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RABIES VACCINE PREPARED FROM THE VIRUS GROWN IN JAPANESE QUAIL EMBRYO CELL CULTURES

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Summary. — Fixed rabies virus strain MNIIVP-74 was grown in Japanese quail embryo cell cultures, concentrated by ultrafiltration and inactivated with β-propiolactone. The resulting vaccine was markedly antigenic and immunogenic for laboratory animals. Human volunteers injected with 2.0 ml vaccine on days 0, 3, 7, 14, 30 and 90 exhibited more intensive and longer antibody production than those injected daily for 14 days.

Key words: rabies vaccine; Japanese quail embryo cell cultures; immunogenicity in human volunteers

Introduction

The rabies vaccines prepared from brains of infected animals or duck embryo tissues and widely used in the world are not completely safe. Sometimes they cause severe allergic complications mostly of nervous system sometimes ending in death. According to Kuwert (1977) the Hempt vaccine which is a suspension of infected adult rabbit brain tissue, caused neurological complications in 1 of 1200—1700 vaccinees in F.R.G.; the number of complications after application of the duck embryo vaccine was 1 : 25,000.

The vaccines prepared from the virus grown in cell cultures are certainly more advantageous. They contain much less impurities and therefore are much less allergenic. Besides, he cultural virus is readily concentrated which considerably increases the efficacy of the vaccine. Currently, two cell culture rabies vaccines have passed epidemiological trials and are produced on a large scale: one from the virus grown in human diploid cell culture and inactivated with β-propiolactone (Koprowsky, 1975), and the other from the virus grown in kidney cells of 2-week-old Syrian hamsters and inactivated by ultraviolet irradiation (Morogova *et al.*, 1973). Vaccines grown in kidney cells of bovine embryos (Atanasiu *et al.*, 1974) and dog kidney cells (Van Wezel, van Steenis, 1978) have also been developed.

In the previous paper (Bektemirova *et al.*, 1979) we described the properties of the fixed rabies virus, MNIIVP-74 strain, adapted to Japanese quail embryo (JQE) cell cultures. Here we describe the preparation of a killed concentrated rabies vaccine from this strain.

Materials and Methods

Viruses. The MNIIVP-74 strain of fixed rabies virus is the Pasteur strain adapted to Japanese quail embryo cell cultures (Bektemirova *et al.*, 1979).

The CVS strain of fixed rabies virus was used as a 10% suspension of infected mouse brain tissue.

The infectivity of viruses was determined by titrations in adult mice weighing 7—10 g. Ten-fold dilutions were prepared in distilled water with 2% calf serum and inoculated intracerebrally (i.c.) in 0.03 ml volumes. The animals were observed for 14 days. Virus titres were calculated by the method of Reed and Muench.

The MNIIVP-74 virus was grown in JQE cell cultures, concentrated 10—30-fold using Amicon P-100. supplemented with stabilizers (human serum albumin to a final concentration of 0.4% and sucrose to 7.5), inactivated with β-propiolactone at a final concentration of 1 : 4,000 for 4 hr at 22° C and 48 hr at 4—6°C and lyophilized.

Vaccine control for the absence of live virus. The liquid vaccine (prior to lyophilization) was tested by inoculation of mice directly as well as after its passage in cell culture to grow more virus. According to the former method, 10 mice weighing 7—8 g were inoculated i.c. with 0.03 ml vaccine each. Five days later 5 mice were sacrificed, a 10% suspension was prepared from their brains and inoculated i.c. into 10 mice which were observed for 14 days. In the case of death of even one mouse due to clinically confirmed rabies, the vaccine was not used. If mice died without any clinical signs of disease, the vaccine was tested in a double number of animals.

The second method was aimed to increase the volume of the virus preparation. The vaccine in a volume of 20 ml was inoculated into JQE cell culture. After 1 hr contact at 37 °C, 80—100 ml maintenance medium was added and the culture was incubated for 7 days. The culture fluid was tested for virus by i.c. inoculation of 0.03 ml amounts into 10 mice weighing 7—8 g. The animals weighing 7—8 g. The animals were observed for 14 days.

Safety test. The vaccine in 0.5 ml amounts was inoculated subcutaneously (s.c.) into the backs of 10 mice weighing 11—12 g and intraperitoneally (i.p.) into two guinea pigs weighing 250-300 g. The animals were obeserved for 14 days.

