A Comparison of the Tolerability of Two Dilution Volumes (0.5 mL and 1.0 mL) of a Purified Chick Embryo Cell Rabies Vaccine Administered Intramuscularly to Healthy Adult Volunteers: A Randomized, Intraindividual, Assessor-Blind Study

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ABSTRACT

Background: The current recommendation of the manufacturer for administering purified chick embryo cell rabies vaccine (PCECV) is to reconstitute the contents with 1.0 mL of water for injection (WFI). However, it has been debated whether a lower volume of WFI (0.5 mL) is likely to cause less pain.

Objectives: The aims of this study were to compare the tolerability of PCECV administered IM at a volume of 0.5 mL versus 1.0 mL of diluent and to determine the immunogenicity of the vaccine when administered according to the World Health Organization–recommended preexposure prophylaxis regimen for rabies immunization.

Methods: This comparative, intraindividual, assessor-blind study was conducted at the Department of Clinical Pharmacology, Topiwala National Medical College and Bai Yamunabai Laxman Nair Charitable Hospital (Mumbai, India). Healthy volunteers aged 18 to 50 years received, by randomized sequence, 3 IM injections of PCECV, diluted in 0.5 mL or 1.0 mL of WFI, on study days 0, 7, and 28. Tolerability was assessed at 30 minutes and 24 hours after injection and included assessments for local and systemic reactions. For immunogenicity assessment, rabies virus–neutralizing antibody (RVNA) titers were assayed at baseline and on day 49 (ie, 3 weeks after the third injection).

Results: Twenty-six subjects (24 men, 2 women; mean [SD] age, 22.4 [2.4] years; mean [SD] body weight, 59.0 [11.3] kg) entered the study. Twenty-five subjects were included in the tolerability assessment; 24 in the immunogenicity assessment. No statistically significant differences were found between dilutions in the frequency of local and systemic reactions. Most reactions were

mild. All subjects developed RVNA titers >0.5 IU/mL (indicative of protection) by day 49.

Conclusions: In this population of healthy volunteers, a full antigenic dose of PCECV in a dilution of 0.5 mL WFI is as well tolerated locally and systemically as in a dilution of 1.0 mL. All subjects developed levels of RVNA far exceeding 0.5 IU/mL, which is indicative of protection against rabies. (*Curr Ther Res Clin Exp.* 2004;65:47–56) Copyright © 2004 Excerpta Medica, Inc.

Key words: rabies vaccine, purified chick embryo cell rabies vaccine, tolerability, volunteer, immunogenicity.

INTRODUCTION

Purified chick embryo cell rabies vaccine (PCECV) is a modern tissue culture rabies vaccine that may be administered either by a preexposure schedule approved by the World Health Organization (WHO) (on days 0, 7, and 28 before exposure) to individuals perceived to be at risk (eg, veterinarians), or by a WHO-approved postexposure schedule on days 0, 3, 7, 14, and 30. Unlike the highly reactogenic nerve tissue rabies vaccines (NTVs), which are still widely used at antirabies clinics in India, PCECV is reported to be well tolerated. NTVs are not intended for preexposure prophylaxis of rabies; hence, no preexposure schedule exists. WHO has recommended that NTVs be phased out worldwide and be substituted with tissue culture vaccines, such as PCECV, because they are more immunogenic, better tolerated, and not known to cause serious neurologic complications.

PCECV is produced by growing the seed virus (Flury low egg passage [LEP]) in primary cultures of chick fibroblasts. The virus is inactivated with β -propiolactone, purified by zonal centrifugation in a continuous sucrose density gradient, and concentrated. The vaccine is lyophilized after the addition of a stabilizer. The WHO requirement for potency of the vaccine is ≥ 2.5 IU/dose.

