Review Article:

EXPERIENCE WITH PURIFIED CHICK EMBRYO CELL VACCINE (FLURY LEP STRAIN) FOR PREVENTING HUMAN RABIES

Ravish H S,[1] DH Ashwath Narayana,[2] M K Sudarshan,[3] Praveen G[4]

ABSTRACT

Modern Cell Culture Vaccines (CCVs), remains the main stay for rabies prophylaxis since their development and has proved to be safe and effective in preventing rabies. Purified chick embryo cell vaccine (PCECV, Flury LEP strain), the world's 1st, second generation CCV, is playing an important role in preventing the disease, since their development more than three decades ago. It has proved to be safe and effective in preventing rabies and is intended for pre-exposure as well as post-exposure prophylaxis by both intramuscular and intradermal route. It is a World Health Organization (WHO) pre-qualified rabies vaccine, most extensively used in the world with over 100 million doses injected and has contributed for saving millions of lives and will continue in the same path for supporting elimination of rabies.

The safety, immunogenicity and efficacy of PCECV (Flury LEP strain), has been well established in more than 100 clinical trials, worldwide. This review article will focus on the experience of using PCECV (Flury LEP strain) for both pre-exposure prophylaxis (PrEP) and post exposure prophylaxis (PEP), by intramuscular as well as intradermal route using various regimens; at the anti-rabies clinic, Department of Community Medicine, Kempegowda Institute of Medical Sciences (KIMS), Bangalore which has the experience & expertise in conducting various clinical trials for over twenty five years.

Rabipur® is a purified chick embryo cell rabies vaccine (PCECV, Flury LEP strain) and it is a registered trademark of the Glaxo Smith Kline Pharmaceuticals Ltd.

Keywords: Purified chick embryo cell vaccine, post-exposure prophylaxis, pre-exposure prophylaxis, rabies, safety, immunogenicity, intradermal, intramuscular.

INTRODUCTION

Rabies is a vaccine-preventable, neglected zoonotic disease (a disease that is transmitted from animals to humans) caused by the rabies virus. The neglected disease indicates that, it is not sufficiently addressed by national and international community, as they are best defined by the people and communities that are affected the most i.e., poor people living in remote rural areas and urban slums of the developing world. It is however, a disease that is amenable to control, as the tools for prevention i.e. post exposure prophylaxis (PEP) and pre-exposure prophylaxis (PrEP) are available. Therefore, it is the first zoonosis on the list of neglected diseases targeted for regional and eventually global elimination by 2030.[1]

The immunological objective of PEP is to neutralize and destroy the rabies virus inoculated immediately following exposure, so that clinical manifestation of rabies does not develop. PEP has three main components: proper wound treatment, rabies immunoglobulin infiltration in all category III exposures and a full course of modern cell culture vaccine administration.[2]

Modern cell culture vaccines (CCVs), remains the main stay for prophylaxis since their development and has proved to be safe and effective in preventing rabies. These vaccines are intended for both pre and post exposure prophylaxis and have been administered to millions of people worldwide. Purified chick embryo cell culture rabies vaccine (PCECV, Flury LEP strain),

[1]Associate Professor, [2]Professor & Head, Department of Community Medicine, [3]Retired Dean& Principal, Kempegowda Institute of Medical Sciences (KIMS), Bangalore. [4]Associate Professor, Department of Community Medicine, Hassan Institute of Medical Sciences, Hassan, Karnataka.

the world's 1st, second generation CCV, is playing an important role in preventing the disease, since their development more than three decades ago (i.e. from 1984).It has proved to be safe and effective in preventing rabies and is intended for pre-exposure as well as post-exposure prophylaxis by both intramuscular and intradermal route.[3]

The PCECV (Flury LEP strain) is a WHO pre-qualified rabies vaccine (since 2002), most extensively used in the world with >100 million doses injected and has contributed for saving millions of lives and will continue in the same path for supporting elimination of rabies by 2030, which is a part of sustainable development goal.

