IMMUNOGENICITY AND SAFETY OF A DTaP-IPV//PRP~T VACCINE (PENTAXIM™) BOOSTER DURING THE SECOND YEAR OF LIFE IN THAI CHILDREN PRIMED WITH AN ACELLULAR PERTUSSIS COMBINED VACCINE

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Abstract. This study assessed the booster immune response to a pentavalent combination vaccine containing diphtheria, tetanus, acellular pertussis, inactivated poliovirus, and conjugated-Hib polysaccharide antigens, (DTaP-IPV//PRP~T, Pentaxim™, an AcXim family vaccine) at 18-24 months of age. Study subjects received a three-dose primary vaccination at 2, 4 and 6 months with a hexavalent vaccine containing the same antigens plus recombinant hepatitis B surface antigen. Antibody concentrations were measured immediately before and one month after vaccination. Reactogenicity and safety were evaluated from parent reports. Before the booster dose, 92.9% of the 156 children included in this study still had anti-PRP antibody titers ≥0.15 μg/ml. Seroprotective concentrations of anti-diphtheria, tetanus and poliovirus antibodies were maintained in 97 to 100% of subjects in the interval between primary and booster vaccination. One month after the booster dose, all subjects had seroprotective anti-PRP (≥1 μg/ml), diphtheria and tetanus (≥0.1 IU/ml) and poliovirus types 1, 2, 3 (≥8 1/dil) antibody levels. At least 92.3% of subjects had 4-fold increases in concentrations of anti-pertussis antigens from pre- to post-booster dose. Geometric mean titers (GMTs) increased from 3.8 to 181.2 EU/ml and from 18.0 to 289.7 EU/ml for anti-PT and anti-FHA, respectively. The anti-PRP GMT increased from 1.6 to 58.0 μg/ml. The pentavalent DTaP-IPV//PRP~T vaccine booster was well tolerated and highly immunogenic, following primary vaccination with a hexavalent vaccine.

INTRODUCTION

Vaccines combining inactivated whole cell *Bordetella pertussis* antigens with diphtheria and tetanus toxoids (DTwP) first licensed during the 1940's, have been used for over 60 years and have been central to the WHO expanded program of immunization (EPI) since its inception in 1974. The EPI originally aimed to protect children against six diseases, tuberculosis, diphtheria, neonatal tetanus, whooping cough, poliomyelitis and measles. Since then other vaccines have been added, including hepatitis B (HB) and *Haemophilus influenzae* type b (Hib). The valences included in combination vaccines reflect current EPI recommendations and can also be adjusted to take into account differences in burden of disease(s) in particular countries, for example hepatitis B, where there is increased risk of transmission from mother-to-child at birth.

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Concerns about the safety and reactogenicity of whole-cell pertussis vaccines, reduced vaccination coverage and increases in disease incidence in some countries, stimulated the development of acellular pertussis (aP) vaccines (Hewlett and Cherry, 1997; Edwards and Decker, 2008). Acellular formulations containing purified *B. pertussis* antigens have been developed that are better tolerated than DTwP combination vaccines, not only for primary vaccination but also for booster vaccinations, and provide similar immunogenicity (Cherry *et al*, 1988; Eglund *et al*, 1994; Decker *et al*, 1995; Edwards *et al*, 1995; Pichichero *et al*, 1997). The clinical protective efficacy of the licensed acellular pertussis vaccines have been documented in a number of controlled trials (Cherry, 1997; Hewlett and Cherry, 1997; Simondon *et al*, 1997; Edwards and Decker, 2008). Currently, acellular pertussis vaccines are generally included in combined vaccines including inactivated poliovirus (IPV), Hib conjugate vaccine and/or HB antigens, and are included in national immunization programs in North America, most western European countries, some Asian countries, *eg*, Japan, South Korea and Australia, and more recently in Mexico and Turkey (MMWR, 1997; Therre and Baron, 2000; WHO, 2005; PHAC, 2006; AAP, 2008).

The WHO position on pertussis vaccines is that "The best aP vaccines have shown similar protective efficacy as the best wP vaccines, and all licensed vaccines have proved to be highly effective in controlling pertussis in infants and young children". (WHO, 2005). Booster vaccination during the second year of life is recommended and practiced in many countries, as the duration of protection against common childhood infectious diseases, including pertussis and Hib wanes over time. According to the WHO, improved control and further decline in incidence of these diseases will require supplementary immunization activities including the administration of additional doses of vaccine (WHO, 1997, 2005).

