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Review Article

Immunogenicity and Efficacy of Different Haemophilus influenzae type b Vaccines

Mojgani*, N.

Department of Biotechnology, Razi Vaccine and Serum Research Institute, Karaj, Iran

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ABSTRACT

Haemophilus influenzae, a major cause of meningitis in young children leading to death and other neurological sequelae. The disease leaves 15 to 35% of the survivors with permanent disabilities, such as, mental retardation or deafness. Despite the availability of new and more powerful antibiotics children with Hib meningitis still suffer from high mortality or morbidity. The emergence of multiresistant Hib strains causes increasing difficulties in selecting proper antibiotics for the treatment. Since 1970, the capsular polysaccharide polyribosylribitol phosphate (PRP) in *H. influenzae* b has been the target for vaccine development. The first Hib polysaccharide vaccine licensed in 1985, proved immunogenic in human adults, but failed to elicit an immune response in children under 2 years of age who were at greatest risk of developing the invasive Hib infection. These factors led to one of the most exciting advances in pediatrics, the development of Hib conjugate vaccines. Unlike most other vaccines for preventing a particular disease which are generally similar for all types, the specific characteristics of the available Hib conjugate vaccines licensed vary from each other in structure and immunological properties. In this review the immunogenicity and efficacy of Hib vaccines including a) PRP vaccine; b) Conjugate vaccines; and c) Combination vaccines is evaluated.

Keywords: *H. influenzae type b,* PRP, meningitis, conjugate, vaccine

INTRODUCTION

Among infants and young children, *Haemophilus influenzae* type b (Hib) is the leading cause of bacterial meningitis deaths and the second leading cause of bacterial pneumonia deaths worldwide and accounts for approximately 400,000 deaths of children each year (Hanna 1990, Saha *et al* 1997, Jackson *et al* 2012). The bacterium causes a wide spectrum of diseases that includes meningitis, epiglottitis, cellulitis, acute pneumonitis, septic arthritis and otitis media. (Watt *et al* 2009). In Iran, the exact burden of disease caused by H.infleunzae is unknown; however a number of reports are available which have indicated observable incidence of Hib meningitis in the country mainly among children below 6 years of age (Haghiashteiani *et al* 2008, Nakhjavani *et al* 2005). In a study conducted on meningitis cases among children admitted in a

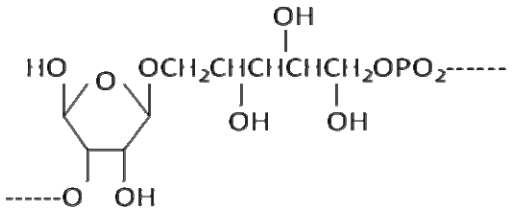
*Author for correspondence.Email: dnmoj@rvsri.ir

Tehran Hospital, the prevalence rates for Hib was reported approximately 52% (capsule type b) (Mojgani *et al* 2011). According to these reports, with regard to disease spectrum meningitis cases accounted for 88% of all the invasive disease caused by Hib in the country.

