THE ABBREVIATED 2 - 1 - 1 SCHEDULE OF PURIFIED CHICK EMBRYO CELL RABIES VACCINATION FOR RABIES POSTEXPOSURE TREATMENT

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Abstract. During August 1988 to January 1990, the immunogenicity and safety of purified chick embryo cell rabies vaccine (PCEC) given by the conventional and abbreviated regimens in 82 vaccinees moderately to severely exposed to laboratory proven rabid animals were studied. The 16 vaccinees received PCEC six doses as conventional schedule on days 0, 3, 7, 14, 28 and 90, the 11 vaccinees received six doses of PCEC plus human rabies immune globulin (HRIG) on day 0. The 29 vaccinees received an abbreviated schedule of PCEC as two doses on day 0, one dose each on days 7 and 21 and the 26 cases received PCEC abbreviated schedule plus HRIG on day 0. The kinetics of the neutralizing antibodies on days 0, 7, 14, 28, 56, 180 and 365 were studied for comparative purpose. All vaccinees had high antibody levels from day 14 which last longer than a year and were safe after one year follow up. The adverse reactions of the vaccine were mild and self-limited.

INTRODUCTION

Purified chick embryo cell (PCEC) rabies vaccine has been introduced in Thailand since October 1985. The conventional six 1 ml doses of PCEC given intramuscularly (im) produce protective antibody response comparable to human diploid cell rabies vaccine (HDCV) (Barth *et al,* 1983, 1984: Bijok *et al,* 1985; Wasi *et al,* 1985, 1986) at a lower cost. However, the expense of the tissue culture rabies vaccine is usually unaffordable by most people in developing countries (Boegel and Mostchwiller, 1986; Wilde *et al,* 1991). Attempts have been made to find the appropriate alternative regimen for economical and compliance reasons. Previous studies demonstrated good antibody responses in subjects who received small doses of vaccines intradermally (Warrell *et al,* 1983, 1984; Harverson and Wasi, 1984; Suntharasamai *et al,* 1987; Nicholson *et al,* 1985, 1987; Trongkamolchai *et al,* 1991; Tanterdtham *et al,* 1991). The abbreviated schedule (2 - 1 - 1) of rabies vaccine immunization by given two injections of full doses intramuscularly on the first day and followed by one dose each on days 7 and 21 were reported to elicit earlier and higher antibody response than the conventional regimen (Vodopija *et al,* 1985, 1986, 1988).

In this report, the effectiveness and tolerability of PCEC rabies vaccine given as abbreviated schedule of 2 - 1 - 1 was compared to the conventional 6 dosses schedule as a primary vaccination.

MATHERIALS AND METHODS

Subjects

The 82 vaccinees were recruited from the patients who visited Siriraj Hospital during August 1988 to January 1990. All were exposed to laboratory proven rabid animals at a moderate to a severe degree. They were 37 females and 45 males with age range from 1 - 16 years (Table 1). At the begining of this study, all subjects were healthy and had no underlying disease. Appropriate local wound treatments were emphasized and given.

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Table 1

Demographic characteristics of each group.

Characteristics	Group 1 (n = 16)	2 (n = 11)	3 (n = 29)	4 (n = 26)
Sex : F/M	9/7	2/9	15/14	11/15
Age (years) :				
Median	19	13	20	22
Range	(1 - 53)	(2 - 25)	(4 - 47)	(2 - 61)

Vaccine and immune globulin

The PCEC vaccine used in this study was lot No. 516053, antigenic value 10 IU/ml and HRIG (Berirab®) were lot No. 004102 and 411030 containing 150 IU/ml supplied by Behring Institute, Marburg, Germany.

Regimen

The PCEC vaccinations were provided within 72 hours after exposure. One milliter of PCEC was injected into the deltoid muscle, at either one or two sites according to the regimen. In the severe exposed cases human rabies immune globulin was also given in a dose of 20 IU/kg by injection around the wound as much as possible and the remaining into gluteal muscles (WHO, 1984).

