

Efficacy of Purified Vero Cell Rabies Vaccine (PVRV) under the Zagreb Regimen in Iran

Pooneh Rahimi ^{1*}, Mohammad Reza Shirzadi ², Firouzeh Farahtaj ³, Vida Fallahian ⁴,
Jamal Sharifian ², Nader Howaizi ³, Mansour Shamsipour ⁵, Rouhollah Vahabpour ¹

1 Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran

2 Center of Disease Control (CDC), Ministry of Health, Tehran, Iran

3 WHO Collaborating Center for Reference and Research on Rabies, Pasteur Institute of Iran, Tehran, Iran

4 Department of Vaccination, Rabies post-exposure prophylaxis, Pasteur Institute of Iran, Tehran, Iran

5 School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran

Received Sep 16, 2013; Accepted May 25, 2014

Abstract:

Introduction: Despite the effective pre- and post-exposure treatments, at least 60,000 deaths from rabies occur worldwide every year. Post-exposure treatment is considered as one of the most significant measures for preventing human deaths in exposed individuals. The 2-1-1 rabies post-exposure treatment schedule known as Zagreb regimen is an abbreviated immunization plan in which a tissue culture rabies vaccine is administered intramuscularly at two sites on day 0 and at one site on days 7 and 21. Objectives: In this study the efficacy of rabies vaccine administration under Zagreb regimen in an Iranian group of patients attending Pasteur Institute of Iran was evaluated. **Methods:** Rabies neutralizing antibody titer was measured in 75 serum samples collected from 25 volunteers receiving post exposure treatment by rapid fluorescent focus inhibition test (RFFIT), and ELISA. **Results:** All patients were negative for rabies antibody in both ELISA and RFFIT tests on day 0. A satisfactory rabies virus antibody response with the titer of ≥ 0.5 IU/ml was detected in all patients on day 21 and two weeks after the completion of vaccination (day 35). **Conclusions:** Rabies immunization with rabies Vero-cell vaccine (PVRV) under the 2-1-1 schedule (Zagreb regimen) could result in an adequate immune response without any adverse effect. However a more comprehensive comparative study is underway to confirm these findings. *Vac Res, 2014, 1 (1): 25 - 27*

Keywords: Zagreb regimen, PVRV, Rabies vaccines, Post-exposure prophylaxis.

INTRODUCTION

Human rabies is a viral encephalitis caused by RNA viruses belonging to the family Rhabdoviridae, genus *Lysavirus* with high mortality that is usually transmitted by the bite or scratch of a rabid animal to humans [1].

Treatment of the exposed person depends on the appraisal of the risk of infection and consists of no treatment for category I, vaccine alone for category II and immediate treatment of category III by vaccination therapy with rabies immunoglobulin [2]. There are two main post-exposure prophylaxis protocols with intramuscular (IM) ad-

ministration of cell culture rabies vaccines recommended by WHO [2, 3]. The Essen protocol is the gold standard for immunization consisting of 5 single doses of vaccine injected intramuscularly over a 28-day period and the Zagreb regimen known as 2-1-1 protocol is administered over a 21-day duration with two doses at two separate

***Corresponding Author:** Pooneh Rahimi

Department of Hepatitis and AIDS, Pasteur Institute of Iran, No. 69, Pasteur Ave, Tehran, Iran, 1316943551.

Email: Prahimi@pasteur.ac.ir

Tel: +98 21 64112240

Fax: +98 21 66969291

sites on D0, followed by single doses on days 7 and 21 [4].

Human diploid cell culture vaccine (HDCV) was used throughout the country prior to 1992, but was replaced by rabies Vero-cell vaccine (Aventis Pasteur, France) under the Essen 5- dose regimen for both pre- and post exposure immunization [5, 6, 7]. Post exposure vaccination using ESSEN regimen has been very successful in reducing the number of human deaths due to rabies however, simplifying the procedure could save time and money and consequently result in better patient compliance especially for those living in remote areas. The 2-1-1 regimen needing only 3 visits to the health centers seems a more acceptable alternative both economically and time-wise. This study was undertaken to assess the antibody level raised in patient volunteers immunized with 2-1-1 schedule.

MATERIALS AND METHODS

Patients. Participants were 25 healthy volunteers with animal bites of the second category who were under treatment in the Department of Vaccine, Rabies Treatment and Prophylaxis, Pasteur Institute of Iran. According to the Helsinki Declaration, the aim of the project and the blood sampling procedures were explained to the patients and the consent form was signed by each volunteer.

