IMMUNOGENICITY AND SAFETY OF PURIFIED VERO-CELL RABIES VACCINE IN SEVERELY RABIES-EXPOSED PATIENTS IN CHINA

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Abstract. The immunogenicity and safety of a purified Vero-cell rabies vaccine (PVRV, VERORAB®; Aventis Pasteur, France) were evaluated in 171 patients treated for severe exposure to rabies (WHO category III contacts) at the Shandong Provincial Antiepidemic Station in Jinan and an EPI center in Ping Yin, China. Post-exposure treatment consisted of a single dose of equine rabies immunoglobulin (ERIG, 40 IU/kg body weight) on Day (D) 0, and intra-muscular administration of PVRV on D 0, 3, 7, 14 and 28. Antirabies antibody levels were evaluated on D 0, 7, 14, 28, 90 and 180 using the rapid fluorescent focus inhibition test. By D 14 all subjects had seroconverted (≥0.5 IU/ml), with a geometric mean titer of 50.3 IU/ml. Antibody titers remained above the seroprotection threshold in all patients for 3 months, and in 98.2% of subjects for 6 months. All patients were still alive 6 months after the start of treatment. PVRV and ERIG were shown to be well tolerated and no serious adverse events were observed. Following PVRV administration, 12 patients (7.0%) had at least one local reaction (mostly pruritus, erythematous rash and pain). Fourteen patients (8.2%) developed local reactions at the site of ERIG administration. Twelve patients (7.0%) developed systemic reactions following post-exposure treatment, the most frequent of which were pruritus, rash and vertigo. This study demonstrates that PVRV is immunogenic and safe in Chinese patients treated according to WHO recommendations for severe rabies exposure.

INTRODUCTION

Rabies is a viral encephalitis that is transmitted to humans from infected animals, most commonly by a bite or a scratch. In untreated cases, the disease is universally fatal and, despite the existence of effective pre-exposure and post-exposure treatments, at least 60,000 deaths from rabies occur worldwide every year (WHO, 1996). Rabies is endemic in the People's Republic of China, particularly in nine provinces including the study region, and accounted for more than 42,000 human deaths between 1983 and 1992 (China Country Report, 1993). Mass animal immunization programs are culturally, economically and administratively difficult to carry out in China, and therefore post-exposure treatment remains one of the most significant measures for combating human rabies in this country (China Country Report, 1993).

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This study was performed at rabies treatment centers in Jinan and Ping Yin, China.

In China, two types of primary hamster kidney cell (PHKC) rabies vaccine (adjuvanted and freeze-dried concentrated) are produced locally. Although these vaccines present a significant advance over the Semple type vaccine used previously, the primary hamster cells used in manufacture are yet to be approved by Western regulatory authorities, and the immunogenicity, and thus efficacy, of the vaccines are not ideal. The failure rate for these vaccines, reported from two provinces in 1988, was between 1 and 4 per 1,000 vaccinations (Fangtao *et al*, 1988a,b; Ren, 1988). Ideally, a rabies vaccine should evoke an early, high and persistent neutralizing antibody response. However, the addition of adjuvant to this vaccine may delay the immune response (Lin, 1990), and up to 10 additional PHKC injections may be required to achieve a persistent response (Ren, 1988). Purified Vero-cell rabies vaccine (PVRV), an alternative to these locally produced vaccines, has been recommended by the World Health Organization (WHO) since 1992 for pre-exposure and post-exposure immunization against rabies (WHO, 1992). Worldwide experience shows that PVRV manufactured by Aventis Pateur (VERORAB®) is highly effective and more than 15 million doses have been

distributed since 1988 for pre-exposure or post-exposure treatment of rabies.

For severe exposure to rabies [*ie* where single or multiple transdermal bites or scratches are present and/or contamination of mucous membranes has occurred (WHO category III contacts)] the WHO recommends a combination of local treatment of the wound, passive immunization with rabies immunoglobulin (RIG) and vaccination with cell culture vaccine (WHO, 1992). The life-saving benefit of adding RIG to post-exposure therapy has been established in such patients (Chutivongse *et al*, 1990, 1991; Fangtao *et al*, 1988a; Wilde *et al*, 1996). However, there have been reports that, under certain conditions, concomitant administration of RIG may partially suppress the immune response to rabies vaccine (Vodopija *et al*, 1988; Wilde *et al*, 1989; Chutivongse *et al*, 1991; Fescharek *et al*, 1991). It is necessary to confirm the immunogenicity of a rabies vaccine in a post-exposure protocol where the vaccine and RIG are given concomitantly.

