A new DTPw-HB/Hib combination vaccine for primary and booster vaccination of infants in Latin America

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ABSTRACT

Objectives. In 1998 the World Health Organization (WHO) recommended the inclusion of Haemophilus influenza type B (Hib) conjugate vaccines in infant immunization programs, whenever in accordance with national priorities. GlaxoSmithKline Biologicals has developed a new pentavalent combined diphtheria-tetanus-wholet cell pertussis-hepatitis B/Hib (DTPw-HB/Hib) vaccine containing 5 µg of polyribosylribitol phosphate (PRP), and we assessed the immunogenicity and reactogenicity of primary and booster vaccination of healthy children with this new vaccine compared with a reference regimen consisting of the licensed DTPw-HB (Tritanrix) and Hib (Hiberix) vaccines given as simultaneous concomitant injections.

Methods. We performed a randomized, double-blind study from September 1998 to August 1999 to establish the immunogenicity and reactogenicity of primary and booster vaccination of healthy children with the new pentavalent combined DTPw-HB/Hib vaccine given as a single injection, compared with the reference regimen.

Results. Both vaccination regimens elicited excellent immune responses, with all subjects in both groups achieving seroprotective anti-PRP antibody concentrations of ≥ 0.15 µg/mL one month after primary vaccination. The combined DTPw-HB/Hib vaccine was non-inferior to the licensed vaccines in terms of seroprotection-seropositivity/vaccine response rates for all antigen components. Persistence of antibodies against all study vaccine antigens up to the time of booster vaccination was comparable between groups, and a marked increase of all antibody concentrations was observed after the booster dose. Both vaccine regimens were similar in terms of their overall reactogenicity profiles.

Conclusions. Our results indicate that the new DTPw-HB/Hib pentavalent combination vaccine provides an efficient and reliable way of implementing WHO recommendations for controlling hepatitis B and Hib infections on a worldwide basis.

Key words. Vaccines, combined; diphtheria-tetanus-pertussis vaccine; hepatitis B vaccines; Haemophilus vaccines; Latin America.

The notion of co-administering multiple antigens in a single injection is now a widely accepted means of maximizing the efficiency and cost-effectiveness of infant immunization
Hepatitis B (HB) and Haemophilus influenzae type b (Hib) infections continue to be endemic in many parts of the world and are still causing disease that could readily be prevented by immunization.

It has been estimated that two billion people in developing countries are now infected with hepatitis B virus and are at high risk of mortality from chronic sequelae (2). Between 70% and 90% of infants infected during the first year of life become chronic carriers (3). These children not only serve as a continuous reservoir for hepatitis B transmission, but are also themselves at risk of developing related diseases, such as cirrhosis and primary hepatocellular carcinoma, later in life (4, 5). For this reason the World Health Organization (WHO) recently reaffirmed its position on hepatitis B vaccination, stating that “universal infant immunization is by far the most effective preventive measure against [hepatitis B virus]-induced disease” (6).

Hib is a principal cause of pneumonia and bacterial meningitis in children, and it is associated with significant childhood morbidity and mortality around the world (3). In developing countries the incidence of Hib disease is higher than in the rest of the world, with infection occurring at a younger age, and with more than half of the cases occurring before 6 months of age (7). In addition, it is often difficult, if not impossible, to establish the exact cause of pneumonia cases. In at least two large field trials, Hib vaccination was shown to provide substantial protection against pneumonia in infants (8, 9). By preventing approximately five times as many nonbacteremic pneumonia cases as meningitis cases, the largest part of the effect of Hib vaccination might be undetectable by routine culture methods. These statistics highlight the need to provide immunization against HB and Hib disease during the first year of life. In 1998, WHO further recommended the inclusion of Hib conjugate vaccines in infant immunization programs, if appropriate and consistent with national capacities and priorities (10).

In 1996 the WHO set an objective for using combined vaccines such as hepatitis B viral antigen with the established diphtheria-tetanus-whole cell pertussis (DTPw) antigens (11). The Tritanrix combined DTPw-HB vaccine (GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium) has been licensed since 1996, and its immunogenicity and reactogenicity profile, when administered according to a three-dose primary course, is well-established (12–15). It has also been shown that Tritanrix can be extemporaneously mixed with the Hibrix conjugated Haemophilus influenzae vaccine (GSK Biologicals, Rixensart, Belgium) and administered as a single injection (16, 17).

