Clin. exp. Immunol. (1986) **63**, 491–497.

An effective economical intradermal regimen of human diploid cell rabies vaccination for post-exposure treatment

SUKATHIDA UBOL & PRAPHAN PHANUPHAK *Science Division, Thai Red Cross Society (Queen Saovabha Memorial Institute), Bangkok, Thailand*

(Accepted for publication 16 October 1985)

SUMMARY

A closely-spaced multisite intradermal regimen of human diploid cell rabies vaccine (HDCV) was evaluated in 39 patients after low-risk exposure to rabies, in comparison to full-dose intramuscular HDCV and sheep brain-derived rabies (Semple) vaccine. The regimen consisted of four intradermal injections, 0·1 ml each of HDCV on days 0, 3 and 7, followed by two booster doses of only 0·1 ml each on days 28 and 91 administered intradermally. Although the total amount of HDCV used in this intradermal regimen was 1·4 ml or one-quarter of the conventional intramuscular regimen, a higher proportion of the recipients of this economical intradermal regimen, as compared to the full-dose intramuscular regimen, developed neutralizing antibodies above the hypothetical protective level of 0·5 iu/ml 7 days after starting immunization. Besides the earlier antibody response, the peak antibody level of the intradermal regimen was also satisfactorily high and not significantly different from that after the intramuscular regimen. Simultaneous administration of inosiplex, an antiviral and immunopotentiating agent, during the first 10 days of intradermal immunization resulted in an even higher antibody response for as long as 91 days. In contrast, but not unexpectedly, Semple vaccine evoked lower, more sluggish and inconsistent antibody responses. The side-effects of intradermal HDCV were mild, mainly local and self-remitting. We therefore recommend our intensive intradermal regimen of HDCV vaccination for safe, effective and economical use in post-exposure rabies immunization.

Keywords intradermal human diploid cell rabies vaccine immunization

INTRODUCTION

Rabies continues to be a major public health problem in many countries throughout the world. Postexposure vaccination is one of the standard measures recommended for treatment of this disease and antibody titres of 0·5 iu/ml are arbitrarily considered protective (Working Group 2, 1978). Nervous tissue-derived vaccines are generally unacceptable due to low immunogenicity and a high incidence of vaccine-derived complications (Vejjajiva, 1967; Vibulbandhitkij, 1980). However, they are still widely used in many countries such as Thailand and India because of their low production cost. Human diploid cell rabies vaccines (HDCV), is the first tissue-culture rabies vaccine and is more immunogenic and less toxic than the nervous tissue-derived vaccines (Aoki *et al.*, 1975). Because of its high cost, HDCV is rarely used in the countries where it is most needed. This has prompted many investigators to examine the efficacy of reduced doses of the HDCV immunogen administered intradermally. However, of all the intradermal regimens reported, none

Correspondence: Dr Praphan Phanuphak, Science Division, Thai Red Cross Society (Queen Saovabha Memorial Institute), Rama IV Road, Bangkok 10500, Thailand.

was as effective as the conventional intramuscular regimen, particularly for the early antibody response (Warrell *et al.*, 1983; Wasi *et al.*, 1983; Bernard *et al.*, 1982).

The ideal regimen for post-exposure rabies vaccination is that which can induce rapid production of a high level of neutralizing antibodies which are maintained throughout the lengthy incubation period of rabies infection. None of the intradermal regimens studied so far can consistently induce antibody production within 7 days of the initial immunization (Warrell *et al.*, 1983; Wasi *et al.*, 1983; Bernard *et al.*, 1982). We report here comparative studies of an intensive multi-site intradermal regimen of post-exposure immunization, with and without inosiplex, an immunopotentiating agent, with the conventional intramuscular regimen. Our results indicate that an intensive reduced-dose intradermal regimen can result in earlier antibody response in a high proportion of the vaccinees and that simultaneous administration of inosiplex during the first 10 days of the immunization regimen can enhance the antibody response to the level achieved by the full-dose intramuscular regimen.