Furthermore, a resurgence of invasive Hib disease among young children has been seen in countries, including the United Kingdom, Ireland and Chile, where only 3 doses of Hib vaccine were administered in the primary series. The most important factor has been identified as the lack of booster vaccination during the second year of life. As a result, the United Kingdom and Ireland now recommend a booster dose of Hib vaccine during the second year of life, and Chile is considering implementing a booster at 12 months (Fitzgerald *et al*, 2005; Cameron and Pebody, 2006; Cruces *et al*, 2006; Johnson *et al*, 2006).

This study evaluated the immunogenicity and safety of a pentavalent acellular pertussis-based combined vaccine, DTaP-IPV//PRP~T (Pentaxim™, an AcXim family vaccine), given as a booster at 18-24 months of age in children primed at 2, 4 and 6 months of age with a hexavalent combined vaccine containing the same antigens plus a recombinant hepatitis B virus surface antigen. Antibody persistence approximately one year after the primary vaccination is also reported.

MATERIALS AND METHODS

Study design and subjects

This open, single center clinical study enrolled subjects at the Chulalongkorn Hospital, Bangkok, Thailand. The study protocol and informed consent form were approved by the institutional review board of the participating center before initiation of the study. This study was conducted according to local regulations, Good Clinical Practice (GCP) and applicable International Conference on Harmonization (ICH) guidelines, and conformed to the ethical principles of

the Declaration of Helsinki, as amended at the time the study was conducted. Written informed consent was obtained from a parent or legally acceptable representative of all subjects before study enrolment.

Healthy children between the ages of 18 and 24 months were eligible if they could comply with the study criteria and timetable. All children included in this study received a single dose of pentavalent vaccine, Pentaxim, described below, at 18-24 months of age following the Thai national recommendation for booster vaccination (MOPH Thailand, 2008; WHO, 2008a). Children were recruited from a group that had received a birth dose of recombinant hepatitis B monovalent vaccine followed by a three-dose primary vaccination at 2, 4 and 6 months of age with a hexavalent vaccine (Hexavac™, sanofi pasteur) containing the same vaccine antigens as the pentavalent study vaccine plus a recombinant hepatitis B virus surface antigen. All of the 262 children who completed the primary vaccination studies were invited to participate in this booster dose study.

Children were excluded from the study if they had: a known or suspected disease of the immune system or other serious illness including neurological disorders, seizures or coagulopathy; fever ≥37.1ºC; a previous booster vaccination against diphtheria, tetanus, pertussis, hepatitis B, Hib infection or poliomyelitis (except for oral poliomyelitis vaccine which was given during a national immunization day campaign before the start of the study). Children were also excluded if they had a history of diphtheria, tetanus, pertussis, Hib infection or poliomyelitis; received any unregistered drug or vaccine in the 30 days before entering the study; had received immunosuppressive therapy, immunoglobulins or any blood products within the previous 14 days or planned to take any such products; had a history of allergic reaction, encephalopathy, hypotonic-hyporesponsive episodes, fever (≥40ºC rectal or ≥39.1ºC axillary) within seven days, seizures or other neurological disorders after previous vaccination against diphtheria, tetanus or pertussis; or were enrolled in another clinical trial.

Vaccines and vaccine administration

The study vaccine, batch number X0364-1, was produced and supplied by Sanofi Pasteur, Lyon, France. Each 0.5 ml dose of vaccine contains ≥30 IU (25Lf) of diphtheria toxoid, ≥40 IU (10Lf) of tetanus toxoid, 25 μg of pertussis toxoid (PT), 25 μg of filamentous hemagglutinin (FHA), 40 D antigen units (DU) of poliovirus type 1 (Mahoney), 8 DU of poliovirus type 2 (MEF-1), 32 DU of poliovirus type 3 (Saukett), and 10 μg polyribosyl-ribitol-phosphate (PRP) conjugated to tetanus protein. The lyophilized PRP~T component was reconstituted with the liquid DTaP-IPV vaccine immediately before vaccination. The vaccine was administered intramuscularly into the left anterolateral external aspect of the upper thigh. This vaccine is licensed as Pentaxim in Thailand, and in European and other countries throughout the world as Pentavac™ or Pentaxim.