In order to ease the implementation of the WHO recommendations, GSK Biologicals has developed a new combined DTPw-HB/Hib vaccine containing 5 μg of polyribosylribitol phosphate (PRP). This study was designed to assess the immunogenicity and reactogenicity of primary and booster vaccination of healthy children with this new pentavalent combined DTPw-HB/Hib vaccine when given as a single injection, compared to a reference vaccine regimen consisting of the licensed Tritanrix and Hibrix vaccines given as simultaneous concomitant injections. The study was conducted in four countries of Latin America.

**MATERIALS AND METHODS**

**Study participants and study design**

The primary-vaccination study was undertaken at four centers in Latin America (Argentina (200 subjects), Colombia (300 subjects), the Dominican Republic (200 subjects), and Nicaragua (300 subjects)) from September 1998 to August 1999. The booster study was undertaken in June–July 2000 at the center in the city of Córdoba, Argentina.

One thousand healthy infants were randomized in a balanced 1:1:1:1 allocation to receive either one of three lots of the candidate combined DTPw-HB/Hib vaccine as a single injection (750 subjects total) or the licensed Tritanrix and Hibrix vaccines given as separate concomitant injections (250 subjects). Three different production lots of the combined DTPw-HB/Hib vaccine were used in a double-blind manner. Because of the two separate injections, blinding could not be maintained for the control group. The vaccines were administered according to the recommendations of the four nations’ national immunization programs, at 2, 4, and 6 months of age.

In order to comply with official recommendations from the Ministry of Health of Argentina, subjects from that country (planned number = 200) were invited for booster vaccination at 18 to 24 months of age, with the same vaccine that they had received during the primary course.

In all four countries, subjects were free from acute disease at the time of the first vaccination, and they were not allowed to participate in other trials or vaccination programs. The exclusion criteria were: a history of allergy to any vaccine component, prior or existing neurological disease, any previous vaccination other than with oral polio and bacillus Calmette-Guérin (BCG) vaccine, and any immune deficiency or immunosuppressive or immunoglobulin therapy.

The study was approved by the ethics committees responsible for each trial center, and was conducted in accordance with the provisions of the Declaration of Helsinki and its amendments. Good Clinical Practice guidelines were observed throughout. Written informed consent was obtained from the subjects’ parents or guardians prior to entry into the study.

**Vaccines**

All the vaccines were manufactured by GSK Biologicals, Rixensart, Belgium. The DTPw-HB (Tritanrix) vaccine contained ≥ 30 international units (IU) diphtheria toxoid, ≥ 60 IU tetanus toxoid, ≥ 4 IU Bordetella pertussis (Bp), 10 μg hepatitis B surface antigen...
(HBsAg), and 0.63 mg aluminum adjuvants (as salts). In the control group the DTPw-HB vaccine was injected concomitantly in separate injections with GSK Biologicals’ licensed H. influenzae type b conjugate vaccine, Hiberix, which contains 10 μg of the capsular PRP polysaccharide conjugated to 20–40 μg of tetanus toxoid. In the candidate vaccine group the liquid DTPw-HB vaccine was used to reconstitute a new lyophilized Hib-conjugate vaccine, containing 5 μg of PRP, conjugated to 10–20 μg of tetanus toxoid and adsorbed onto 0.06 mg of aluminum (as salts).

Serology

Blood samples (3 mL) for serology assessments were taken on entry into the study (Visit 1, at 2 months of age), prior to the third dose of primary vaccination (Post-dose 2, Visit 3, at 6 months of age), and one month after the last primary vaccination (Post-dose 3, Visit 4, at 7 months of age). In the subset of subjects who returned for the booster study, a blood sample was also collected at the time of the booster vaccination (at 18 months of age), and one month after that (at 19 months of age). The AUSUB radioimmunoassay (Abbott Laboratories, Abbott Park, Illinois, United States of America) was used to measure antibodies against hepatitis B surface antigen (anti-HBs), with an assay cutoff set at 10 mIU/mL. Anti-tetanus and anti-diphtheria antibody concentrations were measured by ELISA, with a cutoff of 0.1 IU/mL. Anti-PRP antibody concentrations were measured by enzyme-linked immunosorbent assay (ELISA), with a cutoff of 0.15 μg/mL, and anti-Bordetella pertussis (anti-Bp) antibody concentrations were measured using an ELISA kit (Labsystems, Helsinki, Finland), with a cutoff of 15 ELISA units/mL (EU/mL). With the exception of anti-Bp, antibody concentrations at or above the assay cutoffs were considered to be indicative of protection against disease.

