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Humoral and Cell-mediated Immune Responses to Various Economical Regimens of Purified Vero Cell Rabies Vaccine

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SUMMARY **Purified Vero cell rabies vaccine (PVRV) is a new effective but inexpensive tissue culture rabies vaccine for human use. We investigated if the cost of immunization with PVRV could be further reduced by intradermal immunization. Fifty-eight subjects with low-risk exposure to rabies were randomized into 4 groups to receive full-dose (0.5 ml) intramuscular injection of PVRV on days 0, 3, 7, 14 and 28 or 4, 2 or 1 intradermal injections of PVRV (0.1 ml) on days 0, 3, and 7, followed by another intradermal injection on day 28. Neutralizing antibodies and specific cell-mediated response (CMIR) were sequentially followed up to day 36. The antibody levels in the intradermal groups increased with the number of injection sites and the levels achieved by the 2-site i.d. regimen were not significantly different form those obtained by the full-dose i.m. even though only 1/3 of the amount of PVRV was used. Specific CMIR occurred I week sooner in the 2 and 4-site i.d. regimens than the full-dose i.m. We therefore recommended that our 2-site i.d. regimen of PVRV should be further tested with a view to substituting it for the more expensive full-dose i.m. regimen in order to further reduce the cost of rabies prophylaxis particularly in the developing countries.**

Vaccination is the mainstay for both pre- and post-exposure prophylaxis against rabies. Human diploid cell rabies vaccine (HDCV) has proved to be one of the most effective and safe rabies vaccines currently available for human use.[5] However, the difficult production process makes HDCV very expensive. This constitutes the main obstacle to the wide use of HDCV in most developing countries where it is more needed.

Purified Vero cell rabies vaccine (PVRV) is a new tissue culture rabies vaccine produced in green monkey kidney cells.[3] It was found to be safe and quite as effective as HDCV in the studies conducted in several parts of the world.[1,7,8] The vaccine can be produced on a large industrial scale using the microcarrier system and a fermentor tank for cell culture[3] resulting in a marked reduction in the production cost. We report here a study of the humoral and cell-mediated immune responses to several reduced-dose intradermal regimens of PVRV as compared to the recommended full-dose intramuscular regimen. The studies indicate that a post-exposure regimen of intradermal PVRV using only one-third of the amount of the vaccine is as immunogenic as the full-dose intramuscular regimen.

MATERIAL AND METHODS

Vaccine

Purified Vero cell rabies vaccine (PVRV), lot No. S 1400, produced by the Institut Merieux (Lyon, France) was used in this study. It had a potency of 5.1 IU/0.5ml dose by NIH test.

Subjects

Fifty-eight patients with low-risk exposure to rabies (*e.g.* bitten by a run-away dog or licked by a rabid dog) who attended the Rabies Clinic of the Queen Saovabha Memorial Institute (The Science Division of the Thai Red Cross Society), Bangkok were included in the study. None of the subjects had been previously immunized with rabies vaccine.

Schedule of immunization

The 58 patients were ran-

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domized into 4 study groups as shown in Table 1. Group 1 received intramuscular injection of 0.5 ml (1 dose) of PVRV in the deltoid areas on days 0, 3, 7, 14 and 28. Group 2 received 4 intradermal injections of PVRV, 0.1 ml each, at both deltoids and anterior thighs on days 0, 3 and 7 (4-site i.d.). Group 3 received 2 intradermal injections of PVRV, 0.1 ml each, at both deltoids on days 0, 3 and 7 (2-site i.d.). Group 4 received 1 intradermal injection of 0.1 ml PVRV at the deltoid areas on days 0, 3 and 7 (1-site i.d.). On day 28, every subject in all intradermal regimens received another intradermal injection of 0.1 ml PVRV.

Titration of neutralizing antibodies

Blood was obtained on days 0, 3, 7, 14, 28 and 35 and sera were kept at −70°C until tested. Antibodies to rabies were determined on coded sera by the rapid immunofluorescent focus inhibition test (RIFFIT) according to the method previously described.[6,9] The antibody levels were expressed as international units per millilitre (IU /ml) with reference to an international standard obtained from Behring Institute. The geometric mean titres (GMT) at each time period for each group were determined. Student's *t*-test was used to calculate the significance of the difference between two GMTs. The value of 0.01 IU/ml was assigned to an undetectable titre for computational purpose since the detection limit in our assay system was 0.02 IU/ml.