The vaccinees were assigned into 4 regimens using PCEC vaccine, or PCEC and human rabies immune globulin (HRIG) when indicated (Table 2). In moderate degree exposure, sixteen subjects received PCEC six doses as conventional schedule on days 0, 3, 7, 14, 28 and 90. In severe exposure, eleven subjects received six doses of PCEC plus human rabies immune globulin (HRIG) on day 0. The 29 moderate exposed cases received the abbreviated schedule of PCEC as two doses on day 0, one dose each on days 7 and 21. The 26 severe exposed cases received PCEC abbreviated schedule plus HRIG on day 0.

Neutralizing antibody test

Blood samples were taken from all vaccinees on days 0, 7, 14, 28, 56, 180 and 365. Sera were separated and kept at − 20°C until tested. Neutralizing antibody (NTAb) was determined by rapid fluorescent focus inhibition test (Smith *et al,* 1973). Minimal level of detection in our laboratory was 0.02 IU/ml.

Statistics

Comparison of the antibody levels between groups were assessed by the independent *t*-test with $\log_{10}$ values of titers for normally distributed data and by the Mann-Whitney U test for non-normally distributed data. 95% CI for the efficacy of this regimen was analysed by the exact binomial confidence interval using EPI - PAK version 1.0 (Sullivan, 1990).

Ethical considerations

This study was reviewed and approved by the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine, Siriraj Hospital, Mahidol University.

Table 2

PCEC vaccine regimens.

Group	Regimen	N	Number of sites vaccine given						
			Day0	Day3	Day7	Day14	Day21	Day28	Day90
1	Conventional	16	1	1	1	1	-	1	1
2	Conventional + HRIG	11	1 + HRIG	1	1	1	-	1	1
3	Abbreviate schedule 2-1-1	29	2	-	1	-	1	-	-
4	Abbreviate schedule 2-1-1 + HRIG	26	2 + HRIG	-	1	-	1	-	-

Follw-up

All vaccinees were followed up for at least one year. For vaccine reactions, the signs and symptoms of local and general reactions were recorded by vaccinees and verified by the investigators when the vaccinees came for vaccination or blood collection.

RESULTS

The 82 vaccinees were 45 males and 37 females age between 1 - 61 years (Table 1). They were allocated to 4 treatment groups by their own preference due to the convenient time of the schedules as shown in Table 2. The location of bites and severity were shown in Table 3.

The NTAb levels on days 7, 14, 28, 56, 180 and 365 are shown in Table 4. On day 0, none had rabies NTAb at detectable level, the 100% seroconversion rate was demonstrated on day 7 by all studied regimen. The GMT of vaccinees receiving 6 doses of PCEC as conventional regimen (group 1) was comparable to those who received PCEC as a 2 - 1 - 1 regimen (group 3) as shown is Fig 1. On the contrary the GMT of NTAb in group 4 who received 2 - 1 - 1 plus HRIG on day 0 were significantly higher than the group 2 who received conventional doses plus HRIG. These superior results were demonstrated on days 7, 56 and 365 ($p < 0.01$).

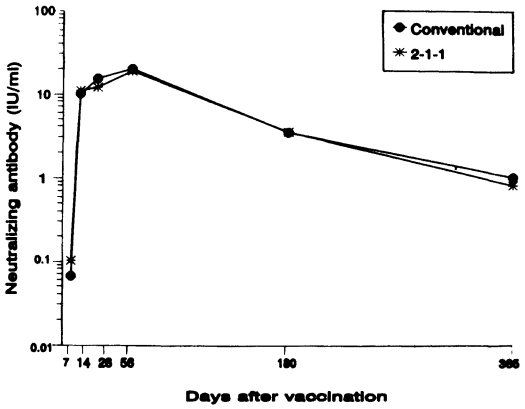

Fig 1—Comparison of the GMTs (IU/ml) of rabies NTAb after PCEC vaccination in conventional 6 doses and 2 - 1 - 1 regimen.