There have been very few studies of rabies vaccines in China, especially in subjects actually exposed to rabies. In order to demonstrate that PVRV is a safe and effective alternative to locally produced rabies vaccines in China, we investigated the immunogenicity of VERORAB® [Aventis Pasteur (AvP)] in patients from the Jinan and Ping Yin regions treated for severe exposure to rabies (WHO category III contacts). According to WHO recommendations for the treatment of such patients, equine rabies immunoglobulin (ERIG; Pasteur Antirabies Serum®; AvP) and PVRV were administered concomitantly on the first day of post-exposure treatment.

MATERIALS AND METHODS

Patients

The study was conducted by the same investigational team at two study centers – the Shangdong Provincial Anti-epidemic Station, Jinan, and the Expanded Program for Immunization Center in Ping Yin, China. Patients with suspected or proven severe exposure to rabies (WHO category III contacts) and presenting within 48 hours of exposure were enrolled. At the time that this study was performed, patients were excluded if there was a positive skin test following intradermal administration of approximately 0.02 ml of ERIG (1 in 10 dilution with normal saline). A positive skin test was defined as erythema (>10 mm), local edema or systemic reaction occurring 15 minutes after the injection. Other exclusion criteria were signs of rabies, acute febrile illness (axillary temperature ≥38.5ºC), immunosuppression as a result of disease or treatment, history of rabies vaccination, history of severe reaction to equine sera, and inability to comply fully with study protocol.

The trial was conducted in accordance with the latest revision of the Helsinki Declaration, Chinese Good Clinical Practice, and local regulatory requirements. The protocol was approved by the National Institute for Control of Pharmaceutical and Biological Products. All patients (and/or their parent or guardian if the patient was under 18 years of age) gave their oral or written informed consent before being included in the trial.

Treatment

Patients received the WHO recommended post-exposure treatment protocol for severe rabies exposure. The wound site was washed abundantly with soap and water, followed by application of 40-70% alcohol, iodine tincture or 0.1% quaternary ammonium solution. Patients received PVRV (0.5 ml) on D 0 (day of inclusion), 3, 7, 14 and 28. Injections were performed intramuscularly, perpendicular to the skin, into the deltoid. Vaccine was used within 1 hour of reconstitution. All patients received a single dose of ERIG (40 IU/kg bodyweight) on the same day as the first injection with PVRV, *ie* D 0. If anatomically feasible, the largest amount possible of the ERIG dose was administered by deep instillation into the wound(s)/exposure sites, as well as by infiltration around the wound(s)/exposure sites, and the remainder of the dose (if any) was administered intramuscularly in the gluteal area.

Products

PVRV and ERIG were manufactured by Aventis Pasteur, France. Lyophilized PVRV (VERORAB®; usual commercial batch no. L0898) was supplied in glass vials, each containing vaccine equivalent to one dose, *ie* ≥2.5 IU of rabies antigen according to the National Institutes of Health test for potency (Seligmann, 1993). Diluent [0.5 ml 0.4% (w/v) sodium chloride] was provided in a pre-filled syringe with a sealed 16 mm needle that was used for injection after reconstitution of the vaccine.

Each 5 ml vial of ERIG (Pasteur Antirabies Serum®; usual commercial batch no. L5983) con-

tained not less than 1,000 IU of specific antirabies equine immunoglobulin [mouse neutralization test (Atanasiu, 1993)], with 50 mg glycine, 25 mg sodium chloride, no more than 15 mg tricresol as preservative, and water to bring the final volume to 5 ml.

Immunogenicity

Blood samples were taken for serological analysis on D 0, 7, 14, 28, 90 and 180. Blood samples were centrifuged and sera frozen at -20ºC until time of analysis. Sera were analyzed at the Aventis Pasteur Clinical Sero-immunology Laboratory (Val de Reuil, France). This establishment undergoes regular quality assurance audits for each assayed antigen, and provides analyses for several WHO reference rabies centers. Rabies neutralizing antibody levels were measured using the rapid fluorescent focus inhibition test (RFFIT). International reference sera were used to express the results in IU/ml. In accordance with WHO recommendations of acceptable seroprotection (WHO, 1992), subjects were considered to have seroconverted for rabies neutralizing antibody if they achieved a RFFIT titer ≥0.5 IU/ml.

Safety evaluation

Patients were observed for 30 minutes after each vaccination for the occurrence of any immediate reactions. Local adverse reactions to ERIG were assessed from D 0 to D 7, and reactions to PVRV were assessed from D 0 to D 28 by patient interview according to a predefined list of reactions. Local and systemic adverse events were classified as non-severe or severe (Table 1). For any systemic event, the investigator reported the date of occurrence, the duration, the severity and the likely causal relationship with the test products.

