THE PROSPECTS OF ANTI-RABIES VACCINE IN DEVELOPING COUNTRIES: A REVIEW

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ABSTRACT

Background: Rabies is a recognized viral zoonosis, and it continues to pose a significant public health threat, mainly in developing nations. It is the most common viral disease in tropical humans, but it is enzootic globally. Despite the fact that it is completely avoidable, it is estimated to cause at least 55,000 annual deaths in most developing countries due to a lack of adequate therapy and mitigation steps. Only immunization can effectively control, prevent, and eradicate rabies in humans and animals. Despite attempts in developing countries since 1919 to develop and manufacture anti-rabies vaccines for human and animal protection against rabies, effective management and prophylaxis through vaccination has been hindered by a lack of modern technology and infrastructure needed for the development and manufacture of safe and effective vaccines.

Aim: Thıs review was desıgned to highlight Anti-rabies vaccine prospects in developing countries

Method: We reviewed publications from internet sources and searched for appropriate documents available in English. Which included Medline, Google scholar and Ajol.

Results: We highlighted the Anti-Rabies Vaccine alternatives, such as Monoclonal antibody RVC20 and adaptation of Vero cell lines for rabies vaccine productionwhich can be used in developing countries, based on our online reviews.

Conclusion: It would be difficult for developing countries to meet the economic demand for the manufacture of a rabies vaccine using recombinant DNA technology. As a result, the use of the Vero cell line should be embraced.

Key words: Anti rabies, Vaccine**,** Zoonosis**,** immunization

INTRODUCTION

Rabies is an infectious illness, a fast progressive and homogenously fatal viral meningoencephalitis affecting human, which is usually overlooked. It is caused by viruses belonging to the order Mononegavirales and genus Lyssavirus in the Rhabdoviridae family (Tordo and Kouknetzoff, 1993). It is an enveloped RNA virus, with negative sense. The virus genome encodes 5 proteins namely; RNApolymerase (L), the matrix protein (M), glycoprotein (GP), Phosphoprotein (P) and Nucleoprotein (NP) (Zhang*et al.,* 2004). The virus glycoprotein is exposed as trimers on the surface of the virus and is responsible for the synthesis of neutralizing antibodies which protect against infection. Rabies is a human and zoonotic viral infection that develops after a scratch or transdermal bite of an infected animal (WHO, 2018).

All mammals are susceptible to rabies, but canine rabies, particularly in Latin America, Asia and Africa, pose the greatest threat to human s. Although Rabies is underreported worldwide, it is estimated to cause at least 55,000 deaths per year (WHO, 2012).

In 99% of cases of infectious rabies, dogs are responsible for its transmission. Although, in the non-endemic areas like the United States and Europe, the frequency of dog bites is approximately 16 per 1000 individual annually *(Gilchrist et al., 2008*). The risk of dog-bites in rural endemic areas is as high as 48 per 1000 annually (Ponsich et al., 2016), while the highest prevalence is among children 6-12 years age (Ponsich*et al.,* 2016). Rabies infection is 100% lethal if no immunoglobulins of the vaccine and rabies virus are administered on time. For immune-compromised people with category II injuries, post-exposure prophylaxis (PEP) for rabies is recommended by the World Health Organization (WHO), which includes comprehensive wound washing and prompt vaccination with one of the three regimens: (i) ''Institut Pasteur Cambodia 2-2-2-0-0" (2-sites intradermal [ID] on days 0, 3 and 7; total duration 7 days), (ii) ''Essen 1-1-1-1-0" (1-site intramuscular [IM] on days 0, 3, 7 and between day 14–28; total duration up to 14–28 days), or (iii) ''Zagreb 2-0-1-0-1" (2-sites IM on days 0 and 1-site IM on days 7, 21; total duration 21 days) (WHO, 2018). In addition, rabies immunoglobulins are recommended for those with category III injuries (WHO, 2018). In spite of the menace of rabies, the advents of cell culture technology have been of immense help in curbing it danger. Cell culture technology was first developed for the production of vaccines, is currently of great importance for the production of biopharmaceuticals (Gupta*et al.,* 2017). More than 50% of the therapeutic bio-products commercialized are generated in mammalian cells (Sanchez-Garcia*et al.,* 2015). A WHO research group has published since 1987the acceptability of Continuous Cell Lines (CCLs) with the potential for an infinite lifetime, for biological development (Barrett *et al.*, 2009). The selection of cell lines used in bio-manufacturing was thus enriched with immortalized cells which were not initially recommended for the development of human bio-pharmaceuticals. This has been done through different studies focused on advanced and perceptive screening technologies (O'Brien*et al.,* 2018). Choosing cell substrate as an integral component of the preparation of viral vaccines is important; it affects the efficiency and output of the processes, as well as the quality of the processed product (Aubrit et al., 2015). Vero cell is an example of CCL, which originates from the kidney of typical adult African Green Monkey spontaneously immortalized (Yasumura and Kawakita, 1963). Low-passage Vero cells (not greater than 150) are commonly used in the manufacturer of human vaccines due to their production and permissiveness to various viruses (Barrett*et al.,* 2009). Since 1986, WHO has licensed vero cell at low-passage for the development of various viral vaccines for human use including rabies vaccine (WHO, 1987).

