

FOUR IS BETTER THAN NINE. A COMBINED DIPHTHERIA-TETANUS-PERTUSSIS-HEPATITIS B-*HAEMOPHILUS INFLUENZAE* TYPE B VACCINE FOR ROUTINE IMMUNIZATION IN MALAYSIA

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Abstract. Malaysian infants would have to receive nine injections during the first few months of life in order to be protected against disease caused by hepatitis B (HBV), diphtheria, tetanus, pertussis and *Haemophilus influenzae* type b (Hib) if single HBV and Hib vaccines were used. We evaluated a combined DTPw-HBV/Hib vaccine administered at 1.5, 3 and 5 months after a birth dose of hepatitis B vaccine (HBV). One month after completion of the primary vaccination, 99% of subjects had seroprotective anti-HBV antibody levels, and at least 98% had seroprotective antibodies against diphtheria, tetanus, and Hib, and were seropositive for pertussis antibodies. The immune response to the combined vaccine was comparable to that induced by separate injections with DTPw, HBV and Hib vaccines. Overall, the DTPw-HBV/Hib vaccine was as well tolerated as separate administration of DTPw, HBV and Hib vaccines. The combined DTPw-HBV/Hib vaccine induces protection against five diseases as recommended in the Malaysian routine vaccination schedule. Use of the combined DTPw-HBV/Hib vaccine can reduce the required number of injections from nine to four in the first few months of life.

INTRODUCTION

Hepatitis B (HBV) infection is endemic in Malaysia and between 3-5% of the total population is estimated to be seropositive for HBV-surface antigen (HBsAg) (Merican *et al*, 2000). Like other countries in Southeast Asia, perinatal transmission is the most important route of disease transmission (Chen *et al*, 2000; Merican *et al*, 2000). In 1989 the World Health Organization (WHO) Expanded Program on Immunization (EPI) was implemented in Malaysia and all infants receive three doses of hepatitis B vaccine commencing at birth, with

subsequent doses administered at 1 and 5 months of age. The seroprevalence of HBsAg in children has fallen from as high as 3.0% in the years prior to implementation of the program, to 0.4% after 1989 (Ng *et al*, 2005). At the time of the study the Malaysian routine childhood vaccination schedule also recommended vaccination with diphtheria-tetanus-whole-cell pertussis (DTPw) at 3, 4 and 5 months of age and was considering adding *Haemophilus influenzae* type b (Hib) vaccines to the immunization schedule. If separate HBV and Hib vaccines were used, Malaysian infants would have to receive nine injections in the first six months of life and attend four vaccination visits during that time. Given the relative complexity of the schedule, it is likely that some vaccination visits may be delayed or missed altogether.

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Vaccines that combine multiple antigens for administration in a single injection provide many advantages in terms of simplifying storage of vaccines, removing complexity from vaccination schedules, reducing the number of visits required to complete primary vaccination and increasing acceptability of vaccination to parents and providers (Capiou *et al*, 2003; Dodd, 2003). We investigated the immunogenicity and safety of a combined DTPw-HBV/Hib vaccine administered at 1.5, 3 and 5 months of age after a birth dose of HBV, compared to separate administration of licensed DTPw and Hib at 3, 4 and 5 months of age and HBV vaccine at birth, 1 and 5 months of age. Persistence of antibodies until 12 months of age was also investigated.

MATERIALS AND METHODS

Study participants and design

This was an open, randomized, clinical trial conducted at two study centers in Malaysia. The study protocol and amendments were approved by the Medical Research Ethics Subcommittee of the Ministry of Health, Malaysia. The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from the parents or guardians of all infants prior to enrolment in the study.

Healthy infants born after a gestational period of 36-42 weeks were enrolled soon after birth and received intramuscular vaccination with hepatitis B vaccine (HBV, *Engerix-B*[™] GlaxoSmithKline Biologicals, Belgium). Infants were randomized to one of two study groups: group DTPw-HBV/Hib received DTPw-HBV vaccine mixed and administered together with Hib vaccine at 1.5, 3 and 5 months of age. Group DTPw + Hib + HBV received HBV vaccine at 1 and 5 months of age, and separate administration of DTPw and Hib vaccines at 3, 4 and 5 months of age.

