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PROPERTIES OF RABIES VACCINE FREED OF NEUROALLERGENIC FACTOR FROM BRAIN TISSUE

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Summary. — Fixed rabies virus in the form of infected sheep brain suspension was freed from approx. 80% of ballast proteins, inactivated by beta-propiolactone and lyophilized. The vaccine thus obtained was devoid of neuroallergenicity when tested on guinea pigs and was highly antigenic and immunogenic. The vaccine caused no generalized reactions in volunteers; local reactions were weak and of short duration. Antibody formation was intensive in all volunteers.

Key words: rabies vaccine; neuroallergenic factor; volunteers

Introduction

Previously (Karakuyumchan *et al.*, 1981) we described a method of purification of rabies virus from infected sheep brain tissue. The method includes suspension of brain tissue in hypertonic buffer solution, shaking and low speed centrifugation. From 65 to 85% ballast prteins are remove with a simultaneous loss of neuroallergenic activity of the material, but practically without loss of virus.

We are reporting the results of testing of a vaccine prepared from thus purified virus in experiments on animals and in volunteers.

Materials and Methods

The strain of fixed rabies virus used, its purification and inactivation with beta-propiolactone, lyophilization, determination of innocuity of the vaccine and its testing for the absence of neuroallergenic activity were described previously (Karakuyumchan *et al.*, 1981).

Innocuity of the vaccine was tested by inoculating 5 mice subcutaneously (s.c.) on their backs each with 0.2 ml of the resuspended vaccine. The animals were observed for 5 days for the appearance of infiltrates at the injection sites and death.

Immunogenicity of the vaccine was assayed by the NIH test (Seligmann, 1973). Mice were inoculated intraperitoneally (i.p.) with 0.5 ml of vaccine diluted 1 : 5, 1 : 25 and 1 : 125, using 10 mice for each dilution. Three parallel groups of mice were injected in the same way with a reference vaccine, obtained from the L. A. Tarasevich Institute of Standardization and Control of Medical Biological Preparations, Moscow. The immunization was repeated after 7 days. After another 8 days the mice were injected intracerebrally with 30—100 LD_{50} of the NIH strain of rabies virus in 0.03 ml volumes. The relative immunogenicity (RI) of the vaccine was determined with reference to the reference vaccine, taking into account that the human dose is 3 ml. The minimal allowed RI is 0.3.

Determination of stability of the vaccine. Lyophilized vaccine was kept for 24 hr at 56 °C, for up to 6 months at 37 °C, 12 months at 22 °C and 18 months at 4—6 °C. Immunogenicity of the vaccine samples was then assayed by the NIH test.

Antigenicity tests in animals. The vaccine was administered to guinea pigs i. p. in doses corresponding to 0.083 ml per kg body-weight daily for 14 days; part of the animals was revaccinated on days 23, 33 and 43. One day before the onset of immunization, some animals were given anti-rabies horse immunoglobulin in a dose of 145 IU per kg body-weight. The antibody titres in guinea pigs were determined in neutralization tests on mice against 30—100 LD_{50} of the CVS strain of rabies virus.

Immunization of volunteers. The vaccine was administered in the abdominal subcutaneous connective tissue in 3-ml volumes daily for 14 days. The temperature of the vaccinees as well as the appearance of reactions was recorded daily. Blood samples were taken before immunization and then on days 8, 14, 30, 45, 60 and 90. Antibody titres in blood serum were determined in neutralization tests on mice.

Results

The protein contents of the final preparations of purified rabies virus vaccine obtained under production conditions varied from 1.4—2.9 mg/ml i.e. from 4- to 8-fold less than Fermi-type commercial rabies vaccines prepared from whole brain tissue of infected sheep.

Our purification method made it possible to free the virus of the neuro-allergenic factor of brain tissue. The final lyophilized preparation caused no death in tests on guinea pigs and on pathomorphological examination the brains from the latter no changes characteristic of allergic encephalitis were observed. When a vaccine from whole brain tissue, in which the virus had been inactivated by ethanol, was subjected to the same tests, from 40 to 100% of the guinea pigs died and the survivors showed a pathomorphological picture of allergic encephalitis.

Inactivation of the purified virus by beta-propiolactone was promising. The final preparation contained no detectable infectious virus. The vaccine proved to be innocuous when subjected to tests prescribed in the U.S.S.R.

The immunogenicity of the purified killed vaccine reached the level of that of Fermi-type vaccine from infected sheep brain tissue: its RI varied from 0.75 to 2.85, in most cases from 1.35 to 2.1.

We based the evaluation of stability of the purified vaccine on the WHO requirements for cell culture rabies vaccines (Netter and Perkins, 1973). According to these requirements, the vaccine should retain a satisfactory immunogenicity under conditions of enhanced degradation test, i. e. after keeping for 1 month at 37 °C. The results of tests on the purified brain vaccine under such and additional temperature conditions are summarized in Table 1. The vaccine remained sufficiently immunogenic after keeping for 24 hr at 56 °C, up to 6 months at 37 °C, 12 months at 22 °C and 18 months at 4—6 °C (the longest interval tested so far).

