

Can *Haemophilus influenzae* type b–tetanus toxoid conjugate vaccine be combined with diphtheria toxoid–pertussis vaccine–tetanus toxoid?

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Objective: To assess the side effects and immune responses after three serial doses of PRP-T vaccine (a *Haemophilus influenzae* type b [Hib]–tetanus toxoid conjugate vaccine) given concurrently or mixed with adsorbed DPT vaccine (diphtheria toxoid–pertussis vaccine–tetanus toxoid).

Design: Multicentre randomized controlled trial.

Setting: Four public health units in western Canada.

Participants: Healthy infants 8 to 15 weeks old at entry who were able to receive routine primary vaccinations. Of 444 infants enrolled, 433 (98%) completed the study.

Interventions: All infants received PRP-T and DPT vaccines at 2, 4 and 6 months of age: half received them mixed in one injection and the others as separate, bilateral injections.

Main outcome measures: Side-effects 24 and 48 hours after each dose and serologic responses to each vaccine component.

Results: Follow-up was obtained after all 1312 vaccinations. Fever was infrequent in the two treatment groups. Local adverse effects of the PRP-T vaccine were infrequent and mild (e.g., redness was noted in 5.9% of cases and the area of redness was more than 2.5 cm in diameter in 0.8%). The incidence rate of local effects of the DPT-containing vaccines was the same in the two groups except for tenderness, which was more frequent in the group given the mixed vaccine (26.6% v. 17.9%, $p < 0.001$). Serologic data were available for 97% of the subjects. After the three doses 98.1% of the subjects had a PRP antibody level of 0.15 µg/mL or more, and 87.9% had a level of 1.0 µg/mL or more, both levels compatible with protection against Hib. Responses to PRP-T were comparable between the treatment groups as were responses to the diphtheria and tetanus toxoids. Pertussis agglutinin titres were reduced after administration of one of two PRP-T lots mixed with DPT vaccine, but responses to four other pertussis antigens were not impaired.

Conclusion: PRP-T vaccine is well tolerated and immunogenic. Combined PRP-T and DPT vaccines performed satisfactorily and may be the preferred method of administration.

Objectif : Évaluer les effets secondaires et les réactions immunitaires après trois doses en série de vaccin PRP-T (un vaccin conjugué d'anatoxine tétanique et d'*Haemophilus influenzae* de type b [Hib]) administrées en même temps ou mélangées à un mélange adsorbé de vaccin DCT (vaccin antidiphthérique, anticoquelucheux et antitétanique).

Conception : Essai contrôlé aléatoire multicentres.

Contexte : Quatre cliniques de santé publique de l'ouest du Canada.

Participants : Nouveau-nés en santé de 8 à 15 semaines au départ qui ont pu recevoir une primovaccination de routine. Sur 444 nouveau-nés inscrits, 433 (98 %) ont terminé l'étude.

Interventions : Tous les nouveau-nés ont reçu des injections de vaccins PRP-T et DCT à 2,

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4 et 6 mois : la moitié les ont reçus mélangés dans la même injection et les autres, sous forme d'injections bilatérales distinctes.

Principales mesures des résultats : Effets secondaires 24 et 48 heures après chaque dose et réactions sérologiques à chaque élément constituant des vaccins.

Résultats : On a effectué le suivi après les 1 312 vaccinations. Les cas de fièvre ont été peu fréquents chez les deux groupes de sujets. Les effets défavorables localisés du vaccin PRP-T ont été peu fréquents et bénins (p. ex., on a noté une rougeur dans 5,9 % des cas et la rougeur avait un diamètre de 2,5 cm ou plus dans 0,8 % des cas). Le taux d'incidence des effets localisés des vaccins contenant du DCT a été le même chez les deux groupes, sauf dans le cas de la douleur, plus fréquente chez les groupes de sujets qui ont reçu le vaccin mixte (26,6 % c. 17,9 %, $p < 0,001$). Des données sérologiques étaient disponibles pour 97 % des sujets. Après les trois doses, 98,1 % des sujets présentaient un titre d'anticorps PRP de 0,15 µg/mL ou plus et 87,9 %, un titre de 1,0 µg/mL ou plus : les deux titres sont compatibles avec la protection contre le Hib. Les réactions au PRP-T ont été comparables entre les groupes de sujets, tout comme les réactions aux anatoxines tétanique et diphtérique. Les titres de l'agglutinine anticoquelucheuse ont diminué après l'administration d'un des deux lots de PRP-T mélangés au vaccin DCT, mais les réactions aux quatre autres antigènes anticoquelucheux n'ont pas diminué.

