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Определение антигенных свойств хиноидного радиотоксина с помощью реакции непрямой гемагглютинации (РНГА)

РЕЗЮМЕ

Актуальность. В статье приведены результаты проведенных опытов по определению антигенных-свойств (способности хиноидного радиотоксина синтезировать антирадиотоксические антитела) растительного радиотоксина с помощью РНГА.

Методы. Для проведения РНГА-теста использовали антигенный вариант эритроцитарного диагностикума (АГЭД), представляющего собой сенсибилизированные лучевыми антигенами эритроциты барана. В качестве контрольных (положительных) противолучевых антител в реакции использовали противолучевые антитела, полученные путем гипериммунизации кроликов лучевым антигеном — радиотоксином, выделенным из печеночной ткани летально облученных овец; испытуемые антитоксические антитела — гипериммунные сыворотки от гипериммунизированных хиноидным (растительным) радиотоксином овец, отрицательная сыворотка, полученная от интактных (необлученных, непривитых никакими вакцинами) овец, гетерологичные сыворотки (противоожоговая, противоколибактериозную). Реакцию ставили на микропланшетах в объеме 50 мкл и учитывали по четырехбалльной системе в крестах.

Результаты. Изучение антигенной активности испытуемого лучевого антигена — растительного хиноидного радиотоксина (ХРТ) — с использованием РНГА-теста показали, что испытуемый антиген обладает высокой антигенной активностью, индуцируя в организме иммунизированных животных (овец) синтез антирадиотоксических антител, титры которых значительно превосходят таковые иммунизированных печеночным (животным) антигеном — радиотоксином животных. Хиноидный радиотоксин, полученный из растительной ткани (клубней картофеля) и конъюгированный с белково-липоидной группой (неполным адъювантом Фрейнда — НАФ), обладает высокой антигенностью, индуцируя синтез специфических антирадиотоксических антител, которые могут быть использованы в качестве важнейшего компонента иммунохимической тест-системы — сенситина, используемого для сенсибилизации микро- и наночастиц бентонита при конструировании противорадиационного антительного варианта бентонитового диагностикума (АТБД).

Ключевые слова: хиноидный радиотоксин, реакция непрямой гемагглютинации (РНГА), антигенные свойства

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Determination of the antigenic properties of quinoidr adiotoxin using the indirect hemagglutination test (IHA)

ABSTRACT

Relevance. The article presents the results of experiments to determine the antigenic properties (the ability of quinoid radiotoxin to synthesize antiradiotoxic antibodies) of plant radiotoxin using IHA.

Methods. An antigenic variant of the erythrocyte diagnosticum (AGED), which is a ram erythrocytes sensitized by radiation antigens, was used to conduct the IHA-test. As control (positive) antiradiation antibodies in the reaction, we used antiradiation antibodies obtained by hyperimmunization of rabbits with a radiation antigen — radiotoxin isolated from the liver tissue of lethally irradiated sheep, tested antitoxic antibodies — hyperimmune sera from sheep hyperimmunized with quinoid (plant) radiotoxin, negative serum obtained from intact (non-irradiated, not vaccinated with any vaccines) sheep, heterologous sera (antiburn, anticolibacillary). The reaction was placed on microplates in a volume of 50 ml and was taken into account according to a four-point system in crosses.

Results. The study of the antigenic activity of the test radiation antigen — plant quinoidr adiotoxin (QRT) — using the IHA-test showed that the test antigen has a high antigenic activity, inducing in the body of immunized animals (sheep) the synthesis of antiradiotoxic antibodies, the titers of which significantly exceed those immunized with liver (animal) antigen — animal radiotoxin. Quinoidr adiotoxin, obtained from plant tissue (potato tubers) and conjugated with a protein-lipoid group (incomplete Freund's adjuvant — IFA), has a high antigenicity, inducing the synthesis of specific antiradiotoxic antibodies, which can be used as an essential component of the immunochemical test system — sensitin used for sensitization of micro- and nanoparticles of bentonite in the design of antiradiation antibody variant of bentonited iagnosticum (ABBD).

Key words: quinoid radiotoxin, indirect hemagglutination test (IHA), antigenic properties

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Introduction

The concept of "radiotoxins" includes a group of substances that are formed under the influence of ionizing radiation in the body, food products, nutrient media and have the ability to mimic and enhance radiobiological effects in the body [1].

The toxic properties of animal blood were first studied in detail by P. D. Gorizontov [2], who gave a broad definition of toxemia in an irradiated organism. B. N.Tarusov [3] was the first to put forward an experimentally substantiated hypothesis about the possible participation of lipid radiotoxins in radiation injury. The work of V.Y. Horgan et al. [4, 5, 6] revealed the high toxicity of lipid peroxides accumulating in the irradiated organism. Low-molecular toxic substances were found in the blood of irradiated animals and plants [7, 8]. These and other works made it possible at the First International Symposium on the initial effects of radiation on cells (1961) for the first time to put forward the concept of the formation in an irradiated organism and plants of polyphenol oxidation products — orthoquinones, which have a radiomimetic effect [9, 10, 11]. The results of extensive research in the field of radiotoxinemia were summarized at the First All-Union Conference of Radiobiologists on the Nature and Role of Radiotoxins (1965), where the term "radiotoxins" was officially recognized [12]. At present, due to the widespread use of radiation biotechnology for the radiation processing of food products, as well as numerous statements that irradiation induces toxic radiolysis products and thereby reduces the nutritional value of products, the FAO/IAEA/WHO Joint International Advisory Group has reviewed scientific research on the assessment of food exposures [13], which emphasizes the relevance of developing highly sensitive methods for monitoring (indicating) radioinduced toxic radiolysis products.

