ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Quantitative Assessment Of Glycoprotein In Rabies Vaccines By Enzyme Immunoassay.

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ABSTRACT

Glycoprotein is the main immunogenic protein of whole virion vaccines for the prevention of rabies. Specific antibodies formed after vaccination have the ability to neutralize the virus in vitro, as well as to protect humans and experimental animals from lethal viral infections. The quantitative content of a specific viral protein - glycoprotein in vaccines is an important indicator of their effectiveness. The purpose of the studies was the development of an enzyme immunoassay method for assessing the activity of rabies vaccines using computer software. As a result, a test system based on the sandwich ELISA variant using monoclonal antibodies to various antigenic determinants of the glycoprotein of the rabies virus has been developed, which ensures high sensitivity and specificity of the method. The creation of an enzyme immunoassay test system for the quantitative evaluation of the specific activity of a rabies vaccine is an important link in the development of a system for monitoring vaccine preparations. A computer program based on the transformation "4-parametric logarithmic logistic method" or "logit-log" is used to construct a calibration curve for the purpose of quantitative determination of the glycoprotein of the rabies virus in the "sandwich" ELISA variant. The sensitivity of the ELISA test system was 0.011125 IU / cm3. The results of the quantitative determination of the glycoprotein of the rabies virus using the sandwich ELISA variant in experimental vaccine samples correlate with the immunogenicity index for NIH in mice (minimum $r = 0.89$; $p < 0.05$ for a monoclonal antibody clone VP3 and maximum $r = 0.975$ $p < 0.05$ for a clone of monoclonal antibodies VP7).

Keywords: rabies, vaccine, glycoprotein, enzyme immunoassay, monoclonal antibodies.

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ISSN: 0975-8585

INTRODUCTION

The intense epizootic situation of rabies in the Russian Federation requires the production of vaccines with high immunogenic activity. In this connection, studies aimed at the development and application of a rapid method for assessing the activity of rabies vaccines based on enzyme immunoassay are very relevant.

Currently, regulatory documents provide for an assessment of the authenticity and specificity of biological products in animal studies, which requires a long time, allowing for the differentiation of vaccine series with low TCD50 (50% tissue cytopathic dose) before the final stages of production. The establishment of a low biological activity of a vaccine preparation excludes it from use. Improving the quality of biological products, their production efficiency and environmental safety is an important task of the biological industry in the Russian Federation [1].

The main indicator of the quality of rabies vaccines is the specific activity determined in the immunogenicity test in mice. It has been established that the main antigenic determinants inducing a protective immune response are on a glycoprotein, the content of which correlates with the immunogenicity of rabies vaccines [10,15]. The method NIH (United States National Institutes of Health) [11] is widely used to determine the immunogenic activity of inactivated rabies vaccines. In assessing the quality of vaccines, the WHO Expert Committee since 1992 proposes the use of alternative in vitro methods [5, 6,8,10,16,17,18].

The aim of the present research is to create a highly specific, sensitive, simple and fast method for quantifying the virus glycoprotein using an enzyme immunoassay and elements of modern computer software for the production of rabies vaccines.

MATERIALS AND METHODS

Outbred white mice weighing 10–12 g were used as experimental animals. [12,13] A fixed rabies virus of the strains Shchelkovo-51, Sheep was used in the work, and the strain CVS was used to infect mice. Used standard culture medium Eagle, 199, DMEM, hydrolyzed lactalbumin solution Earl (GLAE) or Hanks (GLAH), obtained from the Institute of Poliomyelitis and Viral Encephalitis, Moscow; trypsin produced by Merkk, Fluka, bovine serum and fetal serum - BIOLOT LLC (St. Petersburg) and Sigma. At different stages of work, physicochemical, virological, and immunological research methods were used. We used standard methods of solid-phase ELISA on 96 well microplates. For the development of the “sandwich” variant of the ELISA, commercial immunological and chemical reagents of well-known domestic firms Imtek, ZAO NVO Immunotekh, OOO Bialeksa, Moscow were used. The infectivity of the rabies virus was determined by titrating the virus-containing material on white mice weighing 10-12 g. To do this, a 10-fold dilution of the test material was prepared in Hanks solution and 5-7 white mice were infected intracerebrally at a dose of 0.03 cm3. Observation of the animals was carried out for 14 days. Those animals whose death occurred after the 5th day of infection with the manifestation of clinical signs of rabies were believed to have died from rabies. The titer of the virus was calculated according to the method of Reed and Mench and expressed in lg LD50 / cm3 [1,11]. Immunogenicity of the virus-containing material was determined by the NIH method in mice against standard rabies virus, strain “CVS” and relative to the international vaccine standard obtained from the laboratory of biological standards, Denmark, Copenhagen (or industry vaccine standard-reference vaccine, Schelkovsky Biokombinat) and expressed in terms of immunogenicity index or in international units (IU) [11].

