Vaccine Research
ISSN: 2423-4923
eISSN: 2383-2819

Rabies Vaccine: Progress and Prospective

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ARTICLE INFO

Review Article

VacRes, 2023
Vol. 10, No.1, 39 – 44
Received: February 09, 2024
Accepted: February 24, 2024
Pasteur Institute of Iran

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KEYWORDS: Rabies, Rabies Vaccines, Vaccine development, Reverse genetics

ABSTRACT

Rabies is a zoonotic disease, endemic mostly in Asia and Africa. Rabies virus belongs to the family *Rhabdoviridae*, genus *Lyssavirus*. Because infection with rabies virus has no cure and is life-threatening, vaccination is an important preventive measure to combat this disease in humans and animals. On the other hand, considering the limited availability of rabies vaccines in some of less developed countries with a higher rate of human rabies death each year, the need for an alternative strategy to produce a cost-effective and immunogenic vaccine against this disease is essential. In this review, we provide a brief overview of rabies vaccine development and its recent basic research. We also describe how viral vectors such as poxvirus vector, adenovirus replicons, and reverse genetics are manipulated for efficient novel formulated vaccines against this infection and we highlight possible future developments.

INTRODUCTION

Rabies virus (RV) is a highly fatal viral zoonotic disease [1-3] . Rabies is endemic mostly in less developed countries of the world, especially in Asia and Africa [4, 5]. Unfortunately, children below the age of 15 are the main victims of the disease in these countries[6]. According to the World Health Organization (WHO) estimation, more than 29 million people receive post-exposure prophylaxis (PEP), and 59000 cases of rabies lead to fatalities worldwide[7, 8]. Rabies disease is characterized by neurological disorders which in humans manifests in two forms, namely, classic furious rabies and paralytic rabies [9]. Fortunately, effective and safe vaccines have been developed against rabies and the antibody raised by such vaccines can prevent the disease if administered timely and properly [10]. Despite effective vaccines, rabies remains a major health problem in more than 150 countries and there has been an increase in the global incidence of dog bites [11]. The recent WHO strategy on rabies zero by 2030 has resulted in increased activities by countries in key areas towards rabies elimination [12]. Rabies is caused by an enveloped virus with a bullet shape and genomic size of approximately 12 kbp [13]. RV belongs to the family *Rhabdoviridae*, genus *Lyssavirus* and has a single–stranded, negative sense RNA genome that encodes the N (The most abundant protein), P, M, G, and L proteins [14]. Its structural glycoprotein G is responsible for binding to the host cell surface receptors. Furthermore, glycoprotein G harbors different neutralizing epitopes [15, 16] that facilitates entrance to the host, accomplished by a fusion process between the viral envelope and the cell membrane. Meanwhile the NS protein aids viral genome replication.

Lyssaviruses are classified into two phylogroups which all produce rabies disease in humans. The most divergent lyssaviruses are not classified into phylogroup[17]. The genome sequence categorizes phylogroups into different lineages and genotypes. According to the recent taxonomic classification, the genus lyssaviruses are composed of 17 different classified viral species and four related, unclassified viruses [18]. Viral genotypes may differ depending on geographical distribution [19-21].

RV is transmitted to humans mainly by a rabid animal bite[22]. During the infection, the virus enters the body via animal-infected saliva, may replicates locally, and enters peripheral nerves[23]. RV travels by retrograde transport in the neuron's axon to the spinal cord. As a result, the virus multiplies and the virus spreads to peripheral non-nervous tissues. [23, 24].

This article reviews the currently licensed rabies vaccines and other different strategies that have been designed and studied to produce rabies vaccine candidates. In addition, it should be mentioned that besides vaccine development, improvement in

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[DOI: 10.61186/vacres.10.1.39]

vaccination regimens and education of the general population remains an important area in the rabies surveillance system.

History of Pasteur Rabies Vaccine

The first rabies vaccine was developed by Louis Pasteur at the end of the 19th century (Figure 1). Pasteur used homogenates of 14–day, air-dried rabbit origin of nerve tissue inoculated with RV to immunize the exposed patients [25, 26]. Joseph Meister, a French boy was the first person who received this vaccine on July 6th 1885 and his life was saved by a series of vaccination [27]. At that time, without knowledge of rabies etiology, Pasteur's approach had its novelty and was distributed widely throughout the world. This method with some modifications was used for half a century and discontinued in many countries for adverse effects or death in some recipients of the vaccine due to autoimmune reactions [28].