Serology

Blood samples for antibody determination were collected from each subject at 18-24 months of age just before the booster vaccination and one month (30 to 42 days) after the booster. Serologic analyses were done at the Sanofi Pasteur central laboratory in Swiftwater, Pennsylvania, USA. Anti-Hib PRP polysaccharide was measured by a Farr-type radioimmuno assay (RIA) and compared with the American Food and Drug Administration human reference with a lower limit of quantification (LLOQ) of 0.06 μg/ml. Anti-tetanus toxoid was measured by enzyme-linked immunosorbent assay (ELISA)

and compared with the WHO TE3 human standard with a LLOQ of 0.01 IU/ml. Anti-diphtheria toxoid and anti-FHA and anti-PT antibodies were measured by ELISA. Anti-poliovirus was evaluated by serum neutralization following a WHO standardized procedure (*WHO/EPI/GEN 93.9*) with a LLOQ of 4 (1/dil). Predefined seroprotection (SP) levels are an anti-PRP ≥ 0.15 μg/ml, anti-polio ≥ 8 (1/dil); anti-diphtheria and anti-tetanus ≥ 0.01, and 1.0 IU/ml, respectively. Since there are no accepted standards for seroprotection with pertussis antibodies, a good vaccine responses for anti-pertussis antigens was defined as ≥ 4-fold increase in antibody titer from pre- to post-booster vaccination.

Reactogenicity and safety

All subjects who received the study vaccine were included in the reactogenicity and safety evaluation. Children were sent home following a 30-minute observation period to monitor for immediate adverse reactions. Parents or guardians recorded the onset, intensity and duration of a set of solicited local reactions (pain, redness, swelling, induration) and systemic adverse events (fever, unusual drowsiness, irritability) on diary cards daily for the next 8 days. Systemic events were also evaluated for relationship to the vaccination. Other adverse events that occurred between then and the second study visit at 30 to 42 days were also recorded. Serious adverse events were reported throughout the study. Mild, moderate or severe pain was defined as "reacts when injection site is touched", "cries when injection site is touched", and "cries when the leg of the injection site is moved", respectively. For erythema and swelling, a diameter of 2-5 cm was graded as mild, a diameter of 5-7 cm as moderate and a diameter of >7 cm was graded as severe. Mild, moderate and severe fever were defined as a rectal temperature of 38 to 38.9ºC, 39 to 39.9ºC, and ≥ 40ºC, respectively.

Statistical analysis

Seroprotection and vaccine response rates with their corresponding 95% confidence intervals (CI) were calculated for each vaccine antigen using the exact binomial method for the study group. Geometric Mean Titers (GMTs) were calculated with their corresponding 95% CIs using the normal approximation. Reverse Cumulative Distribution Curves (RCDCs) for pre- and post vaccination antibody titers were also calculated for each antigen and time point. Statistical analysis was descriptive only. The numbers and the percentages of subjects experiencing any solicited local reactions or systemic adverse events (AE) were recorded. Systemic events were also evaluated for relationship to the vaccination.

RESULTS

Subject disposition

A total of 156 children from 18 to 24 months of age were enrolled to receive a booster dose of the study vaccine from June 2004 to October 2004. All children had completed a three-dose primary vaccination at 2, 4 and 6 months of age with a hexavalent combined vaccine containing the same antigens plus a recombinant hepatitis B virus surface antigen. Slightly more male (55.1%) than female children (44.9%) were included; the mean (±SD) age was 21.9 (±1.0) months. The full analysis set (FAS) included all 156 children. One subject had received a previous booster vaccination and two children had their second blood sample taken outside the specified 30 to 42 days after booster vaccination, leaving a per protocol set of 153 subjects.

Immunogenicity

Seroprotection, and vaccine response rates and GMTs at 1 month after primary

Table 1
Seroprotection and vaccine response rates for each antigen at post-primary, pre-booster and post-booster vaccination.