Prevention of rabies virus

The most cost effective approach to avoid rabies in human is dog's vaccination. Vaccinating dogs eliminates rabies-related deaths, and the need for PEP as part of dog-bites medical treatment..(WHO,1987). Educating children and adults on dog actions and bite avoidance is an important extensio n of a rabies vaccination program and may minimize both the incidence of human rabies and the financial burden of treating dog bite s. Awareness of rabies prevention and control in communities includes: education and knowledge about responsible petownership, how to avoid dog bites and effective after-bites treatment steps. Community-level engagement, and control of the initiatives increases the scope and take-up of key messages(WHO, 1987).

Preventive vaccination in humans

There are human rabies vaccines available for immunization before exposure. WHO recommended this for individuals that are working in certain high-risk careers, such as laboratory staffs handling rabies and lyssavirus-related viruses; and the veterinarians(WHO, 1987).

Pre-exposure vaccination is also recommended for travelers going to remote areas that are plagued by rabies and are

expected to spend a lot of time outdoors in sports such as caving and mountain climbing.If local access to rabies biologics is limited, expatriates and long-term travelers should be immunized in areas with high rabies exposure risk. Children living in or visiting, rural, high risk areas should also be given immunization. They may get more serious bite when playing with animals, or they may not report bites(WHO, 1987).

Monoclonal antibody RVC20: a primary method for understanding rabies virus

The rabies virus possesses only one surface glycoprotein (Known as G). The rhabdovirus G protein ecto-domain is divided into four different subdomains which are denoted; I, II, III, and IV (Roche*et al.*, 2006).This protein is responsible for the virus entry in to the host cell and is thus the primary target for neutralizing antibodies. RVC20 in G-domain complex is one of the most active neutralizing human monoclonal antibody. Researchers identified the RVC20's antibody crystal structure in contact with its target domain, one of the rabies virus glycoprotein major antigenic site(Jan *et al.,* 2020). The structure shows that RVC20 contains highly conserved viral glycoprotein characteristics, rationalizing its large reactivity among the rabies virus strains. Monoclonal antibody RVC20 prevents fusion of the membrane between the virus and the cell. Hence, the antibody prevents the virus entry into the host cell and further spreads of the virus inside the infected host's body (Jan *et al.,* 2020).

Mechanism of neutralization

Rhabdovirus G; a class III membrane fusion protein that fuses the envelope of the virus with endosomal membranes to carry the viral genome to the cytoplasm (Baquero*et al.,* 2015). Fusion allow G undergo a particular conformation shift when the endosome is exposed to the acidic environment (Gaudin*et al.*, 1991). The rearrangement is considered to be reversible *in vitro*, and for the VSV G ecto-domain, the structures of both the alkaline-pH pre-fusion conformation and the acid-pH post-fusion conformation have been described(Roche *et al.,* 2007).Researches showed that, the interaction between the rhabdovirus Gectodomain and RVC20 slows down as the pH decreases from 8.0 to 7.0 and then to 5.5, but dissociation is slow and essentially uninfluenced by pH. As indicated by the comparison with VSV G, the interaction step's pH dependence is caused by epitope masking through domain rearrangements, since the isolated domain III bound RVC20 was independent of pH. Therefore, the decreased association rate at low pH leads to an equilibrium change towards its acidic-pH post-fusion form (Jan *et al.,* 2020).

Vero cells adaptation to suspension growth in rabies virus production

Vero cells are very safe to use and permit the growth of different viruses, they were thus the first authorized continuous cell line for the development of human viral vaccines (O'Brien and Nolan, 2018). Many viral vaccines for human consumption are currently developed in Vero cells. As adapting anchorage-dependent cells to suspension culture solves scale-up constraints related to monolayer cultures, it is accepted to be an attractive method for industrial usage (Biaggio*et al.,* 2015). Samia*et al.* (2019) showed that disaggregation agents are not required for the adaptation of Vero cells to suspension culture in serum free media. However, their study is did show cell population doubling. In addition to the chemically specified; Hycell-CHO and CD-U5 commercial media, IPT-AFM with reduced amounts of calcium and magnesium is conducive for growing rabies virus in VeroS cells grown in shake flasks (Samia*et al.* 2019). In case of cell growth and rabies virus titer, these media demonstrate comparable out-put to medium containing serum. VeroS cell infection also demonstrates a higher productivity of cells in relative to adherent vero cells.Fig. 1.0 summarizes the steps to be taken in establishing VeroS grown in serum-free media, which will create an alternative method of cell culture for large-scale vaccines production (Samia*et al*. 2019).

