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Evaluation of a human diploid cell strain rabies vaccine: final report of a three year study of pre-exposure immunization

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SUMMARY

The antibody responses of 194 volunteers were studied for up to 3 years after primary immunization with one, two or three doses of human diploid cell rabies vaccine, administered either in 0·1 ml volumes intradermally (i.d.) or as 1·0 ml intramuscularly (i.m.). Sero-conversion occurred in 95% of subjects after the first injection and in 100% after the second. The highest titres and most durable antibody responses were induced by three injections of vaccine.

Booster doses were administered either by the subcutaneous (s.c.) or i.d. route, after 6, 12 or 24 months to randomly grouped volunteers; these induced responses $\geqslant$ 5·0 i.u. per ml in 95% of subjects. The responses were rapid and were neither influenced by the primary regimen nor by the timing and route of the booster dose.

Antibody titres after i.d. immunization were only two-fold lower than those induced by the larger volume of vaccine. The findings suggest that the i.d. route is both effective and economic.

INTRODUCTION

For many years the World Health Organization has recommended immunization of persons at risk of exposure to rabies (WHO, 1957). Vaccines produced either in neural or avian tissue are unsatisfactory for this purpose either because they are poorly antigenic and require multiple injections or because they are associated with unacceptable clinical reactions (Miller & Nathanson, 1977; Turner, 1977). However, significant advances in rabies prophylaxis have occurred since the

development of a vaccine derived from rabies virus grown in human diploid cells (Wiktor & Koprowski, 1965). Inactivated human diploid cell strain (HDCS) vaccine has been thoroughly evaluated in the laboratory and the field and its safety, immunogenicity and protective efficacy is now well established (Plotkin & Wiktor, 1978; Bahmanyar *et al.* 1976; Kuwert, Marcus & Hoher, 1976; Anderson *et al.* 1980; MMWR, 1981). Although it is the vaccine of choice whenever available, high production costs unfortunately restrict its widespread application; in the U.K. each 1·0 ml dose costs £18.40 (~ 40 U.S. Dollars).

In 1974, the Merieux Institute kindly donated a quantity of HDCS vaccine to the Medical Research Council. In a preliminary assessment of the vaccine, we reported that it was well tolerated by the intramuscular (i.m.) and intradermal (i.d.) routes and that the antibody response to primary immunization was excellent (Aoki *et al.* 1975). More recently we showed that neutralizing antibody persisted for at least 2 years after three primary injections, and that substantial titre increases occurred after booster inoculations (Nicholson, Turner & Aoki, 1978). In this final report, we summarize the long-term experience gained from the immunization of numerous subjects in attempts to find simple, effective and economic regimens for antirabies prophylaxis.

SUBJECTS AND METHODS

Volunteers. Results were obtained from 194 volunteers who represented more than 90% of the initial study population. All were potentially at risk of exposure to rabies virus, and gave informed consent to immunization. There were 64 females aged 14–61 years (mean 30 years), and 130 males aged 16–68 years (mean 36 years). No volunteer had previously been immunized against rabies.

Vaccine

The vaccine is prepared from the Pitman Moore strain of rabies virus grown in human diploid cells; it is concentrated, inactivated with β-propiolactone and lyophilized. The batches used, had antigenic values of 1·7 and 5·9 (Lot no. S 0203) and 1·6, 3·9 and 1·1 (Lot No. S 0322) when tested for potency at different periods by the 'NIH' method (Seligman, 1973). Vaccine was stored at 4 °C and reconstituted with pyrogen-free distilled water immediately before use.

Study design

Approval for the study was given by Northwick Park Hospital Ethical Committee. Volunteers were allocated to receive one, two or three primary doses of vaccine by the intramuscular (i.m.) route in 1·0 ml volumes or by the intradermal (i.d.) route in 0·1 ml volumes, on days 0, 28 and 56. Vaccine was inoculated into the deltoid region of the left arm. A booster dose of vaccine was given at 6, 12 or 24 months by the i.d. (0·1 ml) or deep subcutaneous (s.c.) (1·0 ml) routes to randomly selected members of each group (Table 1). Blood samples were taken on day 0 and 1, 2, 3, 6, 12, 24 and 36 months; a further sample was taken one month after the booster dose. The rapidity of the booster response was

