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Reduced dose pre-exposure primary and booster intradermal rabies vaccination with a Purified Chicken Embryo Cell Vaccine (PCECV) is immunogenic and safe in adults

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Abstract

Background

Pre-exposure vaccination of persons at risk with intradermally administered reduced dose cell culture rabies vaccines remains controversial in low-enzootic countries.

Methods

In a prospective clinical trial of adult volunteers (N=25), we studied the immune response to purified chick embryo rabies cell vaccine (PCECV) administered intradermally at a reduced dose (0.1mL) in a three-dose schedule (0, 7 and 21 days). In 10 subjects, immunogenicity of intradermally administered one-dose booster vaccination with 0.1 mL PCECV was investigated.

Results

All participants were seroconverted 3 weeks after primary and 1 week after booster vaccination, (antibody titre >0.5 EU/mL, measured by enzyme linked immunosorbent assay). Local adverse events such as erythema and swelling were moderate and transitory.

Conclusion

The intradermal vaccination route offers an efficacious and cost-reducing strategy to increase the accessibility of cell culture rabies vaccines.

Introduction

Rabies virus is transmitted through contact of saliva of a rabid animal with a person's mucosa or a skin lesion. Infection results in an encephalitis for which currently no antiviral treatment is available [1]. Because of the almost invariable fatal outcome after infection, medical care facilities in high-enzootic areas and travel clinics in non- or low-enzootic areas focus on prevention by vaccination either before a potential or shortly after a possible exposure. Individuals eligible for vaccination are the exposed population living in or travelling to enzootic areas, or persons who may be exposed to rabies by nature of their occupation [2].

Pre-exposure vaccination, which consists of a three-dose schedule on day 0, 7 and 21 (or 28), induces long-lasting memory, eliminates the need for rabies immunoglobulins (RIG), and reduces the number of days of post-exposure vaccination in case of possible exposure to the virus from five to two.

In areas where high rabies virus transmission occurs, intradermal (i.d.) pre- and post-exposure vaccination against rabies with a reduced vaccine dose is a widely accepted, safe, efficacious and cost-reducing strategy to increase the accessibility of more expensive cell culture rabies vaccines, and to phase out the use of nerve tissue rabies vaccines [3-6]. In travel clinics in non- or low-enzootic countries, pre-exposure rabies vaccination takes up an important and relatively expensive part in the prevention of travel-related diseases. Low-budget long term travellers such as backpackers at risk are more inclined to opt for pre-exposure rabies vaccination if vaccine costs are low.

However, western travel clinics are hesitant to implement the i.d. administration of cell culture rabies vaccine with a tenfold reduced dose for pre-exposure prophylaxis. Several reasons could underlie this reluctance to vaccinate more economically: 1) intramuscular (i.m.) vaccination results in higher antibody titres when compared to i.d. administration, even though it has been shown with several cell culture derived rabies vaccines that antibody titres induced by i.d. vaccination with 1/10th of the i.m. dose reach adequate levels as defined by the World Health Organization (WHO) [2, 3, 7-10], 2) i.d. vaccination is technically more demanding than the i.m. route, thus requiring a more trained staff, 3) i.d. rabies vaccination can induce more local adverse events than i.m. vaccination [7, 8, 11] and 4) not all official advisory institutions agree on the interchangeability of i.d. administration of the different cell culture rabies vaccines; i.e., human diploid cell vaccine (HDCV), purified chick embryo cell vaccine (PCECV), purified duck embryo vaccine (PDEV) and purified Vero cell rabies vaccine (PVRV). The Centre for Disease Control (CDC) for example recommends using only

HDCV for i.d. administration and the WHO advocates the i.d. application of any cell culture rabies vaccine, provided that the country adopting this i.d. regimen repeats immunogenicity studies with the selected vaccine in their own population [12, 13].

In the setting of pre-exposure prophylaxis, we investigated the efficacy and safety of pre-exposure i.d. primary (three-dose schedule of 0.1 mL) and booster (one dose of 0.1 mL) rabies vaccination with PCECV, in an adult population.