The most important virulence factor defining pathogenic *H. influenzae* strains is the capsular polysaccharide, of which there are six serotypes, a-f (Booy & Moxon 1991). Among the six serotypes, most invasive is serotype b which accounts for 95% of the invasive Hib clinical isolates. All Hib isolates contain a capsule recognized as a major virulence factor in invasive disease due to the capsule's role in protecting the bacterium from phagocytosis and resistance to the antibacterial action of complement and serum components. The capsule is composed of the polysaccharide polyribosylribitol phosphate (PRP), and responsible for causing sustained bacteremia and subsequent focal infection in non-immune hosts (Saha *et al* 1997, Hanna 1990). The virulence and infectivity of Hib has been demonstrated in many animal models (Mojgani *et al* 2013, Miler 1993, Brodeur *et al* 1986, Kaplan *et al* 1986). The virulent genes involved in the expression of H.influenzae type b capsule are present as a duplication of an approximately 18-kb DNA segment (the Cap b locus). Hib Polysaccharide capsule production relates to the number of copies of the cap locus (Cerquetti *et al* 2006, Corn *et al* 1993, Mojgani *et al* 2013). After the advent of the key role played by PRP in the pathogenesis of invasive disease by this organism, purified type b capsule was used as a vaccine antigen (Jackson *et al* 2012). In 1977, a field study conducted in Finland on PRP vaccine showed 90% efficacy in children 18-71 months (Shapiro & Ward, Peltola *et al* 1977). However, in other countries like UK, USA where peak disease incidence occurs early, PRP failed to demonstrate good efficacy given at 18-24 months of age (Wegner *et al* 1999). The reason for this failure was due to the inability of infants to mount a protective antibody response to PRP. PRP induces a T-cell independent response and consequently is unable to elicit a memory, or booster, immune response and this type of response is immature in infants resulting in poor or no antibody response (Peltola *et al* 1984). This problem was overcome by conjugating PRP to a carrier protein which converted the response to a T-cell dependent response which is well developed in infants resulting in a protective antibody response to PRP. (Force *et al* 1992). PRP has been conjugated to various protein carriers such as diphtheria toxoid (PRP-D), non toxic mutant diphtheria CRM_{197} (HbOC), tetanus toxoid (PRP-T), and meningococcal outer membrane proteins (PRP-OMP). An important aspect in determining the protective effectiveness of these vaccines depends on the choice of conjugate, the number of doses and their timing; together with the burden of invasive Hib disease in that particular region. Although all of above mentioned conjugate vaccines vary in their immunogenicity, as defined by antibody titers, the net effect is the generation of protective immunity in most vaccinated individuals (Goldblatt 2000, Finn 2004, Kelly *et al* 2004). In recent years a number of combination vaccines have been developed by combining Hib vaccine with DPT (quadrivalent), DPaT (quadrivalent), DPT/IPV (pentavalent), DPaT/IPV (pentavalent), DPT/HepB, DPT/IPV/HepB (hexavalent) etc. The studies show similar immune responses to all the components in the combination vaccines compared with the responses to individual components when given separately. The main advantage of combination vaccines is a single injection which save delivery costs and reduce the storage space in the refrigerator (Corbel 1994, Anthony 1995). In Iran, owing to the growing number of cases of Hib disease, a lot of emphasis is laid on development and use of Hib conjugate vaccine. According to recent reports of National Ministry of Health and Medical Education, Hib conjugate vaccine is included among the routine compulsory vaccines and onwards all children in the country would be vaccinated against Hib with other routine vaccines. Acknowledging the importance and benefits of Hib conjugate vaccine many companies and research institutes in the country have approached development of Hib vaccine for achieving

self reliance in the field. The projects for the development are underway and promising and soon we might witness the manufacture of Hib conjugate vaccine in the country which would be highly economical and within reach not only for children in the country but also for neighboring countries. Owing to the availability of different types of Hib vaccines in the global market, in the following section we summarize and compare the immunogenicity and protective efficacy of these Hib vaccines.

Polyribosyl ribitol phosphate (PRP) vaccine. The first Hib vaccine licensed in the USA in 1985 contained capsular PRP alone and was meant for children at least 2 years of age (Center for Disease control 1985). However, PRP failed to induce protection in children below the age of 18 months who were most at the risk of morbidity and mortality. Polysaccharide antigens are large molecules of repeating epitopes (Figure 1), which are not processed by antigen presenting cells and elicits a T-cell independent immune response by acting directly on B-cells to induce the synthesis of humoral antibodies (Robins *et al* 1973, Anderson *et al* 1977).



→3)-β-D-Rib*f*-(1→1)-D-ribitol-5-PO_4-→

Figure 1. The repeating unit structure of the *H. influenzae* type b capsular polysaccharide.

T-cell independent responses generally are not effective in infants less than 18-24 months as this type of response is not well developed at that age. Additionally, the absence of T cell help induces predominantly IgM antibodies which persist for a shorter time and usually low titered. It is well documented that PRP induces a poor immune response that is highly age dependent and does not induce higher antibody levels following booster doses. The PRP vaccine also failed to reduce carriage in individuals chronically infected with Hib. An interesting aspect is that all bacterial capsular polysaccharides do not behave similarly. Some, e.g., group A meningococcal or type 3 pneumococcal capsular polysaccharides, are fairly good immunogen even in infancy, while others, such as E.coli KI, group B meningococcal, or type 6 pneumococcal capsular polysaccharides are hardly immunogenic even in adults (Robins 1978). The limited immunogenicity of PRP vaccine in young infants, and the variable levels of protective efficacy reported from clinical trials provided the impetus for the development of the next generation of vaccines, the conjugate vaccines.