	Post-primary vaccination	Pre-booster vaccination	Post-booster vaccination
Criteria	Rate in % (95% CI)	Rate in % (95% CI)	Rate in % (95% CI)
Anti-PRP ≥ 0.15 μg/ml	100 (97.7-100)	92.9 (87.7-96.4)	100 (97.7-100)
Anti-PRP ≥ 1 μg/ml	93.6 (88.5-96.9)	62.8 (54.7-70.4)	100 (97.7-100)
Anti-Diphtheria ≥ 0.01 IU/ml	100 (97.6-100)	97.4 (93.6-99.3)	100 (97.7-100)
Anti-Diphtheria ≥ 0.1 IU/ml	100 (97.6-100)	51.3 (43.2-59.4)	100 (97.7-100)
Anti-Tetanus ≥ 0.01 IU/ml	100 (97.6-100)	100 (97.6-100)	100 (97.6-100)
Anti-Tetanus ≥ 0.1 IU/ml	100 (97.6-100)	100 (97.6-100)	100 (97.6-100)
Anti-Polio 1 ≥ 8 1/dil	100 (96.7-100)	98.2 (93.5-99.8)	100 (97.7-100)
Anti-Polio 2 ≥ 8 1/dil	100 (96.7-100)	99.1 (94.9-100)	100 (96.6-100)
Anti-Polio 3 ≥ 8 1/dil	100 (96.7-100)	98.1 (93.3-99.8)	100 (96.6-100)
Anti-PT 4-fold increase	99.3 (96.4-100)	NA	99.2 (95.4-100))
Anti-FHA 4-fold increase	99.3 (96.2-100)	NA	92.3 (86.9-95.9)

Table 2
Geometric Mean Titers (GMTs) for each antigen at post-primary, pre-booster and post-booster vaccination.

	Post-primary vaccination	Pre-booster vaccination	Post booster vaccination
Criteria	GMT (95% CI)	GMT (95% CI)	GMT (95% CI)
Anti-PRP μg/ml	11.31 (9.0-14.1)	1.6 (1.3-2.1)	58.0 (47.7-70.4)
Anti-Diphtheria IU/ml	1.6 (1.4-1.8)	0.09 (0.0-0.1)	2.7 (2.4-3.2)
Anti-Tetanus IU/ml	2.4 (2.2-2.6)	0.4 (0.4-0.5)	6.2 (5.7-6.9)
Anti-Polio 1 (1/dil)	1,292.8 (1,022.5-1,634.6)	175.9 (133.7-231.4)	3,138.25 (2,696.5-3,652.2)
Anti-Polio 2 (1/dil)	2,041.5 (1,623.6-2,567.0)	433.3 (315.6-594.8)	5,121.7 (4,329.4-6,059.1)
Anti-Polio 3 (1/dil)	3,320.6 (2,717.0-4,058.3)	289.2 (210.5-397.4)	9,490.4 (7,402.0-12,168.2)
Anti-PT EU/ml	101.2 (93.4-109.6)	3.8 (3.2-4.5)	181.2 (159.4-205.9)
Anti-FHA EU/ml	210.6 (192.5-230.4)	18.0 (15.4-20.9)	289.7 (261.4-321.0)

vaccination, just before the booster at 18 to 24 months of age and 1 month after the booster are summarized in Tables 1 and 2. Following primary vaccination, all children had seroprotection against diptheria and tetanus (antibody titer ≥ 0.1 IU/ml), polio (anti-polio ≥ 8 (1/dil) and Hib (anti-PRP ≥ 0.15 μg/ml). Vaccine response rates were 99.3% for both PT and FHA. The children participating in this study had thus been successfully primed. As expected, antibody titers decreased in the interval between completion of the primary series and the booster, and a strong anamnestic response occurred in the month following booster administration.

Table 3
Incidence of solicited local and systemic symptoms within 8 days after booster dose of the DTacP-IPV// Hib combined vaccine (Pentaxim™) given at 18-24 months of age.

		DTaP-IPV//PRP~T *N* = 156	
Local adverse reaction		*n*	%
Pain	Any	74	47.4
	Severe	2	1.3
Redness	Any	18	11.5
	Severe	0	0.0
Swelling	Any	15	9.6
	Severe	0	0.0
Induration	Any	7	4.5
	Severe	0	0.0
Systemic adverse event			
Fever[a]	Any	45	28.8
	Severe	5	3.2
	Related	38	24.4
Unusual drowsiness	Any	29	18.6
	Severe	1	0.6
	Related	27	17.3
Irritability	Any	49	31.4
	Severe	3	1.9
	Related	47	30.1

N, number of injected subjects with available safety data; *n*, number of subjects with a specific adverse event; %, percentage of subjects with a specific adverse event; [a]any, rectal temperature ≥38ºC; [a]severe, rectal temperature >40ºC

Approximately 1 year after primary vaccination, 92.9% of subjects retained anti-PRP titers ≥0.15 μg/ml and 1 month after the booster, all subjects had anti-PRP ≥1 μg/ml. GMT increased from 1.69 μg/ml before the booster to 58.0 μg/ml after the booster injection, a 34-fold increase. RCDC curves for pre- and post-booster anti-PRP antibody titers show a strong increase in response to the vaccination (Fig 1a).