Methods

Study design

Travellers of 18 years and older, with an indication for pre-exposure rabies vaccination according to Dutch medical travel guidelines [14] were eligible for inclusion. We excluded volunteers with a compromised immunity due to underlying illness or immunosuppressive medication, travellers taking chloroquine or hydroxychloroquine, pregnant travellers and those allergic to chicken eggs. Written informed consent was obtained from each participant. The protocol and consent forms were approved by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC) (protocol number P05.093), the Netherlands. The study was carried out between August 2005 and July 2007. Vaccinations were administered at the travel clinic of the LUMC by the medical travel consultants who were trained in methods of i.d. vaccine administration.

Subjects received 0.1 mL PCECV i.d. in the dorsal side of the right forearm in a 3-dose schedule (0, 7 and 21 days, one vaccination each time). This site of administration was chosen in order to be able to distinguish between adverse events of i.d. rabies vaccination and other vaccines administered in the deltoid muscle, in case of multiple vaccinations for travel purposes. Additionally, the i.d. vaccination in the dorsal side of the forearm facilitated the monitoring of adverse events by the participants (compared to the deltoid region). The syringe that was used for i.d. administration is identical to the syringe used for administration of tuberculin in the Mantoux test. The quality of the i.d. injection was defined by the diameter of the arisen cutaneous wheal (adapted from the tuberculin skin test), with 6 mm being the lowest acceptable diameter [15]. Booster vaccination consisted of one i.d. vaccination with 0.1 mL PCECV, approximately 1.5 years (range 16 – 20 months) after the primary series.

Rabies vaccine

The PCECV used in this study contained ≥ 2.5 IU/mL of Flury low egg passage (Flury-LEP) rabies strain that was grown in chick embryo fibroblasts, inactivated by β -propionolactone, and purified by density gradient centrifugation (Rabipur®, Novartis Vaccines and Diagnostics GmbH & Co KG, Marburg, Germany). Multiple doses (maximally 8) were obtained from one 1.0 mL vial (0.1 mL per i.d. vaccination). After reconstitution, vials were stored at 4°C and discarded after maximally 8 hours.

Data collection

At the time of inclusion, data on demographic and clinical characteristics of the participants were obtained. Blood samples were collected in all primary vaccinated participants before vaccination (day 0), and 3 weeks after their last vaccination (day 42). Rabies vaccination was offered for free and a financial compensation was given for every blood sample collection at completion of the study.

Participants were asked to document local and systemic symptoms after each vaccination in a diary. In case of swelling at the site of injection the maximum diameter was documented by the participant.

Antibody detection against rabies

Antibody titres against rabies were measured using a commercial in vitro diagnostic ELISA (PLATELIA™ RABIES II kit, Bio-Rad, France) according to manufacturer's instructions. Briefly, a 96-well microplate coated with rabies glycoprotein was used. This viral envelope protein is responsible for the induction of neutralising antibodies [16]. The enzymatic conjugate consisted of a protein A from *Staphylococcus aureus* coupled with peroxidase. Positive controls, which are calibrated against WHO standards, allowed the quantitative determination of anti-rabies antibody titre in the serum, which were expressed as Elisa Units (EU) per mL.

The ELISA PLATELIA™ II rabies test reaches 98.6% sensitivity and 99.4% specificity in comparison to the virus neutralisation assay, the rapid fluorescent focus inhibition test (RFFIT). There is a strong concordance between the two methods as demonstrated by the linearity of the correspondence between titres obtained by PLATELIA™ RABIES II and those by RFFIT in the range 0–4 IU/mL ($r^2 = 0.94$), and the cut-off level of 0.5 EU/mL corresponds to the internationally recommended 0.5 IU/mL threshold [17].