This study was approved by the Ethical Committees of Pasteur Institute of Iran, and the committee of Rabies Control, Ministry of Health, Treatment and Medical Education of Iran. Patients were between 18-60 years old and immune-competent, had no history of rabies vaccination, were not taking immune-suppressives or Chloroquine, had not been immunized with other killed or live vaccines within the last 3 weeks of rabies vaccination, and did not have any acute febrile disease. Three blood samples were taken on days 0, 21 and 35.

Assessment of post-immunization immune response. Rabies neutralizing antibody titer was measured using the rapid fluorescent focus inhibition test (RFFIT) as described by WHO [8, 9] using international reference sera as control. Briefly, The BSR cells (a clone of baby hamster ovary cells) were grown and used in the test as described previously [9]. Sera were diluted at 1:3 in the 96-wells micro plates and mixed with a constant dose of challenge virus sufficient to cause infection in 80% of the cells and the sera-virus mixture was incubated at 37°C for 1 h. After incubation, susceptible cells were added to the serum-virus mixtures, and after a further 24 h of incubation, the cell monolayer was acetone fixed and stained with anti-nucleocapsid conjugate (Bio-Rad, France) according to the manufacturer's instructions and the fluorescent foci counted. Calculations were done by the Reed and Muench method [8].

Anti-rabies neutralizing antibody titers were also determined by ELISA (Bio-Rad, USA) according to the manufacturer's instruction.

Statistical Analysis. Data from RFFIT and ELISA were analyzed using the repeated measure analysis of variance. Sex and age were considered as the covariates in this

study and p-values <0.05 were considered significant.

RESULTS

Of the total of 25 patients 15 (60%) were male and 10 (40%) female with the median age of 27.2 and 26 respectively. All patients were negative for rabies antibody in both ELISA and FRRIT tests on D0.

An antibody response significantly higher than >0.5 IU/ml was detected in all subjects on day 21 and on day 35.

Table 1. Rabies antibody titer obtained by rapid fluorescent focus inhibition test (RFFIT)

Vaccination Method	Days post Vaccination	Mean titer
Zagreb	RFFIT-0	ND*
Zagreb	RFFIT_21	15.6
Zagreb	RFFIT_35	27.3

*ND: Not Detectable

The geometric mean titer (GMT) of the RFFIT test was 15.6 and 27.3 IU/ml in serum samples of D21 and D35 respectively (Table 1), and 2.31 and 4.9 in sera of D21 and D35 by ELISA (Table 2).

Table 2. ELISA results of rabies antibody titer

Vaccination Method	Days post Vaccination	Mean titer
Zagreb	ELISA-0	ND*
Zagreb	ELISA_21	2.31
Zagreb	ELISA_35	4.9

*ND: Not Detectable

In this study, there was no significant correlation between sex and age (covariates) and the titer of the anti-rabies antibody ($P > 0.6$), but the difference between the antibody level on D0, D21 and D35 was statistically significant ($P < 0.016$). No adverse effects such as pruritus, erythematous rash, fever, nausea, pain were observed in the vaccines.

DISCUSSION

The onset of clinical symptoms in human rabies invariably results in death, but prompt and appropriate post-exposure prophylaxis prevents the fatalities [2]. Selection of an appropriate regimen for post-exposure treatment from the several approved by WHO is a decision made by individual countries. The efficacy of an immunization protocol is judged by the presence of neutralizing antibody in the sera of the vaccinees to the level of >0.5 IU/ml by day 14 as recommended by WHO [2, 10]. Essen immunization which is the gold standard of rabies post-exposure treatment (PET) consists of 5 intramuscular injection of 0.1 ml out of an 0.5 ml PVRV vial which has to be used within 6-8 h after opening with or without rabies immunoglobulin. The cost of the vaccine, coupled with expenditures incurred for travelling, visits to the clinics and other ser-

vices over a period of 28 days make this protocol a costly one. It has been estimated that the cost of PET in Asia and Africa accounts for 83% of the total budget allocated for rabies control [11]. Zagreb regimen is a shortened version of Essen treatment, reducing the number of injections, visits and duration of the treatment, making it economically more acceptable. Clinical trials and epidemiological studies have demonstrated the efficacy of Zagreb protocol [8, 10, 12]

All patients were negative for anti-rabies antibody prior to treatment (D0) but subsequently showed significant increase in anti-rabies antibody on day 21 (Table 1). In a study conducted in China using locally produced PVRV administered under the 2-1-1 regimen similar anti-body levels were reported [8]. Furthermore, Liu et al. [8] did not observe a significant difference in antibody level between subjects undergoing vaccination under Essen protocol with those of Zagreb method.

