SAFETY AND IMMUNOGENICITY OF A *HAEMOPHILUS INFLUENZAE* TYPE B POLYSACCHARIDE -TETANUS TOXOID CONJUGATE VACCINE COMBINED WITH DIPHTHERIA, TETANUS AND PERTUSSIS VACCINES IN THAI INFANTS

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Abstract. A randomized, open, multicenter trial was conducted to determine the safety and immunogenicity of a *Haemophilus influenzae* type b polysaccharide-tetanus toxoid (PRP-T) conjugate vaccine combined with tetanus, diphtheria and pertussis (DTP) vaccine in 271 Thai infants born to mothers immunized against tetanus during pregnancy. Infants were immunized at approximately 2, 4 and 6 months of age with these vaccines. To determine if elevated levels of anti-tetanus toxin antibodies suppressed the anti-PRP antibody response, a second group of infants were immunized with PRP complexed with outer membrane proteins of *Neisseria meningitidis* (Pedvax HIB®) in one limb at 2 and 4 months of age and DTP vaccine in the other limb at 2, 4 and 6 months of age. A third group of infants received only DTP vaccine at 2, 4 and 6 months of age. The occurence of both local and systemic adverse reactions were comparable in all 3 groups. The geometric mean anti-tetanus antibody titer was > 1 IU/ml at baseline. Approximately 1 month after the administration of the third dose of vaccine, 98.5%, 99.3% and 9.7% of the children immunized with DTP+Pedvax HIB®, DTP-PRP-T or DTP possessed ≥ 0.15 μg of anti-PRP antibody per ml. No child in the DTP group achieved ≥1 μg/ml while 74.2% and 89.3% did so after immunization with DTP+Pedvax HIB®, or DTP-PRP-T, respectively ($p < 0.05$). Immune responses to diphtheria, tetanus and pertussis antigens were similar in all vaccine groups. These results demonstrate that elevated tetanus antibody titers do not diminish the anti-PRP antibody response following immunization with a PRP-T conjugate combined with DTP vaccine.

INTRODUCTION

Invasive disease caused by *Haemophilus influenzae* type b (Hib) is a major cause of morbidity and mortality among infants and young children throughout the world. (Funkhouser *et al*, 1991; Bijlmer *et al*, 1990; Wright, 1989; Munson *et al*, 1992). It is the most frequent cause of bacterial meningitis in Thai infants less than 2 years of age (Chotpitayasunondh, 1994; Lohleka, 1991) and can also cause bacteremia, pneumonia, cellulitis, epiglotitis and septic arthritis (Shapiro and Ward, 1991; Broome, 1987). However, for some unknown reason epiglottitis is a rarely caused by Hib in less developed countries. The recent introduction of routine immunization of infants against Hib in many developed countries have shown a substantial decline in the incidence of disease (Adams *et al*, 1993; Vadheim *et al*, 1994; Santosham *et al*, 1992; Peltola *et al*, 1992). Many clinical trials have demonstrated that a series of two or three immunizations with conjugate vaccines containing polyribosylphosphate (PRP) protects young infants against invasive Hib disease. (Black *et al*, 1991; Santosham *et al*, 1991; Eskola *et al*, 1990). The most recently licensed Hib conjugate vaccine consists of PRP covalently coupled to tetanus toxoid (PRP-T) (Fritzell *et al*, 1992; Decker *et al*, 1990; Watemberg *et al*, 1991; Ferreccio *et al*, 1991; Greenberg *et al*, 1994). For reasons of practicality, it is important to co-administer PRP-T in the same syringe with diphtheria-tetanus-pertussis (DTP) vaccines to facilitate immunization programs, especially in developing areas. (Avandano *et al*, 1993). The peak incidence of invasive Hib infection is approximately 9 months of age in the United States and many European countries but earlier, (5-6 months), in developing countries where the incidence is also high. (Munson *et al*, 1992; Chotpitayasunondh, 1994; Lohleka, 1991; Bijlmer, 1991; Makala *et al*,

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1992).