Table 1. *Distribution of the dose regimens in the volunteers studied*

Primary immunization on	Nos. given no booster inoculation	Nos. given booster inoculation: 6 months i.d.	6 months s.c.	12 months i.d.	12 months s.c.	24 months i.d.	24 months s.c.	Totals
Day 0 only								
i.d.	4	5	5	6	5	4	4	33
i.m.	3	4	4	3	4	2	5	25
Day 0 and 28								
i.d.	3	5	3	7	5	5	5	33
i.m.	1	4	4	4	4	3	4	24
Day 0, 28 and 56								
i.d.	7	—	7	4	5	9	7	39
i.m.	9	—	11	7	6	4	3	40
Total	27	18	34	31	29	27	28	194

examined in five subjects from whom serum samples were taken at 0, 2, 4, 8, 16 as well as 28 days. All volunteers were asked to complete a symptom questionnaire for 10 days after each inoculation, and the injection sites were examined after 48 h by a medical officer.

Titration of serum antibody

1383 sera were tested for virus neutralizing antibodies by the mouse neutralization technique (MNT) (Atanasiu, 1973); the International Standard antirabies serum (WHO International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen, Denmark) was titrated with each batch of sera. Titration endpoints were estimated by the Spearman-Kärber method (Lorenz & Bögel, 1973). Seventy-six sera taken 36 months after immunization were titrated for antirabies IgG using an ELISA technique (Nicholson & Prestage, 1982).

Statistical analysis

Antibody responses to the various regimens were compared by an unpaired *t* test. Booster responses were analyzed with respect to both the route and number of doses used in primary immunization and to the route and timing of the booster doses. For this purpose data from the individual groups were pooled and compared both by titre and by the ratio of mean titre increase.

RESULTS

Antibody response to primary immunization

The geometric mean titre (GMT) of antibody, the range of titres, the proportion of subjects without detectable antibody (< 0·1 i.u./ml) and the proportion with titres less than 0·5 i.u./ml are shown in Table 2.

Immunization on day 0 only. Twenty eight days after immunization, neutralizing

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Table 2. *Antibody titres after primary immunization with HDCS rabies vaccine*

Immunization schedule		Neutralizing antibody (i.u./ml) months after first dose					
		1	2	3	6	12	24
0·1 ml i.d. on day 0	'n'	101	31	—	29	22	9
	GMT	3·4	1·6	—	0·5	0·3	0·4
	Range	< 0·1–32	< 0·1–17	—	< 0·1–8·7	< 0·1–4·6	< 0·1–3·3
	% < 0·1	2	10	—	21	27	33
	% < 0·5	4	19	—	28	41	33
1·0 ml i.m. on day 0	'n'	86	20	—	24	14	7
	GMT	2·8	1·6	—	0·7	0·8	0·9
	Range	< 0·1–44	< 0·1–17	—	< 0·1–8·7	< 0·1–8·7	0·1–4·6
	% < 0·1	3·5	10	—	17	7	0
	% < 0·5	6	20	—	25	29	14
0·1 ml i.d. days 0 and 28	'n'	101	69	—	24	20	8
	GMT	3·4	6·8	—	1·3	0·6	2·6
	Range	< 0·1–32	0·7–83	—	< 0·1–6·3	< 0·1–1·7	0·9–8·7
	% < 0·1	2	0	—	4	10	0
	% < 0·5	4	0	—	12·5	25	0
1·0 ml i.m. days 0 and 28	'n'	86	58	—	21	15	8
	GMT	2·8	14·7	—	6·3	1·7	1·4
	Range	< 0·1–44	0·7–216	—	0·9–32	< 0·1–6·3	< 0·1–6·3
	% < 0·1	3·5	0	—	0	7	12·5
	% < 0·5	6	0	—	0	7	25
0·1 ml i.d. days 0, 28 and 56	'n'	101	69	36	35	28	21
	GMT	3·4	6·8	10·4	3·5	1·5	1·3
	Range	< 0·1–32	0·7–83	2·4–60	0·5–44	0·4–6·3	0·1–12
	% < 0·1	2	0	0	0	0	0
	% < 0·5	4	0	0	0	7	5
1·0 ml i.m. days 0, 28 and 56	'n'	86	58	37	34	24	13
	GMT	2·8	14·7	18·7	6·0	3·4	1·9
	Range	< 0·1–44	0·7–216	0·5–216	0·9–60	< 0·5–31	0·7–41
	% < 0·1	3·5	0	0	0	0	0
	% < 0·5	6	0	0	0	0	0

