

Article

Research Progress of Rabies Vaccine

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ABSTRACT

Rabies is a zoonotic infectious disease caused by rabies virus. Lead to acute fatal encephalomyelitis after rabies virus infection of the central nervous system of warm-blooded animals and human. The development of vaccines that prevent rabies has a long and distinguished history, with the earliest preceding modern understanding of viruses and the mechanisms of immune protection against disease. Since the first development of a rabies vaccine by Pasteur in the late 19th century, second- and third-generation vaccines with improved efficacy and less reactogenicity have been developed for use in humans and animals. The correct application of inactivated tissue culture-derived vaccines is highly effective at preventing the development of rabies. Furthermore, oral vaccination is possible for wildlife, companion animals and livestock. A number of experimental vaccines are under development. These include DNA vaccines, recombinant viral vaccines, and recombinant protein vaccines. Further testing is needed to determine if and which one of these novel vaccines will make their way into mass production and application in the future. This review provide an overview of the past, present and possible future of rabies vaccination.

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Abbreviations: DEV: Duck Embryo Vaccine, HDCV: Human Diploid Cell Vaccine, CVS:Challenge Virus Standard, PCEC: Purified Chick Embryo Cells, LEP:Low Egg Passage, PVRV: Purified Vero cell Rabies Vaccine, PHKCV: Primary Hamster Kidney Cell Rabies Vaccine

Introduction

Rabies is a zoonotic disease, and this infection is transmitted to human by

the animals which already suffering from it. The animals which are mainly reported as causes of rabies are dogs, raccoons,

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skunks, bats, and foxes. Rabies is caused by a virus that attacks on the nerves system and later excreted in saliva. Rabies affects the brain and spinal cord (central nervous system) with initial symptoms like flu, fever, headache, but the infection can progress quickly to hallucinations, paralysis, and eventually death, and no effective therapy. Once the disease occurred, the mortality rate of was almost 100%. Rabies occurs in more than 150 countries and territories. According to an estimation by WHO, almost 55,000 people die because of rabies every year. The Dogs are the major reason behind this, approximately 99% human deaths caused by dog's bites. Developing and under developing countries, both are the victims of rabies. With the post-exposure preventive regimes, 327,000 people can prevent this disease annually^[1].

Rabies was a viral disease which cause by the infection of rabies virus. The rabies virus has bullet morphology (see figure 1) and is the type species of the Lyssavirus genus of the

Rhabdoviridae family. These viruses are enveloped and have a single stranded RNA genome with negative-sense. The RNA genome of the virus encodes five genes whose order is highly conserved. These genes code for nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the viral RNA polymerase (L). The G protein was the major protective antigen^[2]. All extant rabies viruses appear to have evolved within the last 1500 years, and there's only one serotype^[3].

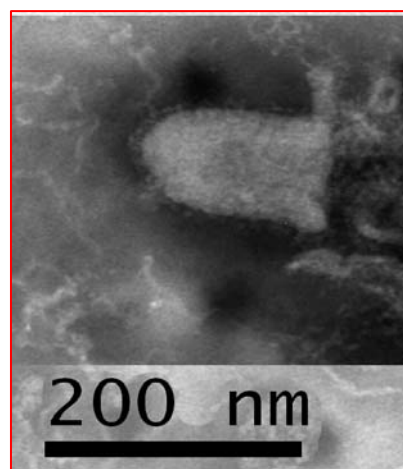


Figure 1: The morphology of rabies virus by negatively stained transmission electron microscope

Origin of rabies vaccine

The word of Rabies derives from the world's oldest languages of Sanskrit,

Rabbahs, meaning "violent". The earliest record of rabies may be as far as 2300 BC. Rabies virus in nature is widely spread. In 1804, Georg Gottfried Zinke first transmitted rabies from a rabid dog to a normal one and from dog to a rabbit and a hen, by injection of saliva. This proved that the disease was infectious. But the species susceptibility was unclear until Victor Galtier demonstrated the transmission from dog to rabbit to rabbit. He then immunized sheep by inoculating rabid saliva intravenously. This did not produce the disease but interestingly, protect the animals from the effects of a further inoculation^[4].