MATERIAL AND METHODS

Vaccine. The antirabies human diploid cell vaccine (HDCV) produced by L'Institute Merieux, Lyon, France was used. The vaccine was of lot no. X 1121 with a potency of 3·16 iu/ml by NIH test.

The Semple vaccine was a product of the Science Division, Thai Red Cross Society. It was a 5% suspension, lot no. 41 with a potency of 81,000 LD_{50} by Habel test.

Subject. Seventy-six patients with low risk exposure to rabies attending the Rabies Clinic of the Queen Saovabha Memorial Institute, Bangkok (The Science Division of the Thai Red Cross Society) were recruited for the study. They were either licked by a rabid dog or bitten by a run-away dog or by a non rabid dog and all insisted on vaccination. None of the subjects had been previously exposed to rabies or immunized with rabies vaccine.

Schedule of vaccination. The patients were randomly assigned to one of the four study groups as shown in Table 1. Group 1, consisting of 22 subjects, 7 males and 15 females, ranging in age from 11 to 64 ($\bar{x} \pm$ s.d. = 30·6 ± 15·6). They were given HDCV by intramuscular injection of 1·0 ml at one site on days 0, 3, 7, 14, 28 and 91. Group 2, consisting of 19 subjects, 6 males and 13 females with age ranging from 9 to 60 ($\bar{x} \pm$ s.d. = 31·6 ± 14·6), received 0·1 ml HDCV intradermally each at four sites (deltoids and anterior thighs) on days 0, 3 and 7. On days 28 and 91 they received 0·1 ml HDCV intradermally at one site (deltoid) only. Group 3, consisting of 20 individuals, 10 males and 10 females, with age ranging from 14 to 64 ($\bar{x} \pm$ s.d. = 30·7 ± 11·5) received the same intradermal HDCV as group 2 but also received inosiplex (Isoprinosine, Newport Pharmaceuticals, Newport Beach, CA, USA, also marketed in other countries as Modimmunal, Delimmun, Imunovir, Viruxan) orally at 50 mg/kg/day in 3–4 divided doses from day 0 to day 9. Group 4, consisting of 15

Table 1. Schedule of immunization

Regimen*	*n*	Dose per site (number of sites) Day 0	Day 3	Day 7	Day 14	Day 28	Day 91	Total dose (ml)
1. HDCV i.m.	22	1·0 ml	1·0 ml	1·0 ml	1·0 ml	1·0 ml	1·0 ml	6
2. HDCV i.d.	19	0·1 ml (4)	0·1 ml (4)	0·1 ml (4)	–	0·1 ml (1)	0·1 ml (1)	1·4
3. HDCV i.d. + Inosiplex	20	0·1 ml (4)	0·1 ml (4)	0·1 ml (4)	–	0·1 ml (1)	0·1 ml (1)	1·4
4. Semple s.c.	15	immunized on days 0–13, 2 ml/day and booster on days 23, 33 and 103						

*HDCV = human diploid cell rabies vaccine.
i.m. = intramuscular.
i.d. = intradermal.
s.c. = subcutaneous.

subjects, 4 males and 11 females, with age ranging from 17 to 68 ($\bar{x} \pm$ s.d. $= 30 \pm 15{\cdot}5$) received daily subcutaneous injection of 2 ml of Semple vaccine for 14 days and a booster on days 23, 33 and 103.

Blood was obtained on days 0, 7, 14, 28, 91 and 182 and sera were stored at $-20°C$ until tested for antirabies antibody.

Serum antibody titration. Rabies antibody was measured by the rapid immunofluorescent focus inhibition test (RIFFIT) (Smith, Yager & Baer, 1973). Before testing all serum samples were heat-inactivated for 30 min at 56°C. For this test, the constant dilution of the challenge standard strain of rabies virus was mixed with equal volume of two-fold dilution of serum samples. After 90 min incubation at 37°C in CO_2 incubator at 37°C, 100 ml of the serum-virus mixture were pipetted into the compartments of Lab-Tek tissue culture chamber slide (Lab-Tek Products, Division of Miles Laboratory, Inc., Naperville, IL, USA) and 300 μl of 5×10^5 cells/ml of BHK-21/C-13 cells were added. The tissue culture chamber slides were incubated overnight in a controlled humidity, CO_2 incubator at 37°C. The medium was then removed and the cells were washed with phosphate-buffered saline, fixed with acetone at $-20°C$ for 30 min and stained for 20 min with fluorescein-labelled anti-rabies globulin (BBL, Becton). The slides were examined with a fluorescence microscope.