As no correlate of protection is established for Bordetella pertussis, a vaccine response to this vaccine component was defined. For primary vaccination, taking into account the decline in maternal antibodies, a pertussis vaccine response was defined as the appearance of anti-Bp antibodies in initially seronegative subjects, or the presence of a postvaccination antibody concentration greater than or equal to the initial prevaccination concentration. A booster response against the pertussis component was defined as the appearance of antibodies in prebooster seronegative subjects, or at least a 2-fold increase in the anti-Bp antibody concentration in subjects seropositive prior to boosting.

Reactogenicity

Details of adverse events were collected on diary cards. Reactogenicity data were collected during a 4-day follow-up period after each vaccination. Solicited local symptoms included pain, redness, and swelling at the site of injection, and solicited general symptoms included drowsiness, fever (defined as axillary temperature ≥ 37.5 °C), fussiness or irritability, and loss of appetite. Intensity of general symptoms (apart from fever) was graded on a 3-point scale, with Grade 1 corresponding to minimal discomfort and Grade 3 corresponding to interference with daily activities. All other symptoms were recorded for a period up to 30 days postvaccination, and serious adverse events (SAEs) were recorded during the entire study period.

Statistical analysis

Statistical analyses were performed for the according-to-protocol (ATP) cohorts. Subjects excluded from the ATP cohorts were identified before data analysis after a review of the individual subject data blinded to group allocation.

It was calculated that 224 evaluable subjects per treatment group would provide 90% power to reject the null hypothesis. The working hypothesis was that the percentage of subjects with anti-PRP antibody concentration ≥ 0.15 μg/mL one month following the immunization regimen would differ by 5% or more in at least two of the three lots. Since it was assumed that up to 10% of subjects might be unevaluable for the ATP analysis, it was necessary to enroll 250 subjects in each vaccine group. In the booster study, all subjects who had completed the primary vaccination at the center in Córdoba, Argentina (planned to be 200), were invited to participate.

Seropositivity/seroprotection/vaccine response rates and geometric mean antibody concentrations (GMCS) were calculated with 95% confidence intervals (CIs) at each blood sampling time point. For GMC calculations, samples with antibody levels below the assay cutoff were arbitrarily assigned a value corresponding to half the cutoff value of the test. The incidence of solicited symptoms was calculated with exact 95% CIs for each type of adverse event.

The difference between groups with respect to seropositivity/seroprotection/vaccine response rates following primary vaccination was calculated with 90% CIs. The 90% CIs were computed to demonstrate non-inferiority, ensuring a 5% type I error when comparing the upper limit to the predefined non-inferiority limit (one-sided test). The response following primary vaccination with the candidate DTPw-HB/Hib vaccine was considered to be non-inferior to that observed with the control vaccines if the upper limit of the 90% CI of the group difference was < 5% for anti-PRP ≥ 0.15 μg/mL and was < 10% for all other antigens.

Statistical tests were computed using SAS 6.12 software (SAS Institute Inc., Cary, North Carolina, United States) and the StatXact-3 (Cytel Inc., Cambridge, Massachusetts, United States) procedure on SAS.

RESULTS

A total of 1 000 infants were enrolled in the primary-vaccination study and were randomly allocated to receive
one of the two vaccine regimens. The disposition of the subjects is shown in Figure 1. Seventy-six participants did not complete the primary-vaccination study: 34 moved from the study area or were lost to follow-up, consent was withdrawn in 21 cases, 12 for other reasons, and 2 infants died during the study. A total of 968 subjects were included in the ATP analysis of safety (29 were excluded because essential data were missing, and 3 for randomization failure). An additional 256 subjects were eliminated from the ATP analysis of immunogenicity (due to noncompliance with the vaccination or blood-sampling schedule or subjects being outside of the specified age range at enrollment), resulting in 712 subjects being included in the ATP cohort for immunogenicity analysis.

