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Primary and booster immunization with a diphtheria, tetanus, acellular pertussis, hepatitis B (DTPa—HBV) and *Haemophilus influenzae* type b (Hib) vaccine administered separately or together is safe and immunogenic

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Summary
Objectives: The aim of this study was to evaluate the safety and immunogenicity of DTPa—HBV and Hib vaccines given mixed or separately to 360 healthy infants at 2, 4, and 6 months of age.
Methods: Immune memory was assessed in lower responders (post-primary anti-PRP <0.545 μg/ml), through administration of plain polyribosylribitol phosphate (PRP) at 12—15 months. All subjects received a DTPa—HBV/Hib booster at 18—19 months.
Results: One month after primary vaccination, 98% had seroprotective antibody levels against HBV and 94—97% against Hib (anti-PRP ≥ 0.15 μg/ml). A statistically significant difference between groups was observed in the proportion of subjects who achieved anti-PRP antibodies $\geq$1.0 μg/ml post-primary vaccination; 68.1% for DTPa—HBV/Hib and 84.5% for DTPa—HBV and Hib. PRP administered to lower responders produced a 7-fold increase in anti-PRP antibodies, indicative of immunological memory. After DTPa—HBV/Hib booster vaccination, 96—100% of subjects had seroprotective antibody concentrations against Hib, hepatitis B, tetanus, and diphtheria and high vaccine response rates against pertussis toxoid, filamentous hemagglutinin, and pertactin.

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Conclusion: A robust and protective Hib response was demonstrated following plain PRP and/or a booster conjugate Hib vaccine in both lower and higher Hib responders.

Introduction

The National Immunisation Program in Australia currently recommends primary vaccination with diphtheria, tetanus and acellular pertussis (DTPa), inactivated polio vaccine (IPV), hepatitis B virus (HBV), *Haemophilus influenzae* type b (Hib), rotavirus and 7-valent pneumococcal conjugate vaccines for infants.[1] A booster dose of Hib is provided at 12 months of age in addition to meningococcal C vaccine and measles, mumps and rubella vaccine. The combination DTPa vaccine was introduced as the standard recommendation in Australia in 1997–1999.[2] A national program for Hib vaccination was begun in 1993, and has resulted in substantial reductions in invasive Hib disease.[3]

The increasing number of vaccines available for immunization against numerous childhood diseases has the effect of making vaccination schedules progressively more complex, which may be a significant barrier to the success of immunization programs.[4] The large number of injections required if each vaccine is administered separately can also be a source of distress to parents and children.[5] The use of combination vaccines can reduce the number of injections, simplify immunization schedules, reduce the risk of delayed doses, improve patient convenience by requiring fewer clinic visits, reduce the perceived pain and distress for the child, and reduce costs associated with vaccine administration.[6] A study of infant immunization in Sydney found that administration of DTP, Hib and HBV vaccines was often fragmented across separate visits, with a risk of missed or delayed doses, and concluded that there was a need for a combination DTPa–HBV–Hib vaccine.[7]

Earlier studies have established the effectiveness of a quadrivalent DTPa–HBV vaccine[8] and the feasibility of combining DTPa–HBV and Hib vaccines in a single injection for primary vaccination in healthy infants.[9–12]

Although the purpose of combination vaccines is to reduce the number of needles administered to children, it is essential that vaccine efficacy is not compromised. It has previously been documented that combining Hib tetanus conjugated vaccines with DTPa vaccines can result in a lower level of circulating antibodies to the capsular polysaccharide polyribosylribitol phosphate (PRP) compared to separate administration of the vaccines. The reasons for the decrease in antibody response in some DTP/Hib combination vaccine studies remain unclear, but the variation in results observed suggests these differences are vaccine-specific.[13] Possible reasons for decreased immunogenicity of Hib in DTPa combination vaccines include direct interference between different antigens when mixed, epitope-specific suppression, and/or variation in adjuvants in vaccines studied.[13] However the variability in response is unlikely to be of any clinical significance as the protective efficacy of DTPa–Hib combination vaccines with lower antibody concentrations has been established.[14,15] In addition, immunological memory has been demonstrated in studies where contact with unconjugated (plain) PRP antigen following priming with a combination Hib vaccine has resulted in induced functional Hib antibody.[16] Plain PRP used in this study and in others as an immunological challenge, is used to mimic exposure to wild-type Hib infection as a method of assessing immunological memory.[17,18]

Data have also shown that when corrected for total antibody level, anti-PRP antibody avidity does not differ with different methods of administration including administration of Hib separately or in combination.[14]

The aim of this study was to investigate the immunogenicity and reactogenicity of a candidate Hib vaccine and quadrivalent DTPa–HBV vaccine given either as a single mixed injection or administered simultaneously in opposite limbs for primary vaccination to healthy infants at 2, 4, and 6 months of age. A follow-up booster study was conducted in which the DTPa–HBV and Hib vaccines were administered as a single injection to the same subjects during the second year of life.

