SPECIAL ARTICLE

A Brief History of the Purified Chick Embryo Cell Human Rabies Vaccine, Chirorab (formerly known as Rabipur)

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ABSTRACT

The development of modern cell culture vaccines revolutionized human rabies prevention since the latter part of the 20th century. ChiroRab (previously known as Rabipur) is an inactivated purified chick embryo cell rabies vaccine, produced in India for the prevention of rabies in humans. Over the past 35 years, this pure, potent, safe and efficacious biologic, when administered by the intramuscular or intradermal routes, in pre- or postexposure prophylaxis, prevented rabies in millions of exposed people in Africa, the Americas, Australia, and Eurasia. Based upon its sound clinical performance to date, ChiroRab will continue to play a major role in the global elimination of human rabies caused from dogs by 2030, particularly throughout the Indian subcontinent.

INTRODUCTION

Rabies, an acute progressive encephalitis, is one of the oldest described infectious diseases – some believe its origins transcend human history (1-3). The etiological agents consist of single-stranded, negative-sense RNA viruses, with more than 17 recognized and putative viral species (4,5). Multiple lyssaviruses utilize bats and other mammals as reservoirs on all continents, except Antarctica (6,7). As quintessential neurotropic viruses, lyssaviruses exploit routine mammalian behaviors to ensure their transmission via the transdermal or mucosal routes (8-11). From the standpoint of concerns in agriculture, biological conservation and public health, rabies virus is the most important lyssavirus and dogs constitute the most prominent reservoir (8,12). Unlike diseases such as smallpox and rinderpest, rabies is not a candidate for eradication (13). However, within the 21st century, rabies can be prevented, controlled and in some cases, selectively eliminated from circulation, over large geographic regions (8,14-16).

Before the age of vaccination, rabies management included human avoidance of animal bites, leashing and muzzling of owned dogs, culling of stray dogs and a wide variety of techniques (e.g., amputation, cauterization, 'quackery', etc.) in an attempt to prevent a productive viral infection after a bite from a rabid animal (17). After Pasteur's historical demonstration of the first human rabies vaccination during the late 19th century, gradual improvements followed, towards further development of nerve tissue vaccines (NTV) produced in animals (e.g., rabbits, sheep, mice, etc.). Unfortunately, NTV suffered from issues related to lower potency and product safety (18). During the mid-20th century, use of avian embryos, primary cell cultures and continuous cell lines for defined viral propagation revolutionized rabies vaccine development from the era of the NTV predominance (Table 1).

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Table 1. Historical examples in the evolution of human rabies vaccines

Type	Substrate	Comments	References
Pasteur, 1885	Adult nervous tissue vaccine (NTV), produced in dried spinal cord of rabbits	Residual rabies virus, high myelin content, required > 14 doses administered subcutaneously in the abdominal area	17
Fermi	NTV	Partial chemical treatment of rabies virus-infected tissue specimens for vaccine preparation, with a combination of both inactivated and replication-competent virions	19
Semple	NTV	Greater inactivation of rabies virus-infected rabbit, sheep or goat tissues, initially by carbolic acid (i.e., phenol), with widespread use, particularly in India, but with residual risk of post-vaccinal encephalomyelitis	20
Hempt	NTV	Ether treatment prior to phenol inactivation and stabilization for distribution over broader geographic areas, especially throughout Central Europe, but with risk of post-vaccinal encephalomyelitis	21
Fuenzalida Palacios	Suckling mouse NTV	Safer than adult NTV, inactivated by ultra-violet (UV) irradiation (after infection with CVS rabies virus), once widely used throughout Latin America	22
Duck embryo vaccine (DEV)	Duck embryos	Infected with PM rabies virus strains, and inactivated by beta-propiolactone (BPL), hence safer than NTV, but poorly immunogenic	23
Primary hamster kidney cell vaccine (PHKC)	Young Syrian hamster kidney cell culture	UV-inactivation of cells infected with the Vnukovo rabies virus strain, used widely in China and the former USSR	24
Rabies vaccine adsorbed (RVA)	Diploid fetal rhesus monkey lung fibroblasts	BPL-treated rabies virus, adsorbed onto aluminum Phosphate, produced originally by the Michigan Department of Health, USA	25
Human diploid cell vaccine (HDCV)	WI-38 cells, later MRC-5 cells	Inactivated by BPL, much safer than NTV, highly potent, immunogenic and efficacious, used in multiple countries, especially in Australia, Europe and North America	26-28