For statistical processing of the results of an enzyme immunoassay using a 4-parameter logarithmic logistic method ("4PL"), a computer program developed by Alkor Bio, St. Petersburg, was used. In the case of the “sandwich” variant of the ELISA, the logarithmic transformation is sufficient to linearize the calibration curve. An example of such a special linearizing transformation is the logit transformation proposed by Rodbard [14]. The vaccine “Nobivac Rabies”, “Intervet International” (Netherlands) of the series A195 AO1 and A225 AO1 was used in the work; industry standard sample of immunogenicity of the rabies vaccine of the FSC “Shchelkovsky Biokombinat” ser.1-15 with immunogenicity of 1.8 IU / cm3 - STO-00494189-0042-2010 “Antirabic reference vaccine” and other domestic vaccine biologics and their semi-finished products with encrypted immunogenicity, predetermined in mice by the NIH method.

ISSN: 0975-8585

RESULTS AND DISCUSSION

In the Department of Molecular Biology and Virology of the FNB "VNITIBP" a "Reagent Kit for Detection of the Glycoprotein of the Rabies Virus by ELISA" has been developed. The main immunological component of the developed test system in the "sandwich" format — ELISA variants are commercial monoclonal antibodies (MCA): isotype: IgG1 for VR6, isotype IgG2a for VR7, isotype IgG2b for VR1, VR3. The isotype IgG2b for VR1 was labeled with horseradish peroxidase and used as a developing complex, the remaining antibodies were used as fixatives or "substrates".

In the immunoassay of the "sandwich" variant at the first stage, monoclonal antibodies specific for the glycoprotein antigen under study were sorbed onto the tablet surface. After removing the unbound antibody molecules, a sample containing the antigen was added. For the detection of the resulting antibody-substrate-antigen complex, second antibodies conjugated with horseradish peroxidase specific to another, spatially-deleted epitope of the antigen are added. The use of the ELISA test system requires standardization of results accounting, which poses the problem of introducing a positive control sample with known specific activity. In this regard, the test-system included a reference vaccine from the Shchelkovo-51 strain produced by the Schelkovo Biokombinat, which is an industry standard immunogenicity sample calibrated according to the International Standard for Anti-rabies Vaccine. Quantitative assessment of the glycoprotein of the virus was expressed in IU / cm3 based on the registration of the signal of the optical density of dilution of the test sample of the vaccine and the projection of its indicators in a calibration curve based on the results of the evaluation of dilutions of the standard reference reference vaccine against rabies with a known index of immunogenicity. For the quantitative statistical processing of the results of the enzyme immunoassay and the calculation of the glycoprotein concentration of the rabies virus in vaccine raw materials and finished preparations, a modern computer program was adapted, which according to the ELISA data automatically determined the activity of the tested vaccine material relative to the standard. At present, such theoretical methods for constructing a calibration graph as the theoretical 4-parametric logarithmic logistic model ("4PL") and the "logit-log" transformation, which is a special case of "4PL" [4], are considered to be the most acceptable.

The figure shows the results of the determination of the glycoprotein of the virus in experimental samples of rabies vaccine manufactured by FSBI "VNITIBP". The sensitivity of the ELISA test system was 0.011125 IU / cm3.

The test results of experimental samples of rabies vaccines "sandwich" with an ELISA variant and the results of vaccine activity determined by the NIH method are presented in the table.

From the data in the table it follows that the results of applying the "sandwich" ELISA variant for quantitative evaluation of the content of the glycoprotein of the rabies virus correlated with the NIH method. These data are consistent with the results of many studies that have established a direct relationship between the quantitative content of the glycoprotein of the rabies virus in a vaccine and its immunogenic activity [2,3,4,9].

Table: Determination of immunogenicity in ELISA and NIH method of rabies vaccines (n = 6)

Vaccine series	Activity in ELISA, IU / cm3			activity by the method of NIH, IU / cm3
	VP3*	VP6*	VP7*	
1	1,41	1,06	1,31	1,2+0,03
2	1,26	1,09	1,26	1,4+0,03
3	1,61	1,65	1,71	1,8+0,03
4	1,79	1,86	1,96	2,2+0,03
5	1,86	1,79	1,86	2,0+0,03
correlation method with NIH,%	0,89 P<0,05	0,913 P<0,05	0,975 P<0,05	-