Materials and methods

Study design and subjects

The primary vaccination study (208140/039) was an open-label, randomized, comparative trial conducted in two centers in Australia. Healthy infants of either sex, and aged between 8 and 12 weeks at the time of first immunization, were randomized to receive either a single vaccination with combined DTPa–HBV/Hib or separate injections of DTPa–HBV and Hib in opposite thighs, at 2, 4 and 6 months of age. Subjects were excluded if they had obvious health problems established by clinical examination and/or medical history, or if they had a history of previous exposure to diphtheria, tetanus, pertussis, hepatitis B, or Hib vaccination or disease.

Subjects completing the primary vaccination study were eligible to enter an open-label booster study conducted at the same centers (208108/043) and to receive a single injection of DTPa–HBV/Hib vaccine at 18–19 months of age. To assess immune memory in subjects potentially at risk (i.e., low anti-PRP response after primary vaccination), 40 subjects with anti-PRP values below 0.545 μg/ml after primary vaccination (lower responders) received a dose of plain PRP at 12–15 months of age following the primary vaccination. The cut-off (0.545 μg/ml) was based on the anti-PRP antibody responses of the first 100 subjects evaluated in the primary vaccination study and was chosen so as not to challenge subjects who had already shown a very high response to primary vaccination.

Both study protocols were approved by the ethics committee at each trial center, and the studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The parent or guardian of each subject provided written informed consent before any study procedure was performed.

Study vaccines

All vaccines were manufactured by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium. The DTPa—HBV licensed vaccine contained, per 0.5 ml dose, diphtheria toxoid ≥30 IU, tetanus toxoid ≥40 IU, pertussis toxoid (PT) 25 μg, filamentous hemagglutinin (FHA) 25 μg, pertactin (PRN) 8 μg, and recombinant surface antigen of the hepatitis B virus (HBsAg) 10 μg. The candidate Hib conjugate vaccine contained, per lyophilized dose, PRP 10 μg conjugated to tetanus toxoid 20—40 μg adsorbed onto aluminum salts as adjuvant, and lactose 10 mg. In addition to the study vaccines all infants received oral polio vaccine in accordance with the Australian Standard Vaccination Schedule.

Immunogenicity assessment

Blood samples for immunogenicity assessment were taken immediately before the first primary vaccine dose and one month after the third dose. In the booster study, blood samples were taken before and one month after the DTPa—HBV/Hib booster vaccine dose. In the subset of subjects receiving plain PRP (10 μg), blood samples were also taken before and 7—10 days after the dose of PRP.

Total antibodies to Hib PRP were measured using a radiolabeled antigen binding assay with a cut-off of 0.15 μg/ml. Antibodies against HBsAg (anti-HBs) were determined using a commercially available radioimmunoassay (AUSAB®, Abbott Laboratories, Abbott Park, IL, USA) with a cut-off of 10 mIU/ml. Antibody concentrations ≥10 mIU/ml were considered as protective. Antibodies against diphtheria and tetanus toxoids were measured by ELISA techniques, with a cut-off level of 0.1 IU/ml. Antibodies (IgG) against PT, FHA, and PRN (pertussis antigens), were measured by ELISA with a cut-off of 5 ELISA units (EL.U)/ml. A vaccine response to PT, PRN, and FHA after the booster dose was defined as appearance of antibodies in initially seronegative subjects or a 2-fold increase in antibody concentration in initially seropositive subjects.

Safety and reactogenicity assessment

Diary cards were distributed to the parents or guardians to record solicited and unsolicited symptoms and adverse events. Reactogenicity was assessed by measuring the appearance of solicited local symptoms (pain, redness, or swelling at the injection site) or general symptoms (irritability/fussiness, fever, vomiting, diarrhea, restlessness, sleepiness, unusual crying, drowsiness, and loss of appetite) during a 4-day follow-up period after each vaccination dose. Unsolicited adverse events (AEs) and serious adverse events (SAEs) were recorded throughout the study period. Intensity was assessed on a three-point scale where grade 3 intensity for solicited symptoms included crying when the limb was moved or a spontaneously painful limb, crying that could not be comforted or that prevented normal everyday activity, drowsiness that prevented normal everyday activity, loss of appetite such that the study subject did not eat at all. Local redness and swelling were assessed by measuring the largest diameter, where grade 3 was >20 mm.

