Acta virol. 32 : 217 – 226, 1988

SPECIFIC ACTIVITY OF TISSUE CULTURE ANTIRABIC VACCINE RABIVAK-VNUKOVO-32 WITH SHORT INTRAMUSCULAR VACCINATION SCHEDULE

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Received February 24, 1987

Summary. — Tissue culture rabies vaccine has been used for subcutaneous immunization of 158 subjects according to official instructions and also for intramuscular immunization of 128 subjects according to a short schedule with booster inoculations. All 286 subjects were either bitten or contaminated with saliva of rabid animals or animals suspected of having rabies. The 1168 serum samples were tested by neutralization test (NT) in mice, by radial haemolysis (RH) and by indirect haemagglutination (IHA). The highest, earliest and longest active post-vaccination immunity was registered after the most intensive subcutaneous vaccination course at a dose of 5 ml for 25 days with 3 booster inoculations. Subcutaneous inoculation of 3 ml vaccine for 12 days (36 ml) failed to produce a satisfactory elevation of antibody titre. After 2 to 4 booster inoculations, however, a satisfactory level of antibody was observed. The tissue culture vaccine was shown to have good prospects for clinical vaccination by intramuscular route. On intramuscular vaccination at 1.5 ml for 9 days with 6 booster inoculations on days 16, 23, 30, 37, 67 and 97 (initial vaccine volume 45 ml) the mean geometric antibody titres (MGT) reached 93, 160, 322 and 165 on days 30, 60, 90 and 112, respectively. The economically efficient and rapid IHA and RH tests were confirmed to be specific and suitable for titration of antirabies antibody.

Key words: Rabivak-Vnukovo-32; Fermi type vaccine; serologic activity; application of the vaccine; booster inoculations

Introduction

In early seventies when Rabivak-Vnukovo-32 was being adopted into practical health service, its field efficiency and reactogenicity was assessed in strict comparison with the Fermi type vaccine which in that times had been in use for 50 years in the U.S.S.R. Therefore, during the last 12 years,

Rabivak-Vnukovo-32 has been applied subcutaneously on a relatively intensive schedule. During this period 14,390.000 doses of Rabivak have been manufactured. Post-exposure immunization of about 1 million people in the U.S.S.R., G.D.R., Czechoslovakia, Bulgaria as well as in some Asian, African and South American countries provided convincing evidence for field efficiency of this vaccine. At present, attempts are being made to improve both production and application methods of the Rabivak vaccine.

The present paper describes the results of comparative studies on serologic activity of Rabivak-Vnukovo-32 after subcutaneous and intramuscular application according to intensive and short schedules.

Materials and Methods

Vaccine. Commercial vaccines from I. A. Mechnikov Institute of Vaccines and Sera, Ufa, with relative potency (RP) 0.32 to 0.7 (9 batches), 0.77 to 1.3 (4 batches) and 3.2 IU (1 batch) were used. RI was determined by the NIH procedure (volumetric variant based on national reference vaccine) (Seligmann, 1973). The antirabic gamma-globulin (AGG) purchased from Bacterial and Viral Preparations Manufacturers Tomsk had a titre of 1100—1200 ME/ml.

Groups of subjects. The vaccines were tested in Frunze Sanitary-Epidemiological Station. People aged from 11 to 50 years bitten or contaminated with saliva of rabid animals or animals suspected of having rabies were immunized subcutaneously, generally according to instructions officially in operation. Some of them, however, were vaccinated subcutaneously or intramuscularly according to a considerably shortened schedule in line with the program approved by the Committee of Vaccines and Sera of the U.S.S.R. Ministry of Health on January 20, 1982. Vaccine at a dose of 3 ml was dissolved in 1.5 ml of distilled water and injected into the upper external quadrant of the gluteal muscle or dissolved in 3 ml of distilled water and injected into subcutaneous fat of abdominal area. The 5-ml dose of the vaccine was injected at 2.5 ml in the morning and evening.