Statistical analysis

All patients who were seronegative for rabies neutralizing antibodies at baseline and who received at least one injection of PVRV were included in the immunogenicity analysis. Geometric mean titer (GMT) values with 95% confidence intervals were calculated on D 0, 7, 14, 28, 90 and 180. The percentage of patients who seroconverted for rabies antibody (RFFIT value ≥0.5 IU/ml) was calculated at each sampling time. For the safety analysis, all patients who received at least one injection of ERIG were included. Statistical analysis was performed by the Aventis Pasteur Biometry Department (Marnes-la-Coquette, France) using SAS Version 6.10 software (SAS Institute Inc, Cary, NC, USA).

RESULTS

Patient population

One hundred and seventy-five patients were enrolled in the study and underwent ERIG skin tests. Four patients (2.3%) developed a local induration 11-18 mm in diameter associated with local edema, indicating a positive ERIG skin test, and were excluded from the trial. One hundred and seventy-one patients were therefore included in the trial and received post-exposure treatment according to the study protocol. The majority of subjects were male (60.2%), and most were over 15 years of age (80.1%).

Table 1
Definition of severe adverse events.

Adverse event	Severe
Local	
Pain	Disabling/limited motion
Erythema	Diameter >2 inches (>5 cm)
Bruising	Diameter >2 inches (>5 cm)
Induration/swelling	Diameter >2 inches (>5 cm)
Site pruritus	Continuous
Regional adenopathy	Diameter >2 cm
Systemic	
Fever	≥40ºC
Other systemic events	Disabling or requiring confinement to bed

Dogs were responsible for the bite in 132 patients (77.2%) and cats in 32 patients (18.7%). The number of bites per patient varied from one (89.5%) to three (1.8%), and the legs (43.9%), hands (32.2%) and arms (14.0%) were the most common sites. In 55.6% of cases the attack was unprovoked. All patients had WHO category III contacts, and >90% had one or more transdermal bites (Table 2).

The interval between rabies exposure and inclusion in the trial varied between 0 and 2 days with a mean of 0.4 days. All patients received non-specific local treatment of the wound. Most commonly, wounds were washed with soap and water (136 patients, 79.5%), and tincture of iodine (158 patients, 92.4%) and/or alcohol (89 patients, 52.0%) was applied. ERIG was administered on the day of the bite for 102 patients (59.6% of cases), 1 day later for 67 (39.2%) and 2 days later for 2 patients (1.2%).

One hundred and sixty-seven patients (97.7%) completed the five-dose post-exposure protocol. Of the four patients who discontinued the trial, two patients refused to continue to participate (last seen D 14), one left the study area for a long period (last seen D 14), while the fourth patient was discontinued because one of the follow-up visits did not comply with the study protocol.

Immunogenicity

After completion of the study, three patients were found to have had baseline rabies antibody levels above the seroconversion threshold (≥0.5 IU/ml) at inclusion, probably as a result of a previous forgotten vaccination, and were excluded from the immunogenicity analysis. Antibody titers rose rapidly following initiation of post-exposure treatment, such that as early as D 7, 27.0% of patients had antibody titers above the WHO-recommended seroconversion threshold (≥0.5 IU/ml). By D 14,

Table 2
Types of wounds found in 171 Chinese patients treated for severe rabies exposure (all WHO category III contacts).

Wound type	Number (%) of patients
Single or multiple transdermal bites	153 (89.5)
Single or multiple transdermal scratches	14 (8.2)
Both of the above	4 (2.3)
Total	171 (100.0)

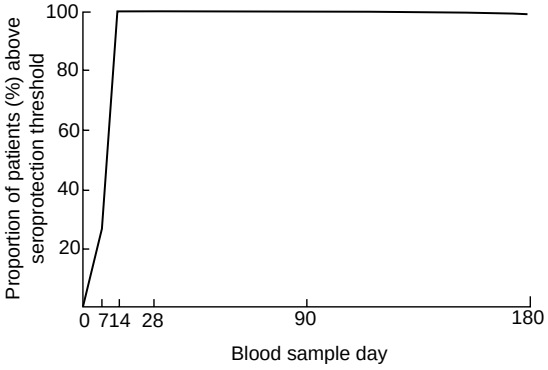

Fig 1–Percentage of Chinese patients with rabies neutralizing antibodies above the WHO-recommended threshold for acceptable seroprotection ((0.5 IU/ml) following administration of purified Vero rabies vaccine. Treatment was commenced within 48 hours after exposure, and vaccine was administered on Days 0, 3, 7, 14 and 28 of treatment.