Statistical analyses

The study was exploratory. Antibody seroprotection/seropositivity and vaccine response rates against vaccine antigens for the according-to-protocol (ATP) cohort were calculated with exact 95% confidence intervals (CI). Geometric mean antibody concentrations (GMC) with 95% CI were calculated from the anti-log of the mean of log-transformed values. Antibody concentrations below the lower limit of detection of the assay were assigned an arbitrary value of half the cut-off for the purpose of GMC calculation.

Reactogenicity was evaluated by calculating the percentage (and 95% CI) of doses followed by a report of at least one solicited or unsolicited local or general symptom during the defined follow-up period after vaccination. For each group, the incidence of each symptom overall and rated as grade 3 was recorded. Values were considered as significantly different between groups if the 95% CI did not overlap.

Results

Demographics

The primary study was conducted between November 1996 and January 1998, and enrolled 360 infants. The booster study took place between January 1998 and January 1999 and included 276 subjects. Figure 1 presents the disposition of subjects in both studies. All but three infants completed the primary vaccination course (all were lost to follow-up). The ATP cohort for immunogenicity comprised 328 (91.1%) infants. Thirty-two subjects were excluded from the ATP analysis (randomization failure (1), initially seropositive or entry status unknown (4), prohibited medication (1), non-compliant with vaccination or blood sampling schedule (21), and essential serological data missing (5)).

In the primary study, the mean age at first dose was 8.6 weeks and 168/328 (51.2%) were male. The mean age at the time of PRP challenge was 13.4 months in the subgroup that received plain PRP vaccination, and the mean age at the time of booster vaccination was 17.9 months and 18.0 months in the subjects primed with DTPa—HBV + Hib and DTPa—HBV/Hib, respectively.

Immunogenicity

Primary vaccination

Anti-PRP and anti-HBs antibody concentrations were evaluated prior to and after the primary vaccination course. Seroprotection rates and GMCs one month after the third dose of primary vaccination are presented in Table 1.

A total of 94.4% (95% CI 89.6—97.4%) of subjects in the combined DTPa—HBV/Hib group had seroprotective antibody concentrations against Hib (anti-PRP ≥0.15 μg/ml) compared with 97.6% (95% CI 94.0—99.3%) in the group receiving separate injections. A higher proportion of subjects in the group that received separate injections reached an antibody level against PRP of ≥1.0 μg/ml (84.5%, 95% CI 78.2—89.6%) compared with the group that received the combined injection (68.1%, 95% CI 60.3—75.3%). The GMC for anti-PRP antibodies was also higher in the group that received separate injections (4.553 μg/ml (95% CI 3.647—5.685 μg/ml))