Newborn infants were excluded from par-

ticipation if they had an immunosuppressive disorder, a family history of an immune disorder or a major congenital defect. At the time of the first study vaccine dose, infants were excluded from further participation in the trial if they had a history of previous diseases or had a history of previous vaccination against diphtheria, tetanus, pertussis or *Haemophilus influenzae* type b, planned administration of other vaccines with the exception of BCG or oral polio vaccine (OPV) during the study period, were receiving chronic drug therapy, had a neurological disease, an immunodeficient condition, past or planned administration of blood products, or allergic disease likely to be exacerbated by any component of the vaccines.

Vaccines

DTPw (*D.T. COQ*[™]) was manufactured by Pasteur Merieux Connaught and was supplied in multi-dose vials. One dose (0.5 ml) contained ≥ 30 IU diphtheria toxoid, ≥ 60 IU tetanus toxoid, ≥ 4 IU *Bordetella pertussis* and thiomersal as a preservative. All other vaccines were manufactured by GlaxoSmithKline Biologicals (Rixensart, Belgium). The combined DTPw-HBV (*Tritanrix*[™]-HepB) vaccine contained ≥ 30 IU diphtheria toxoid, ≥ 60 IU tetanus toxoid, ≥ 4 IU *B. pertussis*, 10 μ g recombinant DNA HbsAg and thiomersal as a preservative. The Hib (*Hiberix*[™]) vaccine contained 10 μ g polyribosyl-ribitol-phosphate (PRP) conjugated to tetanus-toxoid (TT) and was reconstituted for use with either the liquid combined DTPw-HBV vaccine (Group DTPw-HBV/Hib) or with a sterile saline solution in the group receiving separate injections (Group DTPw + Hib + HBV). *Engerix-B*[™] contained 10 μ g recombinant DNA HbsAg and thiomersal as a preservative.

All vaccines were administered intramuscularly. DTPw-containing vaccines were administered into the left antero-lateral thigh. Hepatitis B and Hib vaccine were administered into the right upper and lower right thigh respectively.

Serological assessment

Blood samples were obtained from all subjects at birth and at 3 and 6 months of age to assess the antibody response to the vaccines. An additional blood sample was taken at the age of 1 year to assess antibody persistence.

Anti-HBs antibodies were determined using a commercial radioimmunoassay (AUSAB, Abbott) with an assay cut-off set at 10 mIU/ml. Anti-PRP antibodies were measured by ELISA (enzyme-linked immunosorbent assay) with a cut-off of 0.15 µg/ml. Anti-diphtheria and anti-tetanus antibody concentrations were measured by ELISA with an assay cut-off set at 0.1 IU/ml. Subjects seronegative for anti-diphtheria antibodies by ELISA at 12 months of age were re-tested using an *in vitro* neutralization assay on Vero cells (cut-off of 0.016 IU/ml). Anti-whole-cell *B. pertussis* (BPT) antibody concentrations were measured by ELISA (Labsystems, cut-off of 15 EL.U/ml).

For PRP, hepatitis B, diphtheria and tetanus, an antibody concentration greater than the assay cut-off was defined as seroprotection. Since there is no defined serological correlate of protection against pertussis, a vaccine response to primary vaccination was defined as the induction of measurable antibody in initially seronegative subjects, or maintenance of pre-vaccination antibody concentrations after primary vaccination.

Assessment of reactogenicity

Reactogenicity was actively assessed using diary cards for 4 days (Days 0-3) following each vaccination. Symptoms solicited after vaccination were local symptoms of pain, redness and swelling at the site of injection, and general symptoms of drowsiness, fever (axillary temperature $\geq 37.5^{\circ}\text{C}$), fussiness/irritability and not or loss of appetite. Intensity was graded on a 3-point scale where Grade 3 was defined as: cries when limb is moved/spontaneously painful (pain); a diameter > 20 mm

(swelling and redness); axillary temperature $> 39.0^{\circ}\text{C}$ (fever); not eating at all (loss of appetite); preventing normal activity (other symptoms). All other symptoms that occurred within 30 days of each vaccine dose, and serious adverse events (SAEs) occurring until Month 12, were recorded.