Immunogenic potency test. Immunogenic potency of the vaccine was tested by the method of the National Institutes of Health (NIH) USA (Seligman *et al.*, 1966). The lyophilized vaccine was dissolved in distilled water, and mice weighing 12-14 g were inoculated i.p. twice at 7-day intervals with 0.5 ml of the vaccine diluted 1 : 5, 1 : 25, and 1 : 125. Eight days after the second inoculation, the animals were challenged i.c. with CVS virus at a dose of 50—100 LD_{50}. Comparative immunogenicity was determined in comparison with the reference vaccine of the L.A. Tarasevich State Institute of Standardization and Control of Medical Biologics, Moscow, used at the same dose; the preparation potency was 1 IU/nl.

Antigenic potency of the vaccine in animals. Guinea pigs weighing 350—400 g were inoculated i.p. with 0.033 ml per kg body weight daily for 14 days followed by booster injections on day 23 and 33, or without them. Some of the animals were given rabies horse gamma globulin at a dose of 290 international units (IU) per kg body weight one day before the beginning of active immunization.

Macaca mulatta monkeys weighing 2.0—2.5 kg were inoculated s.c. with 0.033 ml per kg body weight daily for 6 or 14 days.

Serum antibodies were determined by neutralization tests in mice weighing 7—10 g using CVS virus at a dose of 30—100 mouse i.c. LD_{50}. Mixture of the virus and sera was incubated for 1 hr at 37°C.

Vaccine trials in human volunteers. Human volunteers were inoculated with 2.0 ml vaccine s.c. daily for 14 days or 6 times on days 0, 3, 7 and 14 with booster injections on days 30 and 90. Serum-neutralizing antibodies were determined on days 0, 7, 14, 30, 45, 60, 90 and 140 by mouse neutralization tests as described above.

Neuroallergenic activity of the preparation was determined according to Gispen *et al.* (1973).

The content of the protein in the preparation was determined by the method of Lowry *et al.*, (1951).

Results

The infectious titre of the MNIIVP-74 virus grown in JQE cell cultures was 5.75—6.75 LD_{50}/ml. Virus concentration 10- to 30-fold resulted in a rise of its titre by 1.0—1.25 log LD_{50}ml. The content of the protein prior to

Table 1. Comparative immunogenicity of the vaccine tested by the NIH method*

Lot No.	After lyophilization		After 14 days incubation at 37 °C	
	Comparative immunogenicity of the vaccine	CVS strain challenge dose (LD_{50})	Comparative immunogenicity of the vaccine	CVS strain challenge dose (LD_{50})
1	3.0	60	3.0	100
2	8.0	60	5.0	100
3	2.5	60	2.0	100
4	3.4	50	3.6	100
5	10.0	50	5.0	100
6	2.2	50	1.6	100

* Explained in Materials and Methods.

addition of the stabilizer (human serum albumin) was less than 150 μg/ml. The regimen of β-propiolactone treatment of the virus used resulted in its reliable inactivation — live virus was not present in the vaccine. The concentrated cell culture-grown vaccine was markedly immunogenic and sufficiently thermostable (Table 1).

The results of tests for the antigenic potency of the vaccine in guinea pigs are presented in Table 2. The conditions of the test were approximated with those of human vaccination: the vaccine dose was determined according to the amount given to man — 2 ml per 60 kg of body weight. On day 21 the animals had sufficiently high antibody titres. Geometric mean titres in sera of 5 animals were 1 : 64 to 1 : 128. Differences in the titres in individual an mals were rather high, within the range of 1 : 64 to 1 : 512. The maximum level of antibody was achieved on day 40—50 (1 : 417—1 : 958) after which it began to decline although on day 80 it was still high (1 : 104—1 : 256).

Table 2. Antigenic potency of the vaccine in guinea pigs

Group No.	γ-globulin dose IU/kg	Vaccine dose, ml/kg	Vaccination regimen (days)	Geometric mean antibody titres							
				0	13*	20	30	40	50	70	80
1	—	0.033	0—13	< 2	64**	84	182	417	632	256	138
2	—	0.033	0—13 23,33	< 2	37	74	158	417	958	315	256
3	290	0.033	0—13	< 2	32	64	223	512	835	275	104
4	290	0.033	0—13 23,33	< 2	54	128	182	958	588	338	194

Note: each group contained 5 animals.
* days after vaccination
** dilution reciprocals

Table 3. Antigenic potency of the vaccine in monkeys injected with 0.033 ml/kg body weight

Group No.	Days of vaccination	Geometric mean antibody titres					
		0	13	20	30	40	50
1	0—5	< 2	69	150	208	388	117
2	0—13	< 2	82	120	194	302	89

For explanations see Table 2.
Note: each group contained 4 animals.