* '*n*' = the number of sera tested at each time period; GMT, geometric mean titre < 0·1, no detectable antibody.

antibody was present (≥ 0·1 i.u./ml) in 99 of 101 (98 %) persons inoculated i.d. and in 83 of 86 (96·5 %) persons inoculated i.m. There was a higher GMT in the i.d. group (3·4 i.u./ml) than in the i.m. group (2·8 i.u./ml) but the difference was not statistically significant.

The range of the antibody responses was wide (< 0·1—44 i.u./ml); 72 and 78 % of the results differed by less than fourfold from the GMT's for i.m. and i.d. groups respectively. The titres fell rapidly but were similar after both i.m. and i.d. immunization at each of the different time periods; by 6 months 28 % of all vaccinees had titres less than 0·5 i.u./ml, and 19 % had no detectable antibody (< 0·1 i.u./ml).

Immunization on days 0 *and* 28. One hundred per cent of 127 vaccinees had titres of neutralizing antibody greater than 0·5 i.u./ml 28 days after a second injection.

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The range remained wide (0·7 – 216 i.u./ml), but the majority of titres (91 and 76%) again differed by less than fourfold from GMTs of 6·8 and 14·7 i.u./ml for i.d. and i.m. immunization respectively. At 2, 6 and 12 months, the GMTs were 2·2 – 4·8 times higher after immunization by the i.m. route and the differences were statistically significant. The neutralizing antibody persisted for longer and at higher levels after two injections than after one (Table 2). However, at 6 months, 1 of 24 (4%) persons immunized i.d. no longer had detectable antibody; at 12 months, neutralizing antibody was absent from the sera of 2 of 20 (10%) persons inoculated i.d. and 1 of 15 (7%) who were inoculated i.m.

Immunization on days 0, 28 *and* 56. Twenty-eight days after a third injection, the GMTs had increased from 6·8 to 10·4 i.u./ml (1·5-fold), and from 14·7 to 18·7 i.u./ml (1·3-fold) for i.d. and i.m. immunization respectively. The range of the antibody titres was again wide (0·5–216 i.u./ml); 81% of titres after i.d. immunization, and 76% after i.m. immunization, differed by less than fourfold from the GMT. At 3, 6 and 12 months, the GMTs were 1·7–2·3 times higher after immunization by the i.m. route and the differences were stastistically significant. At 24 months, 100% of 34 subjects still had neutralizing antibody. At 36 months, the sera of 5 persons vaccinated, i.d., and 7 of 9 vaccinated i.m., still had antirabies antibody as measured by ELISA.

Antibody response to booster immunization. Sera from a total of 150 persons were taken immediately before and 28 days after re-immunization at 6, 12 and 24 months. A single booster dose resulted in a substantial increase of virus neutralizing antibodies in most subjects; only 2 of 150 persons (1·3%) failed to develop titres greater than 1·0 i.u./ml, and more than 95% had titres greater than 5·0 i.u./ml. Analysis of the aggregated data shows that the route of administration of the primary regimen had no significant effect either upon the titre increase or the titres that were attained, although the antibody levels before re-immunization were significantly lower (~ 2-fold) in persons previously injected by the i.d. route (Table 3A). After one, two or three primary doses, an inverse relationship existed between the pre-immunization titres and the increase in the mean titres which differed significantly from group to group; nevertheless the one-dose regimen was as effective as two- or three-dose regimens when the titres after re-immunization were compared (Table 3B). Antibody titres measured before i.d. and s.c. booster injections were not significantly different, although they tended to be higher in the group subsequently inoculated subcutaneously. While the ratio of the titre increase was higher after re-immunization by the s.c. route the difference was not statistically significant. However, a comparison of the actual titres after re-immunization showed that they were significantly higher (~ 2-fold) after s.c. boosters ($P = \leqslant 0{\cdot}001$) (Table 4A). Analysis revealed no significant differences between the antibody titres of sera taken 28 days after booster injections at 6, 12 or 24 months: however, the ratio of increase was significantly lower in subjects boosted at 6 months (Table 4B) because the titre before boosting was higher.