Sera. Blood sera samples taken from the vein, or in some cases from a finger before immunization and at various times of the vaccination were kept at —20 °C and tested in NT in mice, RH and IHA (Madyarova *et al.*, 1978; Zgurskaya *et al.*, 1984).

Statistical treatment. Virus and antibody titres were determined as LD_{50}/0.03 ml or ED_{50}/0.03 ml according to Reed and Muench (1938). Mean geometric titres (MGT) of antibodies in terms of reciprocal values as well as comparative MGT were calculated by the Student's "t" test and the frequency of coincidences of reaction results was determined according to X^2 criterion for contingency tables 2×2; confidence limits and the frequency of coincidence of antibody titres in NT, IHA and RH were measured according to the binominal distribution formula (Ashmarian and Vorobiev, 1962; Zaks, 1976).

Results

The 1168 serum samples from 286 subjects have been tested in NT in mice, IHA and RH. However, in order not to overburden the description with numerous figures we will first give the MGT of antibodies in NT only, the most suitable technique substantiated by observations over many years. These MGT values will be then compared with IHA and RH findings.

Subcutaneous application

Twenty-four subjects have been immunized using the vaccine batch revealing an average RI activity of 0.58 IU administered at 3 ml doses for 7 days. The MGT of antibodies appeared low. After vaccination at 3 ml doses

Table 1. Titres of virus neutralizing antibodies in subjects subcutaneously vaccinated with Rabivak-Vnukovo-32

Vaccination schedule	Mean RI of the vaccine (IU)	Immune response	Day			
			8th	15th	30th	90th
3 ml for 7 days	0.58	Seroconversion (%)	4.1	85.7	100	24.0
		MGT	10	21.6	19.9	2.2
		Confidence limits		12.32	13.35	1.4—3.2
3 ml for 12 days	0.55	Seroconversion (%)	4.2	85.7	96.2	60.0
		MGT	10	24	37	10
		Confidence limits	—	16—38	26—54	6—17
3 ml for 12 days followed by 2 booster inoculations	0.54	Seroconversion (%)	20.0	88.8	100	100
		MGT	10	32	73	44
		Confidence limits	—	19—54	47—112	29—69
5 ml for 25 days followed by 3 booster inoculations	0.84	Seroconversion (%)	84.2	100	100	100
		MGT	13	93	275	164
		Confidence limits	10—17	52—166	155—467	95—277
AGG 0.25 ml/kg and 5 ml vaccine for 25 days with 3 booster inoculations	1.14	Seroconversion (%)	100	100	100	100
		MGT	16	30	200	224
		Confidence limits	11—25	19—47	117—339	151—331

for 12 days the antibody MGT also appeared unsatisfactory. The RI of the vaccine was 0.55 IU.

The response to booster inoculation was noteworthy. After immunization of 20 subjects (group 1) with the vaccine of mean RI 0.54 IU at a dose of 3 ml for 12 days combined with 2 booster injections on days 22 and 32 of vaccination, MGT of antibodies on days 15, 30 and 90 reached 32, 73 and 44, respectively. On day 30 the level of seroconversions was 100 % (Fig. 1; Table 1). Subjects in group 2 were immunized with the vaccine showing an average RI of 0.45 IU at 3 ml doses for 7 days combined with 2 booster inoculations on days 17 and 27. On day 40 the level of seroconversions appeared to be 100 % in serum samples and MGT of antibodies was 102; the individual lowest titre was 5 and the highest 625. Group 3 consisted of 16 subjects which were immunized with the vaccine of the mean RI of 1.0 IU at 3 ml doses for 5 to 12 days followed by 2 to 4 booster injections at intervals

Table 2. Titres of VN antibodies in humans subcutaneously vaccinated with Rabivak-Vnukovo-32 on a short schedule followed with booster inoculations

