1. Dynamics of the survival of experimental animals for the period from the sixth to the thirtieth days after a single, general, external exposure to γ-radiation (n = 18).

Experimental group	Dose of Preparation		7th day	12th day	21st day	30th day
	DAFS-25к, mg/kg	Monclavit-1, ml				
Intact animals	–	–	0/18	0/18	0/18	0/18
			0 %	0 %	0 %	0 %
Control of exposure	–/oil	–/water	3/18	13/18	15/18	15/18
			17 %	72 %	83 %	83 %
Monclavit-1	–/oil	0.3 ml	2/18	11/18	12/18	12/18
			11 %	61 %	67 %	67 %
DAFS-25к	6.5 mg/kg	–/water	4/18	10/18	10/18	10/18

			22 %	55 %	55 %	55 %
DAFS-25к	1.3 mg/kg	–/water	1/18	6/18	9/18	9/18
			6 %	33 %	50 %	50 %
DAFS-25к + Monclavit-1	6.5 mg/kg	0.3 ml	4/18	6/18	8/18	8/18#
			22 %	33 %	44 %	44%
DAFS-25к + Monclavit-1	1.3 mg/kg	0.3 ml	0/18	5/18	6/18	6/18#
			0 %	28 %	33 %	33%
Note: # – statistically significant differences in comparison with animals from "Control of exposure" group based on Fisher's exact test ≤0.05.						

Diarrhea was noted in 100% of the animals (Table 2). The faeces were from a watery to mushy consistency, with an admixture of blood and mucus, the root of the tail and the hair around the anus were stained with faeces. Similar clinical signs were also recorded in other experimental animals, but the degree and duration of their manifestation was lower. The manifestation of diarrhea in mice on application of the combined use of DAFS-25k at a dose of 6.5 and 1.6 mg/kg and Monclavit-1 for more than 4 days was recorded in 50% ($p \leq 0.05$) and 42% ($p \leq 0.05$), respectively; in animals of other groups – in 67%.

Table 2. Manifestation of diarrhea in mice against the background of exposure to γ-radiation during the period of pronounced clinical signs of radiation pathology (n = 18).

Experimental group	Dose of Preparation		Manifestation of diarrhea for more than 4 consecutive days, number of cases / total number of observed mice (%)
	DAFS-25к, mg/kg	Monclavit-1 ml	
Intact animals			0/18 (0)
Control of exposure	–/oil	–/water	18/18 (100)
Monclavit-1	–/oil	0.3 ml	12/18 (67)
DAFS-25к	6.5 mg/kg	–/water	12/18 (67)
DAFS-25к	1.3 mg/kg	–/water	12/18 (67)
DAFS-25к + Monclavit-1	6.5 mg/kg	0.3 ml	9/18# (50)
DAFS-25к + Monclavit-1	1.3 mg/kg	0.3 ml	8/18#(42)
Note: # – $p \leq 0.05$, according to Fisher's exact test, the incidence of diarrhea is statistically significantly different from the values in control animals			

The quantitative characteristics of the absolute content of leukocytes in experimental animals in the period before irradiation and after irradiation on day 1, day 3, day 12 and day 30 are presented in Table 3.

According to table 3, 24 hours after irradiation in mice that did not receive DAFS-25k, a significant increase in the number of leukocytes in the blood by 7% and 5%, respectively, was recorded compared with healthy animals. In the subsequent periods of observation, the absolute content of leukocytes in all animals as a result of exposure to γ-radiation was significantly lower in comparison with intact mice in the same periods of observation.

The peak of leukopenia in all irradiated animals fell on the 12th day after exposure to γ-radiation, while the number of leukocytes in mice of all experimental groups decreased

(p≤0.05) by 38%, 33%, 34%, 34%, 28%, respectively. 28% compared with healthy animals. At the same time, the severity of leukopenia in animals that use Monclavit-1, including the combination with DAFS-25k by 12 and 30 days, was significantly lower compared with control animals at the corresponding time of the study. By the end of the observation, in all experimental animals, the number of leukocytes in the peripheral blood remained significantly lower in comparison with healthy animals.

The dynamics of recovery of the absolute leukocyte count in peripheral blood in mice in the groups receiving DAFS-25k and Monclavit-1 is more progressive by 30 knocks in comparison with control animals.

The quantitative characteristics of the absolute content of platelets in experimental animals in the period before and after irradiation on day 1, day 3, day 12, day 30 are presented in Table 4.

The absolute content of platelets in the peripheral blood of mice, as a result of total, external, single γ-radiation, progressively decreased in the period from 1st day after exposure to 12th day (Table 4).

At the same time, the number of platelets in the irradiated mice of the experimental groups decreased (p≤0.05 relative to non-irradiated animals) by 7.6 times, 3.8 times, 3.4 times, 3.8 times, 3.1 times, and 3 times, respectively. 3 times.