It may be noted that administration of rabies gamma globulin before vaccination exerted no marked effect on antibody production in the animals.

Monkeys were given 0.033 ml/kg body weight of vaccine daily for 6 or 14 days. No difference between these two vaccination regimens were observed either in the time course of antibody production or in their levels. On day 14 antibody titres were 1 : 69 and 1 : 82 and reached the peak titres on day 40 (1 : 388 and 1 : 302) (Table 3).

The vaccine with the comparative immunogenicity index of 2.0 was used to immunize two groups of human volunteers, 11 and 10 subjects, respectively (Table 4).

The volunteers of the first group received the vaccine daily for 14 days. No systemic reactions to the vaccine were observed. Local reactions were limited to erythema of 1.0 and 1.5 cm in diameter at the site of inoculation in two volunteers 7 and 10 days after injection, respectively. The erythemas disappeared within 24 hr. On day 8 all the vaccinees had antibody titres of 1 : 32 to 1 : 34 (mean geometric titre 1 : 9.8). At 30 days the titres were 1 : 104—1 : 835 (mean geometric titre 1 : 338) and at 45 days 1 : 42 to 1 :1024 (mean geometric titre 1 : 256). At 60 and 90 days most of the volunteers still had rather high antibody titres. The second group of volunteers received 4 injections, on day 0, 3, 7 and 14, and booster injections, on day 30 and 90. No systemic or local reactions to vaccination were observed. Eight days after vaccination antibody titres were 1 : 2—1 : 8 (mean geometric titre 1 : 4.3), at 14 days 1 : 91 to 1 : 417 (mean geometric tirre 1 : 239), at 30 days 1 : 208 to 1 : 1270 (mean geometric titre 1 : 722). By days the antibody titres in most volunteers rose even more (up to 1 : 256—1 : 2048), but in two subjects declined to 1 : 256—1 : 338. Nevertheless, antibody titres in volunteers of the second group at 60 days were much higher than in those of the first group. By 90 days the antibody titre decreased, but the booster injection resulted in a significant rise (at 140 days the mean titre was 1 : 256).

Discussion

The data presented above indicate that the MNIIVP-74 rabies virus grown in JQE cell culture gives a sufficiently potent rabies vaccine. This cell system has been in use for many years for measles vaccine production in the U.S.S.R.

Table 4. Antigenic potency of the vaccine in human volunteers

Group No.	No. of volunteers	Age (years)	Vaccine dose (ml)	Immunization schedule (days)	Geometric mean antibody titres 0	8	14	30	45	60	90	140
1	11	20–39	2.0	0–13	< 2	9.8	208	338	256	169	56	n.d.
2	10	24–44	2.0	0, 3, 7, 14, 30, 90	< 2	4.3	239	722	n.d.	779	60	256

Note: n.d. – not done
For explanations see Table 2.

It is much cheaper than human diploid cells used for rabies vaccine manufacture. The advantage of JQE cells over hamster kidney cells lies in the possibility of producing them any time of the year in unlimited amounts.

Treatment of the MNIIVP-74 virus with β-propiolactone gives a completely inactivated vaccine. In the lyophilized form it was quite stable: no decline in titre was observed after 14 days at 37°C or after a year at 4°C. The vaccine was safe in trials in animals. When human volunteers were inoculated, no systemic reactions were observed, while local reactions were limited to erythema at the site of inoculation in some volunteers.

The vaccine was markedly antigenic and immunogenic. Two vaccination regimens were tested in volunteers: a course of 14 daily injections used in many countries and a course proposed by Kuwert (1977) consisting of 4 injections given on day 0, 3, 7 and 14 with two booster injections on day 30 and 90. At 8 days the titre of virus-neutralizing antibody in the second group was slightly lower than in the group given the vaccine on the daily regimen, but subsequently the antibody titres were higher and persisted at high levels for longer periods than after daily vaccinations.

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