Vaccination schedule	Mean RP of vaccine (IU)	Number of vaccinated subjects	Antibody MGT	Mean virus dose (LD_{50})
3 ml for 7 days followed with 2 booster inoculations	0.45	16	102 (47—224)*	25.0
3 ml for 5—12 days followed with 2—4 booster inoculations	1.0	16	549 (268—1148)*	72.0 (36.3—144)*

* Confidence limits

of 10 and 15 days. Two weeks after finishing the vaccination course a 100 % seroconversion was observed; MGT of antibodies was 549 (268-1148), individual lowest titre being 47 and the highest 3125 (Table 2).

Fifteen days after intensive immunization of 19 subjects at a dose of 5 ml for 25 days followed with 3 booster inoculations on days 35, 45 and 60 (mean RI of the vaccine was 0.84 IU) a 100 % seroconversion was registered; MGT of antibodies on days 8, 15, 30 and 90 was 13, 93, 275 and 164, respectively. The individual lowest and highest titres on day 15 were 10 and 1548, respectively. Upon immunization of 32 subjects using AGG in combination with the vaccine of mean RI 1.14 IU a certain delay of antibody production has been observed in the early immunization period. On days 30 through 90, however, antibody MGT was comparable to those in the previous group of subjects immunized with the vaccine alone (Fig. 1 and Table 1).

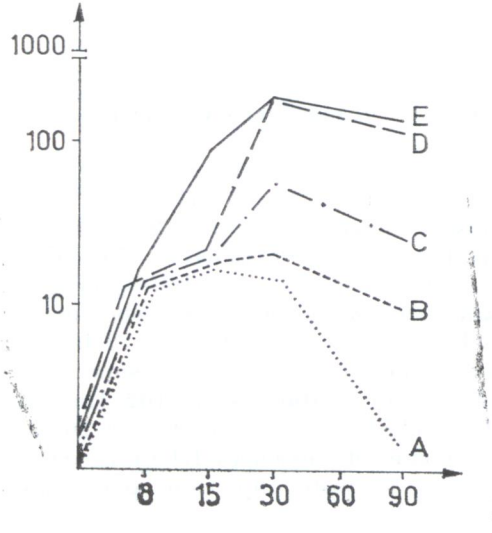

Fig. 1.

Time course of geometric mean antibody titres in subcutaneously vaccinated subjects

A — 3 ml of vaccine for 7 days; B — the same for 12 days; C — the same + 2 booster inoculations; D — 5 ml of vaccine for 25 days + 3 booster inoculations; E — the same + AGG.

Abscissa: vaccination days; ordinate: geometric mean antibody titres (ED_{50}/0.03 ml).

Table 3. Titres of virusneutralizing antibodies in humans intramuscularly vaccinated with Rabivak-Vnukovo-32 on a short schedule

Vaccination schedule	Mean RP of vaccine (IU)	Immune response	Day				
			15th	30th	60th	90th	112th
1.5 ml for 3 days followed with 5 booster inoculations	1.07	Seroconversion (%)	84.8	100	100	100	100
		MGT	8.5	24	38	38	30
		Confidence limits	6—12	17—33	26—53	27—52	21—43
1.5 ml for 6 days followed with 6 booster inoculations	0.85	Seroconversion (%)	77.0	100	100	100	100
		MGT	8	26	107	58	123
		Confidence limits	6—11	19—36	90—177	44—99	78—154
1.5 ml for 9 days followed with 6 booster inoculations	0.85	Seroconversion (%)	100	100	100	100	100
		MGT	39	93	160	322	165
		Confidence limits	32—48	54—103	148—291	153—300	121—238
AGG 0.25 ml/kg and 1.5 ml for 9 days followed with 6 booster inoculations	0.8	Seroconversion (%)	100	100	100	100	100
		MGT	17	18	65	94	111
		Confidence limits	11—26	10—40	40—104	40—171	62—197