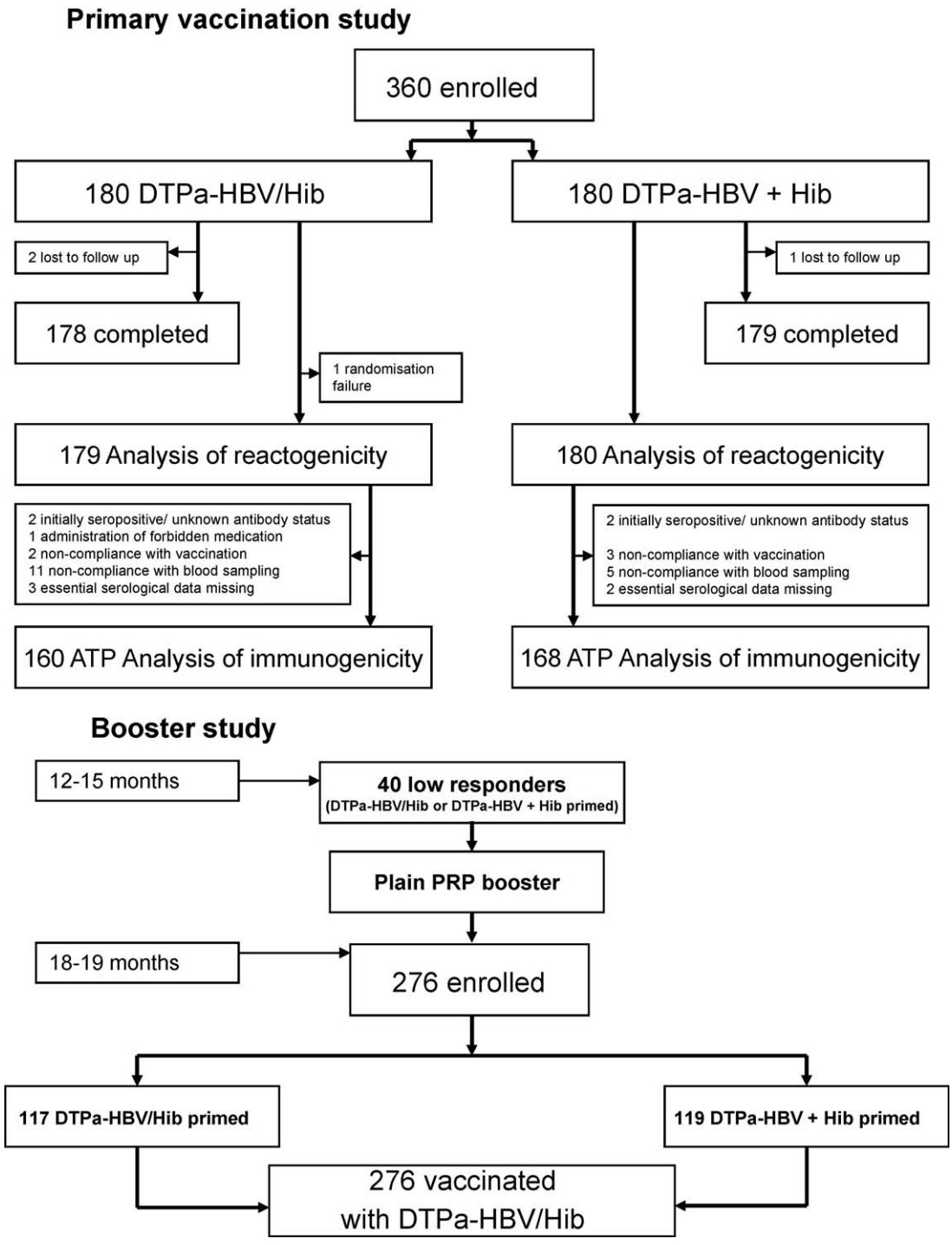

Figure 1 Disposition of subjects included in the primary and booster vaccination trials.

than in the group that received the combined injection (2.034 μg/ml (95% CI 1.574—2.628 μg/ml)).

Anti-HBs antibody levels of ≥10 mIU/ml one month after the third dose were high in both groups, occurring in 98.8% of subjects receiving a single injection and 98.2% of subjects receiving separate injections.

Plain PRP challenge: assessment of immune memory

Infants younger than 18—24 months of age cannot mount a seroprotective response to plain polysaccharides such as PRP unless immune memory has been previously primed. The presence of immune memory was assessed in the subset of 40 subjects that were lower responders, by measuring the increase in anti-PRP antibody concentrations 7 days after the administration of plain PRP at 12—15 months of age. Twenty-five lower responders had received priming with combined DTPa—HBV/Hib vaccine and 15 had received DTPa—HBV + Hib vaccines. The results show that after a PRP challenge, the anti-PRP antibody GMC rapidly increased, indicating that anti-PRP immune memory had been induced regardless of

Table 1 Seroprotection/seropositivity rates and antibody geometric mean concentrations one month after the third dose of DTPa—HBV and Hib vaccines (ATP cohort for immunogenicity)

	DTPa—HBV/Hib (*N* = 160)				DTPa—HBV + Hib (*N* = 168)			
Antigen	%	95% CI	GMC	95% CI	%	95% CI	GMC	95% CI
Anti-PRP								
≥0.15 μg/ml	94.4	89.6—97.4	2.034	1.574—2.628	97.6	94.0—99.3	4.553	3.647—5.685
≥1.0 μg/ml	68.1[a]	60.3—75.3	-	-	84.5[a]	78.2—89.6	-	-
Anti-HBs								
≥10 mIU/ml	98.8	95.6—99.8	920.3	752.1—1126.1	98.2	94.9—99.6	783.6	629.7—975.0

DTPa—HBV, diphtheria, tetanus, acellular pertussis, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; ATP, according-to-protocol; *N*, number of subjects with available results; 95% CI, 95% confidence interval; GMC, geometric mean concentration; anti-PRP, antibodies to polyribosylribitol phosphate; anti-HBs, antibodies to the hepatitis B surface antigen.
[a] Statistically significant difference — 95% CI do not overlap.

