

ORIGINAL RESEARCH ARTICLE

A Novel Recombinant Rabies G Protein Vaccine (Thrabis®) - A VLP based Rabies vaccine

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ABSTRACT

In past many effective rabies vaccines were developed that used live-attenuated strains or inactivated killed pathogens. Live-attenuated vaccine strains are typically highly immunogenic, but have inherent safety concerns. The inactivated or killed vaccine stimulates a weaker immune reaction, and thus may require the administration of multiple dosages, an important practical limitation and increases non-compliance rate. The virus like particle (VLP) technology is an attractive platform for vaccine preparation which improves antigen stability and immunogenicity. The main advantages of VLPs is safety. VLPs cannot replicate, recombine or undergo reassortment because they do not contain infectious DNA or RNA material; therefore, VLPs are safer than traditional vaccines. The Recombinant Rabies G Protein Vaccine (Thrabis® Cadila Pharmaceuticals Ltd., Ahmedabad) is the first novel recombinant nano-particle based Rabies G vaccine prepared by using VLP technology. This technology offers a collective strength of multiple binding sites (avidity) and can provide improved antigen stability and immunogenicity.

Key words: Rabies, VLP, Recombinant

INTRODUCTION:

Rabies is a zoonotic viral disease that is caused by rabies virus (RABV) and claims highest fatality among all infectious diseases. In spite of being a fatal disease, it can be prevented by vaccination. Vaccination against rabies is safe and efficacious however it is not used enough especially in developing countries where socioeconomic factors play a vital role. In past many effective vaccines were developed that used live-attenuated strains of a pathogen, or inactivated killed pathogens. Live-attenuated vaccine strains are typically highly immunogenic, but has inherent safety concerns. The inactivated or killed vaccine stimulate a weaker immune reaction, and thus may require the administration of multiple dosages, an important practical limitation and increases non-compliance rate. Currently available rabies vaccines are live attenuated and have to be given several times, which becomes very burdensome for those living in remote areas. As described by Shankaraiah, the non-compliance rate increases with increase in each dose and it was 40% at the 5th dose. Thus the non-compliance rate for the rabies vaccine is a major challenge.²

DEVELOPMENT OF RABIES VACCINE

The development of rabies vaccines conceded through four chronological stages those are (i) Neural tissue vaccines (ii) Non-neural vaccines (iii) Cell culture based vaccines and (iv) Recombinant DNA and protein based vaccines. During these developmental processes various killed inactivated and live attenuated virus were tested. From these studies it was observed that killed virus vaccines were less efficient due to poor immunogenicity and lack of strong T- and B- cell responses.

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In 1885, Pasteur first developed the live attenuated rabies vaccine. However, this vaccine had various adverse effects such as neurological disorders and sometimes death. The members of the first generation rabies vaccines (vaccines prepared in neural tissues) included: Pasteur vaccine (1885, living attenuated), Hogen Vaccine (1887, Live attenuated) and Puscariu Vaccine (1895, inactivated vaccine), Suckling Mouse Brain Vaccine (1955, Inactivated), Suckling rat Brain Vaccine (1955, Inactivated) and Suckling Rabbit Brain Vaccine (1955, Inactivated). All these vaccines promote autoimmune reactions in the recipients. Due to these reasons the second generation non-neural vaccines were developed, where embryonated eggs were used for culturing the virus. Later the virus was inactivated and used as vaccine. These vaccines were administered by intramuscularly route in the deltoid muscle, or in thigh muscle in case of small children. Total of 6 injections over a period of 90 days were given for protection. Although these vaccines were safer than the first generation vaccine, the major disadvantage of these vaccines was the need for large number of doses to induce sufficient protection beside their serious side effects.³ The third generation of vaccines were developed using animal cell culture technique, where virus was cultivated in Hamster Kidney cells and human diploid cells. The third generation vaccines were economical compared to the 1st and 2nd generation vaccines. However, these vaccines are expensive due to requirement of multiple doses. The major disadvantages of this vaccine is poor immune response in malnourished animals or humans.,.,.,.