Conjugate vaccines. The basis for the conjugate vaccine was laid by the studies of Avery & Goebel (1929), which showed that the immunogenicity of polysaccharides could be enhanced by covalently attaching them to appropriate carrier protein and thus forming a conjugate. The advantages of conjugate vaccine are 3 fold. Firstly, it is effective from 6 weeks of age onwards. Secondly, it induces IgG antibodies and leads to boosting on repeated doses leading to better long term immunity (Force *et al* 1992, Finn 2004). Being T cell dependent it stimulates memory B cells which will lead to an anamnestic response even years later. Lastly it also induces IgG antibodies which are secreted in nasal secretions. This will postpone and reduce the chances of carrier rate leading to herd immunity (Robins & Schneerson 1990, Kelly *et al* 2004, Sharma *et al* 2009).

Types of conjugated Hib Vaccines. Many Hib conjugate vaccines have been developed and licensed by both big pharmaceutical companies and developing country manufacturers. Conjugate vaccines utilize PRP usually purified from *H. influenzae* type b organisms grown in a bioreactor, and chemically linking them to various carrier proteins by the method of conjugation with or without the use of a spacer molecule (Wenger *et al* 1989). These conjugates are able to increase the Immunogenicity of PRP by recruitment of T helper cells (T-cell dependent immune response) resulting in heightened anti-PRP antibody titres. The recruitment of

T helper cells results in isotype switching with the predominant antibody being IgG which characteristically results in memory cells and thus prolonged antibody synthesis, and the production of a booster response (Avery & Goebel 1931, Ward *et al* 1988, Wainwright *et al* 1990, Decker *et al* 1990, McCormick & Molyneux 2011). The four Hib conjugate vaccines licensed in developed countries are PRP-D (diphtheria toxoid; *Cornybacterium diphtheriae*), PRP-T (tetanus toxoid; *Clostridium tetani*), PRP-OMPC (outer membrane protein complex; *Neisseria meningitidis* B) and HbOC (Haemophilus b oligosaccharide conjugate; mutant D toxin CRM_{197}). Although all of the four vaccines are similar in some respects, they differ from one another in several ways (Table 1). The differences involve the size of the PRP molecule, the type of carrier protein, the type of linkage between PRP & carrier protein(conjugation chemistry); presence or absence of an adjuvant in the vaccine (manufacturing process); and the level of protective efficacy (Dick & Beurret 1989).

i) PRP-diphtheria toxoid conjugate (PRP-D) vaccine. This vaccine links diphtheria toxoid as the protein carrier to a medium sized length PRP. The process involves hydrolyzing and lowering the molecular weight of the PRP and activation to an electrophilic species with cyanogen bromide. In a separate process stream, diphtheria toxoid (DT) is functionalized with adipic acid dihydrazide, using the water soluble 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimde (EDAC) and creating the nucleophilic DT derivative. The electrophilic and neutrophilic products are then combined and a conjugate formed (Gordon 1986). Compared to plain PRP polysaccharide vaccine, the immune responses to PRP-D vaccine are demonstrably better and an increased response is seen following a second or third booster dose (Eskola *et al* 1985, Lepow *et al* 1987, Greenberg *et al* 1991). An efficacy study conducted in the USA by Greenberg et al. reported a protective efficacy of 88% (95% CI=42% to 97%) of the aforementioned vaccine in children aged 18-59 months of age (Greenberg *et al* 1991). Older children achieved high concentrations of anti-PRP antibody even after a single dose (Greenberg et al 1991, Lepow *et al* 1987). Although most children developed high antibody levels, the immune response in children 2 to 6 months of age was found to be poor, even after three doses (Eskola *et al* 1985). Although PRP-D was highly efficacious in Finnish infants, it did not have statistical significant efficacy in Native Alaskan infants (Ward *et al* 1990), who are a high risk group. Thirteen of 32 episodes of invasive disease occurred in children

Table 1. Types of Hib conjugate vaccine.