We therefore undertook an open, randomized, comparative multicenter study in Thai infants to : (1) determine the safety and immunogencity of an experimental *Haemophilus influenzae* type b PRP-T conjugate vaccine combined with DTP vaccine; (2) to compare the immune response engendered with a licensed *Haemophilus influenzae* type b PRP- outer membrane protein vaccine (PRP-OMP, Pedvax HIB®, Merck, Sharp and Dohme) and diphtheria-tetanus-pertussis vaccine, and (3) to evaluate whether elevated levels of anti-tetanus antibodies present at baseline, the result of routine immunization of pregnant women against tetanus, would suppress the anti-PRP antibody reponse elicited by the PRP-T vaccine via epitopic suppression.

MATERIALS AND METHODS

Ethical approval

The study was conducted at the Pediatric Department of Udon Thani Hospital, Udon Thani Province and Children's Hospital, Bangkok from February 1994 to December 1995. The study was approved by the Thai Ministry of Public Health Ethical Review Committee and informed consent was obtained from the parents or guardian before enrolment.

Subjects

Healthy 2-month-old infants (1.4-2.9 months of age) with no prior history of immunization against diphtheria, tetanus, pertussis or Hib were eligible for enrolment. All children were examined by the study physicians before each immunization to ensure that no contraindications to vaccination existed. Exclusion criteria included the following: acute febrile illness, a neurological or developemental disorder, history of allergies, treatment with immunosuppressive drugs, immunodeficiency syndrome, significant systemic illness, immunoglobulin therapy, plasma or whole blood transfusion since birth and participation in another clinical trial. All mothers of these infants were vaccinated with 2 doses of tetanus toxoid during pregnancy which is recommended in Thailand to prevent neonatal tetanus.

Vaccines

Each 0.5 ml dose of DTP vaccine (DiTePer Berna™, Swiss Serum and Vaccine Institute, Berne, Switzerland; Lot NO. 12410549) contained 25 Lf of diphtheria toxoid; 10 Lf of tetanus toxoid and 4 IU of *Bordetella pertussis* antigen per dose. The vaccine was adsorbed to aluminum phosphate 0.4% wt/vol and contained 0.01% thimerosal. Each 0.5 ml dose of DiTePer-Hib-T vaccine (Swiss Serum and Vaccine Institute, Berne, Switzerland; Lot NO. PM793008) contained 25 Lf of diphtheria toxoid; 5 Lf of tetanus toxoid, 4 IU of *Bordetella pertussis*; 10 μg of PRP covalently coupled to approximately 10 μg (equal to ~ 5 Lf) of tetanus toxoid. The vaccine was in liquid form adsorbed to aluminum phosphate and packaged in a ready to use syringe. Each 0.5 ml dose of Pedvax HIB® vaccine (Merck, Sharp and Dohme, West Point, PN) contained 15 μg of PRP complexed to *Neisseria meningitidis* outer membrane proteins adsorbed to aluminum hydroxide.

Vaccination regimens

At Children's Hospital, Bangkok, infants were randomized to recieve either 3 doses of DTP vaccine (as control) or DTP-PRP-T vaccine at approximately 2, 4, 6 months of age. At Udon Thani Hospital, infants were randomized to receive either 3 doses of DTP-PRP-T vaccine or 3 doses of DTP concurrently with PedvaxHIB® (a single injection at 2 and 4 months of age according to the manufacturer's recommendation). The two vaccines were given in different limbs. All vaccines were given intramuscularly in the upper thigh muscle. The infants were observed for 30 minutes following each immunization to monitor for immediate type reactions. Local and systemic reactions were recorded at 6 hours 1, 2, 3, 4, 5, 6 and 7 days after each of the three vaccinations by the parents on a standard adverse reaction report form. Parents were specifically requested to note the following symptoms; redness, induration and swelling at the injected site, fever (> 37.5°C), excessive crying, anorexia, vomiting, convulsion and others symptoms which could be associate with immunization. All subjects were concurrently administered oral polio vaccine at age 2, 4, 6 months and the third dose of hepatitis B vaccine at 6 months of age.

Serological testing

Venous blood sample (2-3 ml) were taken immediately prior to the first immunization and 1 month after the third immunization (at approximately 7 months of age). The serum was collected, labeled and frozen until tested for antibody levels in a blinded manner. Anti-diphtheria toxin, anti-tetanus toxin, anti-pertussis toxin and anti-*Bordetella pertussis* filamentous hemagglutinin (FHA) IgG antibody titers were determined by ELISA. Tetanus and diphtheria antibody levels were reported as international units (IU)/ml by comparison to a reference serum. Anti-pertussis toxin and anti-FHA IgG antibody levels were reported as μg of specific immunoglobulin G (IgG antibody)/ml. Total anti-PRP antibody was quantitated using a Farr-type radioimmunoassay (Anderson, 1984) with intrinsically labeled 3H-PRP supplied by University of Rochester, Rochester, NY. The results are expressed in μg/ml. A reference serum with a known amount of anti-PRP antibodies was included as a control for each series of samples tested.

