The Turkish Journal of Pediatrics 1999; 42: 421-427 Original

SAFETY, TOLERABILITY AND IMMUNOGENICITY OF A HAEMOPHILUS INFLUENZAE TYPE b VACCINE CONTAINING ALUMINUM PHOSPHATE ADJUVANT ADMINISTERED AT 2, 3 AND 4 MONTHS OF AGE*

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SUMMARY: Kanra G, Viviani S, Yurdakök K, Özmert E, Yalçın S, Baldini A, Mutlu B, Kara A, Ceyhan M, Podda A. (Infectious Diseases and Social Pediatrics Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey and Chiron Vaccines Clinical Research Chiron SpA, Siena, Italy). Safety, tolerability and immunogenicity of a Haemophilus influenzae type b vaccine containing aluminum phosphate adjuvant administered at 2, 3 and 4 months of age. Turk J Pediatr 1999; 41: 421-427.

The primary aim of this study was to assess the tolerability and immunogenicity of a new Haemophilus influenzae type b (Hib)/$AlPO_4$ (CHIRON, SpA) vaccine, in two-month-old healthy infants. Twenty-three subjects were enrolled and administered the new Hib vaccine containing $AlPO_4$ adjuvant at two, three and four months of age concomitantly with diphtheria-pertussis-tetanus (DPT) and hepatitis B vaccines according to the local program. Children were observed for 30 minutes after each immunization for any immediate local and systemic reactions. An active surveillance for side effects was performed on the 2nd and 7th days following each immunization by telephone. Families also filled out diaries for the first seven days. From the 2nd day to the next immunization only data about adverse events necessitating a physician's visit or about serious adverse events were collected. Blood samples were obtained before the first immunization and one month after the third dose for evaluation of anti-polyribosylribitol phosphate (PRP) antibody response. Local reactions at the Hib site were mild and less frequent compared to those observed at the DPT site. Systemic reactions noted after the three immunizations were fever in 70 percent,

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irritability in 48 percent, persistent crying in 26 percent, change in eating habits in 22 percent, diarrhea in 17 percent, sleepiness in 17 percent, vomiting in 9 percent, and unusual crying in 4 percent of the cases. There was no serious adverse event. One hundred percent and 95 percent of children achieved an anti-PRP antibody response over 0.15 µg/ml and 1.0 µg/ml, respectively. The geometric mean titer was 15 µg/ml and the geometric mean ratio 84. It was concluded that the new (Hib)/$AlPO_4$ vaccine is safe and well tolerated, and induced a good PRP antibody response in healthy two-month-old infants. *Key words: aluminum phosphate adjuvant. Haemophilus influenzae type b vaccine, safety, immunogenicity.*

In those countries where haemophilus influenzae type b (Hib) conjugate vaccines have been used for routine infant vaccination, Hib invasive disease has been virtually eradicated[1-4].

The first generation of Hib vaccines consisted of plain capsular polysaccharide (PRP). These vaccines elicited a fairly good immunological response in adults and children > 18 months of age. Anti-PRP antibody level was, however, of short duration and, in addition, as a PRP a T-cell independent antigen immunological memory was not primed[5,6].

Priming of immunological memory and elicitation of anti-PRP antibody in infants are instead achieved with Hib conjugate vaccines. Plain Hib polysaccharide becomes a T-cell dependent antigen, and is thus able to prime immunological memory by conjugation to a protein[7,8].

Several Hib conjugate vaccines are used worldwide. In the PRP-T vaccine, the plain polysaccharide is conjugated to tetanus toxoid; in the PRP-OMP vaccine, conjugation is made to an outer membrane protein complex of Neisseria meningitidis; in HbOC vaccine, the protein used in CRM_{197}, a non-toxic mutant of diphtheria toxin.

Some years, ago, Chiron Vaccines in Siena, Italy developed a HbOC-like vaccine (VaxemHib®) consisting of oligomers of capsular PRP of Haemophilus influenzae type b conjugated to CRM_{197}[9,10].

The vaccine is presented in two vials for mixing before injection: one containing the Hib conjugate to CRM_{197} protein and the other aluminum hydroxide as adjuvant. In view of the inconvenience of mixing the two vials before injection, an improved formulation has ben recently developed at Chiron Vaccines in Siena. The adjuvant has been replaced by aluminum phosphate which permits formulation of the vaccine in a stable solution presented in a ready-to-use vial.