Table 3
Antibody response to post-exposure treatment in initially seronegative patients treated for WHO category III contacts with PVRV and ERIG.

Immune response	D 0 (N=167)	D 7 (N=163)	D 14 (N=159)	D 28 (N=162)	D 90 (N=162)	D 180 (N=163)
GMT (IU/ml)	0.03	0.25	50.30	42.20	10.60	6.35
[95% confidence intervals]	[0.025; 0.030]	[0.198; 0.309]	[45.0; 56.2]	[38.1; 46.7]	[9.01; 12.5]	[5.32; 7.58]

Abbreviations: ERIG = equine rabies immunoglobulin; GMT = geometric mean titer; PVRV = purified Vero-cell rabies vaccine.

100% of patients had seroconverted (Fig 1), and the GMT was 50.3 IU/ml, 100-fold higher than the seroconversion threshold (Table 3). Antibody levels remained high, such that at D 90 all patients were still above the seroconversion threshold. Six months after the start of treatment, almost all patients (98.2%) were still seroconverted. All patients were alive at the end of the 6-month follow-up period.

Safety

No immediate or delayed serious adverse events occurred following injection of either PVRV or ERIG.

Twelve patients (7%) presented 15 local reactions after PVRV administration, most commonly pruritus or pain at the injection site (Table 4). Most of the local reactions appeared within 24 hours of administration and all except one (non-severe pruritus) disappeared within 24 hours.

Fourteen patients (8.2%) developed a total of 21 local reactions following ERIG administration, of which 13 were severe (7 patients). The most common local reactions induced by ERIG were pruritus and erythematous rash (Table 5). Symptoms resolved within 3 days in all but one patient.

Twelve patients (7%) presented 21 systemic reactions. The most frequent systemic reactions were rash, pruritus and vertigo (Table 6). Most systemic reactions appeared within 3 days and disappeared within 24 hours. Only one case of

Table 4
Number and percentage of Chinese patients with at least one local reaction following injection of PVRV during post-exposure treatment for severe rabies exposure.

	Number (%) of patients		
Local reaction	Non-severe	Severe	Total
Pruritus	4 (2.3)	2 (1.2)	6 (3.5)
Pain at injection site	2 (1.2)	1 (0.6)	3 (1.8)
Erythematous rash	1 (0.6)	1 (0.6)	2 (1.2)
Rash	0 (0.0)	2 (1.2)	2 (1.2)
Lymphadenopathy	1 (0.6)	0 (0.0)	1 (0.6)
Mass at injection site	1 (0.6)	0 (0.0)	1 (0.6)
Total[a]	8 (4.7)	4 (2.3)	12 (7.0)

[a]Some patients had more than one reaction, but are counted only once in the total.
Abbreviation: PVRV = purified Vero-cell rabies vaccine.

Table 5
Number and percentage of Chinese patients with at least one local reaction following injection of ERIG on D 0 of post-exposure regimen for severe rabies exposure.

	Number (%) of patients		
Local reaction	Non-severe	Severe	Total
Pruritus	4 (2.3)	3 (1.8)	7 (4.1)
Erythematous rash	1 (0.6)	5 (2.9)	6 (3.5)
Rash	2 (1.2)	1 (0.6)	3 (1.8)
Lymphadenopathy	1 (0.6)	0 (0.0)	1 (0.6)
Mass at injection site	0 (0.0)	3 (1.8)	3 (1.8)
Urticaria	0 (0.0)	1 (0.6)	1 (0.6)
Total[a]	7 (4.1)	7 (4.1)	14 (8.2)

[a]Some patients had more than one reaction, but are counted only once in the total.
Abbreviation: ERIG = equine rabies immunoglobulin.

Table 6
Number and percentage of Chinese patients with at least one systemic reaction during post-exposure treatment with PVRV and ERIG for severe rabies exposure.

Systemic reaction	Number (%) of patients		
	Non-severe	Severe	Total
Rash	4 (2.3)	0 (0.0)	4 (2.3)
Pruritus	2 (1.2)	1 (0.6)	3 (1.8)
Vertigo	3 (1.8)	0 (0.0)	3 (1.8)
Nausea	2 (1.2)	0 (0.0)	2 (1.2)
Somnolence	2 (1.2)	0 (0.0)	2 (1.2)
Asthenia	1 (0.6)	0 (0.0)	1 (0.6)
Dyspnea	0 (0.0)	1 (0.6)	1 (0.6)
Erythematous rash	1 (0.6)	0 (0.0)	1 (0.6)
Fever	1 (0.6)	0 (0.0)	1 (0.6)
Headache	1 (0.6)	0 (0.0)	1 (0.6)
Edema	0 (0.0)	1 (0.6)	1 (0.6)
Urticaria	0 (0.0)	1 (0.6)	1 (0.6)
Total[a]	11 (6.4)	1 (0.6)	12 (7.0)

[a]Some patients had more than one reaction, but are counted only once in the total.
Abbreviations: ERIG = equine rabies immunoglobulin; PVRV = purified Vero-cell rabies vaccine.

pruritus and one of erythematous rash failed to disappear within 3 days.

