Primary vaccination of infants with diphtheria-tetanus-acellular pertussis–hepatitis B virus–inactivated polio virus and *Haemophilus influenzae* type b vaccines given as either separate or mixed injections

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Objective: The aim of this open, multicenter, randomized trial was to evaluate the immunogenicity and reactogenicity of a candidate combined diphtheria-tetanus-acellular pertussis–hepatitis B virus–inactivated polio virus (DTaP-HBV-IPV) vaccine when given as either a mixed or as separate concomitant injections with *Haemophilus influenzae* type b (Hib) vaccine.

Study design: A total of 359 subjects were randomized to receive either DTaP-HBV-IPV/Hib (mixed administration – 180 subjects) or DTaP-HBV-IPV + Hib (separate administration in opposite limbs – 179 subjects) at 2, 3, and 4 months of age.

Results: After vaccination, seroprotective antibody concentrations against diphtheria, tetanus, hepatitis B, and polio viruses and a high (≥97%) pertussis vaccine response were seen in almost all study participants. All subjects except one in the mixed administration group had postvaccination Hib anti-PRP antibody concentrations ≥0.15 μg/mL. Of subjects in the mixed and separate group, 77.2% (geometric mean antibody concentration, 2.62 μg/mL) and 88.6% (geometric mean antibody concentration, 4.45 μg/mL) had Hib anti-PRP concentrations ≥1 μg/mL, respectively. The addition of the Hib component to the 5-component vaccine did not increase the incidence of local or general reactions.

Conclusion: Both administrations of the candidate vaccine were found to be safe, immunogenic, and well tolerated. Although anti-PRP geometric mean antibody concentrations and the percent of subjects achieving the 1 μg/mL seroprotective level were lower after the mixed administration, they were in the range seen with monovalent Hib vaccines or with other DTaP-based/Hib combinations licensed in some European countries. Therefore both administrations have the potential to simplify childhood immunization. (J Pediatr 2000;137:304-12)

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Combination vaccines are already widely accepted as an effective means of eliciting protection against several diseases at the same time. The lower number of injections reduces trauma to the infant and may lead to an overall improvement in immunization rates. Combination vaccines also offer bene-

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fits in terms of reduced storage and administration costs.[1-4] Diphtheria-tetanus-pertussis vaccines have been in use since the 1940s and have become the cornerstone for vaccination of infants worldwide. The acellular pertussis components were recently developed to reduce the severity of adverse events commonly associated with the older whole cell components.[5,6] Many countries have now adopted the use of acellular pertussis-containing DTaP vaccines, since their clinical efficacy was successfully demonstrated between 1994 to 1996.[6] In recent years vaccination against *Haemophilus influenzae* type b and also against hepatitis B have been introduced into many vaccination programs. Furthermore, an increasing number of countries now recommend the use of inactivated polio virus vaccines rather than oral polio virus vaccines because of the small risk for vaccine-associated paralytic poliomyelitis associated with the latter. To meet these demands, a number of different DTaP combination vaccines with the previously described antigens have been licensed in many countries or are now in the final stages of development.[7]

ATP	According-to-protocol analysis
DTaP	Diphtheria-tetanus-acellular pertussis vaccine
FHA	Filamentous hemagglutinin
GMC	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
Hib	*Haemophilus influenzae* type b
IPV	Inactivated polio vaccine
PRN	Pertactin
PRP	Polyribosyl-ribitol-phosphate
PT	Pertussis toxoid
RCC	Reverse cumulative distribution curves
"+"	Separate concomitant administration of vaccines (DTaP-HBV-IPV + Hib)
"/"	Extemporaneously mixed administration of vaccines (DTaP-HBV-IPV/Hib)

The combination of HBV and IPV with DTaP has had no effect on the immune response of the individual components; however, there has been a consistent pattern of diminished anti-PRP antibody concentrations after Hib is combined with DTaP or DTaP-based combinations.[8-11] These results raise concerns over the possible negative impact on the effectiveness of these vaccines against invasive Hib disease. However, several DTaP-based Hib combinations have subsequently been licensed, 2 of which, DTaP/Hib and DTaP-IPV/Hib, have been in wide use in Germany since 1996. Surveillance data from Germany suggest that such combinations offer an excellent protection against Hib disease.[12] Furthermore, the level of antibody is unlikely to be solely responsible for affording protection, and other factors such as immune memory and specific functional activities, which are less markedly affected by combination with DTaP, are also thought to play an important role.[13]

