Quality and Immunogenicity of MenAfriVac, a Conjugate Vaccine Against Meningococcal Group A Disease

C. Mattick1, A. Martino1, C. Tiengwe2, I.M. Feavers1, S. Viviani2, D. Crane1, K. Burkin1 and B. Bolgiano1

1National Institute for Biological Standards and Control, U.K.; 2Meningitis Vaccine Project, PATH, France.

Introduction

Bacterial meningitis causes significant morbidity and mortality worldwide. The highest burden of meningococcal disease affecting children under the age of 15, occurs within the meningitis belt in sub-Saharan African countries. Major epidemic outbreaks have occurred in Burkina Faso, Chad, Ethiopia, Niger and Nigeria which have been attributable mainly to Neisseria meningitidis serogroup A (Fig.1).

The Meningitis Vaccine Project (MVP), a partnership between WHO and PATH, aims to eliminate epidemic meningitis in Africa. Development of a monovalent MenA polysaccharide- tetanus toxoid (TT) conjugate vaccine, MenAfriVac, has shown promising results in phase I trials and is currently undergoing Phase II. The object of this study was to analyze the quality and immunogenicity of MenAfriVac. The effects of adjuvant binding and the addition of an extra booster dose of polysaccharide/conjugate are also investigated in a mouse model.

Fig 1 - Global distribution of N. meningitidis serogroup A: November 2006-June 2007 Epidemic Season

Methods

Vaccines:

Developmental MenA-TT bulk and MenA polysaccharide are labelled ‘dev bulk’ and ‘native PS’, respectively; MenA-TT clinical Phase I/II, Phase I/II‘c’ and Phase I/II’c’- containing AlPO4, clinical Phase II batch, ‘Phase II’, was tested with and without AlPO4 adjuvant.

Molecular Sizing:

Vaccine molecular size was determined by HPLC Size-Exclusion Chromatography (SEC) with 18-angle laser light scattering (Wyatt Technology). Samples of 50 μg saccharide & TT or 65 μg conjugate (PS-prox) were isotropically eluted in PBS pH 7.4 on a TSK 5000PWXL analytical column. UV absorbance at 280 and 214 nm, refractive index and light scattering signals were collected and used to determine Mw.

Immunochemistry:

Female Balb/c mice (10/group) were immunised with a 1 µg/dose of vaccine following a 3-dose schedule, with injections at 0, 14, 28 days and serum samples taken 14 days after each vaccination. Total MenA-specific IgG response was quantified by ELISA and the mean geometric titre calculated. Vaccine protection induced in the female Balb/c mice (10/group) were immunised with a 1 µg/dose of vaccine following a 3-dose schedule, with injections at 0, 14, 28 days and serum samples taken 14 days after each vaccination. Total MenA-specific IgG response was quantified by ELISA and the mean geometric titre calculated. Vaccine protection induced in the

Results

Molecular Sizing

Fig 2 - Sizing Profile of MenA-TT from 90°Light Scattering (black) and RI (red) detectors.

Table 1 - Weight-Average Molecular Weight of MenA-TT and Components

<table>
<thead>
<tr>
<th>Sample</th>
<th>dextrin used</th>
<th>Mw (Tris)</th>
<th>g/mol</th>
<th>Mw (Tris)</th>
<th>g/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>MenA-TT</td>
<td>0.190 (Jumal)</td>
<td>1.8 x 10⁶ (2%)</td>
<td>0.190 (Jumal)</td>
<td>1.8 x 10⁶ (2%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.256 (Jumal)</td>
<td>1.8 x 10⁶ (2%)</td>
<td>0.256 (Jumal)</td>
<td>1.8 x 10⁶ (2%)</td>
<td></td>
</tr>
<tr>
<td>MenA PS</td>
<td>0.150 (assumed)</td>
<td>1.60 x 10⁶ (1%)</td>
<td>0.150 (assumed)</td>
<td>1.60 x 10⁶ (1%)</td>
<td></td>
</tr>
</tbody>
</table>

Immunogenic response to MenA-TT vaccine

Fig 3 - Total IgG and Bactericidal Titres with 1, 2 and 3 Doses of MenA-TT Phase II/AlPO4

Total IgG and bactericidal titres exhibited a similar correlation after receiving three separate doses of the Phase II MenA-TT vaccine with adjuvant (Fig 3). Titres significantly increased (p<0.05) upon additional vaccination with a second dose. After the third dose the response appeared to increase slightly, but this did not reach statistical significance.

Flash-to-Batch Consistency

Fig 4 - Mouse Immune Response to MenA PS and MenA-TT Developmental/Clinical Batches

Total IgG and bactericidal responses were consistent among various MenA-TT vaccine formulations (Fig.4). Vaccination with native MenA PS alone elicited a significantly lower IgG response.

Adjuvant Effects

Fig 5 - Effect of Adjuvant on MenA-TT Vaccine Response

The inclusion of adjuvant induced a higher MenA-specific total IgG and bactericidal response after two doses compared with the control vaccine lacking adjuvant (Fig 5).

Table 2 – Percentage Adsorption of MenA-TT Conjugates and Carrier Protein to AlPO4

<table>
<thead>
<tr>
<th>Sample</th>
<th>Buffer</th>
<th>pH</th>
<th>% Adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>MenA-TT</td>
<td>Saline</td>
<td>5.4 – 5.6</td>
<td>58%</td>
</tr>
<tr>
<td>TT</td>
<td>PBS</td>
<td>4.3 – 4.4</td>
<td>1%</td>
</tr>
<tr>
<td>MenA PS</td>
<td>PBS</td>
<td>7.1</td>
<td>12%</td>
</tr>
</tbody>
</table>

Binding of the conjugate to adjuvant was measured in Tris-saline (formulation), and PBS (buffer for immunogenicity studies.) Binding to AlPO4 was higher in Tris than in PBS for both TT and conjugate.

Booster Dose

Fig 6 - Effect of a Delayed Booster Dose of Conjugate or Polysaccharide

A fourth dose of conjugate vaccine at 26 or 38 weeks, elicited a higher IgG and functional antibody response compared to polysaccharide with a significant increase above the pre-booster levels (Fig 6).

A polysaccharide booster at 36 weeks led to a further increase in functional antibody titre.

Conclusion

- The MenA-TT Phase II vaccine with adjuvant generated significantly elevated total IgG and bactericidal responses after two doses. A third dose did not significantly boost the response further.
- Immunogenicity data demonstrated consistency among clinical phase I and II conjugate vaccine formulations.
- Persistence of antibody and the ability to boost the response with either polysaccharide or conjugate up to 8 months were demonstrated.

References