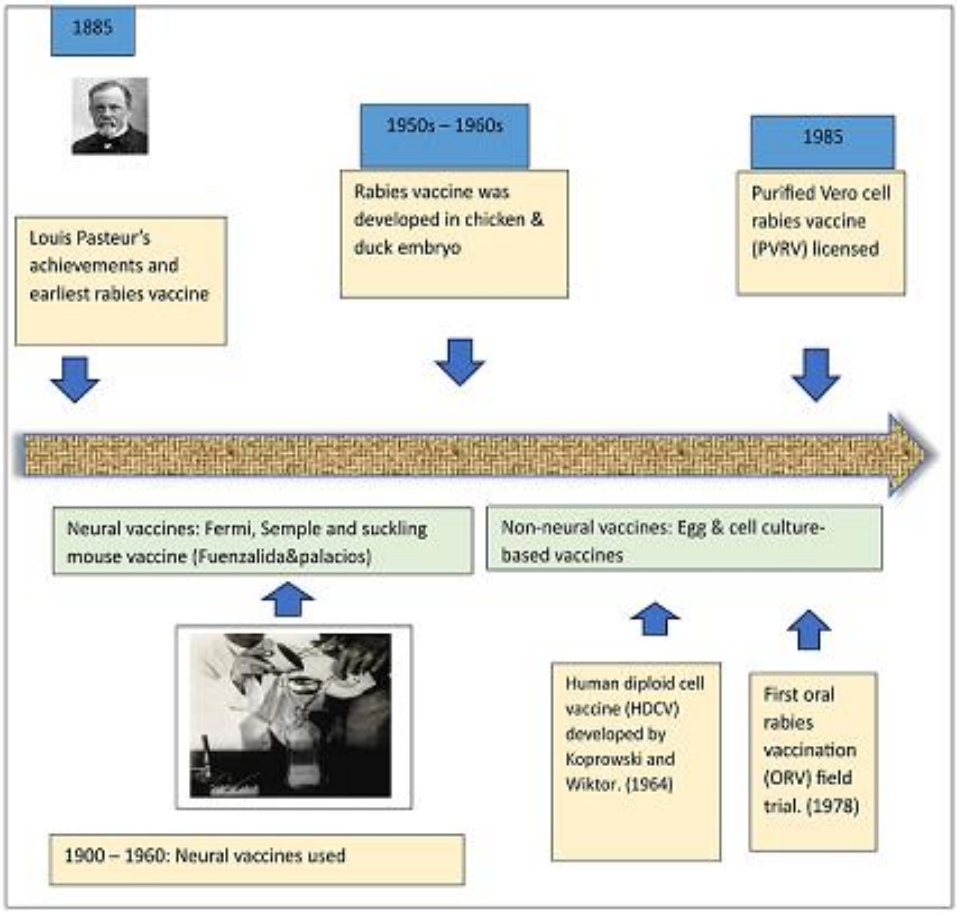

Fig. 1. Schematic diagram of rabies vaccine development.

Other Neural Rabies Vaccines with Modification

The main problems with Pasteur's vaccine were due to its partial inactivation of the virus and difficulty in vaccine preservation for long-term storage. In 1908, Fermi demonstrated that the safety and preservation of Pasteur's vaccine could be improved by adding phenol and incubation at 22°C [27]. The Fermi method was not able to completely inactivate the RV in the vaccine batch. Later in 1911, Semple showed that incubating of Fermi's vaccine at 30°C for 48-72 hours resulted in the complete inactivation of the vaccine [29]. Semple vaccine contained 5-10 % homogenate of an adult sheep or goat brain tissue and was used extensively in many countries of the world but was replaced by modern rabies vaccine in many countries [30]. The suckling mouse brain (SMB) type vaccine was another neural type vaccine developed by Fuenzalida and co-workers in 1955 in South America [27, 31]. The vaccine was prepared from 1% homogenized suckling mouse brain suspension [31]. In comparison with the adult animal neural tissue vaccines, SMB had less amounts of myelin basic proteins; however, the potency of the vaccine was lower [32]. Currently, few countries in South America are still using this type of vaccine [33].

