Safety, Reactogenicity, and Immunogenicity of a Tetravalent Meningococcal Polysaccharide–Diphtheria Toxoid Conjugate Vaccine Given to Healthy Adults

James D. Campbell,1 Robert Edelman,1 James C. King, Jr.,1 Thomas Papa,2 Robert Ryall,2 and Margaret B. Rennels1

Healthy adults, 18–55 years old, were immunized once with a tetravalent (serogroups A, C, Y, and W-135) meningococcal vaccine conjugated to diphtheria toxoid at 1 of 3 doses and were monitored for safety, reactogenicity, and immunogenicity. No immediate reactions were observed. Only 1 of 89 subjects reported fever; only 1 reported any severe reactogenicity (local pain/soreness, chills, arthralgia, anorexia, and malaise). For each serogroup and in each dose group, the geometric mean serum bactericidal antibody (SBA) titer and immunoglobulin G concentration increased after immunization. In the 4- and 10-μg dose groups, all subjects had SBA titers ≥8 against serogroups A and C, and 89% and 93% of subjects had SBA titers ≥8 against serogroups Y and W-135, respectively. The A, C, Y, and W-135 Neisseria meningitidis–diphtheria toxoid conjugate vaccine, when given to healthy adults as a single intramuscular injection of 1, 4, or 10 μg/serogroup, is acceptably tolerated and immunogenic and deserves further development.

Neisseria meningitidis is a common cause of serious bacterial infection around the world. Infants, immunocompromised individuals, and young adults living in close quarters are at a higher risk for invasive meningococcal disease than the general population [1]. Annually, 2500–3500 cases of meningococcal infection are reported in the United States, for a crude population incidence rate of ∼1 case/100,000 persons/year [2].

The meningococcus has 13 serogroups, distinguished by differences in the composition of repeating polysaccharide units of the capsule. Immunologic response to these antigens is serogroup specific and is important in protection from invasive disease [3, 4]. Infections in the United States occur sporadically or in small clusters, and the most common serogroups involved are B, C, and Y [5]. In some parts of the developing world, most notably across the center of Africa, serogroups A and, more recently, W-135 cause severe epidemic disease.

The currently licensed meningococcal vaccine is a tetravalent preparation of the polysaccharide capsules of serogroups A, C, Y, and W-135. It is recommended for at-risk individuals ≥2 years old [6], but it suffers from drawbacks common to vaccines containing polysaccharide as the sole immunogen. These drawbacks, inherent in T cell–independent vaccines, have been overcome in vaccines against Haemophilus influenzae type b and Streptococcus pneumoniae by conjugation of the appropriate polysaccharide immunogen to a protein. These conjugate vaccines are safe, well tolerated, and protective.

Studies of meningococcal conjugate vaccines have been performed with adults [7], adolescents [8], toddlers [8], and infants [9]. In these and other trials, vaccines with monovalent C or bivalent A and C polysaccharide conjugated to either CRM197 or tetanus toxoid have proven to be well tolerated, compared with placebo, meningococcal polysaccharide vaccine, or routine infant vaccines. In addition, these conjugate meningococcal vaccines have elicited significant serogroup-specific serum and mucosal antibody responses, including complement-dependent, putatively protective bactericidal antibody levels. We evaluated, in healthy young adults, the safety, reactogenicity, and immunogenicity of a single immunization of 3 different doses of a tetravalent meningococcal vaccine conjugated to diphtheria toxoid.

Subjects and Methods

Subjects. Ninety healthy adults, 18–55 years old, were recruited from 2 sites in Baltimore in 1997 and 1998. They were
screened for general health according to medical history and a physical examination.

**Trial design and vaccine.** In this phase 1/2, open-label, dose-escalation study, each volunteer received a single intramuscular dose of 0.5 mL of tetravalent meningococcal conjugate vaccine (Pasteur Mérieux Connaught) in the deltoid muscle. The vaccine contained equal amounts of capsular polysaccharide from serogroups A, C, Y, and W-135 conjugated to diphtheria toxoid. The first group of 30 volunteers received vaccine containing 1 μg of each polysaccharide, the second group of 30 volunteers received 4 μg of each polysaccharide, and the third group of 30 volunteers received 10 μg of each polysaccharide. Dose escalation occurred only after the lower dose was deemed to be safe by the investigator and the sponsor. Blood was collected before vaccination and at days 4 and 28 after vaccination.