Louis Pasteur, C Chamberland, PPE Roux, and T Thuillier wrote the first of their papers in 1887, it's the beginning of Pasteur's studies on rabies. In further work, they showed indications of the rabies virus in the blood. "It first grows and multiplies in the spinal cord and brain". He reported: When passed from dog to monkey and then from monkey to monkey, the virulence diminishes with each transmission, and then if inoculated back into dogs, rabbits, or guinea pigs, it remains attenuated.

However, virulence was serially increased when passed from rabbit to rabbit, or from guinea pig to guinea pig. Infected by the virus of the spinal cord, exposed to the air He can produce different virulence of the virus; the virulence gradually diminished with time. Thus, Pasteur produced an attenuated vaccine, and successfully immunized 50 inoculated dogs^[5].

On Monday 6 July 1885, Joseph Meister, aged nine, was brought to him from Alsace having been bitten by a rabid dog on 4 July. With some reluctance, Pasteur was persuaded by Drs Vulpian and Grancher of the Académie de Médecine to give Dr Grancher the emulsion from the cord of a rabbit that had died of rabies on 21 June, and had been kept in dry air for 15 days. The child was given 13 further inoculations in 10 days with portions of the cord that were progressively fresher (more virulent), until after three months and three days he announced that the child's life was now out of danger and his health appeared excellent. On 20 October, he successfully treated another patient infected by a mad dog six days earlier. By 1886, he had treated 350 patients from all over Europe, Russia, and America^[6].

Despite some disagreements and criticism about the safety of the vaccine, which contained virulent virus, the medical community rapidly accepted Pasteur's method of treatment for an otherwise fatal infection. To avoid iatrogenic infection of patients by the vaccine virus, major modifications in the preparation of nerve tissue vaccine were introduced by Fermi and Semple. They partially or completely inactivated the virus by treatment with phenol. Even with those modifications, there were still some cases of paralysis after vaccination caused either by incomplete inactivation of the virus or by reactions to the adult mammalian nervous tissue in the vaccine. This tissue can induce a neuroparalytic reaction in the form of an allergic encephalomyelitis caused by sensitization to myelin basic protein [7]. Despite the potentially serious side effects on the central nervous system, the Semple vaccine is still used in humans in Asia and Africa. Fuenzalida and colleagues introduced the first myelin-free inactivated vaccines to rabies virus prepared from neonatal mouse brains^[8]. This vaccine type is

still widely used in South America and the former Soviet Union.

So the rabies was preventable infectious disease by treated with vaccine, and some kinds of new rabies vaccine were studied successfully.

Duck embryo vaccine

Another vaccine that had less serious reactions was the duck embryo vaccine (DEV) prepared from virus propagated in embryonated duck eggs^[9]. However, DEV was less immunogenic than brain tissue vaccines and did not always protect against rabies. The Swiss Serum and Vaccine Institute later improved the DEV by introducing a purified DEV (PDEV) that contains about 1% of the protein of the DEV therefore reducing the risk of allergic reactions^[11, 12]. Concentration of the virus by density gradient ultracentrifugation also led to a substantial increase of the vaccine's efficacy and its ability to elicit virus-neutralizing antibody responses in humans. The PDEV is still produced today and used for vaccination of humans worldwide.

Cell culture vaccine

Cell culture rabies vaccine as a new progress gradually spread. The first tissue culture vaccine was derived from virus

grown in primary hamster kidney cells [12, 13]. The primary hamster kidney cell rabies vaccine (PHKCV) was developed in Moscow and is now approved for use and produced in China. This was followed by growth of fixed RABV in a human diploid cell line [14]. The lung-derived cell line WI-38 was used initially, but was switched subsequently to the MRC-5 cell line which resulted in the development and licensing of a human diploid cell vaccine (HDCV) in the mid-1970s. The Pitman–Moore strain of rabies virus was then adapted to WI-38 [15] and the free virus was inactivated by β -propiolactone, and concentrated by ultracentrifugation [16]. This human diploid cell vaccine (HDCV) induced much better immune responses in animals and humans than all the other vaccines known at that time [17, 18]. Vaccination with HDCV not only results in higher antibody levels, but also the antibodies appear earlier after vaccination. Using this vaccine, it was for the first time possible to demonstrate that a single injection of the vaccine given several hours after challenge with a virulent virus could

protect animals from rabies [19].