The safety, immunogenicity and efficacy of PCECV (Flury LEP strain) has been well established in more than 100 clinical trials, worldwide. The anti-rabies clinic, Department of Community Medicine, Kempegowda Institute of Medical Sciences (KIMS), Bangalore has been conducting various clinical trials from over twenty five years and is one of the best centres that is approved by Drugs Control General of India (DCGI). This review article will focus on the experience of using PCECV (Flury LEP strain) at Kempegowda Institute of Medical Sciences (KIMS) Hospital, Bangalore for PrEP and PEP, by intramuscular as well as intradermal route by various regimens.

PRE EXPOSURE PROPHYLAXIS (PrEP)

Pre-exposure prophylaxis is recommended for anyone who will be at continual, frequent or increased risk of exposure to the rabies virus, either as a result of their residence or occupation and for travellers.[4] Similarly, children living in or visiting rabies affected areas are at particular risk; since, severe exposures in children make it more difficult to prevent rabies unless access to good medical care is immediately available. Therefore, PrEP in these potential bite victims would prevent a number of unnecessary deaths due to rabies.

PrEP is a safe and effective method of preventing rabies, since the development of immunological memory after pre-exposure vaccination with CCVs has established long lasting immunity against rabies in humans; individuals who had received their primary series 5 does, IM, taken even 21 years previously showed good anamnestic responses after booster vaccination.[5] Similarly, pre-exposure vaccination has also been shown to be safe and effective in children with 5 years follow up and showed that co-administration of rabies PrEP with Diphtheria, Pertussis, Tetanus (DPT) vaccine and inactivated poliomyelitis vaccine at 2 and 4 months and 1 year, elicited satisfactory antibody titters to all antigens with no interference with other antigens used.[6]

PrEP also simplifies the PEP by eliminating the need for RIG, which is costly and may not be available at all time/ place after an exposure and reducing the number of doses of vaccine needed to only two doses (on Days 0 and 3) for immediate PEP. Therefore, WHO encourages incorporating CCVs into the immunization programs of children where canine rabies is a public health problem.

PCECV(Flury LEP strain)as intradermal PrEP in children, 2015

The safety of PCECV (*Flury LEP strain*) was examined, when administered intradermally to 950 children at the Tibetian colony, Kushal Nagar, Madikeri, Karnataka. PCECV was given as pre-exposure prophylaxis on days 0, 7 and 28.The safety evaluation was done only for unsolicited serious adverse events. The study showed, no serious adverse event (SAE) attributed to vaccine administration occurred during the study period and none of them dropped from the complete course due to any adverse drug events (ADEs).

In another study, 720 government school children in Bangalore were administered PCECV (Flury LEP strain) intradermally as PrEP on days 0, 7 & 21. This study also showed that, there were no unsolicited SAEs attributed to vaccine administration during the study period. In summary, PCECV Flury LEP strain was found to be safe and well tolerated in children as intradermal PrEP.

PCECV (Flury LEP strain) as intradermal PrEP in high risk adults, 2014

A safety study was conducted with PCECV (Flury LEP strain) as intradermal pre-exposure prophylaxis for adults belonging to high risk group. The study assessed the safety and tolerability of PCECV (Flury LEP strain) administered as a 3 dose intradermal pre-exposure vaccination on days 0, 7 and 28 in healthy volunteered veterinary students of Government Veterinary College, Bangalore. 122 apparently healthy adults of both sex, between 18 and 30 years of age were involved in the study and 105 (86%) completed all three doses. A total of 342 doses of intradermal vaccine were administered, among which 38 adverse reactions were reported from 19 veterinary students. The adverse reactions were pain at the injection site 7 (2.1%), redness 13 (3.8%), itching at the site of injection 11 (3.2%), induration 6 (1.8%) and headache 1 (0.3%). All reactions subsided without any complication and none of them dropped out from the study because of any adverse drug reactions. In conclusion, it showed that, pre-exposure vaccination is a useful tool for protecting high risk groups and purified chick embryo cell rabies vaccine is safe and well tolerated by intradermal route in adults.[7]