A total of 143 subjects were enrolled in the booster study, out of 200 subjects enrolled for priming in Argentina. All but one subject, who moved away from the study area, completed the booster phase. Three subjects were excluded from the ATP cohort for safety (2 for randomization failure, and 1 with essential data missing), and an additional 6 subjects were excluded from the ATP cohort for immunogenicity (for failure to comply with the blood-sampling schedule).

The mean age of the total cohort at the time of first vaccination was 8.0 ± 1.8 weeks, with a male:female ratio of 1:1. The mean age at the time of the booster dose was 18.0 ± 0.73 months, with a male:female ratio of 0.93:1.

No clinically significant differences were observed among the three different candidate DTPw-HB/Hib vaccine lots used. Therefore, we only show the results of the pooled candidate-vaccine lots.

**Anti-PRP antibody response**

Two months after the second primary-vaccination dose, 98.7% of subjects who received DTPw-HB/Hib had anti-PRP antibody concentrations ≥ 0.15 μg/mL, compared to 92.6% in the control group receiving the licensed DTPw-HB and Hib vaccines separately (Table 1). Following completion of the three-dose primary-vaccination course, this increased to 100% in both groups. In addition, after the third primary-vaccination dose, 99.4% of subjects who received DTPw-HB/Hib had anti-PRP antibody concentrations ≥ 1.0 μg/mL. No significant difference between groups was observed in terms of anti-PRP antibody GMCs after primary vaccination. The anti-PRP response after primary vaccination with the new combined DTPw-HB/Hib vaccine was shown to be non-inferior to that with the licensed vaccines administered separately, since the upper limit of the 90% CI for the difference (control minus new combined DTPw-HB/Hib) in the percentage of subjects achieving anti-PRP antibody levels ≥ 0.15 μg/mL was...
1.1%, and therefore below the predefined clinical limit of 5%.

Prior to the booster dose, at approximately 18 months of age, 99.0% of the subjects in the DTPw-HB/Hib group continued to have seroprotective anti-PRP antibody concentrations ≥ 0.15 μg/mL, compared with 96.9% in the control group. A marked increase in anti-PRP antibody GMC was observed in both groups after booster vaccination (Figure 2).

### TABLE 1. Anti-PRP antibody responses following primary and booster vaccination with licensed DTPw-HB and Hib vaccines given separately or with combined DTPw-HB/Hib vaccine, in four countries of Latin America, 1998–1999

<table>
<thead>
<tr>
<th>Vaccination(s)/Timing (age in months)</th>
<th>No.</th>
<th>Anti-PRP ≥ 0.15 μg/mL</th>
<th>Anti-PRP ≥ 1.0 μg/mL</th>
<th>Anti-PRP GMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separate DTPw-HB + Hib primary vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-vaccination (Month 2)</td>
<td>178</td>
<td>43.8</td>
<td>36.4–51.4</td>
<td>0.175</td>
</tr>
<tr>
<td>Post-dose 2 (Month 6)</td>
<td>176</td>
<td>92.6</td>
<td>87.7–96.0</td>
<td>3.296</td>
</tr>
<tr>
<td>Post-dose 3 (Month 7)</td>
<td>177</td>
<td>100.0</td>
<td>97.9–100.0</td>
<td>34.069</td>
</tr>
<tr>
<td>Separate DTPw-HB + Hib booster</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prebooster (Month 18)</td>
<td>32</td>
<td>96.9</td>
<td>83.8–99.9</td>
<td>2.021</td>
</tr>
<tr>
<td>Postbooster (Month 19)</td>
<td>31</td>
<td>100.0</td>
<td>88.8–100.0</td>
<td>145.306</td>
</tr>
<tr>
<td>Combined DTPw-HB/Hib primary vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-vaccination (Month 2)</td>
<td>529</td>
<td>41.6</td>
<td>37.4–45.9</td>
<td>0.172</td>
</tr>
<tr>
<td>Post-dose 2 (Month 6)</td>
<td>521</td>
<td>98.7</td>
<td>97.3–99.5</td>
<td>6.186</td>
</tr>
<tr>
<td>Post-dose 3 (Month 7)</td>
<td>524</td>
<td>100.0</td>
<td>99.3–100.0</td>
<td>26.858</td>
</tr>
<tr>
<td>Combined DTPw-HB/Hib booster</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prebooster (Month 18)</td>
<td>100</td>
<td>99.0</td>
<td>94.6–100.0</td>
<td>2.652</td>
</tr>
<tr>
<td>Postbooster (Month 19)</td>
<td>99</td>
<td>100.0</td>
<td>96.3–100.0</td>
<td>87.876</td>
</tr>
</tbody>
</table>

