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Original Article

Safety and immunogenicity of the DTP/HB /Hib combination vaccine: phase I study

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Abstract

Background The World Health Organization (WHO) has recommended the introduction of hepatitis B (HB) and *Haemophilus influenza* type b (Hib) vaccines into routine childhood vaccination programs. A new diptheria/tetanus/pertussis (DTP)/hepatitis B/Hib pentavalent combination vaccine has been developed.

Objective To evaluate the safety and immunogenicity of a new combination DTP/HB/Hib liquid vaccine in infants.

Methods An open-label, uncontrolled, prospective intervention phase I study was conducted on 30 healthy infants aged 6−11 weeks. Each subject received 3 doses of DTP/HB/Hib vaccine, formulated by Bio Farma, 0.5 mL intramuscularly at the left anterolateral thigh region using a 25-gauge needle of 25 mm length. Subjects were followed for 1 month after administration of each vaccine dose to evaluate its safety, while serum anti-diphteria, tetanus, HB, Hib, and pertussis antibodies were measured prior to the 1st dose and 1 month after the 3rd dose.

Results Among 30 vaccinated subjects, 18 infants had fever within 24 hours after the first vaccination. Most cases of fever were mild in intensity and resolved within 24 hours. No other systemic or local reactions, or serious adverse events were observed in our subjects during the study. The immunogenicity results after 3rd vaccine dose showed that the geometric mean titer of the anti-polyribosylribitol phosphate (PRP) antibody levels increased significantly from 0.0041μg/mL to 4.37 μg/mL after vaccination, and most infants had a fourfold or greater rise in antibody levels over their pre-injection levels. All subjects who received DTP/HB/Hib liquid vaccine had seroprotective antibodies against tetanus, diphtheria,a and hepatitis B, while 29/30 infants had seroprotective antibodies against pertussis.

Conclusion This new diphtheria/tetanus/pertusis/hepatitis B/Hib combination vaccine has excellent safety profile and antibody responses in infants. These results encourage further clinical evaluation in phase II. **[Paediatr Indones. 2013;53:309-14.]**.

Keywords: *DTP/HB/Hib vaccine, safety, immunogenicity, phase I*

Global strategies for immunization against diphtheria, tetanus, and pertussis (DTP) were adopted in the late 1970s and early 1980s under the auspices of the WHO-sponsored Expanded Program on Immunization (EPI). The target was to achieve 80% global DTP coverage with a three-dose vaccination schedule in infants aged less than one year by 1990. This target has been achieved, though with considerable regional variation within the global target. The new goal set recently by the WHO is to achieve 90% coverage with DTP vaccination of infants aged less than one year by the year 2000.[1] In countries where hepatitis B is endemic, early infant immunization has also been recommended.[2] Since the coverage of hepatitis B (HB) immunization is much lower in Indonesia, a combination of HB with DTP was considered to be a good way to increase the coverage of HB immunization. In Indonesia, the Lombok study (1997−2000), concluded that 8% of children below 2 years of age had Hib in their respiratory tract.[3] In

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addition, a study reported that in subjects from two Bandung subdistricts, of 1,012 throat swab samples, 42% were positive for *Haemophylus influenzae*.[4]

The limited immunogenicity of the polysaccharide anti-polyribosylribitol phosphate (PRP) vaccine in infants and young children led to the development of the Hib protein conjugate vaccine. Conjugation is the process of chemically bonding a polysaccharide, a somewhat ineffective antigen, to a protein carrier, a more effective antigen. This process changes the polysaccharide from a T-independent to a T-dependent antigen and greatly improves immunogenicity, particularly in young children.[5] The Hib-conjugate vaccine is one such vaccine that can be used as part of EPI. It has dramatically reduced the incidence of Hib meningitis. This vaccine is given in a schedule of three doses during infancy, together with DPT, with or without a booster dose at the age of 12-18 months.[3,4] The aim of this trial was to assess the safety and immunogenicity after three dose of DTP/HB/Hib vaccine formulated by Bio Farma.