Lymphocyte transformation test

The antigen-induced lymphocyte transformation test was used as the measurement of a specific cell-mediated immune response following rabies vaccination. The peripheral blood mononuclear cells were cultured for 5 days in the microtitre plate both in the presence and in the absence of rabies antigen. One hundred μl of HDCV at dilution of 1:5 was used as the stimulating antigen in this study. PVRV at 1:5 dilution was equally effective. The lymphocyte proliferative response was assessed by the uptake of tritiated thymidine by the antigen-stimulated lymphocytes according to the method previously described.[4] The lymphocyte reactivity was expressed as Δ cpm, *i.e.*, the difference between stimulated and unstimulated cultures.

Side-effect of vaccination

At each visit, patients were interviewed and examined for any local and systemic side-effects resulting from the preceding immunization.

RESULTS

Antirabies antibody response

No subject had detectable rabies antibody on day 0 or 3. On day 7, two patients in the 4-site i.d. and two in the 2-site i.d. groups had developed rabies antibody. The antibody levels in the 4-site i.d. group were 0.55 and 4.43 IU/ml whereas those in the 2-site i.d. group were 0.34 and 0.45 IU/ml.

All patients in the study had detectable rabies antibody by day 14. In all but 2 of the 1-site i.d. group, the antibody levels on day 14 exceeded the arbitrary protective level of 0.5 IU/ml. The GMTs of

Table 1 Schedule of immunization

Group	Regimen of PVRV*	No	Male/ Female	Age range (yr)	Dose per site (no. of sites) Days 0	3	7	14	28	Total dose (ml)
1.	Full dose i.m.	15	10/5	19 – 58 (31.2 ± 11.4)†	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	2.5
2.	4-site i.d.	14	10/4	15 – 49 (23.7 ± 8.4)	0.1ml(4)	0.1ml(4)	0.1ml(4)	–	0.1ml(1)	1.3
3.	2-site i.d.	15	6/9	18 – 53 (30.5 ± 12.5)	0.1ml(2)	0.1ml(2)	0.1ml(2)	–	0.1ml(1)	0.7
4.	1-site i.d.	14	7/7	14 – 49 (29.6 ± 10.0)	0.1ml(1)	0.1ml(1)	0.1ml(1)	–	0.1ml(1)	0.4

* PVRV = purified Vero cell rabies vaccine
i.m. = intramuscular
i.d. = intradermal
† Mean ± SD in parenthesis

the 4-site i.d. group appeared highest at all times (Fig. 1). It reached 12.66 IU/ml on day 14. This was significantly higher than the 5.95, 5.79 and 2.76 IU/ml of the full-dose i.m., 2-site i.d. and 1-site i.d., respectively ($P < 0.0005$ in all groups) (Table 2). The GMTs of the 4-site i.d. remained significantly higher than all other groups both on days 28 and 35 ($P < 0.01 - P < 0.0005$).

The GMTs of the 2-site i.d. were not significantly different from the full-dose i.m. either on day 14 or day 28. However, they were significantly lower than the intramuscular group on day 35 ($P < 0.0005$) (Table 2). The GMTs of the 1-site i.d. appeared the lowest of all groups (Fig. 1) but were comparable to the 2-site i.d. on day 35 (Table 2). All of the subjects in the 1-site i.d. group developed antibodies above 0.5 IU/ml from day 28 onwards.

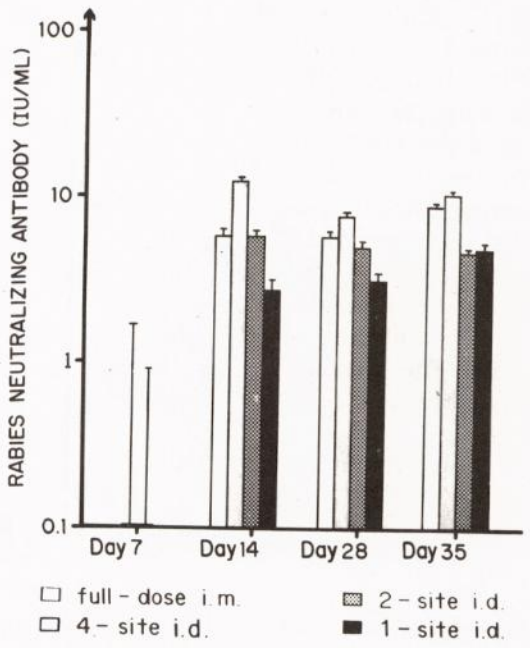

Fig. 1 Neutralizing antibody responses in different regimens of post-exposure purified Vero cell rabies vaccine immunization. Each column respresents the geometric mean titre with bar as SEM.