Table 2 Anti-PRP seroprotection rates and antibody geometric mean concentrations after the plain PRP challenge in subjects classified as lower responders (anti-PRP <0.545 μg/ml) one month after the primary vaccination course

Post-primary antibody concentration		Anti-PRP ≥0.15 μg/ml		GMC (μg/ml)	
	n	%	95% CI	GMC	95% CI
<0.15 μg/ml	7	85.7	42.1—99.6	0.372	0.155—0.893
≥0.15 to <0.545 μg/ml	32	96.9	83.8—99.9	1.296	0.729—2.305
Total	39	94.9	82.7—99.4	1.036	0.624—1.722

PRP, polyribosylribitol phosphate; GMC, geometric mean concentration; 95% CI, 95% confidence interval.

whether the subjects were primed with DTPa—HBV/Hib or DTPa—HBV + Hib (Table 2; Figure 2). Before the plain PRP challenge, 50% (95% CI 33.4—66.6%) of the lower responders had anti-PRP ≥0.15 μg/ml, which rose to 94.9% (82.7—99.4%) 7 days post-PRP challenge (Table 2). Of low responders with post-primary anti-PRP antibodies <0.15 μg/ml, 85.7% achieved seroprotective concentrations after the challenge dose. In low responders the anti-PRP antibody GMC increased 7-fold from 0.146 μg/ml (95% CI 0.113—0.187 μg/ml) pre-vaccination to 1.036 μg/ml (95% CI 0.624—1.722 μg/ml) post-vaccination.

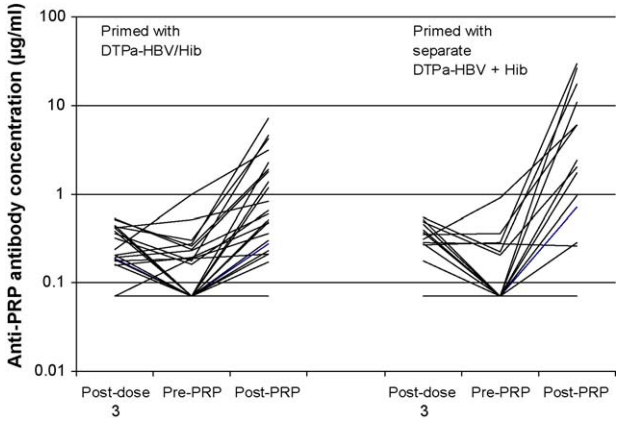

Figure 2 Individual responses to plain PRP challenge by vaccine used for primary vaccination. Post-dose 3 = one month after primary vaccination; Pre-PRP and post-PRP = prior to and 7 days after challenge with plain PRP at 12—15 months of age. Each line represents results from an individual.

Booster vaccination

Prior to booster dosing with DTPa—HBV/Hib, antibody persistence was similar in both groups, irrespective of whether the primary vaccination had been administered using separate or single injections (Table 3). One month after booster vaccination, substantial increases in all antibody concentrations were observed. The percentage of subjects with anti-PRP ≥1.0 μg/ml reached 98.2—100% after boosting. At least 99.1% of subjects had seroprotective antibody concentrations against diphtheria, tetanus and hepatitis B after the booster dose. Vaccine response rates as represented by anti-PT, anti-FHA and anti-PRN antibody concentrations were also high (over 96%). Overall, the immune response to the booster dose was similar for the two comparative treatment groups.

Safety and reactogenicity

Primary vaccination

Primary vaccination was well tolerated in both groups (Table 4). The incidence of grade 3 solicited symptoms was low, and was similar in both groups (Table 4). There were no statistically significant differences in reactogenicity between the two groups.

The total number of unsolicited symptoms reported in the 30-day follow-up period after primary vaccination was 253 in the group receiving the vaccines as a single injection and 228 in the group receiving separate injections.

A total of 27 SAEs were reported during the study. All except one were considered unrelated to vaccination by the investigator. One SAE was assessed as probably related, a hypotonic hyporesponsive episode that occurred in the separate vaccination group. The event lasted for 45 minutes,

Table 3 Seroprotection/seropositivity and antibody geometric mean concentrations before and one month after booster vaccination with DTPa—HBV/Hib vaccine in children aged 18—19 months (total cohort)

