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Research into the use of protective agents in the case of combined radiation and chemical injury

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ABSTRACT

Laboratory and agricultural animals were used to carry out the present study on the specific features of combined injury caused by long-term intake of ecotoxicant cadmium chloride and the exposure of intoxicated animals to gamma rays at half-lethal doses (LD_{50}). The study showed that combined injury in white mice were accompanied by the weakening of their general condition, sluggishness, decreased appetite, diarrhea, adynamy, body weight decrease, falling counts of leukocytes, T- and B- lymphocytes, and protein sulfhydryl groups in parallel with an increase in the levels of malondialdehyde and quinoid radiotoxic. Dissection of dead animals revealed the signs of the hemorrhagic syndrome, hyperemia of blood vessels in internal organs, gastroenterocolitis, and hemorrhage in the intestinal mucosa, liver, kidneys, as well as pulmonary edema and a swollen spleen. The survival rate of animals subjected to combined radiation and chemical exposure was 10% against 55–60% survival among animals subjected to chemical or radiation exposure alone.

As radioprotective and antidote agents, antiradiation therapeutic and prophylactic immunoglobulin (TU 9380-073-00008064-98) and bentonite from Biklyansky field deposit (Tatarstan, Russia) were selected which when used in isolation, have radioprotective and sorption effects on the body.

It was established that a three-dose (after 2, 24, and 48 hours) subcutaneous administration of antiradiation therapeutic and prophylactic immunoglobulin at a dose of 50 mg / kg to animals affected by the two pathological factors, as well as the introduction of bentonite into their diets at the rate of 2% of the ration weight ensured a survival rate in the range of 80–100% in relation to the 55% and 90% death rates among non-treated animals, by reducing the level of toxicant accumulation in organs and tissues and accelerating toxicant elimination from the body. The mechanism of resistance development in injured and treated animals is implemented through the inhibition of pancytopenia, protection of the immune-hematopoietic system, correction of the prooxidant system function, as well as the ion-exchange sorption of the toxicant by bentonite.

Keywords: gamma rays, cadmium chloride, combined injury, enterosorbent, anti-radiation therapeutic and prophylactic immunoglobulin, bentonite

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INTRODUCTION

The biota of ecosystems in industrial regions is currently subjected to combined effects caused by factors of different nature. Thus, the development of processing technologies leads to increased concentrations of heavy metals in the environment. On the other hand, following the accident at the Chernobyl nuclear power plant, the radiation background has increased in some areas of Russia as a result of long-lived radionuclides fallout. Under these circumstances, research into the combined effects of heavy metals and ionizing radiation is very important for conducting risk assessment, radiation dose normalization, and the development of treatment methods and techniques (Ibrahim, I.K. et al., 2000; Grjsicki A., 2005).

Combined injuries are usually severe. In cases of combined radiation injury, the latent phase of the radiation sickness is reduced while the manifest illness period is significantly longer; the hemorrhagic syndrome is more obvious, the percentage of complications increases. The radiation sickness, in turn, aggravates the symptoms of intoxication and infection process. The activity of the bone marrow, lymphoid system elements, phagocytic activity of leukocytes, immunobiological protection mechanisms, barrier properties of tissues is suppressed; tissues permeability increases, the inflammatory response is weakened or inverted, etc.

Regarding the damage caused by cadmium ions and gamma radiation to genetic structures, the formation of DNA–protein cross-links was reported to disrupt the normal functions of nuclear chromatin and may result in serious genetic damage (mutagenesis, carcinogenesis, apoptosis, etc.)

In view of the foregoing, the search for methods and tools for treating combined injury is one of the urgent tasks of radiation biology and toxicology.

MATERIALS AND METHODS

A total of 110 white mice with body weights of 18–20 g, 280 white rats with body weights of 180–200 g, and 27 'Prekos' breed sheep aged 1.5–2 years with body weights of 38–42 kg were used for this study.

In order to simulate the radiation exposure, a "Puma" gamma irradiation facility was utilized with a ^{137}Cs radiation source at the exposure dose power of 3.13×10^{-5} C / (kg·s), the non-uniformity level of the gamma radiation field not exceeding 10%. Cadmium chloride ($CdCl_2$, GOST 4330–66) was used to simulate the chemical exposure. The numbers of erythrocytes, leukocytes, hemoglobin, leukogram, hematocrit, total protein, lipid peroxidation products, T- and B-lymphocytes were determined by methods applied in hematology and immunology; the concentration of sulfhydryl groups in the blood was determined by photocolorimetry method; the level of cadmium was detected on AAnalyst 200 device (GOST 39178–96); the titration of radiotoxic in the serum was performed by the indirect hemagglutination assay (IHA) method according to the developed technique (RF Patent No. 2240137).

Cadmium chloride was administered intragastrically by an atraumatic tube for 5 days at a dose of 1.5 LD_{50} (50.8 mg / kg) and for 20 days at a dose of 5 MPCs (1.5 mg / kg) followed by the irradiation of animals in the "Puma" facility at a dose rate of 6.0 Gy for white mice, 5.5 and 7.0 Gy for white rats, and 3.8 Gy for sheep. Control animals received the equivalent amounts of toxicant-free liquid.

