SÍNTESE E ESTRUTURA DE ADJUVENTES ARTIFICIAIS PARA PREPARAÇÃO DE VACINAS SINTÉTICAS

SYNTHESIS AND STRUCTURE OF ARTIFICIAL ADJUVANT FOR PREPARATION OF SYNTHETIC VACCINES

СИНТЕЗ И СТРОЕНИЕ ИСКУССТВЕННОГО АДЪЮВАНТА ДЛЯ ПОЛУЧЕНИЯ СИНТЕТИЧЕСКИХ ВАКЦИН

S. V. Savina^{1*}, V. M. Skorlyakov¹, D. A. Zayarskiy²

¹Saratov State Vavilov Agrarian University
410012, 1, Teatralnaya pl., Saratov, Russia

²Saratov State University
410054, 77, Politekhnikeskaya str., Saratov, Russia

* *Corresponding author*
savinas77@mail.ru

Received 28 June 2018; received in revised form 26 November 2018; accepted 23 November 2018

RESUMO

O objetivo da pesquisa foi desenvolver uma nova abordagem para projetar uma nova estrutura na criação de vacinas sintéticas. Conseguimos sintetizar um adjuvante polieletrólítico. Como material de partida, utilizou-se cloreto de polidialildimetilamônio modificado com íons de hidrato de halogênio. Os testes pré-clínicos realizados em animais de laboratório não demonstraram desvios em seus parâmetros fisiológicos e toxicológicos.

Palavras-chave: *adjuvante, polímero, linfócito T, linfócito B, anticorpo.*

ABSTRACT

The aim of the research was to develop a new approach to designing a new structure in creating synthetic vaccines. We succeeded in synthesizing a polyelectrolyte adjuvant. As a starting material, we used polydiallyldimethylammonium chloride modified with halogen hydrate-ions. The pre-clinical tests carried out on laboratory animals demonstrated no deviations in their physiological and toxicological parameters.

Keywords: *adjuvant, polymer, T-lymphocyte, B-lymphocyte, antibody.*

АННОТАЦИЯ

Для совершенствования подхода в создании новой структуры для получения синтетических вакцин удалось синтезировать полиэлектrolитный адъювант. Исходным сырьем для создания, которого был использован полидиаллилдиметиламмоний хлористый модифицированного гидрат-ионами галогенов. Проведенные доклинические исследования на лабораторных животных показали отсутствие отклонений в физиологических и токсических параметрах организма.

Ключевые слова: *адъювант, полимер, Т-лимфоцит, В-лимфоцит, антитело.*

INTRODUCTION

Modern biotechnological developments provide for creation of numerous versions of vaccinal preparations.

Achievements in immunology, molecular biology and biochemistry serve as a basis for creation of antibacterial and antiviral vaccines.

Creating synthetic vaccines, it is necessary to obtain such artificial bioorganic complex which can ensure an immune response of a body to the proper antigen notwithstanding its genetically subdued low responsiveness. Only in this case will synthetic vaccines be a significant step forward by comparison with the natural ones that represent preparations of weakened or killed viruses or preparations of antigen substances extracted from them (Petrov & Khaitov, 1979).

There have been created experimental synthetic vaccines for diphtheria, cholera, streptococcosis, hepatitis B, flu, foot-and-mouth disease, tick-borne encephalitis, pneumococcal and salmonella infections.

MATERIALS AND METHODS

An important condition for the development of certain carriers (adjuvant substances) is creation of a preparation that can remain for a long time in a body with an active immune system without any local or general negative reactions in the body.

To develop an effective immune response, in many vaccines it is necessary to add adjuvants (substances) which often cause undesirable side effects (Vorobiev & Vasiliev, 1969; Volpina, 1992).

In the recent decades, research laboratories have been working on a different approach to the problem of synthetic vaccines. Its essence is in the fact that synthetic vaccines must carry on their macromolecules not only the determinate fragments of antigens, but, simultaneously, such a structure which can ensure production of antibodies to various antigens.