		DTPa—HBV/Hib primed					DTPa—HBV + Hib primed				
	Time point	*N*	%	95% CI	GMC	95% CI	*N*	%	95% CI	GMC	95% CI
Anti-PRP	Pre-booster	103	81.6	72.7—88.5	0.464	0.365—0.591	113	86.7	79.1—92.4	0.481	0.383—0.604
≥0.15 μg/ml	Post-booster	114	100.0	96.8—100.0	70.286	55.522—88.977	111	100.0	96.7—100.0	78.544	64.275—95.981
Anti-PRP	Pre-booster	103	24.3	16.4—33.7	-	-	113	23.0	15.6—31.9	-	-
≥1.0 μg/ml	Post-booster	114	98.2	93.8—99.8	-	-	111	100.0	96.7—100.0	-	-
Anti-T	Pre-booster	105	74.3	64.8—82.3	0.158	0.134—0.187	112	88.4	81.0—93.7	0.235	0.201—0.274
≥0.1 IU/ml	Post-booster	114	100.0	96.8—100	5.762	4.956—6.699	111	99.1	95.1—100	8.559	7.364—9.948
Anti-D	Pre-booster	104	43.3	33.6—53.3	0.089	0.078—0.103	112	37.5	28.5—47.1	0.082	0.072—0.094
≥0.1 IU/ml	Post-booster	114	100.0	96.8—100	4.369	3.712—5.143	111	100.0	96.7—100	3.422	2.912—4.022
Anti-HBs	Pre-booster	105	85.7	77.5—91.8	76.7	55.4—106.2	113	88.5	81.1—93.7	70.8	53.8—93.4
≥10 mIU/ml	Post-booster	114	100.0	96.8—100	2365.4	1696.7—3297.8	112	100.0	96.8—100	1568.4	1155.3—2129.3
Anti-PT	Pre-booster	104	48.1	38.2—58.1	5.2	4.3—6.2	113	46.9	37.5—56.5	4.8	4.1—5.5
≥5 EL.U/ml	Post-booster	114	100.0	96.8—100.0	70.3	60.6—81.6	111	99.1	95.1—100.0	61.9	53.0—72.3
VR	Post-booster	104	96.2	90.4—98.9	-	-	106	99.1	94.9—100.0	-	-
Anti-FHA	Pre-booster	101	99.0	94.6—100.0	40.2	33.5—48.1	108	100.0	96.6—100.0	41.1	35.3—47.8
≥5 EL.U/ml	Post-booster	114	100.0	96.8—100.0	822.0	724.6—932.4	111	100.0	96.7—100.0	708.8	614.0—818.2
VR	Post-booster	101	97.0	91.6—99.4			101	98.0	93.0—99.8		
Anti-PRN	Pre-booster	105	91.4	84.4—96.0	16.7	14.1—19.8	113	89.4	82.2—94.4	16.1	13.4—19.3
≥5 EL.U/ml	Post-booster	114	100.0	96.8—100.0	776.4	659.6—913.8	111	100.0	96.7—100.0	632.7	530.3—754.8
VR	Post- booster	105	99.0	94.8—100.0	-	-	106	98.1	93.4—99.8	-	-

DTPa—HBV, diphtheria, tetanus, acellular pertussis, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; *N*, number of subjects with available serology results at the specified time point; %, percentage of subjects with specified antibody concentrations or vaccine response; 95% CI, 95% confidence interval; GMC, geometric mean concentration; anti-PRP, antibodies to polyribosylribitol phosphate; anti-T, antibodies to tetanus toxoid; anti-D, antibodies to diphtheria toxoid; anti-HBs, antibodies to the hepatitis B surface antigen; anti-PT, antibodies to the pertussis toxoid; anti-FHA, antibodies to filamentous hemagglutinin; anti-PRN, antibodies to pertactin; VR, vaccine response (appearance of antibodies in initially seronegative subjects or a 2-fold increase in antibody concentration in initially seropositive subjects); EL.U, ELISA units; IU, international units.

Table 4 Incidence of solicited local and general symptoms within the 4-day follow-up: primary vaccination (overall doses, ATP reactogenicity cohort) and booster vaccination (total cohort)