Conclusion : Le vaccin PRP-T est bien toléré et immunogène. Les vaccins PRP-T et DCT combinés ont donné des résultats satisfaisants et la méthode préférée d'administration peut être celle du jumelage des vaccins.

Invasive infections caused by *Haemophilus influenzae* type b (Hib) have been a major source of illness for children.¹ Principal syndromes include meningitis, epiglottitis, septic arthritis, cellulitis and pneumonia.² The recent availability of Hib vaccines suitable for use at 2 months of age, before the period of peak risk of infection (at 6 to 18 months), holds promise for virtual eradication of such infections. These vaccines are based on the conjugation of the polyriboseryl phosphate (PRP) capsular polysaccharide of Hib to certain carrier proteins to facilitate protective anti-PRP responses.^{3,4} Unlike PRP itself, conjugate vaccines can elicit in young infants responses that are T-lymphocyte assisted; this results in immunologic memory and permits secondary booster responses.^{4,5} The efficacy and safety of currently recommended conjugate vaccines have been well established.⁶⁻⁸ Infants younger than 6 months require a series of primary doses and a booster dose in the second year of life.⁹ When given concurrently with the primary doses of diphtheria toxoid-pertussis vaccine-tetanus toxoid (DPT vaccine), as recommended,⁹ separate injections are required per visit. Given the distress caused by two injections a preparation that combined the two vaccines into one injection would be desirable.

The newest Hib conjugate vaccine to be licensed in Canada is a tetanus-toxoid-based one referred to as PRP-T vaccine (Act-HIB, Connaught Laboratories Ltd., Willowdale, Ont.).³ It is routinely supplied as a lyophilized powder to be rehydrated with a saline diluent. The vaccine was used in this way in young infants in an independent, direct comparison of four available Hib conjugate vaccines, from which it emerged as the most immunogenic after three primary doses.¹⁰ In other studies PRP-T vaccine was reconstituted with liquid DPT vaccine for a single injection.¹¹⁻¹³ PRP-T vaccine combined with a DTP vaccine made in France resulted in

weaker responses to PRP¹² and pertussis than separately injected vaccines.¹³

The performance of mixed vaccines may vary with the DPT preparation used to rehydrate the PRP-T. We report our findings from a study of the compatibility of PRP-T vaccine with Canadian-made DPT vaccine.

Methods

Children were recruited from the clientele of four participating public health units in British Columbia and Calgary. The British Columbia units vaccinate 40% to 60% of infants in their jurisdiction, whereas the unit in Calgary vaccinates virtually all infants. Children born within a 12-week period were identified from birth registration lists, and their parents were sent a letter of invitation outlining the study. Parents expressing interest during a subsequent telephone contact were offered a detailed explanation of the study during a home visit, provided their infants were eligible. Parents were then given time to consult their family physician or relatives. Written consent was obtained at a subsequent visit before the first vaccine dose was given.

Letters were sent to about 1100 families; of the approximately 580 who agreed to an information visit about 75% subsequently enrolled. Recruitment was stopped when the enrolment target of 444 was reached. Children were eligible for enrolment if they were 8 to 15 weeks old, healthy and free of acute symptoms or conditions for which use of DPT vaccine and oral poliovirus vaccine (OPV) is contraindicated.¹⁴ Additional eligibility criteria included availability of a home telephone, ability of the parents to converse adequately in English and absence of plans to relocate within 5 months. The protocol was approved by the ethics committees of the University of British Columbia and the Calgary Board of Health.

The study was conducted by three field teams, two in Vancouver and one in Calgary. Participants were vaccinated at 2, 4 and 6 months of age with adsorbed DPT vaccine, conjugated PRP-T vaccine and trivalent OPV vaccine. Participants were randomly assigned at study entry to receive either (a) combined injections of DPT and PRP-T vaccines or (b) concurrent injections of DPT and PRP-T vaccines in opposite thighs. Randomization sequences were prepared for each field team and were based on tables of randomly generated numbers in balanced (1:1 assignment ratio) blocks of 12. Assignments were built into subjects' study numbers, which were serially assigned at entry and linked to numbered boxes containing the appropriate diluent for the method of vaccination and all three doses of the PRP-T lot to be used, labelled only with the subject's number.