Rapidity of booster responses. Antibody assays on serial, serum samples taken after booster doses of vaccine showed that responses were rapid. An upward trend in mean titre was apparent after 48 h although one of the subjects appeared to be

Table 3. *Effect of route and dosage of primary immunization on the antibody response measured 28 days after a booster dose*

	No. of subjects	G.M.T. (i.u./ml). Before boost	After boost	Mean titre elevation	Range
(A) Route of primary immunization					
i.d.	81	0·9	33·3	35·5	0·7–416
i.m.	69	1·7	43·5	25·1	3·3–954
(B) No. of primary doses					
1	48	0·4	44·5	105·6	0·7–954
2	46	1·4	49·7	36·6	2·4–389
3	56	2·7	23·8	8·8	0·7–316

Statistical significance of the difference between the pairs of figures bracketed is as follows: * denotes $P = < 0{\cdot}05, > 0{\cdot}01$; ** denotes $P = \leqslant 0{\cdot}01, > 0{\cdot}001$; *** denotes $P = \leqslant 0{\cdot}001$.

Table 4. *Effect of route of administration and timing of booster doses on the antibody response measured 28 days after the booster doses*

	No. of subjects	GMT (i.u./ml) Before boost	After boost	Mean titre elevation	Range
(A) Route of booster dose					
i.d.	72	1·0	25·8	25·8	0·7–219
s.c.	78	1·5	51·0	34·9	0·7–954
(B) Timing of booster dose in months					
6	48	1·6	28·1	17·6	2·4–954
12	51	0·9	43·7	46·4	2·4–416
24	51	1·2	38·8	32·2	0·7–158

Significance of difference: ** denotes $P = \leqslant 0{\cdot}01, > 0{\cdot}001$; *** denotes $P = \leqslant 0{\cdot}001$.

Table 5. *The rapidity of the antibody response to booster doses of vaccine*

	Antibody response (i.u./ml) at days after booster dose							
Subject	0	1	2	4	7	8	16	28
RW	2·4	—	8·7	8·7	—	60	83	8·7
JW	6·3	—	17	32	—	32	60	8·7
BC	0·5	0·5	—	17	30	—	60	17
MP	1·7	8·7	4·6	12	—	—	44	5·2
FA	4·6	6·3	4·0	6·3	—	115	32	43
GMT	2·3	3·0	7·4	13	51		53	12·4
Significance (P)	—	NS	0·05–0·1	0·02–0·05	0·01–0·02		< 0·001	0·05–0·1

GMT, geometric mean titres; NS, not significantly different from pre-boost value.

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a slow responder. Four of the five individuals had statistically significant titre increases by 4 days and after 7–8 days very substantial increases had occurred in all subjects. The mean titre elevation at this time was approximately 23-fold but individual responses showed variations between 5 and 60 times their pre-boost levels. The assays also showed that peak antibody titres probably occurred between 8 and 16 days and declined between 16 and 28 days after the booster dose (Table 5).

Antibody titres at 36 months. At 36 months, the MNT and ELISA test detected antirabies antibody in all of 142 persons who had received one, two or three primary doses and a booster. Analysis showed no statistically significant differences between the titres of neutralizing antibody of groups re-immunized after one or two primary inoculations by the i.d. or i.m. routes. However, as found earlier, the titres were significantly greater ($\sim$ 2-fold) when the booster injection was given s.c.

DISCUSSION

The long-term studies reported here supplement our previous observations on the antibody responses to HDCS vaccine. (Aoki *et al.* 1975; Turner *et al.* 1976; Nicholson & Turner, 1978; Nicholson *et al.* 1978; Nicholson *et al.* 1979). Recent recommendations for pre-exposure immunization against rabies advise that a minimum antibody level of 0·5 i.u./ml should be attained 4 weeks after the last inoculation (WHO/IABS, 1978). In the present investigation 96% of subjects given a single dose of 0·1 ml of vaccine i.d., and 94% given 1·0 ml i.m. had titres equal to, or in excess of, this arbitrary level. However, the titres rapidly declined, and by 6 months 28% of vaccinees had titres less than 0·5 i.u./ml, and 19% had no detectable antibody ($<$ 0·1 i.u./ml), clearly indicating that more than one dose is necessary.