Although the lyophilized contents of 1 vial of PCECV dissolve completely in 0.5 mL water for injection (WFI), the manufacturer's current recommendation is to reconstitute the contents with 1.0 mL of WFI. This is because human diploid cell rabies vaccine, which preceded PCECV, was dissolved in 1.0 mL. However, the recommendation for purified vero cell rabies vaccine (PVRV), which followed PCECV, is to add only 0.5 mL of WFI. This lower volume of injection for PVRV has been suggested by some manufacturers to offer the advantage of being less likely to cause pain, especially in small children. With this in mind, and because 0.5 mL of WFI is enough to dissolve PCECV completely, it seemed appropriate to investigate and compare the tolerability of PCECV administered IM in a volume of 0.5 mL versus 1.0 mL. In addition, this study determined the immunogenicity of the vaccine when administered according to the recommended preexposure prophylaxis regimen for rabies immunization.

SUBJECTS AND METHODS

The study was conducted at the Clinical Pharmacology Department of the Topiwala National Medical College and Bai Yamunabai Laxman Nair Charitable Hospital, Mumbai, India. The protocol was approved by the institutional ethics committee. The study was carried out in accordance with the Declaration of Helsinki and in compliance with the International Conference on Harmonisation Good Clinical Practice guidelines. All subjects gave their written informed consent prior to enrollment in the study.

Subjects

Twenty-six apparently healthy volunteers who underwent physical examination and laboratory screening as per departmental protocol were enrolled to ensure that at least 24 would complete the study according to protocol. The volunteers were veterinary students who needed to routinely receive a course of preexposure prophylaxis against rabies. Their selection was based on the inclusion criteria of age 18 to 50 years and mental capacity to understand the nature, scope, and consequences of the study. Exclusion criteria included history of rabies immunization; treatment within the preceding 2 months with antimalarial drugs, oral or parenteral steroids, immunosuppressive drugs, phenytoin, or specific anti-inflammatory drugs; history of allergy to egg protein or PCECV; history of alcohol or other narcotic drug abuse; suspicion or evidence of human immunodeficiency virus infection, autoimmune disease, or other immunologic disorder; surgery planned during study period; and participation or likely participation in a separate, simultaneous study. Pregnant, possibly pregnant, or breastfeeding women were excluded from the study. Women of childbearing age were advised to practice birth control, although the vaccine has no documented teratogenic effects (unpublished data, Chiron Vaccines, Mumbai, India, 2002).

Methods

Vials of PCECV* of a marketed batch (no. 697) containing in each dose, when reconstituted, lyophilized inactivated rabies virus of the Flury LEP strain, with a potency of 9.43 IU/dose of rabies antigen, were used for the study. Two dilutions were used to reconstitute the lyophilized contents of the vial (0.5 mL and 1.0 mL of WFI); the antigen content was equivalent at both dilutions. The vaccine was brought to room temperature prior to administration. The injections were given by a physician, in the deltoid muscle by the same vaccinator using the same sized needle (24G) for all injections.

Randomization

Each subject received 3 injections, 1 each on days 0, 7, and 28. Because each subject had 2 deltoid sites, left (L) and right (R), there were $2^3 = 8$ possible arm

*Trademark: Rabipur® (Chiron Vaccines, Mumbai, India).

sequences for the injection site: LLL, LLR, LRL, LRR, RLL, RLR, RRL, and RRR. Likewise, because 2 dilution volumes were to be used (0.5 mL [5] and 1.0 mL [1]), there were $2^3 = 8$ possible dilution sequences: 555, 551, 515, 511, 155, 151, 115, and 111. Subjects were randomly assigned to these sequences in blocks of 8, so that on each day of injection the distribution of arms and dilution volumes was balanced. The randomization slips for each subject for each day were provided in sealed envelopes. The envelopes were opened just before each injection to see the dilution and the arm to be used. The subjects and the physician assessing the tolerability parameters (the evaluator) were kept blinded to the dilutions used. Because the dose of rabies antigen was equivalent in both the dilutions, each subject received the full immunization dose on days 0, 7, and 28.

