ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
Ramzi N. Nizamov et al /J. Pharm. Sci. & Res. Vol. 11(2), 2019, 653-655

Research of Radioprotective Agents from the Class of Natural Antioxidants

Ramzi N. Nizamov*, Gennady V. Konyukhov, Natalya B. Tarasova, Ilnar R. Yunusov, Nikolai M. Vasilevsky, Marsel Z. Tuhfatullin, Konstantin N. Vagin

Radiobiology laboratory, Federal center for toxicological, radiation and biological safety, Scientific town-2, Kazan city, 420075, Russia

Abstract.

The limited funds for the correction of free radical radiopathology served as the basis for these studies aimed at researching radioprotective agents from the class of natural antioxidants. The experiments used laboratory (white mice, white rats, guinea pigs) and farm (sheep) animals, 61 types of plant, animal and microbial raw materials. Obtaining, preparation and processing of technological raw materials, preparation of extracts was carried out according to the State Pharmacopoeia of the Russian Federation. Simulation of exogenous generation of superoxide radicals using in vitro test system was carried out by irradiating the blood cells with gamma rays at the "Puma" facility at doses of 0.023-0.5 g. The intensity of superoxide radical formation was judged by malondialdehyde (MDA) accumulation in the culture medium. The agent "Era-ZhM" was administered at a dose of 25 mg/kg 24 h before and 24 h after irradiation. The criteria of the radioprotective effect were the 30-day survival rate of irradiated animals and the state of the prooxidant-antioxidant system: (the intensity of MDA synthesis, the content of anti-radical enzymes (superoxide dismutase, catalases). It was found out that a single subcutaneous injection of the agent "Era-ZhM" provides 75-80% protection of lethally irradiated animals.

Key words: acute radiation sickness, superoxide radicals, free radical pathology, malondialdehyde, radioprotection.

INTRODUCTION.

After discovering oxygen, part of the air necessary for life, its toxicity was established, as it is involved in forming the reactive oxygen intermediates [1,2,3,4] which participate in the process of oxidative modification of macromolecules followed by the formation of toxic oxidation products: aldehydes, epoxides, unsaturated fatty acids, lipid radicals, malondialdehyde, ethane, methane, etc. [5,6,7]. Long-term activation of free radical oxidation in the tissues of a living organism inevitably leads to the development of free radical pathology [8,9,10]. This point of view marked the beginning of active development of means for prevention and treatment of radio-induced free radical pathology with the use of antioxidant agents. The issue of the use of amino acids, vitamins, plant- and animal-based agents that contain antioxidant enzymes (catalase, superoxyddismutase) is considered highly significant and less developed in the field of radiobiology [11,12,13].

It was shown that the use of a complex of biologically active agents of plant (alfalfa, clover, carrots, herbal mixture) and animal (mussels, crabs, shellfish, erythrocyte mass) origin leads to the normalization of the prooxidant-antioxidant system [14,15]. However, these studies are occasional and did not go beyond the model experiments, which dictates the need and feasibility of in-depth study of the issue. Taking into account the absence of efficient antioxidant agents – radioprotactive agents made of natural raw materials, the present studies have been undertaken with the purpose to find radioprotective agents from the class of natural antioxidants.

MATERIAL AND METHODS.

The experiments included 250 white mice with a live weight of 18-20 g, 316 white rats with a live weight of 180-200 g, 10 Guinea pigs with a live weight of 250-300 g, 13 Precoce sheep with an average live weight of 35 kg aged 18-24 months old. Animals were fed in accordance with zootechnical requirements. Throughout the whole study period, the experimental and control animals were in the same conditions.

For the simulation of radiation disease for laboratory and farm animals the experiment used gamma-ray unit "Puma" with ^{137}Cs radiation source at the radiation exposure intensity of $3.13^{*}10^{-3}$ C/kg*C and gamma-ray unit "Issledovatel" with ^{60}Co radiation source at the radiation intensity of $1.24^{*}10^{-2}$ C/kg*s.

For hematological, biochemical, toxicological, immunological and microbiological studies, standard solutions, nutrient media, necessary chemical agents were used. Commercial agent "Mexidol" was used as a control antioxidant, antiradiation medical and preventive immunoglobulin was used as a control radioprotective agent, "Erakond", "Era-N" and developed feed additive "Vita-Force" were used as standard plant-based agents.

Obtaining, preparation and processing of technological raw materials was carried out according to the State Pharmacopoeia of the Russian Federation. Maceration, percolation and repercolation methods were used for the selection of optimal conditions for extracting plant-based raw materials.

Simulation of exogenous generation of superoxide radicals using in vitro test system was carried out by irradiating the peripheral blood cells with gamma rays at the "Puma" facility at doses of 0.023 to 0.5 g and subsequent incubation using Igla nutrient medium with the addition of 2 mm of glutamine and 80 µg/ml of gentamicine.

The degree of malondialdehyde accumulation slowdown and superoxide dismutase activity preservation in the incubation medium in the presence of the studied extracts

helped to judge about their antioxidant activity determined using Fridovich method.

