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Improvement Technology Manufacturing Poly Radioprotective Microbial Poliantigen.

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ABSTRACT

The purpose of the real research was improvement of manufacturing techniques of a radio protective polyantigene. Results of the detailed analysis of manufacturing techniques of the known radio protective preparation of a microbic polyantigene (MPAG) have shown that the known technology consists of 4 independent stages of preparation of separate components of a polyantigene. At the same time at the same time repetition of separate stages (receiving a protective antigene and esherikhiozny anatoksin) which are almost identical takes place and differ on terms of engagement of microbic cages and a detoksikant (formalin). Therefore these stages have found it possible to unite in uniform technological process. As for a stage of receiving a radio anti-gene and the subsequent his conjugation with the proteinaceous carrier - esherikhiozny ekzotoksiny, we find possible this stage (and expedient) to exclude from technological process as radiation of microbic biomass in a dose of 150 Gr before a formalinization induces formation of radio toxin (the main specific immunizing polyantigene component) in a microbic cage. Taking into account stated by us 8 options of potential radio protective preparations with use of 4 types of nutrient mediums, the containing benzokhinonguanilgidrazon-tiosemicarbazones (BHGGSK), the Hottinger's broth (HB) and their combinations (HB+BHGGSK), and also 2 types of detoksikant (formalin, aminoform) have been received. Results of preliminary screening researches with use of in vitro of test system (joint cultivation of the irradiated lymphocytes in the presence of examinees of potential radio protectors) have shown that the preparation received on the basis of the Hottinger's broth (HB), BHGGSK and aminoform (GMTM) which provided survival of 69,3% letalno of the irradiated lymphocytes possessed the highest radio protective activity. This preparation is conditionally called by us "MPAG-M". The positive results received in model experiences have formed the basis for carrying out experiences in in vivo conditions - on letalno the irradiated different types of animals. The first series of experiments was made on 60 white mice divided into 3 groups on 20 animals in everyone. An animal of the 1st group for 24 h before lethal radiation (7,7 Gr) once hypodermically in a dose of 0,1 sm^3 injected the examinee a drug – MPAG-M, the 2nd group – the known preparation - MPAG in a similar dose. Animals of the 3rd group didn't immunize (control). Results of researches have shown that single hypodermic introduction of the examinee of a preparation for 24 h before radiation provided 66,6% survival letalno of the irradiated animals. Parallel use of the known radio protective preparation (MPAG) was ineffective – the survival has made 16,7% that concedes to again received preparation by 3,91 times. Formation of the maximum radio resistance at the animals imparted by MPAG-M I came at immunization for 5 days before radiation while when using the known preparation the maximum has been reached at introduction of a polyantigene for 14 days before radiation. The results of researches received on white mice have been repeated in the following series of the experiments made on white rats (a dose of radiation of 9,0 and 11,0 Gr). The mechanism of formation of radio resistance against application of MPAG-M is carried out by strengthening of synthesis of haemoregulatory cytokine, correction of an imbalance of an immunoregulatory index (Th/Ts), the inhibition of generalization of an intestinal autoinfektion and sepsis leading to increase of survival of the irradiated animals.

Keywords: radio protection, polyantigene, E.coli, radio resistance, anatoksin

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INTRODUCTION

Researches on research of radio protective equipment have led to creation of preparations for treatment of sharp radiation sickness [6, 3]. The special direction gets search of antibeam means in recent years among biologically active agents of a natural origin which possess smaller toxicity, influence regulatory systems, possess versatile action, warning an agammaglobulinemiya, stimulating an immunoreactivity, neutralizing products of fabric destruction and inhibiting synthesis of radio toxic components. In recent years when studying various aspects of the mechanism of antibeam action domestic and foreign researches have saved up the experimental material testifying to ability of means of a microbic origin (ekzotoksina, polysaccharides, anatoksina) to increase resistance of an organism to radiation [1, 7].

