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Research Paper

Immunologic response to Hib tetanus toxoid conjugated vaccine coadministered with DTPa either mixed or in two separate injections in toddlers not primed with Hib vaccine

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Abbreviations: DTPa, diphtheria-tetanus-acellular pertussis; Hib, *Haemophilus influenzae* type b; PRP-T, polyribosylribitol phosphate conjugated to the tetanus toxoid; CI, confidence interval; ELISA, enzyme linked immunosorbent assay; GMC, geometric mean concentration;

Key words: Hib, combined vaccines, conjugate vaccines, interference

Abstract

This open randomised study compared the immunogenicity and safety of a diphtheria-tetanus-acellular pertussis (DTPa) and *H. influenzae* polyribosylribitol phosphate conjugated to the tetanus toxoid (Hib-PRP-T) vaccine (mixed prior to administration) with separate injections of DTPa and Hib vaccines in toddlers aged two years. A total of 119 children (60 mixed; 59 separate administration), primed with DTPw, but not with Hib vaccine were enrolled. Prior to immunization only 10.3% of toddlers had anti-PRP antibody titres ≥1.0 μg/ml, compared with all children on Days 7 and 30. The anti-PRP and anti-tetanus antibody geometric mean concentrations were lower after the combined DTPa/Hib vaccine compared to separately administered vaccines (47.16 μg/ml vs 78.36 μg/ml and 24.95 IU/ml vs 40.63 IU/ml, respectively). One month after vaccination all children had anti-tetanus and anti-diphtheria antibody titres above the protective level of ≥0.1 IU/ml. The rates of recorded adverse events were similar and mostly mild or moderate in intensity whether the vaccines were combined as a single injection or given separately. We conclude that in 2-year old children, previously not immunised against Hib, a single dose of DTPa and Hib was safe and highly immunogenic irrespective of whether it was given as a combined vaccine or separate injections. Although the increase in anti-T and early (7–10 days after) anti-PRP concentrations was greater when the vaccine components were given separately than after combined administration, the DTPa/Hib combined vaccine would provide an effective method of delivering primary Hib vaccination in unprimed toddlers.

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Introduction

The combined administration of vaccines included in childhood immunisation schedules as a single injection promotes vaccination compliance and is considered to be more acceptable by the vaccinees and their parents. In general, the tolerability and immunogenicity of combined vaccines is similar to that of their separately administered components. However, decreased antibody response has been reported to the *H. influenzae* (Hib) polyribosylribitol phosphate conjugated to the tetanus toxoid (PRP-T) and oligosaccharide-Hib conjugate conjugated to CRM197 (HbOC) when this was administered in a single injection with diphtheria-tetanus-acellular pertussis (DTPa).[1-4] The factors leading to such immune interferences have been suggested to be associated with the vaccine content (e.g., interaction between different antigens, adjuvants, reconstitution), with the trial design and/or with the subject's characteristics (e.g., immune memory, ethnicity). It has been demonstrated that children who received their first Hib dose with PRP-D or HbOC vaccines in the second year of life achieved lower antibody levels than those who have been primed in infancy.[4] In this latter group the magnitude of antibody response did not depend whether the vaccine was given as a mixture of Hib and DTP or as separate injections.[4] Whether similar phenomena also occur in toddlers administered the Hib PRP-T vaccine is less clear. Thus, we conducted a study to evaluate the immunogenicity and tolerability of the tetanus-toxoid (TT) conjugated Hib vaccine coadministered with DTPa either mixed in one syringe or in two separate injections.

Methods

Design and ethics. This open, randomised, single-centre study was conducted at Tartu, Estonia. The trial was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines with approval of the Ethics Review Committee of Tartu University. Written, informed consent was obtained from parents or guardians before enrolment.