This trial was a feasibility study designed to investigate 2 means of administering diphtheria, tetanus, acellular pertussis, hepatitis B, IPV, and Hib vaccines as a primary vaccination course in infancy. The first 5 components have been combined to give a candidate DTaP-HBV-IPV vaccine. The aim of the trial was to compare the immunogenicity and reactogenicity of DTaP-HBV-IPV and Hib vaccines administered separately and in combination.

METHODS

Design and Subjects

This was an open, randomized, multicenter study conducted at the Department of Pediatrics, Christian-Albrechts-Universität zu Kiel and 12 private pediatric offices in the same locality. Parents provided written informed consent for the study, which had received approval by the appropriate Ethical Review Boards and was conducted according to the guidelines of the Declaration of Helsinki, Good Clinical Practice, and the German Drug Act.

A total of 359 healthy infants between the age of 8 and 16 weeks were enrolled. Subjects were excluded if they were participating in any other clinical trial, had an acute disease, had a history of allergic reaction to any of the vaccine components, were undergoing immunosuppressive therapy, had received any immunoglobulin therapy or blood products before the start of the trial or during the trial, or had been administered any vaccines or experimental drug or vaccine 30 days before the start of the trial or during the trial. Occurrence of any of the contracautionary or precautionary indications for pertussis vaccination after any dose of trial vaccines resulted in exclusion from the trial.[14]

Vaccines

Vaccines were administered as a primary vaccination course at 2, 3, and 4 months of age. A 0.5-mL dose of the candidate vaccine (DTaP-HBV-IPV) was composed of ≥30 IU (25 Lf) of diphtheria toxoid, ≥40 IU (10 Lf) of tetanus toxoid, 25 μg of adsorbed pertussis toxoid, 25 μg of adsorbed filamentous hemagglutinin, 8 μg of adsorbed pertactin, 10 μg of recombinant hepatitis surface B antigen; 40 D-antigen units of type 1 (Mahoney), 8 D-antigen units of type 2 (MEF-1) and 32 D-antigen units of type 3 (Saukett) of the polio virus, and 0.7 mg of aluminum salts (hydroxide and phosphate). The conjugate Hib vaccine was supplied as a lyophilized pellet containing 10 μg of polyribose-ribitol-phosphate conjugated to ~30 μg of tetanus toxoid, 0.12 mg of aluminum phosphate, and 10 mg of lactose. The mixed administration (DTaP-HBV-IPV/Hib) was prepared by reconstituting the lyophilized Hib vaccine pellet with liquid DTaP-HBV-IPV vaccine. For separate concomitant administration (DTaP-HBV-IPV + Hib), the Hib vaccine was resuspended in 0.5 mL of saline solution diluent (9 mg NaCl/mL) as supplied by the manufacturer. All vaccines were administered by deep intramuscular injection into the anterolateral thigh. Single lots of DTaP-HBV-IPV and Hib vaccine were used.

Table I. Anti-PRP seroprotection rates and GMCs 1 month after vaccination

Antibody SP	Mixed (m) N = 145 n/N	Mixed (m) N = 145 %SP (95% CI)	Mixed (m) N = 145 GMC (95% CI)	Separate (s) N = 141 n/N	Separate (s) N = 141 %SP (95% CI)	Separate (s) N = 141 GMC (95% CI)	ΔSP (s-m) (90% CI)
Anti-PRP							
≥0.15 μg/mL	144/145	99.3* (96.2-100)	2.62 (2.1-3.2)	140/140	100.0 (97.4-100)	4.45 (3.6-5.5)	0.69† (-2.8; 5.3*)
≥1 μg/mL	112/145	77.2* (69.5-83.8)	-	124/140	88.6 (82.1-93.3)	-	11.3‡ (3.0; 20.4*)

N, Number of subjects with available results; *n*, number of subjects with seroprotective concentrations; *s*, separate administration; *m*, mixed administration; Δ*SP*, difference in seroprotection rates (separate – mixed).
*The mixed administration was statistically less immunogenic (ie, the upper limit of 90% CI fell outside the a priori limit of clinical noninferiority).
†Secondary end point.
‡Primary end point.

