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Research Article

A COMPOSITE THERAPEUTIC PREPARATION FOR RADIOISOTOPE ELIMINATION: THEORETICAL PRESUPPOSITIONS

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Abstract

After having conducted a targeted search for radioprotective drugs, the domestic and foreign scientists have proposed a number of means of synthetic protectors (cystamine, serotonin, tryptamine, indralin), zooadaptogenic (tissue extracts of animals, poisons of snakes, insects), hormonal agents (estradiol, estrogen, estrone), herbal remedies (ginseng, Siberian ginseng, Chinese magnolia-vine, hydrolyzate from cabbage, mustard, radish, the extracts of mushrooms, lichens, club mosses, and seaweed). As the means of protection from internal exposure the adsorbents (bentonite, zeolite, iodine preparations, ferrocyn, pectin, calcium alginate, algisorb, adsorb, polisurmin) have been offered. However, using a set of the above means of pharmacotherapy and isotopes decorporation is not systematized, the existing recommendations for their application require prolonged courses of treatment with the components of various composition, without a specific dosage and consistency in their application, which creates some difficulties and problems in assessing their effectiveness. Considering the above stated, we have conducted a study aimed at substantiation and investigation of the possibility of constructing a composite radioprotective drug with therapeutic and sorption properties. Based on the conceptual provisions of the pharmacotherapy of radiation pathology and ecopathology, our designed strategy for the construction of composite radioprotective drugs having bifunctional (medical decorporating) effect intended an inclusion of immunotropic (serum and tissue globulins), biogenic stimulants (adaptogens, apical and herbal remedies) and natural sorbents (bentonite, montmorillonite) in the drug composition.

Keywords: Radioprotective activity, sorbents, bentonite, radionuclides.

Introduction. In the wild, both animals and humans can be exposed not only to an isolated external or internal radiation exposure, but also to the combined effect of both factors [1]. It is assumed that the external and internal exposure in all cases are summed up [2].

According to Japanese and domestic researchers, the radiation injury resulted from the combined external and internal exposure is more severe than the general irradiation [3]. Currently, there are difficulties in curing the radiation pathologies, which dictates the need to find more effective ways and means of radiation protection. In the process of this research, involving the construction of a unified single agent having both curative and decorporating effect, we were guided by the conceptual position of radiation pharmacology that multicomponent mixtures of phytozoopreparation extracts have universal properties, and have a beneficial effect on the immune, hematopoietic, endocrine, prooxidant-antioxidant system, reducing thereby stress reactions to pathological agents, i.e., showing an adaptive effect on the body [4-6] and, secondly, promotes excretion of exo- and endo-toxins, heavy metals, and radionuclides through stimulating the metabolism [7-9]. As a result of dedicated research aimed at search for the means of radiation protection, we had previously developed a production technology of radioprotective means based on zoogenic (blood serum, lymph, globulins), microbial (radioprotective poliantigen), apiphytogenic (Vita-Force) and detoxification-desorbing agents (superfine bentonite fractions) (RF patent No.2338546 of 10.09.2006; RF patent No.2324361 of May 20, 2008). Given that the developed products have a high radiotherapeutic effect in isolated external irradiation (anti-radiation immunoglobulin, apiphytopreparation “Vita-Force"), and the mineral sorbent (bentonite) has a decorporating effect, we set forth a scientific idea that the combined use of these drugs may have a therapeutic effect in combined radiation injury. Previously, a research was conducted to study the therapeutic action of these drugs in their combined use on the background of combined radiation toxicity [10] and radiation-biological injury [11].

One of the key mechanisms of radioprotective effect of immunotropic substances (vaccines, sera, globulins) is the regulation of prooxidant-antioxidant protection system by inactivation of superoxide radicals and activation of antioxidant enzymes (catalase, superoxide dismutase); the activation of the antioxidant defense system is performed by increasing the synthesis of cytokines, initiated by immunotropic means that trigger the cytokine synthesis [12,13]. Cytokines, by capturing and neutralizing superoxide radicals induced by ionizing radiation during external and internal (incorporated) irradiation of the body, have myelostimulating effect, preventing thereby an apoptotic death of the lymphocytes and hematopoietic bone marrow cells [13,14].