ISSN: 0975-8585

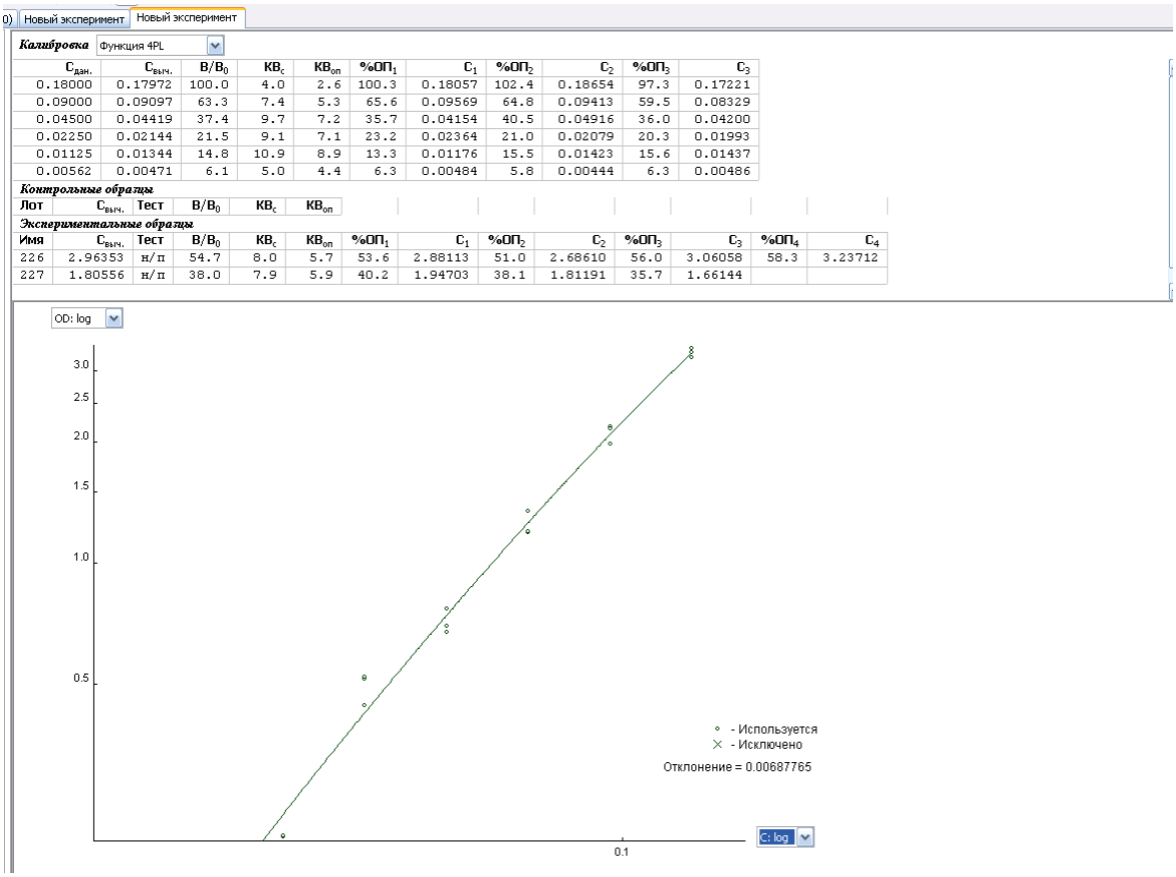

Figure: Determination of the glycoprotein of a virus in experimental samples of rabies vaccines; on the y-axis - the logarithm of the values of optical density, on the x-axis - the logarithm of the concentration of glycoprotein, expressed in IU / cm3

Using the developed ELISA test system, the amount of the glycoprotein of the rabies virus in the vaccine "Nobivac Rabies", the Netherlands was determined. Prior to ELISA, the glycoprotein of the rabies virus was pre-desorbed in the Nobivac Rabies vaccine from aluminum phosphate, and centrifuging 1000 µl of the test vaccine sample at 6000 rpm for 5 minutes at room temperature. From 900 µl of the supernatant, a series of serial dilutions were prepared and ELISA was performed to determine the amount of glycoprotein in the supernatant (N1 value). The precipitate was dissolved in 900 µl of buffer solution for desorption, thoroughly mixed and left overnight at a temperature of from 2 ° C to 8 ° C. Buffer for desorption of rabies virus from aluminum phosphate contained gelatin, sodium phosphate disubstituted, Trilon B (EDTA), tween-20 and purified water. Then it was centrifuged at 6000 rpm for 5 minutes at room temperature. A series of serial dilutions were prepared from the supernatant and the amount of glycoprotein (N2 value) was determined by ELISA.

The amount of glycoprotein in a foreign vaccine was equal to the sum of the values of N1 and N2. Ultimately, according to the results of the ELISA and the use of a 4-parametric logarithmic logistic method for constructing a calibration curve, the total glycoprotein content in the Nobivac Rabies vaccine (N1 and N2) was 2.0 IU / cm3 as stated by the manufacturer.

CONCLUSION

The principal possibility of quantitative determination of glycoprotein content in rabies vaccines using enzyme immunoassay is shown. The coefficient of variation in optical density does not exceed 10%. The sensitivity of the test system was 0.01125 IU / cm3. It was shown that the results of the quantitative determination of the glycoprotein of the rabies virus using the “sandwich” ELISA variant in experimental vaccine samples correlate with the immunogenicity index for NIH in mice (minimum r = 0.89; p <0.05 for the VP3 clone and maximum r = 0.975 r <0.05 for a VP7 clone). The advantage of using ELISA is to eliminate the work of laboratory staff with the live rabies virus strain “CVS”, avoid using a large number of animals, reduce

execution time, obtain quantitative characteristics of the content of the main protein immunogenicity in the study of vaccine material, which contributes to standardization of antigen at all stages of vaccine production.

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