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DNA vaccination for rabies: Evaluation of preclinical safety and toxicology

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ABSTRACT

The worldwide incidence of rabies and high rates of therapy failure, despite availability of effective vaccines indicate the need for timely and improved prophylactic approaches. DNA vaccination based on optimized formulation of lysosome-targeted glycoprotein of the rabies virus provides potential platform for preventing and controlling rabies. As per the pre-clinical requirements, listed in guidelines of Schedule Y, FDA and that of The European Agency for evaluation of Medicinal Products; we evaluated the acute (single dose – 14 days) using three dosing levels, that is, the therapeutic (1×), average (5×) and high dose (10×) intramuscular toxicity in the rodent model Swiss Albino mice. Furthermore, the chronic intramuscular toxicity (repeated dose – 43 days with another 14 days for satellite groups) was investigated using broad dosing levels ranging from low (7×), mid (14×) to high (28×) in Wistar rats. A range of parameters including physical, physiological, clinical, immunological, hematological along with histopathology profiles of target organs was monitored to assess the impact of vaccination. There were no observational adverse effects despite high dose administration of the DNA vaccine formulation. Thus, this study indicates the safety of next generation of vaccines as well as highlights their potential application.

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Introduction

Human rabies is a significant public health concern with approximately 55,000 deaths estimated by the World Health Organization (WHO) every year [1]. The Indian scenario is grave with about 36% of the world's rabies deaths, most of those when children come into contact with infected dogs [2]. Though Rabies virus causes a fatal infectious disease, effective protection may be achieved with immediate administration of rabies immunoglobulin (RIG) followed by rabies vaccination. WHO also recommends pre-exposure prophylaxis for high risk group like veterinarians and animal handlers. However, their availability, requirement of multiple boosters and high cost often leads to failure of therapy. Also, the lack of knowledge on the appropriate treatment of bite wounds contributes towards high incidence of fatal rabies. Dimaano et al. examined the largest cohort of rabies patients reported and observed the majority of patients were from lower socioeconomic groups who lacked knowledge, resources, or both, which compromised their access to appropriate medical care [3]. Development of alternative counter-measures, preferably a single dose of more immunogenic and cost effective rabies vaccine along with awareness on apt treatment post-exposure, is highly desirable under the current scenario. A preventative vaccine used for the immunization of children, especially those in high incidence countries, would be expected to lower fatality rates.

The advancement of recombinant DNA technology has given way to several new vaccination strategies, notably DNA vaccination. Several DNA vaccine candidates are currently under developmental phase. The challenge of preclinical development is to evaluate their safety, pharmacologic, metabolic, and toxicological properties. This also calls for development of standard regulatory guidelines for assessing the candidates. We previously reported the development of a DNA vaccine formulation based on lysosome-targeted glycoprotein along with Emulsigen-D, which conferred complete protection on pre-exposure prophylaxis and post-exposure efficacy analysis [4,5]. This vaccine formulation is

Abbreviations: Ab, antibody; Ig, immunoglobulin; gp, glycoprotein; LAMP-1, lysosomal-associated membrane protein-1.

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first of its kind in India, as it is free of any viral component and thus, averts the various side-effects associated with conventional rabies vaccines. As a pre-requisite for conducting animal and human clinical trials, the present study was undertaken to evaluate the pre-clinical toxicology profile, that is, acute and chronic toxicity test of DNA vaccine formulation, in rodent species based on the Schedule Y guidelines [6].

Materials and methods

As per the regulatory guidelines, the study was designed for acute and chronic toxicity tests using different dose levels in Swiss Albino mice and Wistar rats, respectively. The study was used to evaluate physical, physiological, clinical, haematological and histopathological parameters.

Materials

Test formulations

Rabies DNA vaccine (RDV) was prepared using GMP compliant facilities at School of Biotechnology, JNU. Emulsigen-D (MVP Laboratories, Inc., Omaha, NE, USA) was used as an adjuvant for RDV.

Test dose

For 1× single dose, 125 μg RDV was mixed with adjuvant at 20% and volume was made up to 200 μl in 1× PBS. Various dose groups for acute toxicity were low dose (LD) group containing 125 μg (1×) RDV, mid dose group (MD) containing 625 μg (5×) RDV and high dose (HD) group containing 1250 μg (10×), prepared in a desired volume of PBS, decided according to number of animals in each group. The vaccine control (VC) group was immunized with 1× PBS and the adjuvant control (AC) group was immunized with 20% adjuvant made in 1× PBS.