Types of vaccine					Properties
PRP-T	**PRP-OMPC**	**HbOC**		**PRP-D**	
		With adjuvant	**No adjuvant**		
Large	Medium	Medium	Small	Medium	Polysaccharide source
10	15	10		25	Polysaccharide dose (µg)
Tetanus toxoid	OMP of *N.meningitidis*	CRM197 mutant Diphtheria toxoid		Diphtheria toxoid	Carrier protein
150	37	62		62	Protein mol wt (KD)
6-carbon	Thioether	Adipic acid	No spacer	6-carbon	Chemical linkage
None	Al (OH)2	Al (OH)2	None	None	Adjuvants
Thimerosal	Thimerosal	Thimerosal		Thimerosal	Preservative
Lyophilized	Lyophilized	Liquid		Liquid	Form
2,4,6,12-15	2,4,12,15	2,4,6	2,4,6,12-15	>15	Age of vaccination (months)
Good, after 2nd dose	Moderate, after 1st dose	Good, after 2nd dose		Moderate, after 2nd dose	Antibody response In infancy
Yes	Yes	Not recommended	Yes	Yes	Booster dose
ActHib	Pedivax	Vaxem hib	Hib Titre	Prohibit	Brands
Active	Active	Inactive		Inactive	Vaccine status

who had been immunized, with eight of these having received the full course of three vaccinations at 2, 4 and 6 months of age. Vaccine efficacy in this group of children was estimated to be as low as 35% (95% CI= -57 to 73%). It has been proposed that a higher incidence or earlier development of Hib disease in this population, or other undefined factors, may be responsible for reduced efficacy in these children (Ward 1991, Force *et al* 1992). PRP-D was licensed in the US in December 1987 (Update 1988), for use as a single dose in children 18 months of age or older and not licensed for use in infants below this age due to conflicting results of the clinical trials of the protective efficacy of this vaccine mainly in infants. However, PRP-D has been licensed for use in infants in both Germany and Iceland, countries in which the epidemiology of Hib infections is similar to that in Finland.

ii) *H. influenzae* type b oligosaccharide conjugate (HbOC) vaccine. The production methodology for HbOC involves size reduction of the PRP polymer by cleavage of the glycol functionality in the ribitol moiety using sodium metaperiodate, and then covalently linking the PRP oligosaccharide to a non-toxic mutant diphtheria toxin known as CRM_{197}, by the method of reductive amination (Funkhouser *et al* 1991, Ward 1991). A three dose course of the vaccine has been demonstrated to provide protective concentrations of antibody for a minimum period of a year in most young children, with reports in some series of protective levels lasting 2-4 years (Leston *et al* 1988, Rothstein *et al* 1991). The HbOC vaccine efficacy in population of 61,080 children from Northern California has been reported to be 84%, when infants were vaccinated at 3, 5 and 7 months of age (Black *et al* 1991). During the course of study, only three vaccine failures were reported in children who had received a single dose of vaccine, but prior to the second vaccine. Twenty six percent protective levels of antibody were observed after single dose in these children, while was 100% after three doses. Fourth dose of vaccine given at 18 months of age produced a significant booster response. In another study, HbOC vaccine was evaluated in a group of British infants, by giving the vaccine in one of two doses, either 2 pg or 10 μg, at 3, 5and 9 months of age (Tudor-Williams *et al* 1989). After a month, regardless of the dose administered, 98% of those immunized had protective levels of antibody. Similarly a study conducted on Native American children, using three dose series (6.5 μg to 15 μg) to immunize them at 2, 4 and 6 months of age, indicated similar antibody levels 2 months after the first dose in control and placebo groups. However, a booster response was observed following second and third doses of the vaccine and a month after the final dose over 90% of the children had protective concentrations of antibody. A booster dose at 15-18 months of age was recommended due to observed decline in antibody levels in the months following administration of the final dose (Leston *et al* 1988).