Statistical analysis and sample size

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

At each blood sampling time point, Geometric Mean Concentrations (GMCs) were calculated by taking the anti-log of the mean of the log concentration transformations. Antibody concentrations below the cut-off of the assay were given an arbitrary value of half the cut-off for the purpose of GMC calculation. Seroprotection rates for anti-HBs, anti-D, anti-T and anti-PRP antibodies with exact 95% confidence intervals (CI), seropositivity rates for anti-BPT, and their exact 95% CI were tabulated. Safety over the vaccination course encompassed data following two doses of DTPw + Hib and one dose of DTPw + Hib + HBV vaccines in DTPw + Hib + HBV group, and for three doses of DTPw-HBV/Hib vaccine in DTPw-HBV/Hib group. The percentage of doses followed by each individual solicited local and general symptoms (any or Grade 3), or by unsolicited symptoms, were calculated by group with exact 95% CIs.

The primary objective of the study was to demonstrate the non-inferiority of the HBV response after vaccination with the combined DTPw-HBV/Hib vaccine compared to the routine vaccination schedule of HBV administered at 0, 1, and 5 months of age. A clinical limit for non-inferiority was pre-defined: the lower limit of the asymptotic standardized 95% CI for the group difference (the DTPw-HBV/Hib

minus the DTPw + Hib + HBV group) in the proportion of subjects with an anti-HBs antibody concentration ≥ 10 mIU/ml should be above -10%. Assuming that 97.5% of subjects were seroprotected against HBV after vaccination, a sample size of 100 evaluable subjects per group would have at least 80% power to meet the primary objective (one-sided test based on 95% exact confidence intervals on proportion of difference, $\alpha=5\%$, simulations on 1,000 replicates).

RESULTS

The study took place between 2000 and 2002. Two hundred forty infants were enrolled, of which 21 (11 in the DTPw-HBV/Hib group) failed to complete the primary vaccination study (three doses of DTPw-HBV/Hib at 1.5, 3 and 5 months of age); two due to SAEs (see details below); three due to protocol violation, consent withdrawal by 10 subjects; and six subjects were lost to follow-up or migrated out of the study area. At Month 12, 17 subjects did not return for follow-up blood sampling.

Of 240 subjects enrolled in the study, one was eliminated for receiving a vaccine of the group other than the group to which the subject was randomized. Hence, 239 subjects were included in the ATP safety analysis. An additional 33 subjects (17 in the DTPw-HBV/

Hib group) were eliminated from the ATP immunogenicity analysis: one subject received blood products during the study period, 12 subjects did not comply with the required timings for vaccination or blood sampling, 20 subjects (ten in each group) did not have serological results available at all three post-vaccination blood sampling time points.

The demographic characteristics of the ATP immunogenicity cohort at the time of the first vaccination with a DTPw-containing vaccine are presented in Table 1.

Immunogenicity

Anti-HBs antibody response. Subjects in the DTPw-HBV/Hib group received 4 doses of hepatitis B vaccine: monovalent HBV at birth and three subsequent doses of HBV as combined DTPw-HBV/Hib at 1.5, 3 and 5 months of age. Subjects in the DTPw + Hib + HBV group received three doses of monovalent HBV vaccine at 0, 1 and 5 months of age. At screening, the proportion of mothers of study subjects who had anti-HBs antibody concentrations ≥ 10 mIU/ml was similar in each group: 26.2% in the DTPw-HBV/Hib group and 27.5% in the DTPw + Hib + HBV group.