### FIGURE 2. Anti-polyribosylribitol phosphate (anti-PRP) antibody geometric mean concentrations (GMCs) following primary and booster vaccination with licensed diphtheria-tetanus-whole cell pertussis-hepatitis B (DTPw-HB) vaccine and Haemophilus influenzae type B (Hib) vaccine given separately or with a new combined DTPw-HB/Hib vaccine, in four countries of Latin America, 1998–1999

Response to other vaccine components

Tables 2 and 3 present the immune response to the other vaccine antigens. The majority (> 98%) of subjects in both groups developed seroprotective antibody concentrations against diphtheria, tetanus, and hepatitis B, and demonstrated a vaccine response against pertussis after primary vaccination (Table 2). For all the antigens, the seroprotection/vaccine response rates following primary vaccination with the candidate DTPw-HB/Hib vaccine were non-inferior to those following priming with the licensed control vaccines: The upper limit of the 90% CIs for the differences in the percentage of subjects achieving seroprotective antibodies/vaccine response for each antigen was below the predefined clinical limit for non-inferiority (2.6% for anti-HBs, 0.7% for anti-diphtheria, 1.1% for anti-tetanus, and 1.6% for the vaccine response to Bp).

After primary vaccination, anti-HBs antibody GMCs were significantly lower after DTPw-HB/Hib vaccination than after DTPw-HB + Hib vaccination (Table 3, evidenced by non-overlapping
### TABLE 2. Seroprotection rates for anti-tetanus, anti-diphtheria, and anti-HBs antibodies and vaccine response rates for anti-Bp following primary and booster vaccination with licensed DTPw-HB and Hib vaccines given separately or with combined DTPw-HB/Hib vaccine, in four countries of Latin America, 1998–1999\(^a,b,c,d\)

<table>
<thead>
<tr>
<th>Vaccination(s)/Timing</th>
<th>Anti-tetanus ≥ 0.1 IU/mL(^e)</th>
<th>Anti-diphtheria ≥ 0.1 IU/mL</th>
<th>Anti-HBs ≥ 10 IU/mL</th>
<th>Anti-Bp VR(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>95% CI</td>
<td></td>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>95% CI</td>
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<td>No.</td>
<td>%</td>
<td>95% CI</td>
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<td></td>
<td>No.</td>
<td>%</td>
<td>95% CI</td>
<td></td>
</tr>
</tbody>
</table>

**Separate DTPw-HB + Hib primary vaccination**

- **Prevaccination**
  - 179 96.6 92.8–98.8
  - 179 53.1 45.5–60.6
  - 174 4.0 1.6–8.1

- **Post-dose 2**
  - 179 87.2 81.3–91.7
  - 179 89.9 84.6–93.9
  - 179 97.8 94.4–99.4

- **Post-dose 3**
  - 176 100.0 97.9–100.0
  - 177 98.9 96.0–99.9
  - 177 99.4 96.9–100.0

**Separate DTPw-HB + Hib booster**

- **Prebooster**
  - 32 78.1 60.0–90.7
  - 32 62.5 43.7–78.9
  - 32 90.6 75.0–98.0

- **Postbooster**
  - 32 100.0 89.1–100.0
  - 32 100.0 89.1–100.0
  - 32 100.0 89.1–100.0

**Combined DTPw-HB/Hib primary vaccination**

- **Prevaccination**
  - 530 95.5 93.3–97.1
  - 524 48.1 43.7–52.5
  - 518 5.4 3.6–7.7