The high positive effect is found at introduction before radiation of optimum doses of the killed corpuscular microbic antigenes the gramotritsatelnykh of bacteria (salmonellas, escherichias, klebsiella) [4, 2, 5]. This situation has formed the basis for development of FGBNU "FTsTRB-VNIVI" of a radio protective polyantigene by employees on the basis of E.coli (MPAG) and the offer him for specific prevention of radiation defeats of an organism.

However, despite the high radioprotective efficacy, the drug has a number of disadvantages (complexity of manufacturing technology, multi-component, multi-stage, large labor input) that restrains the serial production radiovaktsiny for widespread use in practice, even though the need for the said drug in emergency situations (at nuclear reactor accidents, nuclear power plants, submarines, etc.) is obvious.

Told defines relevance of a problem and dictates need of improvement and unification of manufacturing techniques of the known radio protective preparation.

MATERIALS AND METHODS

In experiences 219 white mice with a live weight of 18-20 g, 136 white rats with a live weight of 180-200 g, 15 guinea pigs with a live weight of 300-350 g, 51 rabbits of breed "Chinchilla" with a live weight of 2,5-3,5 kg have been used. As test microbes used a production strain of "PL-6" of E.coli, an epizootic strain of "PZ-3" № 1150/15 E.coli - the causative agent of esherikhiozny diarrhea of calfs, laboratories received from a collection of strains on studying of causative agents of especially dangerous diseases of FGBNU "FTsTRB-VNIVI"; for determination of lysozyme activity used standard culture of M.lysodeicticus. As a standard radio protectant used a microbic polyantigene (MPAG) made according to TU 383-032-00492474-02, and as potential radio protective equipment - experimental series of preparations of own production according to advanced technology.

Modeling of radiation sickness of heavy degree at animals carried out by radiation them on a gamma-ray irradiation plant "Puma". Animals were irradiated once at the power of an exposition dose of the radiation of $3{,}13 \times 10^{-5}$ Kl / (кg's) in doses of 7,7 Gr (white mice), 9,0 Gr (white rats) and 11,0 Gr (rabbits).

The strain producer was grown up on standard liquid (MPB, Hottinger's broth, GPEM) and dense (MPA) nutrient mediums, with use of a growth factor of E.coli - 1,4 benzochino guanilgidrazontiosemikarbazon (a.s. USSR № 1682394). Varying technological parameters of cultivation (time, pH, nutrient mediums, various on structure), received 8 options of experimental series of potential radio protective preparations.

Unification of technology of receiving a radio protective preparation was carried out by the comparative analysis of technological operations, their compatibility, duration and a possibility of an exception of separate technological parameters. For the emergency assessment of antibeam activity of the received options of experimental series of potential radio protective preparations used in vitro test system – joint cultivation irradiated (5 Gr) lymphocytes of peripheral blood and examinees of radio protective equipment. As criterion of antibeam activity of examinees of preparations served preservation of viability of cages in comparison with a control preparation. Determination of toxicity and harmlessness of the received potential radio protective preparations was carried out according to "Recommendations about an assessment of harmlessness and toxicity of vaccinal and serumal preparations", using the IPM-test – change of a gain of weight.

About efficiency of examinees of radio protective equipment judged by kliniko-hematologic indicators: determined the maintenance of erythrocytes, hemoglobin, leukocytes, a leykoformula by the standard methods in hematology [6]. About change of immunological reactivity of an organism against application of examinees of preparations judged by a state humoral (synthesis of immunoglobulins) and cellular (T - B-lymphocytes and their subpopulations) immunity links.

The assessment of a condition of antimicrobic protection of an organism against application of examinees of preparations was carried out by studying of factors of nonspecific protection: phagocytic activity of neutrophils, lysozyme activity of serum of blood, bactericidal activity - in vitro-test on reduction of number of viable bacteria of E.coli after their contact with serum of blood of laboratory animals.