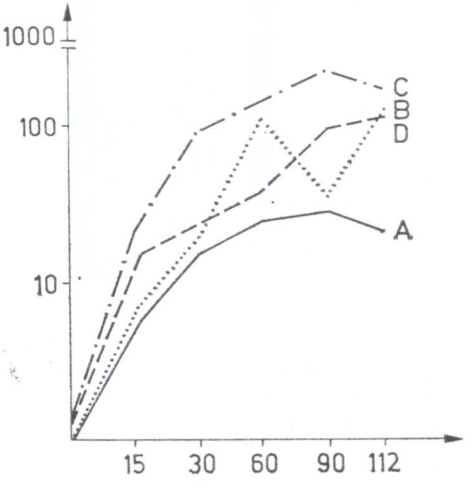

Fig. 2.

Time course of MGT of VN antibodies inintramuscularly vaccinated subjects

A — 1.5 ml of vaccine for 3 days + 5 booster inoculations; B — 1.5 ml of vaccine for 6 days + 6 booster inoculations; C — 1.5 ml of vaccine for 9 days + + 6 booster inoculations; D — the same + AGG.

Abscissa: vaccination days; ordinate geometric mean neutralizing antibody titres (ED_{50}/0.03 ml).

Table 4. Coincidence of IHA and RH results

RH	IHA					
	10	10–20	40–80	160–320	320	Total
<10	138	7	1			146
10–20		141	4	1		146
40–80		2	183	1		186
160–320				65	5	70
≧320				1	14	15
Total	138	150	188	68	19	563

Intramuscular application of the vaccine according to a short schedule

A short course of intramuscular immunization at a dose of 1.5 ml for 3 days followed by 5 booster injections on days 10, 17, 24, 30 and 45 (33 subjects were immunized, the mean RI of the vaccine was 1.0 IU) resulted in a delayed and relatively low antibody production (Fig. 2, Table 3). By increasing the basic immunization course up to 6 daily inoculations at a dose

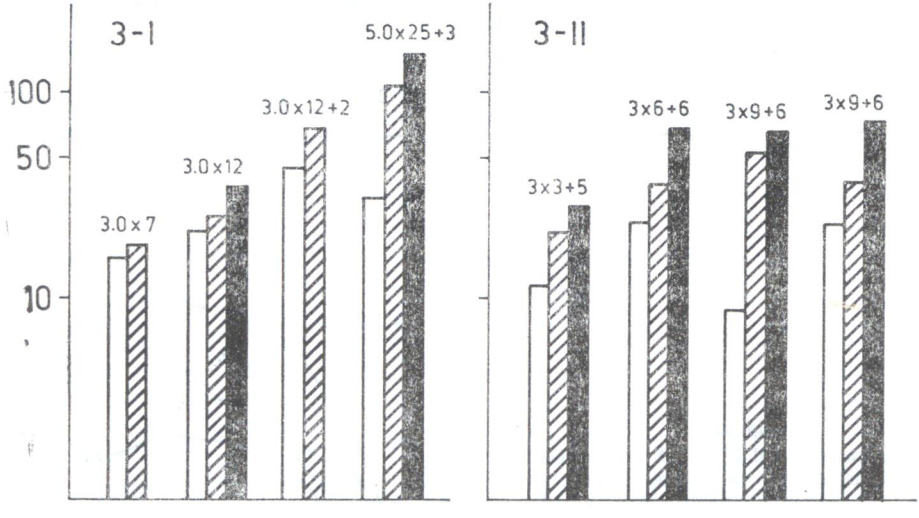

Fig. 3

Comparison of MGT of antibodies and indices of immunogenic activity of the vaccine (serum samples prepared on day vaccination 30). I — subcutaneous application, II — intramuscular application. Ordinates: MGT of HA inhibing antibodies (ED_{50}/0.03 ml). Empty columns — RP of the vaccine from 0.3 to 0.4 IU, shaded columns — RP of vaccine from 0.7 to 0.9, black columns — RP over 1.0 IU.