The international standard antiserum (kindly provided by Dr Urich Bijok, Behring Institute, FRG) to rabies virus was titrated with each batch of serum specimens. Antibodies' results are expressed as international units per millilitre.

Statistical analyses. The geometric mean titres (GMT) at each time interval were calculated for each group. The 95% confidence interval was calculated from the s.e.m. by Student's distribution. Student's *t*-test was used to calculate the significance of the difference between two GMTs. The value of 0·1 iu/ml was assigned to an undetectable titre for computational purposes since the lowest limit of the antibody detectable in our assay system was 0·2 iu/ml.

Side effect of vaccination. At each visit patients were interviewed and examined for any local and systemic side-effects that could be related to vaccination.

RESULTS

The antibody titres of all groups are shown in Fig. 1. No subjects had detectable rabies antibody on day 0.

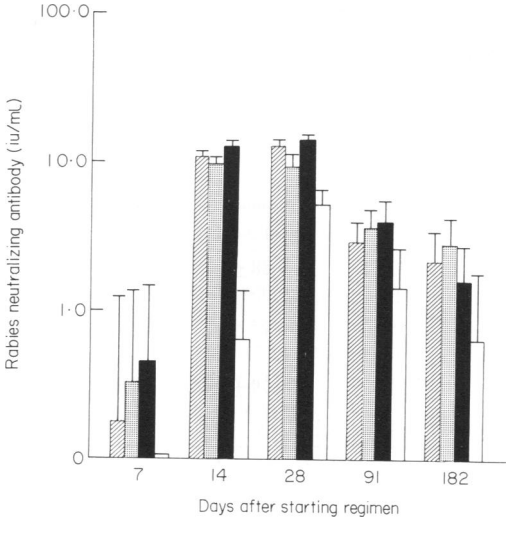

Fig. 1. Neutralizing antibody responses in different regimens of post-exposure rabies immunization. (■) HDCV, intramuscular; (▨) HDCV, intradermal; (■) HDCV, intradermal + inosiplex; (□) Semple.

Group 1 (22 individuals receiving intramuscular HDCV)
By day 7, six of the 22 patients developed detectable antibody levels, and all of these six subjects had antibody exceeding 0·5 iu/ml. The antibody of this group reached the peak on day 28 with a geometric mean titre of 12·96 ± 1·2 iu/ml. All of the patients had antibody higher than 0·5 iu/ml on day 182 with a geometric mean titre of 2·32 ± 1·13 iu/ml.

Group 2 (19 individuals receiving intradermal HDCV)
Ten of the 19 patients developed detectable antibody by day 7. All but one of the 10 individuals had antibody levels higher than 0·5 iu/ml. The peak GMT of this group was 9·88 ± 1·19 iu/ml on day 14. All patients had antibody higher than 0·5 iu/ml on day 182.

Group 3 (20 individuals receiving intradermal HDCV and inosiplex)
Twelve of the 20 patients developed detectable antibody by day 7. All but two of these 12 individuals had antibody levels higher than the protective level of 0·5 iu/ml. The peak antibody level of this group was reached on day 28 (13·50 ± 1·2 iu/ml). The geometric mean antibody titres on days 14 and 91 were 12·25 ± 1·2 and 3·81 ± 1·26 iu/ml respectively. All patients had antibody titres higher than 0·5 iu/ml on day 182 with a geometric mean titre of 1·6 ± 1·2 iu/ml.