Radio protective activity of examinees of preparations was determined by an integrated indicator - 30-day survival of the animals irradiated in a dose of $LD_{100/30}$. At the same time determined the content of lipidic radio toxins, activity of antioxidant enzymes, a condition of cytokine system.

Statistical processing of the digital material received during experiments was carried out on the computer with use of the application programs Microsoft Excel, Word 2010.

RESULTS OF RESEARCHES

At the first stage experiments on production of experimental samples of preparations with use of the known and modified technology by cultivation of a strain of "PL-6" of E.coli on MPA, washout of biomass, cultivation to concentration $1,2 \bullet 10^{10}$ m.k./sm^3, gamma irradiations in a dose of 150 Gr with the subsequent termostatirovaniye of culture during 3,5-4,5 h, formalin addition (0,5%), a repeated termostatirovaniye within 10-12 days, standardization of preparations by quantity of microbic cages – $2,5 \bullet 10^{10}$ m.k./$sm^3$and sterilizations of a product by gamma irradiation in a dose of 3000 Gr have been made.

The preparation (MPAG) received on the known technology was used as a standard (control) radio protectant. When receiving an improved version used both usual (MPA), and a special nutrient medium (2,5-3,5 mas.% agar+0,5-1,5 mas. % peptone+0,0008-0,1 mas. % of 1,4% of benzokhinonguanilgidrazontiosemikarbazon - BHGGSK), and also a liquid nutrient medium (Hottinger's broth without and with addition 0,0008-0,1 mas. % 1,4% of BHGGSK). For the translation of an esherikhiozny enterotoksin in anatoxin as the inactivator used the regulated preparation – formalin, and also its analog – aminoform (GMTM).

As a result of the conducted researches 8 options of potential radio protective equipment with use of 4 types of nutrient mediums and 2 types of detoksikant have been received.

Results of studying of harmlessness of preparations by intraperitoneally introduction and 1,0 sm^3 have respectively shown to white mice and guinea pigs in doses of 0,5 sm^3 that mortality after an injection of preparations it wasn't observed, the general condition of animals remained satisfactory. Results of the hematologic researches conducted on guinea pigs have shown that quantity of leukocytes, erythrocytes and a leykoformula had no reliable differences from those at control animals. Therefore, examinees potential radio protective preparations didn't exert negative impact on an organism that gives the grounds to consider them harmless.

The assessment of radio protective activity of the received preparations was carried out on 48 white mice divided into 4 groups on 12 animals in everyone. An animal of the 1-3rd of groups once hypodermically injected the drug MPAG-M received by cultivation of E.coli on MPA with addition of a growth factor – BHGGSK and use of detoxifying agent - hexamethylenetetramine (GMTM) instead of formalin; an animal of the 2nd group in similar conditions injected the drug MPAG-M received on Hottinger's circle with addition of the specified growth factor and detoksikant; an animal of the 3rd group – the regulated radio protective polyantigene (MPAG) (control of a preparation). An animal of the 4th group preparations didn't enter, they served as biological control. Preparations entered in a dose 0,1 cm3 ($2,5 \bullet 10^9$ m.k./kg) respectively. In 24 h after immunization of animal 1st, 2nd and 3rd groups irradiated on a gamma-ray irradiation plant "Puma" in a dose of 7,7 Gr. Observed animals within 30 days after radiation, registering quantity fallen and survived.

Results of researches have shown that single hypodermic introduction of examinees of preparations for 24 h before radiation provided 66,6% survival letalno of the irradiated animals. Parallel use of the known radio protective polyantigene (MPAG) at the same time was ineffective - the survival made 16,7% that concedes to again received preparation by 3,91 times.