Such works were started in the 1970s by R. V. Petrov and R. M. Khaitov (1979), V. A. Kabanov and others (1982), and in the 90s by O. M. Volpina et al. (1992).

For actualization of immune correction they managed to start the development of complex antigens – such artificial macromolecular compounds which include both necessary antigen

determinants and a stimulating (adjuvant) structure ensuring an immune response.

During the study of the action mechanism of synthetic polyelectrolytes on immunogenesis, it was established that carbon-chain polyelectrolytes – poly-4-vinylpyridine and polyacrylic acid (PVP, PAA), as well as polyoxidonium, stimulate proliferation and migration of hemopoietic stem cells, expansion of T-lymphocytes from the thymus and B-lymphocytes from the bone marrow into the peripheral lymphatic organs and cause a considerable increase in the output of the emerging antibody producers. (Petrov et al., 1984).

In the works of O. M. Volpina (1992) it was established that the synthetic complexes obtained by addition to polyelectrolyte carriers-adjuvants of a poorly immunogenic protein antigen ensure induction of an increased immune response to synthetic antigens.

M. A. Kokhovich et al. (2010) conducted a comparative evaluation of the influence of different types of adjuvants, such as aluminium hydrate (AH), ISCOM and MontanideGel, on the antigen strength of the ERA-CB 20 M rabies vaccine, both in an experimental monovalent antirabies vaccine and in the composition of a multivalent vaccine. In small domestic animals, the maximum titer of antirabies antibodies (14.58 IU/ml) was observed when using the ISCOM adjuvant in the monovalent antirabies vaccine, while with ISCOM in the multivalent vaccine the antibody titer constituted 7.2 IU/ml.

I. I. Chernikova, O. S. Kashirina and Y. M. Vasiliev (2015) studied the immunogenicity of adjuvants of different nature and the action mechanism of inactivated flu vaccines. Added to the vaccine as adjuvants were aluminium hydrate, CpG oligonucleotide, complete Freund's adjuvant, microparticles of poly (lactide-co-glycolide), monophosphoryl lipid A and polyoxidonium, as well as 2 adjuvants based on the specified substances of chitosan with different physical and chemical properties and 2 experimental complex preparations (multicomponent adjuvant and emulsion of the type of oil in water on the basis of squalen and tocopherol). It has been established that different adjuvants increase the vaccine immunogenicity against the homologous strain in a different way. The experimental complex preparations had the highest immunogenicity; the antibody titer increased by 48-96 times as compared to the vaccine without an adjuvant. High immunogenicity was noted for chitosan-based

adjuvants. Not all adjuvants increased immunogenicity significantly, and in some cases after their addition there was even some reduction of the antibody titer.

Thus, a polyelectrolyte and antigen introduced into a body, during the time of active interaction with blood components and lymphoid system, contribute to the formation of durable intercellular and intermolecular contacts, which results in strengthening the antigen-specific immune response.

To improve this approach to creation of a new adjuvant structure, a group of scientists from Saratov State University and Saratov State Vavilov Agrarian University managed to synthesize an adjuvant representing a complex of a synthetic polyelectrolyte.

RESULTS

The starting material for creation of polyazolidinammonium (PAA) modified with iodine hydrate-ions is polydiallyldimethylammonium chloride (PDDA). The polymer's structural formula is presented in Figure 1.

To enhance PDDA with new antimicrobial properties, chloride ions are replaced with halogen hydrate-ions, namely, in our case, with iodine hydrate-ions (IOH⁻). This modification is performed in alternating electric field under the influence of light with a certain wave-length (more exact modification parameters shall not be disclosed). According to the results of combinational scattering spectroscopy, no structural transformations in the polyelectrolyte molecule were registered.

It is necessary to add that, when being in a bound state in the polyelectrolyte molecule, the iodine hydrate-ions do not show any activity, which is testified by the absence of "the blue reaction" with starch.