Cell-mediated immuned response against PVRV

Antigen-stimulated Lymphocyte Transformation Test (LTT) was used to measure the specific cell-mediated immune response (CMIR)

Table 2 Sequential neutralizing antibody response to different regimens of PVRV

Regimen	Geometric mean antibody titre (in IU/ml) ± SEM					
	Days					
	0	3	7	14	28	35
Full dose i.m.	0	0	0	5.95 ± 0.71 (0.60 – 22.40)*	5.95 ± 0.51 (1.60 – 19.00)	8.99 ± 0.38 (4.20 – 20.09)
4-site i.d.	0	0	0.02 ± 1.72 (0 – 4.43)	12.66 ± 0.73 (2.30 – 235.22)	7.76 ± 0.46 (3.20 – 24.92)	10.73 ± 0.46 (3.30 – 40.05)
2-site i.d.	0	0	0.01 ± 0.93 (0 – 0.45)	5.79 ± 0.66 (0.69 – 22.40)	5.07 ± 0.49 (1.12 – 12.10)	4.69 ± 0.72 (0.85 – 25.86)
1-site i.d.	0	0	0	2.76 ± 0.97 (0.19 – 10.36)	3.38 ± 0.75 (0.75 – 20.80)	4.96 ± 0.44 (1.50 – 10.96)

* Antibody ranges

following PVRV immunization. Results are shown in Fig. 2. None of the patients had any lymphocyte stimulation prior to immunization (day 0). The antigen-stimulated tritiated thymidine uptake in the group with full-dose i.m. was first evident on day 14 which was also the peak response in this group. However, for the 4-site and 2-site i.d., LTT was first evident as early as 7 days after starting immunization. The response in these groups peaked at day 14-28. Similar to the full-dose i.m. group, the 1-site i.d. also developed the first evidence of CMIR on day 14. The response peaked at day 28 and became negative again 7 days later inspite of another i.d. injection on day 28 (Fig. 2).

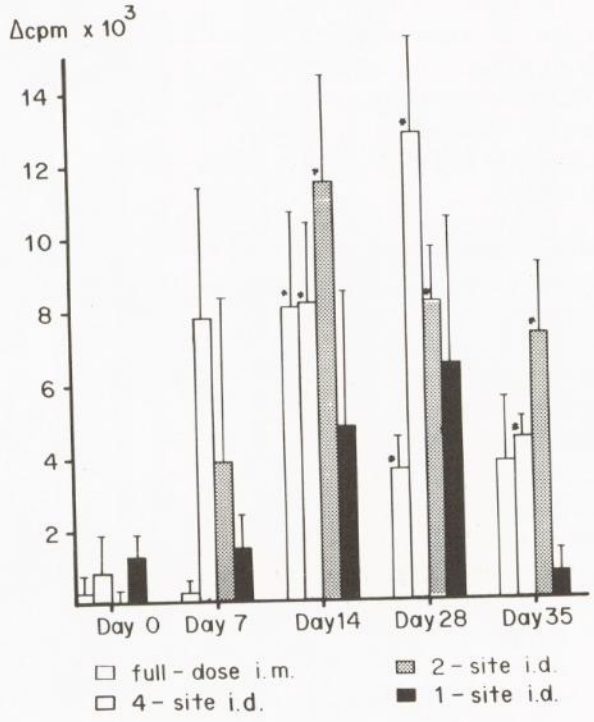

Fig. 2 Kinetics of the development of specific cell-mediated immune response following intramuscular and intradermal immunizations with purified Vero cell rabies vaccine. Each column represents the mean △ cpm (stimulated cpm – unstimulated cmp) with bar as SEM. Asterisks denote significant difference from day 0.