Approximately 1-1.5 months after the second dose of hepatitis B-containing vaccine (*ie*, at Month 3 of the study), groups were comparable in terms of the proportion of subjects

Table 1
Demographic characteristics of the ATP cohort for immunogenicity at the time of the first dose of the DTPw-based vaccine.

| Group ^a | Total | Female | Age (weeks) | | | Oriental race |
|--------------------|----------|--------------|-------------|------|-------|---------------|
| | <i>N</i> | <i>n</i> (%) | Mean | SD | Range | <i>n</i> (%) |
| DTPw-HBV/Hib | 103 | 44 (42.7%) | 6.3 | 0.48 | 5-8 | 18 (17.5%) |
| DTPw + Hib + HBV | 103 | 60 (58.2%) | 12.4 | 0.73 | 11-14 | 26 (25.2%) |

N = number in the ATP cohort for immunogenicity, *n* (%) = number (percent) of subjects in the specified category, SD = standard deviation, Min = minimum age, Max = maximum age.

^aSubjects in the DTPw-HBV/Hib group received DTPw-HBV/Hib vaccine at 1.5, 3 and 5 months of age, subjects in the DTPw + Hib + HBV group received DTPw + Hib at 3, 4, 5 months of age.

Table 2
Hepatitis B antibody seroprotection rates and GMCs in subjects vaccinated with Engerix-B™ at birth, followed by DTPw-HBV/Hib at 1.5, 3 and 5 months or Engerix-B™ at 1 and 5 months of age.

| Antibody (cut-off) | The DTPw-HBV/Hib group | | | | | The DTPw + Hib + HBV group | | | | | | | | | |
|------------------------|------------------------|-----|------|-------|-------|----------------------------|-------|---------|-----|-------|------|-------|---------|---------|---------|
| | Time-point | N | %SP | 95%CI | GMC | N | %SP | 95%CI | GMC | 95%CI | | | | | |
| Anti-HBs (≥ 10 mIU/ml) | Pre(M0) | 96 | 24.0 | 15.8 | 33.7 | 11.8 | 8.2 | 17.0 | 96 | 22.9 | 15.0 | 32.6 | 11.6 | 8.3 | 16.2 |
| | PII(M3) | 103 | 63.1 | 53.0 | 72.4 | 28.9 | 20.9 | 40.1 | 102 | 74.5 | 64.9 | 82.6 | 35.5 | 25.7 | 49.2 |
| | PIII(M6) | 101 | 99.0 | 94.6 | 100.0 | 1,042.2 | 810.3 | 1,340.4 | 103 | 99.0 | 94.7 | 100.0 | 1,514.9 | 1,132.1 | 2,027.1 |
| | PIII(M12) | 82 | 92.7 | 84.8 | 97.3 | 212.9 | 150.6 | 301.1 | 88 | 95.5 | 88.8 | 98.7 | 358.6 | 256.4 | 501.5 |

N = Number of subjects with available results, %SP = percent of subjects with antibody concentration above the specified seroprotection cut-off, GMC = geometric mean concentration, 95%CI = 95% confidence interval, Pre (M0), PII (M3) PIII (M6), PIII (M12) = blood sample collected at birth, 3 months of age (corresponding to 2 months after the second dose of HBV in the DTPw + Hib + HBV group, and 1 month after the second dose of HBV in the DTPw-HBV/Hib group), 1 month after completion of primary vaccination at 6 months of age, and at 12 months of age, respectively.

with seroprotective anti-HBs antibody concentrations, and in anti-HBs antibody GMCs (Table 2). One month after completion of the primary vaccination course (Month 6), all but one subject in each group had seroprotective antibody concentrations of anti-HBs. The standardized asymptotic 95% CIs for this difference between groups (DTPw-HBV/Hib minus DTPw + Hib + HBV) with respect to anti-HBs seroprotection rates at Month 6 were -4.4% and 4.5%, respectively. As the lower limit of the 95% CI was above the pre-defined limit of -10, the non-inferiority of the combined DTPw-HBV/Hib vaccine in terms of the hepatitis B immune response was demonstrated. Further Fig 1 shows a similar distribution of anti-HBs antibodies in both groups at the same time point.

The vast majority of subjects in both groups (92.7% and 95.5% in the DTPw-HBV/Hib and DTPw + Hib + HBV groups, respectively) continued to have seroprotective anti-HBs antibody concentrations 7 months after primary vaccination, at 12 months of age.