Methods

This phase I of intervention study was conducted by the Department of Child Health at Hasan Sadikin Hospital, from April to August 2011 at the primary health center (*puskesmas*) in Garuda, Bandung. Ethical clearance was obtained from the Ethics Committee of the Hasan Sadikin Hospital. The clinical trial protocol and vaccine were also approved by the Indonesian National Regulatory Affair. This trial was conducted in accordance with the latest Edinburgh, Scotland revision of the Declaration of Helsinki, *International Conference on Harmonisation* (ICH), Good Clinical Practice guidelines[6-8] and local regulatory requirements.[9] The 30 subjects involved in this study were healthy infants aged 6−11 weeks whose parents agreed to join the study, understood the nature of the study and signed the informed consent form. Each subject received 3 doses of DTP/HB/Hib vaccine, 0.5mL intramuscularly at the left anterolateral thigh region using a 25-gauge needle of 25mm length. Each 0.5-mL dose of the DTP-HB/Hib vaccine contained purified diphtheria toxoid (≥30 international units (IU)), purified tetanus toxoid (≥60 IU), inactivated *B. pertussis* whole-cell suspension (≥4 IU), 10 mcg recombinant HBsAg protein, 1.5 mg aluminum phosphate, 4.5 mg sodium chloride, and 0.025 mg thimerosal (produced by Bio Farma; batch number 501040). The interval of each dose was 28 days.

Systemic and local reactions were evaluated at 30 minutes, 24, 48 and 72 hours, as well as 28 days after immunization. Subjects were evaluated at the primary health center for the first three days after the first immunization dose for adverse events. The reactions between days 4 and 28 were recorded in the diary cards collected and confirmed by the investigators at the subjects' last visits. Reactions after the second and third doses were recorded on the diary card for 24, 48 and 72 hours, as well as 28 days after immunization.

Blood specimens were taken before the 1st immunization, and 28 days after the last immunization. Antibody titers to PRP/T or Hib, diphtheria, and tetanus were evaluated using the enzyme-linked immunosorbent assay (ELISA) method, and pertussis antibodies were tested using a microagglutination method. All four methods had been validated and were done at the Clinical Trial Department of Bio Farma after blinding procedure. Anti-HB antibodies were tested using a kit from Abbott in a commercial laboratory which had been audited by Quality Assurance from Bio Farma. The original identity of the samples was concealed to ensure unbiased antibody testing.[10]

The number of subjects protected from Hib, diphtheria, tetanus, pertussis, and hepatitis B were calculated in percentages. Protective levels of Hib antibodies are 0.15 μg /mL for a short-term protection and 1 μg /mL for long-term protection.[11-13] For tetanus and diphtheria, protective antibody levels are 0.01 IU/mL for short-term protection and 0.1 IU/mL for a long-term protection, while that for HB is 10mIU/mL.[11] Seroconversion rates are calculated by the percentage of subjects with a transition from seronegative to seropositive, or the percentage of subjects with four fold antibody increase after immunization. Geometric mean titers prior to immunization were compared to those after immunization.

Results

A total of 30 subjects enrolled in this study and had a median age of 8 weeks. Fifteen subjects were male. All

subjects received three doses of DTP/HB/Hib liquid vaccine, and met the protocol criteria.

Adverse events reported after immunization are summarized in **Table 1**. No subjects presented with immediate local reactions or systemic events from 0 to 30 minutes after immunization. However, 18 subjects presented with delayed systemic events mainly in the period from 31 minutes to 24 hours after immunization. Furthermore, no subjects presented with immediate local reactions or systemic events from >24 hours to 28 days after immunization and no serious adverse events were observed during the study.

After the 3rd immunization dose, 29 infants were considered to be protected against Hib, as the geometric mean titer (GMT) of anti-Hib increased from 0.0041 μg/mL pre-immunization to 4.37 μg/mL post-immunization **(Table 2)**.

The proportions of infants who had post-immunization anti-diphtheria titers of ≥0.01 IU/mL, and ≥0.1 IU/mL, were 30/30 and 28/30, respectively. The GMT of anti-diphtheria antibodies was 0.259 μg/mL post-immunization. The proportions of infants who had post-immunization anti-tetanus titers of ≥0.01 IU/mL and ≥0.1 IU/mL, were 30/30 and 27/30, respectively. The GMT of anti-tetanus antibodies was 0.480 μg/mL post-immunization. Furthermore, the proportions of subjects with >4-fold increased antibody titers were 28/30 for anti-diptheria and 5/30 for anti-tetanus. We also observed that 30/30 infants underwent an anti-diphtheria transition from seronegative to seropositive **(Table 3)**.