	Primary vaccination						Booster vaccination with DTPa—HBV/Hib			
	DTPa—HBV/Hib (*N* = 533)		DTPa—HBV + Hib (*N* = 538)				DTPa—HBV/ Hib-primed (*N* = 116)		DTPa—HBV + Hib-primed (*N* = 117)	
			DTPa—HBV		Hib					
Symptom	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Pain	19.5	16.2—23.1	16.5	13.5—20.9	12.6	10.0—15.7	50.0	40.6—59.4	53.8	44.4—63.1
Grade 3[a]	0.2	0.0—1.0	1.1	0.4—2.4	0.4	0.0—1.3	6.0	2.5—12.0	4.3	1.4—9.7
Redness	38.6	34.5—42.9	35.1	31.1—39.3	25.1	21.5—29.0	70.7	61.5—78.8	69.2	60.0—77.4
>20 mm	2.3	1.2—3.9	1.7	0.8—3.2	0.9	0.3—2.2	29.3	21.2—38.5	26.5	18.8—35.5
Swelling	25.3	21.7—29.2	23.4	19.9—27.2	11.0	8.5—13.9	54.3	44.8—63.6	56.4	46.9—65.6
>20 mm	4.5	2.9—6.6	3.7	2.3—5.7	0.4	0.0—1.3	25.9	18.2—34.8	25.6	18.0—34.5
Diarrhea	17.1	14.0—20.5	16.2	13.2—19.6	8.6	4.2—15.3	9.3	4.7—16.1		
Grade 3[b]	0.4	0.0—1.3	0.4	0.0—1.3	0.0	0.0—3.1	0.0	0.0—3.1		
Fever[c]										
≥37.5 °C	14.4	11.6—17.7	11.2	8.6—14.1	18.1	11.6—26.3	16.9	10.7—25.0		
>39.0 °C	0.4	0.0—1.3	0.7	0.2—1.9	0.9	0.0—4.7	2.5	0.5—7.3		
Fussiness	52.0	47.6—56.3	54.6	50.3—58.9	51.7	42.3—61.1	44.1	34.9—53.5		
Grade 3[b]	0.9	0.3—2.2	2.0	1.3—3.6	2.6	0.5—7.4	0.0	0.0—3.1		
Loss of appetite	14.1	11.2—17.3	15.1	12.1—18.4	31.0	22.8—40.3	22.0	14.6—30.6		
Grade 3[b]	0.4	0.0—1.3	0	0.0—0.7	1.7	0.2—6.1	1.7	0.2—6.0		
Restlessness	31.5	27.6—35.7	31.8	27.9—35.9	26.7	18.9—35.7	31.4	23.1—40.5		
Grade 3[b]	0.4	0.0—1.3	0	0.0—0.7	4.3	1.4—9.8	0.8	0.0—4.6		
Sleeping more than usual	28.1	24.4—32.2	28.3	24.5—32.3	19.8	13.0—28.3	16.1	10.0—24.0		
Grade 3[b]	0.2	0.0—1.0	0.2	0.0—1.0	0.9	0.0—4.7	0.8	0.0—4.6		
Unusual crying	37.0	32.9—41.2	40.7	36.5—45.0	10.3	5.5—17.4	7.6	3.5—14.0		
Grade 3[b]	0.4	0.0—1.3	0.6	0.1—1.6	2.6	0.5—7.4	0.0	0.0—3.1		
Vomiting	14.1	11.2—17.3	13.9	11.1—17.2	7.8	3.6—14.2	4.2	1.4—9.6		
Grade 3[b]	0.4	0.0—1.3	0	0.0—0.7	0.9	0.0—4.7	0.0	0.0—3.1		

ATP, according-to-protocol; DTPa—HBV, diphtheria, tetanus, acellular pertussis, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; *N* = total number of diary cards returned following all doses (results presented from subjects who did not receive PRP challenge); 95% CI, 95% confidence interval; PRP, polyribosylribitol phosphate.

[a] Grade 3 pain at injection site = pain such that the infant cries when the limb is moved.

[b] Grade 3 = symptom that prevents normal everyday activities.

[c] Fever = axillary temperature.

after which the subject recovered. No subject withdrew from the study due to an AE.

Booster vaccination

Local symptoms were reported more commonly after the booster dose, with pain redness or swelling reported by up to 70.7% of subjects (Table 4). In contrast, with the exception of loss of appetite and fever, the incidence of general solicited symptoms tended to be lower than that following primary vaccination. Fever >39.0 °C and grade 3 loss of appetite were uncommon. There was no appreciable difference between groups (primed with DTPa—HBV/Hib or DTPa—HBV + Hib) in terms of the incidence or intensity of solicited symptoms that occurred after the booster dose of DTPa—HBV/Hib. Unsolicited clinical events were reported by 102 subjects in the DTPa—HBV/Hib-primed group and 119 subjects in the DTPa—HBV + Hib-primed group. One event (otitis media) was of grade 3 intensity, but was not considered by the investigator to be related to the booster dose. A total of nine SAEs were reported, none of which were considered related to the study vaccine. All subjects recovered, and none withdrew from the study due to a SAE.

Discussion

A large range of combination vaccines, including DTPa—HBV, DTPa—IPV, and DTPa—HBV—IPV (GlaxoSmithKline Biologicals) have been developed, any of which can be reconstituted with lyophilized conjugate Hib vaccine to provide protection

against up to six diseases in a single injection.[10] The efficacy and safety of such DTPa-based combinations have been established in a range of clinical studies in infants, toddlers, and school-age children.[11,19–22]

The study reported here evaluated the immunogenicity and safety of DTPa–HBV and Hib vaccines administered as a single mixed injection or as separate injections in opposite limbs for primary vaccination in infants aged 2, 4, and 6 months. A follow-up study investigated the immunogenicity and safety of booster vaccination with DTPa–HBV/Hib as a single injection given in the second year of life. This study is original in that plain PRP was used to demonstrate an anamnestic response to a primary course of Hib vaccine in children identified as non-responders and low responders to Hib, prior to a booster Hib vaccination.

