Original Article

Safety and Non-Suppressive Effect of a Purified F(ab')$_2$ Equine Rabies Immunoglobulin in WHO Category III Rabies Exposed Patients

Jatuporn Sirikun MD[1], Ploypailin Ratanachinakorn MD[1], Somphon Roeksomtawin MD[1], Athiwat Primsirikunawut DVM[2]

[1] Division of Trauma Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand
[2] National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Background: Early management of rabies post-exposure patients with rabies immunoglobulin [RIG] is highly recommended in WHO category III of rabies exposure. Equine rabies immunoglobulin [ERIG] is as economical and effective as compared to human rabies immunoglobulin [HRIG] but more affordable to patients in developing countries.

Objective: To evaluate safety and suppressive effect of a F(ab')$_2$ ERIG (VINRAB®) in WHO category III rabies exposed patients.

Materials and Methods: Eligible patients received wound treatment, single dose ERIG around the wound and intramuscularly [IM], and 5-dose purified chick embryo cell [PCEC] rabies vaccine (Essen regimen, 1.0 ml IM on days 0, 3, 7, 14, and 28). Sera before immunization were tested for rabies viral neutralizing antibodies by RFFIT on days 0, 7, 14, and 28. The adverse reaction was monitored for 60 days.

Results: The results showed on day 14, 100% of the patients had seroconverted without suppressive effect. No serious adverse reactions or immediate reaction were observed.

Conclusion: The purified F(ab')$_2$ ERIG is considered well tolerated and can be utilized in parallel with standard IM dose of the PCEC rabies vaccine.

Keywords: Rabies, Safety, Non-suppressive effect, ERIG, Post-exposure prophylaxis, Rabies antibodies, RFFIT

J Med Assoc Thai 2018; 101 (5): 563-8
Website: http://www.jmatonline.com

Human rabies has the highest case-fatality rate in endemic countries despite rabies biologicals for prevention and treatment are available[1,2]. In Thailand, the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, reported four patients died of rabies in the first quarter of 2016. The World Health Organization [WHO] highly recommends for category III rabies exposed patients, besides wound treatment, passive immunization by rabies immunoglobulin [RIG] to provide immediate protection during the first one to two weeks post exposure from development of rabies since there is a lag period before the full development of active immunity following vaccination (i.e., not until day 14 following vaccination)[3]. WHO recommended that RIG should be given within seven days after the first vaccine dose or before the administration of the third dose to avoid suppression of vaccine-induced neutralizing antibody production[4]. Human RIG [HRIG] is no doubt the product of choice for post-rabies exposure prophylaxis [PEP]; however, it is not affordable by most patients in the developing world because its cost is four to six times higher as compared to equine RIG [ERIG]. Lately, it has been shown that several ERIGs have undergone significant improvement in purification techniques and decreased its level of protein content. This caused less adverse events than previously. Thus, the evaluation of economical PEP, applied adequately with clinical insignificant adverse events, is needed to be able to give more patients to this treatment. The F(ab')$_2$ ERIG (VINRAB®) produced by VINS Bioproducts Ltd., Mahaboobnagar, India, was used in the present study. VINRAB® has been widely used in endemic countries, i.e., India, Nepal, Sri Lanka, Philippines, and Thailand, etc. The product under WHO GMP certified manufacturing process involves purification technique of pepsin and caprylic acid to

Correspondence to:

Sirikun J. Trauma Division, Department of Surgery, Faculty of Medicine Siriraj Hospital, 2 Wang Lang Road, Siriraj, Bangkoknoi, Bangkok 10700, Thailand.
Phone: +66-2-4197727, **Fax**: +66-2-4197730
Email: j_sirikun@hotmail.com

How to cite this article: Sirikun J, Ratanachinakorn P, Roeksomtawin S, Primsirikunawut A. Safety and non-suppressive effect of a purified F(ab')$_2$ equine rabies immunoglobulin in WHO category III rabies exposed patients. J Med Assoc Thai 2018;101:563-8.

get the purified F(ab')$_2$ segment. The unpublished post-marketing study of the F(ab')$_2$ VINRAB® ERIG showed that the product was found to be safe and efficacious. Most patients that returned their reply postcard had no adverse effect, one developed fever and pain at injection site, and another complained of itching at ERIG infiltrated site. The eight patients confirmed exposure to rabies and survived following treatment after a 16-month follow-up[5]. Despite that the product has been commercially available in Thailand, it has never been studied in Thais. The result of this study is expected to guide the healthcare provider in using this purified ERIG in Thai patients.

The present study aimed to assess the safety and suppressive effect of PEP using the F(ab')$_2$ ERIG manufactured by VINS Bioproducts Ltd., VINRAB®, with concomitant intramuscularly [IM] purified chick embryo cell [PCEC] rabies vaccine administered in WHO grade III exposed patients.