The received positive results have formed the basis for continuation of experiments by an assessment of radio protective activity of preparations depending on an interval between immunization and radiation. For the solution of this task experiments were made in 14 options (7 terms of immunization of animals by the examinee – MPAG-M and control – MPAG) on 102 white mice to whom for 3, 5, 7, 10, 14, 28 and 60 days before radiation in a dose of 7,7 Gr once hypodermically injected drugs in a dose on 0,1 cm3 ($2,5 \bullet 10^9$ m.k./kg) respectively.

Results of experiences have shown that immunization of mice of MPAG-M for 5 days before radiation protected from radiation death 83,3% of animals while in group of mice, the immunized the known preparation (MPAG), percent of protection didn't exceed 33,3%, SPZh at the animals imparted by MPAG-M for this term made 9,6 days against 5,1 in control of MPAG and 3,9 days – in radiation control.

Radio resistance came at the immunized of animals at application of the known preparation (MPAG) in 14 days prior to radiation.

Repetition of experiments on 136 white rats and 51 rabbits irradiated in doses of 9,0 and 11,0 Gr respectively and imparted by preparation MPAG-M for 1, 3, 5, 7, 10, 14, 28 and 60 days before lethal radiation has shown full coincidence of results that confirms advantage of the offered preparation manufacturing techniques before known and expediency of its further application by production of a bioradio protector of new generation.

When carrying out the real researches we considered that the haemo tire-tread and miyeloprotektorny action mediated through system of regulators of an immunohematopoiesis - tsitokin is the cornerstone of the mechanism of radio protective effect of substances of a microbic origin. Taking into account told, studied reaction of system of blood and a condition of blood formation at the irradiated and prevented animals.

The studying of reaction of system of blood including morphological changes of cells of peripheral blood and a kletochnost of marrow, spleen and a timus, which is carried out in 7, 14, 21 days after radiation was shown that immunization of animals before radiation warned a post radiation devastation of bodies of a hematopoiesis (marrow, a spleen and a timus) and I accelerated processes of restoration of marrowy blood formation, rendering thereby, haemo - and miyeloprotektorny effect.

Results of biochemical researches of serums of blood of white rats with use of TBK - test systems have shown that the tire-tread effect correlated with anti-radio toxic action of a preparation.

It is established that preliminary immunization of animals of MPAG-M led to essential braking of synthesis of one of key toxic radicals - lipidic radio toxins which target of attack are cells of peripheral blood (lymphocytes) and cages of an immunohematopoiesis - marrow haematopoietic stem cells.

The inhibition of synthesis of products of oxidative modification of macromolecules (radio toxins) in immunized MPAG-M an organism before radiation has been caused by prevention of decrease in level of antioxidant enzymes: catalases (KAT) and superoxiddismutases (SOD) [12,11, 14].

According to literary data, the mechanism haemo - and miyeloprotektorny, and also immunotherapy action of preparations on the basis of substances of a microbic origin consists in regulation of subpopulation structure of the T-lymphocytes participating in cooperation with macrophages in synthesis of haemo regulatory tsitokin of IL-1, IL-3, IL-6, [9,10,13,11] of a colony stimulating factor – KSF [8], and also interferon.

Taking into account stated by us researches on studying of subpopulation structure of the lymphocytes of peripheral blood irradiated against preventive application of preparation MPAG-M on rabbits have been conducted.

It is established that lethal (11,0 Gr) radiation of rabbits led to essential change of a quantitative ratio both T - and B-cages, and subpopulations of T-lymphocytes. So, already since 3 days after radiation of rabbits reliable decrease T-helperov at simultaneous increase T-supressorov has followed.

The specified divergence of change of subpopulation structure of T - cages at impact on an organism a stress factors (pathogenic, toxic, physical agents) is the most important indicator of functioning of a cellular link of immunity and is characterized as a helperno-supressorny ratio (Th/Ts) or as an immunoregulatory index. It is shown that in process of development of OLB sharp reduction has followed (in 2 and more times) ratios of Th/Ts which wasn't restored up to death of animals. Sharp reduction of a helperno-supressorny ratio of T-lymphocytes in the third stage of OLB is connected with development in the irradiated organism of an endogenous infection as reduction of this indicator demonstrates weakening of factors of natural resistance to infections [8].