With regard to the pertussis antigens, 94.8% of subjects had detectable anti-FHA and 52.1% had detectable anti-PT antibody titers of ≥5 EU/ml before the booster. One month after the booster injection, all children had anti-PT and FHA titers ≥25 EU/ml. Vaccine response rates (≥4-fold increase in antibody titer) were 99.2% for PT and 92.3% for FHA (Table 1). Ratios of post- to prebooster GMT (GMTR) were 48 for PT and 16 for FHA. The GMTs are shown in Table 2. RCDCs showed a strong increase in pertussis antibody titers post-booster vaccination (Fig 1b and 1c). Seroprotection rates for anti-diphtheria and anti-tetanus antibody (titers ≥ 0.01 IU/ml) before the booster dose were 97% and 100%, respectively. All children had post-booster anti-D and anti-T antibody titers ≥ 0.1 IU/ml. Anti-diphtheria and anti-tetanus GMTs increased, from 0.1 to 2.8 IU/ml and from 0.5 to 6.3 IU/ml, respectively.

A total of 46 children included in the study received one or two doses of OPV during a national poliomyelitis immunization day (NID) campaign that was conducted by the health authorities in Thailand in 2004. Just before the booster, 98.2, 99.1, and 98.1% of the 110 subjects who received only the DTaP-IPV//PRP~T vaccine were still seroprotected against poliovirus types 1, 2, and 3, respectively. All children who received only the study vaccine had anti-polio 1, 2 and 3 antibody titers ≥8 1/dil one month after the booster dose (Table 1). Strong anamnestic responses to IPV occurred; anti-poliovirus GMTs increased 18-fold for type 1, 12-fold for type 2 and 33-fold for type 3. Children who received OPV as part of the NID program had higher prebooster anti-polio titers than those who received only the study vaccine, but comparison of 95% CIs did not indicate any differences in booster responses to IPV response except for poliovirus type 1, where the post-booster titers were higher in children who

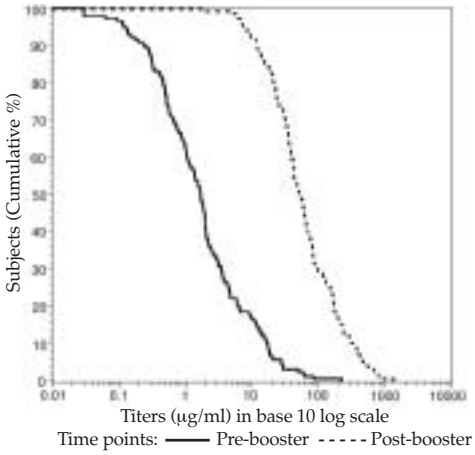

Fig 1a. Anti-PRP (RIA - μg/ml)

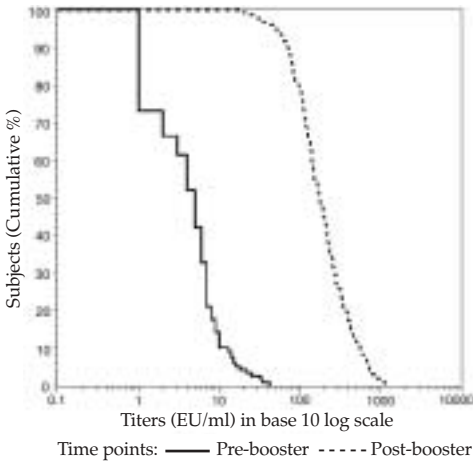

Fig 1b. Anti-PT (EU/ml)

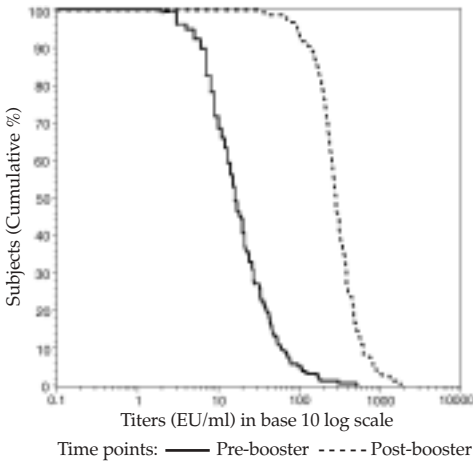

Fig 1c. Anti-FHA (EU/ml)