Materials and Methods

The present study was a single center, prospective cohort, open-label conducted in accordance with the latest revision of the Declaration of Helsinki, with Good Clinical Practices, and with local regulatory requirements. The protocol was approved by the Siriraj Institutional Review Board (IRB approval number Si178/2015).

This is an investigator initiated study and the authors are solely responsible on protocol development, study conduct and implementation, data collection and analysis, as well as manuscript preparation. VINS Bioproducts Ltd., India, and Biogenetech Co., Ltd., Thailand supported only F(ab')$_2$ ERIG and no involvement with the subject matter, materials, or methods discussed in the manuscript apart from those disclosed.

Subjects

Sixty Thai females or males aged 18 years and above who were in WHO category III of rabies exposure and required rabies post exposure treatment screened at the Trauma division, Siriraj Hospital, Bangkok, Thailand. Exclusion criteria were positive skin test to ERIG or known hypersensitivity to ERIG or its excipients, co-enrolled with other studies, participated in other investigational drug studies, use of other investigational drugs within four weeks or five times the half-life of the investigational drug, currently pregnant or breast feeding, presenting with wound at eye(s) or eye lid(s), received rabies vaccination more than seven days for this exposure, history of complete pre-exposure or post-exposure regimen with at least three doses, known allergy to egg or poultry meat, history of previous exposure to equine sera (anti-tetanus, snake anti-sera, ERIG, diphtheria, etc.), significant illness that might harm or increase the risk to the patients, and history of drug abuse or alcoholism.

ERIG and rabies vaccine administration products

All patients received the skin test, which consisted of an intradermal [ID] injection of a 1:100 dilution of ERIG (VINRAB® lot 08AR14012, 0.02 ml) on the lateral aspect of the forearm to obtain a wheal in the skin induration 3 mm in diameter. A control ID injection of physiological saline solution was used as a control on the other side of the arm. The evaluation performed 20 minutes later was considered to be positive if erythema (≥10 mm in diameter), local edema, or a systemic reaction were observed while the control was negative. If the wheal was doubly positive, for the ERIG and the control, the test was considered certain only if there was a wheal of 10 mm in diameter at the ERIG site. All subjects with a positive skin test were excluded from the study. The F(ab')$_2$ ERIG (available in the strength of 1,000 IU/5 ml vial) was administered in a single dose of 40 IU/kg body weight, of which maximum possible amount was infiltrated locally around and into the wound and the remained was given IM.

Rabies vaccine (Rabipur®), an inactivated rabies virus, Flury LEP type, PCEC, from Chiron Behring Vaccines Pvt Ltd., Ankleshwar, India was chosen because it was extensively investigated in PEP and had a well-established immunogenicity and tolerability profile[6].

Study procedures

Each patient had screening, baseline, and four subsequent follow-up visits at days 3, 7, 14, and 28, and two phone-call visits at days 1 and 60. At screening, all patients had local wound cleaning and dressing done. We evaluated the exposure risk, and reviewed eligibility before obtaining consent prior to any other procedure performed. Enrolled patients were reviewed for additional history taking, demographic, medical history, rabies exposure history, rabies vaccination history, wound details, and the details of the animal responsible for the bite. Single dose of ERIG and the first dose of rabies vaccine were administered on the first visit. On days 3, 7, 14, and 28, the patients were IM administered the second to fifth doses of rabies

vaccine. The supportive treatments such as anti-tetanus, antimicrobial, and supportive treatment were provided at the discretion of the study doctors.

Determination of rabies viral neutralizing antibodies

Blood samples (5 ml) were taken before immunization on days 0, 7, 14, and 28 in tubes with anticoagulant under sterile conditions. Serum aliquots were sent to the National Institute of Health, Department of Medical Sciences, Thailand, for analysis. The level of rabies viral neutralizing antibodies [RVNA] activity against the challenge virus standard 11 [CVS-11] strain of rabies virus in human serum samples were measured by rapid fluorescent focus inhibition test [RFFIT]. Two-fold serial dilutions of heat-inactivated serum samples were incubated with the CVS-11 strain in 96-well tissue culture plate for 90 minutes at 37°C. Mouse neuroblastoma cells were then added to the serum-virus mixture and incubated for an additional 20 to 24 hours at 37°C with 5% CO_2. The 96-well tissue culture plates were then acetone fixed and stained with an anti-rabies FITC conjugate and were examined using a fluorescence microscope at x200 magnification to score the virus-infected cells (foci). The number of positive fields with rabies-infected cells per well was recorded. The neutralization endpoint titer was defined as the highest sample dilution at which 50% of the observed microscopic fields contain one or more infected cells. The RVNA titers are mathematically interpolated using the Reed and Muench method or a Reed and Muench chart for assigning a RFFIT titer[7].

