

***Haemophilus influenzae* Type b/Polyribosylribitol Phosphate-Tetanus (Hib/PRP-T) Vaccine Safety, Phase I Study**

Kusnandi Rusmil,¹ Eddy Fadlyana,¹ Rachmat Gunadi,² Novilia Sjafrri Bachtiar,³ Hadyana⁴

¹Department of Child Health, Faculty of Medicine, Universitas Padjadjaran-Dr. Hasan Sadikin General Hospital

²Department of Internal Medicine, Faculty of Medicine, Universitas Padjadjaran-Dr. Hasan Sadikin General Hospital

³Surveillance and Clinical Trial Division, Bio Farma, Bandung, Indonesia

⁴Departement Epidemiology and Biostatistic, Faculty of Medicine, Universitas Padjadjaran-Dr. Hasan Sadikin General Hospital

Abstract

Objective: To assess the safety and immunogenicity of the *Haemophilus influenzae* type b/polyribosylribitol phosphate-Tetanus (Hib/PRP-T) liquid vaccine in healthy adults.

Methods: An open label prospective intervention phase I study was conducted in Dr. Hasan Sadikin General Hospital from November to December 2010. Healthy adults aged 18–40 years were eligible to participate. Participants received one dose of Hib/PRP-T liquid vaccine. Blood samples were taken before, 4 days, and 1 month after vaccination. For a 28-day period following vaccination, solicited adverse events were recorded in the subjects' diary and assessed afterward.

Results: No local reactions or immediate systemic events were observed during the 30-minute period after immunization. There were no serious local or systemic reactions in this study. All of local and systemic reactions observed were slight, transient, self-limiting, and lasting no more than 72 hours after the administration of the vaccine. These reactions resolved without any medical intervention. Hematological and biochemical indices before and 4 days after vaccination were in normal limits. All subjects reached protective levels of antibodies (seroprotectivity) against Hib. All subjects demonstrated antibodies performing high bactericidal activities 1 month after immunization.

Conclusions: This study demonstrates that liquid Hib/PRP-T vaccine is highly immunogenic and beneficially safe when administered to healthy adults.

Keywords: Adults, Hib vaccines, immunogenicity, safety

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Introduction

Before the year of 1985, *Haemophilus influenzae* type b (Hib) was the leading cause of bacterial meningitis and also a common cause of other invasive diseases (e.g., epiglottitis, pneumonia, septic arthritis, cellulitis, purulent pericarditis, and bacteremia) among U.S. children aged <5 years.¹ Meningitis occurred in approximately two thirds of children suffering from invasive

Hib disease; 15–30% of survivors had hearing impairment or severe permanent neurologic sequelae. Approximately 4% of all cases were fatal.² The first polysaccharide Hib vaccine was introduced in the United States in 1985, followed by conjugate Hib vaccines in 1987 and 1989. During the period of 1989–2000, the annual incidence of invasive Hib disease in children aged <5 years decreased by 99% to less than one case per 100,000 children.³ During the period of 2000–2012, the average annual incidence rate of invasive Hib disease in children aged <5 years in the United States remained below the Healthy People 2020 goal of 0.27/100,000.⁴

In the period of 1989 to 2008, there were

Correspondence:

Kusnandi Rusmil, Department of Child Health, Faculty of Medicine, Universitas Padjadjaran-Dr. Hasan Sadikin General Hospital
Jl. Pasteur No. 38, Bandung, Indonesia
e-mail: kusnandi@hotmail.com

7,559 cases of *H. influenzae* disease reported from Active Bacterial Core surveillance sites. Small increases in the incidence of serotypes a, e, and f were observed during 1989–2008. The largest of these increases was in serotype f and was primarily among adults aged ≥ 18 years.² In Indonesia, based on Lombok study in the 1997–2000 period, it was concluded that 4.6% of children below the age of 2 were found to have Hib in their respiratory tract.⁵ Based on the study in two subdistricts in Bandung, of 1,012 throat swab samples, 42% were positive for *H. influenzae*. However, serotyping to find the strain was not performed.⁶

The first vaccine available for the prevention of Hib disease was purified polyribosylribitol phosphate (PRP) polysaccharide vaccine used for children 18 to 60 months of age, licensed in the United States in April 1985. The immune response of children to PRP polysaccharide vaccine is strikingly age related. Young infants respond infrequently and with low antibodies level but the immune responses then improve with age. The limited immunogenicity of the polysaccharide PRP vaccine in infants and young children has led to the development of the Hib protein conjugate vaccine. Conjugation refers to the process of chemically bonding a polysaccharide, which is somewhat ineffective antigen, to a protein carrier which is a more effective antigen. This process transforms the polysaccharide from a T-independent into a T-dependent antigen and greatly improves immunogenicity, especially in young children.⁷