Table 3. The absolute content of leukocytes ($\times 10^9$/л) in the blood of experimental animals in the period before irradiation and after irradiation on day 1, day 3, day 12, day 30. Me [25%; 75%], n = 10

Day	Intact animals	Control of exposure	Monclavit	DAFS-25к 6.5 mg/kg	DAFS-25к 1.3 mg/kg	DAFS-25к (6.5 mg/kg) Monclavit	DAFS-25к (1.3 mg/kg) Monclavit
Radiation background	10,8 [10,1;11,4]	10,6 [10,1;11]	10,8 [10,1;10,9]	10,8 10,1;11,1	10,5 [10,2;11]	10,6 [10,3;11,6]	10,7 [10,3;11,6]
1	11,1 [10,6;11,6]	11,9* [11,7;12]	11,6* [11,4;12,1]	9,5*# 9,1;10,1	10,6# [10;11]	9,8# [9,3;10,1]	10,5# [10,3;11,0]
3	11,0 [10,3;11,5]	8,9* [8,6;9,4]	9,5*# [9,2;10,1]	9,0* 8,4;9,3	9,1* [8,6;9,5]	9,2* [9,0;9,7]	9,4* [8,8;10,0]
12	10,9 [10,3;11,2]	6,8* [6,3;7,0]	7,3*# [7,2;7,6]	7,2* 6,7;8,1	7,2* [6,5;8,2]	7,8*# [7,3;8,2]	7,8*# [7,3;8,2]
30	11,2 [10,7;11,3]	8,2* [7,8;8,4]	8,1* [7,7;8,5]	8,6* [8,3;9,0]	9,1*# [8,9;9,3]	9,1*# [8,9;9,1]	9,3*# [9,1;9,5]
Note: * – p≤0.05 (Mann-Whitney U-test) relative to intact animals; # – p≤0.05 (Mann-Whitney U-test) relative to the control animals.							

The number of platelets in animals receiving DAFS-25 by 12th day and 30th day was significantly higher than in animals of the "Control of exposure" group in the same observation periods by 36-100%.

Table 4. The absolute content of platelets ($\times 10^{12}$/л) in the blood of experimental animals in the period before irradiation and after irradiation on day 1, day 3, day 12, day 30. Me [25%; 75%], n = 10

Day	Intact animals	Control of exposure	Monclavit -1	DAFS-25к 6.5 mg/kg	DAFS-25к 1.3 mg/kg	DAFS-25к (6.5 mg/kg)+ Monclavit	DAFS-25к (1.3 mg/kg)+ Monclavit

Radiation background	274 [253;315]	269 [255;312]	282 [258;302]	263 [243;281]	260 [237;287]	268 [248;291]	255 [232;293]
1 day	290 [270;310]	250* [217;273]	236* [210;273]	251 [235;283]	244* [218;264]	237* [214;267]	233* [209;257]
3day	286 [277;302]	208* [195;218]	179* [127;205]	219* [200;278]	212* [191;230]	206* [198;233]	209* [187;232]
12day	297 [280;310]	39* [23;65]	78*# [45;11]	87*# [74;100]	78*# [65;106]	95*# [83;102]	88*# [73;107]
30day	295 [277;321]	109* [101;122]	165*# [124;202]	132*# [119;139]	130*# [125;144]	139*# [124;146]	137*# [127;162]
Note: * – p≤0.05 (Mann-Whitney U-test) relative to intact animals; # – p≤0.05 (Mann-Whitney U-test) relative to the control animals.							

4 Discussion

High doses of selenium in the DAFS-25k feed additive, in particular 6.5 and 1.6 mg/kg, which is 1/2 and 1/8 of LD_{16} (13.0 mg/kg), caused signs of poisoning in animals, which were characterized by a decrease in the total body temperature by 0.9–1.1°C (p≤0.05 relative to control animals) against the background of exposure to γ-radiation in the period 3 hours after irradiation and up to 2 days. In addition, significant leukocytopenia was noted by 1 and 3 days after irradiation, while in animals irradiated without selenium subsidies, moderate leukocytosis was recorded by 1st day, followed by a slight decrease in the absolute number of leukocytes by 3rd day. The described clinical manifestations against the background of increased doses of selenium are consistent with those described in the literature and obtained by us in preclinical studies [7, 8].

The combined use of DAFS-25k and Monclavit-1 had the most favourable effect on the course and outcome of acute radiation injury in mice, increasing the survival rate to 56% and 67%. A possible mechanism of the radioprotective and radiotherapeutic action of the studied compounds may be the arrest of the link of the indirect action of γ-radiation, in particular of peroxide compounds, including on the thyroid gland.

Selenium-dependent proteins such as glutathione peroxidases, thioredoxin reductases, oxidoreductases and selenoproteins determine the main physiological effects of this essential trace element [9, 10, 11]. In particular, the thioredoxin reductase-glutathione peroxidase system is involved in DNA repair, gene transcription, and also affects cell proliferation, differentiation, and apoptosis [3, 9, 12].

Another possible mechanism of radiotherapeutic efficacy may be that Monclavit-1 not only normalizes the function of the thyroid gland through donations of iodine, which is part of this drug, but also as an antiseptic with pronounced antimicrobial properties, this contributes to the relief of gastrointestinal syndrome appeared as a result of exposure to γ-radiation, which was clinically manifested by a significant decrease in the frequency of diarrhea in irradiated animals to 50 and 42% versus 100% in the control of irradiation. The data revealed in this experimental study are consistent with those obtained earlier.

5 Conclusion

Thus, the combined use of DAFS-25k at a dose of 6.5 mg/kg and 1.3 mg/kg 3 hours before the total, external, single exposure to γ-radiation at a dose of 6.0 Gy, followed by ten-fold intragastric administration of Monclavit-1, previously mixed with water in a ratio of 1: 1, with a total volume of 0.3 ml/animal/administration, with an interval of 48 hours, increases the survival rate of mice to 56% and 67%, respectively, versus 17% in the control of exposure.

Application of Monclavit-1 to irradiated mice reduces the incidence of gastrointestinal syndrome in mice exposed to γ-radiation at a dose of 6.0 Gy to 33%. This effect is potentiated by intragastric administration of DAFS-25k 3 hours before irradiation at a dose of 6.5 and 1.3 mg/kg; the frequency of gastrointestinal syndrome in this case is reduced to 50 and 42%, respectively, against 100% in the control of exposure.

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