As therapeutic agents for treating combined radiation and chemical injury in animals, antiradiation therapeutic and prophylactic immunoglobulin and bentonite were used, the former applied at a 10% concentration by subcutaneous administration 2, 24, and 48 hours after the combined exposure at dose of 50 mg/kg body weight in accordance with its guideline for use. Bentonite was used in the amount of 2% of the weight of feed beginning from the first day of cadmium chloride administration.

Results and Discussion. Experiments were carried out using white mice which, according to the principle of analogs, were divided into 6 groups of 10 animals each. Animals from Group 1 were exposed to gamma radiation at a dose of 6.0 Gy; mice from Group 2 were given cadmium chloride for 5 days at a dose of 1/5 LD_{50}; white mice from Group 3 received cadmium chloride for 20 days at a dose of 1.5 LD_{50} (5 MPCs); animals from Group 4 were given cadmium chloride for 5 days at a dose of 1/5 LD_{50} and a consequent irradiation at a dose of 6.0 Gy; mice from Group 5 were given cadmium chloride for 20 days at a dose of 1.5 mg/kg and then exposed to gamma rays at a dose of 6.0 Gy; animals from Group 6 served as biological controls.

The clinical signs of combined injury in white mice within the initial 2–3 days after exposure included weakening of their general condition, sluggishness, and decreased appetite which progressed in parallel with a decrease in body weight. Survival rates in Group 1, Group 2, Group 4, and Group 5 were 60%, 80%, 30%, and 50%, respectively against 100% survival in Group 3 and Group 6.

In the second set of experiments 80 white rats were divided into 4 groups: animals from Group 1 were given cadmium chloride for 5 days at a dose of 1/5 LD_{50}; rats from Group 2 received cadmium chloride for 5 days at a dose of 1/5 LD_{50} and a consequent gamma irradiation at a dose of 7.0 Gy; white rats from Group 3 were irradiated at a dose of 7.0 Gy; Group 4 served as biological controls.

The animals exposed to combined effects of cadmium chloride and gamma rays presented with the weakened general condition, diarrhea, decreased food intake, labored breathing, and reduced mobility.

The survival rate of the white rats exposed to gamma irradiation at a dose of 7.0 Gy was 55%, their mean life spans accounted for 12.2 days; the survival rate among animals which had received cadmium chloride was 90%, with the mean lifespans of 5.5 days. 18 out of 20 animals with combined injury died, which corresponded to 90% of the total number of animals in the group.

Further research focused on specific features of combined damage caused by the effects of cadmium chloride and gamma radiation to 14 "Prekos" breed sheep aged 18–24 months. The animals were divided into 5 groups: animals from Group 1 were given cadmium chloride for 20 days at a dose of 5 MPCs; animals from Group 2 were given cadmium chloride for 20 days at a dose of 5 MPCs followed by gamma irradiation at a dose of 3.8 Gy; sheep from Group 3 were irradiated at a dose of 3.8 Gy; Group 4 served as biological controls. The experiment revealed that the administration of cadmium chloride (Group 1) and the exposure to gamma irradiation (Group 3) led to the development of a generally oppressed condition replaced by agitation in the animals. The function of the gastrointestinal tract was disrupted; feces of some animals contained mucus and blood admixtures. A significant loss of body weight was observed on the 28th day after exposure. Prior to the death of animals, increases in their body temperature and heart rate were recorded; the pulse was weak and poorly palpable. The recovery was slow: the survival rate among sheep with combined injury was 60%, mean life spans of fallen animals constituted 13.0 ± 0.1 days. Effects of a radiation exposure or chemical intoxication alone were associated with a survival rate of 100%.

Studies on the effectiveness of pharmacological agents for treatment of radiation and chemical injuries was conducted using white mice divided into 5 groups of 10 animals each: animals from Group 1 received with cadmium chloride for 5 days at a dose of 1/5 LD_{50} and a consequent irradiation at a dose of 6.0 Gy; animals from Group 2 were given cadmium chloride at a dose of 5 MPCs for 20 days and gamma irradiation at a dose of 6.0 Gy; white mice from Group 3 were given cadmium chloride for 5 days at a dose of 1/5 LD_{50} and received treatment with antiradiation therapeutic and prophylactic immunoglobulin and bentonite; animals from Group 4 were given cadmium chloride for 20 days at a dose of 5 MPCs, exposed to gamma radiation at a dose of 6.0 Gy, and received treatment with antiradiation therapeutic and prophylactic immunoglobulin and bentonite; animals from Group 5 served as biological controls.

The use of the specified agents lead to an increase in the survival of mice up to the rates of 80–100%; the survival in the non-treated group constituted 30–50%. In injured animals that had received treatment, the intensity of clinical signs of combined injury reduced and life spans increased.