All vaccines were manufactured by SmithKline Beecham Biologicals, Rixensart, Belgium.

Immunogenicity Analysis

Blood samples were taken immediately before the first dose and 21 to 42 days after the final dose and stored at <–20°C until analysis was conducted at SmithKline Beecham Biologicals laboratories in a blinded fashion. Antibodies against diphtheria, tetanus, pertussis antigens (PT, filamentous hemagglutinin, and PRN), and polioviruses were measured by standard techniques as described before.[10,15] An enzyme-linked immunosorbent assay was used to measure the anti-PRP antibodies elicited by the Hib vaccine. All antibody concentrations except for *B. pertussis* at or above the assay cutoffs were considered to correlate with protection, because there is no generally accepted, well-defined antibody concentration to single antigens that correlates with protection. Therefore a vaccine response was defined as in previous studies.[16,17]

Reverse cumulative distribution curves have been used to compare antibody concentrations between trials.[18] Reverse cumulative distribution curves for all 3 anti-pertussis antibody concentrations induced by either the mixed or separate vaccine administration were compared with the immunogenicity data from an earlier efficacy in which the same DTaP component (Infanrix) as used here was shown to have an efficacy of 88.7% (95% CI 76.6 to 94.6).[19] In this efficacy trial all lots provided a similar degree of protection. We used the data from the lots from the efficacy trial that induced the highest (DTaP101A2) and lowest (DTaP117B2) antibody titers for comparison with the data from this study.

Reactogenicity Analysis

Diary cards were used by parents or guardians to solicit local reactions (pain, redness, and swelling) and general adverse experiences (fever, fussiness, vomiting, diarrhea, loss of appetite, restlessness, sleepiness) on the day of vaccination and for 3 subsequent days. Symptoms were graded from 1 to 3 in intensity. Total incidences and grade 3 are reported. Fever was defined as rectal body temperature ≥38.0°C and grade 3 fever as a temperature ≥39.5°C. Grade 3 pain was defined as being present if a child cried when his or her limb was moved. Redness or swelling at the injection site >20 mm was defined as grade 3. Grade 3 fussiness was defined as inconsolable, persistent crying. For all other symptoms grade 3 was defined as preventing normal daily activities.

Statistical Analysis

The primary objective of this study was to demonstrate that no decrease occurred in responses to the mixed administration (DTaP-HBV-IPV/Hib compared with separate administration (DTaP-HBV-IPV + Hib) (ie, demonstrate clinical noninferiority) according to the guidelines described elsewhere.[20] The noninferiority of the mixed administration compared with that of the separate administration with respect to the seroprotection/vaccine response rates and postvaccination GMTs/GMCs was defined by the upper limit of the 90% CI of the difference in seroprotection/vaccine response rates (separate – mixed) or GMT/GMC ratio (separate/mixed) being below the prespecified (a priori) limit defining clinical noninferiority. The a priori limit was set at a 10% decrease in the percent of subjects with anti-PRP titer ≥1 μg/mL (primary end point), the D, T, HBV, IPV seroprotection rates, and the pertussis vaccine response rates (secondary end points). For the percent of subjects with anti-PRP titer ≥0.15 μg/mL, a 5% decrease was set as the a priori limit (secondary end point). A decrease in the pertussis GMT/GMC ratio was set at 1.5 (secondary end point). The 90% CI for the vaccine differences in seroprotection/vaccine response rate was obtained from StatXact 3.0, whereas the CI for the GMT/GMC ratio was derived from a 1-way analysis of variance model on the log-transformed antibody titers. The 95% CIs were calculated for all seroprotection, pertussis vaccine response rates, postvaccination GMTs/GMCs, and the percentage of subjects having adverse reactions (descriptive analysis).