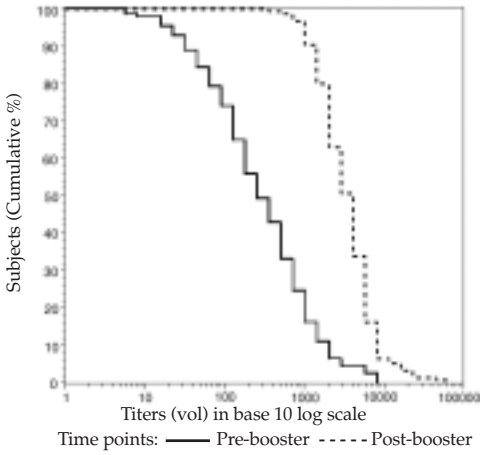

Fig 1d. Anti-Polio 1 (SN - 1/dil)

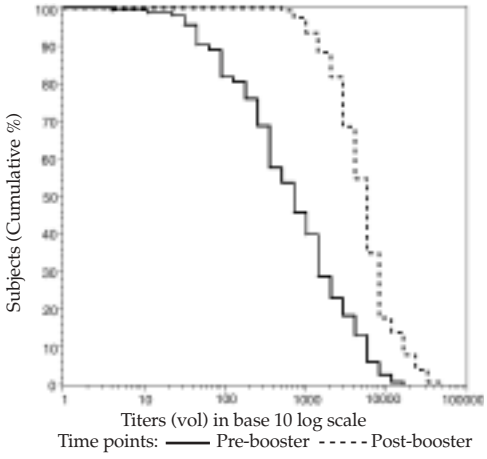

Fig 1e. Anti-Polio 2 (SN - 1/dil)

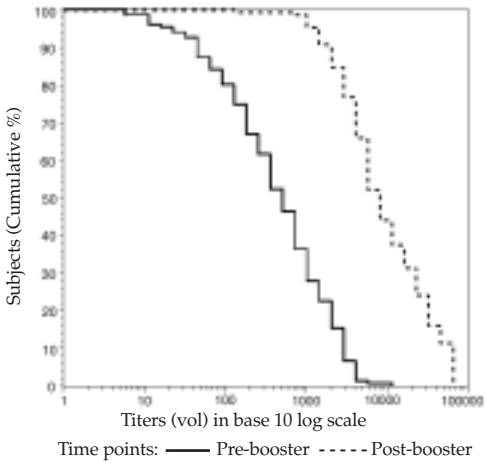

Fig 1f. Anti-Polio 3 (SN - 1/dil)

Fig 1–Reverse cumulative distribution curves pre- and post-booster vaccination with DTaP-IPV//PRP~T.

had received OPV (data not shown). RCDCs illustrate the strong booster response to IPV observed in all children (Fig 1d,e,f).

Reactogenicity and safety

A total of 38 subjects (24.4%) experienced injection site reactions within 30 minutes of vaccination. The most frequently reported were pain in 29 subjects (18.6%), redness in 13 (8.3%), swelling in 5 (3.2%) and induration in one (0.6%). Systemic reactions within 30 minutes of the booster injection were observed in 22 subjects (14.1%), including irritability in 19 subjects (12.2%), fever in two children (1.3%) and unusual drowsiness in two (1.3%).

The solicited local reactions and systemic adverse events that occurred within 8 days after vaccination are summarized in Table 3. Most AEs occurred within 3 days of vaccination, and only a few subjects experienced severe AEs. The most frequently reported local reaction was pain (47.4% of subjects), but severe pain occurred in only two of the 156 (1.3%). There were no reports of severe redness, swelling or induration at the injection site. The most commonly reported solicited systemic events were irritability (31.4% of subjects) and fever (28.8% of subjects). Fever was rated as severe (rectal temperature >40ºC) in five subjects, but was considered to be related to vaccination in only two of these subjects. Irritability related to vaccination occurred in 47 subjects (30%) and severe irritability occurred in only two subjects (1.3%).

Of the 156 subjects, 23 (14.7%) experienced an unsolicited AE within 30 days after vaccination. Most of these subjects presented with respiratory disorders, including 14 with upper respiratory tract infection and 4 with pneumonia. All unsolicited AEs were considered by the investigators as not related to the vaccination, except for one subject, who experienced mild eczema lasting 2 days. Six SAEs were observed in six patients during the study. None were considered to be related to the vaccination. There were no reports of hypotonic hyporesponsive episodes or seizures. No withdrawals occurred because of AEs or SAEs.