In Lombok study, it was discovered that the Hib vaccine effectiveness for pneumonia was high in absolute number, i.e. 884 children per 100,000 child annually, but small in terms of proportion. The proportion of meningitis and pneumonia was 22% and 3%, respectively.⁸ World Health Organization (WHO) position paper on Hib conjugates vaccines stated: "In view of their demonstrated safety and efficacy, conjugate Hib vaccines should be included in all routine infant immunization programs. The lack of local surveillance data should not delay the introduction of these vaccines, especially in countries where regional evidence indicates there is a high burden of disease".⁷ Diphtheria, tetanus, pertussis, hepatitis B and *H. influenzae* carry a high burden in terms of morbidity and mortality, especially in the developing world. Vaccination has been shown to be effective to control these diseases when mass immunization programs covering a significant proportion of the population is implemented. Successful vaccination programs are very much influenced by the acceptability of the vaccine

and of the administration schedule to the target population. With the increasing number of vaccines to be concomitantly administered, usually to the very young children, combined vaccines become a key element to maximize the uptake of any mass vaccination program. Trivalent diphtheria tetanus pertussis (DTP) vaccine, monovalent hepatitis B (HB) vaccine and monovalent Hib conjugated vaccines are logical choices for the development of a new combined vaccine that will reduce the number of injections required to immunize a child.⁹ Before developing a new combination DTP/HB/Hib vaccine of, the safety profile of this new Hib antigen in adults should be evaluated first.

Methods

A study, which was an open label prospective intervention phase I (descriptive) study, was conducted at the Clinical Trial Unit of Dr. Hasan Sadikin General Hospital, Bandung from November to December 2010. An ethical clearance was obtained from the Health Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran-Dr. Hasan Sadikin General Hospital. The clinical trial protocol and vaccine were also approved by the Indonesian National Regulatory Affair. Twenty-five subjects involved in this phase I study were healthy adults, 18–40 years of age, who had agreed to participate in the study, understood the nature of the study, and signed the informed consent form. Each subject received 1 dose of Hib vaccine, 0.5 mL (10 μ g PRP-T) intramuscularly in the left deltoid region using gauge number G23 with a needle length 25 of mm (Bio Farma batch number CHL099-200310).

Systemic and local reactions 30 minutes, 24, 48, and 72 hours, and 28 days after immunization were evaluated.¹⁰ The subjects attended the Clinical Trial Unit of Dr. Hasan Sadikin General Hospital for the first three days after immunization to meet the investigators and received observation and examination for the adverse events. The reactions between days 4 and 28 were recorded in the diary cards were collected and confirmed by the investigators in the last visit.

Blood were sampled before immunization and on days 4 and 28 after immunization. Antibody titer to PRP/T or Hib was evaluated using the enzyme-linked immunosorbent assay (ELISA) method at the Clinical Trial Department of Bio Farma after blinding.¹⁰ The

original identity of the samples was concealed to ensure non-bias antibody testing, Hib polysaccharide conjugated to human serum albumin (HbO-HA) was used to coat the microtiter plates. After overnight incubation at room temperature and subsequent washing, references and samples that had been diluted using the sample buffer were respectively added into the first well in the first row. Serial two fold dilutions were performed and the plates were later incubated. Conjugate, substrate solution and stopping solution were subsequently added after incubation and washing for each step except for the substrate and stopping solutions. The optical densities were measured using the ELISA, they were calculated using kinetically calculation. This method had been validated and was performed using the International reference from Food and Drug Administration (FDA), lot No.1983.¹⁰ The blinding would be opened after Bio Farma has formally released the antibody results.

The number of subjects protected from Hib was calculated in percentage, with the protective level of antibody 0.15 µg/mL for a short-term protection and 1 µg/mL for a long-term protection.^{10,11} Geometric mean titer was be compared before and after immunization.

Results

Of the thirty subjects screened, five were excluded due to hematological and biochemical abnormal results. A total of 25 subjects were enrolled in this study with a median age of 30 years: 13 males and 12 females. Hematological and biochemical indices before and 4 days after vaccination were within normal limits. During the trial, 10 delayed local reactions (pain) and 5 systemic events (muscle pain) occurred between 31 minutes and 72 hours after immunization were reported (Table 1). No serious adverse event was observed during this study, all reactions were mild and resolved spontaneously.