Response to other vaccine antigens.

Subjects in the DTPw-HBV/Hib group received three doses of combined DTPw-HBV/Hib vaccine at 1.5, 3 and 5 months of age, whereas subjects in the DTPw + Hib + HBV group received separate administration of DTPw and Hib vaccines at 3, 4 and 5 months of age.

One month after completing the primary vaccination course (Month 6), at least 98.0% of subjects in both groups had seroprotective antibody concentrations against tetanus, diphtheria and Hib. All but two subjects in the DTPw + Hib + HBV group and all subjects in the DTPw-HBV/Hib group were seropositive for anti-BPT antibodies (Table 3). These data indicate that overall, there was no difference between groups in the percentage of seroprotected/seropositive individuals after vaccination.

Table 3
Antibody seroprotection/seropositivity rates and GMCs in subjects vaccinated with DTPw-HBV/Hib at 1.5, 3 and 5 months or with DTPw + Hib + HBV at 3, 4 and 5 months of age.

| Antibody (cut-off) | Time-point | The DTPw-HBV/Hib group | | | | The DTPw + Hib + HBV group | | | | |
|---|-------------------------|------------------------|--------|-------|-------|----------------------------|--------|-------|-------|-------|
| | | N | %SP/S+ | GMC | 95%CI | N | %SP/S+ | 95%CI | GMC | 95%CI |
| Anti-PRP (≥ 0.15 µg/ml) | Pre (M0) | 103 | 62.1 | 0.39 | 0.28 | 102 | 62.7 | 0.54 | 0.35 | 0.26 |
| | PIII (M6) | 102 | 100.0 | 28.51 | 23.34 | 103 | 99.0 | 34.82 | 21.01 | 16.41 |
| | PIII (M12) | 83 | 100.0 | 5.46 | 4.48 | 89 | 100.0 | 6.67 | 5.85 | 4.51 |
| Anti-diphtheria (≥ 0.1 IU/ml) | Pre (M0) | 101 | 31.7 | 0.09 | 0.07 | 101 | 33.7 | 0.11 | 0.09 | 0.07 |
| | PIII (M6) | 102 | 98.0 | 2.03 | 1.66 | 103 | 100.0 | 2.50 | 2.24 | 1.89 |
| | PIII (M12) ^a | 82 | 89.0 | 0.20 | 0.16 | 88 | 93.2 | 0.26 | 0.31 | 0.25 |
| Anti-tetanus (≥ 0.1 IU/ml) | Pre (M0) | 103 | 100.0 | 9.63 | 7.59 | 103 | 100.0 | 12.2 | 8.97 | 7.12 |
| | PIII (M6) | 102 | 100.0 | 2.99 | 2.48 | 102 | 100.0 | 3.61 | 1.20 | 1.03 |
| | PIII (M12) | 82 | 96.3 | 0.76 | 0.60 | 82 | 95.5 | 0.96 | 0.51 | 0.43 |
| Anti-BPT (≥ 15 EL U/ml) | Pre (M0) | 103 | 32.0 | 12.0 | 10.4 | 102 | 32.4 | 13.8 | 11.9 | 10.3 |
| | PIII (M6) | 102 | 100.0 | 110.0 | 98.4 | 103 | 98.1 | 122.9 | 63.6 | 56.0 |
| | PIII (M12) | 82 | 47.6 | 13.5 | 11.6 | 89 | 62.9 | 15.6 | 17.9 | 15.3 |

N = Number of subjects with available results, %SP/S+ = percent of subjects with antibody concentration above the specified seroprotection/seropositivity (for anti-BPT) cut-off, GMC = geometric mean concentration, 95%CI-95% confidence interval, Pre (M0), PIII (M6), PIII (M12) = blood sample collected at birth, 1 month after primary vaccination at 6 months of age, and at 12 months of age, respectively. %SP at Month 12 for diphtheria by ELISA or Vero cell testing (cut-off 0.016 IU/ml).