Statistical analysis

The statistical analysis of adverse reactions was performed as follows. Comparison between groups was performed by Bartlett's test for homogenicity of variance, Student's *t*-test and Kruskal-Wallis test (equivalent to Chi-square test). Differences between geometric mean titers (GMT) were determined by the Wilcoxon-Mann-Whitney-U test while seroconversion rates were compared by Chi-square analysis.

RESULTS

A total of 326 infants were enrolled from both study sites. Of these, 271 (83%) completed the 3 dose immunization regimen and returned adverse reaction report forms while 268 (82%) provided paired serum samples. The reasons for failing to complete the protocol were as follows: 1) immunization with DTP vaccine at local health clinic, 2) families who moved away from the trial areas, or 3) unwillingness of the parents to have the second blood sample drawn. There were no drop outs due to severe adverse reactions. There were no significant differences between gender, age and weight between the study groups either at baseline or subsequent vaccinations.

Adverse events associated with immunization are shown in Table 1. The frequency and severity of local reactions were comparable between groups with the exception of redness and induration which was significantly ($p < 0.05$) higher only after the first immunization with the DTP-PRP-T combined vaccine. Similarly, there was little difference between the rate of systemic events elicited between the 3 vaccines except for low-grade fever (≥37.5°C) which occurred more frequently in the group immunized with DTP alone. Six episodes of febrile seizures were reported within 24 hours of immunization, 1 in the DTP group, 1 in the DTP+Pedvax HIB® group and 4 in the group immunized with DTP-PRP-T. However, only one child was brought in for clinical observation. For all 6 children, there was no evidence of any neurological sequelae upon subsequent examinations.

The anti-PRP antibody response engendered by vaccination is shown in Table 2. There were no significant differences ($p > 0.05$) in baseline anti-PRP antibody levels between the 3 groups. As would be expected, there was a substantial decline in anti-PRP antibody levels in the group immunized with DTP only reflecting a decrease in maternal antibody. The percentage of infants who attained ≥ 0.15 μg/ml of anti-PRP antibody was 99.3% and 98.5% after immunization with DTP-PRP-T or DTP+Pedvax HIB®, respectively ($p > 0.05$). The percent who achieved ≥ 1 μg/ml was 89.3% and 74.2%, ($p < 0.05$), after vaccination with DTP-PRP-T or DTP+PedvaxHIB®, respectively. The geometric mean anti-PRP antibody titer was significantly higher ($p < 0.05$) for those infants immunized with DTP-PRP-T versus DTP+PedvaxHIB® (5.6 versus 2.1 μg/ml, respectively).

The humoral immune responses to the diphtheria, tetanus and pertussis vaccine components are shown in Table 3. There were no significant differences ($p > 0.05$) in baseline values for any antibody measured. Most infants possessed protective levels of anti-tetanus antibodies (≥ 0.1 IU/ml) at baseline reflecting the fact that 2 doses of tetanus toxoid are routinely administered to mother during pregnancy in Thailand. All 3 vaccine components engendered a vigorous antibody response. After immunization, all infants possessed protective levels of anti-tetanus toxin antibodies while virtually all had protective levels of anti-diphtheria toxin antibodies.

Table 1

Summary of adverse events associated with immunization[1].