Subjects. Healthy toddlers aged between 22 and 26 months and previously primed according to routine practices (at age of 3, 4.5 and 6 months) with three doses of whole cellular DTP (DTPw) and

Table 1 **Anti-PRP, anti-D and anti-T seroprotection rates and GMCs (ATP immunogenicity analysis)**

Antibody	Group	Timing	N	≥0.15 μg/ml			≥1.0 μg/ml			GMC		
				n	%	95% CI	n	%	95% CI		95% CI	
Anti-PRP	DTPa + Hib	Pre	52	14	26.9	15.6 41.0	6	11.5	4.4 23.4	0.133	0.098	0.181
		Day 7	25	25	100.0	86.3 100.0	25	100.0	86.3 100.0	43.753*	25.754	74.330
		Day 30	54	54	100.0	93.4 100.0	54	100.0	93.4 100.0	78.360	55.400	110.837
	DTPa/Hib	Pre	55	26	47.3	33.7 61.2	5	9.1	3.0 20.0	0.162	0.123	0.213
		Day 7	24	24	100.0	85.8 100.0	24	100.0	85.8 100.0	17.116	8.275	35.402
		Day 30	55	55	100.0	93.5 100.0	55	100.0	93.5 100.0	47.158	31.768	70.006
					≥0.1 IU/ml					GMT		
				n	%	95% CI					95% CI	
Anti-D**	DTPa + Hib	Day 30	54	54	100.0	93.4 100.0				13.473	10.796	16.815
	DTPa/Hib	Day 30	55	55	100.0	93.5 100.0				14.069	11.372	17.406
Anti-T	DTPa + Hib	Pre	52	46	88.5	76.6 95.6				0.467	0.339	0.641
		Day 30	54	54	100.0	93.4 100.0				40.632*	31.738	52.019
	DTPa/Hib	Pre	55	53	96.4	87.5 99.6				0.448	0.344	0.582
		Day 30	55	55	100.0	93.5 100.0				24.949	21.029	29.600

*p<0.05; **pre-vaccination concentrations were not determined.

oral poliomyelitis vaccine, but not with Hib, were recruited in 1998 in Estonia. The required interval between last DTPw and study vaccines was at least 15 months.

Subjects were equally randomized (1:1) using an algorithm of pseudo random numbers to receive either separate injections of DTPa and Hib vaccines concomitantly into opposite thighs (Group 1) or combined DTPa/Hib vaccine, which was mixed together prior to vaccination, as a single injection into the left thigh (Group 2).

Vaccines. Each 0.5ml dose of the combined DTPa vaccine [*Infanrix*™, GlaxoSmithKline Biologicals (GSK), Rixensart, Belgium] contained 25 μg pertussis toxoid (PT); 25 μg filamentous haemagglutinin (FHA); 8 μg pertactin (PRN); ≥30 IU (25 Lf) diphtheria toxoid (DT); ≥40 IU (10 Lf) TT; 0.7 mg aluminium salts. The lyophilised Hib-TT conjugate vaccine (*Hiberix*™; GSK) contained after reconstitution 10 μg PRP; 20–40 μg TT and 10 mg lactose per 0.5 ml dose. The Hib vaccine was reconstituted as appropriate with either liquid DTPa vaccine (combined group) or saline diluent (separate group).

Immunogenicity evaluation. Blood samples were taken in all subjects before and one month after vaccination. In addition a subset of subjects (n = 25 in Group 1 and n = 24 in Group 2) were also bled between 7–10 days after vaccination. All samples were stored locally at -20°C and then analysed at GSK laboratories in Rixensart, Belgium. The anti-tetanus antibody titres were measured in the samples taken before, and one month after the vaccination and anti-diphtheria antibodies at one month post vaccination by ELISA (as described previously; Refs. 5, 6) the cut-off value for both antibodies was 0.1 IU/ml. The PRP antibodies were measured from samples taken before, 7-10 days and one month after immunisation using a Radio Labelled Antigen Binding Assay with the cut-off value of 0.15 μg /ml (as described by Claesson et al Ref. 7).