It is assumed that in mixing the polyelectrolyte with antigenic substances a part of iodine ions disengages, and the antigen is bonded instead. The formation of the core-shell structures is most often conducted by the method of successive polyelectrolytes adsorption onto the surface of a solid core (Kabanov et al., 1982; Petrov et al., 1983). As core can any solid substance be used, which is insoluble in water

and has the dimensions from a few microns to a few tens of nanometers. Most often, for core formation, substances with high biological activity or pharmaceutical preparations are used. Polyelectrolytes have low toxicity and high LD50 value, which is generally more than 3 g/kg.

It has been shown that the average size of the molecule constitutes 60-80 nm, and the value of electrokinetic potential is not lower than 35 mV. The obtained substance is characterized by high aggregative stability and does not flocculate for a long time.

So, using the method of molecular engineering, there has been synthesized a version of a polyvalent synthetic subunit which will be used as an adjuvant.

The preclinical trials of polyazolidinammonium conducted on laboratory animals with acute and chronic toxicity and calculated LD50 showed absence of deviations of clinical and toxic parameters.

The intramuscular injection of the adjuvant during 5 days did not cause any changes in the animals' state, their hematologic parameters and condition of their inner organs (necropsy was performed).

Studies of local irritating and allergenic (sensitizing) effect of polyazolidinammonium were conducted during conjunctival and dermatic tests.

The visual assessment of the state of the conjunctiva, eye keratoderma and blepharon established that polyazolidinammonium modified with hydrate-ions does not cause irritation of the conjunctiva and does not produce any allergenic (sensitizing) effect on it.

The skin tests did not reveal any injury in the form of an erythema or edema. The application of the preparation did not cause a hyperemia, desquamation, allergic eczema or dermatitis.

Clinical blood analyses (hematologic, biochemical, etc.) are widely used as they help evaluate the work of inner organs (liver, kidneys, pancreas, gall bladder, etc.), obtain information on metabolism (of lipids, proteins, carbohydrates), establish the need for mineral nutrients and diagnose diseases.

DISCUSSION

The studies of peripheral blood parameters testify to the fact that, independently of the way of the adjuvant administration, hematological indices correspond to the physiological norm for a given animal species. Our data agree with the normal hematological indices of CD (Sprague Dawley) rats' blood and CD 1 mice's blood, obtained by I. N. Kravchenko and others (2008); the biochemical values correspond to those received by I. V. Ananich, M. A. Derkho and T. A. Tkachuk (2008). Besides, no inflammatory or allergic processes were noted in the place of injection.

The research results showed that the polyelectrolyte is not mutagenic according to the results of AMES tests, not teratogenic (does not cause abnormalities and teratisms), NOEL = 25 mg/kg.

The tests conducted in the Research Institute of Veterinary Virology and Microbiology (Pokrov, Moscow Oblast) on inactivated rabies antigen in compliance with STOO 00495549-0020-2006 according to standard technical regulations showed high immunogenic activity in groups 3 (tested vaccine + polyethylsiloxane), 4 (reference-vaccine + polyethylsiloxane) and 5 (inactivated antigen without G0A + polyethylsiloxane), 1.82IU/cm³, 2.18IU/cm³, and 2.26IU/cm³, respectively.

The conducted pre-clinical tests prove a possibility of the adjuvant (polyelectrolyte) use in preparing test batches of vaccines.

CONCLUSIONS:

The prepared adjuvant showed promising results in the conducted tests. It was established that polyazolidinammonium modified with hydrate-ions (adjuvant – antigen-carrier) does not produce any local irritating effect. The conducted hematologic, biochemical research has not revealed any physiological deviations. No signs of allergenic (sensitizing) effect on the skin and conjunctiva were noted. High immunogenicity was established in the pre-clinical tests.

The positive results make it possible to consider that the prepared carrier-adjuvant will be harmless for animals.

A further study of the immunogenic properties of the developed vaccine is planned.

