A PHASE IV OPEN LABEL STUDY TO ASSESS THE SAFETY, TOLERABILITY AND IMMUNOGENICITY OF A *HAEMOPHILUS INFLUENZAE* TYPE B (HIB) CRM_{197} CONJUGATED VACCINE ADMINISTERED TO HEALTHY INFANTS AT 2, 4, AND 6 MONTHS OF AGE

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Abstract. The objective of this prospective clinical study was to evaluate the safety, tolerability and immunogenicity of Chiron Hib vaccine (Vaxem Hib®) in Thai infants. This trial was conducted at Bhumibol Adulyadej Hospital, Bangkok, Thailand from June to November 1999. Three intramuscular injections of the vaccine were given to 119 infants at 2, 4 and 6 months of age. Reactions and adverse events after the vaccination were recorded. Blood samples for anti-PRP antibody were collected before the first immunization, and after the second and third immunizations. After the second dose, 91% and 58% of the subjects had anti-PRP antibody titers of ≥0.15 μg/ml and ≥1.0 μg/ml, respectively. After the third dose, 99% and 90% of the subjects had anti-PRP antibody titer ≥0.15 μg/ml and ≥1.0 μg/ml, respectively. Local and systemic reactions were mild and transient. The study indicates that Vaxem Hib vaccine is safe and well tolerated. Three doses of the vaccine are necessary to achieve adequate protection in infants.

INTRODUCTION

Haemophilus influenzae type B (Hib) causes serious and life threatening invasive diseases such as meningitis and pneumonia. The capsule, composed of a repeating polymer of ribosyl and ribitol phosphate (PRP), is the main virulence factor, and antibodies against PRP are protective at a level of 0.15 μg/ml (Ward and Zangwill, 1999).

Vaccines are produced through conjugation of poly- or oligosaccharides derived from the bacterial capsule of Hib to a protein carrier. The Hib conjugate vaccine used in this study contains Hib oligosaccharides conjugated to a nontoxic mutant of diphtheria toxin CRM_{197} (Costantino *et al*, 1991). In the final vaccine formulation, aluminum hydroxide was used as an adjuvant. The clinical development program of Vaxem Hib has shown its safety, tolerability, and immunogenicity (Galli *et al*, 1993). The vaccine was licensed in Italy in 1996 for pediatric immunization, and then was marketed in several countries.

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The objective of this trial was to evaluate the antibody response to Vaxem Hib in infants by measuring anti-PRP antibody titers after the second and third doses, in order to assess when protection in infants is obtained.

MATERIALS AND METHODS

Subjects

Healthy infants of both sexes, 6-12 weeks (42-84 days) of age were enrolled at the vaccination center of Bhumibol Adulyadej Hospital, Bangkok, Thailand, from June to November 1999. Written informed consents were obtained from the parents or legal guardians of the subjects.

Vaccine

Three doses of the study vaccine (Chiron Hib vaccine with aluminum hydroxide adjuvant, Vaxem Hib® - Lot No. 2704) were administered at 2, 4, and 6 months of age in the mid-anterolateral part of the right thigh.

The Vaxem Hib vaccine came in two containers: one vial containing 0.3 ml of vaccine and one pre-filled syringe containing 0.3 ml of aluminum hydroxide suspension. After mixing the contents of the vial with the contents of the prefilled syringe, one dose of 0.5 ml was obtained

containing the active ingredient, 10 μg of capsular oligosaccharide of Hib conjugated to approximately 25 μg of CRM_{197} protein. The excipients included 0.05 mg of thimerosal, 1 mg of aluminum hydroxide and up to 0.5 ml of sodium phosphate buffer pH 7.

Concomitant vaccines were administered according to the local schedule. Chiron DTaP Vaccine (Acelluvax DTP) and locally available oral polio vaccine (OPV) were administered concomitantly with each Hib immunization. Local hepatitis B vaccine (HBV) was administered concomitantly with the first and third Hib immunization.

Blood sampling

Blood samples for anti-PRP antibody, measured by the enzyme-linked immunosorbent assay (ELISA) method, were collected: (1) before the first injection, (2) before the third injection, and (3) 4-6 weeks after the third infection. Protective anti-PRP antibody titers were considered ≥0.15 μg/ml and ≥1.0 μg/ml.