Primary immunization with the combined vaccine resulted in high levels of seroprotection (94%) against the Hib PRP antigen (defined as anti-PRP antibody $\geq$0.15 μg/ml) and 97% seroprotection for separate injections. Similarly for hepatitis B, no statistically significant difference was observed between the combined or separate methods of vaccine administration with overlapping 95% CI for seroprotection rate. However the proportion of subjects with anti-PRP antibody levels of $\geq$1.0 μg/ml, and anti-PRP antibody GMC, was higher in the group that received separate injections. Concentrations of specific anti-PRP antibody $>$0.15 μg/ml have traditionally been associated with short-term protection against natural infections, whereas concentrations $>$1.0 μg/ml have been associated with long-term protection.[23,24] It is well documented that the combined administration of Hib tetanus-conjugated vaccines with DTPa-based vaccines can reduce the level of circulating antibodies to PRP compared to separate administration of the Hib vaccine.[11,14,25] However, it has also been shown that the functional nature of the antibodies against Hib produced by combined DTPa-based/Hib vaccines is the same as those induced by separate injections, and that immunological memory is induced.[15,16]

The results of the present study confirm these findings and in addition demonstrate induction of immune memory to the PRP antigen even in the lower responders. Indeed, a subset of subjects with lower anti-PRP responses to primary vaccination (defined using an arbitrary cut-off to identify the 10% of lowest responders in the primary vaccination study) who received an injection of plain PRP at age 12–15 months showed an increase in mean anti-PRP GMC of over 7-fold. This response to PRP challenge, at an age where no significant response to the polysaccharide is expected, is indicative of immunological memory and confirms the findings of other studies.[22] The dose of plain PRP in this study, mimicking Hib infection, was used to demonstrate effective priming and immunological memory in subjects who developed a lower antibody response to Hib. The anamnestic response observed following plain PRP challenge and the booster DTPa–HBV/Hib dose confirm results from other studies with DTPa–Hib combination vaccines showing induction of immune memory.[17,25,26] In addition studies that have examined the functional and qualitative characteristics of antibodies have shown no difference between separate or mixed Hib vaccine administration.[15]

The importance of a booster dose of Hib conjugate vaccine in achieving effective and long-term immunity is well appreciated. Missing the recommended booster dose was associated with an increase in Hib disease in Germany[27] and with a reduction in prevention of Hib colonization.[28] Immunity after primary vaccination without booster was shown to wane over time in the UK, with a fall in vaccine effectiveness to 37.3% two years post-vaccination.[28,29] Concerns about the efficacy of Hib in DTPa-based/Hib combination vaccines have been negated by epidemiological data showing that when these vaccines have been included in routine infant schedules they have been highly successful in prevention of Hib disease.[30]

In terms of other vaccine antigens, the proportion of subjects who developed seroprotective antibody concentrations against diphtheria, tetanus, and hepatitis B or a vaccine response to pertussis antigens after the booster dose was high, and robust increases in antibody concentrations were observed regardless of the vaccine administered for the primary vaccination course.

Mixing of the DTPa–HBV and Hib vaccines did not result in increased reactogenicity for either primary or booster vaccination. In the booster study reported here, the combined DTPa–HBV/Hib booster vaccination was found to be safe, with no SAEs that were considered to be related to treatment, and no withdrawals due to SAEs. There was however, a notable increase in grade 3 pain and redness and swelling $>$20 mm following the booster vaccinations in both groups. Booster DTPa vaccination has been shown to be associated with a higher rate of extensive local reactions than primary vaccination, the pathogenesis of which is likely to be multifactorial.[31,32] However, these local reactions have been shown to resolve spontaneously without any resulting sequelae.[33]

In conclusion, DTPa–HBV and Hib vaccines have been shown to be safe and immunogenic whether administered as a single injection or as separate injections for primary vaccination of infants at ages 2, 4, and 6 months. After booster vaccination with combined DTPa–HBV/Hib vaccine at age 18–19 months, 96–100% of subjects showed seroprotective/seropositive levels of antibodies against all vaccine antigens, and induction of immune memory to PRP was demonstrated in low–moderate responders.

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