- **Post-dose 2**
  - 529 99.1 97.8–99.7
  - 529 87.9 84.8–90.6
  - 532 95.1 92.9–96.8

- **Post-dose 3**
  - 525 100.0 99.3–100.0
  - 525 99.8 98.9–100.0
  - 524 99.0 97.8–99.9

**Combined DTPw-HB/Hib booster**

- **Prebooster**
  - 102 97.1 91.6–99.4
  - 102 65.7 55.6–74.8
  - 100 93.0 86.1–97.1

- **Postbooster**
  - 101 100.0 96.4–100.0
  - 101 99.0 94.6–100.0
  - 100 95.0 88.7–98.4

\(^a\) Anti-HBs = hepatitis B surface antibody.

\(^b\) Bp = Bordetella pertussis.

\(^c\) DTPw-HB = diphtheria-tetanus-whole cell pertussis-hepatitis B vaccine.

\(^d\) Hib = Haemophilus influenzae type B.

\(^e\) IU = international units.

\(^f\) VR = vaccine response.

\(^g\) No. = number of subjects tested.

\(^h\) % = percentage of subjects above the specified cutoff.

\(^i\) CI = confidence interval.

\(^j\) NA = not applicable.

### TABLE 3. Antibody geometric mean concentration (GMC) for anti-tetanus, anti-diphtheria, anti-HBs, and anti-Bp following primary and booster vaccination with licensed DTPw-HB and Hib vaccines given separately or with combined DTPw-HB/Hib vaccine, in four countries of Latin America, 1998–1999\(^a,b,c,d\)

<table>
<thead>
<tr>
<th>Vaccination(s)/Timing</th>
<th>Anti-tetanus</th>
<th>Anti-diphtheria</th>
<th>Anti-HBs</th>
<th>Anti-Bp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>GMC 95% CI</td>
<td>No.</td>
<td>GMC 95% CI</td>
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<td></td>
<td>No.</td>
<td>GMC 95% CI</td>
<td>No.</td>
<td>GMC 95% CI</td>
</tr>
</tbody>
</table>

**Separate DTPw-HB + Hib primary vaccination**

- **Prevaccination**
  - 179 1.529 1.294–1.807
  - 179 0.185 0.149–0.228
  - 174 5.9 5.2–6.6

- **Post-dose 2**
  - 179 0.352 0.300–0.414
  - 179 0.569 0.471–0.688
  - 179 502.4 402.3–627.3

- **Post-dose 3**
  - 176 2.863 2.452–3.343
  - 177 2.516 2.161–2.929
  - 177 22 826.7 11 332.2–45 980.6

**Separate DTPw-HB + Hib booster**

- **Prebooster**
  - 32 0.305 0.191–0.489
  - 32 0.171 0.111–0.262
  - 32 145.4 75.8–279.0

- **Postbooster**
  - 32 7.013 5.415–9.083
  - 32 22 826.7 11 332.2–45 980.6

**Combined DTPw-HB/Hib primary vaccination**

- **Prevaccination**
  - 530 1.469 1.323–1.631
  - 524 0.163 0.144–0.184
  - 518 6.0 5.6–6.5

- **Post-dose 2**
  - 529 0.739 0.680–0.803
  - 529 0.580 0.515–0.653
  - 532 402.3 347.4–465.8

- **Post-dose 3**
  - 525 4.605 4.263–4.973
  - 525 2.568 2.370–2.782
  - 524 12 382.1 8 994.0–17 046.6

**Combined DTPw-HB/Hib booster**

- **Prebooster**
  - 102 0.436 0.365–0.520
  - 102 0.157 0.111–0.196
  - 102 72.5 55.8–95.2

- **Postbooster**
  - 101 11.438 10.359–12.520
  - 101 6.308 5.294–7.382
  - 101 12 382.1 8 994.0–17 046.6

\(^a\) Anti-HBs = hepatitis B surface antibody.

\(^b\) Bp = Bordetella pertussis.

\(^c\) DTPw-HB = diphtheria-tetanus-whole cell pertussis-hepatitis B vaccine.

\(^d\) Hib = Haemophilus influenzae type B.