Vaccines were supplied by Connaught Laboratories. One lot of DPT vaccine was used (3905-21/31). This contained 25 Lf of diphtheria toxoid, 5 Lf of tetanus toxoid, 4 to 12 PU of pertussis vaccine, 1.5 mg of aluminum phosphate and 0.01% thimerosal preservative per 0.5 mL of vaccine. Two lots of PRP-T vaccine (S2189 and S2226) were used. These will be referred to subsequently as lots 1 and 2 respectively. They were manufactured by Pasteur Mérieux Sérums et Vaccins, Lyon, France, as described elsewhere.¹⁵ The PRP-T vaccine was supplied in lyophilized form in single-dose vials. Each dose contained 10 µg of PRP covalently linked to 20 µg of tetanus toxoid. Vaccines from the two lots were assigned to half of each treatment group by the same randomization process. The OPV vaccine was derived from several lots and was given at 2 and 4 months of age. All vaccines were stored according to the manufacturer's recommendations.

Vaccination was performed in a standardized fashion by research nurses. When a child was to receive a combined injection of DPT and PRP-T vaccines the dose of liquid DPT was drawn into a syringe and injected into the PRP-T vial. The mixture was shaken to ensure dissolution of the PRP-T and then drawn into a syringe for injection. When PRP-T vaccine was to be given by separate injection it was rehydrated with saline diluent (4 g/L) supplied by the manufacturer; the full contents of the vial (0.4 to 0.6 mL) were removed for injection. Injections were administered intramuscularly in the anterolateral portion of the thigh. The preferred needle was 25 gauge and 22 mm in length. When two injections were given concurrently parents were not told which vaccine was which. PRP-T vaccine was preferentially given on the left side to minimize recording errors.

Use of acetaminophen prophylaxis (15 mg/kg given 0, 4 and 8 hours after vaccination) was recommended to parents following each dose of DPT vaccine, in compliance with routine policies of the health units.

Each child was observed for 15 minutes after vaccination to detect and treat any anaphylactic reactions. For 48 hours after each dose parents were asked to look for

and record in a simple diary any changes at the injection site(s) or in their child's health or behaviour. Digital thermometers were supplied to parents with instructions on their use (rectal method encouraged). Body temperature was to be taken one to two times daily and whenever parents suspected fever. Celluloid rulers, consisting of sized circles (1 to 5 cm in diameter) and a linear scale, were supplied to help parents measure areas of redness or swelling at injection sites. Parents' observations were reviewed systematically during telephone interviews with study staff members 24 and 48 hours after vaccination. Parents were encouraged to report any severe adverse event during the study period. Nurses were authorized to extend follow-up for 7 to 10 days if troublesome symptoms were still present 48 hours after vaccination.

Blood was obtained from infants before the first and third doses and 1 month after the third dose. Blood was collected from heel or finger punctures using Microtainer collector-separator devices (Becton Dickinson, Rutherford, NJ). Serum was separated promptly and stored at -20°C before testing. Code-labelled serum samples were tested for diphtheria antitoxin by microneutralization assay, pertussis agglutinins by microagglutination method, pertussis antitoxins by microneutralization assay with Chinese hamster ovary cells, tetanus antitoxin by enzyme-linked immunosorbent assay (ELISA) and PRP antibody by a Farr-type radioimmunoassay. The last test was performed at Connaught Laboratories Inc., Swiftwater, Penn.; the others were performed at Connaught Laboratories Ltd., Willowdale.

Subsequent to analysing these data we chose to perform assays for additional pertussis antibody responses using remaining serum. ELISA assays were used to measure antibodies directed against pertussis toxin, filamentous hemagglutinin, 69kDa outer-membrane protein and fimbriae. These assays were performed at Connaught Laboratories in Willowdale. Reference serum samples were provided by the FDA Pertussis Laboratory, Rockville, Md.

Compliance with the protocol was closely scrutinized at each enrolment centre by a study monitor. Data were assembled and analysed by one of us (D.S.). Case report forms were checked for accuracy and completeness upon receipt and corrections effected if necessary. Accuracy of data entry into the custom-designed database was verified through programming checks and manual verification of key data for all files.