Materials and methods. The first stage of the study included the experiments on the production of immunoglobulins. Serum donors were rabbits divided into 3 groups, each of 3 animals. The animals of the first group received subcutaneously a radioprotective poliantigen at a single dose of 0.5 cm^3; animals of group 2 were exposed to twofold external gamma-irradiation at a dose of 0.1 Gy (adapting dose) and after 24 hours - 10.9 Gy (test dose); the animals of group 3 received a lethal dose (11 Gy) of radiation, and after 24 hours received subcutaneously an anti-radiation therapeutic and prophylactic immune globulin at a single dose of 1.0 cm^3. After 10, 20, and 30 days of immunization and treatment the serum was obtained and the globulins were isolated by salting out with ammonium sulfate. The resulted experimental series of globulins were standardized on a dry weight basis, adjusting the globulins concentration to 10% and storing at 6±2°C. To obtain tissue globulins the hemogenization and extraction were applied followed by salting-out of globulins and fractionation according to the conventional method [15]. As one of the component of the potential multifunctional composition the apiphytopreparation “Vita-Force" was used - a natural dietary supplement containing a mass fraction of honey (1.5-2.0), propolis (2.0-2.5), pollen (30-2.0), pollen pellet (15-17), bee venom (0.5-1.5), bee brood at different stages of development (5.5-6.0), wax moth and its larvae (2.3-3.0), wax (5.0-7.0), dead bees (7.0-8.0), grass meal (the rest). Due to the fact that the product contains both highly active treatment components (honey, pollen, bee venom, male bee brood) and absorbent components (chitin, chitosan), the drug in its composite form was used as an antiradiation therapeutic agent, and its components were used either combined or separately as the potential decorporants of cesium-137 radionuclides. We used a natural mineral bentonite as a decorporating agent in the potential therapeutic and decorporating composite preparation.

Results. As a result of the first stage of research, antisera were obtained by immunization of animals with the radioprotective polyantigene (MPAG), twofold irradiation with gamma rays at an adapting (0.1 Gy) and test (10.9 Gy) doses, and treatment with anti-radiation treatment and prevention immunoglobulin (ARTPIG), which were further used to isolate the immunoglobulins serving as a component of polyfunctional medico-decorporating monopreparation. The same animals, sacrificed after completion of hyperimmunization, irradiation and treatment, were used to obtain samples from their internal organs (liver, kidney, spleen, lymph nodes, heart, skeletal muscle, skin, colon and small intestine, ovaries, testis, and bone marrow) and to prepare the aqueous tissue extracts for further isolation of tissue globulins. Conditions of physical and biological effects on donor animals and the number of globulins obtained were as follows: group 1 (5 rabbits) - MPAG single subcutaneous immunization at a dose of 0.5 cm^3 and blood sampling in 10, 20, and 30 days; group 2 (5 rabbits) - twofold (10.9+0.1 Gy) gamma radiation and

blood sampling in 10, 20, and 30 days; group 3 (5 rabbits) - treatment with anti-radiation treatment and prevention immunoglobulin after 24 hours of lethal (11.0 Gy) irradiation by a single subcutaneous drug administration at a dose of 25 mg/kg. As can be seen from the above data, the physical and biological influence on the donor animals resulted in 9 variants of antisera and 36 variants of tissue globulins obtained (12 organs for each period: 10, 20, 30 days after the 3-factor influence). The results of screening studies with the use of the *in vitro* model test system (co-incubation of peripheral blood lymphocytes irradiated at lethal dose (5.0 Gy) with the test serum and tissue globulins showed that 3 drugs of 45 tested variants of immunoglobulins had relatively high radioprotective activity providing 69.7-74.9% survival of lethally irradiated lymphocytes (Table 1).

Table 1. Radioprotective effect of serum and tissue globulins on the 5.0 Gy irradiated lymphocytes (incubation time - 48 hours).

variant	lymphocytes incubation in a medium containing:	lymphocytes survival rate, (%)
I	Globulins from antisera to MPAG (GAS MPAG)	74.9
II	Globulins from sera of treated ARTPIGs of lethally irradiated animals	74.5
III	Globulins from the small intestine of the MPAG immunized animals	69.7
Control	Anti-radiation therapeutic and preventive immunoglobulin (ARTPIG)	75.0

As can be seen from Table 1, the most active radioprotective properties were shown by globulins from antisera to MPAG (GAS MPAG), globulins from sera of treated ARTPIGs of lethally irradiated animals, globulins from the small intestine of the MPAG immunized animals that were subsequently used as potential components of the therapeutic and decorporating agent. The next part of the experiments involved testing of the decorporating activity of drugs (Table 2) in respect of cesium-137. Potential sorbent were used to prepare samples in the amounts recommended for their evaluation *in vitro* - from 0.1 to 1.0 g.

Sorption activity of the test drugs was determined by the formula:

$$C_a = (A_1/A_0)\cdot 100, \text{ where:}$$

C_a– sorption ability, %;

A_0– ^{137}Cs standard solution activity, equal to 6.16 kBq/ml;

A_1– ^{137}Cs activity in the filtrate, kBq/mg.

The results of the determined sorption ability of the tested sorbents in respect of ^{137}Cs radionuclide are shown in Table 2. As can be seen from Table 2, the most active phyto-drug was “Vita-Force”, and among mineral sorbents - a purified bentonite fraction.