Reactogenicity evaluation. All children were closely monitored for 30 minutes after vaccination by study personnel. Thereafter during a four-day follow-up period all solicited local and general symptoms were recorded by parents/guardians using diary cards. The unsolicited and serious adverse events occurring within 30 days after vaccination were recorded. Investigators graded symptom intensity 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 > 39.5°C (fever); interfering with normal activities (other symptoms). Irritability of grade 3 intensity was further investigated for evidence of persistent crying; defined as crying which was continuous and unaltered ≥ 3 hours. All other symptoms, including serious adverse events (SAE) occurring within 30 days of vaccination, were recorded.

Statistical methods. The according to protocol (ATP) analysis included subjects who completed the study according to the protocol (i.e., met the inclusion criteria and complied with protocol specified procedures). For each time the antibody concentrations were measured, geometric mean concentrations (GMC) were calculated and between-group comparisons were made using the t-test. All between group comparisons were exploratory. The percentages of subjects with antibodies titres ≥ the cut-off were calculated and analyzed by SAS software, using a type I error of 5%.

The incidence of each local and general solicited symptom during the 4-day follow up period after vaccination was calculated together with the severity and relationship to vaccination. For the total incidence calculation, a local reaction was counted once, even if it was recorded at both injection sites for the separate administration.

Results

Of 119 subjects recruited three withdrew consent leaving 116 subjects to complete the study. A total of 109 subjects (54 in the separate group and 55 in the combined group) were included in the ATP immunogenicity analysis. All demographic characteristics in both groups were similar; the mean age was 24.0 ± 0.74 months and there were 55 males.

Immunogenicity. The antibody concentrations before and after vaccination are presented in (Table 1). Prior to vaccination, no differences in anti-tetanus and anti-PRP antibody titres between

Table 2 **Incidence of solicited local and general adverse events (Total cohort analysis)**

		DTPa + Hib (n = 60)		DTPa/Hib (n = 59)
Symptom	**Total % (95%CI)**	**DTPa %**	**Hib %**	
Any Local symptoms	66.7 (53.3; 78.3)	58.3 (44.9; 70.9)	53.3 (40.0; 66.3)	72.9 (59.7; 83.6)
Pain at injection site	40.0	36.7	31.7	59.3
Grade 3	10.0	8.3	5.0	1.7
Redness	48.3	38.3	41.7	50.8
>20 mm	8.3	6.7	5.0	8.5
Swelling	13.3	11.7	8.3	18.6
>20 mm	8.3	5.0	3.3	8.5
Any General symptoms	61.7 (48.2; 73.9)			69.5 (56.1; 80.8)
Fever	11.7			16.9
Grade 3	3.3			1.7
Fussiness	36.7			35.6
Grade 3	0			1.7
Loss of appetite	25.0			20.3
Grade 3	1.7			0
Restlessness	20.0			16.9
Grade 3	0			0
Sleeping more than usual	18.3			23.7
Grade 3	0			0
Diarrhoea	15.0			16.9
Grade 3	0			0
Vomiting	3.3			3.4
Grade 3	0			0

Grade 3: pain, cries when limb is moved; redness and swelling, diameter >20 mm; fever (axillary temperature) >39.0°C; fussiness, persistent crying and cannot be comforted; all other symptoms, preventing normal activity.

