Development of polysaccharide-based conjugate vaccine against *Haemophilus influenzae* type b infection

Adriansjah A.
Bio Farma, Indonesia
May 15 2007
Haemophilus influenzae

- Gram-negative, coccobacillus bacterium
- Six serotypes (a – f) of encapsulated strains
  - *H. influenzae* type b (Hib) responsible for over 90% infections in children under age of 5 years
- Present in nose and throat, transmitted in droplet through sneezing and coughing.
- Clinical manifestations of Hib infections
  - Bacterial meningitis
    - 40% cases in developing countries fatal
  - Pneumonia
  - Epiglottitis
  - Septicaemia
Public Health aspects of Hib disease

- 3 million cases of severe Hib disease (meningitis and pneumonia) per year predominantly in developing countries
- > 400,000 deaths/yr (predominantly young children)
- Risk of disease highest for children 6 months – 2 years of age
Hib meningitis and pneumonia burden

Estimated rate (children < 1)
- > 200/100,000
- 100-200/100,000
- 45-100/100,000
- < 45/100,000
Immunization against Hib

• Immunization is one of the most successful and cost-effective health interventions in reducing morbidity and mortality

• Introduction of Hib vaccines in the market
Hib vaccine

• Initial studies have demonstrated that antibody to capsular polysaccharide of *H. influenzae* type b, polyribosyl-ribitol-phosphate (PRP), was the primary component of immunity

• Hib polysaccharide (PRP) vaccine available in 1985 - 1988
Structure of PRP

*H. influenzae b*

\[ \rightarrow \text{O)}-\text{P-(O} \rightarrow \text{3)}-\beta\text{-D-Ribf-(1} \rightarrow \text{1)}-\text{D-Ribol-(5} \rightarrow \text{)} \]

\[ \text{C}_{10}\text{H}_{19}\text{O}_{11}\text{P} = 346.228 \]

\[ \text{C}_{10}\text{H}_{18}\text{NaO}_{11}\text{P} = 368.210 \]
**Haemophilus influenzae** type b polysaccharide vaccine

- Not effective in children <18 months of age
- Effectiveness in older children variable
- Poor immunogenicity and efficacy of PRP vaccine due to T-cell-independent nature of PRP as an immunogen
- Failure to stimulate T-cell interaction led to little or no primary immunologic response in infant immune systems and failure to prime cells for subsequent booster response to additional doses.
Polysaccharide conjugate vaccines

- Stimulates T-dependent immunity
- Enhanced antibody production, especially in young children
- Repeat doses elicit booster response
Hib conjugate vaccine

• Derived from the *Haemophilus influenzae* type b polysaccharide (PRP) covalently coupled (conjugated) to a protein carrier

• Numerous Hib conjugate vaccines available eg.:
  – PRP-tetanus toxoid (PRP-T)
  – PRP-diphtheria toxoid (PRP-D)
Impact of Hib conjugate vaccination on Public Health

• Wide spread use of Hib conjugate vaccine in industrialized countries has virtually eliminated the Hib disease from Western Europe, North America, Australia, New Zealand

• The Gambia trial shows that Hib conjugate vaccine is similarly effective (90% against meningitis) in a developing-country setting
Global status of countries using Hib vaccine in national immunization system, 2005

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Routine Hib implementation status

- Orange: Yes
- Pink: GAVI/Vaccine Fund Supported
- Gray: No

Source: WHO/IVB database, 2005
Data as of August 2005
Problems in introduction of Hib vaccine in developing countries

• Hib disease is not perceived to be a major problem: under-recognition of disease burden
• Vaccine is too expensive
• Competing EPI targets like polio eradication, measles, increase vaccine coverage, HBV, neonatal tetanus, etc
WHO position paper

- WHO position paper on Hib conjugate vaccine, Nov. 2006:
  “Hib conjugate vaccines should be included in all routine infant immunization programmes. Lack of local surveillance data should not delay the introduction of the vaccine, especially in countries where regional evidence indicates a high disease burden.”
Development of Hib conjugate vaccine in Indonesia

• Bio Farma has developed Hib conjugate vaccine in anticipation of demand particularly in Indonesian market.
Bio Farma