Table II. Anti-pertussis rates vaccine response rates (VR) and GMCs one month post vaccination

Antibody VR	Mixed (m) N = 145 n/N	%VR (95% CI)	GMC (95% CI)	Separate (s) N = 141 n/N	%VR (95% CI)	GMC (95% CI)	Δ (s-m) VR (90% CI)
Anti-PT	134/135	99.3	53.0	125/127	98.4	58.1	–0.83*
VR		(95.9-100)	(48.1-58.5)		(94.4-99.8)	(52.2-64.7)	(–6.2; 3.8)
Anti-FHA	129/130	99.2	151.4	128/129	99.2	154.4	0.0†
VR		(95.8-100)	(137-168)		(95.8-100)	(138-173)	(–4.8; 4.7)
Anti-PRN	133/134	99.3	161.7	127/130	97.7	170.8	–1.56*
VR		(95.9-100)	(142-185)		(93.4-99.5)	(149-196)	(–7.1; 3.3)

N, Number of subjects with available results; *n*, number of subjects showing a VR; *VR*, appearance of antibodies above the assay cutoff (5 EL.U/mL) in initially seronegative subjects and at least maintenance of prevaccination antibody titers in initially seropositive; *s*, separate administration; *m*, mixed administration; Δ *VR*, difference in VR rates (separate – mixed).
Ratio GMTs†: s/m (90% CL) – anti-PT: 1.10 (0.9-1.2); anti-FHA: 1.02 (0.9-1.2); anti-PRN: 1.06 (0.9-1.2).
*The mixed administration was statistically less immunogenic (ie, the upper limit of 90% CI fell outside the a priori limit of clinical noninferiority).
†Secondary end point.

Table III. Anti-diphtheria, anti-tetanus, and anti-hepatitis B surface seroprotection rates and GMCs, and anti-polio seroprotection and GMTs 1 month after vaccination

Antibody SP	Mixed (m) N = 145 n/N	%SP (95% CI)	GMC/GMT (95% CI)	Separate (s) N = 141 n/N	%SP (95% CI)	GMC/GMT (95% CI)	Δ SP (s-m) (90% CI)
Anti-HBs	143/145	98.6	393	139/141	98.6	524.9	–0.04†
≥10 mIU/mL		(95.1-99.8)	(316-489)		(95.0-99.8)	(424-650)	(–4.9; 4.6)
Anti-diphtheria	141/141	100.0	1.71	134/134	100.0	1.81	0.0*
≥0.1 IU/mL		(97.4-100)	(1.48-1.98)		(97.3-100)	(1.56-2.11)	(4.0; 3.7)
Anti-tetanus	141/141	100.0	1.54	134/134	100.0	1.96	0.0*
≥0.1 IU/mL		(97.4-100)	(1.36-1.73)		(97.3-100)	(1.72-2.22)	(4.0; 3.7)
Anti-polio 1	104/104	100.0	330.9	99/99	100.0	404.7	0.0*
≥1:8		(96.5-100)	(2.56-428)		(96.3-100)	(308-532)	(–5.4; 4.9)
Anti-polio 2	97/98	99.0	161.2	97/97	100.0	165.7	1.02*
≥1:8		(94.4-100)	(118-217)		(96.3-100)	(122-225)	(–3.8; 7.8)
Anti-polio 3	102/102	100.0	683.5	98/98	100.0	913.9	0.0*
≥1:8		(96.4-100)	(567-824)		(96.3-100)	(753-1109)	(–5.4; 5.1)

N, Number of subjects with available results; *n*, number of subjects with seroprotective antibody titers/concentrations; *s*, separate administration; *m*, mixed administration; Δ *SP*, difference in seroprotection rates (separate – mixed).
*The mixed administration was statistically less immunogenic (ie, the upper limit of 90% CI fell outside the a priori limit of clinical noninferiority).
†Secondary end point.

RESULTS

Of the 359 subjects enrolled, only 10 failed to complete the study; 8 were lost to follow-up: 5 from the mixed group (DTaP-HBV-IPV/Hib) and 3 from the separate group (DTaP-HBV-IPV + Hib), 1 migrated from the study area (mixed group), and another dropped out because of a protocol violation (separate group). No subjects dropped out as a result of an adverse event.