Type	Substrate	Comments	References
Purified vero cell rabies vaccine (PVRV)	Vero cells	Rabies virus (PM strain) inactivated by BPL, much safer than NTV, highly potent, immunogenic, efficacious, and economical to produce for global use	29,30
Purified chick embryo cell vaccine (PCEC)	Chicken fibroblasts	Rabies virus (LEP strain) inactivated by BPL, much safer than NTV, highly potent, immunogenic, efficacious, and economical to produce for global use	31-33

Whereas the first duck embryo vaccine (DEV) was an important milestone in safety over the NTV progenitors, potency remained low (23,34). Later, at the Wistar Institute of Anatomy & Biology, the human diploid cell vaccine (HDCV) became the first modern cell culture vaccine with both high safety and potency (26-28). Despite its overall superiority to NTV and DEV, higher production costs of HDCV limited its availability largely to the developed world. This drawback was overcome in part by the effective production of a purified vero cell vaccine, PVRV (29,30). Similarly, by repurposing the utility of avian tissues for rabies virus propagation, a purified chick embryo cell vaccine (PCEC) joined the vanguard for economic human rabies prevention in the developing world (31-33, 35,36). These modern cell culture rabies vaccines resulted in far fewer serious adverse events than the NTV, were highly immunogenic in the rapid induction of rabies virus neutralizing antibodies (VNA), were suitable for delivery via the intramuscular (IM) or intradermal (ID) routes by a variety of regimens, and could be used for the preexposure (PrEP) immunization of persons at risk of occupational rabies virus exposure (e.g., laboratory workers, veterinarians, etc.) as well as for postexposure prophylaxis (PEP) for the greater population at large after rabies virus exposure (i.e., typically after a rabid animal bite). Since their advent, several different brands of HDCV, PVRV and PCEC vaccines were marketed (8). In the interest of clarity, the objective of this communique is to provide a brief history of the PCEC vaccine, Rabavert, known today as ChiroRab (Figure 1).

Figure 1 ChiroRab Rabies Vaccine (NB: please insert a better product image if available)

PURIFIED CHICK EMBRYO CELL VACCINE

The adaption of different rabies viruses, isolated from nature and serial passage within animals, primary cells or continuous cell cultures, serve as vaccine seed strains (e.g., CVS, PM, PV, ERA/SAD, etc.). The Flury rabies virus strain was isolated from a child infected through exposure to a rabid dog in Georgia during 1939 (37). This isolate was attenuated by serial brain passages within chicks and chick embryos (38). Historically, these were differentiated as the Flury low egg-passage virus

strain (i.e., LEP, < 50 passages) or the high-egg passage virus strain (I.e., HEP, > 179 passages). During the 1950-1960s, HEP and LEP rabies viruses were considered for use in both human and veterinary vaccine applications (39-44). Given the prior utility of the LEP virus propagated in avian tissues as a biologic, further investigations began during the late 1970s in Marburg, Germany, and during the 1980s, the PCEC vaccine was developed. In a series of pre-clinical tests, PCEC vaccine demonstrated comparable potency and safety to HDCV. Thereafter, in human clinical studies, PCEC vaccine showed similar immunogenicity to HDCV and was subsequently licensed in Germany during 1984.

Over the past 35 years, Rabipur (today known as ChiroRab), has been used effectively for both PrEP and PEP (Table 2). In a recent meta-analysis of PCEC vaccine, nearly all individuals had rabies VNA above the minimum WHO suggested value, as indicative of an adequate immune response to vaccination (i.e., $\geqslant$ 0.5 IU/ml by day 14) and in the vaccinated population at large, had an acceptable safety profile (36).

Table 2. Comparative clinical use of Rabipur (AKA today, ChiroRab)