The anti-pertussis (PT) GMT was 148.936 l/dL, while 28/30 of infants presented with anti-PT titers ≥40 (1/dL), 25/30 presented with anti-PT titers ≥80 (1/dL), and 10/30 presented with anti-PT titers

Table 1. Summary of adverse events following the first dose of DTP/HB/Hib vaccine

Adverse events	N = 30
Immediate reactions from 0 to 30 min after immunization	
Local reactions	0
Systemic events	0
Delayed adverse events from 31 min to 24 hours after immunization	
Local reactions	0
Systemic events	18
Delayed adverse events from >24 to 48 hours after immunization	
Local reactions	0
Systemic events	0
Delayed adverse events from >48 to 72 hours after immunization	
Local reactions	0
Systemic events	0
Delayed adverse events from 72 hours to 28 days after immunization	
Local reactions	0
Systemic events	0

DTP = diphtheria-tetanus-(whole cell) pertussis; HB = hepatitis B; Hib = *Haemophilus influenzae* type b

Table 2. Antibody responses to the Hib component, pre- and post-vaccination with DTP/HB/Hib

Antibody responses	Pre-vaccination N=30	Post-vaccination N=30
Anti-PRP >0.15 μg/mL, n	8	29
95% CI (%)	12.3 to 45.9	82.8 to 99.9
Anti-PRP >1.0 μg/mL, n	0	26
95% CI (%)	0 to 11.6	69.3 to 96.2
Anti-Hib GMT, μg/mL	0.0041	4.37
95% CI (%)	0.0015 to 0.0108	2.0635 to 9.2858
Antibody titer ≥4-fold increase, n	-	27
95% CI (%)		73.5 to 97.9
Seronegative to seropositive transition, n	-	21
95% CI (%)		50.6 to 85.3

GMT = geometric mean titer; 95% CI = confidence interval

≥320 (1/dL). In addition, 25/30 infants had ≥4-fold increased antibody titers **(Table 4).**

The proportion of infants with anti-hepatitis B titer >10 mIU/L post-immunization was 30/30, the GMT was 547.898mIU/mL. Proportion of subjects with increasing antibody titer ≥ 4 fold was 29/30, and 27/30 subjects experienced transition of seronegative to seropositive **(Table 5)**.

Table 3. Serological responses to diphtheria and tetanus toxoid antigens, pre- and post- vaccination with DTP/HB/Hib

Antibody response	Pre-vaccination N=30	Post-vaccination N=30
Anti-diptheria		
≥0.01 IU/mL, n	11	30
≥0.1 IU/mL, n	2	28
GMT, IU/mL	0.0031	0.259
95% CI for GMT	0.0017 to 0.0058	0.2068 to 0.4233
Anti-tetanus		
≥0.01 IU/mL, n	29	30
≥0.1 IU/mL, n	25	27
GMT, IU/mL	0.5098	0.480
95% CI for GMT	0.287 to 0.9057	0.3349 to 0.6891
Antibody titer ≥ 4-fold increase, n		
- Anti-diptheria		28
95% CI (%)	-	77.9 to 99.2
- Anti-tetanus		5
95% CI (%)	-	5.6 to 34.7
Seronegative to seropositive transition, n		
- Anti-diptheria	-	19
95% CI (%)		43.9 to 80.1
- Anti-tetanus	-	n.a.

95% CI = confidence interval. IU = international units. 4-fold increase=4 fold increase over pre-booster liters (applicable only for post-booster seroconversion rates).
n.a: percentage of infants presenting with anti-tetanus transition of seronegative to seropositive is not applicable, since all the subjects were protected before immunization

Table 4. Antibody responses to the pertussis component, pre- and post-vaccination with DTP/HB/Hib vaccine

Antibody response	Pre-vaccination N=30	Post-vaccination N=30
GMT, l/dL	11.215	148.936
95% CI (%)	9.517 to 13.213	104.448 to 212.3244
Anti-PT ≥40 (1/dL)	2	28
95% CI (%)	0.8 to 22.1	77.9 to 99.2
Antibody titer ≥4-fold increase, n (%)	-	25
95% CI (%)		65.3 to 94.4

