Comparison and correlation of Neisseria meningitidis serogroup A immunologic assay results following one dose of Meningococcal serogroup A conjugate vaccine in healthy Indian adults

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Introduction

The Meningitis Vaccine Project (MVP) has been created in 2001 by a grant from the Bill and Melinda Gates Foundation as a partnership between WHO and PATH to develop and introduce affordable meningococcal conjugate vaccines for elimination of meningococcal epidemics in African countries [1].

A serogroup A meningococcal conjugate vaccine using tetanus toxoid as a carrier protein (PsA-TT) has been developed at the Serum Institute of India Ltd., using a new conjugated vaccine technology from Biometrics Evaluation and Research/Food and Drug Administration. Immunogenicity and antibody persistence and safety in healthy volunteers aged 18-35 years was demonstrated in a double-blind, randomised controlled phase I study [2].

Khosravi et al. [2] reported immunogenicity as determined by SBA using baby rabbit complement and standardised ELISA for capsular antigen total IgG.

The aim of the study was to analyse sera by additional immunological assays, in order to investigate the relationship between different serogroup A immunologic assays.

Methods

Study group

Healthy adult volunteers (18-35 years) were recruited from three sites within India [2] and randomised in a double-blind fashion to receive intramuscularly in the deltoid region one dose of

- PsA-TT conjugate vaccine (Serum Institute of India, Pune, India)
- Meningococcal polysaccharide Vaccine ABC/cTM (Sanofi Pasteur, Lyon, France, PSI)
- Tetanus Toxoid AdsorbedTM (SII, Pune, India)

Blood samples were taken for immunogenicity studies on the day of immunisation and 4, 24, and 48 weeks later.

The immune response to PsA-TT and PsAC was compared in 6 immunological assays. Data from the Tetanus Toxoid vaccine group is not included in this analysis

Immunological assays

- Serum bactericidal antibody (SBA) assay
- SBA assays were performed against the serogroup A target strain, CDC no FB382 (phenotype A-4211-20.9) [3]
- Complement source used was either:
  - serum from 2-4 week old rabbits (Pel-Freez Biologicals, WI) [Performed at HPA, Manchester, UK]
  - pooled human complement (from individual serum with no intrinsic killing of FB382, SBA titres ≥ 8 and total IgGAM < 30 μg/mL) and showed no intrinsic complement activity against FB382 and showed preservation of complement activity as determined by CH50 titres as described by CH50 titres (performed at PDA, Bethesda, USA)
- SBA titres were expressed as the reciprocal serum dilutions yielding ≥ 50% killing after 60 min for SBA and 90 min for hSBA

- Serogroup A-specific IgG

Serogroup A-specific IgG levels were determined using:

- standardised ELISA using CDC 1992 and mouse monoclonal-PAN anti-human IgG Fc labelled with horseradish peroxidase [4] performed at CDC, Atlanta, GA, USA
- Multiplex bead assay [5] performed at HPA, Manchester

Briefly, meningococcal serogroup A polysaccharide was attached to poly-L-lysine and then covalently attached to carboxylated microspheres using a two-step carbodimide reaction. Serum and beads were incubated for 20 min at room temperature of a plate shaker followed by washing and incubation with R-phycocerythrin-conjugated anti-human IgG. The plate was then read on a Bio-plex reader and data analysed using Bio-plex Manager. Data for unknown test serum was generated from a 5-parameter logistic standard curve of CDC 1992 and converted to pg/mL

- Oropharyngocytic activity (OPA)

The oropharyngocytic activity (performed at the CDC) was measured by incubating serogroup A polysaccharide conjugated to fluorescent beads, with serum dilutions followed by the addition of baby rabbit complement and HU60 cells which had been chemically induced into monocytess [6].

Results

• Titres were reported as the reciprocal of the highest serum dilution yielding ≥ 50% of the maximum phagocytic uptake (calculated from a 5-FL curve of % uptake vs dilution).
• Samples with a maximum phagocytic uptake of ≥20% were considered negative and were reported to have a titre of 4.
• Oropharyngocytic activity (performed at the CDC) was measured as the respiratory burst using human complement and live serogroup A strain FB382 [7]. The highest reciprocal serum dilution giving ≥ 50% respiratory burst of the PMNs is recorded as the serum titre.
• Data analysis

Results from all assays, at each time point were log transformed and geometric means calculated with 95% confidence intervals (95% CI).
• Differences in GMTs or GMCs between groups were expressed as ratios with 95% CI.
• GMTs and GMCs between time points were significantly different when the confidence intervals did not include 1.0.
• The relationship between the different immunological assays was determined using the Pearson correlation coefficient.

Table 1. SBA

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<thead>
<tr>
<th>Groups</th>
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<th>95% CI</th>
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Table 2. Serogroup A-specific IgG

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Conclusions

• The proportion of subjects determined to be putatively protected by hSBA (titres ≥ 4) and SBA (titres ≥ 8) and also ≥ 4 fold rises similar.
• Four weeks post-vaccination the vaccine groups were the same (74.6% for PsA-TT and 72.7% for PsAC).

Table 3. OPA

<table>
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<th>Vaccine</th>
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The CDC OPA assay showed a significant decline pre- to 48 weeks post-vaccination (p<0.001).

Four weeks post-vaccination there was a significant difference between the CDC OPA GMT of the two vaccine groups (p=0.03) which was not seen with the CDC OPA GMTs (p>0.05) (data not available at 1 year for hSBA).


Figure 1. Percentage of subjects before and 4 weeks following vaccination with either PsA-TT in serum bactericidal titre cutoffs of <4, 8, <16, ≥ 128 and ≥ 256

4 weeks post-vaccination the vaccine groups differed by hSBA titres (p=0.01) although there was not seen with the rSBA GMTs (p=0.05) which was not seen with the rSBA GMTs (p=0.05) (data not available at 1 year for hSBA).

There was a good correlation between subjects demonstrating a four-fold rise in the hSBA and SBA for both groups (71.4% for PsA-TT and 72.7% for PsAC).

Figure 2. Correlation of serogroup A-specific IgG determined by ELISA and multiplex bead assay (both vaccine groups and all visits combined).

The proportion of subjects with putatively protective titres (hSBA ≥ 8) as determined by both SBA assays were similar regardless of the vaccine administered.

Table 4. Pearson correlation coefficients for the PsA-TT (white shading) and PsAC (values shaded grey) vaccine groups

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Correlations between immunological assays were calculated for all visits combined therefore covering a wide range of antibody levels.

There was a strong correlation between serogroup A-specific IgG (by either method) and the hSBA in the PsA-TT group.

The two OPA methods correlated weekly in both vaccine groups. For both OPA methods there was no correlation to any other immunological assay.

References