Observation	Comments	References
Safety	Rabipur was well tolerated by human subjects in multiple studies in China, Europe, India, Thailand & the USA	35
PrEP	Kinetics, height of the response and persistence of VNA over two years were virtually identical after Rabipur and HDCV administration	45
Comparable immunogenicity	Rabipur was as effective as HDCV at inducing an antibody titre of > 0.5 IU/mlml in all Indian subjects, irrespective of their age and sex	46
Duration of VNA after Zagreb regimen	All vaccinees had high VNA levels from day 14, which lasted more than a year and the adverse reactions of the Rabipur vaccine were mild and self-limiting	47
Concomitant ID vaccination and RIG administration	Healthy volunteers received either ID Rabipur vaccine alone (0.1 ml at each of two sites on days 0, 3 and 7, and at one site on days 28 and 90), or similar vaccine with one dose of HRIG IM on day 0 and VNA was detectable in all subjects, in both groups, from day 14 to day 365, but no significant suppressive effects of RIG were observed.	48
Vaccine volume	In this population of healthy volunteers, a full dose of Rabipur in a dilution of 0.5 mL was as well tolerated locally and systemically as in a dilution of 1.0 mL and all subjects developed levels of VNA exceeding 0.5 IU/mL	49
Safety and efficacy in Thai patients using a dose-sparing regimen	Rabipur was safe and highly immunogenic when administered ID in 0.1-ml doses using a two-site method (i.e., on days 2,2,2,0,1,1)	50
Efficacy after exposure to confirmed rabid animals	A multi-site regimen of Rabipur, with or without passive immunization, prevented the development of rabies encephalitis in patients bitten by confirmed rabid dogs, over a 3-year observation period	51
Comparative response of different regimens with or without immune globulin	All participants given Rabipur ID achieved adequate VNA concentrations regardless of regimen or co-administration of RIG, but VNA concentrations were consistently lower in adults with RIG administration than in those without	52
Comparability via regimen	Vaccination with Rabipur under a 2-1-1 regimen was as safe and immunogenic as under a traditional 5-dose Essen regimen in healthy adult Chinese and Indian subjects	53, 54
Longevity after ID vaccination	All subjects had positive VNA in excess of 1 year	55
PEP and iD administration	Rabipur was safe and effective during PEP by the ID route in 43 Indian dog bite cases	56

Observation	Comments	References
Abbreviated, ID regimen	All subjects, who had nursed or casually handled a rabies patient and administered Rabipur, experienced seroconversion and all developed VNA levels higher than the adequate level	57
Effect of reduced volume of vaccine by the ID route	Adequate antibody response obtained with Rabipur when administered by the TRC regimen, even after reducing the quantity of vaccine from 0.2 ml to 0.1 ml per intradermal dose	58
Immunogenicity by number of ID site administration	Both a 2-site and 8-site ID regimens with purified chick embryo cell vaccine produced adequate levels of VNA	59
Cost-effective, dose sparing efficacy	Rabipur efficacy confirmed using the TRC regimen in 113 exposed patients in the Philippines, where vaccine was well tolerated, no vaccine-related serious adverse events occurred, and all patients were alive at least 1 year after initial exposure	60
Comparison to the Thai Red Cross regimen in India	Rabipur was safe and highly immunogenic in Indian subjects when administered intradermally as 0.1 mL/site using the "2-2-2-0-1-1" PEP regimen	61
ID vaccination using the Kempegowda Institute of Medical Sciences (KIMS), Bangalore, India, regimen	In healthy volunteers, PEP with Rabipur, administered using the KIMS-ID regimen, was well tolerated and immunogenic for a year	62
Comparative utility to PVRV	Rabipur was immunogenic, efficacious, and well tolerated when administered in the TRC regimen in ID doses of 0.1 mL compared to PVRV	63
Relationship of potency to immune response	No significant linear relationship between antigenicity and immunogenicity of Rabipur compared to HDCV and PVRV vaccines, when administered by the intradermal route	64
Comparability with other CCV	Rabipur was found safe, tolerable, and immunogenic as PDEV and PVRV in exposed Indian patients	65
Effect of prior route of administration	Rabipur was safe and immunogenic following booster vaccination, after cross-over from the ID to the IM route and vice versa	66
Switches in vaccine brand during prophylaxis	PEP was safe and immunogenic, despite changes in the route of administration and brand/type of vaccine (e.g., Rabipur, PVRV, etc.)	67
Shortened regimens	In a clinical comparison of Rabipur and PVRV, a new "one week ID regimen" was both immunogenic and safe in healthy Indian volunteers	68
Comparison of vaccine booster number	Clinically insignificant benefit of using 1 vs. 2 boosters of PCEC vaccine	69
Anamnestic responses	Anamnestic responses to booster doses of Rabipur demonstrated at least 14 years after the last vaccination	70
Pregnancy	Rabipur well tolerated with no reports of systemic or local AEs and the exposed patients had normal deliveries of healthy babies with no evidence of congenital abnormalities	71
Effect of age and regimen	Vaccination with Rabipur following a Zagreb regimen induced immune responses non-inferior to those of the Essen regimen, and had a similar safety and tolerability profile to the Essen regimen in Chinese children, adolescents, and adults over 51 years	72

