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Production of Cell Culture Based Anti- rabies Vaccine in Ethiopia

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Abstract

Prevention and control of rabies in the world will require international efforts to increase the availability and use of high quality cell-culture rabies vaccines for use in human and veterinary. An important aspect of activities to ensure such availability is transfer of technologies to developing countries for production of these vaccines. Methods for Rabies Virus manipulation have changed fundamentally from random attenuation to defined modifications. In 2001, WHO issued a resolution for the complete replacement of nerve tissue vaccines by 2006 with cell-culture rabies vaccines. However, sheep brain derived Fermi type rabies vaccine is still being manufactured and utilized for the majority of exposed patients in Ethiopia. Therefore, production of a safer and effective cell culture based anti-rabies vaccine is needed. Currently the Ethiopian government has heavily invested in upgrading the facilities required to produce a rabies vaccine in keeping with WHO recommendation. Rabies virus suspensions were obtained from vero cells cultivated on roller bottles after infection with the Pasteur virus strain (PV) and Evelyn Rokitniki Abelseth (ERA). Initially the titer of the obtained virus and multiplicity of infection of the viruses had to be optimized; therefore in rabies virus infected cultures, higher virus yields was obtained when infected with 0.001ERA virus/cell and incubated at 37^0C in 5% CO_2 for 96hr and 0.01PV/cell incubated at 37^0C in 5% CO_2 for 48hr. Based on the results it is conclude that, ERA virus 0.001ID/cell with incubation period of 96h and was selected as best titer for rabies vaccine production.

Keyword: ERA;PV; fermi; cell culture; Multiplicity of infection

1. Introduction

Although rabies vaccines have been in use since 1885, much remains to be done in the prevention and control, both in humans and animals. The early rabies vaccine derived

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from nervous tissue has now been superseded in purity, potency, and safety by products prepared in cell cultures. Every effort should be made to discontinue the use of vaccines derived from nervous tissue of adult animals, and to use these improved products (1).
Different animal species can be responsible for viral circulation and rabies transmission in different continents and countries, worldwide rabies in dogs is the source of 99% of human infections and poses a threat to >3.3 billion people (2). There are an estimated 60,000 human rabies related deaths worldwide each year. Of these most cases occur in Asia and Africa (3). In 1992/1993 human rabies in Addis Ababa, the capital city of Ethiopia, was surveyed and 464 rabies cases were identified. The 1992/1993 Addis Ababa data on human rabies cases was extrapolated to estimate the actual magnitude of human rabies throughout Ethiopia by using an assumption that there is a uniform distribution of dog rabies and of human exposure and cases throughout the country. Thus, with an estimated 1993 population of 2.5 million in Addis Ababa and an estimated countrywide population of 55 million people, it was estimated that approximately 10,000 persons die of rabies in Ethiopia each year, and that more than 40,000 persons may require human rabies post exposure treatment (PET) (4). Ethiopia's current population is estimated at 85 million. The fatal human cases in 2001-2009 were 386 humans with annual range of 35 to 58. In the last ten years a minimum of 6,263 and a maximum of 21, 832 doses of the human rabies vaccine were produced and distributed every year (5).
Pasteur developed the first vaccine against rabies in 1885, and then the production of this vaccine followed the technological improvement towards immunogenicity and safeguards. Rabies vaccines produced in mammalian neural tissues have the disadvantage of causing severe adverse reactions, at a rate estimated as 0.3–0.8 per thousand treated patients (WHO, 2004). World Health Organization (WHO) has recommended since the 90s that they have to replaced by vaccines produced in substrates free from animal nervous tissues, as the latter are more immunogenic and, more importantly, safer (6); (7). The vaccines recommended by WHO include those produced in Vero cells, available since the 1980s. Unfortunately, the cell culture rabies vaccines are expensive and not readily available to individuals living in developing countries where rabies is endemic in dogs (8). Today, the rabies vaccines for both human and animal use are produced using different cell lines in cultures (9); one of the most widely used cell lines for human vaccine production is the non-tumorigenic Vero cell line (10. Sheep brain derived Fermi type rabies vaccine is still being manufactured and utilized for the majority of exposed patients in Ethiopia, even though this vaccine has been discouraged by the WHO. The high costs of tissue culture vaccine and inertia have been the main barrier to the replacement of Fermi type vaccine. Currently EHNRI is working to revolutionize anti-rabies vaccine production by changing the nervous tissue vaccine (Fermi type) to cell culture based vaccine.

The important challenge of prevention and control of rabies in the world will require international efforts to increase the availability and use of high quality cell culture rabies vaccines for human and animals. An important aspect of activities to ensure such availability is the transfer of technologies to developing countries, for production of

such vaccines. Extending the production of vaccine, using both existing and new manufacturing units, requires that careful attention be given to maintaining high standards of vaccine safety and efficacy by application of appropriate standardization and control procedures. A variety of cell-culture systems are available for the preparation of inactivated rabies vaccines for use and veterinary. Vaccines produced are inactivated by treatment with beta-Propiolactone for human use (1).