In efforts to produce vaccines at lower cost with similar potency as HDCV, a whole series of different cell culture vaccines have been developed since then worldwide. The rabies vaccine adsorbed (RVA) is made from a challenge virus standard (CVS) rabies virus strain adapted to a diploid rhesus monkey lung fibroblast cell line. The vaccine virus is inactivated by β -propiolactone and concentrated by adsorption to aluminum phosphate [20, 21]. It is considered to be equal to HDCV with regard to efficacy and safety [22].

An alternative to HDCV was the use of purified chick embryo cells (PCEC) [23]. PCECV is prepared from the rabies virus strain Flury low egg passage (LEP) grown in primary cultures of chick embryo fibroblasts. The virus is inactivated with β -propiolactone and then purified and concentrated by zonal centrifugation in a sucrose gradient. Comparative trials with HDCV showed no significant differences between the two vaccines [24, 25]. These vaccines are now used successfully worldwide.

A continuous African green monkey kidney cell line called Vero was used to produce another rabies vaccine [26]. The

purified Vero cell rabies vaccine (PVRV) is based on the Pitman man–Moore strain of the rabies virus, which is inactivated with β -propiolactone and concentrated and purified by zonal centrifugation and ultrafiltration. Antibody responses after primary and booster vaccination are comparable to those seen after vaccination with HDCV [27]. PVRV is licensed for use in humans in Europe and in many countries in the developing world.

Animal vaccine

The first vaccine used for mass vaccination of dogs was based on the Semple vaccine modified by Umeno and Doi. In contrast to a perceived need for completely inactivated vaccines for use in humans, live attenuated viral vaccines were developed for veterinary use. Cell culture-derived vaccines can be used for the parenteral vaccination of companion animals and livestock, and have also been used to develop oral vaccines for wildlife immunization [28]. The combination of high titers of attenuated strains of RABV with an oral bait attractive to wildlife vectors

such as the red fox (*Vulpes vulpes*) have been highly effective at eliminating rabies from western Europe and remain in use throughout eastern Europe and Turkey [29, 30].

The easiest way to immunize wildlife animals such as foxes and raccoons is via oral vaccination. With the development of the Street Alabama Dufferin (SAD B19) attenuated rabies vaccine, this option became feasible. This virus is propagated on a baby hamster kidney cell line and is stable even at high temperatures. Because SAD B19 retains residual pathogenicity for rodents, the more attenuated strain SAG2 has been developed and is now used for vaccination of wildlife animals [31].

Future vaccines of rabies

Despite the undoubted success of current commercial vaccines against rabies there have been numerous attempts to develop alternatives, all taking advantage of the genetic manipulation revolution. Antibodies have been shown to be critical for protection against the spread of RABV [32]. The key target for antibodies is virus glycoprotein. Glycoprotein is the only surface-exposed protein on the virion particle, and a

number of antigenic sites to which neutralizing monoclonal antibodies bind have been identified on this protein [33, 34]. The ability to clone the RABV glycoprotein into bacterial plasmids and then express the protein in a range of systems has led to a number of alternative approaches with the potential for new vaccines against rabies. In each case the recombinant protein, expressed in a range of vectors, has been shown to be protective in mouse models of vaccination and virus challenge. Despite the ability to rapidly induce high titers of RABV neutralizing antibodies, effective at preventing infection in small animal models, they have been unable to challenge existing vaccines, principally on cost and acceptance for human use.

A new avenue for research on RABV biology and rabies vaccine development was opened with the ability to manipulate the viral genome. An attenuated, fixed strain of RABV, SAD B19, a European derivative of an American SAD strain, was recovered from a plasmid-encoded genome by Conzelmann and Schnell. This has

paved the way for a range of developments in vaccination biology through manipulation of the RABV genome.

The persistent challenge in the field of RABV therapy is how to treat patients who have developed rabies beyond palliative care. Experimental models suggest that the mammalian host produces a vigorous innate immune response to RABV infection in the brain, however, there is strong evidence that this response is antagonized to some extent by the viral phosphoprotein [35, 36]. This subversion may be the reason for the inability of the innate and adaptive immune response to control RABV replication in the central nervous system and leads ultimately to the death of the host. It is therefore encouraging that therapeutic treatment with modified rabies genomes appears to attenuate infection with a more virulent strain [37], and offers the future possibility that these may form the foundation of a future successful treatment to ameliorate the worst outcomes of RABV infection.

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