The sample size was calculated to provide approximately 90% power to reject the null hypothesis if serologic response rates to any of the constituent vaccines differed by 10% or more between separate and combined modes of administration ($\alpha = 0.05$, two-sided). Such a sample provided 80% power to recognize differences in immunogenicity of 15% or greater between lots of PRP-T ($\alpha = 0.05$, two-sided) for each mode of vaccination. Finally, the cumulative observations over three doses were expected to provide 90% power to recognize

differences in rates of local and systemic adverse effects of 50% or more ($\alpha = 0.05$, two-sided) between modes of vaccination. Incidence rates of fever and local redness were selected as the primary outcome measures because they are most likely to be ascertained reliably by parents. Analyses of intergroup differences in antibody responses were based on absolute titres achieved as well as the proportion exceeding predetermined threshold titres. Geometric mean titres (GMTs) were calculated on log-transformed values, negative values being assigned an arbitrary value just below the lower detection limit. Means of normally distributed data were compared using Student's *t*-test. The χ^2 test with continuity correction was preferred for comparison of proportional data. Calculations were performed using SAS/STAT software (SAS Institute, Cary, NC).

Results

A total of 444 infants were enrolled, but one set of twins was withdrawn by their mother immediately after the first dose. Of the remaining 442 infants 437 (98.9%) received two doses and 433 (98.0%) received all three doses. Vancouver site A enrolled 202 subjects and site B enrolled 84 subjects, and the Calgary unit enrolled 156 subjects. Participants at each site were alike in terms of sex ratio (51% to 53% female), age at the first dose (97.0% to 100% were 8 to 12 weeks old) and average birth weight. Only nine infants (2.0%) weighed less than 2500 g at birth, and seven were born at less than 36

weeks' gestation. Demographic data are summarized for the four treatment groups in Table 1; the mean age at the first dose was similar in the four groups, varying from 9.1 to 9.3 weeks. In total 220 infants received PRP-T lot 1 and DPT vaccine (combined in 109 and as separate injections in 111), and 222 received PRP-T lot 2 and DPT vaccine (combined in 111 and as separate injections in 111). A dose interval of 7 to 10 weeks was achieved for 93% of the subjects over the vaccination series.

Adverse reactions

Follow-up information was provided by parents after all 1312 vaccinations. The frequency and severity of adverse effects might have been influenced by the administration of acetaminophen, which was given prophylactically after 95.7% of the doses. Additional acetaminophen treatment was reported during the first 24 hours after 33% of the doses.

Local adverse reactions to the PRP-T vaccines were infrequent and mild (Table 2). No significant difference was evident between the two lots or with successive doses (data not shown). Local redness was noted in 5.9% of cases 24 hours after vaccination; an area of redness of 25 mm in diameter or greater was reported in 0.8% of cases. Redness resolved within 48 hours in 79.5%. Swelling was reported in 4.1% of cases at 24 hours after vaccination and in 0.5% at 48 hours. Tenderness at the injection site was noted in 10.8% of cases at 24 hours; in only 10 cases (1.5%) was the tenderness reported as being severe.

Table 1: Demographic characteristics of children given one of two lots of *Haemophilus influenzae* type b (Hib)-tetanus toxoid conjugate vaccine (PRP-T vaccine) given concurrently or mixed with adsorbed diphtheria toxoid-pertussis vaccine-tetanus toxoid (DPT vaccine), by treatment group

Characteristic	Treatment group; no. (and %) of children			
	Separate injections		Combined injection	
	Lot 1	Lot 2	Lot 1	Lot 2
No. of subjects	111	111	109	111
Male	46 (41.4)	54 (48.6)	57 (52.3)	56 (50.5)
Female	65 (58.6)	57 (51.4)	52 (47.7)	55 (49.5)
Birth weight, g				
< 2500	1	0	4	4
2500-2999	15	15	14	14
3000-4000	76 (68.5)	83 (74.8)	77 (70.6)	78 (70.3)
> 4000	19	13	14	15
Length of pregnancy, wk				
30-35	1	3	2	1
≥ 36	110	108	107	110
Age at first dose, wk				
8-10	94 (84.7)	94 (84.7)	98 (89.9)	91 (82.0)
11-12	14 (12.6)	15 (13.5)	10 (9.2)	18 (16.2)
13-15	3 (2.7)	2 (1.8)	1 (0.9)	2 (1.8)
Dose received				
First	111	111	109	111
Second	110	110	109	108
Third	110 (99.1)	106 (95.5)	109 (100.0)	108 (97.3)

