Safety and immunogenicity of two *Haemophilus influenzae* type b conjugate vaccines

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Objectives. *Haemophilus influenzae* type b (Hib) infection remains a major public health problem in the developing world. We evaluated the safety and immunogenicity of a new PRP-CRM_{197} conjugate Hib vaccine (Vaxem Hib, Chiron Vaccines), compared with the HibTITER vaccine (Wyeth-Lederle Vaccines), following the World Health Organisation (WHO)'s accelerated schedule which allows 4-week intervals between doses.

Study design. A phase II, observer-blind, multicentre, randomised, controlled, non-inferiority study.

Methods. In total, 331 babies were immunised with either Vaxem Hib (N = 167) or HibTITER (N = 164) vaccine at 6, 10 and 14 weeks of age, in parallel with oral polio, diphtheria-tetanus-pertussis and hepatitis B vaccines. Post-immunisation reactions were recorded after each immunisation and at follow-up visits. Anti-polyribosyl-ribitol phosphate (PRP) antibodies were measured using enzyme-linked immunosorbent assays (ELISAs) before and 1 month after the third immunisation.

Results. Overall, there was no significant difference in the anti-PRP levels between the two groups. One month after the third immunisation, 76% of vaccinees in the Vaxem Hib group and 70% in the HibTITER group had anti-PRP antibody titres ≥ 1.0 µg/ml, while 96% of the Vaxem Hib group and 90% of the HibTITER group demonstrated anti-PRP antibody titres ≥ 0.15 µg/ml. The geometric mean titre at day 90 was 3.77 µg/ml for the Vaxem Hib and 3.0 µg/ml for the HibTITER groups. Although the Vaxem Hib vaccine produced more redness (6% versus 1%; p = 0.006) and swelling (5% versus 1%, p = 0.037), overall it was well tolerated compared with the HibTITER vaccine. There was no significant difference in vaccine-related elevated temperature (≥ 38°C) between the two groups (p = 0.11).

Conclusion. Both vaccines showed comparable safety and immunogenicity profiles when administered to South African babies at 6, 10 and 14 weeks of age.

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Haemophilus influenzae type b (Hib) remains the principal cause of invasive bacterial diseases in under 5-year-olds in developing countries.[1-3] In those few developing countries where Hib vaccines are being used, the vaccines have proven to be effective in reducing transmission rates and protecting children at risk from invasive Hib diseases.[4,5]

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South Africa introduced universal Hib vaccination into the Expanded Programme on Immunisation (EPI) in July 1999.[6] A recent pre-immunisation study,[7] conducted in Cape Town, indicated that Hib accounted for 86.5% of all the *H. influenzae* cases, 97.3% of meningitis cases, 71.4% of pneumonia cases and 50% of septicaemia cases.[7] The incidence rates for all invasive Hib infections were found to be 169 and 47 per 100 000 population for children < 1 and < 5 years, respectively.[7] These figures are no different from those in many other developing countries where there is a high burden of Hib diseases.[3,4]

Hib vaccines contain a capsular polysaccharide, polyribosyl-ribitol phosphate (PRP), which is the major virulence factor,[2] and a target for eliciting protective antibodies (anti-PRP) against Hib disease.[8-10] Current Hib vaccines contain polysaccharide antigens chemically conjugated to a carrier protein (derived from either *Corynebacterium diphtheriae, Clostridium tetani,* or *N. meningitidis*) to enhance their immunogenicity and boost immunological memory.[11-13]

South Africa has adopted the World Health Organisation (WHO)'s accelerated 4-week interval immunisation schedule, whereby children are vaccinated against polio, diphtheria-tetanus-pertussis, hepatitis B and Hib diseases at the ages of 6, 10 and 14 weeks. In this study, we evaluated the safety and

immunogenicity of a new PRP-CRM_{197} conjugate Hib vaccine (Vaxem Hib, Chiron Vaccines), compared with the HibTITER vaccine (Wyeth-Lederle Vaccines), following the WHO's accelerated immunisation schedule (4-week intervals between doses).