Immunogenicity

Subjects were eligible for the immunogenicity analysis if a postvaccination sample had been taken and they had complied with the criteria specified in the protocol (the according to protocol analysis). A total of 73 subjects were eliminated from the immunogenicity analysis: 35 from the mixed group and 38 from the separate group. One subject was lost to follow-up after the first dose, and therefore no blood sample was taken (this was the only subject eliminated

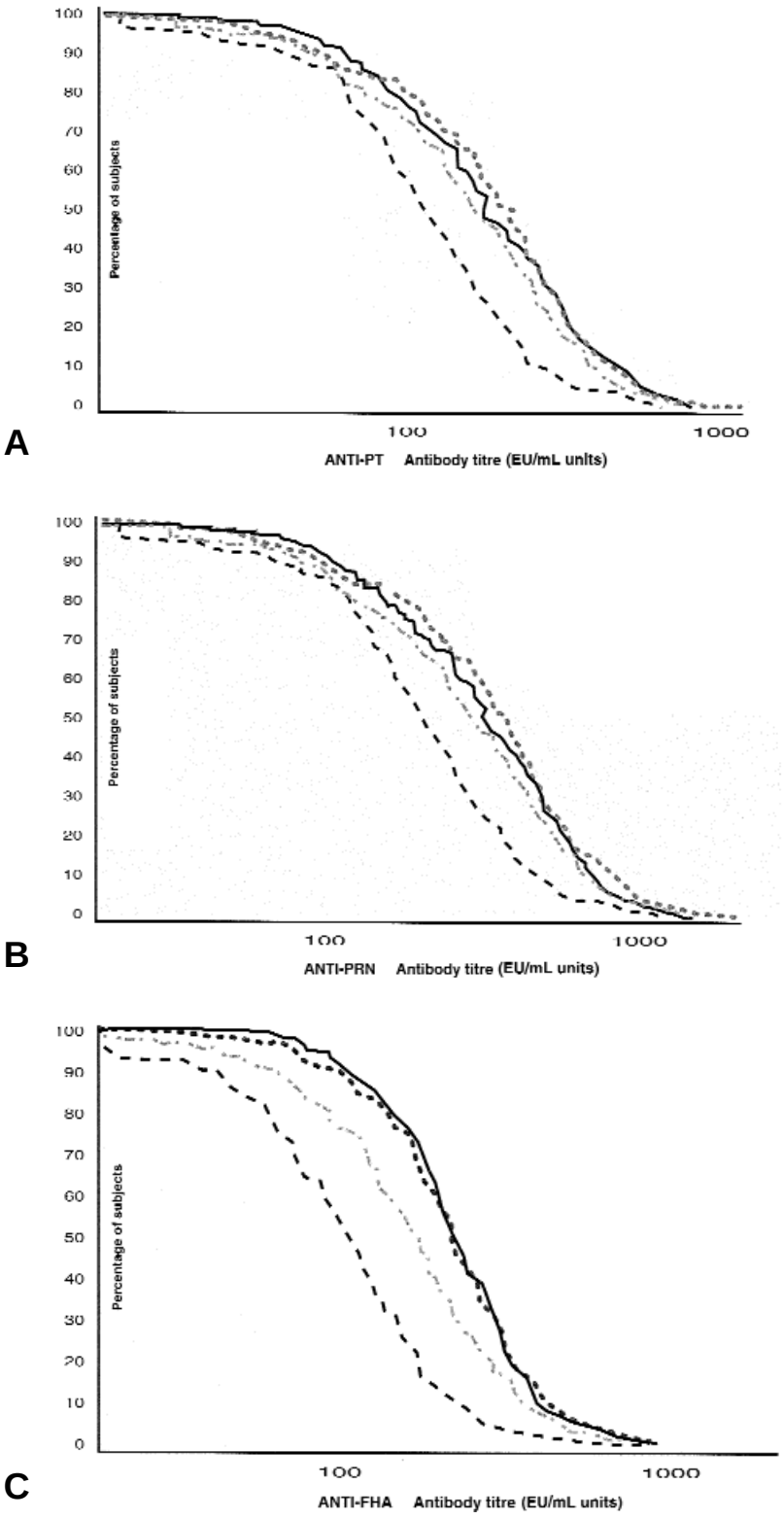

Figure. Reverse cumulative distribution curves (*RCC*) for anti-pertussis antibodies derived from subjects receiving mixed and separate administrations of DTaP-HBV-IPV and Hib vaccines (candidate vaccine group) compared with those derived from subjects receiving DTaP in earlier efficacy study (reference group). **A,** Anti-PT; **B,** anti-PRN; **C,** anti-filamentous hemagglutinin. Reference group (Efficacy trial): — · — APV-39/DTaP 101 A2 most immunogenic lot; - - - - APV-39/DTaP 117B2 least immunogenic lot. Candidate vaccine groups: ····· DTaP-HBV-IPV + Hib (separate); —— DTaP-HBV-IPV/Hib (mixed).

