

Evaluation of a Novel Adjuvant in Rabies Vaccine Formulation

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Abstract— There are different types of vaccines against rabies worldwide. In this laboratory study we evaluated a nanodendrimer as adjuvant in vaccine against rabies infection and tested on mice to detect antibody titers in sera of subjects. In our study, subjects (mice) were divided in to control group and groups receiving vaccine with 0.5, 1 and 1.5 mg/dose of dendrimer. One week after the last vaccination, blood samples were obtained and antibody titers in the sera isolated by RFFIT measurement method. Data were analyzed using One Way ANOVA. The results showed that vaccine with 0.5 mg/dose of dendrimer had highest antibody response. The findings show that our designed vaccine can increase antibody at the appropriate dose, so, is of importance in clinical area of immunization against rabies infection.

Index Terms— Rabies vaccine, vaccination, mice

I. INTRODUCTION

RABIES virus, a rhabdovirus of the Lyssavirus genus, is a negative-strand virus containing a nonsegmented RNA molecule. Like vesicular stomatitis virus (VSV), the model system for rhabdoviruses, rabies virus has a bullet-shaped morphology. The RNA is found to be associated with the nucleoprotein (N), forming the nucleocapsid core. The M1 protein as well as the transcriptase activity, presumably supplied by the L protein, are also associated with the nucleocapsid [1],[2]. M2 is associated with the lipid envelope, and a glycoprotein is found on the outer surface of the virion [3] – [5].

Rabies is spread when an infected animal scratches or bites another animal or human. Saliva from an infected animal can also transmit rabies if the saliva comes into contact with the mouth, nose, or eyes. Overall dogs are the most common animal involved.[6] More than 99% of rabies cases in countries where dogs commonly have the disease are caused by dog bites [7] In the Americas, bat bites are the most common source of rabies infections in humans, and less than 5% of cases are from dogs[7]. Rodents are very rarely infected with rabies. The rabies virus travels to the brain by following the peripheral nerves. The disease can only be diagnosed after the start of symptoms [6].

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Almost all human cases of rabies were fatal until a vaccine was developed in 1885 by Louis Pasteur and Émile Roux. Their original vaccine was harvested from infected rabbits, from which the virus in the nerve tissue was weakened by allowing it to dry for five to ten days [8]. Similar nerve tissue-derived vaccines are still used in some countries, as they are much cheaper than modern cell culture vaccines [9].

The human diploid cell rabies vaccine was started in 1967. Less expensive purified chicken embryo cell vaccine and purified vero cell rabies vaccine are now available [10]. Recombinant vaccine called V-RG has been used in Belgium, France, Germany, and the United States to prevent outbreaks of rabies in undomesticated animals [11]. Immunization before exposure has been used in both human and nonhuman populations, where, as in many jurisdictions, domesticated animals are required to be vaccinated The Missouri Department of Health and Senior Services Communicable Disease Surveillance 2007 Annual Report states the following can help reduce the risk of contracting rabies [12].

1. Vaccinating dogs, cats, rabbits, and ferrets against rabies
2. Keeping pets under supervision
3. Not handling wild animals or strays
4. Contacting an animal control officer upon observing a wild animal or a stray, especially if the animal is acting strangely
5. If bitten by an animal, washing the wound with soap and water for 10 to 15 minutes and contacting a healthcare provider to determine if post-exposure prophylaxis is required. September 28 is World Rabies Day, which promotes the information, prevention, and elimination of the disease [13]. In this laboratory study we designed a vaccine against rabies infection and tested on mice to detect antibody titers in sera of subjects.

II. MATERIAL AND METHODS

One dose of inactivated rabies virus (1ml) was prepared and 0.5, 1 and 1.5 mg of dendrimer were added under sterile condition in laminar hood. PBS was added to control solution. The solutions were incubated in 4⁰ centigrade on magnetic stirrer rotating in 30/minute. Vaccines (0.5 ml) were injected intraperitoneally to subjects (mice) in the days 0 and 7. To determine the amount of antibody produced by any of the vaccines tested, one week after the last vaccination were bled and the titers of antibodies according to the following procedure (test RFFIT) were measured:

1. Preparing three dilutions of serum and serum isolated from the standard 96-well plate (Add 50 ml of serum dilution to 100 ml and 50 ml of MEM medium mixed with 100 ml medium.