Materials and methods

Study design and study area

This was a phase II, observer-blind, multicentre, randomised, controlled, non-inferiority study, conducted in three different clinics: Phedisong 1 (Ga-Rankuwa), Hebron (Hebron) and Boekenhout (Mabopane), all of which are situated north-west of Pretoria, South Africa.

Vaccines

The investigational vaccine was the Hib b conjugate vaccine, Vaxem Hib (Chiron Vaccines, Siena, Italy). The chemistry of this vaccine was based on controlled hydrolysis of the PRP polysaccharide to oligosaccharides, followed by selective activation of the reducing end and subsequent coupling to the carrier protein, through an adipic acid spacer.[14] The non-toxic mutant of diphtheria toxin, CRM_{197}, was used as a protein carrier. In the final vaccine formulation, aluminium hydroxide was used as an adjuvant.

The pre-clinical and clinical development of this vaccine showed a high level of tolerability and immunogenicity,[14,15] and allowed it to be registered for use in infants and children under the age of 12 months, with an 8-week interval between doses. Each dose of Vaxem Hib contains 10 µg of capsular oligosaccharide of Hib conjugated to approximately 25 µg of carrier protein, mutant diphtheria CRM_{197}, and the final formulation contains 0.05 mg of thiomersal, 1 mg of aluminium hydroxide and sodium phosphate buffer (pH = 7) in a 0.5 ml volume.

The comparative vaccine was the American Home Product HibTITER vaccine (Wyeth-Lederle Vaccines, USA). Each 0.5 ml dose contains 10 µg of purified Hib capsular antigen and approximately 25 µg of diphtheria CRM_{197} protein conjugate, dissolved in 0.9% sodium chloride. The HibTITER is indicated for the immunisation of babies and children aged 6 weeks - 5 years, against Hib diseases. The HibTITER vaccine has the same carrier protein, CRM_{197}, as the Vaxem Hib vaccine, but lacks the aluminium hydroxide adjuvant in the final formulation.

Ethics and consent

The study protocol was approved by the Research and Ethics Committee of the Faculty of Medicine of the Medical University of Southern Africa (MEDUNSA) and the Medicines Control Council of South Africa. Parents or legal guardians of the subjects were adequately informed of the study, and only babies of parents and legal guardians who signed consent forms were recruited into the study.

Subjects' enrolment and selection criteria

A total of 331 healthy South African babies who were candidates for primary vaccination according to the EPI schedule were recruited, and received either Vaxem Hib vaccine (N = 167, 89 male and 78 female, mean age 47.5 days), or HibTITER vaccine (N = 164, 77 male and 87 female, mean age 47.4 days). Subjects were included if they were 6 - 8 weeks of age and in good health, as determined by medical history, physical examination and clinical assessment.

Vaccination protocols

Each of the study vaccines was injected intramuscularly in the anterolateral muscle of the right thigh. The other routine infant EPI vaccines (i.e. diphtheria-tetanus-pertussis (DTwP), hepatitis B vaccine (HBV) and oral polio vaccine (OPV)), were administered concomitantly, but separately, following standard procedures. DTwP was given as an intramuscular injection in the anterolateral muscle of the left thigh, HBV was administered in the right deltoid region, and OPV was given orally.

Randomisation and blinding

Randomisation was done using a 1:1 ratio and was stratified by immunisation centre. To ensure observer blinding, only designated unblinded study staff who had no access to the study subjects or to their records had access to the randomisation list for vaccine assignment. The unblinded staff were instructed not to reveal (except in an emergency) the identity of the study vaccines to the parents or legal guardians or to the other blinded personnel (investigator, study nurse, etc.) involved in monitoring or conducting the study.

Evaluating post-immunisation reactions

Babies were observed for signs and symptoms of local or systemic reactions at the clinic (30 minutes after each of the three vaccine administrations) by the study personnel and at home (2 - 3 days after immunisation) by the parent or legal guardian and/or study nurse at follow-up visits.