DISCUSSION

This study evaluated the immunogenicity and safety of an acellular pertussis combined vaccine including IPV and conjugated Hib polysaccharide antigen (PRP~T) as a booster at 18-24 months of age. All children had received a hexavalent vaccine containing the same antigens as the study vaccine plus a recombinant hepatitis B surface antigen at 2, 4 and 6 months of age. They had also received a dose of hepatitis B vaccine at birth, according to the national vaccination schedule in Thailand. The persistence of antibodies against the antigens included in the booster vaccine one year after the primary vaccination was also evaluated and found to be high. The seroprotection rates, vaccine response rates and GMTs at 1 month following the third dose of the primary series of immunizations indicated that priming had been satisfactory. Strong anamnestic responses occurred in the month following booster administration.

The antigens included in the study vaccine are well known as monovalent vaccines. The PRP~T vaccine is licensed worldwide under the trade name of ActHIB™, and the IPV is licensed as Imovax Polio™. Both are WHO prequalified vaccines (WHO, 2008a). The immunogenicity and safety of the DTaP vaccine combined with IPV (Tetraxim™), as well as the pentavalent DTaP-IPV//PRP~T vaccine have been extensively evaluated in various clinical studies (Lagos *et al*, 1998; Carlsson *et al*, 1998, 2002; Kanra *et al* 2000; Mallet *et al*, 2000; Capeding *et al*, 2008). The pentavalent vaccine was first licensed in

1997 in Europe as Pentavac™ and is currently licensed in over 85 countries throughout the world under the trade names Pentaxim and Pentavac. As discussed below, the effectiveness of this vaccine for prevention of pertussis infection has been documented by the National Surveillance System in Sweden following over 10 years of routine use in that country (Olin and Hallander, 1999; Hessel *et al*, 2004; Gustafsson *et al*, 2006; SMI, 2006).

After primary vaccination, all subjects had anti-PT titers ≥25 EU/ml, and, 52% of subjects had anti-PT antibody titers ≥5 EU/ml before the booster. The booster response was strong, with an increase in anti-PT GMT from 3.8 to 181 EU/ml and a four-fold increase in titer in all children. Such waning of serum antibody responses in children vaccinated with both acellular and whole cell vaccines is well documented. (Grimprel *et al*, 1996; Guiso *et al*, 2007; Edwards and Decker 2008), but these vaccines provide relatively long lasting immunity. Evidence from an animal model of *B. pertussis* infection shows that cell-mediated immune responses can provide protection from infection in the absence of antibodies (Ryan *et al*, 2000). Acellular pertussis vaccine antigens thus induce both strong humoral and cellular responses. Anti-PT antibodies induced by pertussis vaccines decay with time until boosted by an additional vaccine dose or exposure to *B. pertussis*, but the T-cell count increases and remains elevated between doses (Grimprel *et al*, 1996; Ryan *et al*, 2000; Guiso *et al*, 2007). Since PT is known to be specific for *B. pertussis*, our results also suggest that children were not exposed before the booster dose (Guiso *et al*, 2007).

The majority of children in our study maintained seroprotective antibody titers against diphtheria, tetanus, polio types 1, 2 and 3 and Hib in the year following primary immunization, and the high antibody concentrations observed after the booster dose provide good, long-term protection. The antibody persistence in this group of children in Thailand was comparable to or higher than in Swedish children primed with this combined vaccine at 2, 4 and 6 months of age (Carlsson *et al*, 1998), but the antibody persistence in our study was measured approximately 16 months after completion of the primary series, whereas in the Swedish study the gap was approximately 7 months.

Following the booster, all children achieved anti-PRP antibody levels ≥1.0 μg/ml, anti-diphtheria and -tetanus levels ≥0.1 IU/ml, and anti-polio type 1, 2 and 3 levels ≥8 1/dil. The booster response to the pertussis antigens was also strong; a four-fold increase from pre- to post-booster vaccination in anti-pertussis (PT and FHA) antibody titers occurred in at least 92.3% of subjects. Anti-PRP GMTs increased from 1.69 μg/ml to 58.01 μg/ml. Anti-diphtheria and anti-tetanus GMTs also increased, from 0.09 to 2.79 IU/ml and from 0.47 to 6.29 IU/ml, respectively. Considerable increases in GMT were also observed for polio types 1, 2 and 3 and pertussis antibodies.