- State-owned enterprise under the auspices of the Ministry of State-owned Enterprise.
- Sole vaccine manufacturer in Indonesia
- Mission:
  - Support National Immunization Program through supply of vaccines
  - Provide shareholder (GOI) with dividends
- One of a few WHO prequalified vaccine manufacturers in the world
- Export vaccines through Unicef and other countries
Bio Farma

- Core products:
  - DTP, TT, DT, BCG
  - OPV, Measles, Hepatitis B
  - Combination vaccines: DTP-HepB

- Other products:
  - ATS
  - ADS
  - ASV
Production of Hib conjugate vaccine

Production involves the following steps:

- Production of polysaccharide (PRP)
  - Cultivation of *H. influenzae* type b in fermentors
  - Isolation/purification of PRP

- Conjugation of PRP to carrier protein
  - Basically the adhesion of PRP to carrier protein
  - Numerous conjugation chemistries have been used

- Formulation

- Filling
Overview of Conjugation Technology

Native polysaccharide → Activated polysaccharide

Carrier protein → Activated carrier protein

Carrier protein chemistry

Non activated carrier protein

Carbohydrate activation chemistry

Conjugation chemistry

Final conjugate
Conjugation Chemistry

- Types of PRP activation chemistry
  - Cyanogen bromide activation
  - Periodate oxidation
  - Etc.

- Types of carrier protein
  - Tetanus toxoid
  - Diphtheria toxoid
  - CRM 197 (a genetically detoxified variant of diphtheria toxin)
Hib conjugate production based on cyanogen bromide activation

• Basic steps
  – Activation of PRP with Cyanogen Bromide (CNBr)
  – Spacer Adipic Acid Dihydrazide (ADH)
  – Carrier protein (Tetanus Toxoid – TT) preparation
  – Coupling of modified PRP with TT by 1-ethyl-3(3-dimethylaminopropal) carbodiimide (EDAC)
Flow Scheme of Conjugation Process

1. PRP solution
2. Concentration
3. Activation with CNBr
4. Addition of Linker (ADH)
5. Purification (PRP-ADH)
6. EDC
7. Conjugation (PRP-ADH-TT; PRP-ADH)
8. Purification
9. Formulation
10. Hib conjugate vaccine
Cyanogen Bromide Activation Chemistry

R-OH $\xrightarrow{\text{CNBr}}$ R-O – C≡N $\xrightarrow{\text{ADH}}$ R-O $\xrightarrow{\text{EDAC}}$ \text{protein carrier}
Conjugation of PRP-ADH with TTd HP-GPC analysis (UV detection)
Flow chart of Hib production

Cultivation of Hib
↓
Harvesting and Inactivation
↓
Concentration of culture supernatant
↓
Concentrated Bulk PRP
↓
Purification
↓
Purified PRP
↓
Conjugation and Purification
↓
Concentrated Bulk Conjugate
↓
Formulation
↓
Formulated final Bulk
↓
Final Product

Test on Purified PRP

Test on Bulk conjugate

Purified Tetanus Toxoid

Test on final Bulk

Test on Bulk conjugate
FLOW CHART Hib PRODUCTION

1. Hib CULTIVATION

2. SEPARATION

3. CONCENTRATED PRP CRUDE

4. PRP PURIFICATION

5. PRP CONJUGATION WITH TETANUS TOXOID

6. PURIFICATION OF Hib CONJUGATE

FORMULATION
Evaluation of Hib vaccine developed

- Stability testing completed
  - stable for at least 2 yrs
- Preclinical studies completed
  - Product safe
- Phase 1 clinical trial of Hib vaccine in adults completed
  - To assess safety, local and systemic reactions, adverse events following immunization
  - To assess sero-protectivity
- Clinical trial of pentavalent DTP-HepB-Hib vaccine in infants
Result of phase 1 clinical trial in adults

- Hib conjugate vaccine (produced by Bio Farma) is safe and immunogenic
- All subjects (32 individuals) were sero-protected
- No local nor systemic reactions found 72 hours and 4 – 28 days after immunization.
Clinical trial of pentavalent DTP-HB-Hib in infants
Summary of immunogenicity results
Investigational and control products

• Products:
  – DTP-HepB vaccine (Bio Farma)
    • Suspension
    • Composition of active ingredient per dose (0.5 ml)
      – D 20 Lf
      – T 7.5 Lf
      – P 12 OU
  – Hib conjugate vaccine (Bio Farma)
    • Lyophilized
    • Composition of active ingredient per dose (0.5 ml)
      – PRP-T 10 ug