REFERENCES:

1. Ananich IV, Derkho M A, Kontsevaya SY (2008): Biochemical indices of rats' blood. *Veterinarnaya Klinika* 10, 35-37 (in Russian).
2. Aguilar J. C. Vaccine adjuvants revisited / J.C. Aguilar, E. G. Rodriguez // *Vaccine*. – 2007. - № 25. - P.372.
3. Chernikova MI, Kashirina OS, Vasiliev YM (2015): Comparative study of immunogenicity of adjuvants of different nature and action mechanism on the model of inactivated flu vaccine. *Zhurnal Mikrobiologii, Epidemiologii i Immunologii* 6, 63-67 (in Russian).
4. Derek T O'Hagan MF59 is a safe and potent vaccine adjuvant that enhances protection against influenza virus infection / Derek T O'Hagan // *Expert Review of Vaccines*, – 2007. - Vol. 6, No. 5. – P. 699.
5. Edelman R. Vaccine Adjuvants // *Rev. Infect. Dis.*-1980 - Vol.2- P.383.
6. Kabanov VA, Petrov RV, Khaitov RM (1982): A new principle of synthetic immunogen creation. *Zhurnal Vsesoyuznogo Khimicheskogo Obshchestva*, 27, 57-68 (in Russian).
7. Kokhnovich MA, Gribencha SV, Mukhin AN, Seliverstov A, Verkhovskiy OA, Nepoklonova IV, Aliper TI (2010): Estimation of different adjuvants' influence on antigenic activity of rabies vaccine for small domestic animals. In: *Materials of the 18th Moscow International Veterinary Congress on Diseases of Small Domestic Animals*. Moscow (in Russian).
8. Kravchenko IN, Khokhlova ON, Kravchenko N N, Puzhalin A N, Diachenko IA, Murashev AN (2008): Hematological indices of free from pathological flora rats CD (Sprague Dawley) and mice CD1 n. *Biomeditsina*, 2, 20-31(in Russian).
9. Petrov RV, Khaitov RM (1979): Immune response to synthetic antigens. *Dostizheniya Sovremennoi Biologii*, 88(6), 307-321 (in Russian).
10. Petrov RV, Khaitov RM, Ataulakhanov RI (1983): Immunogenetics and synthetic antigens. Moscow: *Meditina* (in Russian).
11. Petrov RV, Khaitov RM, Ataulakhanov RI (1984): Molecular mechanisms of activation

by oxitovpolyions. 1. Modification of transmembrane transport of cations. *Biologicheskiye Membrany*, 1(8), 599-604 (in Russian).

12. Petrov R.V., Kabanov V.A., Khaitov R.M. *Immunology*. 1986. No. 1. p.5.

13. Savina S.V., Ivashchenko S.V., Skorniyakov V.M., Murtaeva V.S. The effect of synthetic adjuvant on the formation of the immune response. *International research journal*. 2016. № 8-2 (50). C. 42-44.

14. Savina S. V., Skorniyakov V. M., Chastov A. A., Veselovsky S. Yu. Evaluation of reactogenic properties of chemical polyelectrolyte substance-adjuvant in experiment, *International research journal*. 2017. № 5-2 (59). C. 103-106.

15. Savina S.V., Skorniyakov V.M. Study of the influence of chemical polyelectrolyte substance-adjuvant on embryotoxicity and teratogenicity of laboratory animals, *Agricultural scientific journal*. 2017. № 12. C. 48-50.

16. Schijns,V. Immunological concepts of vaccine adjuvant activity / V. Schijns. // *Curr. Opin. Immunol.* – 2000. – Vol. 12. – P. 456-463.

17. Vogel,F. R. Improving vaccine performance with adjuvants / F.R. Vogel. // *Clin. Infect. Dis.* – 2000. – Vol. 30. – Suppl. 3. – S. 266-S270.

18. Vogel, F.R. Adjuvants in perspective / F.R. Vogel // *Dev. Biol. Stand.* – 1998. – Vol. 92. – P. 241-248.

19. Volpina OM (1992): Theoretical and practical approaches to creation of a synthetic foot-mouth disease vaccine on the basis of synthetic peptides. Dissertation. Moscow (in Russian).

20. Vorobiev AA, Vasiliev AA (1969): Adjuvants. Moscow: Meditsina (in Russian).

Fig. 1. Polyazolidinammonium (PAA) and polydiallyldimethylammonium chloride (PDDA) structural formulas