In line with the WHO recommendations, antibody titers of ≥ 0.5 IU/ml was considered indicative of seroconversion and in this study, this value was almost 3 times higher; therefore seroconversion was 100% by day 21. In conclusion, this study showed that rabies vaccination with PVRV under the 2-1-1 schedule (Zagreb regimen) could result in satisfactorily seroconversion without any adverse effect.

ACKNOWLEDGEMENTS

We wish to thank the staffs of the WHO Collaborating Center for Reference and Research on Rabies, Pasteur Institute of Iran. We are also grateful to Dr. Mohammad Mehdi Gouya for his administrative help and support. This project was financed by Center for Disease Control (CDC), Ministry of Health, Tehran, Iran under the grant number 1621.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Lyles DS, Rupprecht CE. Rhabdoviridae. In: Knipe D, Howley P, et al., editors. *Fields virology*. 5th ed. Philadelphia: Lippincott, Williams, and Wilkins, 2007; 1363-408.
2. Hampson K, Cleaveland S, Briggs D. Evaluation of Cost-Effective Strategies for Rabies Post-Exposure Vaccination in Low-Income Countries. *PLoS Negl Trop Dis*, 2011; 5 (3): e982.
3. WHO Expert Committee on Rabies, Eighth Report 1992. Geneva, World Health Organization, 1993; *Australian Veterinary Journal*, 70: 432.
4. The World Health Report 1996 - Fighting disease, Fostering development. World Health Organization, 1996; 57-8.
5. Chutivongse S, Wilde H, Fishbein DB, Baer GM, Hemachudha T. One-year study of the 2-1-1 intramuscular postexposure rabies vaccine regimen in 100 severely exposed Thai patients using rabies immune globulin and Vero cell rabies vaccine. *Vaccine*, 1991; 9 (8): 573-6.
6. Fayaz A. Two Decades Experiences of Using Cell Culture Rabies Vaccine in an Endemic Country New Approach to Vaccine Development 97 (NAVD 97); 1997. Universitat Wien, Wien, Osterreich.
7. Fayaz A, Pourtaghva M, Osouli M, Simani S, Rouintan B. Post exposure treatment with HDC vaccines in Iran for ten years, 1988; *Zoonoses Congress*, Tehran.
8. Liu H, Huang G, Tang Q, Li J, Cao S, Fu C, Cao Q, Liu B, Pan H, Wang M. The immunogenicity and safety of vaccination with purified Vero cell rabies vaccine (PVRV) in China under a 2-1-1 regimen. *Hum Vaccin*, 2011; 7 (2): 220-4.
9. *Laboratory Techniques in Rabies*, edited by Meslin F-X, Kaplan MM, Koprnwsk H. 4th ed, WHO, Geneva, 1996.
10. Warrell MJ. Current rabies vaccines and prophylaxis schedules: Preventing rabies before and after exposure. *Travel Med Infect Dis*, 2012; 10 (1): 1-15.
11. Wera E, Velthuis AG, Geong M, Hogeveen H. Costs of rabies control: An economic calculation method applied to flores island. *PLoS One*, 2013; 8 (12): e83654.
12. Rupprecht CE, Briggs D, Brown CM, Frank R, Katz SL, Kerr HD, Lett S, Levis R, Meltzer MI, Schaffner W, Cieslak PR. Evidence for a 4-dose vaccine schedule for human rabies post-exposure prophylaxis in previously non-vaccinated individuals. *Vaccine*, 2009; 27 (51): 7141-8.
13. Jackson AC, Warrell MJ, Rupprecht CE, Ertl HC, Die-tzschold B, O'Reilly M, Leach RP, Fu ZF, Wunner WH, Bleck TP, Wilde H. Management of rabies in humans. *Clin Infect Dis*, 2003; 36 (1): 60-3.
14. WHO expert consultation on rabies, First report. WHO Technical Report Series 931, Switzerland. 2004.