The second set of experiments was performed on 100 white rats divided into 5 groups: animals from Group 1 received cadmium chloride for 20 days at a dose of 5 MPCs followed by gamma irradiation at a dose of 5.5 Gy; Group 2 was treated with cadmium chloride for 5 days at a dose of 1/5 LD^{50} and then exposed to gamma radiation at a dose of 7.0 Gy; mice from Group 3 received cadmium chloride at a dose of 5 MPCs and a consequent irradiation at a dose of 5.5 Gy followed by treatment with anti-radiation therapeutic and prophylactic immunoglobulin and bentonite; animals from Group 4 received cadmium chloride for 5 days at a dose of 1/5 LD_{50} and a consequent gamma irradiation at a dose of 7.0 Gy followed by the treatment with antiradiation therapeutic and prophylactic immunoglobulin and bentonite. White rats from Group 5 served as biological controls.

The clinical manifestations in animals that had received treatment were mild; their general condition was satisfactory. The progression of the radiation sickness occurred later than in non-treated groups of animals with combined injuries. The death rate among white rats from non-treated groups was in the range of 5–20%.

The next set of experiments was performed on 13 "Prekos" breed sheep aged 18–24 months, which were divided into three groups: Group 1 (5 animals) were given cadmium chloride for 20 days at a dose of 5 MACs and a consequent gamma irradiation at a dose of 3.8 Gy; Group 2 (5 animals) received cadmium chloride for 20 days at a dose of 5 MACs and a consequent gamma irradiation at a dose of 3.8 Gy followed by the treatment with antiradiation therapeutic and prophylactic immunoglobulin and bentonite; Group 3 (3 animals) served as biological controls.

It was found that clinical features of combined radiation and chemical injuries in sheep treated with anti-radiation therapeutic. Prophylactic immunoglobulin, bentonite showed less marked changes, and the sheep were more active. Disorders of the gastrointestinal tract and respiratory system were less intense and did not occur in all treated animals. The survival rate among sheep having received treatment was 100%, with a 40% death rate in the untreated group.

Atomic absorption spectroscopy of the samples derived from the organs and tissues showed that the level of cadmium chloride in the peripheral blood of animals with combined injuries reached its maximum 3 days after the start of the experiment and constituted 0.183 ± 0.074 mg / kg. The use of bentonite and antiradiation therapeutic and prophylactic immunoglobulin contributed to the less intensive accumulation of cadmium (0.087 ± 0.006 mg/kg) and its rapid excretion. Beginning from the 14th day, the level of cadmium in the peripheral blood of sheep that had received therapy did not differ significantly from that of controls, whereas these differences remained elevated in untreated animals for up to 45 days of the experiment.

The mutually aggravating effect of these factors was established by clinical and hematological indicators, as well as by the integral indicator of the body - survival, which is in agreement with the data obtained by P.N. Rubchenkova et al. (2004). The synergistic effect of the two pathological factors is based on the induction of the formation of reactive oxygen species, activation of lipid peroxidation, depletion of antioxidant processes; inhibition of sulfhydryl groups, and a decrease in cellular and humoral immunity factors.

The results of the biochemical examination of the blood serum of animals subjected to combined radiation and chemical effects suggest that the pathology being studied is a free-radical-initiated pathology, accompanied by intense accumulation of secondary products of lipid peroxidation causing an increase in the process intensity due to depletion of antioxidant protection reserves and are also considered in the works of N. Duran (1982), H. Nohe (1986), A.Kh. Kogan et al. (1996).

The study showed high effectiveness of radioprotective therapeutic and prophylactic immunoglobulin and bentonite from Biklyansky deposit in regard to treating combined radiation and cadmium injuries, which was evident from the reduction in the residual amount of cadmium in the peripheral blood, organs, and tissues of sheep; sorption of toxic substances coming from food and water consumed; prevention of allergic reactions, decreasing the functional load on the organs of detoxication, correction of metabolic processes, which is consistent with the data obtained by K.Kh. Papunidi (2005).

As is apparent from the literature data, there is a correlation between the survival rate of white mice irradiated and treated with cadmium chloride, and the increase in the level of metallothioneins (MT) in the bone marrow (Espejo C., Martinez-Caceres E.M., 2005), which act as interceptors and dampers of free oxygen radicals. According to contemporary research data, MTs may produce a protective effect against radiation-induced gene- and cytotoxicity (Thirumoorthy N. et al. 2007; Ngu N., Naganuma A., 2008).

CONCLUSION

The development of the technology for designing a bifunctional mono-preparation with both therapeutic and sorption properties opens up new prospects for treating radiation and chemical injury. For the first time metilloglobulin was obtained, i.e. a bentonite diagnosticum for indicating radiotoxic in the serum of irradiated animals. Metalloglobulin is a globulin-bentonite complex consisting of antiradiotoxic globulin conjugated with a highly dispersed (600–900 μm) fraction of bentonite.

The introduction of bentonite into the sheep diet and the use of anti-radiation therapeutic and prophylactic immunoglobulin for treating combined radiation and chemical injury contribute to lower accumulation levels and rapid elimination of cadmium from organs and tissues, thereby ensuring the production of feedstuff and alimentary products compliant with approved safety standards (Sanitary Rules and Regulations 2.3.2.1078-01).

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