Our results are consistent with earlier studies by Mallet *et al* (2004) and Langue *et al* (2004) on the immune response to a booster dose of the same DTaP-IPV//Hib vaccine during the second year of life in children who had been primed with either DTaP- or DTwP-based combined vaccines. An additional study, conducted in Sweden evaluated the booster responses to Pentaxim at 12 months of age following a two-dose primary vaccination at 3 and 5 months of age and at 13 months in children who had received three primary vaccinations; at 2, 4 and 6 months of age (Carlsson *et al*, 1998). The seroprotection and vaccine response rates achieved with either schedule in the Swedish trial were also comparable to those achieved in this study. In the Swedish chil-

dren, 89, 93, 96-99 and 97% retained seroprotective antibody concentrations 4.5 years after the booster dose against diphtheria, tetanus, poliomyelitis types 1, 2 and 3, and Hib, respectively. With both regimens, 94% of children had detectable antibodies against FHA and 44% against PT (Carlsson *et al*, 1998). Since the immunogenicity results of this study are consistent with those previously obtained with the same vaccine, it is reasonable to expect the children in our study to maintain protective antibody levels until at least the age of 4 to 6 years, when a second booster dose with pertussis vaccine is recommended in Thailand (WHO, 2008b).

A corresponding long-term impact of Pentaxim on pertussis incidence has been documented over the past ten years by the National Surveillance Program in Sweden (Olin *et al*, 1999; Hessel *et al*, 2004; Gustafsson *et al*, 2006; SMI, 2006). With a vaccination coverage at 2 years of >98% for 3 doses at 3, 5 and 12 months of age, the overall case incidence of culture or PCR confirmed pertussis , dropped from 113-150/100,000 in 1993-1995 to 11-16/100,000 person-years in 2001-2004 (Gustafsson *et al*, 2006) The reported incidence of laboratory confirmed cases was 14 per 100,000 person-years (95% CI :13, 16 per 100,000 person-years) in children born between October 1, 1997 and September 30, 2006 and followed until December 2006 (SMI, 2006). Among the children in this cohort who received only Pentavac™/ Pentaxim, the incidence of laboratory confirmed pertussis was 13 per 100,000 person-years (95% CI: 11,16 per 100,000 person-years). Furthermore, a 91% decrease in incidence was seen after 2 doses of the vaccine as compared to no vaccination, and a 95% reduction was reported after the third dose. The surveillance data shows that routine vaccination of children resulted in a marked decrease in the annual case incidence of pertussis, as compared to no vaccination. Protection began with the second dose and continued after the third dose for at least 5 to 6 years (Gustafsson *et al*, 2006) Thus, given the immunogenicity seen in this study, the available surveillance data suggests that the study vaccine may be used routinely in Thailand to achieve high protection against pertussis.

The epidemiologic data from national surveillance is consistent with a waning immunologic response that has been observed with both whole-cell and acellular pertussis vaccines (Grimpel *et al*, 1996; Guiso 2007). The data suggest a need for an additional booster dose of aP-containing vaccines in children around 4 to 6 years of age, which has been introduced in a number of countries including Thailand (WHO, 2008b).

The severity of the reactions with DTaP-based vaccines has been reported to be significantly lower than with DTwP-based vaccines (Cherry *et al*, 1988; Pichichero *et al*, 1997). As expected, overall reactogenicity was low with the study vaccine. Very few cases of clinically significant (severe) pain, and no instances of severe redness, swelling, or induration were seen. Similarly, severe systemic adverse events related to vaccination were reported in no more than 3 (1.9%) of the 156 subjects. No hypotonic hyporesponsive episodes or seizures were reported. No dropouts occurred because of AE or SAE.

In summary, booster vaccination during the second year of life has been recommended and is now practiced in many countries with the aim to control the incidence and to further reduce the burden of childhood infectious diseases. According to the WHO, achieving these goals will require supplementary immunization activities that involve the administration of additional doses of vaccine (WHO, 1997, 2005). These additional doses may be given as part of a routine program as boosters, where the main

purpose is to increase the duration of protection offered. This study confirms that Pentaxim administered at 18-24 months of age to children primed with a DTaP-based combined vaccine, is well tolerated and induces high antibody responses to all the vaccine antigens. The timing of this booster was appropriate as pre-booster antibody titers were at a satisfactory level.

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