GMT = geometric mean titer; 95% CI = 95% confidence interval

Table 5. Antibody responses to the hepatitis B component, pre- and post-vaccination with DTP/HB/Hib vaccine

Antibody response	Pre-vaccination N=30	Post-vaccination N=30
Anti-hepatitis B >10 mIU/L	3	30
95% CI (%)	2.1 to 26.5	88.4 to 100
GMT, mIU/mLb	0.0021	547.898
95% CI	(0.0007 to 0.0061)	(381.5926 to 786.8646)
Antibody titer ≥4-fold increase, n	-	29
95% CI (%)		82.8 to 99.9
Seronegative to seropositive transition, n	-	27
95% CI (%)		73.5 to 97.9

DTPw-HepB-Hib = diphtheria-tetanus-whole cell pertussis-hepatitis *Haemophilus influenza* type B; IU= international unit; 4-fold increase = 4 fold increase over pre-booster liters (applicable only for post-booster seroconversionrates); CI = conficence interval

Discussion

The availability of combined vaccines containing protective antigens against the majority of, and ideally all, diseases for which universal immunization is recommended in infancy would simplify the implementation, increase the acceptance, reduce the global cost of immunization programs, and improve disease control, while offering the possibility of disease elimination or even pathogen eradication.[14] Vaccine development proceeds through discovery, process engineering, toxicology, and animal studies to human phase I, II, and III trials. The human trials initially focus on safety, involving small groups of people (phase I); then progress to moderate-sized "target" populations (persons close to the age and other characteristics for whom the vaccine is intended) to determine both safety and the stimulation of an immune response (phase II); and finally to large target populations to establish whether a vaccine actually prevents a disease as intended (efficacy) (phase III).[15]

A total of 30 subjects received the investigational vaccine containing DTP/HB/Hib for the first time in our phase I trial. All participants were intensively examined at the primary health care center during the first 72 hours after injection. The results of this phase I study demonstrated good safety and immunogenicity profiles for the new combined DTP/HB/Hib vaccine. There were no serious local or systemic reactions in this study. All observed reactions were slight, transient, and did not last more than 24 hours after the administration of the vaccine and resolved without medical intervention.

The anti-PRP antibody GMT significantly increased after the 3rd vaccination dose (4.37 μg/mL), compared to the concentrations found in pre-vaccination sera, and most infants had a 4-fold-or-greater rise in antibody levels over their pre-injection levels. All subjects who received DTP/HB/Hib had seroprotective antibodies against tetanus, diphtheria, and hepatitis B. We observed good immunogenicity and reactogenicity profiles for the new combined DTP/HB/Hib vaccine.

Combination vaccines have numerous advantages, including a decreased number of injections, increased compliance, better coverage, and simplified logistics. They also result in substantial reductions in program costs, since the indirect costs of immunizing a child (e.g., logistics, materials involved, payment of medical staff, and child transportation) are far greater than the vaccines themselves. A new DTPw-HB/Hib combination vaccine for primary and booster vaccination study of infants in Latin America showed that both vaccination regimens elicited excellent immune responses, with all subjects in both groups achieving seroprotective anti-PRP antibody concentrations of $\geq$0.15 μg/mL one month after primary vaccination. The vaccination regimens consisted of DTP/HB/Hib was given in one shot immunization in the left thigh of subject, and DTP/HB and Hib was given in two shots at the different thigh (e.e combined DTPw-HB was given in left thigh (usually), and Hib was given in right thigh). The combined DTPw-HB/Hib vaccine was not inferior to licensed vaccines in terms of seroprotection, seropositivity, and vaccine response rates for all component antigens. Persistence of antibodies against all study vaccine antigens up to the time of booster vaccination was comparable between groups, and a marked increase of all antibody concentrations was observed after the booster dose. Both vaccine regimens were similar in terms of their overall reactogenicity profiles.[16]

The use of the new DTP/HB/Hib vaccine provides protection against five major childhood pathogens and will ease the implementation of global pediatric immunization programs, with a minimum of injections and potentially improved immunization coverage.[16]

In conclusion, the excellent safety profile and antibody responses in the infants observed during this study encourage us to proceed to further clinical evaluations in phase II.

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