Various dose groups for repeated dose toxicity were low dose (LD) group containing 875 μg (7×) RDV, Mid dose (MD) group containing 1750 μg (14×) RDV and high dose (HD) group containing 3500 μg (28×), prepared in a desired volume of PBS, decided according to number of animals in each group. 1× PBS was administered to the vaccine control (VC) group and Emulsigen-D with PBS was administered to the adjuvant control (AC) group. Three additional groups were vaccine control satellite (VCS), adjuvant control satellite (ACS) and high dose satellite (HDS) groups, which were administered the same dose as their respective non-satellite groups but were observed for an additional 14 days.

Test species

For acute dose toxicity studies, Swiss Albino mice (*Mus musculus*) aged between 4–6 weeks, males weighing between 24–28 g and females weighing between 20–22 g were obtained from National Centre for Laboratory Animal Sciences, NIN, Hyderabad, India. For repeated dose toxicity, 6 weeks old Wistar rats (*Rattus norvegicus*) with males weighing 170–190 g and females weighing 135–150 g were also procured from NIN, Hyderabad, India. The animals were maintained in animal holding facility in BSL3 laboratory (School of Biotechnology, Jawaharlal Nehru University, Delhi, India) at temperature 21–25 °C and relative humidity of 55–60%. 12-hourly cycle was set and conventional pelleted diet purchased from commercial supplier was provided *ad libitum* to animals along with reverse osmosis grade pure drinking water. For Wistar rats, corn cob was used as the bedding material.

Methods

Test details

All tests were done according to Schedule Y guidelines, Ministry of Health and Family Welfare, Government of India. All experimental procedures were approved by Institutional Animal Ethics Committee, JNU.

Acute dose toxicity study

Male and female Swiss Albino mice were acclimatized for 7 days in animal holding facility in BSL3 laboratory, following which they were randomized and allocated to various groups. Ten mice (5 males and 5 females) of LD, MD and HD groups were administered a single low dose, single mid dose, and single high dose, intramuscularly respectively on day 0 with a dose volume of 100 μl. The adjuvant along with PBS was administrated to the AC group. Only PBS was administered to the VC group. All the mice were observed for 14 days for the test item related toxic signs and symptoms, body weight and mortality.

Repeated dose toxicity study

Male and female Wistar rats were acclimatized for 7 days in animal holding facility in BSL3 laboratory, following which they were randomized and 5 male and 5 female rats were allocated to various groups. All the animals were dosed on the intended dosing schedule i.e., day 0, day 21 and day 42, intramuscularly with a dose volume of 300 μl. All the animals in the study were observed for 43 days, except the satellite groups which were further observed for additional 14 days. The animals were observed for clinical signs of toxicity, mortality, change in body weight, feed consumption, changes in hematological and biochemical parameters. Gross necropsy and histopathology of vital organs was also carried out to rule out any vaccine induced organ toxicity.

Study parameters

Clinical signs of toxicity. In both acute and repeated dose toxicity tests, various general clinical signs, food and water intake, general behaviour and mortality were observed daily. Body weight was observed every alternate day for acute dose toxicity study and weekly for repeated dose toxicity study.

Hematological parameters. In repeated dose toxicity tests, hematological parameters like total leukocyte count (TLC), differential leukocyte count (DLC), red blood cell (RBC) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, packed cell volume (PCV) were determined in blood samples drawn into tubes containing EDTA at various time points, as indicated in the test details and analysed on an automated blood cell counter.

Blood biochemical parameters. In repeated dose toxicity tests, all the mice from each group were bled from retro-orbital plexus at the end-point of study and sera were prepared by standard procedure. Liver function tests (AST, ALT, ALP) and renal function tests were done. Plasma glucose, albumin, globulin, calcium, triglycerides, sodium, potassium, chloride, calcium, phosphorus and total bilirubin were estimated using automated chemistry analyzer.

Gross necropsy and histopathology. In acute dose toxicity test, all the mice were sacrificed on day 14 after completion of experimental period, while in repeated dose toxicity study, all the study animals were sacrificed after completion of 43 days and after additional 14 days for the satellite group animals. An incision was made ventrally on each animal's body to access all vital organs and gross examination of the organs and tissues (viz, brain, adrenal, heart, testes/ovaries, epididymis/uterus, kidneys, liver, lungs, spleen, spinal cord, trachea, thyroid, thymus, stomach, duodenum, jejunum, colon, thigh muscle, lymph node, urinary bladder, prostate and seminal vesicles) were done. Absolute weights of these organs were recorded on electronic weighing balance (Sartorius)

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