Side-effects

Recipients of intradermal PVRV had, as a group, higher incidence of local inflammatory reaction, itching and lymphadenopathy than those receiving the vaccine intramuscularly (Table 3). The reactions were local, relatively mild and self-limiting after 3-5 days. Other side-effects such as fever, malaise and headache were equally distributed in all groups.

DISCUSSION

Purified Vero cell rabies vaccine (PVRV) is a safe and effective new tissue culture rabies vaccine lower in price than the prototype human diploid cell rabies vaccine (HDCV).[1,7,8] The antibody response in PVRV was comparable to that in HDCV although certain differences existed.[7]

The peak GMT of the full-dose intramuscular PVRV in our study was 8.99 IU/ml obtained on day 35. This was considerably lower than results obtained by Svjetlicic *et al.*[8] of 26 IU/ml on day 14, by Chadli *et al.*[1] of 11.7 IU/ml on day 30 or

Table 3 Side-effects from various PVRV vaccination regimens

		I.d.		
	I.m. (n = 15)	4-site (n = 14)	2-site (n = 15)	1-site (n = 14)
Febrile reaction	3	0	1	1
Local inflammatory reaction	0	3	3	1
Local itching	0	9	7	7
Lymphadenopathy	1	4	3	3
Headache	1	1	2	1
Malaise	4	2	2	0
Weakness	2	2	2	0
Dizziness	1	1	0	0
Nausea, vomiting	0	0	1	0
Others (one each with palpitation and hand tremor)	0	0	1	1

by Suntharasamai *et al.*[7] of 14.9 IU/ml on day 28. The discrepancies may be due to differences in the patient population, the potency of the vaccine or the assay technique. Unfortunately, we did not have a parallel comparative study with HDCV. However, the peak GMT of the intramuscular HDCV previously reported by our laboratory was 12.96 IU/ml on day 28.[9]

The important finding in our study is that the 2-site i.d. regimen of PVRV was as effective as the intramuscular regimen. It has the advantage over the intramuscular regimen in that only one-third of the amount of PVRV is needed and the patients attend one visit less. More interestingly, our 4-site i.d. gave the highest antibody response although only half of the vaccine was needed compared to the full-dose i.m. and again the patient made one visit less. The antibody titres of the 1-site i.d. were generally lower than the other groups and could not be recommended for post-exposure vaccination since 2 out of the 14 subjects developed lower than 0.5 IU/ml neutralizing antibody on day 14. Nevertheless, the antibody response of the 1-site i.d. PVRV in this study was considerably higher than that obtained from Semple vaccine in our previous study.[9]

Our study is the first to investigate the cell-mediated immune response (CMIR) in intramuscular and intradermal immunizations with PVRV. Similar to our previous studies with HDCV,[4] we found that intradermal PVRV (either 4-site or 2 site) induced earlier (about 1 week earlier) and stronger specific CMIR than the full-dose i.m. regimen. One of the reasons is that intradermal immunization is a more efficient route of immunization for T Cell response, probably by acting via the depot effect of the dermis.[2]

Our studies indicate that the reduced-dose 2-site i.d. regimen was as immunogenic as the full-dose i.m. in the induction of neutralizing antibody. In addition, it was more efficient in the induction of specific CMIR. We, therefore, suggest that our 2-site i.d. regimen of PVRV can substitute for the full-dose i.m. in post-exposure rabies prophylaxis, particularly in that it can further reduce the cost of immunization in the developing countries. This will result in the increase in the number of patients who can afford the tissue culture rabies vaccine, thus minimizing the incidence of post-vaccinal neurologic complications. In addition, the 1-site i.d. regimen is, in our experience, superior to immunization with the nervous tissue-derived rabies vaccines and most patients would be able to afford the cost of 1 dose of PVRV to cover the complete course of the 1-site i.d. immunization. The side-effects of intradermal immunization are mainly local, mild and well tolerated.

We stress that our study was a short-term one. A longer follow-up of the antibody titre is certainly needed. It should answer the question as to whether a booster dose on day 90 is necessary.[7] In addition, a long-term and larger-scale follow-up of the protective efficacy of the 2-site i.d. PVRV in high-risk post-exposure group is needed. The suppressive effect if any, of passively administered rabies immune globulin on the antibody response to this 2-site i.d. regimen should also be investigated in the same manner as has been done with intradermal HDCV.[10]

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