Ninety-six point one percent (95%CI 90.3; 98.9) of subjects in the DTPw-HBV/Hib group had a vaccine response to BPT one month after the complete vaccination course (Month 6) as compared to 93.1% (86.4; 97.2) in the DTPw + Hib + HBV group. Anti-tetanus and anti-BPT antibody GMCs after primary vaccination were higher in the DTPw-HBV/Hib group than in the DTPw + Hib + HBV group (non-overlapping 95% CIs). The clinical significance of this finding is unclear, particularly as 100% of subjects achieved the seroprotective threshold of 0.1 IU/ml for anti-tetanus antibody concentrations after vaccination.

At 12 months of age, corresponding to approximately 7 months after completion of primary vaccination, 100% of subjects continued to have seroprotective anti-PRP antibody levels in the DTPw-HBV/Hib group. More than 89.0% had seroprotective anti-diphtheria and tetanus levels ($\geq 0.016 \mu\text{g/ml}$ measured by neutralization assay on Vero cells for diphtheria) and 47.6% continued to be seropositive for anti-BPT antibodies (Table 3).

Reactogenicity

The overall reactogenicity profiles of the study vaccines were similar (Fig 2). The point estimates for the incidence of local symptoms of pain, redness and swelling were higher at

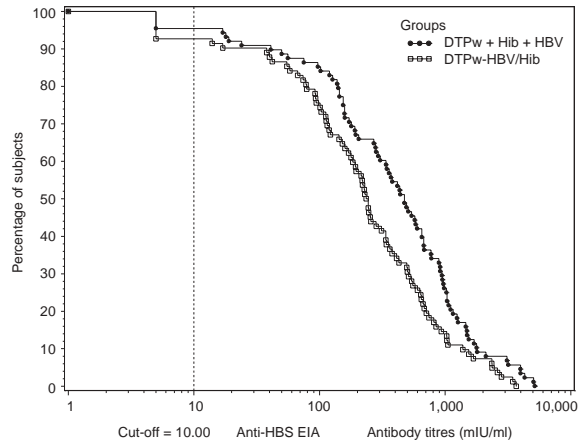


Fig 1—Reverse cumulative curve for anti-HBs antibody concentrations one month after the complete vaccination course (Month 6, ATP immunogenicity cohort).

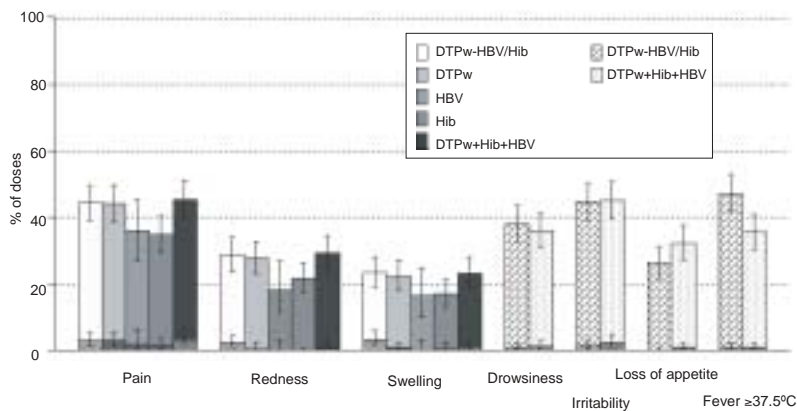


Fig 2—Solicited symptoms after vaccination with a DTPw-containing vaccine.

The DTPw-HBV/Hib group received DTPw-HBV/Hib at 1.5, 3 and 5 months and the DTPw + Hib + HBV group received DTPw + Hib at 3 and 4 months, and DTPw + Hib+ HBV at 5 months of age. *Percentage of doses followed by a symptom at any of the DTPw, Hib or HBV (for dose 3) injection sites. Temperature recorded via axillary route. Hatched regions = grade 3 symptoms. Grade 3: pain = cries when limb is moved/ spontaneously painful, redness and swelling = diameter >20 mm, fever = axillary temperature >39.0°C, loss of appetites = not eating at all, all other symptoms = preventing normal activity.

the DTPw-HBV/Hib and DTPw sites than at the Hib and HBV sites of vaccination. Pain was the most commonly reported locally occurring symptom after vaccination.