Statistical analysis

Student's t-test was performed to compare geometrical means of antibody titres and occurrence of adverse events after primary and booster vaccination. Correlation

between antibody titres after primary and titres after booster vaccination, and between the occurrence of adverse events and the height of the antibody response were analyzed by Pearson correlation on logarithmically transformed antibody titres. Calculation of the population size was based on a pilot study we performed preceding this study. In order to show immunogenicity in all participants (with $\alpha = 0.05$ and $1 - \beta = 80\%$) expressed as an antibody titre above 0.5 EU/mL, 25 participants were to be included, taking into account a withdrawal of 20%. Statistical analysis was performed using a computer-assisted software package (SPSS version 12.0, SPSS, Inc., Chicago, IL).

Results

Demographical characteristics of study cohort

Twenty five participants with a median age of 25.5 years (range 22-59 yrs) were included to receive the primary i.d. vaccination series. Nine of these primary vaccinated participants were male. Ten participants could be contacted after 1.5 years for the revaccination. Their median age was 24.5 years (range 23-59 yrs) at time of inclusion, and two of these participants were male.

Intradermal vaccination

The mean diameter of the arisen cutaneous wheal measured after vaccination was 8 mm (range 7-10 mm), indicating that all i.d. vaccinations (N=85) were performed correctly according to our standard.

Immunogenicity after primary and booster vaccination

Primary i.d. vaccination with PCECV in a three-dose 0.1 mL regimen induced antibody titres ≥ 0.5 EU/mL in 25/25 participants. Booster vaccination with one dose 0.1 mL PCECV induced protective titres in 10/10 participants (table 1). The geometric mean titre (GMT) after booster vaccination was significantly higher when compared to the GMT following primary vaccination ($p = 0.02$), indicating a good anamnestic response. Half of the boosted participants showed an antibody titre above 30 EU/mL (table 1), which is considered predictive for a longer duration of seroconversion after i.m. vaccination [17].

Correlation between immunogenicity after primary and booster vaccination

The divergent antibody responses to primary and even more to booster vaccination (ranges 2.9 – 52.4 EU/mL and 3.9 – 94.0 EU/mL, respectively), allowed to investigate if a high immunologic response after primary vaccination could predict a high

Table 1 Immunogenicity after (3-dose) i.d. primary and (1-dose) i.d. booster vaccination with PCECV (0.1mL/dose)

Vaccination	Time after vaccination	GMT	Minimum (EU/mL)	Maximum (EU/mL)	95% CI of the GMT	n/N titre > 0.5 EU/mL	n/N titre > 30 EU/mL
Primary	Day 0	0.0	-	-	-	0/25	-
	Day 42	10.7	2.9	52.4	8.3 – 13.1	25/25	4/25
Booster	Day 550	0.9	0.2	2.5	0.0 – 3.4	8/10	0/10
	Day 7 PB	4.8	0.9	19.0	2.1 – 7.5	10/10	0/10
	Day 14 PB	23.9	3.9	94.0	21.2 – 26.6	10/10	5/10

Booster vaccination was performed approximately 1.5 years after primary vaccination (day 550, or day 0 PB). PB = post booster vaccination

response after booster vaccination. However, no correlation was observed (coefficient = 0.2, $p = 0.6$) (data not shown) when logarithmically transformed antibody titres after primary vaccination were plotted against the titres after booster vaccination. This lack of intra-individual consistency as far as the height of the antibody response after vaccination is concerned, is demonstrated by the multiple crossing lines (figure 1).

Safety of primary and booster vaccination

Local erythema and swelling at the site of injection occurred in 96% of participants after primary vaccination and in all subjects after booster vaccination. A trend towards more severe local adverse events was documented after booster vaccination, e.g. a mean diameter of swelling twice the diameter after primary vaccination (table 2).

In addition, we investigated if the severity of adverse events corresponded with the height of the vaccine induced antibody response. For primary as well as for booster vaccination, no correlation was found between the severity of adverse events and the height of the antibody titre (correlation coefficients of 0.06, $p = 0.8$ and 0.2, $p = 0.6$, respectively) (data not shown).