Post exposure prophylaxis (PEP)

Rabies is practically a 100% vaccine-preventable disease. A combination of large human and dog populations in congested habitable areas, combined with widespread poverty has led to more rabies exposures in WHO's South East Asia region than in any other part of the world. Therefore, it continues to be a major public health and economic problem throughout the South East Asian Region. Therefore, in rabies endemic regions, where every animal bite is potentially suspected as a rabid animal bite, the PEP should be started immediately. Because of long incubation period, which is typical of most cases of human rabies, it is possible to institute PEP to ensure that the individual will be immunized before the rabies virus reaches the nervous system. Proper wound management and simultaneous administration of RIG combined with prompt administration of rabies vaccine is almost invariably effective in preventing rabies, even after high risk exposure.

PCECV (Flury LEP strain) as PEP by intra-muscular Essen regimen, 2009

A multi centric, phase IV, randomised, comparative, clinical study on safety and immunogenicity was conducted using PCECV (Flury LEP strain), purified vero cell rabies vaccine (PVRV) and purified duck embryo vaccine (PDEV) administered intramuscularly by Essen regimen i.e. one dose of vaccine administered on days 0, 3, 7, 14 & 28. Blood samples were collected on Days 0, 14, 28, 90 & 180 and analysed for rabies virus neutralizing antibodies (RVNA) using the rapid fluorescent focus inhibition test (RFFIT).The study showed that, among 152 subjects belonging to category II & III exposures, the reported adverse drug reactions (ADRs) were 2.0%, 2.8% & 3.1 % for PCECV, PVRV & PDEV respectively and the common ADRs were pain & itching at the site of injection and generalised weakness. All the study subjects receiving different CCVs showed protective RVNA titres of > 0.5 IU/ml from day14 up to day 180.The study concluded that, all the three modern CCVs were safe, well tolerated and immunogenic to prevent rabies.[8]

PCECV (Flury LEP strain)as PEP by intramuscular Zagreb regimen, 2014

In a multi-centric, randomised, simulated PEP study, using PCECV (*Flury LEP strain)*, administered according to the WHO recommended 3-visit,Zagreb vaccination regimen was compared with immunogenicity and safety of standard 5-visit, Essen regimen in Indian subjects.250 healthy adults were enrolled and randomized into Zagreb or Essen group, each receiving PCECV (*Flury LEP strain)* according to their respective regimen. Blood samples were collected on Days 0, 7, 14 and 42 and analysed for RVNA using RFFIT. By Day 14, all subjects across both the groups attained RVNA concentrations of ≥ 0.5IU/ml. The Zagreb regimen was found immunologically non-inferior to the Essen regimen by Day 14, which was the primary endpoint of the study. No safety issues were noted and the occurrence of adverse events (AEs)was similar in both groups with 4% and 11% for Zagreb and Essen group respectively. The most

commonly reported AEs in both the Zagreb and Essen regimens were pain at the injection site (2% and 7% of subjects respectively) and fever (2% of subjects in each regimen). No SAEs or deaths occurred in the study.[13] In conclusion, PCECV (Flury LEP strain) was found to be safe and immunogenic when administered by either Zagreb or Essen regimen as intramuscular PEP.[9]

PCECV (Flury LEP strain) by intradermal route using updated Thai Red Cross (TRC) regimen for PEP, 2006 & 2012