Fever $\geq 37.5^{\circ}\text{C}$ was reported more commonly in subjects who received the DTPw-HBV/Hib vaccine (95% CIs do not overlap). However, fever $> 39.0^{\circ}\text{C}$ was uncommon and occurred in 0.9% of doses for both groups. Overall, the incidence of other solicited general symptoms that occurred after vaccination with the DTPw-based vaccine were similar between the groups.

Forty-six subjects (18 in the DTPw-HBV/Hib group) reported SAEs from birth until the completion of the study at Month 12. None were considered by the investigator to have a causal relationship to the study vaccines. Forty-one SAEs occurred in the period between birth and the first DTPw vaccine dose. Five subjects (three in the DTPw-HBV/Hib group) reported SAEs during the DTPw vaccination course and in the follow-up period until Month 12. These were: anemia on the day of Dose 3, persistent cough and nasal congestion 14 days after Dose 2, bronchial asthma secondary to bronchopneumonia 46 days after Dose 3, bronchiolitis after Dose 3, right knee cellulitis after Dose 3.

Two subjects (both in the DTPw + Hib + HBV group) experienced an SAE that led to discontinuation of vaccination: one had pneumonia, seizures and aspiration pneumonia that began 29 days after the second dose of HBV vaccine. The second child developed jaundice 45 days after the second dose of the HBV vaccine. The final diagnosis was biliary atresia.

DISCUSSION

This study evaluated the immunogenicity and reactogenicity of the combined DTPw-HBV/Hib vaccine compared with separate administration of DTPw, HBV and Hib vaccines, and also assessed the immune re-

sponse when the combined vaccine was administered in an alternative 1.5, 3, and 5 month vaccination schedule in infants. Given the endemicity of hepatitis B in Malaysia and the excellent progress made to date in control of hepatitis B using the 0, 1, 5 month vaccination schedule, the response to hepatitis B induced by the combined vaccine was of particular interest (Ng *et al*, 2005).

Although three injections of HBV are all that are necessary to provide high levels of seroprotection against hepatitis B, the WHO acknowledges a total of four doses may be given when a birth dose is administered, and when combined vaccines are implemented (WHO, 2004). We have demonstrated that the response to hepatitis B one month after completion of primary vaccination with DTPw-HBV/Hib was not inferior to that induced by the standard schedule using monovalent HBV vaccine. In both groups 99% of individuals achieved seroprotective antibody levels against hepatitis B one month after completion of the vaccination course (Month 6) and were comparable in terms of both seroprotective rate and GMC at Month 3 of the study. Furthermore, at 12 months of age, the proportion of subjects in each group with persisting anti-HBs antibodies ≥ 10 mIU/ml was similar in both groups.

Although the 95% CIs overlapped, the point estimate of the anti-HBs GMC was higher in subjects who received three doses of monovalent HBV vaccine at 0, 1 and 5 months of age than in subjects who received four doses of HBV vaccine at 0, 1.5, 3 and 5 months of age (2,027 mIU/ml versus 1,340 mIU/ml). This finding is in keeping with generally higher antibody GMCs that are known to occur when the interval between vaccine doses is increased (WHO, 2004). Accordingly, since the proportions of subjects with seroprotective anti-HBs antibodies after primary vaccination and at Month 12 were comparable in both groups, any potential differences in GMCs is unlikely to be of clinical importance.

In terms of the response to the other vaccination antigens, the immune response induced by the combined DTPw-HBV/Hib vaccine administered at 1.5, 3 and 5 months of age was comparable to the DTPw and Hib vaccines administered separately at 3, 4 and 5 months of age. This is in line with results from other studies that demonstrate that the DTPw, HBV and Hib vaccines can be successfully combined without adversely affecting either the immunogenicity or reactogenicity of the separate vaccines (Win *et al*, 1997; Aristegui *et al*, 2003; Kanra *et al*, 2006; Tregnaghi *et al*, 2006).