Most reactions were non-severe according to predefined criteria. A total of four patients (2.3% of the studied population) developed at least one severe local reaction after PVRV injection (Table 4); all of these reactions resolved within 24 hours. For ERIG injection, seven patients (4.1%) developed at least one severe local reaction (Table 5), only one reaction (urticaria) failed to disappear within 3 days.

In terms of systemic tolerability, one patient (0.6%) developed four severe systemic reactions after the PVRV and ERIG injections on D 0 (Table 6), all of which disappeared within 24 hours.

DISCUSSION

At present, two types of rabies vaccine, derived from primary hamster kidney cells (PHKC), are produced by local manufacturers in China. However, these vaccines are prepared on cells that are not yet pharmaceutically accepted by European nor US health authorities. Furthermore, the possibility of a delayed immune response with adjuvanted PHKC vaccines has been reported (Lin, 1990), in addition to the difficulty in achieving persistence of immunity (Ren, 1988). In view of the short incubation period of the disease, the development of rapid and persistent immunity to rabies following vaccination is essential. Importantly, a failure rate of 1 to 4 per 1,000 vaccinations has been observed with the PHKC vaccine (Fangtao *et al*, 1988b; Ren, 1988). PVRV provides a safe and effective alternative to locally produced vaccine and is recommended by the WHO for pre-exposure and post-exposure immunization against rabies (WHO, 1992).

Few studies of rabies vaccination in China have been carried out, especially in patients exposed to rabies. Trials of the efficacy of rabies vaccines (*eg* Quiambao *et al*, 2000) require laboratory confirmation of the rabid status of the biting animals; however, in China this confirmation is difficult to obtain for at least two reasons. Firstly, Chinese culture dictates that obviously rabid animals are killed and buried, so the attacking animal is often not available for observation and testing for rabies. Secondly, appropriate local laboratory facilities for performing the standardized fluorescent antibody test are often not available.

In the rabies endemic region of China where our study was performed standard fluorescent antibody testing was not possible. Consequently, the rabies status of the biting animals could not be confirmed at the time of patient enrolment and it was not possible for us to perform a “true” rabies

vaccine efficacy study. Nevertheless, patients enrolled in this study were selected on the basis of the severity of their wounds, all of which corresponded to WHO category III. According to WHO recommendations, this category of wound requires immediate treatment, including RIG administration, without waiting for confirmation of the rabies status of the biting animal. Our study was thus conducted under real conditions of clinical practice, in a population of patients seeking treatment for suspected severe rabies exposure.

While the absence of laboratory confirmation of the true rabies status of the biting animals prevents us from making any statement on the efficacy of rabies postexposure treatment in this population, we can confirm the excellent immunogenicity and safety of PVRV when used in post-exposure prophylaxis in patients with WHO category III wounds under field conditions in China.

In our study, patients demonstrated an extremely rapid immune response to vaccination, with all Chinese patients seroconverting within 14 days of the first injection and maintaining antibody levels over 0.5 IU/ml for at least three months after treatment. This indicates that the good immunogenicity of PVRV is maintained even in the presence of RIG, which has been reported in some cases to impair the immune response to rabies vaccine (Vodopija *et al*, 1988; Wilde *et al*, 1989; Chutivongse *et al*, 1991; Fescharek *et al*, 1991). This is important in view of the short incubation period of the disease, with most deaths from rabies occurring in the first month after exposure (Wilde *et al*, 1989). Furthermore, the vaccine was well tolerated, with only 2.3% of patients experiencing severe local reactions, all of which resolved within 24 hours. One patient (0.6%) developed severe systemic reactions after administration of PVRV and ERIG but recovered within 24 hours.

The present results are therefore consistent with previous reports from other Asian countries in showing that PVRV can achieve rapid and persistent seroprotection (Chutivongse *et al*, 1986; Thongcharoen *et al*, 1989), and confirm the safety and immunogenicity of PVRV.

CONCLUSION

PVRV was highly immunogenic and safe under field conditions in Chinese patients treated according to WHO recommendations for severe rabies exposure.

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