According to their chemical nature, known radiotoxins can be assigned to the following classes: hydroperoxides and peroxides; polyphenols, semiquinones and quinones, ketoaldehydes; polypeptide proteins; biogenic amines with cytotoxic properties (inhibition of division, formation of chromosomal aberrations, cell death).

An essential characteristic of radiotoxins is their ability to exhibit antigenic properties when they enter the body.

To date, antigens have already proven their importance in the prevention and diagnosis of various infectious diseases. On the basis of antigens, reference antigenic preparations and test systems for immunodiagnostics are being developed, and vaccines for immunoprophylaxis are being created. Antigens are used to immunize animals to obtain polyclonal and monoclonal antibodies, as well as to standardize immunoglobulin diagnostic test systems [14, 15, 16].

The processes of oxidation of o-phenols that occur in an irradiated organism proceed in the presence of proteins and their decay products, inevitably leading to the formation of quinone adducts with peptides and proteins, i.e., unconjugated autoantigens.

The formation of such adducts of quinones with peptides and proteins will have the most important consequences for understanding the processes occurring in the irradiated organism during the post-radiation period, which consists in the stabilization of quinones, which ensures the remote effect of radiation, the communication of cytotoxic properties inherent in o-quinones to proteins with the carrier of quinoid groups, changes in antigenic properties of proteins and the formation of a new antigen with a determinant group of o-quinone.

The general concept of radiation toxicology indicates the appearance in the irradiated organism of specific (radiation) antigens detected by the method of anaphylaxis with desensitization according to L.A. Zilber [17].

After many years of failure, Lev Alexandrovich Zilber developed a method called the anaphylaxis with desensitization method, which made it possible to determine specific tumor antigens in a wide variety of animal and human tumors.

If indeed autoimmunization in irradiated animals occurs under the influence of proteins bearing a quinoid group as a determinant, then quinoidradiotoxins (QRT) should be specific allergens for animals that have undergone radiation sickness.

According to modern concepts, the main thing in an antigen is its antigenicity (immunogenicity), the ability to induce a specific immune response of the body: the formation of homologous antibodies and specific sensitization of lymphoid cells.

It is postulated that antigenicity is determined mainly by five major provisions:

- foreignness of the antigen for the body,
- its ability to be assimilated by the organism,
- high molecular weight of the antigen (at least 10,000 daltons),
- colloidal state of the antigen,
- the chemical nature of the antigen

From the above postulates regarding the beam antigen (radiotoxin) we received, it can be seen that it does not meet one of the requirements — the molecular weight, which, as mentioned above, is 2500 daltons for quinoidradiotoxin, which is significantly inferior to full-fledged antigens (10,000 daltons) . However, as mentioned above, immunochemical reactions occur in the irradiated organism with the formation of proteins that carry quinones as a determinant group, i.e., radioinduced beam antigens consisting of adducts of quinones with peptides and proteins, forming conjugated antigens in the irradiated organism.

Radioinduced conjugated antigens, carrying out autoimmunization in the irradiated organism, induce the synthesis of autoantibodies. From the foregoing, two important points emerge:

- under in vitro conditions, it is possible to obtain conjugated beam antigens by converting low molecular weight quinoid antigens into full-fledged protein-lipoid antigens with determinant and protein groups of antigens,
- byhyperimmunization of animals with a full-fledged conjugated quinoid-lipoid (or protein) antigen, to receive hyperimmune therapeutic and diagnostic antiradiotoxic sera.

Materials and methods

In view of the foregoing, we conducted experiments to determine the antigenic (the ability of QRT to synthesize antiradiotoxic antibodies) properties of plant radiotoxin obtained by the method of S.K. Melnikova and and V.A. Kopylov [18] (irradiation of plant materials was carried out on the “Researcher” gamma installation at a dose of 400 Gy), with the help of RIHA. Taking into account that quinoidradiotoxin is a low-molecular chemical compound having a molecular weight of 0.6 kD, i.e. an inferior antigen — a hapten, it was conjugated with a protein carrier — an incomplete Freund’s adjuvant (IFA). To do this, a solution of quinoidradiotoxin with a content of 10 mg/ml was mixed with incomplete Freund's adjuvant (IFA) in a ratio of 1:1, the mixture was homogenized on a Schuttel apparatus for 30 min at 110 oscillations/min. The mixture of the conjugated

quinoid-lipoid antigen preheated to a temperature of 42 °C was administered four times intramuscularly in doses of 3 cm^3 to three sheep in the region of the inner surface of the thigh on both sides under aseptic conditions on the 1st, 7th, 14th, 28th and 49th day. On the 7th day after the last injection of the antigen, antibodies were detected in the blood serum using RIGA. To determine the immunological activity of hyperimmuneantiradiotoxic sera obtained after hyperimmunization of animals with the test antigen-plant quinoidradiotoxin (QRT), an indirect hemagglutination test (RIHA) was performed on polystyrene microplates with U-shaped "wells".