Transfer of the ability to manufacture improved rabies vaccines for use in animals or humans is dependent on two factors: a commitment by the government of the recipient country to a long-term programme on the prevention and control of rabies; and an assurance that appropriate resources are, or will be, available to support production of rabies vaccines for use in animals and humans, at a level necessary to meet national needs. Currently Ethiopian government has heavily invested in upgrading the facilities required to produce a rabies vaccine in keeping with GMP standards. The Ethiopian Government recognizes that rabies is a serious problem and vaccine production is one of the strategic focus areas to prevent and control rabies.

The objective of the programme is to establish cell culture based rabies vaccine in Ethiopia for use in animals and humans according to WHO recommendation.

Methods

Vero and BHK- 21 cell lines were used for the manufacturing of the rabies vaccine. Vero cell line initially obtained from the National Polio Laboratory; EHNRI and BHK 21 cell line obtained from National Veterinary Institute (NVI), Bishoftu, Ethiopia by donation. Evelyn Rokitniki Abelseth (ERA) and Pasteur Virus (PV) fixed rabies strain were kindly donated by Canter for Disease Control and Prevention, Atlanta (CDC). Working seed virus was prepared from the master seed virus for the production of the test vaccine. For the determination of rabies virus titer (1) monolayers of Vero cells on 96 well microtiter plates were infected with sample dilutions and incubated at 37 °C with 5% CO_2 for 48, 72 and 96 hr. Cells were then fixed in acetone, washed with phosphate buffered saline (PBS) and incubated with FITC (fluorescein isothiocyanate conjugated) antibodies against rabies virus nucleocapsid (Russia, Pokrov) for 1 h at 37 °C.

Production

Production of cell culture based rabies vaccine was achieved using roller bottles. Initially, the multiplicity of infection of the virus was optimized on cell line.

Results

Rabies virus culture samples were harvested and the rabies virus titers determined individually at different incubation period after virus infection with 0.1, 0.01 and 0.001

moi (table 1). For the determination of rabies virus titer (1) monolayers of Vero cells on 96 well microtiter plates were infected with sample dilutions and incubated at 37 °C with 5% CO_2 for 48, 72 and 96 hr. Cells were then fixed in acetone, washed with phosphate buffered saline (PBS) and incubated with FITC (fluorescein isothiocyanate conjugated) antibodies against rabies virus nucleocapsid (Russia, Pokrov) for 1 h at 37 °C. The microtiter plates were then observed in a fluorescence microscope and the titer expressed as fluorescence focus doses 50% (FFD_{50}) as calculated by the Spearman–Ka¨rber method. Table 1 shows the results of titration of ERA rabies virus strain obtained from cultivation in Vero cells in flasks with different multiplicity of infection and incubation period.

Table 1. Multiplicity of infection and incubation of period of ERA rabies rabies virus strain

Multiplicity TCID/cell	Incubation period 48 hours	72 hours	96 hours
0.1	$10^{6.75}$	$10^{5.75}$	$10^{6.75}$
0.01	$10^{6.50}$	$10^{5.50}$	$10^{6.25}$
0.001	$10^{4.50}$	$10^{5.50}$	$10^{7.25}$

As shown in table 1, the higher rabies titers were obtained from the Vero cell at multiplicity of 0.001 viruses per cell with incubation period of 96 hours. Since the rabies virus shows a non-lytic cycle of replication, the vaccine process of production is based on the collection of several supernatant harvests from infected cultures, which are pooled for further purification. From the above performed rabies virus production, with the view to establish our own vaccine production facility and protocol it is concluded that multiplicity of infection with 0.001 viruses for 96hr was result with the highest virus titer and we recommend for the rabies vaccine production.

As the same time Pasteur Virus Strain is also used in our experiment for the production of cell culture based. Table 2 shows the results of titration of PV (CDC) rabies virus strain obtained from cultivation in Vero cells in flasks with different multiplicity of infection and incubation period.

Table 2. Multiplicity of infection and incubation period of ERA rabies virus strain

Multiplicity TCID/cell	Incubation period 48 hours	72 hours	96 hours
0.1	$10^{6.5}$	$10^{7.5}$	$10^{7.00}$
0.01	$10^{7.25}$	$10^{7.5}$	$10^{6.00}$
0.001	$10^{5.75}$	$10^{6.00}$	10^{5}

As shown in the above table, Tissue Culture infectivity dose of 0.01 per cell with 72hr incubation was selected as the best titer for rabies vaccine production. Large scale production of tissue culture derived vaccines was achieved using monolayer cultures in roller bottles. Although this method is labour intensive, it requires only modest investment in equipment.

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