Purified Embryonated Chicken and Duck Eggs Rabies Vaccine

After observing RV's ability to infect chicken embryos, studies have focused on the production of rabies vaccine in these cell types since the 1960s. The FLURY-LEP strain of RV is adapted to propagate in specific pathogen-free (SPF) primary chick embryo cell cultures [32]. After the virus purification, the concentration of antigen is done by continuous density gradient centrifugation and inactivated by β-Propiolactone. Studies approved its immunogenicity both in humans and animals and currently, the PCECV vaccine is one of the most widely used rabies vaccines in the world, especially in India. Randomized controlled trials revealed that the vaccine is safe and its Intra-muscular or the Intra-dermal administration route should be effective in preventing rabies. Seroconversion rate after one year of vaccine administration was 95% [34-36].

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Human Diploid Cell Rabies Vaccine (HDCV)

Until the early 1950s, there was little information about the cultivation of viruses in cell culture systems. Technologies related to the cultivation of viruses in cell culture systems helped scientists to apply them for vaccine production purposes [37]. In this regard, the Nobel Prize in 1954 was awarded to three scientists for discovering the ability of poliovirus cultivation in a cell culture system [32, 38, 37]. Finding that RV can propagate in human WI-38 diploid cells opened a new area of investigation in the field of rabies HDCV vaccine [32]. Sanofi Pasteur introduced the first rabies HDCV vaccine, propagated in MRC-5 cells in 1974 [39, 40]. Two diploid fibroblastic cell lines known as WI-38 and MRC-5 are used for the production of rabies vaccine [41, 42]. WI-38 cells were derived from elective abortion of a 3-month-old fetus in 1962 [43]. MRC-5 cells are lung cells that were obtained from the elective abortion of a 14-week-old Caucasian male embryo in September 1966 [44]. Because diploid cells contain intact genomic materials, rabies vaccines produced in this cell type are safe and considered the reference vaccine by WHO [45].

Purified Vero-Cell Rabies Vaccine (Pvr)

One of the limitations of rabies HDCV vaccine production is challenges in industrial large-scale production which make this vaccine expensive [32]. This is an important issue due to extensive demands for vaccines in developing countries where rabies is still endemic and the cost is a limiting factor. Compared to the HDCV vaccine, the PVR vaccine is more widely available and cost-effective. The first Vero cell line was introduced in 1962 from African green monkey kidney cells by Yasumura and Kawakita at Chiba University in Japan [46, 47]. Its advantage in comparison with diploid cells is the possibility of its industrial large-scale production for vaccine purposes which can reduce the costs. Furthermore, lyssaviruses can replicate in the Vero cell line [32]. The pitman-Moore strain is used for the production of these vaccines. The first Vero cell-based rabies vaccine was developed in 1985 [26] and currently alongside the PCEC vaccine is one of the widely used rabies vaccines, globally.

Rabies DNA Vaccine

Among the advantages of DNA vaccines are their thermo-stability in ambient temperature, lower production costs and ease of preparation and scaling-up [48, 49]. DNA vaccines are thermo-stable because their only specific gene or genes encoding desired antigens are incorporated inside a plasmid. Although from the field's standpoint, these prpoperties are great for the developing countries, the current RABV DNA vaccine candidates do not show adequate immunogenicity; thus, further improvement of DNA vaccines platforms is warranted. In one study by Osinubi *et al.*, constructed a DNA vaccine encoding the modified G gene of the Evelyn-Rokitnicki-Abelseth (ERA) strain and showed that the DNA vaccine encoding the modified G gene was more effective than the native G gene in mice [50]. Moreover in another study, a DNA vaccine construct combined of four plasmids harboring ectodomain of rabies glycoprotein plus trans membrane domain was shown to be able to produce RV neutralizing antibody [51].

Plant-Based Rabies Vaccines

For more than three decades, plants have provided innovative and cost-effective strategies for the production of therapeutic proteins using genetic engineering technologies [52, 53]. Several clinical trials have shown that plants can be used in the production of vaccine candidate and so far, two plant-derived vaccines (influenza vaccine and Newcastle disease vaccine) have been approved for commercial use [53, 54]. The main advantages of plant-derived vaccines are their oral or topical (local) route of administration, being easy to scale-up and their low-cost of production[53]. However, despite these achievements and advantages, concerns about the Genetically Modified Organisms (GMOs), have limited this approach [55, 56]. Furthermore, the problems of glycosylation and antigenic load concerns remain to be solved with respect to rabies vaccins [33]. RV's G protein produced in transgenic plants and used for immunization of animals is reported to protect them from lethal challenge[57].