Table 1 Distribution of Subjects with Local Reaction and Systemic Event after Immunization (from 31 minutes–72 hours)

Any Delayed Adverse Event	n
Local reaction (pain)	10
Systemic event (muscle pain)	5

Table 2 *Haemophilus influenzae* Type b/ Polyribosylribitol phosphate-Tetanus Seroprotection Pre- and Post-Immunization

Description	Before	After
	Number	Number
<0.15 µg /mL	12	-
>0.15 µg /mL	4	25
>1 µg /mL	9	25

Twelve subjects were not protected before immunization. After immunization, all subjects were protected with a seroconversion rate of 100%. The long-term protection level of antibody was also achieved by these subjects (Table 2). It was also apparent that the geometric mean titre (GMT) had increased from 0.68 to 30.16 µg /mL, showing an approximately 44 times increased in these subjects (Table 3).

Table 3 Geometric Mean Titre of Anti *Haemophilus influenzae* Type b/ Polyribosylribitol phosphate-Tetanus Vaccine before and after Immunization

Description	Before	After
	n=25	n=25
GMT (µg /mL)	0.6834	30.160
(95% CI)	0.314–1.562	19.253–46.402

Discussion

Vaccines are the only public health tool that can reduce the incidence of Hib dramatically in industrialized and developing countries. Serious Hib disease has been practically eliminated within few years in most countries where immunization against this disease has been introduced into the national immunization program. Preventing Hib disease by way of immunization has become more important than ever, owing to the increasing bacterial resistance to some of the most effective antibiotics.⁷ Hib vaccine in this trial was prepared through chemical conjugation of the Hib capsular polysaccharide to tetanus toxoid (PRP-T), with the final vaccine preparation often in a lyophilised form. Each dose of lyophilised PRP-T vaccine is formulated to

contain 10 mg of polyribosylribitol phosphate chemically conjugated to 24 mg of tetanus toxoid.^{12,13}

World Health Organization recommends that all countries adopt Hib vaccine into routine child immunization programs. They estimate that Hib globally is responsible for 400,000 deaths each year in children under five years of age and around 3 million cases of serious illness resulting in long-term consequences, such as deafness, learning disabilities, paralysis, and mental retardation.¹⁰ In Indonesia, based on the Lombok study in the 1997–2000 period, it was concluded that 4.6% of children below 2 years of age were found to have Hib in their respiratory tract.⁷

In this clinical trial, a total of 25 adult volunteers enrolled received, for the first time, the investigational vaccine containing the Hib/PRP-T antigen. All participants attended to the Clinical Trial Unit Dr. Hasan Sadikin General Hospital for the first three days after immunization to meet the investigators and received observation and examination for the adverse events. They were also submitted to a very rigorous follow-up including hematological, renal, and hepatic parameters. As a result, an excellent safety profile was observed. There were no serious local or systemic reactions in this study. All reactions observed were slight, transient, self-limiting in time, without lasting for more than 72 hrs after the administration of the vaccine, and resolved without any medical intervention. All subjects reached protective levels of antibodies (seroprotectivity) against Hib. The GMT was increased from 0.68 µg/mL to 30.16 µg/mL. This study demonstrates that the liquid Hib/

PRP-T vaccine is highly immunogenic and has a beneficial safety when administered to healthy adults.

In 2004, Bio Farma had conducted a clinical trial of Hib vaccine in adult from freeze dried formula. There was no immediate local reaction found in 30 minutes after immunization. Forty eight hours after immunization, pain as a local reaction was found in 5 subjects and indurations in 1 subject. No local or systemic reaction was found in 72 hrs. and 4–28 days after immunization. 94% of subjects were already protected from Hib before immunization, and 100% were protected 28 days after immunization with the increased GMT from 3.041 µg/mL to 76.12 µg/mL.¹²

A previous study by Verez-Bencomo *et al.*¹³ clinical trial phase 1 Hib vaccine in adults, children, and infants in Camaguey, Cuba reported no adverse reactions. Based on single-dose studies, average PRP-specific IgG levels and percent of patients achieving seroconversion were comparable when the sPRP-TT was administered with or without aluminum phosphate, and both were comparable to commercial Hib vaccine.

The excellent safety profile and also the preliminary antibody responses in the adults observed during this study were determinant factors to encourage the implementation of further clinical evaluations in children and infants.

In conclusions, this study demonstrates that the liquid Hib/PRP-T vaccine is highly immunogenic and has a beneficial safety when administered to healthy adults.

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