2. Adding 50 ml of CVS virus to each well (80 % Rqty of virus that can infect cells Plate).
3. Incubating at 37 ° C for one hour.
4. Adding 50 ml of MEM medium with FBS% 40 BSR cell suspension to each well (50000 cells / well)
5. 24 hours of incubation at 37 ° C with % 5CO₂
6. The cells were washed three times with PBS (from this point onwards do not need to sterilize it before cell fixation because the live virus , there should be under the hood work)
7. Fixing the cells with cold 80% acetone for half an hour in the fridge temperature (better once the cells are washed with cold 80% acetone)
8. The cells were washed three times with PBS
9. Immunofluorescence staining cells with anti-nucleocapsid antibodies conjugated with FITC. Add 50 ml of conjugate to each well
10. 10- Hour incubation at 37 ° C
11. The cells were washed three times with PBS
12. Add 60 % glycerol in PBS to each well made
13. View the plate under a fluorescence microscope using a lens Flvtar
14. Determine the percentage of infected cells in each well
15. The antibody titers determined using the Reed-Muench method or the Spearman-Karber

III. RESULTS

Figure I represents serum levels of antibody in vaccinated mice. The results showed that a dose of 0.5 mg of dendrimer had highest antibody response.

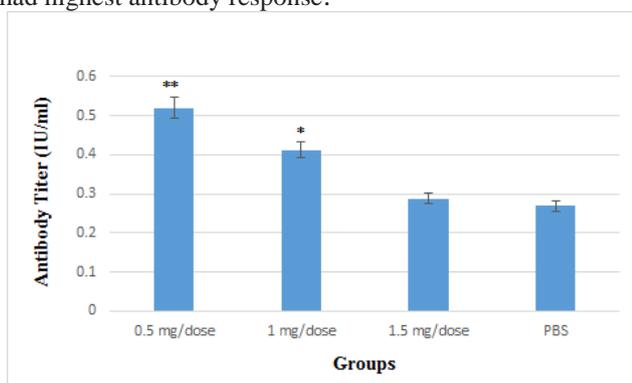


Fig 1 Serum levels of antibody in vaccinated mice. Data are presented as Mean \pm SD. Significant amount difference is compared with PBS group (p value <0.01 **, p value <0.05 *).

IV. DISCUSSION

Our findings showed that the vaccine we prepared in Pasteur Institute was effective on Rabies infection induced in mice resulting in significant increased antibody titer. There are other reports indicating that vaccines prepared in other research centers have had significant effect on Rabies infection. The most commonly used technique for detection of protective level of rabies antibodies in sera of animals and humans is the rapid fluorescent focus inhibition test (RFFIT) developed by Smith [14]. Over the years, several types of anti-rabies vaccines have been developed, produced and used for protection of man and animal against rabies. Pasteur's basic approach to vaccine development such as attenuation and inactivation are still key pillars of vaccinology. In modern technology however, purification of target microbial components, genetic engineering and

enhanced knowledge of immune defence to enable creation of attenuated mutants, expression of vaccine proteins and polysaccharide [15]. Safe and potent rabies vaccines produced on different cell lines such as MRC5 cells (a human fibroblast cell line), heteroploid cells like Vero cells (a continuous cell line of vervet monkey kidney) and chicken embryo cells were developed two decades ago and are currently available in developed countries. Today, Vero cells are considered as a more suitable substrate for the production of viral vaccines. This cell line presents several advantages over primary and diploid cell substrates [16]. Vero cells can be used in microcarrier and suspension cultures for largescale production in bioreactors. Moreover, virus titer achieved is higher than that reached using other types of cell substrates [17].

V. CONCLUSION

The findings show that our designed vaccine can increase antibody at the appropriate concentration, so, is of importance in clinical area of vaccinology

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