Group 4 (15 patients receiving Semple vaccine)
No antibody could be detected until day 14. The antibody levels of six patients on day 14 and three patients on day 91 were lower than the protective level of 0·5 iu/ml. The highest antibody titre was reached by day 28 (5·01 ± 1·18 iu/ml). All patients had antibody titre higher than protective level on day 182.

Table 2 illustrates all the numerical data of the geometric mean antibody titres of each group. The GMTs of both intradermal regimens on day 7 were significantly higher than the intramuscular regimen ($P < 0·05$). The peak GMT of either intradermal regimen were not statistically different from that of the intramuscular regimen. Similarly, although the GMTs of the intradermal HDCV plus inosiplex group appeared higher than those of intradermal HDCV without inosiplex during the first 91 days of immunization, the difference was not statistically significant. However, the GMTs of all HDCV regimens were significantly higher than those of the Semple group at all time intervals ($P < 0·05$) (Table 2).

Table 2. Sequential neutralizing antibody response to different regimens of rabies vaccination

		Day 7	Day 14	Day 28	Day 19	Day 182
Group 1	HDCV i.m. ($n=22$)	0·17 ± 1·2* (0–1·3)‡	10·99 ± 1·2 (1·5–71·60)	12·96 ± 1·2 (1·4–60·73)	2·8 ± 1·2 (0·23–9·2)	2·32 ± 1·13 (1·45–7·5)
Group 2	HDCV i.d. ($n=19$) ($n=19$)	0·32 ± 1·3 (0–1·64)	9·88 ± 1·19 (4·09–48·45)	9·34 ± 1·18 (3·71–30·96)	3·57 ± 1·2 (1·04–10·42)	2·49 ± 1·2 (1·17–4·78)
Group 3	HDCV i.d. + Inosiplex ($n=20$)	0·45 ± 1·2‡ (0–2·74)	12·25 ± 1·2 (2·23–73·34)	13·50 ± 1·2 (2·57–40·77)	3·81 ± 1·26 (1·2–10·6)	1·6 ± 1·2 (0·66–4·31)
Group 4	Semple ($n=15$)	0	0·79 ± 1·04‡ (0·2–4·5)	5·01 ± 1·18‡ (2·27–40·77)	1·41 ± 1·3 (0·3–12·24)	0·63 ± 1·2‡ (1·95–10·52)

* Geometric mean titre (in iu/ml) ± s.e.m., determined by RIFFIT test.
† Antibody ranges.
‡ Significantly different from group 1 ($P < 0·05$).
The rest of the data was not significantly different from group 1 and there was no significant difference between groups 2 and 3.

Table 3. Side-effects from various rabies vaccination regimens

	Group 1 HDCV (i.m.) ($n=22$)	Group 2 HDCV (i.d.) ($n=19$)	Group 3 HDCV (i.d.) +inosiplex ($n=20$)	Group 4 Semple (s.c.) ($n=15$)
Febrile reaction	4	4	3	3
Local inflammatory reaction	0	8	7	12
Local itching	0	4	2	4
Lymphadenopathy	1	4	4	2
Headache	6	0	2	2
Malaise	1	1	0	1
Dizziness	1	0	3	0
Nausea, Vomiting	1	0	2	0
Others	2*	0	0	0

* One case each with weakness and thickened tongue.

Side-effects

Inflammatory reaction and itching at the sites of intradermal injection were the most frequently reported side effects (Table 3). However this reaction was generally mild and self remitting. This was in contrast to the local reaction from Semple vaccine which was generally more severe.

Local lymphadenopathy was associated with the more intense inflammatory reaction at the injection site. It was more pronounced in the inguinal regions than elsewhere and the glands were just palpable and slightly tender. This invariably started on days 4 or 5 of immunization and lasted until day 8 to day 10. Lymphadenopathy did not recur when intradermal injection was repeated on days 28 and 91.

Febrile reaction, headache, malaise, dizziness, nausea and vomiting occurred equally in all vaccination groups (Table 3). The two cases of nausea and vomitting in the inosiplex group could be attributed to the gastrointestinal irritation following inosiplex administration.

