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Early diagnosis of radiation injuries by methods of immunochemical and EPR analyses

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ABSTRACT

The diagnosis of radiation injuries involves a complex of clinical and hematological, biochemical, cytomorphological, and other tests. None of these methods, however, allows the objectives of diagnosis, rapid assessment of severity, and prognosis of radiation injuries to be achieved. The aim of the present study was to investigate the possibility of using immunochemical test methods; indirect hemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA), and a physical method (electron paramagnetic resonance (EPR)) for the rapid diagnosis of radiation injuries. Antigens (radiotoxic of quinoid nature), globulins derived from hyperimmunized rabbits and sheep, erythrocyte and enzyme-labeled conjugates were used as specific components. Sensitization of erythrocytes and labeling of globulins with enzyme horseradish peroxidase were carried out in accordance with methods applied in immunochemistry. After having been tested for activity, specificity, and stability, the diagnostics were used in immunochemical test systems for indicating radiotoxic in the blood serum, tissues, and organs of irradiated animals. To confirm the results of the immunochemical tests, physical analysis of the samples was performed in parallel by the method of electron paramagnetic resonance (EPR). According to the outcomes of the study, it is as early as on day 1–3after radiation exposure that radiotoxic with titers in the range of 1:4–1:16aredetected in the animal blood serum byELISA and IHA test systems. As acute radiation syndrome (ARS) develops, the antigen titers increase; at higher titer values (1:16–1:32) ARSappears in its severe and fatal form.

Due to their high activity and specificity, the produced antiradiation diagnostics, and EPR test is applicable for the indication of radiation and toxic antigens in animal blood serum, lifetime diagnosis and prognosis of ARS, as well as for indicating toxic compounds in the meat and meat from irradiated animals.

Key words: immunochemical analysis, electron paramagnetic resonance (EPR), quinoid radiotoxins, IHA and ELISA tests, erythrocyte diagnosticum, immunoenzyme conjugate

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INTRODUCTION

Recently, researchers in the field of radiation biology have been paying close attention to immunological methods of detection of radio-induced antigens and radiotoxins. In this regard, the working hypothesis was based on reports stating that ionizing radiation, by affecting the immune system, induces auto-antigens of protein, lipoid, or quinoid nature which cause auto-immunization followed by the synthesis of autoantibodies and may be detected by immunological reactions.[2] The emergence of these substances in the blood serum occurs as early as during the initial hours after irradiation.[4,7] Consequently, the detection of incipient, specific, and immunologically significant antigenic and toxic substances in the irradiated organism is the basis for the development of promising rapid methods for early diagnosis of radiation injury.[6]

The possibility of a serological diagnosis of acute radiation syndrome (ARS)using immunochemical test systems (IHA and ELISA) was first demonstrated by N.A. Kartashova[2] and A.Z. Ravilov.[4]

The feasibility of recording changes in the concentration of paramagnetic centers and appearance of EPR signals in the serum as well as in the organs and tissues of irradiated animals were reported by M.I. Ibragimova andM.K. Pulatova.[1,5]

Due to the significance of the problem and potential benefits of further research in this direction, we conducted the present studies aimed at the use of immunochemical test systems for early diagnosis of radiation injuries in the organism.

MATERIALS AND METHODS

At the initial stage of the study we carried out research aimed at designing the main components of the immunochemical test systems:an antibody variant of the erythrocyte diagnostics for the indication of radiation antigens in IHA test, and an immune enzyme conjugate for conducting ELISA essay. Tissue extracts derived from organs and tissues of lethally irradiated white rats were used as a source of radiation antigens for obtaining

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hyperimmune sera. White rats were irradiated at a dose of 9.0 Gy in a "Puma"(Yes, the so-called gamma irradiation facility?) gamma irradiation facility at the exposure dose of 0.26×10^{-4} C/(kg·s). On day 3–4 post exposure, livers were removed from the animals to be used for preparing tissue antigens by the technique of J.P.Hellstrom and E.K.Brown.[3] The obtained tissue extract containing radiation antigens was standardized to the concentration of 5 mg/ml and used for hyperimmunization of animals. The hyperimmunization was performed according to the scheme commonly applied in immunology. Seven days after the end of hyperimmunization animal blood was obtained, and the serum was received. Following titration of antibodies in the serum, the latter was used a raw material for making diagnostic preparations.

In the production process of the antibody variant of erythrocyte diagnostic, sheep erythrocytes, formalinized and tannin-treated according to L. Chizmas,[8] were sensitized with the globulins derived from hyperimmune sera and then used as the primary immunochemical component for indicating radiation antigens in the IHA test system. Immunoenzyme conjugates for indicating radiation antigens in ELISA were obtained by conjugating antiradiation globulins with horseradish peroxidase according to R. Clark and E.Engvall.[7]

The obtained immune enzyme conjugates were tested for activity and specificity by ELISA test (direct and indirect "sandwich" formats). The results were recorded by spectrophotometry at a wavelength of 492 nm (on a scanning spectrophotometer produced in Sweden or on the "Dinatech" unit manufactured in the United States) by the specificity coefficient which was equal to or more than 2.0. Homologous and heterologous antigens served as controls.