from the ATP reactogenicity analysis because no diary card was returned). Five subjects were above the age limit specified in the protocol, 37 and 27 subjects failed to comply with vaccination and blood sampling schedules, respectively, and a further 3 failed to provide postvaccination blood samples. The results of the ATP analyses are presented in Tables I, II, and III. A reanalysis of all subjects from whom data had been collected (intention-to-treat analysis) indicated that no bias was introduced by focusing on the ATP population (data not shown).

RESPONSE TO HIB ANTIGEN (TABLE I). After vaccination all subjects except 1 infant in the mixed group had anti-PRP titers ≥0.15 μg/mL. A total of 77.2% and 88.6% in the mixed and separate groups, respectively, also had antibody concentrations ≥1 μg/mL. After vaccination the GMCs were 2.62 μg/mL (95% CI 2.1 to 3.2) and 4.45 μg/mL (95% CI 3.6 to 5.5) in the mixed and separate groups, respectively. With the specifically predefined criteria, statistical noninferiority of the mixed administration compared with the separate administration could not be established. The difference in the percentage of subjects with seroprotective concentrations (≥0.15 μg/mL) between the groups was only 0.69%; however, the upper limit of the 90% CI (5.3%) fell marginally outside the a priori limit (5%).

RESPONSE TO PERTUSSIS ANTIGENS (TABLE II). The mixed administration was shown to be at least as immunogenic as the separate administration with respect to the vaccine response rates and postvaccination GMCs against all 3 pertussis antigens (secondary end points). The Figure shows the comparison of the anti-pertussis reverse cumulative distribution curve derived from subjects (both groups) in this study with historical data from subjects in an earlier efficacy trial who

Table IV. Incidence of local and general symptoms per subject in groups receiving mixed (DTaP-HBV-IPV/Hib) and separate administration (DTaP-HBV-IPV + Hib) of vaccines

	Mixed (N = 179)	Separate (N = 179)		
Symptom	Total % (95% CI)	Total % (95% CI)	DTaP-HBV-IPV % (95% CI)	Hib % (95% CI)
Local symptoms				
Pain	24.0 (18.0-31.0)	28.5 (22.0-35.7)	24.0 (18.0-31.0)	23.5 (17.5-30.4)
Grade 3	1.1 (0.1-4.0)	1.1 (0.1-4.0)	0.0 (0.0-0.2)	1.1 (0.1-4.0)
Redness	43.0 (35.7-50.6)	46.9 (39.4-54.4)	43.0 (35.7-50.6)	35.8 (28.7-43.2)
>20 mm	3.9 (1.6-7.9)	6.1 (3.1-10.7)	4.5 (1.9-8.6)	2.8 (0.9-6.4)
Swelling	36.9 (29.8-44.4)	39.7 (32.4-47.2)	35.2 (28.2-42.7)	26.8 (20.5-33.9)
>20 mm	5.6 (2.7-10.0)	5.6 (2.7-10.0)	4.5 (1.9-8.6)	2.2 (0.6-5.6)
General symptoms				
Fever	17.7 (14.5-21.2)	17.4 (14.3-20.9)	—	
≥39.5˚C	0.4 (0.0-1.4)	0.4 (0.0-1.3)	—	
Fussiness	19.2 (15.9-22.8)	16.1 (13.1-19.5)	—	
Grade 3	1.5 (0.7-2.9)	0.9 (0.3-3.2)	—	
Restlessness	22.8 (19.3-26.6)	16.1 (13.1-19.5)	—	
Grade 3	0.9 (0.3-2.2)	0.8 (0.2-1.9)	—	
Sleepiness	22.8 (19.3-26.6)	22.1 (18.7-25.9)	—	
Grade 3	0.6 (0.1-1.6)	0.6 (0.1-1.6)	—	

%, Percentage of subjects reporting a specific symptom.
For pain grade 3 was defined as child cries on moving limb; for fussiness grade 3 defined as inconsolable crying; for all other symptoms grade 3 defined as preventing normal daily activities (if not stated otherwise). Fever defined as rectal temperature ≥38.0°C. For total local symptoms after separate concomitant administrations: a symptom was counted once even if reported at both sites.