Symptom	%Reporting reaction								
	1st dose			2nd dose			3rd dose		
	DTP+Pedvax HIB®	DTP-PRP-T	DTP	DTP+Pedvax HIB®	DTP-PRP-T	DTP	DTP+Pedvax HIB®	DTP-PRP-T	DTP
Local	22.1	35.7[2]	23.8	26.4	24.3	20.6	23.5	30.7	25.4
Redness (≥ 1cm)									
Swelling (≥ 1cm)	32.3	43.6	38.1	29.4	34.3	23.8	36.8	37.1	34.9
Induration (≥ 1cm)	30.9	49.3[2]	39.7	30.9	41.4	28.6	41.2	35	30.2
Systemic									
Fever ≥ 37.5°C	33.8	35	57.1[3]	38.2	34.3	46	25	43.6	50.8[4]
≥ 39°C	8.8	2.9	6.3	7.3	3.6	6.3	2.9	5.0	6.3
Fussiness	61.8	66.4	73	70.6	64.3	68.2	57.3	65.7	76.1
Anorexia	20.6	25.7	22.2	41.2	25	38.1	30.9	31.4	38.1
Crying	60.3	64.2	73	69.1	60.7	63.5	50	60	65.1
Pain	54.4	66.4	60.3	58.8	57.1	53.6	51.5	57.1	52.4
Nausea/vomiting	17.6	18.6	23.8	20.6	14.3	17.5	13.2	16.4	17.5
Seizure	0	1.4	1.6	1.5	0.7	0	0	0.7	0

[1] Represents data for the 271 infants (DTP+Pedvax HIB®; N = 68, DTP-PRP-T; N = 140, DTP; N = 63) who completed the 3 doses vaccination regimen.
[2] $p < 0.05$ for DTP-PRP-T versus DTP+Pedvax HIB® and DTP.
[3] $p < 0.05$ for DTP versus DTP+Pedvax HIB® and DTP-PRP-T
[4] $p < 0.05$ for DTP versus DTP+Pedvax HIB®

Table 2

Anti-*H. influenzae* type b PRP antibody response following immunization.

Vaccine	Baseline			7 months[1]		
	GMT	% ≥ 0.15 μg/ml	% ≥ 1.0 μg/ml	GMT	% ≥ 0.15 μg/ml	% ≥ 1.0 μg/ml
DTP (N = 62)	0.15	46.6	8.1	0.07	9.7	0
DTP-PRP-T (N = 140)	0.14	43.6	6.4	5.6[2]	99.3	89.3[2]
DTP+Pedvax HIB® (N = 66)	0.14	37.9	6.1	2.1	98.5	74.2

[1] Infants were immunized with DTP and DTP-PRP-T at 2, 4 and 6 months of age, or with DTP+Pedvax HIB® at 2 and 4 months of age and DTP at 6 months of age. Blood was obtained immediately before the first vaccination, and 1 months after the third immunization.
[2] $p < 0.05$ versus DTP+Pedvax HIB®

Table 3

Anti-diptheria toxin, anti-tetanus toxin, anti-pertussis toxin, anti-FHA and pertussis agglutinating antibody response after immunization.[1]

Antibody	Vaccine	Baseline		7 months	
		GMT	% ≥ 0.1 IU/ml	GMT	% ≥ 0.1 IU/ml
Anti-Diphtheria toxin (IU/ml)	DTP+Pedvax HIB®	0.06	21.2	3	100
	DTP-PRP-T	0.06	29.3	2.6	100
	DTP	0.05	21	1.9	95.2
Anti-Tetanus toxin (IU/ml)	DTP+Pedvax HIB®	1.1	92.4	7.1	100
	DTP-PRP-T	1.3	95	7.1	100
	DTP	1.1	97	9.4	100
Anti-Pertussis toxin (μg IgG/ml)	DTP+Pedvax HIB®	1.34	NA	6.2	NA
	DTP-PRP-T	1.78	NA	7	NA
	DTP	1.33	NA	7.7	NA
Anti-FHA (μg IgG/ml)	DTP+Pedvax HIB®	0.5	NA	0.91[2]	NA
	DTP-PRP-T	0.5	NA	1.51	NA
	DTP	0.5	NA	1.72	NA
Pertussis agglutinating antibody (μg IgG/ml)	DTP+Pedvax HIB®	10.5	NA	262.1	NA
	DTP-PRP-T	10.5	NA	211.1	NA
	DTP	10.5	NA	185	NA

[1] see footnote for Table 1

[2] $p < 0.05$ DTP-PRP-T and DTP versus DTP+Pedvax HIB®

There were no significant differences between groups subsequent to vaccination. Immunization engendered a significant ($p < 0.05$) rise in antibody levels to all three *B. pertussis* antigens measured. The geometric mean anti-FHA antibody level was significantly lower ($p < 0.05$) for the group immunized with DTP+Pedvax HIB®.