\(^e\) IU = international units.

\(^f\) VR = vaccine response.

\(^g\) No. = number of subjects tested.

\(^h\) % = percentage of subjects above the specified cutoff.

\(^i\) CI = confidence interval.

\(^j\) NA = not applicable.
Reactogenicity

The occurrence of local and general symptoms after primary and booster vaccination is shown in Figure 3. The incidence and intensity of symptoms were comparable in both vaccine groups, as demonstrated by overlap of 95% CIs.

Pain at the injection site was the most commonly reported solicited local symptom after both primary and booster vaccination, and irritability/fussiness was the most frequently reported solicited general symptom. The incidence of local symptoms was higher following the booster dose than after the primary course.

During the primary-vaccination course, unsolicited symptoms considered by the investigators to be probably related to vaccination occurred after 0.8% of the licensed vaccine doses and after 1.2% of the combined DTPw-HB/Hib vaccine doses. These were application site reactions, childhood infections, or gastrointestinal symptoms.

Fourteen SAEs were reported during the primary-vaccination study; one was determined to be probably vaccine-related. This infant received DTPw-HB/Hib and developed cellulitis at the injection site after Dose 2, which required treatment with cephalixin. The third dose of vaccine was given without any recurrence of infection. There were two deaths during the study. One was due to diarrhea, vomiting, and convulsions; it occurred 52 days after the infant received the first combination vaccine dose. The other death, due to pneumonia and sepsis, happened 8 days after the second DTPw-HB/Hib dose. The investigators did not consider either death to be related to vaccination. The remaining 11 SAEs were mainly due to infectious diseases such as otitis, respiratory tract infections, meningitis, or diarrhea.

Two SAEs were reported during the booster study. One event (cellulitis that developed at the DTPw-HB injection site following the booster dose of the licensed vaccine) was considered by the investigators to be probably vaccine-related. The event resolved within 10 days.

DISCUSSION

The results of this study demonstrate a good immunogenicity and reactogenicity profile for the new combined DTPw-HB/Hib vaccine containing 5 μg PRP per dose. Statistical comparisons based on the results following primary vaccination showed that, in terms of the antibody response to the PRP antigen, the combined DTPw-HB/Hib vaccine was clinically non-inferior to the licensed DTPw-HB and Hib vaccines. Persistence of anti-PRP antibodies until the age of booster vaccination was within the same range in both groups, and the booster dose induced anti-PRP antibody concentrations ≥ 1 μg/mL in 100% of vaccinees. The substantial increases in anti-PRP antibody levels following the booster dose are indicative of effective priming and induction of immune memory, regardless of the amount of PRP administered in the primary series.

This study also demonstrated non-inferiority of the candidate DTPw-HB/Hib vaccine, compared to the licensed-vaccines control group, in terms of the antibody responses to the other vaccine antigens. Although the anti-HBs antibody GMCs after primary vaccination were significantly lower with DTPw-HB/Hib than with DTPw-HB + Hib, the clinical relevance of this observation is likely to be limited, given the high anti-HBs seroprotection rates observed following priming and maintained until the time of booster vaccination. The observed pre-booster antibody persistence following priming with the combined candidate DTPw-HB/Hib vaccine was at least as good as the observed pre-booster antibody persistence following primary vaccination with the licensed vaccines administered separately. Marked increases in antibody GMCs for all vaccine antigens were found in both groups.

There was no difference between the groups in terms of the tolerability of the combined DTPw-HB/Hib vaccine compared to the separate administration of the licensed vaccines. In both groups, local symptoms were reported with a higher frequency with the booster dose than with the primary course. Such an increase has previously been seen after booster vaccination with DTPw, as well as with diphtheria-tetanus-acellular pertussis combination vaccines (18–21).