Discussion

Reduced dose intradermal pre-exposure vaccination with PCECV resulted in protective antibody titres in all primary and revaccinated healthy adult volunteers. This finding is consistent with a study performed in children aged 5 to 8 in Thailand by Kamoltham

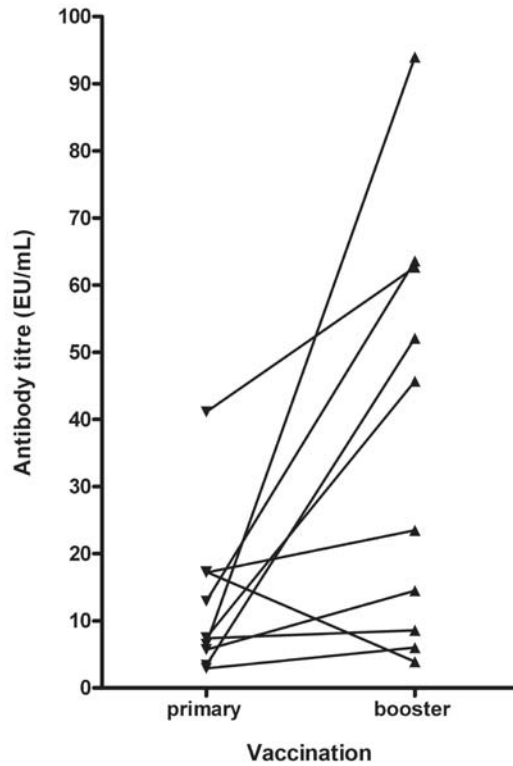


Figure 1 Correlation between antibody response after primary and booster vaccination within participants (N=10)

et al., in which the efficacy of i.d. PCECV vaccination has been shown with a simulated two-dose booster vaccination post-exposure schedule [19].

In addition, the i.d. booster after 1.5 years resulted in an antibody titre above 30 EU/mL in half of the participants, reflecting the possibility of a long-lasting immune response. According to Strady et al. [18] these 'good responders' have an almost 100% probability of staying protected during the following 10 years. This is of importance for those at continuous risk of exposure to rabies virus. Consistent with the population boosted intramuscularly with HDCV or PVRV by Strady et al., i.d. booster with PCECV elicited poor and good responders (figure 1). This dichotomy was

Table 2 Adverse events after i.d. primary and i.d. booster vaccination (day) with PCECV. Lymph node swelling occurred in the ipsilateral axilla

Adverse event			Vaccination preceding adverse events			
			Primary vaccination (day)		Booster vaccination	
			0	7	21	
Local	Erythema	n/N	24/25	24/25	24/25	10/10
		N days (mean)	4.6	5.5	4.3	6.6
		95% CI of mean	3.8 – 5.4	3.9 – 7.1	3.2 – 5.4	5.8 – 6.4
	Swelling	n/N	22/25	20/25	21/25	10/10
		N days (mean)	4.3	4.9	4.3	5.5
		95% CI of mean	3.3 – 5.3	3.1 – 6.7	2.7 – 5.9	3.9 – 7.1
		Diameter (mm)	17.0	15.1	20.3	40.1
		min – max (mm)	0 - 40	0 - 45	0 - 80	5 - 100
		95% CI of mean	11.7 – 22.3	8.5 – 21.6	9.7 – 30.9	18.7 – 61.5
	Pain	n/N	4/25	4/25	6/25	2/10
		N days (mean)	0.5	0.5	0.7	0.6
		95% CI of mean	0.0 – 1.0	0.0 – 1.0	0.2 – 1.2	0.0 – 0.9
Systemic	Pruritus	n/N	12/25	3/10		
		N days (mean)				
		95% CI of mean				
	Myalgia	n/N	2/25	2/25	1/25	1/10
		N days (mean)	0.3	0.3	0.2	0.3
		95% CI of mean	0.0 – 0.7	0.0 – 0.7	0.0 – 0.6	0.0 – 0.9
	Fever	n/N	0/25	1/25	1/25	0/10
		N days (mean)	0.0	0.0	0.1	0.0
		95% CI of mean	-	-	0.0 – 0.3	-
	Lymph node swelling	n/N		2/25		1/10
	Overall severity of adverse events*			+/-	+	+
Range of severity of adverse events*			- to +	- to ++	- to ++	+/- to ++