iii) PRP outer membrane protein conjugates (PRP-OMPC) vaccine. This vaccine contains medium sized lengths of PRP polysaccharide linked to its protein carrier; the outer membrane protein complex of group B *Neisseria meningitidis* referred to as OMPC (Funkhouser *et al* 1991, Ward 1991). The manufacturing process involves derivatization of PRP and then treatment with oxalic acid to reduce its molecular weight to approximately 80,000 daltons (Marburg *et al* 1986). Later, the outer membrane protein of N. meningitidis (OMPC) is randomly cross linked to modified PRP. PRP-OMP is highly immunogenic and in contrast to other Hib conjugate vaccines, a dramatic antibody response is seen in response to the first dose, even in infants as young as 2 months of age (Kayhty *et al* 1983). The estimate of the protective efficacy of a single dose of the vaccine was reported 100% in a group of Native American infants who were immunized at 2 and 4 months of age (Santosham *et al* 1988). According to the observations, the vaccines protective efficacy in children receiving two full doses and at 8 months after the second dose was estimated 93%. The recommendation that the booster dose be administered at 12 months was made based on these

clinical data. However, based on subsequent data on the persistence of antibodies after the two doses, the recommended age for a booster dose was changed to 12-15 months (Snood *et al* 1991, Center for Disease control and Prevention 1991).

iv) PRP tetanus toxoid conjugate (PRP-T) vaccine. In this vaccine the native large polysaccharide polymer (PRP not size modified) is linked to a tetanus toxoid protein carrier via 6 carbon linkage or carbodimide condensation (Clemens *et al* 1992). The vaccine is effective from 6 weeks of age onwards, and has been reported to be immunogenic in children with sickle cell disease and malignancy (Kaplan *et al* 1992). PRP-T is an excellent and potent vaccine with proven field efficacy in many trials (Ferreccio *et al* 1991, Watemberg *et al* 1991). In 1989, a double blind randomized clinical trial of PRP-T begun in infants in Southern California was prematurely halted in 1990 due to licensure of HbOC for infants (Shapiro & Ward 1991, Fritzell & Poltkin 1992). In this study three cases of invasive Hib infections occurred among the controls and none occurred among the vaccines. Later, in 1991 another group of researchers from Oxford estimated the protective efficacy of the vaccine to be 95% (95% CI=75% to 100%) (Booy *et al* 1994). Only one vaccine failure was identified in immunized children while 18 vaccine failures were seen in control children followed for 20 months. These results were achieved without the use of a booster dose in the second year. Consequently, in 1993 PRP-T was evaluated by FDA based on its immunogenicity and was licensed for use in infants and older children. Reports from South America and Africa indicated that PRP-T conjugate vaccine is equally immunogenic in young children from developing countries. Lagos et al. (1996) reported an estimated vaccine efficacy of 92% in Chilean children who completed a 3 dose schedule at 2, 4 and 6 months. In 1997, a double blind randomized trial in the Gambian children was conducted, which stated the protective efficacy of the vaccine in the children immunized with PRP-T conjugate vaccine at 2, 3 and 4 months to be 95% (Mulholland *et al* 1997, Garcia *et al* 2012). Table 2 indicates the prelicensure trials of the protective efficacy of the four conjugate vaccines in fully vaccinated infants.

Table 2. Protective efficacy of conjugate Hib vaccines in fully vaccinated infants.

Vaccine	Approximate age intervals of follow-up (months)	No of Hib infections/ No of subjects		Protective Efficacy (%)
		Vaccines	Controls	
PRP-D	6.4-17.3	4/58000	39/56,000	90
PRP-T	6.7-20+	7/915	12/883	90
HbOC	8.5-18	0/12,949	12/11,335	100
PRP-OMPC	4-18	1/1913	14/1929	93

Combined vaccines. A combined vaccine is one which contains two or more vaccines administered in a single injection (Ellis et al 1994, Begg et al 1995). Combination vaccines available for many years include diphtheria and tetanus toxoids and whole-cell pertussis vaccine (DTwP); measles-mumps-rubella vaccine (MMR); and trivalent inactivated polio vaccine (IPV). It has been demonstrated that combined vaccines are acceptable to both health professionals and parents, and can be as safe and as effective in preventing disease as their component vaccines. Clinical evaluation of new combination vaccines containing Hib conjugate vaccine, such as Hib-DTP, Hib-DTP-IPV and Hib-DTP-HBV-IPV have been conducted (Scheifele *et al* 1992, Black *et al* 1993, Gold et al 1994, Jones *et al* 1998, Paradiso *et al* 1993). Begg and colleagues (1995) compared the safety and immunogenicity of giving combined PRP-T/DTP and HbOC/DTP vaccines with giving the combinations separately at different sites using the standard UK immunization schedule (at 2, 3 and 4 months of age). The protective levels of anti-PRP antibody were attained with both types of combined Hib conjugate vaccines and that no significant differences in post-vaccination titers were seen between the four different groups. According to their reports, the combined vaccine did not result in increased adverse reactions. Similarly, Jones and his colleagues (1998), using a randomized controlled trial approach, reported