This study has some limitations. It was not possible to make the study double-blind, due to the different number of injections in the control group. Therefore, there is a possibility of bias in the reporting of reactogenicity. Subjects were recruited from four countries within Latin America, and it cannot be excluded that the perception of reactogenicity of the vaccines may have varied from country to country. Finally, the use of the new DTPw-HB/Hib vaccine in “field” conditions, for example including use in immunocompromised subjects, was not assessed in this trial. However, since the
FIGURE 3. Incidence of any and of Grade 3 solicited local and general symptoms following primary and booster vaccination with licensed diphtheria-tetanus-whole cell pertussis-hepatitis B (DTPw-HB) vaccine and Haemophilus influenzae type B (Hib) vaccine given separately or with a new combined DTPw-HB/Hib vaccine, with 95% confidence intervals, in four countries of Latin America, 1998–1999a

*a The Grade 3 definitions were: pain: cries when the limb is moved/spontaneously painful (preventing normal daily activity); redness/swelling: diameter > 20 mm; fever: axillary temperature > 39°C; drowsiness: preventing normal daily activity; irritability: crying that cannot be comforted/preventing normal daily activity; loss of appetite: not eating at all.
DTPw-HB/Hib vaccine is a combination of well-known and commonly used antigens, it is unlikely that the performance of the new vaccine would be different from that of the currently licensed products.

Fractional doses of conjugate Hib vaccines have been shown to be as immunogenic as current licensed vaccines (22–27), and the antibodies appeared to have functional characteristics (antibody avidity) that were at least as good as those induced by licensed products (28, 29).

This study demonstrates the immunogenicity and tolerability of the new combined pentavalent DTPw-HB/Hib vaccine. This vaccine will provide protection against five major childhood pathogens and will ease the implementation of global pediatric immunization programs, with a minimum of injections and a potentially improved immunization coverage.

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REFERENCES

Objetivos. En 1998, la Organización Mundial de la Salud (OMS) recomendó que se incluyeran vacunas conjugadas contra *Haemophilus influenzae* tipo B (Hib) en los programas de vacunación de niños menores de un año, siempre que ello estuviera en concordancia con las prioridades nacionales. La compañía GlaxoSmithKline Biologicals ha creado una nueva vacuna pentavalente que es una combinación de la vacuna contra la difteria (D), el tétanos (T) y la tos ferina (P) (con antígeno tosferínico a base de células completas) y las vacunas contra la hepatitis B (HB) y contra *Haemophilus influenzae* tipo B (Hib) (DTPw-HB/Hib), con un total de 5 μg de fosfato de polirribosilribitol (FPR). Hemos evaluado la inmunogenia y reactogenia observadas al aplicarse las dosis primaria y de refuerzo de esta nueva vacuna a niños sanos y las hemos comparado con las observadas al aplicar un régimen de referencia a base de las vacunas autorizadas DTPw-HB (*Tritanrix*) y antiHib (*Hiberix*) en forma de inyecciones simultáneas.

Métodos. Llevamos a cabo un estudio aleatorizado y con doble enmascaramiento de septiembre de 1998 a agosto de 1999 para establecer la inmunogenia y reactogenia observadas a los niños sanos a la nueva vacuna combinada pentavalente (DTPw-HB/Hib) en una sola inyección, y compararlas con las observadas con el régimen de referencia.

Resultados. Se obtuvieron excelentes respuestas inmunitarias con ambos regímenes. Todos los niños vacunados en ambos grupos alcanzaron concentraciones séricas protectoras de anticuerpos antiFPR ≥ 0,15 μg un mes después de recibir la dosis primaria. La vacuna combinada DTPw-HB/Hib no dio resultados inferiores a los obtenidos con las vacunas autorizadas en términos de los porcentajes de seroprotección, seropositividad y respuesta frente a todos los componentes antígenicos de la vacuna. La persistencia de anticuerpos contra todos los antígenos contenidos en ella hasta el momento en que se administró la dosis de refuerzo fue parecida en ambos grupos, y se observó un marcado aumento de las concentraciones de todos los anticuerpos después del refuerzo. La reactogenia general observada con ambos regímenes de vacunación fue parecida.

Conclusiones. Nuestros resultados indican que la nueva vacuna combinada pentavalente DTPw-HB/Hib ofrece una manera eficiente y confiable de poner en práctica las recomendaciones de la OMS para el control de la hepatitis B y de las infecciones por Hib en el mundo entero.

Palabras clave Vacunas combinadas, vacuna difteria-tétano-pertussis, vacunas contra hepatitis B, vacunas contra Haemophilus; América Latina.