Purified Vero cells Rabies Vaccine (PVRV)

Since 1985, the PVRV has been used in the prevention of rabies. Evolving patterns in rabies vaccination, including short intradermal regimens with reduced quantity, together with guidelines from the WHO for intradermal administration have guided recent intradermal PVRV regimen evaluations (Thomas*et al.,* 2020). Given that there is usually less need to ensure that Rabies virus neutralizing antibodies (RVNA) titers with Pre-exposure prophylaxis (PrEP) increase rapidly, assessing immunogenicity within the first few days after initiation or completion of the vaccine is less necessary, as reported in most studies.Nevertheless, some research recorded data over the first year for one or two selected points of time. The PVRV potency used for PrEP ranged across studies from 2.5 to 8.7 IU/0.5 ml.whereas, the 0.5 IU/mL RVNA threshold achieved with intradermal PVRV PrEP may not be retained upto one year after vaccine initiation, the priming induced regardless of the protocol is ideal for a robust RVNA response to PEP. Intradermal PVRV PEP also rapidly induces RVNA titers which exceed the threshold of0.5 IU/mL (Thomas*et al.,* 2020).

Vaccine growth opportunities

Recent developments in peptide chemistry and genetic engineering have allowed higher quantities of pure antigens to be engineered and generated the basic building blocks of vaccines (Freeman and Robbins, 1991). It indicates that, the advancement of genetic engineering and molecular biology has played a major role in the advancement of vaccines (Plotkin, 2005). This offers higher incentives for inactivated antigen development and for organisms to be rationally attenuated by direct mutation. If scientists can identify the infecting organism's antigen that activates immunity, an effective vaccine would likely be developed (Freeman and Robbins, 1991). It is reported that scientists are on the verge of developing a novel DVA vaccines that are promising to combat diseases, with lesser side effects (Diamond, 2011). The new vaccines are however costly and project financing can be difficult to obtain(Diamond, 2011). Such concept strategies replaced the trial and error that was used to produce vaccines in the past (Freeman and Robbins, 1991). The goal of the latest anti-rabies vaccine production is to produce safe, efficient and cheap vaccines that can be administered with brief immunization schedule (Ishaya*et al.,* 2013).However, it has been shown that a purified chick embryo cell vaccine confer immunity in both guinea pigs and monkey after the parenteral rabies virus challenge. The vaccine purification is achieved by continuous zonal centrifugation, while its inactivation is done with beta-propriolactone. This is a standard procedure for the elimination of allergenic substances from vaccine preparations. Researches also came up with highly purified and optimized formulations of standard duck embryo vaccines (DEV) (Barth*et al.*, 1985). These formulations are less allergenic and more immunogenic than their predecessors (Ishaya*et al.,* 2013). One of the premier technique used for rabies virus studies is tissue culture, presently there are numerous continuous cell lines available such as Vero, CER and BHK-21, which are usedin studies on diagnosis, vaccine production and pathogenesis of rabies virus (King, 1996).Rabies cell culture vaccines are safer, without egg proteins and lipids, they are more effective with longer shelf-life and will increase the amount of vaccine produced. A continuous, aneuploid cell line originating from the kidney of vervet monkey is an alternative cell culture media, known as Vero (WHO, 1984). This method allows for higher vaccine antigen yields than the human diploid cell vaccine (HDCV) approach and may be less expensive and more suitable for use in developing countries (Ghosh, 2005).

Nonetheless, attenuation of the Flury virus resulting from the harmonic co-evolution of G and L elements may be more valuable knowledge for producing healthier and more efficient live rabies vaccines (Birhanu*et al*., 2013), whilethe correction of it G-protein discrepancy created by site-directed mutagenesis using recombinant DNA technology seems possible, with this technique it is possible to produce a totally synthetic vaccines (Faber *et al.,* 2005). Such a vaccine does not contain entire particles of the virus or reactogenic elements of cell culture vaccines. Inactivation procedure would therefore be unrequired, and less complicated techniques of purification would be feasible. A recombinant vaccinia virus was also produced which expresses the rabies G protein (Wiktor*et al.,* 1984).Evidently there is also the development of four glycoprotein and nucleoprotein genetic DNA vaccines for South African Mokola virus to vaccinate against Mokola virus (Nel*et al.,* 2003).Humans are actively seeking refuge as a result of globalization, which exposes them to zoonotic diseases such as rabies and with the concept of spillover, a mutation in RBV can pose serious threat to public health, necessitating the development and improvement of more efficient vaccines, As a result, the above-mentioned alternative can be utilized.

CONCLUSION

Vaccines established over the first two centuries since Edward Jenner's lifetime have significantly reduced illnesses and disease wherever they have been used.Purification of microbial components, genetic engineering, and enhanced technical knowledge of immune defense now allow for mutant attenuation, vaccine protein expression in live vectors, purification and synthesis of microbial antigens, and induction of a variety of immune responses throughmanipulations of nucleic acid and proteins. Despite the fact that developing countries cannot afford any of these technical innovations, they can accept the use of the vero cell line in vaccine development.

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