Severity of adverse events was documented as - (absent), +/- (mild), + (moderate) or ++ (severe), according to participants experience. *Severity concerned local adverse events.

not observed after primary vaccination and may therefore be attributed to a difference in induction of memory after primary vaccination.

The population size of this study was adequate to demonstrate the immunogenicity of primary and booster i.d. vaccination with 0.1 mL PCECV, but insufficient to determine differences between adverse events after primary and booster vaccinations.

Furthermore, no comparison was made with intramuscularly vaccinated participants since i.d. and i.m. rabies vaccinations have previously been compared with other vaccines than PCECV [4, 7-11].

Local adverse events occur more frequently after i.d. than after i.m. vaccination, as has been demonstrated with various vaccines such as influenza [20, 21], yellow fever (unpublished data), and also with rabies vaccine [7, 8, 11]. Because the local adverse events after i.d. PCECV administration may hamper the acceptance of this vaccination route, recipients should be informed on the occurrence of transitory local erythema and swelling. Systemic symptoms such as myalgia and fever have not been reported more frequently after i.d. vaccination with PCECV [7]. In our experience adverse events are seldom a reason for vaccinees to discontinue the i.d. vaccination.

The severity of adverse events was not correlated with the height of the antibody response after primary as well as booster vaccination. Neither does the antibody level after primary vaccination predict the response to booster vaccination, implying that poor or good responders to rabies vaccine cannot be identified until boosted.

Although widely used for Mantoux testing and BCG vaccination, i.d. vaccination is technically more demanding. To ensure correct i.d. vaccination, we introduced a minimal cut-off diameter of the cutaneous wheal following i.d. vaccination. If the wheal does not meet the required diameter; vaccination should be repeated.

In conclusion, the findings of this study have the following practical implications: 1) up to eight times as many individuals can be vaccinated intradermally with 0.1 mL PCECV compared to i.m. vaccination. By clustering travelers who will be at risk of exposure to rabies virus, travel clinics in low enzootic countries can adopt this method of economic pre-exposure vaccination without changing their vaccination schedule 2) introduction of the diameter cut-off for the wheal after i.d. vaccination allows for control of i.d. vaccine delivery, and 3) the cost-saving strategy should further encourage pre-exposure immunisation in high-enzootic countries, where focus on rabies prevention is still mainly on post-exposure prophylaxis.