The affordability to rabies vaccine for intramuscular administration in post exposure prophylaxis is a major constraint. Therefore, in countries, where there are financial constraints, WHO recommends intradermal rabies vaccination that reduces the quantity and cost of vaccination. It reduces the volume of vaccine and direct cost required for PEP by 60% when compared with intramuscular vaccination and therefore, largely benefits the poor & needy who visits the Government hospitals. In this background, Drug Controller General of India (DCGI) in 2006, based on the WHO recommendation and Indian council of medical research (ICMR) feasibility study, approved intradermal administration of rabies vaccination by updated TRC Regimen (2-2-2-0-2) especially in Government hospitals and approved PCECV (*Flury LEP strain)* for intradermal usage.[10]

In this background, a pioneer study was conducted to clinically evaluate the safety and immunogenicity of PCECV (*Flury LEP strain)* administered intradermally using updated TRC regimeni,e. 0.1 mL of reconstituted vaccine administered intradermally on both deltoids on days 0, 3, 7 & 28 (2-2-2-0-2) in adult animal bite cases. Eighty one subjects with category II & III exposures were included in the study. Blood samples were collected on Days 0, 14, 28, 90 and 180 and analysed using the RFFIT for RVNA. All the study subjects attained the geometric mean RVNA concentration of ≥ 0.5IU/ml from Day 14 and persisted up to day 180 i.e.,5.83, 7.92, 7.20, 3.4 on Days 14, 28, 90 and 180 respectively. The occurrence of ADEs in the study was 4.9% and no SAEs were noted. In conclusion, PCECV (Flury LEP strain), when administered intradermally using updated TRC regimen (2-2-2-0-2) is safe & produced adequate RVNA titers among all animal bite cases.[11]

Another study was done to evaluate the safety and immunogenicity of indigenously produced PCECV (Pittman Moore strain) in comparison to a WHO pre- qualified, PCECV (Flury LEP strain) rabies vaccine with demonstrated efficacy when administered by intradermal route using updated TRC regimen. Eighty-six dog bite cases were randomly given either PCECV (Pittman Moorestrain) group (n=43) or PCECV (Flury LEP strain) group (n=43) as post exposure prophylaxis. The rabies virus neutralizing antibody concentrations on days 14, 28, 90, and 180 were tested by modified RFFIT. All the study subjects had adequate RVNA concentration of ≥0.5 IU/ mL from day 14 onwards till day 180. The geometric mean RVNA concentration were 13.29 IU/mL, 11.77 IU/ mL, 9.73IU/mL and 8.12IU/mL in PCECV (Pittman Moore strain) group and 13.73IU/mL, 11.38 IU/mL, 9.71IU/mL and 8.27 IU/mL in PCECV (Flury LEPstrain) group on days 14, 28, 90, and 180 respectively. The geometric mean RVNA concentration of both the groups were compared using t- test and was found that, P value > 0.05 on all days, thus showing no significant difference between the 2 groups. The adverse drug events were also compared using Z-test and was found to be not statistically significant (Z = 1.476, P = 0.139)[12] This showed that PCECV was immunologically efficacious by both Pitman Moore and Flury LEP strains.

PCECV (Flury LEP strain) by intradermal route using KIMS-ID regimen for PEP, 2003

A randomised, active controlled, comparative study using PCECV (Flury LEP strain) administered as simulated PEP was conducted among the healthy volunteers who received either KIMS intradermal regimen i.e., 0.1 ml of vaccine administered intradermally over 2 deltoids on days 0,3,7,14 & 28 (2-2-2-2-2) or the standard Essen regimen i.e., one dose of vaccine intramuscularly

on days 0,3,7,14&28 (1-1-1-1-1). Ninety one subjects were included in the study and followed up for 1 year. The rabies virus neutralizing antibody concentrations on days 14, 28, 90, and 180 were tested by modified RFFIT. All the study subjects had adequate RVNA concentration of ≥0.5 IU/ mL from day 14 onwards till day 365.The geometric mean RVNA concentration of both the groups were compared using t- test and was found that, *P* value was > 0.001 on all days, thus showing no significant difference between the 2 groups. The adverse drug events reported were 3.1% & 4.3% for intradermal and intramuscular regimen respectively. The difference between the 2 groups was also compared and was found to be not statistically significant.[13] This showed that the KIMS ID regimen was as safe and immunologically efficacious as intramuscular Essen regimen.