The DPT vaccine caused local adverse effects frequently (Table 2). The combined injection of PRP-T and DPT did not result in more reports of redness or swelling than were seen after DPT vaccination alone (Table 2). Compared with the DPT vaccine alone, the combined vaccine resulted in local tenderness being reported more often at 24 hours (26.6% v. 17.9%, $p < 0.001$) and rated moderate or severe more often (10.1% v. 5.9%, $p < 0.05$).

Assessment of systemic adverse effects was confounded by the occurrence of intercurrent illness, mostly cough and cold syndromes, 48 hours after 79 (6.0%) of the vaccinations. These illnesses occurred at a similar rate among the four treatment groups and the three doses. Nine infants were taken to a physician because of them. All subjects were retained in the analysis of systemic symptoms.

Body temperature was measured within 4 to 6 hours after vaccination in 1256 instances (95.7%) and

within 24 hours in 1275 (97.2%). There were only 27 reports (2.1%) of a temperature of 39.0°C or greater. Rates of such temperatures did not differ significantly between the treatment groups (Table 3), but the overall rate of temperature of 38.0°C or greater was significantly lower among children given lot 1 of the PRP-T vaccine by separate injection than among those given the combined injection (13.6% v. 20.1%, $p < 0.03$).

Reports of irritability, more crying than usual, lethargy and vomiting were obtained at similar frequencies regardless of the PRP-T vaccine lot or mode of administration (Table 3). One hypotonic-hyporesponsive episode was reported in a child after a combined injection containing PRP-T vaccine lot 2. No seizures were reported. Overall, the parents rated adverse events as moderate or severe slightly more often after the combined injection than after the separate injections (12.4% v. 8.8%, $p < 0.05$). Only seven infants experienced ad-

Adverse effect	Vaccine; no. (and %) of children				
	PRP-T vaccine		DPT vaccine (n = 658)	Combined vaccines	
	Lot 1 (n = 331)	Lot 2 (n = 327)		Lot 1 (n = 327)	Lot 2 (n = 327)
Redness					
Any					
At 24 h	17 (5.1)	22 (6.7)	220 (33.4)	85 (26.0)	82 (25.1)
At 48 h	4 (1.2)	4 (1.2)	85 (12.9)	24 (7.3)	23 (7.0)
≥ 25 mm in diameter	2 (0.6)	3 (0.9)	12 (1.8)	7 (2.1)	8 (2.4)
Swelling					
Any					
At 24 h	16 (4.8)	11 (3.4)	127 (19.3)	46 (14.1)	48 (14.7)
At 48 h	2 (0.6)	1 (0.3)	64 (9.7)	28 (8.6)	19 (5.8)
≥ 25 mm in diameter	6 (1.8)	3 (0.9)	33 (5.0)	12 (3.7)	20 (6.1)
Tenderness					
Any					
At 24 h	33 (10.0)	38 (11.6)	118 (17.9)	89 (27.2)	85 (26.0)
At 48 h	5 (1.5)	3 (0.9)	28 (4.3)	18 (5.5)	15 (4.6)
Moderate or severe	13 (3.9)	15 (4.6)	39 (5.9)	32 (9.8)	34 (10.4)

Adverse event	Treatment group; no. (and %) of children			
	Separate injections		Combined injection	
	Lot 1	Lot 2	Lot 1	Lot 2
Fever < 24 h after dose	(n = 316)	(n = 320)	(n = 323)	(n = 316)
≥ 38.0°C	43 (13.6)	65 (20.3)	65 (20.1)	68 (21.5)
≥ 39.0°C	4 (1.3)	7 (2.2)	6 (1.9)	10 (3.2)
Symptom 24 h after dose	(n = 331)	(n = 327)	(n = 327)	(n = 327)
Irritability	142 (42.9)	153 (46.8)	159 (48.6)	172 (52.6)
Increased crying	108 (32.6)	117 (35.8)	100 (30.6)	131 (40.1)
Nonstop crying for 3 h or more	1 (0.3)	0	1 (0.3)	0
Lethargy	128 (38.7)	139 (42.5)	131 (40.1)	131 (40.1)
Vomiting	11 (3.3)	12 (3.7)	8 (2.4)	11 (3.4)
Increased no. of stools	36 (10.9)	28 (8.6)	37 (11.3)	29 (8.9)
Physician seen < 48 h after dose	3 (0.9)	3 (0.9)	0	3 (0.9)
Severe symptoms (rated by parents)	2 (0.6)	1 (0.3)	1 (0.3)	3 (0.9)