The endpoint neutralization titer of the test serum is then transformed into international units [IU]/ml values by calibration against the endpoint neutralization titer of the second International Standard for anti-RIG in human, which was measured in the same assay run, with an assigned potency value of 1.0 IU/ml[7].

Calculation of virus-neutralizing antibody titers

Residual virus is detected using a fluorescence microscope. The serum neutralization endpoint titer is defined as the dilution factor of the highest serum dilution at which 50% of the observed microscopic fields contain one or more infected cells. This value may be obtained by mathematical interpolation. Alternatively, a 100% neutralization titer may be determined by recording the highest serum dilution at which 100% of the challenge inoculum is neutralized and there are no infected cells in any of the observed fields. The titer of antibody in the test serum can be obtained by comparison with the titer of the international reference standard included in each test. Results were expressed in IU per ml.

Safety analysis

Local reactions at or near injection sites, systemic, neurological, and other reactions were carefully recorded over the 60-day follow-up. Systemic reactions were classified by body systems, skin/mucosal, cardiovascular, respiratory, and gastrointestinal. At baseline, adverse reaction was recorded at 1-hour and 30-minute after ERIG and rabies vaccine administration, respectively.

Statistical analysis

Descriptive analysis was used to report median, interquartile range [IQR], frequency, and proportion as appropriate for characteristic and demographic variables and adverse reactions. The geometric mean titer of RFFIT titer with 95% confidence interval and antibody titer range were analyzed.

Results

Demographics

Forty-nine adult patients that had never been rabies vaccinated or incomplete post-exposure regimen were enrolled. The patient and animal characteristics at baseline are shown in Table 1. All patients were

Table 1. Patient characteristics at baseline (n = 49)

Category	Characteristics	Frequency (%)
1	Patient characteristics	
	Female:male	35:14 (71:29)
	Age (years)*	38 (30 to 48)
	BMI (kg/m^2)*	24 (20 to 31)
2	Exposure characteristics	
	Severity of exposure, Grade III	49 (100)
	Type of exposure, bite	49 (100)
	Area of exposure	
	- Hands	16 (33)
	- Arms	7 (14)
	- Body	3 (6)
	- Legs	20 (41)
	- Feet	9 (18)
3	Animal characteristics	
	Dog:cat	32:17 (65:35)
	Animal age	
	- 3 to 6 months	1 (2)
	- 6 to 12 months	5 (10)
	- More than 1 year	21 (43)
	- Do not know	22 (45)
	No owner or unknown	21 (43)
	No or unknown of rabies vaccination	37 (75)

BMI = body mass index

* The figures are expressed as median and IQR, and not the frequency

exposed by a bite from dogs or cats (65% vs. 35%), unknown animal age (45%), no owner or unknown (43%), and no or unknown rabies vaccination history (75%). They received wound cleaning prior to ERIG and 5-dose IM rabies vaccine course and all recovered. All 49 patients were ERIG administered by local infiltration around the wound and 37 patients were also IM administered. The mean dose of ERIG given by local infiltration around the wound was 1,158 IU (±950) and by IM route was 1,882 IU (±768).

Adverse reactions

No patient developed immediate reactions to ERIG administration, serious adverse reactions, or required steroid therapy for their adverse reactions. Only mild and transient adverse reactions were observed in five patients such as erythematous rash, pruritis, local reaction at injection site, and lip swelling (bitten site) (Table 2). One patient experienced rash and itching, which may be due to allergy to anti-microbials. There was no instances of pain, fever, and severe systemic reaction in these patients. Within the follow-up period of 60 days none of the patients developed signs or symptoms of rabies.

RFFIT serology results

The lower limit of detection of antibody was 0.03 IU/ml, while the threshold for a positive result was 0.5 IU/ml or more, as naïve sera can range between 0.07 and 0.46 IU/ml. The anti-rabies titers are shown in Table 3. The results were 35% and 100% of patients had seroconversion above 0.5 IU/ml at day 7 and day 14, respectively.