The fourth generations of rabies vaccines were developed based on recombinant DNA technology where either the DNA vaccine or the protein subunit vaccines were developed. Rabies virus consists of five genes; nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the viral RNA polymerase (L). Among these proteins, the G and N proteins are known to be important for immunogenicity against rabies virus. Many approaches are explored since DNA recombinant technology became available to express the immunogenic recombinant rabies virus glycoprotein. Unfortunately, in humans, DNA vaccines were found to be poorly immunogenic. Rabies G spike proteins play important role in the virion binding to host cells through specific recognition of the cell surface receptor molecules. It is the major antigen in the formation of neutralizing antibodies (Nab) against rabies. Studies indicate that the rabies G protein plays a crucial role in the pathogenesis of rabies and is the most antigenic and immunogenic surface protein involved in viral attachment. Virus neutralizing antibodies (VNAs) exclusively binds to the rabies G protein. WHO experts suggests the specific amount of VNAs should be present in the serum after 7-14 days of virus exposure which is 0.5 IU/mL. The G protein activates the CD4+ T cells which are vital to release long lived antibodies in the blood. Therefore, G protein can be considered the ideal human anti-rabies vaccine. Therefore, for the development of subunit rabies vaccine, strategically, *Spodoptera frugiperda* (Sf-9) cell line is used to produce native proteins as particles commonly known as Virus like particles (VLP's). Thrabis® (Cadila Pharmaceuticals Ltd., Ahmedabad) is the first novel recombinant rabies G vaccine prepared by using VLP technology.

VIRUS-LIKE PARTICLE (VLP) TECHNOLOGY

Virus-like particle (VLP) technology is a very powerful method for developing vaccines. VLPs are multisubunit self-assembly-competent protein structures with identical or highly related overall structure to their corresponding native viruses which does not carry any viral genetic material such as nucleic acids or infectious viruses. The non infectious nature of VLP significantly improves their safety profile over live-attenuated vaccines, while also possessing advantages when compared to other forms of subunit, killed, or particulate vaccines. VLPs have great potential for a number of applications in addition to vaccines. VLP technology might be useful for the treatment of many hereditary and acquired genetic disorders. Moreover, gene therapy is largely based on viral vectors and synthetic liposomes, although both have a number of limitations, such as restricted packaging capacity, production difficulties, and undesirable immunological properties. However, due to their construction flexibility, VLPs can be engineered to overcome these disadvantages, which include the use of chemical modifications to reduce unwanted immunological responses after repeated use.¹⁸

VLP STRUCTURE

VLP are supramolecular assemblies with the same or similar structure as native virions of about 10–200 nm in diameter.¹⁹ VLPs are made up of copies of one or more viral proteins that self-assemble into nanoparticles. VLPs do not contain any genetic material therefore non infectious to vaccinated individuals and safer than whole-pathogen based vaccines such as those containing attenuated viruses.^{20,21,22} Due to the above, VLPs do not have the drawback of replicating, recombining, reassortment or reverting to virulent stage as may occur with traditional vaccines.²³ In terms of immunity, VLPs represent pathogen associated molecular patterns (PAMPs) due to their multimeric structures with conformation that resemble that from wild type viruses. VLPs can be discriminated against by the host, and therefore may induce an adaptive immune response.²⁴

IMMUNE RESPONSES INDUCED BY VLPs

VLPs can directly interact with antigen presenting cells (APCs). VLPs are sensed by pathogen recognition receptors (PRRs) which are expressed on the surface²⁵ or endosomes²⁶ from dendritic cells (DCs). VLP uptake prompts DC maturation and presentation of peptides loaded into major histocompatibility complex (MHC) class I or class II molecules for priming CD8+ or

CD4+ T cell responses, respectively. CD4+ T cells help B cells to produce antibodies (Th2 cells) and enhance CD8+ T cells (Th1 cells) cytotoxicity.^{27,28} Since VLPs contain multimeric epitopes on their surface, they can promote cross-linking of B cell receptors (BCRs). Simple cross-linking of BCRs by VLPs can be strong enough for priming B cells and induce the production of antibodies even without the help of CD4+ T cells. B cells can work as APCs, taking up VLPs, processing and presenting them to T cells.²⁹ Therefore, VLP-based vaccines are capable of inducing both humoral and cellular immune responses, and due to their multimeric nature, adjuvant co-administration is not needed in most cases, but the use of adjuvant improves their immunogenicity.