To determine the immunological activity of hyperimmuneantiradiotoxic sera obtained after hyperimmunization of animals with the test antigen-plant quinoidradiotoxin (XRT), an indirect hemagglutination test (IDHA) was performed on polystyrene microplates with U-shaped "wells".

As control (positive) anti-radiation antibodies in the reaction, we used anti-radiation antibodies obtained by hyperimmunization of rabbits with a beam antigen — radiotoxin isolated from the liver tissue of lethally irradiated sheep, tested antitoxic antibodies — hyperimmune sera from sheep hyperimmunized with quinoid (plant) radiotoxin, negative serum obtained from intact (non-irradiated, not vaccinated with any vaccines) sheep, heterologous sera (anti-burn, anti-colibacillary).

The reaction was placed on microplates in a volume of 50 µl. To do this, 50 µl of physiological saline in phosphate buffer with pH 7.2 was poured into all wells. Then, 50 µl of positive serum was added to the 1st well and serial dilutions were made. Subjects, control negative and heterologous sera were diluted similarly.

The reaction was taken into account according to a four-point system and expressed in "crosses":

++++ — agglutination of erythrocytes occupies half of the bottom of the well in the form of an umbrella with jagged edges and complete clarification of the supernatant,

+++ — agglutination of erythrocytes occupies one third of the bottom of the hole,

++ — agglutination of erythrocytes occupies one third of the bottom of the well, forming a precipitate in the form of a ring with a lumen inside, the clarification of the supernatant is incomplete,

+ — agglutination occupies one third of the bottom of the well, forming a discontinuous ring with fuzzy edges,

– — erythrocytes settle evenly in the form of a compact dot or button.

Results and discussion

The results of studying the antigenic activity of the tested radiation antigen — plant quinoid radiotoxin (QRT) using the RIHA test are presented in Table 1.

Table 1 shows that the test antigen — plant quinoid radiotoxin — has a high antigenic activity, inducing the synthesis of antiradiotoxic antibodies in the body of immunized animals (sheep), the titers of which significantly exceed those of animals immunized with the liver (animal) antigen — radiotoxin.

The organ antigen — quinoidradiotoxin, obtained from the liver of irradiated animals, has a less pronounced antigenicity — the titers of RIHA of antiserum to hepatic quinoidradiotoxin were 1.34 times inferior to those of antisera to QRT ($P < 0.05$).

The data obtained, presented in Table 1, also indicate that hyperimmune antiradiotoxic sera, along with high serological activity, are also highly specific — they do not react with heterologous (burn and bacterial — colibacillary) antigens. Unlike the test sera, antisera to liver radiotoxin are less specific — they react with heterologous (microbial and burn) antigens in titers of 1.3-1.7 log2, which reduces their diagnostic value.

Therefore, quinoidradiotoxin obtained from plant tissue (potato tubers) and conjugated with a protein-lipoid group (incomplete Freund's adjuvant — IFA) has a high antigenicity, inducing the synthesis of specific anti-radiotoxic antibodies, which can be used as an essential component of an immunochemical test system. — sensitin used for sensitization of micro- and nanoparticles of bentonite in the design of anti-radiation antibody variant of bentonite diagnosticum (ABBD).

Conclusion

Thus, as a result of the studies, we obtained a radioantigen from potato tubers irradiated with gamma rays at a dose of 400 Gy — quinoidradiotoxin (QRT), which is chemically o-quinones with a molecular weight of 2500 daltons, which has a high biological (radiomimetic, allergenic , toxic, antigenic) activity. To convert a low-molecular compound into a high-molecular one in order to obtain a full-fledged antigen, a protein-lipoid group, an incomplete Freund's adjuvant, is attached to the hapten part of the antigen, and as a result, a complete antigenic complex (QRT-IFA) is obtained, which is a conjugated quinoid-lipoid antigen, which has the ability to induce the production of antiradiotoxic antibodies with a titer of 8.33 log2 (1:256 — 1:512).

Table 1. **Serological activity of hyperimmune antiserum to radiation antigen — quinoid radiotoxin (QRT) in RIHA-tes**

Antigenname	Antibody titer in RIHA of the tested antiserum to log_2			
	quinoid (plant) radiotoxin	hepaticquinoid (animal) radiotoxin	burnantigen	colibacillaryantigen
Quinoid (plant) antigen — radiotoxin	8,33±0,50x	4,7±0,7xx	-	-
Quinoid (animal) antigen — radiotoxin	7,7±0,5	6,3±0,5x	0,5±0,01	0,3±0,01
colibacillaryantigen	-	1,7±0,1	0,3±0,03	0,5±0,03
Burnantigen	-	1,3±0,05	6,3±0,7	0,5±0,003
Footnote: x — $P < 0.05$				
xx — $P < 0.01$				

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