of 1.5 ml and followed by 6 booster injections, on days 13, 20, 27, 34, 48 and 78 (32 persons, mean RI of the vaccine 0.96 IU) the MGT of antibodies appeared to be relatively good: on days 60, 90 and 112 being 107, 58 and 123, respectively. The individual lowest titre was 5 and the highest amounted 810; a 100 % seroconversion was observed starting from vaccination day 30. The decrease of antibody titres on day 90 is unclear; it is probably related to an irregular neutralization reaction in mice. A marked serologic response was detected upon increasing the basic course up to 9 daily inoculations at a dose of 1.5 ml followed by 6 booster injections on days 16, 23, 30, 37, 67 and 97 (48 subjects immunized the mean RI of the vaccine was 0.85 IU). On day 15 a 100 % seroconversion was registered; MGT of antibodies was 93, 160, 322 and 165 on days 30, 60, 90 and 112, respectively. Combined vaccinations with AGG and vaccine with mean RI of 0.8 IU (15 subjects immunized) was followed by a certain inhibition of vaccinal immunity. This was especially marked from day 15 through 30; MGT of antibodies on days 90 and 112 reached 94 and 111 respectively, which indicates a relatively long-persisting immunity.

Comparative assessment of NT, IHA and RH findings

Altogether 1168 serum samples have been simultaneously tested in NT, IHA and RH. The frequency of coincidence of antibody titres in RH and IHA varied from 92.8 to 98.5 % (Table 4). NT and IHA data at serum dilutions from 10 to 640 coincided in 38 to 90.1 % of cases, and those of NT and RH in 35.2 to 92.8 % of cases. This indicates that economically efficient IHA and RH are specific and suitable for rapid titration of antirabies sera.

Reactogenicity and tolerability of vaccine

Local reactions such as short-lasting and limited erythema, infiltrates, painfulness and itch were registered in 16 % of immunized subjects after subcutaneous application, and in 12.5 % of subjects after intramuscular application. Systemic reactions, such as indisposition, headache, rash and fever were observed in 4.2 to 5.0 % of subjects. After combined immunization with AGG and vaccine, serum complications were registered in 21.8 % of subjects. During intramuscular application generalized adverse reactions were observed in 3.0 to 6.2 % of subjects, and serum complications in 20 % of cases. So, subcutaneous and intramuscular routes did not show any significant differences in terms of reactogenicity or tolerability.

Discussion

The available literature provides no data as to the therapeutic preventive antibody titre, whereas an antibody titre of 0.5 IU is considered effective for pre-exposure immunization, which presumably corresponds to serum dilution 1 : 50 (Lemon *et al.*, 1984). However, seroconversion itself is commonly acknowledged to be indicative of immune rearrangement in the organism and of specific activity of the vaccine. Moreover, individual immune

response to antigen is greatly dependent on the genotype of the macroorganism; our studies provided further convincing evidence for this dependence.

Presented experiments have demonstrated that the antibody level depends on the dose, on total amount of the vaccine administered on the application route, on the number of inoculations and on the intervals between them. The highest, earliest and longest post-vaccination immunity developed after the most intensive subcutaneous vaccination course with a dose of 5 ml for 25 days with 3 booster inoculations (altogether 140 ml vaccine), as well as during immunization with AGG combined with intensive vaccination course; such vaccination course entirely prevented the inhibitory action of passively administered antibodies by days 30 to 90.

Subcutaneous vaccination at a dose of 3 ml for 7 to 12 days failed to produce adequate immunity. The character of serologic response was noticeably changed after booster inoculations of vaccine. Short vaccination schedules with a dose of 3 ml for 7 days with 2 booster inoculations, or 3 ml dose for 5 to 10 days with 2 booster inoculations and the 3 ml dose for 12 days with 2 booster inoculations induced relatively good antibody production (Tables 1 and 2).