PRODUCTION METHODS

The rabies virus G protein has been expressed in various expression systems. The G protein expressed in *E coli* was insoluble and non-immunogenic and failed to confer protection against rabies.³⁰ Similarly, the G protein expressed in *S cerevisiae* resulted in an incorrectly folded version which could protect only against an intra-muscular challenge and not against an intra-cerebral challenge.³¹ The baculovirus expression vector system (BEVS) is one of the most powerful and versatile eukaryotic expression systems available to produce the functionally authentic recombinant proteins.³² When mammalian proteins are expressed in insect cells, the protein folding and processing are more authentic than in other prokaryotic expression systems, although there are differences in glycosylation.³³ The G protein expressed using the BEVS was antigenically conserved with a three-dimensional structure and biological features similar to those of the native protein.^{34,35}

For generation of Thrabis[®] using VLP platform the genetic sequences encoding the rabies G protein sequence is selected. The genes are cloned into baculovirus. The recombinant baculovirus are made to infect insect cells (sf9) wherein the recombinant Baculovirus are amplified into insect cells and protein is purified using multiple purification steps. The protein antigens produced by the this technology are processed with post-translational modifications that ensure proper folding and induce an immune response in recipients that confers protection as measured by preclinical challenge model studies, cell culture virus neutralization studies, and surrogate measures achieved in clinical studies. Protein nanoparticles assembled in oligomeric format are designed to achieve repetitive antigen display. Most of nanoparticle antigens are in the size range of 20-200nm. Nanoparticle vaccines produced in the Sf9/baculovirus system have been adjuvanted successfully with oil-in water emulsions, with or without added TLR-4 agonists; with saponin adjuvants, and with aluminium salts. Saponin adjuvants, aluminium, and a TLR-4 agonist have been tested in rodents, non-human primates, and human trials. Syrian golden hamster challenge model was used to evaluate a VLP based vaccine efficacy and can stimulate both humoral and cellular immune system antigenically similar to parental virus.³⁶ Several studies describe efficient induction of antibody mediated immune responses by VLPs containing rabies glycoprotein-G.^{37,38} The target antigens is expressed in the Sf9 cells which are purified using various chromatographic techniques. The purified target antigen either a multimeric nanoparticles. These particles assemble into native configuration like virus. Unlike traditional vaccines that 'mimic' viruses, nanoparticles can be engineered to elicit differentiated immune responses, increasing vaccine efficacy.