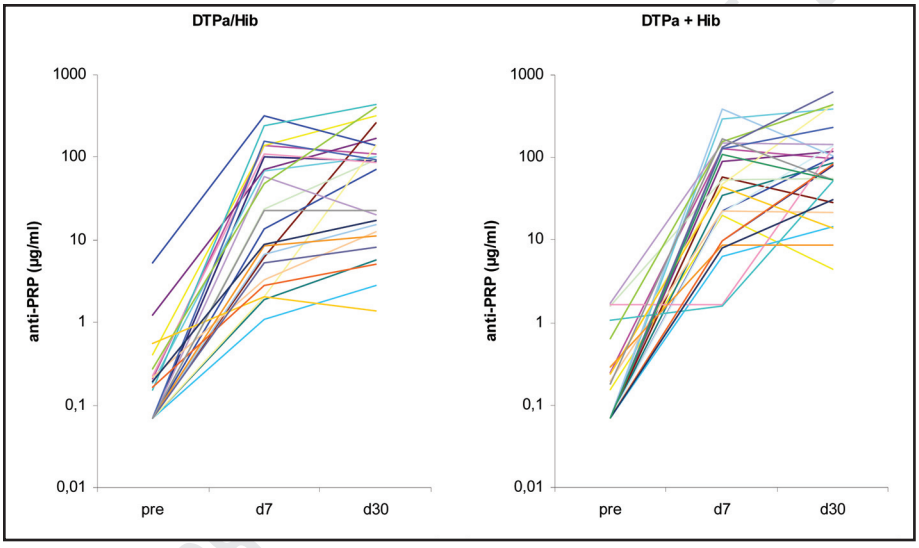

Figure 1. Anti-PRP immune response in Group 1 (n = 26) and Group 2 (n = 25) (total vaccinated cohort).

study groups was seen; a total of 37.4% of subjects had anti-PRP titres ≥0.15 µg/ml and 10.3% ≥1.0 µg/ml. As shown in (Fig. 1), by Day 7 large increases relative to pre-vaccination levels in anti-PRP antibody GMCs were already observed and all children had levels ≥1.0 µg/ml. In comparison to separate administration the mean antibody concentrations were significantly lower when Hib was given together with DTPa (43.75 µg/ml vs 17.12 µg/ml; p = 0.0351). One month after vaccination, however, the significant difference between two groups had disappeared. The antibody response in children with baseline anti-PRP concentrations below 0.15 µg/ml was similar to those who had levels above this cut-off value (55.7 µg/ml vs 70.8 µg/ml). The anti-tetanus IgG GMCs were significantly higher after separately administered than after combined vaccines one month after the booster dose (40.63 IU/ml vs 24.95 IU/ml; p = 0.0016).

Reactogenicity. The recorded adverse events such as redness, swelling, injection site pain and fever were mostly mild to moderate in intensity and self-limiting; no difference between study groups was observed (Table 2). If both sites were considered, the overall incidence of local events tended to be higher after combined than after separate administration (72.9% [CI 59.7; 83.6] vs 58.3% [CI 44.9; 70.9] for DTPa in combined and separate groups respectively and 53.3% [CI 40.0; 66.3] for Hib vaccine. Approximately 10% of children in the separate administration had severe local pain as compared with 1.7% in the combined group. A total of four SAE due to hospitalisation were reported, all unrelated to the vaccination.

Discussion

If first given on the second year of life one dose of conjugated Hib monovaccines has resulted in good antibody response in all subjects.[4] To our best knowledge this is the first time that the immunogenicity of separately or mixed Hib-PRP-TT conjugated and DTPa vaccine has been compared in previously non-primed toddlers. However, this study only looked at the short-term immune response and did not address the consequences of mixing on the duration of immunity and vaccine failure. In several studies irrespective of whether the vaccine was used for primary or booster dosing, the combination of DTPa/Hib in one syringe has resulted in significantly lower anti-PRP antibody titres as compared to separate administration of DTPa and Hib components.[1-3] Similar lower anti PRP responses were also seen in this study on Day 7 after immunisation but one month later such differences had disappeared. However, the difference on Day 7 might have been influenced by the limited number of subjects studied.

The reasons for lower antibody response after combined as compared with separate administration of vaccine components, and especially of PRP, are not fully understood; several mechanisms have been proposed. Dagan et al.[8] compared antibody responses when using DTPw, PRP-T and pneumococcal TT- conjugated (PncT) vaccine and showed that post-immunisation both anti-TT and anti-PRP antibody concentrations were inversely correlated with TT content. The authors concluded that the antigenic interactions between vaccine components were most likely due to the carrier-protein induced epitopic suppression. However, the results of a more recent study by Ocampo et al.[9] with DTPw, Hib-PRP-T and PncTD showed that lower antibody response did not depend on carrier protein overload alone and that further investigations were needed to understand the interplay of vaccine antigens.