Table V. Incidence (%) of local symptoms and fever per subject at each dose

	Dose 1			Dose 2			Dose 3		
	DTaP-HBV-IPV/Hib	DTaP-HBV-IPV	Hib	DTaP-HBV-IPV/Hib	DTaP-HBV-IPV	Hib	DTaP-HBV-IPV/Hib	DTaP-HBV-IPV	Hib
Pain	16.8	14.0	14.0	14.7	11.8	7.9	10.3	8.5	8.5
Redness	21.2	20.7	16.2	27.7	29.8	20.2	32.6	26.1	25.6
Swelling	19.0	17.3	10.6	24.3	21.9	11.8	24.6	23.3	18.2
Fever	16.2	19.0*		19.2	17.4*		16.0	13.2*	

*Incidence of fever after DTPa-HBV-IPV + Hib.

had received the licensed DTaP (Infanrix) vaccine.[20] The pertussis response induced in subjects receiving the mixed and separate administrations of the candidate and Hb vaccines in this trial was comparable to that seen in subjects receiving DTaP in the earlier efficacy trial.

RESPONSES TO DIPHTHERIA AND TETANUS TOXOIDS, HEPATITIS B SURFACE ANTIGEN, AND POLIO ANTIGENS (TABLE III). One month after the third dose was administered, all subjects had seroprotective antibody concentrations against diphtheria, tetanus toxoids, and polio virus types 1 and 3. One subject, who was in the mixed group, failed to produce protective titers against polio virus type 2. A total of 98.6% of subjects in both groups had seroprotective anti-hepatitis B surface titers. With respect to the seroprotection rates (secondary end points), the mixed administration was not inferior to the separate administration. In addition, no relevant differences in post-vaccination GMC/GMTs were observed.

Reactogenicity

Table IV shows the incidence of local and general symptoms solicited. For the total incidence calculation, a local reaction was counted once, even if it was recorded at both sites (for the separate administration); no difference between vaccine administrations was noted. If both sites are considered, the

overall incidence was lower after the mixed administration (1 site). Local reactions at the injection site of the 6-component vaccine were similar to those observed at the injection site of the 5-component vaccine. A similar incidence in systemic reactions, fever (≥38°C), fussiness, restlessness, and sleepiness was reported for both groups (Table IV). This was also true for diarrhea, loss of appetite, and vomiting (data not shown). Most systemic reactions occurred within the first 2 days of vaccination and were mild and self-limiting.

The incidence of pain decreased with each successive dose, whereas swelling and redness increased somewhat, except for redness with the third dose of separate administration (Table V). The incidence of fever decreased with each successive dose after the separate administration; with mixed administration it was highest with the second dose.

Reports of unsolicited symptoms were received after 472 doses; only 13 were considered to be related to vaccination (9 and 4 in the mixed and separate groups, respectively). Four of these reports were related to local reactions, which extended past the 4-day follow-up period, and 9 were general systemic reactions. However, all resolved uneventfully. A total of 8 serious adverse events occurred, 7 of which were judged to be unrelated to vaccination. One SAE (pneumonia, gastroenteritis, and respiratory obstruction) was reported as "unlikely to be related to vaccination" by the study physician. There was no report of hypotonic hyporesponsiveness episodes or of seizures.

DISCUSSION

This trial was designed to investigate the feasibility of giving the Hib conjugate vaccine either as a mixed or as a separate concomitant administration with a DTaP-HBV-IPV vaccine. Both the mixed and the separate administrations were immunogenic in the vast majority of subjects inducing seroprotective postvaccination antibody concentrations against diphtheria, tetanus, hepatitis B, and polioviruses, and Hib titers ≥0.15 μg/mL. An equivalently high pertussis vaccine response was seen between groups. In addition, the vaccines were well tolerated, with an obvious reduction in the total number of injection site reactions when given as a mixed administration.