The simulation of acute radiation syndrome was performed by irradiation of white rats, rabbits, and sheep at sub-lethal and lethal doses. Blood samples were derived 1, 2, 3, 5, 7, 10, 14, 28, and 35 days after irradiation; then the presence and concentration of the radiotoxic in the serum were determined by IHA, ELISA, and EPR test systems. EPR spectra of the blood obtained from irradiated animals were recorded on a "Varian E-12" spectrometer (USA) at a frequency of 9.5 GHz, at an SHF power of 50 mW, with the amplitude of 1Gs and temperature of 77°K according to the technique[9] in our modification.[1]

RESULTS AND DISCUSSION

It has been found that it is already within the initial 24 hours after radiation exposure that radiotoxic in dilutions 1:4–1:8emerge in the serum of laboratory (white rats, rabbits) and agricultural (sheep) animals irradiated at sub-lethal and lethal doses. Gradually increasing, a number of radiotoxic reaches their maximum level by day 7–10 of the experiment. As ARSprogresses, the antigen level gradually decreases and becomes equivalent to the initial level in survived animals by the end of the experiment.

The antigens were not uniformly distributed in the organs, and tissues which is summarized in the following sequence of organs arranged in order of decreasing content of radiotoxic: liver> spleen> kidneys> bone marrow>lungs> lymph nodes> large intestine> small intestine> stomach> skin.

The figure shows the dependence of EPR signal intensity from transferrin in the peripheral blood of sheep 24 hours after irradiation. The use of this dependence (EPR signal) enables the assessment of the severity of radiation injuries in animals.

Immunological methods using diagnostics for IHA and ELISA tests allow detecting radiation antigens, the concentration of which depends on the time elapsed after exposure, the absorbed dose, and the individual radiosensitivity of the organism. An increase in the titers of radiation antigens during the initial24 hours after exposure is only reported in severe cases of ARS. The diagnosis of its mild and moderate cases by immunochemical methods areis not possible until day 3 after irradiation. The EPR method allows the evaluation of the body response to radiation effects within the initial hours postexposure. EPR spectra contain paramagnetic centers. At temperatures below 200°Kresonance lines with $g \approx 6$, $g \approx 4.3$, $g \approx 2.05$, $g \approx 2.01$are recorded and identified as signals from methemoglobin (MetHb), Fe^{3+} -transferrin (Tf), ceruloplasmin (Cp), and free-radical centers.[1]
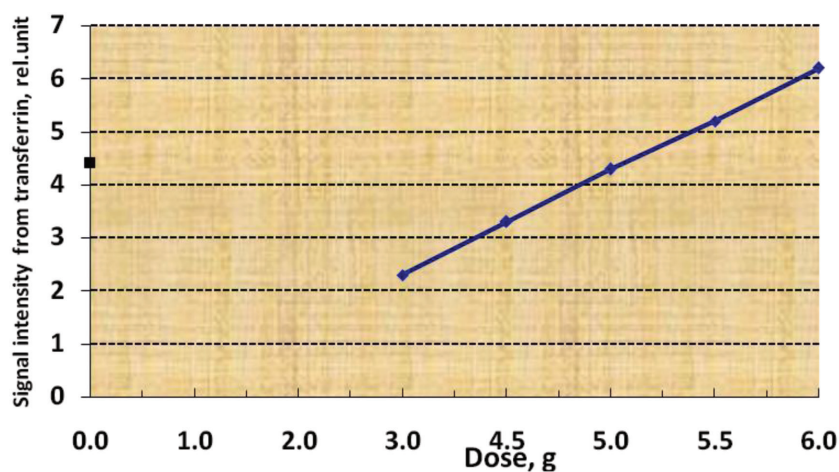

Figure   Intensity of EPR signals from transferrin in the peripheral blood of sheep 24 hours after irradiation

Another paramagnetic plasma protein is Cu^{2+} ceruloplasmin which is able to oxidize Fe^{2+} ions to Fe^{3+} ions, control the incorporation of ions into apo-transferrin, and influence the synthesis of copper-containing proteins.[5] As the studies showed, the degree of radiation injury severity can only be assessed by the changes in Cp level in the blood within 24 hours after irradiation. The dynamics of changes in Cp concentration in the blood in the further period of time enables reliable determination of severe ARS only, whereas the clinical features of its mild and moderate cases are difficult to identify. The level of Cp in the blood reaches its maximum on the third day after the exposure of sheep to a lethal dose of irradiation and then decreases within a week, its consequent levels exceeding the initial one. Therefore, a combined use of immunochemical and physical methods for early diagnosis of radiation injury is advisable since these methods are mutually complementary.

CONCLUSION

Based on the comparative analysis of immunochemical test results, criteria were determined for assessing ARS severity degrees and predicting its outcome by results of IHA and ELISA tests:an increase in the titers of specific radiation antigens during the early post-radiation period to the dilutions of 1:8–1:16 indicates the presence of radiation injury in the body. An increase in the titers of radiotoxic to 1:16–1:32within the 2–3 days after exposure indicates the development of moderate ARSand probable death of animals within the following 2–3 weeks.

The agreement was established between the results obtained by IHA test and those received from ELISA; differences were only reported in relation to the titer levels at the indication of radiation antigens. Therefore, the use of only one of the immunochemical test systems is sufficient for indicating radiotoxic in the case of radiation injury.

EPR analysis may be applied for rapid diagnosis of ARS within 24 hours after radiation exposure, as well as for ARS confirmation on day 3 post exposure.

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