verse events rated as severe: no distinction was made between intercurrent illnesses and adverse effects in these instances. All symptoms and signs that were of concern to the research staff 48 hours after vaccination had resolved when the parents were contacted 5 to 8 days later. The frequency with which centres elected to make follow-up contacts differed substantially: Vancouver site A, 6 calls (2.0%); Vancouver site B, no calls; and Calgary, 24 calls (10.6%).

Serologic data

Serum samples were obtained from 434 (98.2%) of the subjects before the first dose and from 422 (97.5% of the remaining 433) following the third dose. Ten samples taken after dose 3 were omitted from the main analysis because they were obtained more than 45 days after vaccination.

Anti-PRP levels are summarized in Table 4. The distribution of anti-PRP levels at study entry, which reflected maternally derived antibodies, was comparable among the four treatment groups. After two doses of the PRP-T vaccine, separate or combined, 75.1% of the subjects had an anti-PRP level of 0.15 µg/mL or greater; the GMT for the group was 0.53 µg/mL (95% confidence interval [CI] 0.45 to 0.62). Performance of the two PRP-T vaccine lots mixed with DPT vaccine was unaltered for lot 1 but was significantly reduced for lot 2 in terms of anti-PRP levels of 1.0 µg/mL or greater (in 29.0% of cases after mixed v. 51.4% after separate injections,

$p < 0.01$) and the GMTs (0.40 v. 0.83 µg/mL, $p < 0.01$). After three doses no significant difference in immunogenicity was evident between the PRP-T lots or treatment regimens (Table 4). In aggregate, 98.1% of the subjects had an anti-PRP level of 0.15 µg/mL or greater, 87.9% having a level of 1.0 µg/mL or greater. The GMT was 4.24 µg/mL (95% CI 3.75 to 4.80).

For responses to the DPT vaccine components, analysis was limited to serologic data obtained after the third dose. All of the subjects exceeded the minimum protective level for diphtheria antitoxins. GMTs ranged from 0.3 to 0.4 IU/mL between the groups. No reduction in response was evident after use of the combined vaccines. All of the subjects were apparently protected against tetanus: over 98% had titres of 0.10 IU/mL or more, and nearly 50% had titres of 1.00 IU/mL or more. Responses were similar in each treatment group. Pertussis agglutinins were detected in all but two subjects (99.6%). Over 98% had titres of 32 or greater. The two groups given separate injections of DPT had virtually identical responses to the pertussis toxoid (GMTs were 416.9 and 428.4). For both lots of PRP-T vaccine, combination with the DPT vaccine resulted in lower GMTs of pertussis agglutinins (lot 1, 368.0; lot 2, 291.3), but the difference was statistically significant only for lot 2 (32% reduction in GMT, $p < 0.02$). Pertussis toxin neutralization titres were relatively low, the GMT for all participants (416 assayed) being 5.4 (95% CI 5.0 to 5.9). The antitoxin titre was 4 or less in 242 subjects (58.2%). Response rates and GMTs were not significantly different