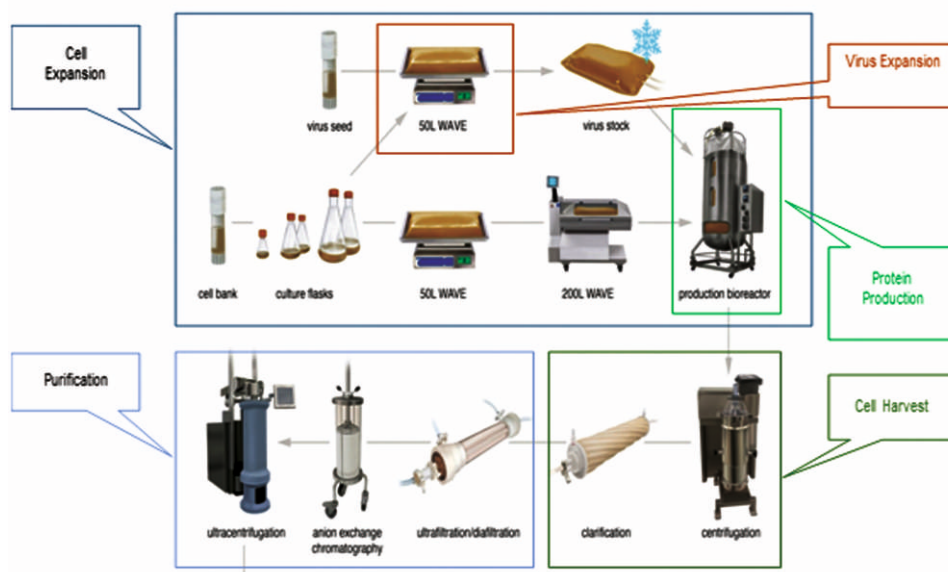


Figure 1: Scale-up of Vaccine production using VLP technology

Manufacturing process is based on single-use technology which can reduce the time for new product implementation. Bulk manufacturing (wave bioreactors) and fill/finish facilities are also available. Quality management system ensures compliance with local, federal, and international regulations and guidelines pertaining to the manufacturing, testing, holding, and distribution of products for human use, as well as the quality oversight of suppliers, contract manufacturers, and laboratories.

ADVANTAGES OF VLPs

The traditional attenuated vaccines contains pathogens that can replicate into the host cells may leads to various side effects some time may results in new pathogenic strains.³⁹ Like traditional vaccines, VLPs are immunogenic and excellent to control infectious diseases. One of the main advantages of VLPs is safety. VLPs cannot replicate, recombine or undergo reassortment because they do not contain infectious DNA or RNA material; therefore, VLPs are safer than traditional vaccines.⁴⁰ VLP-based commercially available vaccines are produced in yeast⁴¹ and baculovirus expression systems.⁴² Baculoviruses are often found in vegetables and fortunately, they are not capable to replicate in mammalian cells⁴³; therefore, they are not pathogenic for mammals⁴⁴. VLPs possess several advantages over the products that are produced by chemical synthesis such as smaller size, which ranges from 10 to 2000 nm, availability of high-resolution three-dimensional (3D) models of their structure, construction flexibility, high- production yields, and structural uniformity of each type of virus or VLP.⁴⁵ The VLP's are stable unlike the virus at room temperature for over six months and the immunogenicity is as potent as viral vaccine.⁴⁶

LIMITATIONS OF VLPs

Currently, VLPs are as good as traditional vaccines but with the advantage of being safer. However, some drawbacks need to be highlighted. Some vaccines based on VLPs are very complex, and therefore, their price is high.⁴⁷ VLPs require a purification process where the use of density gradients or even chromatography are necessary⁴⁸ and therefore, the cost of these downstream processes is high and time consuming.⁴⁹ The use of VLPs as antigen carriers can be complicated due to several difficulties: an adequate viral construct must be selected for incorporating proteins or peptides on their structure, a new particle needs to be designed for each disease, the adjuvant effect as well as the inflammatory response must be evaluated before use in each new VLP.⁵⁰ For the case of chimeric VLPs, the insertion of exogenous peptides is very restrictive. The insertion of more than 20 amino acids may damage the self-assembly properties of VLP proteins. In addition, although the design of chimeric VLPs can be based on the predictions of the structure, this process is often empirical.⁵¹

CLINICAL IMPLICATION OF RECOMBINANT RABIES G PROTEIN VACCINE (THRABIS®)

- Recombinant rabies G protein vaccine provides a collective strength of multiple binding sites (avidity) and thus improved antigen stability and immunogenicity.
- Since it is produced using recombinant technology, it provides better control over antigen content and immunogenicity compared to inactivated virus containing vaccines available currently.
- Currently five-dose regimen (administered on days 0, 3, 7, 14 and 28) are recommended for post-exposure vaccination. Compliance remains a major challenge with 5 days schedule in the post exposure prophylaxis of Rabies. The novel recombinant rabies G protein vaccine (Thrabis®) is the first 3 dose rabies vaccine which may help to improve compliance towards full course of immunization due to unique advantages like reduced dosage schedules, less number of injections and reduced number of visits to clinic/hospital.⁵²

CONCLUSION

Safety of rabies vaccines are prime concern because of the near 99% mortality rate of infections with pathogenic virus strains. For this reason, only killed vaccines have been approved for use in humans. Thus, numerous approaches have been pursued to develop alternatives to traditional live rabies virus vaccines. Recombinant rabies G protein vaccine (Thrabis®) is developed by using VLP technology. VLP are considered as safe and effective candidate of therapeutic vaccines. Therefore, recombinant rabies G protein vaccine (Thrabis®) can be considered the ideal human anti rabies vaccine with improved antigen stability and immunogenicity.

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