For the emergency assessment of radioprotective activity, the microbial cells (E.coli, PL-6) irradiated at doses of 0.25-1.0*10^4 g were incubated in the presence of the tested extracts, taking into account their survival.

The effect of the "Era-N" agent on the animal body was evaluated using clinical and hematological parameters, content of hemoglobin, erythrocytes, thrombocytes, T-, B-lymphocytes, T-helpers, T-suppressors, nonspecific resistance indicators (lysozyme and bactericidal activity, phagocytic activity of neutrophils), bone marrow and spleen cellularity, as well as stress protective action in the immobilization test.

Evaluation of the radioprotective effect of the developed composition "Era-ZhM" in vivo was carried out by a single total irradiation of laboratory (white mice, white rats) and farm (sheep) animals at doses of 8.0, 9.0 and 4.2 g respectively, and a single subcutaneous injection of 10% dealcoholized extract of the agent at a dose of 25 mg/kg 24 h before and 24 h after irradiation. As a criterion of radioprotective effect, 30-day survival of irradiated animals, the state of prooxidant-antioxidant system by the level of TBA-reactive substances, the content of superoxide dismutase, catalase – by Fridovich and glutathione peroxidase – by Ames were used.

The received digital material was subjected to statistical processing using conventional parametric methods, the degree of reliability of differences between the compared indicators was determined by the Student's t-test using the Microsoft Excel application package.

RESULTS AND DISCUSSION.

It was found that among the tested 56 types of plant-based agents the highest antioxidant properties belonged to herbal tea extracts (a mixture of nettle, dill, carrot leaves) which inhibited the concentration of malondialdehyde by 99.8 %. Similar antioxidant activity characterized the extracts of birch fungus and fir needles (99.7 %), leaves and flowers of Saint-John's-wort (99.6%), berries of mountain ash (99.3 %), bilberries (95.6 %), sunflower heads (91.3%), and berries of snowball tree (99.1 %). Among substances of animal and microbial origin the most active from the point of view of antioxidant properties were the extracts of erythrocytes (99.7 %), culture liquid B.bifidum (99.6 %), "Vita-Force" extract (99.3 %), and culture liquid of Bac.subtilis (96.7 %). Antioxidant activity of mexidol was 91.5 %, antioxidant activity of anti-radiation medical and preventive immunoglobulin was 89.5 %.

When cultivating lethally irradiated cells of E.coli PL-6 in the presence of the studied extracts it was found that among the tested 61 types of extracts 19 types had radioprotective activity, ensuring the survival of irradiated cells in the range from 23.4 to 72.1 %. At the same time, the highest radioprotective activity characterized extracts of "Vita-Force" (72.1 %) and herbal tea (a mixture of nettle, dill, carrot leaves) (71.9%), birch fungus (67.1%), St. John's wort herb (69.3%), berries of snowball tree (65.5%), metabolic products of B.bifidum (63.5%), Bac.subtilis (63.9 %), fir needles (63.5 %), erythrocyte mass (62.5 %), berries of mountain ash (58.8 %), needles of silver fir (53.9 %), arborvitae (54.3 %) and pine (53.7 %), extracts of asp bark (49.9 %), berries of bilberry (45.9 %). Among the most active antioxidants were selected 6 substances of plant, 2 - animal and 2 - microbial origin, on the basis of which dry (powder) and liquid (oral and injectable) dosage forms of the agent were developed, the agent conventionally called "Era-ZhM" (plant-, animal-based, and microbial extract) which was further tested for radioprotective effect.

Table 1 - Survival of lethally irradiated mice depending on the scheme of "Era-ZhM" agent use in %

Experience option	Group	Observation period, days					
		3	6	10	14	21	30
I	1st (single subcutaneous administration of injectable agent form 24 h before irradiation)	20 100	20 100	19 95	18 90	16 80	15 75
II	2nd (single subcutaneous administration of injectable agent form 24 h after irradiation)	20 100	20 100	19 95	18 90	17 85	16 80
III	3rd (intragastric administration of the agent solution 15 days before and within 15 days after irradiation)	20 100	20 100	16 80	14 70	13 65	12 60
IV	4th (feeding with the powder agent form during 30 days - during 15 days before and 15 days after irradiation)	20 100	20 100	17 85	15 75	13 60	13 65
V	5th (feeding with the oral form during 30 days before irradiation)	20 100	20 100	18 90	15 75	13 65	12 60
VI	6th (feeding with oral agent form during 30 days after irradiation)	20 100	20 100	17 85	13 65	8 40	7 35
VII	7th (control of irradiation)	20 100	18 90	11 55	4 20	1 5	0 -
VIII	8th (biological control)	20 100	20 100	20 100	20 100	20 100	20 100

Numerator is the number of survived animals, denominator is % of survival

Table 2 - Hematological parameters of sheep subjected to radiation oxidative stress and treatment with the "Era-ZhM» agent

