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Reduced dose pre-exposure primary and booster intradermal rabies vaccination with a purified chick embryo cell vaccine (PCECV) is immunogenic and safe in adults

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ABSTRACT

Pre-exposure vaccination of persons at risk with intradermally administered reduced dose cell culture rabies vaccines remains controversial in low-enzootic countries. In a prospective clinical trial of adult volunteers ($N = 25$), we studied the immune response to purified chick embryo cell vaccine (PCECV) administered intradermally at a reduced dose (0.1 mL) 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. All participants were seroconverted 3 weeks after primary and 1 week after booster vaccination (antibody titre $\geq$0.5 EU/mL, measured by enzyme linked immunosorbent assay). Local adverse events such as erythema and swelling were moderate and transitory. The intradermal vaccination route offers an efficacious and cost-reducing strategy to increase the accessibility of cell culture rabies vaccines.

1. 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 Center 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

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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.

2. Methods

2.1. 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 Center (LUMC) (protocol number P05.093), the Netherlands. The study was carried out between August 2005 and July 2007. Vaccinations were 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 three-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.

2.2. 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 eight) 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 h.

2.3. 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.

2.4. 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 neutralizing 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 neutralization 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 PLATELLA™ 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].

2.5. Statistical analysis

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). 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.

3. Results

3.1. Demographical characteristics of study cohort

Twenty-five participants with a median age of 25.5 years (range 22–59 years) 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 years) at time of inclusion, and two of these participants were male.

3.2. 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.

3.3. 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 boostered participants showed an antibody titre above 30 EU/mL (Table 1), which