Antigenicity of the vaccine was assayed in guinea pigs. The conditions of the experiment as far as possible approached those of curative vaccination of men against rabies. The dose of vaccine for guinea pigs was calculated from the amount administered to humans, namely 5 ml per 60 kg body-weight per one injection. The guinea pigs were immunized daily for 14 days and

Table 1. Stability of purified brain killed lyophilized vaccine at various temperatures

Vaccine kept at °C	for	Vaccine lot no.	Relative immunogenicity I	II
56	24 hr	2	1.06	1.2
		6	1.6	0.7
		4	2.37	1.2
	4 weeks	2	1.06	1.5
		3	1.6	0.99
		4	2.37	1.41
37	6 months	2	1.06	0.54
		3	1.6	0.9
22	12 months	2	1.06	0.75
		3	1.6	1.65
4–6	18 months	1	1.35	1.5

I and II — at the onset and end of experiment.

a part was revaccinated on days 23, 33 and 43. The results concerning antibody formation are summarized in Table 2.

On day 30, considerable levels of antibody were detected in the animals. In the group of guinea pigs that received no immunoglobulin, the antibody titres at this interval were somewhat higher than in those given the immunoglobulin. Subsequently, this difference became smaller. The highest antibody titres reached after 14 immunizing doses were observed on day 40; following revaccination, there was a further increase in antibody titre on day 50.

We immunized 13 volunteers, 10 men and 3 women, aged 18-37 years, with the purified vaccine. The reactions and antibody titres observed in them are listed in Table 3. No generalized reactions were recorded after vaccination. Four vaccinees showed hyperaemia at the injection site, persisting for 1-5 days, on days 7-13 after onset of immunization. On days 6-12, in four volunteers infiltrates appeared at the injection site; they disappeared after 1, 1, 2 and 5 days, respectively.

On day 7, antibody was demonstrated only in one woman who, as revealed subsequently, had been vaccinated against rabies in 1951 (she had an antibody titre of 2 before the onset of the present immunization). On day 13, antibody

Table 2. Antigenicity of the vaccine in guinea pigs

Animal group No.*	Vaccine administered on days	Geom. mean antibody titre on day 0	30	40	50
1	0–13	< 2	205	478	174
2	0–13, 23, 33, 43	< 2	283	704	811
3	0–13	< 2	192	405	160
4	0–13, 23, 33, 43	< 2	171	448	256

*Each group consisted of 5 animals; those in groups 3 and 4 were given immunoglobulin (see text). The RI of the vaccine was 0.9, its protein contents 1.25 mg/ml; it was administered in doses of 0.083 ml per kg body-weight.

Table 3. Reactogenicity and antigenicity of the vaccine in volunteers

No.	Volunteer Sex	Age (years)	Reaction on days hyperaemia	Reaction on days infiltrate	Antibody titres on days 0	8	14	30	45	60	90
1	M	19	—	9	< 2	< 2	128	208	158	64	64
2	M	21	—	—	< 2	< 2	74	182	28	6.5	13
3	M	18	—	6—7	< 2	< 2	49	128	147	18.4	13
4	M	18	—	—	< 2	< 2	91	194	194	64	79
5	M	18	13—14	—	< 2	< 2	84	112	18.4	6.1	22.6
6	M	21	—	12	< 2	< 2	64	79	64	13	8
7	F	20	—	—	< 2	< 2	19.4	194	32	18.4	NT
8	F	27	7	—	< 2	< 2	22.6	91	30	12.1	16
9	F	37	0	—	2	11.3	362	≧ 1450	≧ 1450	722	362
10	M	18	—	—	< 2	< 2	49	≧ 1450	128	32	21.1
11	M	17	—	—	< 2	< 2	22.6	632	588	256	104
12	M	25	7—11	7—10	< 2	< 2	19.7	256	91	32	NT
13	M	20	—	—	< 2	< 2	91	632	512	158	45

NT — not tested.
The RI of the vaccine was 1.06, its protein contents 2.7 mg/ml.

was demonstrated in all vaccinees. They reached the highest level on days 30-45, the titres varying from 79 to $\geqq$ 1450. Subsequently, the antibody titres decreased, but on day 90 antibody was still demonstrated in all vaccinees.

Discussion

Rabies vaccines from animal brain offer certain advantages as compared with those from other sources. They possess a higher immunogenicity than vaccines from avian embryo tissues; their preparation is less complicated and less costly than of cell culture vaccines. The disadvantage of brain vaccines is their neuroallergenic activity which in some vaccinees may cause neurological complications.

We developed a method of purifying rabies virus propagated in brain tisue from a considerable part of ballast substances (Karakuyumchan *et al.*, 1981). The vaccine obtained from thus purified virus has an advantage over other rabies vaccines of brain origin available at present, namely that it is devoid of neuroallergenicity (when tested in guinea pigs). It is justified to assume that this preparation will prove less dangerous for humans than other rabies vaccines of animal brain origin.

Administration to guinea pigs of the vaccine in doses corresponding to those used for immunization of humans was accompanied by intensive antibody formation. Injection of heterologous (horse) anti-rabies immunoglobulin somewhat inhibited antibody formation in the first weeks. Reports by various authors on the effect of the administration of anti-rabies immunoglobulin on the formation of active immunity are ambiguous. For example, Kuwert (1977) observed an inhibitory effect of homologous immunoglobulin on antibody formation in volunteers in the first 10 days after onset of active immunization, while Nicholson *et al.* (1979) reported on the absence of interference between passive and active immunization.

When the purified vaccine was tested in volunteers, it produced no generalized reaction; local reactions appeared irregularly and were less marked and of shorter duration than after immunization with Fermi vaccine prepared from whole infected sheep brain tissue. All vaccinees reacted to vaccine administration by intensive antibody formation.

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