However, consistent with our earlier observation[21] and those of others since,[8-10,22-25] the mixed administration elicited a lower anti-PRP response than the separate administration. The question arises as to whether the lower immunogenicity may result in reduced protection against invasive Hib disease. In this study only 1 subject in the mixed group failed to elicit an anti-PRP-antibody response ≥0.15 μg/mL, and the GMCs reached in this group were similar to those reported for other DTaP-based/Hib vaccines[7,10,15,24,25] licensed in many European countries and even for monovalent Hib conjugate vaccines.[26-29] In addition, the use of 0.15 μg/mL and 1 μg/mL as threshold of short and long-term protection to assess conjugate Hib vaccines or Hib-based combinations[30] is increasingly being questioned.[13] First, these serologic markers were based on the waning response after the administration of unconjugated plain polysaccharide vaccines, which crucially lack the ability to induce memory.[31] Second, if solely based on these values alone and especially the long-term marker, the concentrations induced by conjugate vaccines would not predict the protective efficacies seen in recent field trials.[26-28]

Consequently, recent attention has focused on the importance of immunologic memory when the anti-PRP response after DTaP-based combinations is evaluated. The existence of a PRP-polysaccharide specific B-cell-memory has successfully been demonstrated after both combined and separate administration of Hib vaccines.[10] Other researchers have conclusively demonstrated the existence of immune memory in a number of ways, for example, by observing an anamnestic booster response that clearly exceeds levels reported in unprimed infants[11,24,25] or by the predominance of immunoglobulin G over immunoglobulin M antibodies after a booster thereby, indicating a true secondary type immune response.[22] Furthermore a number of studies also suggest that the functional activity of the anti-PRP antibodies is also not impaired by combination, as reviewed by Eskola et al.[13]

Based on these considerations, DTaP/Hib vaccines and DTaP-IPV/Hib vaccines were licensed in Germany at the end of 1996. Currently, approximately 90% of all DTaP vaccines are given as DTaP/Hib-based combinations, yet the number of invasive Hib cases continues to decline, and DTaP/Hib vaccine failures are exceedingly rare in fully immunized children.[12] Thus it is our overall conclusion that the somewhat lower anti-PRP titers may be of less concern than originally thought.

Of note, a high degree of seroprotection against diphtheria, tetanus, hepatitis B, and the 3 polioviruses was seen in both study groups. Furthermore with respect to the end points mentioned previously, the mixed administration was found not to be less immunogenic than the separate administration. Serologic markers for protection against pertussis, however, are not well defined. Although 2 recent studies have independently associated antibodies against PRN, fimbriae, and PT as serologic markers of protection for acellular pertussis vaccines, definite serologic correlates of protection for all vaccines have not yet been established.[32,33] Nevertheless, study participants in this trial had equivalent anti-PT, anti-filamentous hemagglutinin, and anti-PRN concentrations to those observed in subjects in an earlier DTaP efficacy trial, in which protection from pertussis defined by the World Health Organization was shown to be 88.7%.[19]

The DTaP vaccine used in this efficacy trial was identical to the DTaP component of the candidate DTaP-HBV-IPV in this investigation. Additional efficacy trials with DTaP-based combination vaccines are no longer feasible, and in this situation it is reasonable to assume that a similar immunogenicity will result in a similar rate of protection.

The incidence of severe reactions was similar to that reported for DTaP vaccines.[6,16,34] In addition, the inclusion of the Hib component to the DTaP-HBV-IPV vaccine in the mixed administration did not result in any further increase in local reactions over the incidence observed at the DTaP-HBV-IPV site (separate administration). In 2 earlier reports[16,34] swelling and redness increased with successive doses; however, in this study this tendency was less marked. In terms of general symptoms, the vaccines administered to both study groups were similarly well tolerated, and no increase in fever occurred with successive doses.

Recent years have shown an increasing number of antigens to be added to the list of vaccines to protect children. Combination vaccines are urgently needed to attain high levels of vaccine coverage. Both administrations of the candidate vaccine investigated in this feasibility trial may contribute to the achievement of this goal.

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