Response Assessment

Tolerability

Local and systemic tolerability were assessed by an evaluator who was different from the vaccinator and who was blinded to the treatment received by the subject. The assessments were performed at 30 minutes and at 24 hours after each injection. The following variables were noted or measured and defined: pain, on a 10-point visual analog scale (VAS) as 0 = no pain, 1–3 = mild pain, 4–6 = moderate pain, 7–10 = severe pain. Depending on the VAS score, pain severity was labeled as 1 = mild (self-limiting), 2 = moderate (requiring treatment), or 3 = severe (interfering with activities of daily living); erythema, as 1 = mild (<2 cm), 2 = moderate (2–7 cm), or 3 = severe (>7 cm); induration, as 1 = mild (<2 cm), 2 = moderate (2–7 cm), or 3 = severe (>7 cm); fever, as 1 = mild (38.0°C–38.5°C), 2 = moderate (38.6°C–39.0°C), or 3 = severe (>39.0°C); myalgia, as 1 = mild (ambulant), 2 = moderate (in bed), or 3 = severe (in bed with distress); malaise, as 1 = mild, 2 = moderate, or 3 = severe; headache, as 1 = mild (minimal interference with normal activity), 2 = moderate (significant interference with normal activity), or 3 = severe (requiring bed rest); allergic reactions, as 1 = mild, 2 = moderate, or 3 = severe; and any other adverse effect (AE) or complaint, with the intensity being noted.

Immunogenicity

On days 0 and 49 (21 days after the third injection), 5 mL of venous blood was collected by a laboratory technician from all subjects for a rapid fluorescent focus inhibition test (RFFIT) of rabies virus–neutralizing antibody (RVNA) titers, and 2 aliquots of serum were separated and frozen at –20°C. Because each subject was likely to receive both of the study dilutions (according to the randomization sequence on days 0, 7, and 28) no comparison was possible between the 0.5-mL and 1.0-mL dilutions with respect to the RVNA titers.

The RVNA titers were assayed in the Department of Neurovirology of the National Institute of Mental Health and Neuro Sciences (NIMHANS) (Bangalore, India) using RFFIT as advocated by WHO,¹ with some modifications. We used

challenge virus standard 11 obtained from the Central Research Institute, Kasauli, India. The cell line used was Neuro2A obtained from the National Centre for Cell Sciences (Pune, India). The procedure followed was essentially that advocated by WHO, except that instead of 8-well tissue culture chamber slides, we used 96-well, flat-bottom tissue culture plates (Nunc GmbH & Co. KG, Wiesbaden, Germany). The rabies fluorescein isothiocyanate conjugate obtained from Central Research Institute, Kasauli, was used for staining the cells by fluorescent antibody technique. Antibody titers were expressed as international units per milliliter (IU/mL) compared with a local reference antirabies serum calibrated against the second international reference rabies immune globulin having a potency of 30 IU/mL.

Statistical Analysis

Interval variables were summarized as mean (95% CI). The incidence of reactions with the 2 dilutions of PCECV was tabulated as the frequency of each reaction, and the differences were tested for significance using the Fisher exact test. The difference in VAS scores for pain was tested for significance by analysis of variance (ANOVA) with subject, day, subject arm, and volume as the main factors. We used Stata version 7 (Stata Corporation, College Station, Texas) to calculate the data.

RESULTS

Enrolled subjects were 24 men and 2 women. Their mean age was 22.4 years (95% CI, 21.4–23.4) and their mean body weight was 59.0 kg (95% CI, 54.4–63.5). None of the subjects had a history of allergy to egg protein or PCECV or animal bite, and none had any current illness or were taking any medication.

The **figure** shows the disposition of the 26 subjects who were enrolled in the study. One male volunteer aged 22 years developed fever on day 5, was suspected of having malaria, was treated with chloroquin, and recovered, but was withdrawn from the study. One female volunteer received all 3 injections but did not return on day 49 for the blood sample. These 2 subjects were not assessable for immunogenicity. Furthermore, only 76 injections were available for assessment of tolerability because the subject with suspected malaria did not receive the second and the third injections. The total number of pain assessments was 152 (76 at 30 minutes and 76 at 24 hours).