References

1. Plotkin SA, Rupprecht CE, Koprowski H. Rabies Vaccine. In: Plotkin SA and Orenstein WA, editors. *Vaccines 3rd edition* Philadelphia, W.B. Saunders Company, 1999:743-766.
2. World Health Organization. *Weekly Epidemiological Record*. Rabies Vaccines 2007; 82: 425–436.
3. Turner GS, Nicholson KG, Tyrell DA, Aoki FY. Evaluation of a human diploid cell strain rabies vaccine: final report of a three year study of pre-exposure immunization. *J Hyg (Lond)* 1982; 89:101-110.
4. Warrell MJ, Warrell DA, Suntharasamai P, Viravan C, Sinhaseni A, Udomsakdi D, et al. An economical regimen of human diploid cell strain anti-rabies vaccine for post-exposure prophylaxis. *Lancet* 1983; 2:301-4.
5. Madhusudana SN, Sanjay TV, Mahendra BJ, Sudarshan MK, Narayana DH, Giri A, et al. Comparison of safety and immunogenicity of purified chick embryo cell rabies vaccine (PCECV) and purified vero cell rabies vaccine (PVRV) using the Thai Red Cross intradermal regimen at a dose of 0.1 mL. *Hum Vaccin* 2006; 2:200-4.
6. Quiambao BP, Dimaano EM, Ambas C, Davis R, Banzhoff A, Malerzcyk C. Reducing the cost of post-exposure rabies prophylaxis: efficacy of 0.1 ml PCEC rabies vaccine administered intradermally using the Thai Red Cross post-exposure regimen in patients severely exposed to laboratory-confirmed rabid animals. *Vaccine* 2005; 23:1709-14.
7. Nicholson KG, Farrow PR, Bijok U, Barth R. Pre-exposure studies with purified chick embryo cell culture rabies vaccine and human diploid cell vaccine: serological and clinical responses in man. *Vaccine* 1987; 5:208-10.
8. Dreesen DW, Fishbein DB, Kemp DT, Brown J. Two-year comparative trial on the immunogenicity and adverse effects of purified chick embryo cell rabies vaccine for pre-exposure immunization. *Vaccine* 1989; 7:397-400.
9. Briggs DJ and Schwenke JR. Longevity of rabies antibody titre in recipients of human diploid cell rabies vaccine. *Vaccine* 1992; 10:125-9.
10. Malerzcyk C, Briggs DJ, Dreesden DW, Banzhoff A. Duration of immunity: an anamnestic response 14 years after rabies vaccination with purified chick embryo cell rabies vaccine. *J Travel Med* 2007; 14:63-4.
11. Aoki FY, Tyrell DA, Hill LE. Immunogenicity and acceptability of a human diploid cell culture rabies vaccine in volunteers. *Lancet* 1975; 1:660-662.
12. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *Human Rabies Prevention - United States, 1999*; 48(RR-1):1-21
13. World Health Organization Department of Communicable Diseases Surveillance and Responses. *Current WHO guide for Rabies pre- and post-exposure treatment in humans*. http://www.who.int/rabies/en/WHO_guide_rabies_pre_post_exp_treat_humans.pdf (accessed on 24-08-2007).
14. Landelijk Coördinatiecentrum Reizigersadviesing (LCR). *Dutch national guidelines for traveler's vaccinations and advices*. 2007
15. Center for Disease Control, *Mantoux Tuberculosis Skin Test Facilitator Guide*. <http://www.cdc.gov/tb/pubs/Mantoux/part1.htm> (accessed 3rd of September 2007).

16. Wiktor T, Gyorgy E, Schlumberger H, Sokol F, Koprowski H. Antigenic properties of rabies virus components. *J Immunol* 1973; 110:269-276.
17. Feysaguet M, Dacheux L, Audry L, Compoint A, Morize JL, Blanchard I, et al. Multicenter comparative study of a new ELISA, PLATELIATM RABIES II, for the detection and titration of anti-rabies glycoprotein antibodies and comparison with the rapid fluorescent focus inhibition test (RFFIT) on human samples from vaccinated and non-vaccinated people. *Vaccine* 2007; 25:2244-51.
18. Strady A, Lang J, Lienard M, Blondeau C, Jaussaud R, Plotkin SA. Antibody persistence following preexposure regimens of cell-culture rabies vaccines: 10-year follow-up and proposal for a new booster policy. *J Infect Dis* 1998; 177:1290-5.
19. Kamoltham T, Thinyoung W, Phongchamnaphai P, Phraisuwan P, Khawplod P, Banzhoff A, et al. Pre-exposure rabies vaccination using purified chick embryo cell rabies vaccine intradermally is immunogenic and safe. *J Pediatr* 2007; 151:173-7.
20. Belshe RB, Newman FK, Cannon J, Duane C, Treanor J, van Hoecke C, et al. Serum antibody response after intradermal vaccination against influenza. *N Engl J Med* 2004; 351:2286-2294.
21. Kenney RT, Frech SA, Muenz LR, Villar CP, Glenn GM. Dose sparing with intradermal injection of influenza vaccine. *N Engl J Med* 2004; 351:2295-2301.