On day 7, NTAb levels in vaccinees receiving HRIG plus PCEC (group 2, 4) were higher than the groups receiving PCEC alone (group 1, 3). This was the result of passive immunization. High antibody levels were seen in all vaccinees on day 14 and onward. Peak NTAb was shown on day 56

Table 3

Location of bites, severity of exposure and expected mortality.

(Hattwick, 1974)

Lacation of bites	No. of patients	Severity of exposure		Expected mortality	
		Moderate*	Severe**	%	Cases
Face	3	-	3	45	1.35
Head	3	-	3	45	1.35
Arms	8	2	6	3	0.24
Hands	19	6	13	15	2.85
Fingers	9	2	7	15	1.35
Legs	14	3	11	3	0.42
Feet	14	3	11	3	0.42
Multiple	12	5	7	15	1.80
Total	82	21	61	3 - 45	9.78

* One biting cut wound at any part of the body, except head.

** More than one biting cut wound and licking of mucosa, or one bite involving face, head, finger, or neck.

then decline. At a year, all vaccinees still had satisfactory level of NTAb (range 0.56 - 1.26 IU/ml).

Although high levels of antibody were observed in all groups, the suppressive effect of HRIG was demonstrated on day 14 (group 1 vs group 2, $p < 0.05$ and group 3 vs group 4, $p < 0.05$). After day 28, NTAb levels in all groups were comparable with no suppressive effect (Fig 3, 4).

All exposed vaccinees were in a healthy state, no rabies case occurred when followed up for at least 1 year. On the basis of historic controls (Hattwick, 1974), about 10 of the people in this study would have been expected to die of rabies. Compared to these, the efficacy of this regimen was 100% (95% CI = 95.6 − 100%, Sullivan, 1990).

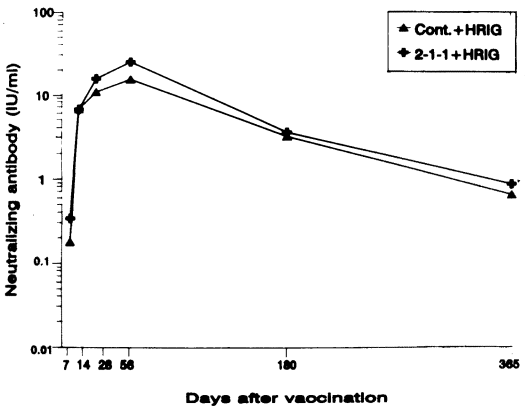

Fig 2—Comparison of the GMTs (IU/ml) of rabies NTAb after HRIG and PCEC vaccination (Conventional 6 doses and 2 - 1 - 1).

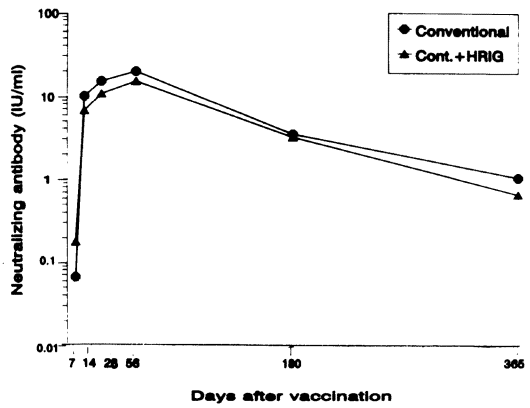

Fig 3—Comparison of the GMTs (IU/ml) of rabies NTAb after PCEC conventional vaccination with and without HRIG.

Only mild pain at infection sites were complaint in few vaccinees, no other adverse reactions were reported.

DISCUSSION

The efficacy of PCEC vaccine given as the abbreviated 2 - 1 - 1 regimen was confirmed in this study. Early antibody response was demonstrated in the vaccinees who initially received double doses of vaccine (Vodopija, 1985). In this study, on day 7, vaccinees who received PCEC two doses alone had higher NTAb than those who received one dose. The group receiving PCEC two doses plus HRIG showed higher level of NTAb (Table 4). Combination of HRIG with PCEC vaccination in either conventional or abbreviated schedule did not show any suppressive effect on the antibody production except on day 14.