Twenty eight days after a second injection by either the i.m. or i.d. routes, 100% of vaccinees had titres in excess of 0·5 i.u./ml. These results confirm our preliminary observations and agree with those of many similar studies conducted in Europe where two doses of Merieux-HDCS vaccine were given 28 days apart (WHO/IABS, 1978). Although the present study shows that the titres were significantly higher using the i.m. route, the proportion of vaccinees who were without antibody 6–24 months after immunization were similar whether the i.d. or i.m. routes were used and it is questionable whether the significantly higher titres which develop after i.m. immunization are clinically important. Our results suggest that two doses of vaccine given 28 days apart by the i.d. (0·1 ml) or i.m. (1·0 ml) routes are adequate for pre-exposure prophylaxis where the risk of exposure is low. Such categories might include certain veterinary surgeons, workers in quarantine facilities, customs officials, and persons, especially children, living in or visiting countries where rabies is endemic. Our results also suggest that with HDCS vaccine of adequate potency (antigenic value $>$ 2·5) there is little justification for assaying the antibody responses of low-risk subjects (MMWR, 1981*b*). The necessity for frequent booster injections also appears doubtful.

A well monitored and solid immune response is probably more important for

persons working with live rabies virus in research, diagnostic laboratories, and in vaccine production facilities where risks of accidental exposure may be higher (MMWR, 1977). Our study showed that although antibody titres increased by only 1·3 to 1·5-fold for i.m. and i.d. immunization 28 days after a third dose, this latter injection reinforced the humoral response so that antibody was still present in 100% of vaccinees at 2 years and in 86% at 3 years. Nevertheless, a small proportion of vaccinees (5/69, 7%) only had titres in the range 0·5–1·0 i.u./ml 3 months after their third injection. Thus, it is probably necessary to monitor antibody titres of individuals at high risk of exposure at 6-monthly intervals and give booster injections when required. In our experience, a single booster injection generally induced a rapid and marked anamnestic response irrespective of the primary regimen or the timing or route of re-immunization (Rosanoff & Tint, 1979; Simona *et al*, 1979). The rapidity of the booster response is of some significance in previously immunized subjects who are subsequently exposed. Our results indicate that in general, responses begin at 48 h and are substantial 96 h after a single dose. Peak values will probably occur after 8–16 days, well within the average incubation period in human rabies.

However, the small percentage of persons who evidently have slow or inadequate responses must be considered. With exposures not involving the head and neck, two doses of HDCS vaccine, one immediately and one 10–20 days later, would seem appropriate for persons with a clear history of seroconversion (> 0·5 i.u./ml). For severe exposures (multiple deep wounds and head and neck exposures), and in cases of previous inoculation of a vaccine with proven immunogenic value (antigenic value > 2·5) but without determination of neutralizing antibody, we believe that the WHO expert Committee's recommendation of vaccination on days 0, 10, 20 and 90 should be followed (WHO, 1973).

An estimated 80000 persons have been treated with HDCS vaccine throughout the world, but only one has developed the Guillain–Barre syndrome as a possible neurological complication to vaccination (Boe & Nyland, 1980). This observation, and many other detailed clinical studies reported, indicate that anaphylaxis, neuroparalysis, and severe systemic reactions are exceedingly rare side effects of this product.

The possible development of adverse clinical reactions after repeated immunizations by the i.d. route has been the subject of some concern (Cox & Schneider, 1976). However, many hundreds of people throughout the world have received two or more immunizations by the i.d. route (Turner *et al*. 1976; Nicholson & Turner, 1978; Ajjan *et al*. 1980; Klietman *et al*. 1980; Furlong & Lea, 1980), and we know of none who have developed hypersensitivity reactions similar to those described by Cox & Schneider (1976).

This long term report, together with the studies of other workers, provides ample evidence that adequate responses to HDCS vaccine can be achieved much more economically than at present.

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