RABIES VIRAL VECTORS

Poxvirus Vectors

Advances in molecular and cell biology have resulted in the design and development of vector-based vaccines and other therapeutic products for the target community [58-60]. On the other hand, several features of poxvirus vector systems make them attractive as vaccine carriers for researchers. These include their large capacity for gene incorporation, relative stability in cold-chain independent conditions, and being a potent inducer of humoral and cellular immunity [61, 62]. The rabies recombinant vaccinia virus (VR-G) was the first recombinant Poxvirus-based vaccine that received a license for vaccination [63-65]. This vaccine has been used in several countries for immunizing wildlife. VR-G is not safe for use in humans, as serious adverse effect has reported after contact with vaccine[66, 67]. Although their efficient response is observed in many hosts like raccoons, gray and red foxes, and coyotes, their protective immunity was observed to be inadequate in dogs and skunks by a single immunization [68]. Furthermore, in that area of bait distribution, two human exposures with adverse reactions have been reported so far. However, several issues, such as frequent adverse local and systemic reactions were observed in the vaccination area that have led to the study and use of attenuated or host-restricted forms of vaccinia vectors [66]. In an attempt by Cadoz *et al.*, rabies glycoprotein G was cloned in canary pox (an avian poxvirus) and its safety along with efficacy was tested in some animal species and also in humans [69].

Adenovirus Replicons

Adenoviruses (*Adenoviridae*), are a member of double-stranded DNA viruses which have been widely used as replicons for vaccine or gene therapy by expressing foreign genes [70, 71]. For this purpose, the viral structural genes are replaced by desired gene or genes [72]. As the structural proteins are not expressed, the anti-vector response is low. Due to their ease of genetic manipulation and high capacity of gene expression, the adenoviral vectors are preferred to other viral vectors. In a study by Yarosh *et al.*, a human adenovirus type-5-based vaccine expressing rabies glycoprotein elicited rabies-neutralizing antibodies by parenteral or oral routes in animals [73]. In another study, intramuscular immunization of dogs with low and high doses of a chimpanzee adenoviral vector-based rabies vaccine induced a strong immune response and protected dogs from lethal challenge [74].

Subunit Vaccines

Recombinant technology enables researchers to produce a desired vaccine without transporting infectious particles. Preparation of inactivated traditional RV vaccines requires high biosafety containment facilities which increase the vaccine production costs [75, 76]. Furthermore, the use of chemicals to inactivate viruses may modify virus epitopes and alter their

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[DOI: 10.61186/vacres.10.1.39]

antigenicity. One of the advantages of subunit vaccines is that the host immune system is targeted against specific pathogen components or epitopes [77]. Rabies surface glycoprotein is one of the main candidate proteins for the development of subunit vaccines. Glycoprotein G is a trimeric form on the surface of the virion and most host-neutralizing antibodies recognize and bind to epitopes of this glycoprotein [69]. Studies have shown that the incorporation of the extra G gene in candidate vaccines can increase the immunogenicity of the vaccine[78].

Reverse Genetics Serving Rabies Vaccine Development

Since the expansion of human rabies fatality around the world, the prevention of rabies has become a global public health priority. In this regard, reverse genetics are considered as useful tools for the study and understanding of different aspects of lyssavirus biology and also rabies vaccine development. Reverse genetics rely on the possibility of rescuing viable viruses containing desired genetic changes [79, 80]. Using reverse genetics, Schnell *et al [81, 82]*, generated infectious RV from SAD B19 strain of rabies from cDNA transcripts and were able to release the virions into the supernatant. Furthermore, the yielded particles were shown that their infectivity was blocked by anti-G monoclonal antibodies, generated by standard RV[82] . Moreover different attempts like gene rearrangement, deletions and duplication have been reported by other researchers [83-85]. This method is useful for researchers since the requirement for handling contagious or dangerous viruses is removed.

CONCLUSION

The rabies vaccines are currently dominated by HDCV, PCECV, and PVRV, (inactivated whole viruses) that are potent and safe. Considering the cost of the vaccine, the production of the vaccine in serum-free cell culture media can resolve the cost factor and safety issues. Furthermore, with the increasing identification of other genotypes of RV, a vaccine protecting all genotypes of RV should also be considered. Indeed, approximately 150 countries have been identified as endemic regions of rabies disease worldwide. Undoubtedly, this type of vaccine would also contribute to the rabies elimination strategies.

ACKNOWLEDGMENT

The authors received no financial support for preparation of this article.

CONFLICT OF INTEREST

The authors declare they have no conflict of interests.

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