As preclinical studies on stability and immunogenicity showed promising results, an expanded pilot study has been carried out.

We present here results of a clinical pilot study to assess the safety of the new Hib formulation in two-month-old healthy infants, and to obtain preliminary data on immunogenicity.

Material and Methods

This trial was designed as an open label, controlled, single center, pilot study. The study was approved by the Ethic Board of Hacettepe University, Medical Faculty (Ankara, Turkey) and by the General Directorate of Medicine and Pharmacy, Ministry of Health, Ankara, and written informed consent was obtained from the parents.

Study subjects were recruited in July 1998 at the Department of Pediatrics Infectious Diseases Unit of Hacettepe University, among two-month-old healthy infants eligible to receive routine EPI vaccines. At the time of the study, the Hib vaccine was not part of the EPI in Turkey. Excluded from the study were infants who had previously received one dose of Hib vaccine, had presented a previous disease potentially related to Hib, had household contact and/or intimate exposure in the previous 30 days to an individual with ascertained Hib disease, had experienced fever >38 °C within the past three days, had significant acute or chronic disease or known or suspected congenital or acquired immune suppression, had received parenteral immunoglobulin, had a history of anaphylaxis or any serious vaccine reaction or had participated in another trial within 30 days.

Infants were immunized with Hib/$AlPO_4$, diphtheria-pertussis-tetanus (DPT) and oral poliovirus vaccine (OPV) vaccines according to the two, three and four months schedule. Two doses of hepatitis B vaccines were administered at the 2^{nd} and 3^{rd} months, and a third dose was administered at 9-11 months of age after the completion of the study.

The Hib/$AlPO_4$ vaccine under investigation (Chiron Vaccines SpA, Siena Italy, lot number N32P18H1) contained 10 µg CRM_{197}- Hib conjugate as oligosaccharide, 0.3 mg aluminium phosphate, 0.01% thimerosal and 0.005% polysorbate (Tween) 80 and qs 0.5 ml phosphate buffered saline. It was provided as a single dose pre-filled syringe and was administered as an intramuscular injection in the left thigh. The concomitant DPT (Pasteur Merieux Connaught) was administered as an intramuscular injection in the right thigh and hepatitis B vaccine (HBV) (Smith Kline Beecham) as an intramuscular injection in the right deltoid.

After vaccination, subjects were observed for 30 minutes for local signs and symptoms (redness, induration, pain) at the injection site and for systemic reactions (rash, drowsiness, change in eating habits, unusual cry, persistent cry, vomiting, diarrhea). Parents were instructed to measure rectal temperature and to complete a diary card to describe local reactions and systemic reactions that developed within six hours after each immunization and daily for a total of seven days. All adverse events regarding any visit or consultation during the

study period were noted. All subjects were followed up the entire study period for the use of prescribed medications. Telephone calls were made two and seven days post-immunization to obtain reaction data and to assess the subject's clinical status. Diary cards were collected at the following visits. A serious adverse event was defined as any experience that suggested a significant hazard, contraindication, side effect or precaution. It included any experience fatal or life threatening, permanently disabling, or requiring or prolonging in-patient hospitalization, or any congenital anomaly or cancer. Blood samples were obtained from subjects on the day of first immunization and four to six weeks after the third immunization. Sera samples were stored frozen at –20 °C until serological testing.

IgG antibody response to PRP was measured by ELISA technique adapted from the FDA ELISA method[11] used for measuring Hib antibodies. The FDA standard reference serum, with known antibody titers as determined by radioimmunoassay (RIA), was used as standard. Immunogenicity measures were the percentage of subjects with an anti-PRP ELISA titer ≥ 1.0 µg/ml one month after the third immunization. The proportion of subjects with an anti-PRP ELISA titer ≥ 0.15 µg/ml and geometric mean titers for PRP antibodies were also evaluated.

Local and systemic reactions were evaluated for each of the three immunizations as well as for the three immunizations combined. If a subject experienced multiple adverse events that mapped to the same assigned term, the adverse event was counted only once.

All statistical analyses were performed using SAS® version 6.12 (SAS Institute, Cary, NC). Percentages of subjects experiencing local or systemic reactions, after any immunization, were calculated.