Even more promising in terms of efficient application of the Rabivak-Vnukovo-32 seems intramuscular application in a shortened basic course with increasing number of booster inoculations. During this vaccination schedule the highest production of virus-neutralizing antibodies was registered when the basic course with the 1.5 ml dose lasted for 9 days followed with 6 booster inoculations (45 ml initial vaccination volume). The antibody level decreased with the decreasing number of vaccinations and with the amount of the vaccine injected: 1.5 ml for 6 days with 6 booster inoculations and 1.5 ml for 3 days with 5 booster inoculations. A very short 3-day basic course of intramuscular immunization with 5 booster inoculations failed to provide adequate immunity. The extent of primary, basic immunization course seems to be of great importance for efficiency of the vaccination schedule (Gribencha and Selimov, 1972). Subcutaneous immunization of 3 ml vaccine for 7 days with 2 booster inoculations appeared to be more efficient than a 12-days daily course without booster injections. The time course of antibody MGT during subcutaneous immunization at a dose of 3 ml for 12 days with 2 booster inoculations did not differ significantly from that during intramuscular vaccination at a dose of 1.5 ml for 6 days with 6 booster injections although the total quantity of the vaccine was reduced to 36 ml.

Our results confirm the long-established fact of the dependence of vaccination effect on vaccine quantity (Gamaleya, 1956) which is a special case of dose-response relationship. They also provide convincing evidence for the importance of repeated vaccinations, and the booster effect reported by many investigators (Atanasiu *et al.*, 1961; Kovalev *et al.*, 1971). Third, they stress the advantage of intramuscular route when injecting the vaccine into amply innervated araes (Akker, 1938; Anina-Radchenko *et al.*, 1975; Techn. Rep. Ser., No. 709, WHO, Geneva, 1984). It is known that since the very first

trials and after adoption into clinical practice the concentrated tissue culture antirabic vaccines have been applied only intramuscularly (Roumiantzeff *et al.*, 1985; Selimov *et al.*, 1982).

Booster inoculations appeared to be quite suitable for achievement of persistent vaccinal immunity. A certain delay of antibody production during immunization at a dose of 1.5 ml for 6 days followed with 6 booster inoculations is unlikely to produce an undersirable effect during treatment of patients with mild exposure, when the incubation period of hydrophobia is rather long-lasting.

Rabivak-Vnukovo-32 with RP $\geqslant$ 1.0 IU naturally caused a relatively higher antibody production. A correlation has been established between the RI of the vaccine 0.3—0.4, 0.7—0.9 and 1.0 IU or higher, and the MGT of antibodies (Fig. 3). This undoubtedly indicates that the lower RI limit of this vaccine should be raised. Meanwhile, Rabivak-Vnukovo-32 with mean RP of 0.45 IU had a certain, in general specific, activity in this experiment (Table 3). This vaccine with immunogenic activity over 0.3 ME presently applied in the U.S.S.R. according to a rather intensive subcutaneous schedule and in the G.D.R. and Czechoslovakia on a somewhat shorter schedule (Sinnecker *et al.*, 1984; Začková *et al.*, 1984) has a marked field efficiency. Thus, for example, a spot test of the material was carried out in the U.S.S.R. 175,217 vaccinated subjects (15,823 of these were bitten by various wild or domestic animals) and also in 215 subjects with dangerous bites of wolves (rabies diagnosis was confirned by laboratory or clinical data). The vaccinations appeared to be 100 % efficient if performed in conformity with official directions.

The described results seem promising for substantial improvement of Rabivak-Vnukovo-32. In our opinion intramuscular application of this vaccine on the short schedules applied in our study deserves special attention for gradual adoption into clinical practice. Combined inoculations of AGG and vaccine for post-exposure treatment of patients with dangerous bites should be further investigated.

Acknowledgement. The authors wish to thank M. A. Shtykova for technical assistance.

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