At the 12 month follow-up visit, there was no evidence for any long-term differences between the vaccines and schedules assessed in this study, either in terms of the response to hepatitis B, or in the long-term response to the other vaccine antigens administered.

The combined DTPw-HBV/Hib vaccine was well tolerated with a reactogenicity profile that was similar to the separately administered vaccines. Although fever occurred more commonly after vaccination with DTPw-HBV/Hib, this is unlikely to be clinically significant given that the same percentage of doses (0.9%) in each group was followed by a fever >39.0°C. The incidence of local adverse events across the full vaccination course was similar, however this does not take into account that symptoms may occur at each injection site, representing a potential three-fold higher incidence in the group receiving separate vaccinations. Overall, the combined DTPw-HBV/Hib vaccine may be considered to be better tolerated than separately administered DTPw, Hib and HBV vaccines since symptoms that occur at multiple injection sites are avoided.

We have shown that the immunogenicity and reactogenicity of the DTPw-HBV/Hib vaccine is comparable to separate administration of HBV, DTPw and Hib vaccines. Furthermore, our results have shown that DTPw-HBV/Hib

when administered at 1.5, 3 and 5 months of age is comparable to monovalent HBV given at 0, 1 and 5 months of age, and to DTPw and Hib given at 3, 4 and 5 months of age. The combined vaccine allows recommended antigens to be given in three, rather than four visits after birth, with associated potential reductions in health care costs and improvements in patient compliance. The DTPw-HBV/Hib vaccine can be easily adapted to existing immunization programs to reduce patient visits and to simplify national vaccination programs, without compromise to the immune response or tolerability of vaccination.

Combined with a birth dose of HBV vaccine, the DTPw-HBV/Hib vaccine induces high levels of protection against hepatitis B, with the added advantages of a simpler schedule, easier vaccine storage and potentially better vaccine coverage as a result of fewer doctor visits and a reduced number of injections required – four versus nine – to complete the primary vaccination course.

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REFERENCES

- Aristegui J, Usonis V, Coovadia H, *et al*. Facilitating the WHO Expanded Program of Immunization: the clinical profile of a combined diphtheria, tetanus, pertussis, hepatitis B and *Haemophilus influenzae* type B vaccine. *Int J Infect Dis* 2003; 7: 143-51.
- Capiou C, Poolman J, Hoet B, Bogaerts H, Andre F. Development and clinical testing of multivalent vaccines based on a diphtheria-tetanus-acellular pertussis vaccine: difficulties encoun-

- tered and lessons learned. *Vaccine* 2003; 21: 2273-87.
- Chen CJ, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. *J Gastroenterol Hepatol* 2000; 15 (suppl): E3-6.
- Dodd D. Benefits of combination vaccines: effective vaccination on a simplified schedule. *Am J Manag Care* 2003; 9 (suppl): S6-12.
- Kanra G, Kara A, Demiralp O, *et al.* Safety and immunogenicity of a new fully liquid DTPw-HepB-Hib combination vaccine in infants. *Hum Vaccin* 2006; 2: 155-60.
- Merican I, Guan R, Amarapuka D, *et al.* Chronic hepatitis B virus infection in Asian countries. *J Gastroenterol Hepatol* 2000; 15: 1356-61.
- Ng KP, Saw TL, Baki A, Rozainah K, Pang KW, Ramanathan M. Impact of the Expanded Program of Immunization against hepatitis B infection in school children in Malaysia. *Med Microbiol Immunol (Berl)* 2005; 194: 163-8.
- Tregnaghi M, López P, Rocha C, *et al.* A new DTPw-HB/Hib combination vaccine for primary and booster vaccination of infants in Latin America. *Rev Panam Salud Publica* 2006; 19: 179-88.
- Win KM, Aye M, Han HH, Safary A, Bock HL. Comparison of separate and mixed administration of DTPw-HBV and Hib vaccines: immunogenicity and reactogenicity profiles. *Int J Infect Dis* 1997; 2: 79-84.
- World Health Organization. Hepatitis B vaccines. WHO position paper. *WER* 2004; 79: 253-64.