Tolerability

The frequency of various local and general adverse effects is shown in **Table I**. None of the differences in the incidence of adverse effects between the 0.5-mL and 1.0-mL dilutions were statistically significant. The reactions either were self-limiting or could be controlled with an analgesic, such as paracetamol. All of the AEs were treatment related except malaria. None of the AEs were severe.

Figure. Disposition of subjects receiving 2 dilution volumes of purified chick embryo cell rabies vaccine, from baseline to study end. M = male; Rx = prescription; F = female; RFFIT = rapid fluorescent focus inhibition test. N = 76 injections, 152 pain assessments for tolerability.

A detailed comparison of the incidence and degree of pain at 30 minutes and at 24 hours is shown in **Table II**. None of the subjects complained of severe pain at any time with either dilution. Fewer subjects complained of mild to moderate pain with the 0.5-mL dilution than with the 1.0-mL dilution, but the difference was not statistically significant.

Table I. No. (%) of subjects experiencing ≥ 1 local and generalized treatment-related adverse effect with 2 dilution volumes of purified chick embryo cell rabies vaccine.*

Adverse Effect	0.5 mL (n = 38)	1.0 mL (n = 38)
Local pain at 24 h (mild)	3 (7.9)	4 (10.5)
Local pain at 24 h (moderate)	2 (5.3)	4 (10.5)
Myalgia (mild)	3 (7.9)	3 (7.9)
Malaise (mild)	2 (5.3)	4 (10.5)
Fever (mild)	1 (2.6)	2 (5.3)
Headache (mild)	1 (2.6)	1 (2.6)
Local pain at 30 min (mild)	0 (0.0)	1 (2.6)

*No significant differences were found between dilution volumes. None of the adverse effects were severe.

Table II. Severity of pain with 2 dilution volumes of purified chick embryo cell rabies vaccine. (Data are expressed as no. [%] of subjects/events.)

Pain Severity	Subjects with Pain at 30 Minutes		Subjects with Pain at 24 Hours		Total Pain Events	
	0.5 mL (n = 38)	1.0 mL (n = 38)	0.5 mL (n = 38)	1.0 mL (n = 38)*	0.5 mL (n = 76)*	1.0 mL (n = 76)*
None	38 (100.0)	37 (97.4)	33 (86.8)	30 (78.9)	71 (93.4)	67 (88.2)
Mild	0 (0.0)	1 (2.6)	3 (7.9)	4 (10.5)	3 (3.9)	5 (6.6)
Moderate	0 (0.0)	0 (0.0)	2 (5.3)	4 (10.5)	2 (2.6)	4 (5.3)
Severe	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

*Percentages do not total 100% due to rounding.

The VAS pain score at 24 hours was examined by ANOVA with subject, subject arm, day, and volume as the main factors. As shown in **Table III**, there was no significant difference between the 0.5-mL and 1.0-mL dilutions, or between right and left arms, but a significant difference between days was found. The 1.0-mL dilution caused similar pain as the 0.5-mL dilution, and the left arm had pain similar to that in the right arm. No subjects complained of pain on day 28 at either 30 minutes or 24 hours, in contrast to days 0 and 7 ($P = 0.04$).

Immunogenicity

All subjects achieved an RVNA titer >0.5 IU/mL, which is recognized as the minimum protective level. The geometric mean titer (GMT) was 13.3 IU/mL, which is higher than the minimum protective level. The lowest value was

Table III. Pain visual analog scale* (VAS) scores in subjects receiving 2 dilution volumes of purified chick embryo cell rabies vaccine.