All vaccinees had high NTAb levels from day 14 onwards. The NTAb levels in the groups receiving 2 - 1 - 1 were comparable with those receiving the conventional regimen. In severely exposed groups, when HRIG was given on day 0, the 2 - 1 - 1 schedule gave better an antibody response than the conventional schedule. The initial double doses of vaccine are critical to elicit a high and early antibody response. In spite of the reduction in total volume of vaccine used and number of visits to clinic, the 2 - 1 - 1 regimen has shown superior results compared to the conventional schedule, a result, which is also of economic importance.

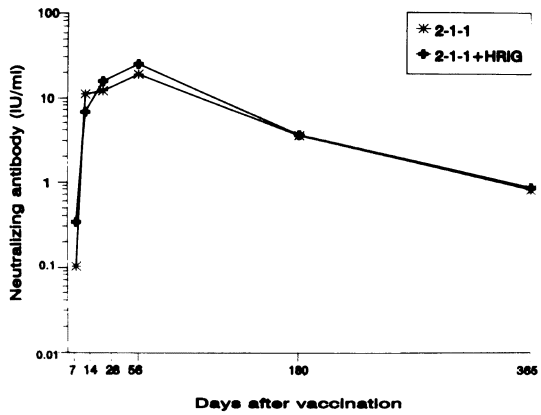

Fig 4—Comparison of the GMTs (IU/ml) of rabies NTAb after 2 - 1 - 1 PCEC vaccination with and without HRIG.

Table 4

Neutralizing rabies antibody (IU/ml) in vaccinees receiving various regimens of PCEC vaccine.

Group	No.	GMT of days					
		7	14	28	56	180	365
1	16	0.07 (0.05 - 0.10)	10.17 (8.33 − 12.43)	15.41 (12.16 − 19.52)	20.07 (15.96 − 25.23)	3.52 (3.06 − 4.05)	1.04 (0.85 − 1.26)
2	11	0.18 (0.15 − 0.22)	6.81 (5.04 − 9.21)	10.87 (8.32 − 14.21)	15.31 (11.93 − 19.64)	3.22 (2.86 − 3.61)	0.66 (0.56 − 0.78)
3	29	0.10 (0.08 − 0.13)	11.8 (8.70 − 14.12)	12.04 (7.66 − 18.92)	18.76 (15.40 − 22.84)	3.55 (3.04 002 4.14)	0.83 (0.69 − 1.00)
4	26	0.34 (0.30 − 0.39)	6.78 (4.89 − 9.40)	15.62 (12.13 − 20.13)	24.76 (20.70 − 29.63)	3.61 (3.28 002 3.97)	0.88 (0.76 − 1.02)

( ) 95% confidence intervals.

The dose of HRIG recommended by WHO in 1984 (WHO, 1984) is generally accepted to be appropriate. Since excessive doses of immune serum may be detrimental (Warrell *et al,* 1985), this suppressive effect was seen only on day 14 but not on the other days.

One study of 2 - 1 - 1 regimen using the purified vero cell rabies vaccines and rabies immune globulin (ERIG) in severely exposed Thai patients showed that the high antibody (> 0.5 IU/ml) persisted in only 50% of cases after a year (Chuti - vongse *et al,* 1991). This might reflect the suppressive effect of ERIG and the lower antigenicity of vaccine used as 3.17 IU/ml in that study compared to 10 IU/ml in this report.

The regimen of 0.1 ml multisite intradermal vaccination with PCEC vaccine resulted in good antibody response and is very economic in centers with many vaccinees within a day, However, there exists a technical problem with intradermal infection, especially in children. Also the use of 0.1 ml of the vaccine per dose may not be economical if the number of vaccinees are too few in the clinic. The 2 - 1 - 1 schedule given as a full dose intramuscularly is more appropriate because it is economic, simple and convenient in clinical practice.

Since early immune response and sustained long lasting immunity to cover the incubation period is the ultimate goal for rabies postexposure prophylaxis, this abbreviated schedule with or without HRIG showed an advantage over the conventional schedule.

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