Observation	Comments	References
PrEP in children	Rabipur was safe and immunogenic when administered for PrEP in Thai and Indian children	73, 74
Concomitant administration with Japanese encephalitis vaccine	After concomitant administration of Rabipur and Japanese encephalitis vaccine to Thai toddlers, adequate rabies and Japanese encephalitis VNA were demonstrated by day 49, 7 days after a booster at 1 year, and in a majorly at 3 years postvaccination	75
Potential compatibility with other travel vaccines	Raipur and Japanese encephalitis vaccine, administered together, with or without a quadrivalent meningococcal glycoconjugate vaccine, did not compromise immunogenicity or safety of the individual products	76
Malnourished subjects	No significant difference in VNA between malnutrition categories identified (i.e., immune response to Rabipur was independent of the extent of malnutrition) and no SAEs reported, indicating that the vaccine was well tolerated	77
Immune-compromised patients	Rabipur was immunogenic in HIV-1-infected patients with CD4+ cell counts below 200 cells/microL when administered in a modified eight-site intradermal PEP regimen	78
Comparability by commercial site of vaccine production	Noninferiority in immunogenicity and tolerability of the Rabipur produced in India to German vaccine batches	79
Exposure to bat lyssaviruses	Sera from volunteers who received Rabipur in a simulated ID PEP regimen contained adequate neutralizing antibodies to European and Australian bat lyssaviruses	80

Based upon such extensive studies, one may summarize the clinical experience with this PCEC vaccine:

• ChiroRab, administered using the recommended WHO PrEP schedule (e.g., days 0, 7, 21 or 28), provides an adequate concentration of VNA at ⩾ 0.5 IU/mL by day 28, or earlier, in all subjects.

• ChiroRab can be administered safely IM or ID, using various schedules for PrEP and PEP and as booster vaccinations, in all age groups.

• ChiroRab is highly immunogenic, as 98% to 100% of subjects achieved VNA concentrations ⩾ 0.5 IU/mL after PEP on day 14, regardless of the administration route (IM or ID).

• ChiroRab is efficacious, with regards to 1-year survival (100% survival seen in >120 subjects).

• ChiroRab displays a rapid anamnestic response, even years after primary vaccination, providing increased immunity and VNA of ⩾ 0.5 IU/mL.

• ChiroRab has an established safety profile, with a positive benefit-risk profile, and no documented cases of true vaccine failure (i.e., fatal rabies virus infection after correct PEP administration) were identified, in contrast to the few cases where infection after PEP occurred, because prophylaxis had been inadequate (i.e., not according to WHO recommendations).

BASIC DESCRIPTION OF CHIRORAB VACCINE

ChiroRab is a sterile, freeze-dried vaccine obtained by replicating the fixed-virus strain Flury LEP in primary cultures of chicken fibroblasts using specific pathogen-free eggs in compliance with current applicable pharmacopeia and with WHO requirements. The rabies virus seed strain Flury LEP was obtained from the American Type Culture Collection at the 59th egg passage. Propagation of the LEP rabies virus occurs in a synthetic cell culture growth medium, with the addition of human albumin, polygeline (processed bovine gelatin), and antibiotics. The LEP rabies virus is inactivated by β-propiolactone and purified via continuous density-gradient centrifugation. Lyophilization of the vaccine occurs after addition of a stabilizer

solution, consisting of buffered polygeline and potassium glutamate. A dose of the reconstituted vaccine contains ⩽ 12 mg polygeline (processed bovine gelatin), ⩽ 0.3 mg human serum albumin, 1 mg potassium glutamate, and 0.3 mg sodium EDTA. Small amounts of bovine serum are used in the cell culture process. The bovine components originate from Australia, New Zealand, and the USA. Minimal amounts of avian protein may be present in the final product. The ovalbumin content is ⩽ 3 ng/dose (1 mL), based on ELISA testing. Antibiotics (e.g., neomycin, chlortetracycline, amphotericin B), added during cell and virus propagation, are largely removed during subsequent steps in the production process. Neomycin is present at ⩽ 10 mcg, chlortetracycline at ⩽ 200 ng, and amphotericin B at ⩽ 20 ng per vaccine dose. The vaccine is intended for intramuscular (IM) or intradermal (ID) administration. The vaccine contains no preservative and should be used immediately after reconstitution with the supplied Sterile Diluent (Water for Injection). The potency of the final vaccine product is determined by the NIH test. The potency of 1 dose (1.0 mL) of vaccine is equal to at least 2.5 IU of rabies virus antigen. This biologic is a white, freeze-dried vaccine for reconstitution with a diluent prior to human parenteral use. The reconstituted vaccine is a clear to slightly opalescent, colorless to slightly pink suspension. Until recently, PCEC vaccine was produced at two WHO pre-qualified manufacturing facilities: Germany and India. Batches of vaccine from the two sites were clinically comparable (79). Today, ChiroRab is produced only in Ankleshwar, India. Besides being efficacious against rabies virus variants, VNA elicited after ChiroRab immunization also cross react against other lyssaviruses, which is important given the opportunity for their potential emergence in India (80,81)