Application of radio protective preparation MPAG-M before radiation rendered immunocorrective effect on an organism, preventing immunosupression development.

Continuing researches on studying of influence of again received radio protective preparation on system of immunity of the irradiated rabbits, in parallel in serum of blood determined the content of immunoglobulins, lysozyme, bactericidal activity of serum, and also studied functional activity of lymphocytes and phagocytes.

Results of researches have shown that immunization of rabbits before lethal radiation prevented oppression of factors of nonspecific resistance of an organism, keeping the initial level of activity of the studied indicators. Preservation of blast transforming activity of lymphocytes under the influence of a preparation has found reflection on their main function – synthesis of immunoglobulins which content at the immunoprevented and irradiated animals to 20 days of experience slightly differed from a reference level.

Immunotropny action of a microbic radio protector - MPAG-M has found confirmation when studying a condition of anti-infectious resistance of an organism which adequately reflects level autoflor of intestines, excrements, integuments. At the same time we considered that stressful impact of the ionizing radiation on an organism is followed by essential change of immunobiological reactivity, one of the main indicators of which is development of an endogenous infection at radiation sickness.

Results of microbiological researches have shown that application of preparation MPAG-M before lethal radiation inhibited reproduction of opportunistic intestinal microflora, stimulating anti-infectious protection with the struck ionizing organism radiation. At the same time reliable decrease in quantity of anaerobe bacterias, in particular, of bifidobacteria and lactobacilli, didn't happen that has found reflection on survival letalno of the irradiated animals. The obtained data will be coordinated with results of the previous researches on studying of factors of nonspecific resistance of an organism, stimulated application of the examinee of a radio protective preparation.

Considering that substances of a microbic origin are inductors of mediators of an immunohematopoiesis – the tsitokin and interferon developed by stimulated lymphocytes and macrophages, and rendering radio protective effect in the finishing series of experiences conducted researches on determination of content of the major mediators of immunogenesis – interferon and interleykina-1.

It is established that immunization of rabbits preparation MPAG-M for 10 days before radiation exerted the regulating impact on cytokinin system, preventing the radio induced synthesis oppression interleykina-1 and interferon.

Thus, hypodermic immunization of animals the preparation MPAG-M received on advanced technology rendered radio tire-tread effect, providing 60-80% protection of animals against radiation death. At the same time it is established that advantage of a new radio protectant before the known MPAG is the possibility of induction of the emergency resistance of animals before radiation (for 24 h before radiation).

CONCLUSION

By an exception of separate stages (isolation, cleaning, radiotoxins conjugation to a protein carrier) and associations of stages of receiving a protective antigene and an anatoksin of E.coli in continuous process, unification of production of the known radio protective preparation – a microbic polyantigene is carried out.

Single hypodermic introduction of preparation MPAG-M to white mice, white rats and rabbits in a dose of 0,02 ml/kg for 1-60 days before lethal radiation formed radio resistance, providing 60-80% survival of the irradiated animals.

Dynamics of formation of radio resistance against application of preparation MPAG-M had three-phase character: increase of this indicator to 3 days (60% protection), achievement of a maximum (80% protection during the period from 5 to 20 days), decrease in an indicator to initial level (60% in 30 days) which kept at this level to 60 days (supervision term).

The mechanism of formation of radio resistance at the animals imparted by preparation MPAG-M was realized by increase of haemo regulatory tsitokin (IL-1, INF), correction of an imbalance of an immunoregulatory index (Th/Ts = 2,59 against 1,13 in radiation control), lysozyme preservation (0,38 units against 0,15 at irradiated), bactericidal (0,98 against 0,30 at irradiated) and phagocytic activity (33,9 against 1,5% in radiation control), decrease in quantity of conditionally pathogenic enterobacteria (2,1 log2 against 4,3 log2 at irradiated) in intestines, preventions of generalization of an intestinal autoinfektion and the sepsis leading to increase of survival letalno the irradiated animals.