Safety evaluation

Measures of vaccine safety include data from the physical examination and observed local and systemic reactions, and adverse events after vaccinations. The subjects were observed for 30 minutes after each immunization for immediate reactions. An active surveillance of study subjects on the second day following each immunization was set up to collect data on local (tenderness, redness, swelling) and systemic (fever) reactions. Serious adverse events and/or adverse events necessitating a physician's visit and/or adverse events resulting in a subject's withdrawal from subsequent vaccinations or from follow-up were collected during the trial.

Ethics

The study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University and the Ethics Committee of Bhumibol Adulyadej Hospital, Bangkok, Thailand.

RESULTS

Study population

A total of 119 subjects were enrolled. The mean age of the subjects upon enrollment was 69.3 days (range 48-88 days). The sample was distributed between male and female subjects (53% male, 47% female).

One subject (1%) was withdrawn due to loss to follow-up. The remaining 118 subjects completed the study protocol. All 119 subjects received at least one immunization and were included in the safety evaluation; 118 subjects were included for immunogenicity evaluation.

Immunogenicity

Table 1 reports the percentage of subjects with anti-PRP antibody titers ≥0.15 μg/ml and ≥1 μg/ml for each dose. The geometric mean titer (GMTs) of anti-PRP antibody titers at baseline was 0.15 μg/ml. After the second dose, the anti-PRP GMT was 1.3 μg/ml and, after the third dose it was 8.8 μg/ml.

Vaccine safety

Local and systemic reactions were mild and transient. The most frequent local reaction was tenderness (48%), followed by redness (37%). No subject reported swelling. The frequency of rectal temperature ≥38ºC was 5%. (Table 2). There were no adverse events possibly or probably related to treatment reported in the study.

DISCUSSION

Hib is a major cause of bacterial meningitis, and/or pneumonia in several Asian countries. Recent studies have shown that Hib vaccination is effective in preventing invasive disease in chil-

Table 1
Percentage of subjects with a protective level of anti-PRP antibody titer before the first immunization, two months after the second dose of vaccine and one month after the third dose of vaccine.

	N	Anti-PRP antibody			
		≥0.15 μg/ml		≥1.0 μg/ml	
		% protected	95% CI	% protected	95% CI
Day 0	119	43	34-52	14	9-22
Day 120	118	91	84-95	58	49-67
Day 150	118	99	95-100	90	83-95

Table 2
Local and systemic reactions within 2 days following any of the three immunizations.

Post-immunization reactions (Day 0-2)	(N=119) n (%)
Local (at the injection site)	
Tenderness	
any	57 (48)
cried when injected leg was moved	0
Redness	
any	44 (37)
>50 mm	0
Swelling	
any	0
>50 mm	0
Systemic	
Rectal temperature ≥38ºC	6 (5)

dren in developing countries (Lolekha *et al*, 2000). Our study confirms previous studies concerning the safety and immunogenicity of Hib conjugate vaccines among children in several developing countries (Chotpitayasunondh *et al*, 1997; Phadke *et al*, 1997; Araujo *et al*, 2000; Chub-uppakarn, 2000; Fernandej *et al*, 2000; Gylca *et al*, 2000; Lolekha *et al*, 2001).

Concerning the reactogenicity profile of the studied Hib vaccine, it appeared to be well tolerated and safe. Both local and systemic reactions were mild and transient. No serious adverse events related to the vaccine were reported.

Concerning immunogenicity, the three doses of the Hib vaccine were highly immunogenic; after two doses, only the level of protection was unsatisfactory. After the second dose, only 58% of children were protected adequately. These findings are consistent with a previous study (Kanra *et al*, 2000) demonstrating the need for three doses of Vaxem Hib to protect >90% of vaccinees adequately.

The Hib vaccine may be used seperately or in combination with the diphtheria, tetanus, and pertussis (DTP) vaccine in infants aged 2, 4, and 6 months in Thailand and in other Asian countries. The epidemiologic data of Hib disease in this area of the world should be extensively evaluated in order to assess the cost/benefit ratio of this vaccine.

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