similar results for a study conducted in a different part of the UK. Results from these studies and several others worldwide indicate that there is a reduction in the anti-PRP antibody response as well as tetanus and pertussis antitoxin responses in combined vaccines containing PRP-T or PRP-D and DTP (Ferreccio *et al* 1991, Watemberg *et al* 1991, Scheifele *et al* 1992, Black *et al* 1993, Paradiso *et al* 1993, Gold *et al* 1994, Jones *et al* 1998). Reasons for these reduced responses are not known but it has been proposed that it may be due to carrier suppression (Murphy 1997). The clinical significance of the reduced response to combined vaccines is yet to be determined. In future, combination vaccines might include increasing numbers of components in different arrays to protect against these and other diseases, including hepatitis A, *Neisseria meningitidis*, *Streptococcus pneumoniae*, and varicella. With more and more parenteral vaccines on the horizon, the reasons for combining a number of these vaccines into a single injection becomes attractive (Paradiso *et al* 1993, Gold *et al* 1994, Lagos *et al* 1996, Mulholland *et al* 1997). Combination vaccines not only reduce the number of visits of infant and parent and also the number of injections, but from the public health perspective, a reduction in the number of inoculations would reduce the cost of administration, be more practical and contribute to improved vaccine uptake (Murphy 1990, Blanchard & Pollard 2008).

Vaccination schedule for Hib conjugate vaccines. It is recommended that all infants including those born prematurely should receive a primary series of Hib conjugate vaccine, beginning at 2 months of age. The number of doses in the primary series depends on the type of vaccine used and the booster dose is recommended at 12-15 months regardless of the vaccine used for the primary series. The recommended interval between primary series doses is 8 weeks with a minimum interval of 4 weeks (Fitzwater *et al* 2010). Hib conjugate vaccine is not recommended for infants younger than 6 weeks of age as it may induce immunologic tolerance to subsequent dose of Hib vaccine. The number of doses of Hib conjugate vaccine required by a child depends on the current age and all children 15-59 months of age need at least one dose of this vaccine. Hib vaccine may be given simultaneously with all other vaccines.

Conclusion

Prior to the licensing of the conjugated Hib vaccine in 1987 and its widespread use by 1990, Hib was estimated to cause 27,000 annual cases of meningitis in developed countries and 330,000 in developing countries. Data from 11 countries in Africa, the Middle East, and Asia showed rates of Hib meningitis from >50 cases per 100,000 children >5 years in Ghana and Uganda to <15 per 100,000 in Iran, Jordan, and Uzbekistan. By October 2011, 171 of the 193 Member States (89%) had adopted the vaccine in their routine immunization programmes. As a consequence, invasive Hib disease is practically eliminated in many industrialized countries and its incidence dramatically reduced also in many parts of the developing world. The decline in incidence seen following the introduction of the vaccine has been observed not only in immunized children but also in unimmunized children. This phenomenon, known as the herd immune effect, occurs as a result of the presence of immune individuals in the population providing indirect protection to non-immune individuals. The WHO and the Children's Vaccine Initiative (CVI) have recommended that Hib conjugate vaccines be included in infant immunization programs, and are currently considering how best to incorporate these vaccines into the EPI program. In Iran, however routine vaccination against Hib has yet not been fully defined in its National Immunization Program. Although, i*n a study conducted on cost-benefit* and *cost-utility* of running an Hib vaccination program in the country has indicated that vaccination against Hib is a *cost*-effective health intervention and allocating resources for routine vaccination against Hib seems logical.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted review article.

Conflict of Interests

I hereby declare "no conflict of interest exists" regarding my submitted review article.

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