DISCUSSION

HDCV is the tissue culture rabies vaccine in most common use at the present time. It has proved safe and very effective but it is expensive. Its high price has stimulated the interest of several groups of investigators to investigate other more economical regimens of HDCV administration, particularly multi-site intradermal regimens (Cox & Schneider, 1976; Ajjan *et al.*, 1980; Lemon *et al.*, 1984). Our regimen of closely spaced multi-site intradermal immunization during the early phase of immunization (days 0, 3 & 7) appears superior to other previously reported intradermal regimens for the induction of early antibody response. Approximately half of the subjects in our regimen developed rabies antibodies reaching the arbitrary protective level ($\geqslant 0{\cdot}5$ iu/ml) by day 7 of immunization whereas this was rarely achieved by other regimens (Warrell *et al.*, 1983; Wasi *et al.*, 1983). We believe that four-site intradermal injection is probably as effective as the eight-site intradermal injection but additional immunization on day 3 should accelerate the antibody response. We are now comparing these different intradermal regimens in a single study protocol in order to confirm our initial findings.

Besides the more rapid antibody response, we found in a parallel study, that our intradermal immunization regimen was more effective than the intramuscular regimen in inducing prompt cell-mediated immune response to the virus as assayed by the the antigen-stimulated lymphocyte transformation test (Ratanavongsiri *et al.*, 1985). In addition, the peak antibody level achieved by

our intradermal regimen was as high as the full-dose intramuscular regimen although only one quarter of the vaccine was needed.

We therefore advocate the use of our intradermal regimen in all cases of post-rabies exposure and particularly for those with moderate income, able to afford two but not the full six doses of HDCV. Such a regimen may not be practical in a centre where only occasional patients are treated with HDCV since the vaccine may lose its potency soon after reconstitution; although we have found that the potency of HDCV remained unchanged up to at least 2 weeks after reconstitution when stored at 4–6°C (K. Trakunchang, personal communication). Furthermore, the degree of susceptibility of this intradermal regimen to the suppression by the concomitant administration of rabies immune globulin should also be tested. We are currently undertaking this investigation in our Institute.

Inosiplex, an antiviral drug with immunopotentiating activity, was found to enhance the antibody response to HDCV somewhat, when given during the first 10 days of intradermal HDCV immunization, although the geometric mean titre was not statistically different from the intradermal group without inosiplex ($P = 0{\cdot}1$–$0{\cdot}2$). The weak immunopotentiating effect of inosiplex when given to immunocompetent individuals (e.g. recipients of various vaccines) requires further evaluation. Its *in vivo* immunopotentiating effect has been described primarily in immunoincompetent patients such as patients with lepromatous leprosy (Saint-Andre *et al.*, 1982) and alopecia areata (Galbraith *et al.*, 1984). If the immunopotentiating effect of inosiplex in immunocompetent individuals is proven then its use as an adjunct is warranted with individuals suffering from a disease where a rapid and high antibody response following active and passive immunization can prevent morbidity and mortality, such as rabies and hepatitis B infection.

The level of antibodies induced by Semple vaccine in various patients were variable, low and slow to develop (Fig. 1). This is in agreement with previous reports (Warrell *et al.*, 1983; Wasi *et al.*, 1983; Trivedi, 1981). Although we did not encounter any vaccine-related neurological complications in our study, it has been estimated from our Institute that of 59,597 individuals who received Semple vaccination between 1961 and 1970, 156 developed neurological complications, a 1 in 400 incidence. It is clear that Semple vaccine should be replaced by other more effective rabies vaccines.

The results of this study, demonstrate that our closely-spaced, multisite, intradermal regimen is an economical regimen that can be recommended. The local reactions resulting from the intradermal injections was generally mild and self remitting. No other serious adverse effects were observed. Our regimen is not only economical and effective, it is also superior to the conventional intramuscular regimen in that early antibody response is achieved.

We wish to acknowledge the helpful advice of Dr David Warrell and Dr Mary Warrell and the support of May & Baker (Thailand) and Pacific Health Care (Thailand). We also wish to thank Miss Maneerat Thaweepathomwat for her excellent help and Dr Bruce Reynolds for his critical review of the manuscript.