Table 2. Number and percentage of subjects with at least one adverse reaction (n = 49)

Adverse reactions	Number (%)
Local reactions	
Itching at injection site	2 (4.08)
local reaction at injection site	2 (4.08)
Systemic reactions	
Rash	2 (4.08)
Generalized erythema	3 (6.12)
Generalized pruritis	1 (2.04)

Discussion

The key objective of PEP is to neutralize the rabies virus inoculated by ERIG and to immunize the patient for rabies neutralizing antibody from the vaccination immediately following exposure so that clinical manifestation of rabies does not develop. The recommendations for PEP depend on the type of contact with the suspected rabid animal and the vaccination status of the individual based on three categories of risk and exposure (I-III)[1,2]. In the present study, only patients with category III severity were recruited (transdermal bite, scratches, or contamination of mucous membrane, which would require use of RIG plus vaccine). Wound treatment, vaccine administration (5-dose IM regimen), and ERIG administration were done to provide immediate protection against rabies before active immunization is high enough to the protective level. On day 7, the proportion of seroconverted patients rose to 35% compared to 6.0% to 58.3% in other studies with no antibodies suppressive effect observed[8,9]. One hundred percent of the patients reached protective level of 0.5 IU/ml from day 14 to day 28 of testing, which RFFIT titer at 0.5 IU/ml is currently thought to be the sero-protective level for human subject immunized against rabies[8]. Previously, the simultaneous administration of RIG together with rabies vaccine for PEP raised concern on possible interference of RIG on the patient's own active immune response to the vaccine[5,10-12]. In some studies, HRIG indicated more suppress immunogenicity of rabies vaccine than ERIG when administered by 2-1-1 regimen, with note that it was probably because of the longer half-life of HRIG compared to ERIG[11-13].

In WHO expert consultation on rabies in 2013, skin tests are not recommended before administration of ERIG[14]. However, the present study performed skin test as per Thai guideline and manufacturer recommendation, with full preparation to manage

Table 3. RFFIT serology results for patients who received F(ab')2 ERIG and 5-dose IM rabies vaccine for PEP (n = 49)

	Rabies RFFIT titer on days			
	Baseline	Day 7	Day 14	Day 28
Geometric mean titers (IU/ml)	Negative	0.36	3.93	6.31
95% CI	-	0.28 to 0.46	3.01 to 5.12	5.15 to 7.74
Titer range (IU/ml)	0.07 to 0.46	0.07 to 6.15	0.74 to 53.46	0.95 to 33.04
Number of patients with antibody titer more than or equal to 0.5 IU/ml, n (%)	-	17 (35)	49 (100)	49 (100)

ERIG = equine rabies immunoglobulin; IM = intramuscularly; PEP = post-rabies exposure prophylaxis; RFFIT = rapid fluorescent focus inhibition test

anaphylaxis[15,16]. Patients with positive skin test (3%) were excluded from the present study and were given HRIG instead of ERIG. No pain and fever were reported, nor was immediate adverse reactions in the present study group. Few mild and transient adverse reactions were observed in five patients (10.2%), which could be attributed either to the ERIG or the PCEC vaccine. They were comparable to those reported in several ERIG PEP studies (0.9% to 22.9%)[5,9,17-19]. Difference in manufacturing, purification process, and protein content in ERIG products may cause significant differences in adverse reaction rates. Modern ERIG products are safe and effective when compared to crude equine sera or ERIG products in the past, as were $F(ab')_2$ ERIG product used in the present study. However, anaphylaxis or serum sickness may not always develop, even in patients severely exposed to rabies that have positive ERIG skin test[20].

A couple of months of follow-up of patients is not sufficient to confirm the effectiveness of ERIG since the incubation period could be longer[1]. However, none of the recruited patients reported any signs and symptoms of rabies within the study period. Engagement among global and local health organizations and institutions have been formed over three decades in the prevention and control of rabies, particularly in low- and middle-income countries with aim to become rabies-free in 2020 to 2025. Widespread use of ID PEP regimen could become a widespread practice in other countries in Asia and elsewhere. Therefore, further studies of the $F(ab')_2$ VINRAB® ERIG with ID vaccine regimen is worth to investigate to find more alternative cost saving and safety PEP in patients in developing countries.

Conclusion

The $F(ab')_2$ ERIG (VINRAB®) is safe when given in severe exposures to rabies simultaneously with WHO standard 5-dose rabies vaccine IM regimen. All patients had seroconverted at day 14 without antibody suppressive effect.

What is already known on this topic?

The unpublished post-marketing study of the $F(ab')_2$ VINRAB® ERIG showed that the product was found to be safe and effective. Most patients who returned their reply postcard had no adverse effect, while one developed fever and pain at injection site and another complained of itching at ERIG infiltration site. The eight patients that had confirmed exposures to rabies survived following treatment after 16-months follow-up[8].

What this study adds?

Despite the product is commercially available in Thailand, it has never been studied in Thai patients. Result is expected to guide the healthcare provider in using this purified ERIG in Thai patients.

Acknowledgment

The authors are grateful to the nurse team for recruitment and treatments and the National Institute of Health, Department of Medical Sciences, Thailand, for carrying out the antibody determinations.

Financial disclosure

The present study was financial supported by VINS Bioproducts Ltd., India, and Biogenetech Co., Ltd., Thailand.

Potential conflicts of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials disclosed in the manuscript apart from those disclosed.

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