Factor	Mean VAS Score	95% CI	$p^{\dagger}$
Volume			
0.5 mL (n = 38)	0.46	0.00–0.93	0.38
1.0 mL (n = 38)	0.72	0.19–1.25	
Arm			
Left (n = 38)	0.83	0.20–1.46	0.07
Right (n = 38)	0.36	0.07–0.64	
Study day			
0 (n = 26)	0.85	0.21–1.49	0.04
7 (n = 25)	0.92	0.12–1.72	
28 (n = 25)	0.00	0.00	

*Scale: 0 = no pain, 1–3 = mild pain, 4–6 = moderate pain, 7–10 = severe pain.

† Analysis of variance.

7.5 IU/mL and the highest was 25.6 IU/mL. As the highest value was an outlier and the data did not have a normal distribution ($P < 0.001$ [Shapiro-Wilk test]), the 5th and 95th percentiles were computed to define the 90% range, which was 7.5 IU/mL to 15.9 IU/mL.

DISCUSSION

This study was designed to ensure that all the subjects received the recommended full immunizing dose of PCECV for preexposure prophylaxis against rabies, as recommended by WHO,¹ on days 0, 7, and 28. This was possible because only the volume of the diluent differed (0.5 mL or 1.0 mL) depending on the randomization but not the dose of the antigen.

Two studies^{2,3} that examined the factors associated with muscle pain in humans after hypertonic, isotonic, and hypotonic saline solution administration reported increased IM pressure and pain with hypertonic saline and postulated that pain activation in skeletal muscle is related to a higher concentration of sodium. Another study⁴ found that sodium chloride solution was less painful than iso-osmolar glucose solution, indicating that discomfort is not attributable solely to high osmolality and that ionic low-osmolality solutions are less painful to receive than nonionic low-osmolality solutions. A fourth study⁵ indicated that following SC administration, pain is related to the volume of the injection, with 1.0 mL being more painful than 0.5 mL.

However, it has also been established that local tolerability of IM injections of vaccines or vaccinelike suspensions is very good over a range of osmolality, from 300 to 1100 mOsm/kg, and that there is no clinically significant difference in local pain or burning sensation at the site of injection over this range.⁶ When the vaccine used in our study was tested for osmolality at dilutions of 0.5 mL and 1.0 mL, the osmolality was 905 mOsm/kg and 403 mOsm/kg, respectively, suggesting that there might be no clinically significant difference in local tolerability with either dilution.

Prior to the present study, a toxicology study (data on file, Institute of Toxicological Studies, Mumbai, India, 2002) was conducted in rabbits to determine whether there was a clinically significant difference in the local tolerability of 0.5 mL and 1.0 mL of PCECV, administered IM. No abnormal clinical signs or signs of local pain were observed in any of the treated animals throughout the period of the study at either dilution. No gross pathologic changes were observed at the site of injection in animals treated at the 2 concentrations. Histopathologic examination of muscles at the site of injection also did not reveal any abnormality due to the injection of PCECV reconstituted with either 0.5 mL or 1.0 mL of WFI.

In our study, no statistically significant difference was observed in the tolerability parameters between the 0.5-mL and 1.0-mL dilutions of PCECV administered to volunteers in the deltoid muscle. None of the subjects at any time complained of severe pain, and no subject complained of pain 30 minutes after

being administered 0.5 mL. The RVNA in all the volunteers 3 weeks after the third dose of PCECV was highly satisfactory. The GMT was 13.3 IU/mL, as against a titer of 0.5 IU/mL, which is considered to be indicative of protection against rabies.

In view of minimal pain at the injection site found with PCECV at both dilutions as demonstrated in healthy adult volunteers, as well as the high degree of immunogenicity, it seems justified and appropriate to further study the immunogenicity and tolerability in children, for whom the smaller volume is intended.

CONCLUSIONS

In this population of healthy volunteers, a full antigenic dose of PCECV in a dilution of 0.5 mL WFI is as well tolerated locally and systemically as in a dilution of 1.0 mL. All subjects developed levels of RVNA far exceeding 0.5 IU/mL, which is indicative of protection against rabies.

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