REFERENCES

AJJAN, N., SOULEBOT, J.P., TRIAU, R. & BIRON, G. (1980) Intradermal immunization with rabies vaccine, inactivated Wistar strain cultivated in Human Diploid cells. *JAMA.* **244,** 2528.

AOKI, F.Y., TYRRELL, D.A.J., HILL, L.E. & TURNER, G.S. (1975) Immunogenicity and acceptability of a human diploid-cell culture rabies vaccine in volunteers. *Lancet,* **i,** 660.

BERNARD, K.W., ROBERTS, M.A., SUMNER, J., WINKLER, W.G., MALLONEE, J., BAER, G.M. & CHANEY, R. (1982) Human diploid cell rabies vaccine, effectiveness of immunization with small intradermal or subcutaneous doses. *JAMA.* **247,** 1138.

COX, J.H. & SCHNEIDER, L.G. (1976) Prophylactic immunization of human diploid cell culture vaccine. *J. clin. Microbiol.* **3,** 96.

GALBRAITH, G.M.P., THIERS, B.H. & FUDENBERG, H.H. (1984) An open-label trial of immunomodulation therapy with inosiplex (Isoprinosine) in patients with alopecia totalis and cell-mediated immunodeficiency. *J. Am. Acad. Dermatol.* **11,** 224.

LEMON, S.M., MILLER, R.N., PANG, L.W., PRIER, R.E. & BERNARD, K.W. (1984) Failure to achieve predicted antibody responses with intradermal and intramuscular human diploid cell rabies vaccine. *Lancet,* **ii,** 1098.

RATANAVONGSIRI, J., SRIWANTHNA, B., UBOL, S. & PHANUPHAK, P. (1985) Cell-mediated immune response following intradermal immunization with

human diploid cell rabies vaccine. *Asian Pacific J. Allergy Immun.* **3,** 187.

SAINT-ANDRE, P., FERRACCI, P., RIDEL, P.R., BAQUILLION, G. & BONCHER, P. (1982) The use of an immunomodulator, Isoprinosine, in the treatment of multi-bacillary leprosy, preliminary study. *Acta Leprologica,* **86–87,** 113.

SMITH, J.S., YAGER, P.A. & BAER, G.M. (1973) A rapid reproducible test for determining rabies neutralizing antibody. *Bull. WHO Suppl.* **48,** 535.

TRIVEDI, C.R. (1981) Profile of dog bites, rabies and default in antirabic immunization at V.S.G. Hospital, Ahmedabad. *J. Indian Med. Assoc.* **76,** 134.

VEJJAJIVA, A. (1967) Neurological sequelae of antirabies inoculation *J. Med. Assoc. Thailand,* **50,** 806.

VIBULBANDHITKIJ, S. (1980) Work report. Data from the rabies patients at Bamratnaradul Hospital between 1971 and 1977. In: *Rabies* (ed. by P. Thongcharoen) p. 235. Aksarasamai, Bangkok, Thailand.

WARRELL, M.J., WARRELL, D.A., SUNTHARASAMAI, P., VIRAVAN, C., SINHASENI, A., UDOMSAKDI, D., PHANFUNG, R., XUEREF, C., VINCENT-FALQUET, J.-C., NICHOLSON, K.G., BUNNAG, D. & HARINASUTA, T. (1983) An economical regimen of human diploid Cell strain anti-rabies vaccine for post-exposure prophylaxis. *Lancet,* **ii,** 301.

WASI, C., CHAIPRASITHIKUL, P., THONGCHAROEN, P., RUNGPITARUNGSI, V., THARACHAN, C. & LIKANONTSAKUL, S. (1983) Protective antibodies after vaccination with human diploid cell rabies vaccine. *Asian Pacific J. Allergy. Immun.* **1,** 125.

WORKING GROUP 2 (1978) Vaccine potency requirements for reduced immunization schedules and pre-exposure treatment. *Dev. Biol. Stand.* **40,** 268.