PCECV (Flury LEP strain) **by intradermal route using one week-ID regimen for PEP, 2012-14**

A study was conducted to evaluate the immunogenicity and safety of "one week intradermal regimen" as simulated PEP. A total of 80 healthy adult volunteers were enrolled and allocated randomly either to PCECV (Flury LEP strain) rabies vaccine or PVRV, 40 in each group. Each subject received intradermally one of the vaccines, using the one week regimen (4-4-4). Blood samples were collected on Days 0, 7, 14, 28,180 and 365 for estimation of RVNA concentration. The sera samples were analyzed by RFFIT. All subjects in both the groups had adequate RVNA concentration of 0.5 IU/mL from day 14 to till day 180 and the difference between the two groups was not significant (P > 0.606). Further to assess the immunological memory produced by this new regimen, a "single visit four site" intradermal booster vaccination was given to those who did not have adequate RVNA concentration on day 365. This resulted in a quick and enhanced RVNA concentration in these subjects thus denoting a successful anamnestic response. The incidence of adverse events was 8.3% in PCECV (Flury LEP strain) group and 1.6% in PVRV group and the regimen was well tolerated without any dropouts. In conclusion, the new "one week intradermal regimen" is immunogenic and safe for rabies PEP and needs to be further evaluated in persons exposed to rabies.[14]

Therefore, in continuation, another study was conducted to assess the safety and immunogenicity of 2 WHO-prequalified rabies vaccines administered by one week, 4 site intra dermal regimen (4-4-4-0-0) in animal bite cases. It was a comparative, open label, phase III, randomized clinical trial. Ninety subjects with category II/III animal bites/exposures were enrolled. Equine rabies immunoglobulin was administered to all category III exposures. 0.1 mL of either PCECV (*Flury LEP strain)* or PVRV was administered intradermally into 4 sites on days 0, 3 and 7 to all the study subjects. Serum of subjects collected on day 0, 14, 90 and 365 were analyzed for RVNA. The incidence of ADRs in PCECV (*Flury LEP strain)* and PVRV group was 2.96% and 1.14% respectively. In PCECV (*Flury LEP strain)* group, geometric mean concentration (95% confidence interval) of RVNA was 14.5, 11.7 and 5.9 IU/mL on days 14, 90 and 365 respectively; In PVRV group geometric mean concentration (95% confidence interval) of RVNA was 14.4, 11.9 and 5.6 IU/mL on days 14, 90 and 365 respectively. In conclusion, PCECV (Flury LEP strain) and PVRV were found to be safe, immunogenic and comparable with each other, when administered using one week, 4 site intradermal regimen (4-4-4-0-0) in animal bite cases.[15] These studies showed that PCECV (Flury LEP strain) administered intradermally in any of the regimen is safe and immunogenic.

In conclusion, prevention is the only public health intervention for a fatal and neglected zoonotic disease like rabies. The PCECV (Flury LEP strain), a WHO pre-qualified rabies vaccine, most extensively used in the world with >100 million doses administered as PEP &PrEP by both intramuscular as well as intradermal route by different regimens is safe & immunogenic and has contributed for saving millions of lives; It will continue to contribute in the same path for

supporting elimination of dog mediated human rabies by 2030, which is a part of sustainable development goal.