Based in part upon global demand, product supply, regulatory compliance and SAGE recommendations, rabies as a vaccine preventable disease was listed as a high priority candidate by the WHO during 2018-2020, in which Rabipur (and in the near future, ChiroRab) played a prominent role

(https://extranet.who.int/pqweb/vaccines/eligibility).

ASCENDENCY OF CHIRORAB®

In the 1980s, Rabipur was manufactured in Marburg, Germany by Behringwerke (a constituent of the Hoechst Group) and was exclusively imported and marketed in India (marketed by Hoechst India Ltd., Bombay). During 1989, a facility was opened at Ankleshwar, Gujarat for manufacturing and production of Rabipur in India, in technical collaboration with Behirngwerke. In 1996, the vaccine business of Behringwerke (including that of Rabipur® vaccine and its technology) was transferred to the Chiron Corporation, known as Chiron Behring GmbH & Company, Germany. Consequently, in India too, new entity M/s Chiron Behring Vaccines Private Limited (CBVPL) was incorporated to take over existing manufacturing facility (including technology) from Hoechst India Limited. Further during 1998, Novartis Group took over the Global Chiron Corporation, thereby CBPL became the subsidiary of Novartis Group. In 2014, Novartis sold its vaccine business to GlaxoSmithKline (GSK).

In 2019, Bharat Biotech acquired legal entity Chiron Behring Vaccines from GSK, along with all technology, rights regarding clinical development, manufacturing process, QC processes, distribution and sale of rabies vaccine. All licenses and regulatory approvals obtained for Rabipur® have been fully transferred to ChiroRab®, including WHO prequalification which is in process. Chiron Behring Vaccines is one of the largest manufacturers of rabies vaccines in the world.

As can be seem production of ARV has continued past 3 decades at same plant with same technology, although there has been change in managements / Trade name. Such emphasis on vaccine supply is critical, given international aspirations for rabies prevention and control. For example, during 2015, the WHO announced a program for the global elimination of human rabies caused by dogs (GEHRD) via mass canine vaccination and human rabies prophylaxis, according to an increased emphasis on PrEP together with new ID dose-sparing and shortened PEP regimens (82-89). Clinicians have a major responsibility in selection of the ideal rabies biologics for their patients, to prevent deaths from a zoonosis with the highest case fatality of any infectious disease and to remain alert for counterfeit vaccines (90). Considering its considerable public health benefits shown to date, ChiroRab's future legacy includes a prominent role in progressing toward the GEHRD by 2030, especially throughout the Indian subcontinent and the surrounding region.

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National Guidelines for Rabies Prophylaxis

Summary of Vaccination Schedule

The summary of vaccination schedule as per route is as under :

Type of Prophylaxis	Route of Administration	Dose of Vaccine	Day of Dose	No. of injections	Total No. of Per Visit	Site of Injection Visits
Post Exposure Prophylaxis	Intra Dermal	0.1 ml per dose	Day 0, 3, 7 and 28	2	4	**Adult:** Deltoid Muscle **Infants and Small Children:** Anterolateral Thigh
	Intra Muscular	1 entire vaccine vial	Day 0, 3, 7, 14 and 28	1	5	
Pre Exposure Prophylaxis	Intra Dermal	0.1 ml per dose	Day 0, 7, and booster on either day 21 or 28	1	3	
	Intra Muscular	1 entire vaccine vial	Day 0, 7, and booster on either day 21 or 28	1	3	
Re-exposure	Intra Dermal	0.1 ml per dose	Day 0 & 3	1	2	
	Intra Muscular	1 entire vaccine vial	Day 0 & 3	1	2	