DISCUSSION

The inclusion of Hib conjugates into primary DTP immunization regimens has resulted in a dramatic decrease of invasive disease, especially meningitis (Micheals *et al*, 1993; Anonymous, 1995; Shinefield and Black, 1995). To date, such vaccines have seen little use in developing countries. This is due to several factors including cost and, until very recently, an unknown disease incidence. It is now generally accepted that Hib disease is also a significant cause of infant and childhood illness in developing areas of the world. The peak incidence of Hib disease occurs at a somewhat earlier age in developing countries. While the precise reason for this is not known, we observed in the current study that, by 7 months of age, fully 90% of infants not immunized against Hib possessed non-protective levels of anti-PRP antibody.

Several Hib conjugate vaccines are currently licensed. To render the PRP immunogenic in infants, a variety of carrier proteins, including diphtheria toxoid, *Corynebacterium diphtheriae* CRM^{197}, tetanus toxoid, and *Neisseria meningitidis* outer membrane proteins, are used (Decker *et al*, 1990; Granoff *et al*, 1992; Greenberg *et al*, 1995; Capeding *et al*, 1996; Mulholland *et al*, 1993; Lenoir *et al*, 1987). One specific concern regarding the use of tetanus toxoid-PRP conjugates in developing countries is

the fact that pregnant women are often immunized to prevent neonatal tetanus, so that, infants born to these mothers have very high levels of anti-tetanus toxin antibodies which may reduce the anti-PRP immune response through epitopic suppression (Barington *et al*, 1994; Claesson *et al*, 1989). Therefore, in the present study we compared the anti-PRP antibody response engendered by conjugate vaccines employing either tetanus toxoid or *N. meningitidis* outer membrane proteins as carrier entities administered simultaneously with DTP vaccine. A group of infants who recieved only DTP vaccine was included to determine if combining the PRP-T conjugate in the same syringe as DTP vaccine would influence the immune response to diphtheria toxoid, tetanus toxoid and various pertussis antigens.

No evidence was seen to suggest that elevated anti-tetanus toxin antibody levels, present at the time of initial immunization, suppressed the anti-PRP immune response elicited by the PRP-T conjugate vaccine. Similar results have been recently reported in Finnish children with high preimmunization anti-tetanus toxin antibodies concentration and immunized with PRP-T vaccine at 1-2 months of age. (Kurikka *et al*, 1996). After immunization with either vaccine according to the manufacturers recommended schedule, > 98% of infants attained ≥ 0.15 μg/ml of anti-PRP antibody. However, both the geometric mean anti-PRP antibody titer and the percentage of infants reaching ≥ 1.0 μg/ml of anti-PRP antibody was significantly ($p < 0.05$) higher for the group immunized with DTP-PRP-T versus DTP+PedvaxHIB®. Similar results have been reported elsewhere. (Decker *et al*, 1990; Greenberg *et al*, 1995; Capeding *et al*, 1996).

The immune response to the diphtheria and tetanus vaccine components was comparable in all 3 study groups. This was also found to be the case for anti-pertussis toxin and anti-*B. pertussis* agglutinating antibody levels. However, the geometric mean anti-FHA antibody titer was significantly lower in infants immunized with DTP+Pedvax HIB® compared to DTP alone or combined with PRP-T.

The overall rate of local and systemic reactions observed were similar to what has previously been reported with comparable vaccine combinations (Watemberg *et al*, 1991; Ferreccio *et al*, 1991; Avandano *et al*, 1993; Kaplan *et al*, 1994; Paradiso *et al*, 1993). The fact that redness and induration occurred more frequently following the first dose of DTP-PRP-T can be attributed to the fact that 21 infants inadvertently were given the first dose subcutaneously. Virtually all of these subjects presented with redness and induration. While several episodes of seizure were reported, only 1 child was seen by a physician. There were no sequelae associated with these events.

In conclusion, immunization of infants with elevated anti-tetanus toxoid antibody titers did not suppress that anti-PRP antibody response following immunization with a PRP-T Hib conjugate vaccine. Also, this study confirms the safety and immunogenicity of combined DTP-Hib conjugated vaccine administered simultaneously in the same syringe. Studies to expand the valency by the addition of hepatitis B and inactivated polio vaccines are underway. Hopefully, such vaccines will improve vaccine utilization and coverage in developing areas of the world by simplifying vaccination regiments and reducing costs.

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