Indicator	Group	Result	Research period, days				
			3	7	14	21	28
Hemoglobin, g/l	1	97.0±4.1	96.6±3.1	68.0±4.0	33.0±9.5	-	-
	2	97.1±4.5	97.3±3.3	96.1±3.7	97.0±2.1	96.7±7.7	94.9±2.1
	3	96.9±3.1	96.8±3.5	101.1±5.3	101.0±7.5	97.8±2.8	97.1±7.7
Erythrocytes, * 10^{12}/l	1	6.3±0.5	6.4±0.1	5.6±0.1	5.4±0.3	-	-
	2	6.5±0.7	5.9±0.5	5.8±0.2	5.9±0.1	5.9±0.3	6.8±0.5
	3	6.1±1.3	5.9±0.2	5.9±0.3	6,01±0,1	6.1±0.3	6.1±0.1
Thrombocytes, 10^9/l	1	3.1±0.5	1.6±0.7	2.0±0.1	2.1±0.3	-	-
	2	3.1±0.9	2.9±0.5	2.9±0.5*	2.8±0.3*	2.9±0.1	2.9±0.5
	3	3,2±0,1	3.1±0.5	3.0±0.7	3.1±0.5	3.0±0.7	3.1±0.9
Leukocytes, 10^9/l	1	9,4±0,5	4.4±1.2*	5.7±0.9	5.1±0.7*	-	-
	2	9.6±0.5	8.1±0.3*	7.7±0.3	8.1±0.5	8.5±0.7	9.1±0.5*
	3	9.5±0.3	9.6±0.5	9,5±0,2	9.4±0.9	9.5±0.7	9.6±0.1
Lymphocytes, %	1	60.1±2.1	40.1±2.1*	39.0±2.3*	31.1±3.9*	-	-
	2	60.1±1.9	50.1±3.5*	49.9±1.9*	48.7±3.3*	47.5±4.3*	47.1±4.9*
	3	59.8±2.5	58.1±4.1	55.9±2.5	56.7±3.1	59.3±5.3	60.1±3.7

The results of studies on laboratory and farm animals showed that a single subcutaneous injection of "Era-ZhM" at a dose of 0.25 ml/kg 24 h before and 24 h after the lethal irradiation ensured the survival of 75 and 80% of animals while 100% of the control animals died (Table 1).

As a result of the comparative analysis of different schemes of agent use, it was found that the most convenient and efficient one is parenteral (subcutaneous) use of the developed agent both before (24 h) and after (24 h) irradiation of animals, which ensured the survival of 75% of mice receiving the agent in the preventive variant (group I).

Increased survival of lethally irradiated laboratory and farm (sheep) animals against the background of medical and preventive use of "Era-ZhM" agent was accompanied by a lighter clinical course of acute radiation sickness; less inhibition of the content of leukocytes (1.6 times), erythrocytes, lymphocytes compared with irradiated animals without treatment (Table 2); reduced activity of antioxidant enzymes while inhibiting the concentration of superoxide radicals.

CONCLUSION.

On the basis of substances of plant, animal, and microbial origin, the composition "Era-ZhM" has been developed, which has an antioxidant and radioprotective effect, implemented by the mechanism of interception and neutralization of toxic products of macromolecules modification and, thereby blocking the launch of radio-induced apoptosis.

REFERENCES

[1] Kulinsky V.I., Metabolism of reactive oxygen intermediates. Advances in modern Biology. 1993, 113, 107-112.
[2] Rutkovskaya Zh.A., Correction of disorders of the antioxidant system of animal body caused by the introduction of ^{137} Cs, a new complex of B carotene and vitamin A, E, C. Pushchino. 1997, 1, 236-240.
[3] Ames B.N., Oxidants antioxidats and degeneration diseases of aging. Proc. Nat. Acad. Sci. USA. 1993, 90, 155-165.
[4] Fridovich I., Superoxide anion radical, superoxide dismutazes and related matters. 1972, 72, 18515-18517.
[5] Fridovich I., Superoxide dismutase. Ann. Red. Biochem. 1975, 44, 147-159.
[6] Gally H.F., Total antioxidant capacity and loid peroxidation luring liker transplantation. Clin. Sci. Colch. 1995, 89, 329-332.
[7] Harman D., Free radical thery of aging: History. Basel: Birkhanser. 1992, 2, 1-10.
[8] Harman D., Aging: Minimizing free radical dameg. J. Anti-Aging Med. 1999, 2, 15-36.
[9] Hassan H.M., Free Radicals in molecular biology, ageing and disease. Raven Press. 1984, 11, 77-86.
[10] Kritharides L., The use of antioxidant supplement sin coronary heart disease. Atherosklerosis. 2002, 164, 211-213.
[11] Legkovits I., Immunological Metods. New York: Acad. Press. 1981, 1, 57.
[12] Marini F., Laltra Faccia Dellossigeno. Chirital. 1985, 37, 517-524.
[13] Mc Cord, J.M., Superoxide radical controversies, contradiction and paradoxes. Proc. Soc. Exp. Biol. Med. 1995, 209, 112-117.
[14] Suzuci V.J., Regulation of antioxidant enzymes. Free Radical. Biol. Med. 1996, 22, 269-285.
[15] Pryor W.A., Free Radical in biology. New York: Acad. Press. 1976, 1, 239-277.