There is also a suggestion that adsorption of PRP-T to an aluminium hydroxide adjuvant may reduce immune response to both the PRP and tetanus toxoid components in the conjugate vaccine.[1] In contrast such depression of the antibody response to PRP has not been observed in certain studies when lyophilized Hib vaccines were reconstituted with DTPw vaccine or when a fully liquid (not reconstituted) combination of DTaP and Hib was administered to infants.[10]

The clinical relevance of reduced antibody response to Hib has often been questioned. In all studies, including this one, even if the antibody titres were significantly lower with combined as compared to separate administration, the anti-PRP antibodies clearly exceeded the proposed protective levels of 0.15 μ/ml. Furthermore, in countries such as Germany where the combined DTPa/Hib vaccine has been implemented into national immunization programme, no increase in the incidence of Hib infections in comparison with time when Hib was given as a separate injection has occurred.[11]

Another interesting finding described also in some previous studies[1-3,12] was a significantly lower and most likely clinically irrelevant antibody response to TT after combined administration of DTPa and Hib as compared with separate administration. Reasons and mechanisms of such interference have been extensively studied in animal models and showed that suppression of PRP is a plausible result of interaction between the Hib and TT components.[13,14] In contrast, such an impact on the immune response to TT was not seen with DTPa-IPV/Hib or DTPa-HBV-IPV/Hib vaccines both of which are based on the same DTPa component as the one used in our study.[15,16]

The interesting findings here were much higher than in previous studies anti-TT and anti-PRP antibody titres (40.632 IU/ml vs 24.949 IU/ml and 47.158 μg/ml vs 78.36 μg/ml after combined and separate administration, respectively) in the population primed with TT but not with Hib vaccine.[3,12,15] The latter contrasts to (previous report by Eskola et al. ref. 4) who showed that in unimmunised children a single dose of Hib induced antibody levels of 1.0–10.0 μg/ml whereas in previously immunised children booster responses to HbOC, PRP-D and PRP-T were in range of 20.0 to 60.0 μg/ml. A few explanations for such antibody differences exist. Firstly, the PRP-T vaccine, which has been demonstrated to be one the most immunogenic of all conjugated Hib vaccines was used in our study, whereas the less potent HbOC and PRP-D vaccines were tested in toddlers by others.[4,17,18] Secondly, differences in vaccine responses between populations have been reported before; children from less industrialized countries appear to have higher anti-PRP and anti-TT antibody titers than those from developed western countries.[19,20] The fact that environmental factors play an important role in IgG responses to TT-antigens was recently shown in twin studies conducted in Gambia but the exact mechanisms of this is not completely understood.[21] In studies conducted with Hib vaccines in less privileged countries, it has been suggested that natural priming is associated with a greater antibody response to PRP.[22-24] This natural priming effect, however, cannot explain the appearance of greater anti-TT antibody levels since environmental exposure to *Clostridium tetani* does not influence TT-specific antibodies.[25] It is tempting to speculate that greater exposure to various microorganisms commonly seen in less privileged countries might be an important factor in stimulation of an immune-regulatory network and B-cell response.[26] Further studies should investigate importance of high antibody levels and show whether this results in longer duration of immune response and thus allows implementation of different immunisation schedules in different populations.

As observed by other workers, the occurrence of reported local and general reactions in our study, were comparable in both groups and their incidence was similar to that seen in children after fourth dose of DTPa/Hib.[3,12,15] However, in contrast to the other studies, the severe injection site pain was more common in children who received two separate injections than in those given one shot.

In conclusion, in toddlers not previously immunised with Hib vaccine both DTPa/PRP-T as a single injection or separately administrated DTPa and PRP-T resulted in antibody responses that were significantly higher than proposed protective levels. Although, on Day 7 the achieved antibody concentrations after separate administration were significantly greater than when these components were given as a single injection this is not likely to be clinically significant and thus the DTPa/Hib combined vaccine would provide an effective method of immunizing toddlers not primed with Hib vaccine in infancy. However, prior the implementation of this vaccine into immunisation programs, the duration of the immune response after a single dose needs to be evaluated.

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