REFERENCES

1. WHO Expert Consultation on Rabies. Second report. Technical Report Series No. 982. Geneva: World Health Organization; 2013.
2. World Health Organization. Rabies vaccines: WHO position paper, Weekly Epidemiological Record, No. 32. 2010; 85:309-20.
3. WHO South East Asia region: Strategic Framework for Elimination of Human Rabies Transmitted by Dogs in the South-East Asia Region: World Health Organization, Regional office for South East Asia; 2012.
4. WHO Expert Consultation on Rabies. First report. Technical Report Series No. 931. Geneva: World Health Organization; 2005.
5. Suwansrinon K, Wilde H, Benjavongkulchai M, Banjongkasaena U, Lertjarutorn S, Boonchang S, et al. Survival of neutralizing antibody in previously rabies vaccinated subjects: A prospective study showing long lasting immunity. Vaccine 2006; 24:3878-80.
6. Lang J, Duong GH, Nguyen VG, Le TT, Nguyen CV, Kesmedjian V, et al. Randomised feasibility trial of pre-exposure rabies vaccination with DTP-IPV in infants. Lancet 1997; 349:1663-5
7. Ravish HS, Aravind M, AshwathNarayana DH, Yannick P, Phaneendra MS. Safety of intradermal rabies vaccine as pre-exposure prophylaxis among veterinary students. International Journal of Community Medicine and Public Health *2017;4(2):396-9.*
8. DH AshwathNarayana, Shampur N Madhusudana, GadeySampath, DM Sathpathy, RanjithMankeshwar, Ravish HS. A comparative study on the safety and immunogenicity of PDEV, Vaxirab with PCEC, Rabipur and PVRV, Verorab. Vaccine 2010;28:148-151.
9. BJ Mahendra, DH AshwathNarayana, SharadAgarkhedkar, HS Ravish, BR Harish, ShalakaAgarkhedkar et.al., Comparative study on the immunogenicity and safety of a purified chick embryo cell rabies vaccine (PCECV) administered according to two different simulated post exposure intramuscular regimens (Zagreb versus Essen). Human Vaccines &Immunotherapeutics 2015;11(2):428-34.
10. National Rabies Control Programme, National guidelines for rabies prophylaxis, National Centre for Diseases Control, Ministry of Health and Family Welfare, New Delhi, India. 2015. p. 7-12.
11. AshwathNarayana DH, Praveen G, Gangaboraiah, Ravish HS, A clinical evaluation of safety and immunogenicity of purified chick embryo cell rabies vaccine (PCECV) administered intradermally using updated TRC regimen in animal bite cases. A report of a clinical study at anti rabies clinic, KIMS, Bangalore 2007.
12. H S Ravish, VeenaVijayashankar, Shampur N Madhusudana, Mysore K Sudarshan, D H AshwathNarayana, Gangaboraiah, et.al., Safety and Immunogenicity of purified chick embryo cell rabies vaccine administered intradermally as post exposure prophylaxis. Human Vaccines &Immunotherapeutics 2014;10(8):1–5.
13. MK Sudarshan, SN Madhusudana, BJ Mahendra, DH AshwathNarayana, Anandagiri MS, O Popova. Evaluation of a new five–injection, two-site, Intradermalschedule for purified chick embryo cell rabies vaccine: A Randomized, Open Label, Active-Controlled Trial in Healthy Adult Volunteers in India. Current therapeutic research 2005;64(4):323–34.
14. MK Sudarshan, DH AshwathNarayana, SN Madhusudana, Ramesh Holla, BYAshwin, Gangaboraiah, HS Ravish. Evaluation of a One Week Intradermal Regimen for Rabies Post-Exposure Prophylaxis: Results of a Randomized, Open Label, Active-Controlled Trial in Healthy Adult Volunteers in India.Human Vaccines &Immunotherapeutics 2012;8(8):1077–81.
15. AshwathNarayana, Aravind M, Madhusudana SN, Sudarshan M K,Gangaboraiah,Ravish H S, et.al. Comparison of safety and immunogenicity of 2 WHO prequalified rabies vaccines administered by one week, 4 site intra dermal regimen (4-4-4-0-0) in animal bite cases. Human Vaccines &Immunotherapeutics 2012;11(7):1748–53.