Table 2. Sorption activity of tested sorbents in respect to ^{137}Cs radionuclide.

sorbent	initial solution activity, kBq/ml	residual solution activity, kBq/ml	sorption activity, %
Alfalfa powder	6.16	5.75	6.71 ± 0.59
Grass meal	6.16	5.84	5.19 ± 0.32
Pine meal	6.16	5.79	5.93 ± 0.51
“Era-N” powder	6.16	5.78	6.01 ± 0.77
“Erakond” powder	6.16	5.81	5.57 ± 0.81
Chaga mushroom powder	6.16	5.74	6.79 ± 0.75
Dried blood	6.16	5.86	4.72 ± 0.33
Crab chitosan	6.16	5.75	6.65 ± 0.41
Bee chitosan - apisan	6.16	5.71	7.25 ± 0.63
Propolis	6.16	5.84	5.15 ± 0.21
Bee pollen	6.16	5.91	4.12 ± 0.45
Dead bees	6.16	5.66	8.11 ± 0.95
Royal jelly	6.16	5.85	5.03 ± 0.71
Wax	6.16	5.90	4.21 ± 0.19
Wax moth	6.16	5.89	4.33 ± 0.25
“Vita-Force”	6.16	5.61	8.85 ± 0.13
B.subtilis powder	6.16	5.84	5.15 ± 0.55
B.bifidum powder	6.16	5.92	3.93 ± 0.47
Native bentonite (control I)	6.16	2.03	67.01 ± 0.85
Purified bentonite	6.16	1.42	77.00 ± 0.93
“Bifege” (control II)	6.16	0.91	85.19 ± 2.35
“ХЖ-90” (control III)	6.16	1.65	73.25 ± 0.76

During the next stage, we made a composition of sorbents: “Vita-Force” + purified bentonite (VF + PB). The ratio of the components was 1:1. The resulted composition was tested for sorption ability in *invitro* test system using the above technique. The used control sorbents were traditional sorbents “ХЖ-90" and “Bifege". The results of the determined sorption ability of the tested sorbent compositions in respect of ^{137}Cs radionuclide are shown in Table 3.

Table 3. Sorption activity of the tested sorbent compositions in respect of ^{137}Cs radionuclide in *in-vitro* test system.

Sorbent composition ratio 1:1	**Composition sorption activity, %**
“Vita-Force” + purified bentonite fraction (VF + PBF).	85.11 ± 1.93
Control III (“ХЖ-90”)	73.25 ± 0.76
Control II (“Bifege”)	85.19 ± 2.35

As can be seen from the table, the combination of composite apiphytopreparation “Vita-Force" with purified (highly dispersed, suspension-forming) fraction of bentonite (montmorillonite) has led to maximum increase in the sorption capacity of the sorbent composition (“Vita-Force" + PBF), providing 85.11% binding of cesium-137 in the *in vitro* test system, and reaching the level of the most active (control) sorbent - “Bifege". The results served the basis for the use of the composition of “Vita-Force" + bentonite (VF+B) as a sorbent for the removal of cesium-137, and its inclusion in the composition of potential therapeutic and decorporating agent.

Discussion. The results of the conducted study allowes us to make the following conclusion:

1. A theoretical basis for the design of therapeutic and decorporating monopreparation is the use of protein-polysaccharide and vitamin complexes of vegetable origin (grass meal, alfalfa), having antioxidant effect and blocking the accumulation of radioactive cesium in the body. 2. Apiproducts in the therapeutic and decorporating composition show antibacterial, antitoxic, adaptogenic, and radioprotective metabolism-stimulating properties. 3. Ionizing irradiation is accompanied by immunopathology, and one of the most effective anti-radiation means in this case are homo- and heterogeneous immunoglobulins, and the inclusion of serum and tissue globulins (histoglobulins) in the therapeutic and decorporating drug is logical and justified. 4. We have established the possibility of combining globulins with herbal remedies (in particular, “Era-N", "Erakond") and an apipreparation “Vita-Force", which results in the increased globulin radioprotective activity with simultaneous reduction in the consumption of pharmacological agents. 5. We have determined a principle possibility of joining globulin to a mineral immunosorbent - bentonite, and producing a highly sensitive diagnostic drug - antibody bentonite diagnostic agent (ABBDA), and a high-performance decorporating agent in the incorporated irradiation of the organism with radionuclides, such as cesium isotopes. 6. Induction of an additive radioprotective effect during the combination of plant and animal substances, as well as strengthening of a decorporating effect of these substances in the presence of natural montmorillonite sorbent is the essential foundation for the construction of the therapeutic and decorporating monopreparation for the treatment of the externally and internally irradiated body.

Conclusion. The analysis of the operation mechanism of different classes of radioprotectors, the investigation of their compatibility and the establishment of the possibility of their synergistic action when combined have theoretically proved the possibility and feasibility of designing the bifunctional composite monopreparation having therapeutic and decorporating properties. Taking into account the available single literature data about drugs with therapeutic and decorporating properties, we have obtained the components of phyto-, zoo- and apiproducts and mineral sorbents, which showed a sufficiently high sorption and radioprotective properties in the *in vitro* test system. Considering also the compatibility of the selected components with each other and in certain ratios of the components, we have obtained a new property such as bifunctionality, providing both radioprotective and sorption (decorporating) effect. The research results will be further used to construct a composite bifunctional therapeutic and decorporating monopreparation.

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