Laboratory methods

Collection of specimens

Samples of venous blood (2 - 3 ml) for antibody assays were drawn before the first immunisation and 4 - 6 weeks after the third immunisation. The clotted blood was stored at 2 - 8°C and centrifuged within 12 hours. Serum samples were stored at –20°C until shipped in dry ice to Chiron laboratories, Siena, Italy.

Measurement of serum antibody titres

The anti-PRP antibody assays were performed at the Chiron laboratories, Siena, Italy, using a modified enzyme-linked immunosorbent assay (ELISA) technique.[16] The Food and Drug Administration (FDA) standard reference sera, with known antibody titres, were used. Measures of immunogenicity were anti-PRP antibody titres ≥ 0.15 µg/ml (for protection) and ≥ 1.0 µg/ml (for long-lasting protection).

Statistical methods

This was designed as a non-inferiority study, adequately powered to exclude differences in percentage of subjects with post-immunisation anti-PRP titres ≥ 1.0 µg/ml greater than 15% by means of a two-sided 90% confidence interval (CI).[17]

Descriptive statistics (mean, standard deviation, median, minimum and maximum) were calculated. Incidences of local and systemic reactions were compared between vaccine groups using the chi-square test, or Fisher's exact test. The geometric mean titres (GMTs) and 95% CIs were calculated by exponentiating (base 10) the least squares means of the logarithmically transformed (base 10) titres and their 95% CIs obtained from a one-way analysis of variance (ANOVA) with factor for vaccine group. The geometric mean ratios (GMRs) and 90% CIs were calculated by exponentiating the difference between the least squares means and the associated 90% CI from the same ANOVA.

Results

Immunisations schedules

Initially, 167 babies received Vaxem Hib vaccine and 164 received HibTITER vaccine. However, 17 subjects (10%) in the Vaxem Hib group were eventually excluded after receiving the first dose, 4 because of initial inappropriate enrolment and 13 because they were lost to follow-up. Similarly, 32 subjects (20%) in the HibTITER group were eventually excluded, for several reasons: 24 because they were lost to follow-up, 6 because of protocol deviation or inappropriate initial enrolment, 1 due to an adverse event, and 1 because of withdrawal of consent. Therefore, a total of 150 subjects (90%) in the Vaxem Hib group and 132 subjects (80%) in the HibTITER group completed the immunisation protocols, and of these babies, 143 (86%) in the Vaxem Hib group and 126 (77%) in the HibTITER group were included in the immunogenicity analyses (Fig. 1).

Primary and secondary post-immunisation response

At baseline there was no significant difference between study subjects with anti-PRP antibody titres ≥ 1.0 µg/ml in the two groups (14% v. 15%) in the subset of babies evaluated for immunogenicity (Fig. 1). At day 90, 1 month after the third

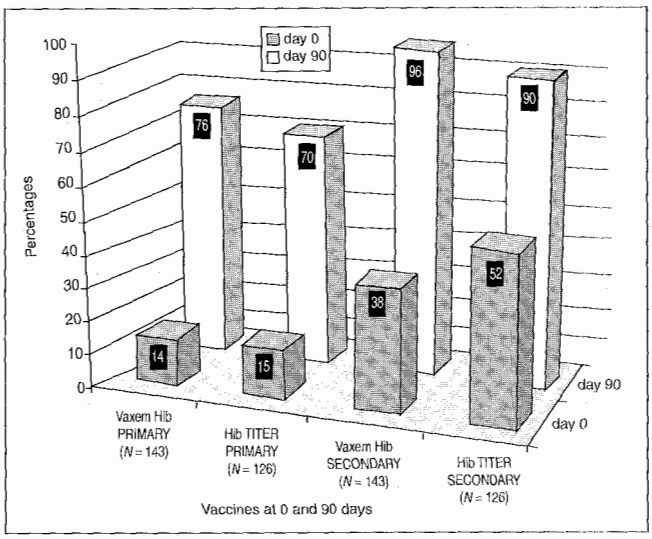

Fig. 1. Primary (anti-PRP (1.0 µg/ml) and secondary (anti-PRP ≥ 0.15 µg/ml) immunogenicity measures.

immunisation, 76% of vaccinees in the Vaxem Hib group and 70% of those in the HibTITER group demonstrated a titre ≥ 1.0 µg/ml, and this difference was not statistically significant. Since the lower limit of the CI was well above the pre-specified value of –15% (90% CI: –3% - 15%), non-inferiority was demonstrated.