Table 4: Anti-PRP levels, by treatment group

Anti-PRP level, µg/mL	Treatment group; no. (and %) of children			
	Separate injections		Combined injection	
	Lot 1	Lot 2	Lot 1	Lot 2
Before vaccination	(n = 108)	(n = 109)	(n = 107)	(n = 110)
< 0.06	61 (56.5)	64 (58.7)	62 (57.9)	76 (69.1)
0.06–0.14	10 (9.2)	10 (9.2)	8 (7.5)	7 (6.4)
0.15–0.99	33 (30.6)	28 (25.7)	30 (28.0)	26 (23.6)
≥ 1.00	4 (3.7)	7 (6.4)	7 (6.5)	1 (0.9)
GMT*	0.11	0.11	0.11	0.08
95% confidence interval (CI)	0.09–0.13	0.09–0.13	0.09–0.14	0.07–0.10
After two doses	(n = 108)	(n = 107)	(n = 107)	(n = 107)
< 0.06	16 (14.8)	13 (12.1)	20 (18.7)	22 (20.6)
0.06–0.14	9 (8.3)	6 (5.6)	8 (7.5)	13 (12.1)
0.15–0.99	51 (47.2)	33 (30.8)	44 (41.1)	41 (38.3)
≥ 1.00	32 (29.6)	55 (51.4)	35 (32.7)	31 (29.0)
GMT*	0.48	0.83	0.51	0.40
95% CI	0.36–0.64	0.61–1.13	0.37–0.69	0.29–0.53
After three doses	(n = 108)	(n = 105)	(n = 107)	(n = 102)
< 0.06	0	1 (1.0)	2 (1.9)	2 (2.0)
0.06–0.14	1 (0.9)	1 (1.0)	1 (0.9)	0
0.15–0.99	14 (13.0)	9 (8.6)	11 (10.3)	9 (8.8)
≥ 1.00	93 (86.1)	94 (89.5)	93 (86.9)	91 (89.2)
GMT*	3.89	4.24	4.50	4.37
95% CI	3.05–4.96	3.36–5.36	3.46–5.86	3.39–5.63

*GMT = geometric mean titre; for antibody levels less than 0.06 µg/mL the value was assumed to be 0.05 µg/mL.

after combined or separate injections (e.g., GMT 6.0 v. 5.1 respectively for lot 1 and 5.8 v. 4.9 for lot 2). None of the four supplementary pertussis antibody tests showed significant response differences between the groups receiving separate or mixed injections (data not shown).

Discussion

Our findings convincingly demonstrate that PRP-T vaccine can be safely administered with DPT vaccine. Follow-up observations after 1312 vaccinations involving concurrent or mixed injections revealed no serious adverse events. Although a group receiving only DPT vaccine was not included to permit measurement of the incremental effect of PRP-T vaccine, this was not felt to be necessary because others have shown PRP-T vaccine to have minimal reactogenic effects.^{16,17} The incidence of reported adverse effects may have been reduced by the near-universal use of acetaminophen prophylaxis among the subjects.¹⁵ This is the current standard of care in our area for infants receiving DPT vaccine, and we elected not to depart from it. On the other hand, intercurrent illnesses such as colds developed in 79 infants (i.e., after 6.0% of the doses) within 48 hours after vaccination and might have added to the morbidity attributed to vaccines.

Local adverse effects of PRP-T vaccine reconstituted with saline were infrequent and mild (Table 2), as has been shown in other evaluations to date.^{10,12} No important difference was evident between the two lots or with successive doses. The rates of local reactions to the DPT vaccine were typical.¹⁸ Local redness and swelling occurred at the same rates with the combined DPT and PRP-T vaccines as with the DPT vaccine alone, but tenderness was reported more frequently after the combined injections and was more often judged to be moderate or severe by the parents. The differences were statistically but not clinically significant. Most instances of tenderness resolved within 48 hours. In other assessments of mixed DPT and PRP-T vaccines local adverse effects were indistinguishable from those of DPT vaccine alone.^{11,12}

Systemic adverse effects of PRP-T vaccination could not be assessed in our study because all the subjects received DPT vaccine at the same visit. With routine acetaminophen prophylaxis, fever is expected after DPT vaccination in about 27% of infants, the temperature exceeding 39.0°C in 3%.¹⁵ The mixed PRP-T and DPT vaccines were not associated with a higher incidence of fever or other systemic effects than the separately injected vaccines were. The lower rate of fever among the children given lot 1 of the PRP-T vaccine by separate injection (Table 3) than among those in the other treatment groups was statistically significant ($p < 0.03$) but not clinically significant; the difference in actual rates was small (7%) and not associated with a lower risk of higher temperature. No serious adverse effects were encountered. One child experienced a brief hypotonic-hyporesponsive episode after a dose of mixed

vaccines, with prompt recovery. In a study of this size one case of this syndrome after 1312 doses is not unexpected. The incidence of hypotonic-hyporesponsive spells observed in a much larger study was 1:1750 doses.¹⁸ Although our observation was uncontrolled it suggests that routine use of acetaminophen prophylaxis does not prevent such spells.

