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An accelerated rabies vaccine schedule based on toll-like receptor 3 (TLR3) agonist PIKA adjuvant augments rabies virus specific antibody and T cell response in healthy adult volunteers

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ABSTRACT

Background: Rabies is a fatal disease where post-exposure prophylaxis (PEP) is crucial in preventing infection. However, deaths even after appropriate PEP, have been reported. The PIKA Rabies vaccine adjuvant is a TLR3 agonist that activates B and T cells leading to a robust immune response.
Methods: We conducted a phase I, open label, randomized study in healthy adults to assess the safety and immunogenicity of the PIKA Rabies vaccine and an accelerated vaccine regimen. Thirty-seven subjects were randomized into 3 groups: control vaccine classic regimen, PIKA vaccine classic regimen and PIKA vaccine accelerated regimen. Subjects were followed up for safety, rabies virus neutralizing antibodies (RVNA) and T cell responses.
Results: Both the control and PIKA Rabies vaccine were well tolerated. All adverse events (AEs) were mild and self-limiting. Seventy-five percent of subjects in the PIKA accelerated regimen achieved a RVNA titer $\geqslant$0.5 IU/mL on day 7, compared to 53.9% in the PIKA classic regimen ($p = 0.411$) and 16.7% in control vaccine classic regimen ($p = 0.012$). The PIKA rabies vaccine elicited multi-specific rabies CD4 mediated T cell response already detectable *ex vivo* at day 7 after vaccination and that was maintained at day 42.
Conclusion: The investigational PIKA rabies vaccine was well tolerated and more immunogenic than the commercially available vaccine in healthy adults.
Clinical trial registry: The study was registered with clinicaltrials.gov NCT02657161.

1. Background

Rabies is a zoonosis, transmitted from animal to human via a contaminated wound with saliva-borne rabies virus. It is a lethal infectious disease [1–5], with an estimated 60,000 deaths annually - 95% of which occurs in Asia and Africa [6]. Disease is preventable by immediate local wound treatment and PEP [3]; occasionally co-administration of rabies immunoglobulins (RIG) is needed [7].

PEP induces antibodies against rabies virus, hence a full course should be administered as soon as possible. The two common PEP regimens are the Essen (four injections administered on days 0, 3, 7, 14) [8] and the Zagreb schedule (two injections administered on day zero and one injection on days 7 and 21 each) [9]. In the Zagreb schedule, antibody titers of 0.5 IU/mL were achieved in all patients by day 14 [10].

Abbreviations: ACIP, Advisory Committee on Immunization Practices; AEs, adverse events; CSIRO, Commonwealth Scientific and Industrial Research Organization; CTCAE, Common Terminology Criteria for Adverse Events; ELISPOT, enzyme-linked immunosorbent spot; FAVN, Fluorescent antibody virus neutralization; GlyRab, rabies glycoprotein; IPRV, Inactivated and Purified Rabies Virus; PBMCs, Peripheral Blood Mononuclear cells; MAbs, monoclonal antibodies; MedDRA, Medical Dictionary for Regulatory Activities; mITT, modified intent-to-treat; PEP, post-exposure prophylaxis; PIKA, Polyinosinic-Polycytidylic Acid Based Adjuvant; PP, per-protocol; RIG, rabies immunoglobulins; RVNA, rabies virus neutralizing antibodies; SAEs, Serious Adverse Events; SFU, spot-forming units; TLR3, Toll-like receptor 3; WHO, World Health Organization.

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However, in spite of PEP, death after vaccination occurs. This is likely due to extremely short incubation periods from severe bites to highly innervated areas like face, neck or hands before a protective immune response can be achieved by vaccination to protect against disease onset and death.[11,12]. Thus, an accelerated vaccine regimen after exposure may potentially improve vaccination efficacy. The PIKA adjuvant is a synthetic analogue of a dsRNA and a refined form of Polyinosinic-Polycytidylic Acid stabilized with kanamycin and calcium [13–15]. As a TLR3 agonist, it activates potent antigen presenting cells like dendritic cells, leading to a more robust adaptive immunity [14,15].

Mice challenged with a lethal rabies dose were fully protected when treated with PIKA vaccine and achieved higher IgM and IgG titers after immunization compared to aluminum adjuvant [16]. Recent studies of infected mice also showed that PIKA rabies vaccine administered using an accelerated regimen enhanced survival in mice from 67.7% to 80% compared to the standard regimen [17].

Thus, in this study we sought to address the compelling medical need for a more potent, rapid and robust post exposure vaccine with a shorter vaccine regimen and higher immunogenicity that would be beneficial. We evaluated the investigational PIKA rabies vaccine for its B and T cell immunogenicity, described its safety profile compared with a commercial rabies vaccine and further tested an accelerated regimen to evaluate its ability to induce earlier and higher titer of RVNA, and T cell mediated immunity in the early stages of rabies infection.