• Cocktailed/seperated
# DTP-HepB-Hib clinical trials (1)

<table>
<thead>
<tr>
<th>Trials</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study site</td>
<td>Bandung, West Java</td>
<td>Bandung, West Java</td>
</tr>
</tbody>
</table>
DTP-HepB-Hib clinical trials (2)

<table>
<thead>
<tr>
<th>Trials</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Open labeled</td>
<td>Open labeled randomized</td>
</tr>
<tr>
<td></td>
<td>Non randomized</td>
<td>Single blind, controlled</td>
</tr>
<tr>
<td></td>
<td>Non controlled</td>
<td></td>
</tr>
<tr>
<td>Sample Size</td>
<td>25 infants 8-11 months of age</td>
<td>50 infants, each group 6-11 months of age</td>
</tr>
</tbody>
</table>
DTP-HepB-Hib clinical trials (3)

<table>
<thead>
<tr>
<th>Trials</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Objective</td>
<td>To assess the safety</td>
<td>To assess the safety &amp; immunogenicity</td>
</tr>
<tr>
<td>Immunization schedule</td>
<td>3 doses with interval 4 weeks</td>
<td>3 doses with interval 4 weeks</td>
</tr>
</tbody>
</table>
Study design
Clinical Trial Phase 2

Infant age | 0 Mo | 2 Mo | 3 Mo | 4 Mo
---|---|---|---|---
Randomization

DTP-HB / Hib
DTP-HB / Hib
Subjects who completed the trials

<table>
<thead>
<tr>
<th>Trials</th>
<th>DTP-HB-Hib Phase I</th>
<th>DTP-HB-Hib Phase II (A)</th>
<th>DTP-HB+Hib Phase II (B)</th>
<th>DTP-HB Phase II (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects involved in the trial</td>
<td>25</td>
<td>48</td>
<td>50</td>
<td>140</td>
</tr>
<tr>
<td>Subjects completed the trial</td>
<td>24</td>
<td>48</td>
<td>48</td>
<td>133</td>
</tr>
<tr>
<td>N of drop out subjects</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>
# Overall results

<table>
<thead>
<tr>
<th>Protective antibody level</th>
<th>Phase 1 DTPHepB-Hib</th>
<th>Phase 2 DTPHepB-Hib</th>
<th>Phase 2 DTPHepB+Hib</th>
<th>DTPHepB</th>
</tr>
</thead>
<tbody>
<tr>
<td>D &gt;0.01 IU/ml N (%)</td>
<td>24 (100)</td>
<td>48 (100)</td>
<td>48 (100)</td>
<td>128 (96.24)</td>
</tr>
<tr>
<td>T&gt; 0.01 IU/ml N(%)</td>
<td>24 (100)</td>
<td>48 (100)</td>
<td>48 (100)</td>
<td>133 (100)</td>
</tr>
<tr>
<td>P&gt; 1/40 N(%)</td>
<td>24 (100)</td>
<td>41 (85.4)</td>
<td>45 (93.8)</td>
<td>115 (86.47)</td>
</tr>
<tr>
<td>HepB &gt;10 IU/ml N(%)</td>
<td>23 (96)</td>
<td>46 (95.8)</td>
<td>45 (93.8)</td>
<td>129 (96.9)</td>
</tr>
<tr>
<td>Hib &gt;0.15µg/ml N(%)</td>
<td>24 (100)</td>
<td>45 (93.7)</td>
<td>46 (95.83)</td>
<td>-</td>
</tr>
</tbody>
</table>
Conclusion

• This DTwP-Hep B-Hib vaccine for primary immunization was generally well-tolerated and provided immune response adequate as a strategy to reduce the number of injections.
Conclusions

• Hib conjugate vaccine manufacture requires complex technology

• Hib conjugate vaccine produced by Bio Farma has proven to be safe and efficacious

• Development of pentavalent (DTP-HepB-Hib) vaccine (combination vaccines) inline with WHO’s strategy:
  – Reduce number of injections
  – Reduce drop-outs

• Availability of pentavalent vaccine by Bio Farma and expanding the market size in the world may lead to lower Hib vaccine costs
Thank you for your attention