The emergency formation of radio resistance against application of preparation MPAG-M is provided due to the accelerated synthesis of interceptors of toxic radicals - cytokines induced radio inactivated E.coli microbial cells.

REFERENCES

[1] Andrushhenko, V.N. Protivoluchevoe dejstvie veshhestv mikrobnogo proishozhdenija //Radiacionnaja biologija. Radiojekologija. - 1996. - T. 36. № 2. - S. 195-208.

[2] Budagov, R.S. Vlijanie sredstv mikrobnogo proishozhdenija na uroven' citokinov v syvorotke krovi i vyzhivaemost' myshej pri kombinirovannyh radiacionnyh porazhenijah / R.S.Budagov, L.P.Ul'janova // Radiacionnaja biologija. Radiojekologija. 2001. – T. 41 - № 1. – S. 38-42.

[3] Il'in, L.A. Protivoluchevye sredstva v sisteme radiacionnoj zashhity personala i naselenija pri radiacionnyh avarijah /L.A.Il'in, I.B.Ushakova, M.V.Vasin //Radiacionnaja bezopasnost'. - 2012. - T. 57. - S. 26-31.

[4] Mal'cev, V.N. Vlijanie bakterial'nyh preparatov na vyzhivaemost' obluchennyh zhivotnyh /V.N.Mal'cev, K.K.Gucenko, N.V.Emchenko i dr. //Radiacionnaja biologija. Radiojekologija. - 1994. - T. 34. - Vyp. 4-5. - S.578-581.

[5] Ainsworth E., Chase H. Effect of microbial antigens on radiation mortality in mice //Proc. Soc. Exp. Biol. 1968. № 2. P. 483-486.

[6] Bacq, Z.M. Chemical Protection Against Ionizing Radiation /Z.M.Bacq //Spring. Fold: Tomas Press. - 1965. - 263 p.

[7] Behling, U. Beneficial Effect of Endotoxins. /U.Behling //N.Y.: Pergamon Press. - 1983. - P. 127-148.

[8] Ehlers, S. Gamma interferon is essential for clearing Mycobacterium genavense infection /S.Ehlers, F.Richter //Infect. Immune. - 2000. - V. 68. - P. 3720-3723.

[9] Neta, R. Role of cytokines in radioprotection /R.Neta //Pharmacol. Ther. - 1988. - V. 39. - №1. - P. 261-266.

[10] Neta, R. In lethally irradiated mice interleukin – 12 protects bone marrow but sensitizes intestinal tract to damage from ionosong radiation /R.Neta, S.M.Stiefel, N.Ali //Ann. N. Y. Acad. Sci. - 1995. - V. 762. - P. 274-280.

[11] Renard P., Zachary M. D., Bougelet C. et al. Effect of antioxidant enzime modulation on interleukin-1-induced nuclear factor kappa B activation //Biochim. Pharmacol. 1997. V. 53. P.149-160.

[12] Speciale, A. Nutritional antioxidants adaptive cell responses: an update /A.Speciale, J.Chirafisi, A.Saija, F.Cimino //Cur. Mol. Med. - 2011. - V. 11. - P. 770-789.

[13] Watanabe, K. Effect of central injection of angiotensin-converting-enzyme inhibitor and angiotensin type 1 receptor antagonist on the brain NF-kappaB and AP-I activities of rats ginen LPS /K.Watanabe, M.Taniguchi, M.Miyochi et al. //Peptides. - 2006. - V.27. - № 6. - P. 1538-1546.

[14] Wu, S.D. Radioprotection of the intestinal crypts of mice by recombinant human interleukin-1-alpha /S.D.Wu, T.Miyamoto //Radiat. Res. - 1990. - V. 123. - № 1. - P. 112-115.