2. Material and methods study design

This was a Phase I, single-centre, open label, randomized study which involved healthy adult subjects aged 21–65 years in Singapore, with no prior history of rabies vaccination and undetectable RVNA at baseline. The study was designed to assess the safety, tolerability and immunogenicity of the PIKA rabies vaccine as well as the immunogenicity of an accelerated vaccine regimen. Study approval was obtained from Singapore Health Sciences Authority (HSA CTC1400532) and Centralized Institutional Review Board (CIRB Ref: 2014/747/F). The study was performed in agreement with the International Conference on Harmonization guidelines on Good Clinical Practices, laws and regulatory requirements in Singapore. Informed consent was obtained from each subject prior to screening. Subjects were first enrolled on 02 February 2015 with the last subject visit on the 23 July 2015.

Forty-four subjects were screened with 37 subjects randomized into three groups at a ratio of 1:1:1; using randomly generated numbers in pre-sealed envelopes. In order to have at least 10 healthy volunteers reaching the primary end point in each of the three groups, it was planned to recruit 12 per group for a total of 36 subjects. This was based on an estimate of 20% dropout rate from the recruited cohort. The RVNA titer levels were batched tested in Commonwealth Scientific and Industrial Research Organization (CSIRO)-Australian Animal Health Laboratory at the end of the last subject visit. Subject disposition in the study is illustrated in Fig. 1a.

Group A received the control vaccine, a commercially available rabies vaccine (Novartis RABIPUR®) (Batch No.: 548011H) containing 5.9 IU/ vial of inactivated Rabies virus), while Group B received the investigational PIKA rabies vaccine (containing 2.0 IU of inactivated Rabies virus). Both groups were vaccinated using the classic 4-dose regimen (1–1–1–1), whilst Group C received the investigational PIKA rabies vaccine with an accelerated regimen (2–2–1). (Fig. 1b)

The primary endpoints were safety and immunogenicity response, induced by the PIKA rabies vaccine. All AEs were captured in the three groups. Safety data was recorded throughout the study, up to day 42. Immunogenicity was measured using the RVNA titer level from serum at Day 0, 7, 14 and 42 after the first vaccination, with a level of at least 0.5 IU/ml according to World Health Organization (WHO) requirement, as evidence for protection.

The secondary endpoints were detectable specific T cell mediated immune response on day 0, 7, 14 and 42 and the RVNA titer on day 0, 7, and 14 and 42 in the accelerated regimen compared to the classic regimen with control vaccine. Rabies-specific T cell response at Day 0, 7, 14 and 42 were tested directly *ex vivo* using ELISPOT assay.

2.1. Subjects and study procedures

Participants 21 to 65 years of age, with satisfactory baseline medical assessment and laboratory values within the normal ranges were eligible. The full list of inclusion and exclusion criteria is shown in supplementary Table 1.

All clinical data were collected on structured case report forms and entered into a secure, web-based database maintained by Clinactis Pte. Ltd., a commercial clinical research organization. Investigators had no access to the database until completion of the trial.

2.2. Vaccine production

The investigational PIKA rabies vaccine was manufactured by Liaoning Yisheng Bio-Pharmaceutical Co., Ltd in Daxiner Village, Cailuo Town, Xinchengzi District, Shenyang in China. These facilities meet the GMP standards for quality assurance and control.

The CTN-1 rabies virus strain was obtained by isolating a strain of wild virus from the brain tissue of a human rabies death case in Shandong, China in 1956 (named CTN-1S), It was passaged in Vero cell lines, which are the most commonly used cell culture for rabies vaccine today [18,19].

The investigational PIKA rabies vaccine is a combination of the Inactivated and Purified Rabies Virus (IPRV) (CTN-1 strain virus antigen that is being produced in Vero cell line) and the PIKA adjuvant combined by mixing in a phosphate buffer solution. The ratio of antigen and adjuvant mix has been optimized to reduce the dose without compromising the potency in pre-clinical animal tests. The final product is in freeze-dried form by adding human albumin as a stabilizer and other excipients, namely maltose and dextran. The formulation of one vial of the freeze-dried PIKA rabies vaccine from a standard volume (1 ml) of the vaccine comprises of 2.0 IU of inactivated purified rabies antigen; 1.0 mg of PIKA adjuvant; 3ug of human albumin; 30ug of maltose and 50ug of dextran.

2.3. Safety assessments

Monitoring for adverse events included a medical assessment within 2 h prior and post vaccination. Systemic and local injection site AEs were documented by study personnel up to 2 h after each injection. All subjects were provided with a patient diary to record all solicited local and systemic AEs and any other unsolicited AEs up to day 42. Pre-specified haematology and biochemistry blood tests and urine analyses were taken at all study visits. (Supplementary Tables 1 and 2)

All AEs were classified according to the Medical Dictionary for Regulatory Activities (MedDRA), version 18 and coded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.