Geometric mean titers (GMTs) of anti-PRP antibodies and 95% confidence intervals (CIs) were constructed by exponentiating (base 10) the means and the lower and upper limits of the 95% confidence intervals of the logarithmically transformed (base 10) titers. For statistical analysis, antibody levels less than minimum level of detection were set to half of that limit.

Results

Twenty-three healthy infants whose parents had signed informed consent were enrolled into the study. Their mean age was 63.1 days (range 44-83) and 52 percent were males. They all simultaneously received three doses of Hib, DPT and OPV. The second and third immunizations were given 31 (range 31-33) and 64 (range 62-76) days after the first immunization, respectively.

Local and systemic post-immunization reactions are reported in Table I. There were no deaths, serious adverse events or adverse events leading to premature

withdrawal from the study. One subject was noted to have agitation, anorexia and vomiting for eight days starting the day of the third immunization, classified as possibly related to vaccine.

Anti-PRP antibody titers before and after vaccination with Hib vaccine are shown in Table II. After three doses, 100 percent of infants (95% CI 85-100%) had a PRP antibody titer ≥ 0.15 µg/ml versus 59 percent before vaccination (95% CI 36-79%), and 95 percent had a PRP antibody titer ≥ 1.0 µg/ml (95% CI 77-100) versus 5 percent before vaccination (95% CI 0-23%). GMT of anti-PRP antibody increased from 0.18 (95% CI 0.11-0.32%) before immunization to 15 (95% CI

Table I: Percentages of Subjects Reporting Local and Systemic Reactions within Seven Days after the Three Immunizations

Reaction	n=23
Local reactions at	
Left thigh (Hib)	
Tenderness	9%
Erythema	13%
Induration	0%
Right thigh (DTwP)	
Tenderness	22%
Erythema	35%
Induration	61%
Systemic reactions	
Rash	0%
Change in eating habits	22%
Sleepiness	17%
Unusual crying	4%
Persistent crying	26%
Irritability	48%
Vomiting	9%
Diarrhea	17%
Rectal temperature ≥ 38 °C	70%

Table II: Anti-PRP Antibody Titers Before the First and after the Third Immunization

	n	Titer ≥ 0.15 µg/ml %	(95% CI)	Titer ≥ 1.0 µg/ml %	(95% CI)	GMT µg/ml	(95% CI)	GMR ratio GMT	(95% CI)
Day 0	23	59	(36-79)	5	(0-23)	0.18	(0.11-0.32	–	
Day 84	23	100	(85-100)	95	(77-100)	15	(8.21-29)	84	(36-196)

GMT: geometric mean titer GMR: geometric mean ratio CI: confidence interval

8.21-29%) after immunization, which corresponds to a geometric mean ratio (GMR) of 84.

Discussion

This was a pilot study to primarily evaluate the tolerability of a new Hib vaccine with $AlPO_4$ as adjuvant. Adjuvants have been used to augment the immune response in vaccinations for more than 60 years, but they may be responsible for some of the vaccine side effects[12,13]. The most commonly used adjuvants for human vaccines are aluminum hydroxide, aluminum phosphate and calcium phosphate[12]. In animals it has been shown that aluminum phosphate may be a more potent adjuvant than aluminum hydroxide for several antigent[14,15]. In addition, the aluminum hydroxide adjuvant may lead to catalytic depolymerization of PRP[16]. The substitution of the aluminum hydroxide adjuvant has also led to a more convenient presentation of the vaccine which can be formulated in a single container.

In this study there was no serious side effect or death reported during the study period. The local side effects at the Hib site were less frequent and mild compared to those observed at the DPT site. Systemic reactions were more frequent than local reactions, including persistent crying, irritability, sleepiness, and febrile reactions most likely attributable to the DPT vaccine[17].

The anti-PRP antibody response after three doses of vaccine was highly satisfactory. All infants achieved an anti-PRP level over 0.15 µg/ml, which is considered protective, and 95 percent achieved an anti-PRP antibody level over 1.0 µg/ml, which is indicative of long-term protection. The GMTs were comparable to those published earlier with vaccines proven to be protective[18-20] despite the presence of maternal antibodies.

The results of this study indicate that three doses of Hib/$AlPO_4$ (CHIRON SpA) vaccine are safe and well tolerated and induce a good PRP antibody response. A further large scale clinical trial to assess the safety and immunogenicity of this vaccine is in progress.

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