As to the key issue of the compatibility of mixed DPT and PRP-T vaccines, we observed no effect on anti-PRP responses after three doses of mixed products, as compared with responses to separately injected vaccines. A significant reduction in the anti-PRP level existed after two doses of lot 2 of the PRP-T vaccine (Table 4) mixed with DPT vaccine; however, this was not evident with lot 1, and the difference disappeared after the third dose. Our results contrast with those from a recent study in Chile using DTP vaccine made in France, in which responses to PRP-T vaccine were reduced by more than 50% after three doses of mixed vaccines, as compared with separately injected vaccines.¹² The differing results most likely reflect compositional differences between the DPT products used. It has not been determined whether the more subtle compositional differences that exist between lots of a particular DPT vaccine¹⁹ will be relevant to the compatibility of mixtures with PRP-T vaccine. Our study and the Chilean one involved single lots of DPT vaccine.

Equally relevant to the compatibility question is whether mixing with PRP-T vaccine impairs the response to any component of the DPT vaccine. Our data and those from other reports^{11,12} indicated no impaired response to the diphtheria and tetanus toxoids as a result of mixing the vaccines. However, the data for responses to the pertussis component are conflicting. Waternberg and associates¹¹ detected no impaired pertussis agglutinin response in Israeli infants given PRP-T vaccine mixed with a DTP vaccine made in France. In contrast, Clemens and collaborators¹³ detected significant impairment in Chilean infants given single lots of the same mixed products. Pertussis agglutinin responses after three doses were moderately reduced (GMT 1995.3 after separate injections and 1230.3 after combined injection, $p < 0.05$); however, responses to pertussis toxin and filamentous hemagglutinin were equivalent between these groups. Surprisingly, both groups given PRP-T vaccine had lower responses to pertussis agglutinins and pertussis toxin than a control group given only DTP and placebo. The explanation for the different results of the Israeli and Chilean studies is uncertain: each was well controlled but used different lots of both products. It could reflect differences in the lots, in the hosts or even in the routes used for vaccination (intramuscular in the Israeli study, subcutaneous in the Chilean one).

Our results fell between the outcomes in those two studies: only one of the two lots of PRP-T vaccine mixed with the Canadian-made DPT vaccine resulted in reduced pertussis agglutinin responses. The observed

difference in GMTs, although relatively small, was comparable in magnitude to the difference seen in the Chilean study (32% v. 38%). It is not apparent whether the difference observed in our study resulted from differences in the PRP-T vaccine lots or in the response capacities of infants assigned to each group. The pertussis toxin neutralization, antitoxin, filamentous hemagglutinin, 69kDa protein or fimbrial antibody levels did not differ between the groups.

The biologic significance of the reduced pertussis agglutinin titres after the use of the mixed DPT and PRP-T vaccines is uncertain.¹³ Over 98% of our subjects still had a titre of 32 or more, and 64% had one of 256 or more. The reduced values fall within the range usually seen after the use of noncombined DPT vaccine. The mixed vaccines still meet regulatory requirements for potency of the pertussis component. The ability to respond to pertussis bacteria on exposure may be more important than the peak values after three doses, because titres generally fall rapidly between 7 and 18 months of age to near baseline values,¹³ but protection usually persists.²⁰ Responses to other pertussis components believed to be important for protective immunity were unaltered by the use of the mixed vaccines. The effect of booster doses of mixed DPT and PRP-T vaccines on pertussis or PRP antibody levels has not been published to date. Separate doses of PRP-T at 18 months elicited strong increases of anti-PRP levels in children given primary doses as young infants.¹⁶

We conclude that PRP-T vaccine is safe and highly immunogenic. Mixed with DPT vaccine for single injections it is well tolerated, and responses to the PRP, diphtheria and tetanus toxoids are unimpaired. With one of two lots tested, pertussis agglutinin responses were reduced following the use of the mixed vaccines, but the biologic significance of this is uncertain. Responses to four other antigens considered relevant to protection were equivalent between the lots. Our data support the use of mixtures of PRP-T and DPT vaccines but underscore the need for additional studies of compatibility between different lots of both products. The effect of booster doses at 18 months will also be relevant. Compatibility of PRP-T vaccine combined with DT vaccine or DPT-IPV (inactivated poliovirus vaccine) has not been determined using Canadian products.

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