At baseline there was a significant imbalance for both percentage of subjects with anti-PRP titres ≥ 0.15 µg/ml and anti-PRP GMT, with higher values in the HibTITER group. After vaccination, the Vaxem Hib vaccine group was slightly more immunogenic when anti-PRP antibody titres ≥ 0.15 µg/ml were used as a measure 1 month after the last dose. It was found that 96% in the Vaxem Hib group and 90% in the HibTITER group had anti-PRP antibody titres ≥ 0.15 µg/ml 1 month after the third immunisation in the same subset of subjects evaluated for immunogenicity (Fig. 1). Moreover, the post-vaccination GMT for the Vaxem Hib group was 3.77 µg/ml and 3.0 µg/ml for the HibTITER group, and the 90% CI for their ratio included 1, i.e. the difference was not statistically significant. The ratio of Vaxem Hib vaccine to HibTITER vaccine GMRs was 1.77 (90% CI: 1.1 - 2.85).

Post-immunisation reactions

In both vaccine groups, local and systemic reactions were mild and transient and resolved without sequelae. Although the Vaxem Hib vaccine produced more redness (6% versus 1%, $p = 0.006$) and swelling (5% versus 1%, $p = 0.037$) than the HibTITER vaccine, overall it was well tolerated. Finally, 6% of subjects in the Vaxem Hib group demonstrated a post-vaccination fever (temperature ≥ 38°C) versus 2% in the HibTITER group, which was not statistically significant ($p = 0.11$).

Discussion

The widespread application of Hib conjugate vaccines in the USA has resulted in the decline of the number of reported Hib invasive cases by 99% between 1987 and 1997.[18] In The Gambia, the efficacy of the conjugate vaccine was 95% for the prevention of all invasive Hib diseases.[4] These are encouraging results and clearly demonstrate the feasibility of eliminating Hib diseases through effective immunisation programmes. However, significant reduction in childhood morbidity and mortality from Hib diseases will only occur when vaccination coverage rates with Hib conjugate vaccines have been achieved in most of the developing world. The major constraint on introducing these vaccines is cost. One study investigated the potential of maximising the use of these vaccines in poorer countries by diluting a Hib conjugate vaccine 10-fold in a multidose vial of DTwP vaccine, and the diluted vaccine was found to be as immunogenic and safe as the full dose.[19]

Many developing countries vaccinate infants according to the WHO's accelerated 4-week interval schedule. An ideal Hib conjugate vaccine for the developing world should be easily integrated into this schedule, without compromising its immunogenic potency, and should be compatible with other childhood vaccines. We found comparable immunogenicity results for the Vaxem Hib vaccine and the HibTITER vaccine for anti-PRP antibody titres ≥ 1.0 μg/ml before and after the immunisation course, and non-inferiority of the Vaxem Hib vaccine was demonstrated (Fig. 1). However, overall the Vaxem Hib vaccine was slightly more immunogenic than the HibTITER vaccine, perhaps due to aluminium hydroxide adjuvant, which is not present in the HibTITER.

Further, the Vaxem Hib vaccine was well tolerated, despite producing slightly more redness and swelling at the site of injection compared with the HibTITER vaccine. Increased local reaction at the injection site is a well-known and normal consequence of an adjuvant, in this case included in the Vaxem Hib vaccine but not in the HibTITER vaccine.

In conclusion, Vaxem Hib and HibTITER vaccines demonstrated comparable safety and immunogenicity profiles when administered to South African infants at 6, 10 and 14 weeks of age. Infants in countries such as South Africa with a high endemicity of Hib invasive diseases need to be protected with an optimal immunogenic vaccine in early childhood as provided by the WHO accelerated immunisation schedule.

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