**Safety and reactogenicity.** Investigators observed subjects for 30 min after vaccination to record and treat any immediate side effects. On the day of vaccination and for the next 3 days, subjects recorded reactogenicity data, including oral temperature, in a diary. Local and systemic effects were recorded and graded on a scale from 0 to 3. A score of 0 represented no reaction; a score of 1 referred to mild awareness of symptoms, easily tolerated and not interfering with daily activities; a score of 2 referred to moderate discomfort leading to interference with some daily activities; and a score of 3 referred to severe effects that disabled the volunteer and required bed rest. Adverse events and serious adverse events were evaluated and recorded for the entire study period. Safety laboratory tests (complete blood count, blood chemistry analyses, liver function tests, and urinalysis) were done immediately before vaccination and at 4 days and 4 weeks after vaccination.

**Immunogenicity.** Serum was collected immediately before and at 28 days after immunization for evaluation of the immune response. ELISAs for IgG antibodies [10] and serum bactericidal antibodies (SBAs) [11] against each of the vaccine-associated serogroup-specific polysaccharides were performed at Pasteur Mérieux Connaught (Swiftwater, PA). The ELISA standard reference serum was CDC 1992, and the source of complement for the SBA titer was baby rabbit serum. The SBA titer is defined as the reciprocal of the highest dilution leading to at least 50% killing. The primary serologic end points for each dose group were the proportion of subjects with SBA titers ≥8, the SBA geometric mean titer (GMT), and the geometric mean concentration (GMC) of anticapsular IgG for each serogroup found in the vaccine. The percentage of subjects with a 4-fold increase in SBA titer was also reported.

**Statistical methods.** Data were analyzed by both intent-to-treat and per-protocol analysis. For adverse reactions, proportions of subjects were compared among the dose groups by 2-sided Fisher’s exact test and 2-sided Cochran-Armitage trend test. The same tests were used to compare the proportions of subjects who had an SBA titer ≥8. The pre- and postimmunization SBA titers and ELISA antibody concentrations were compared using analysis of variance (ANOVA) with multiple test comparisons and analysis of covariance (ANCOVA). P < .05 was considered to be significant.

**Results**

**Subjects.** Twenty-nine men (32%) and 61 women (68%) were enrolled in the study. The mean age of the subjects was 29.5 years (range, 19–54 years). All were analyzed for immediate postimmunization reactions, but 1 volunteer in the 1-μg group was noncompliant for subsequent reactogenicity evaluation; ensuing safety and reactogenicity were analyzed in the remaining 89 subjects. Eighty-one subjects fulfilled the criteria for per-protocol immune analysis.

**Safety and reactogenicity.** No volunteer experienced any immediate reaction to the vaccine (table 1). The proportion of subjects experiencing any local reaction to the vaccine increased with increasing dose (P = .004, Fisher’s exact; P = .001, Cochran-Armitage test for trend). The most commonly reported injection-site reactions were erythema and swelling/hardness. Most subjects who reported local reactions rated them as mild, and most local reactions resolved by the third day after vaccination. In each dose group, 41%–50% of participants reported ≥1 systemic reaction, but there was no statistical difference between dose groups (P = .8333, Fisher’s exact test). The most commonly reported postvaccination systemic events were headache and malaise. Symptoms were most often rated as mild and resolved by the third day after immunization. Only 1 subject reported fever, an oral temperature ≤38.9°C on the day after vaccination with a 10-μg dose.

Severe reactions were reported by a single volunteer. After receiving a 4-μg dose, he reported severe local pain/soreness, chills, arthralgia, anorexia, and malaise, as well as moderate local tenderness, vomiting, mild headache, and adenopathy. All symptoms resolved by day 2 without sequelae. Three other volunteers reported chills and malaise, but of only mild or

**Table 1.** Reports of local and systemic side effects in the 4 days after vaccination with tetravalent meningococcal vaccine conjugated to diphtheria toxoid, by dose level.

<table>
<thead>
<tr>
<th>Side effects</th>
<th>1-μg dose (n = 29)</th>
<th>4-μg dose (n = 30)</th>
<th>10-μg dose (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any local reaction</td>
<td>17 (58.6) [39.1–75.9]</td>
<td>25 (83.3) [64.5–93.7]</td>
<td>28 (93.3) [76.5–98.8]</td>
</tr>
<tr>
<td>Local reaction of grade &gt;1*</td>
<td>2 (6.9) [1.2–24.2]</td>
<td>3 (10.0) [2.6–27.7]</td>
<td>8 (26.7) [13.0–46.2]</td>
</tr>
<tr>
<td><strong>Systemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-grade fever, temperature &lt;39°C</td>
<td>0 (0) [0.0–11.9]</td>
<td>0 (0) [0.0–11.6]</td>
<td>1 (3.3) [0.1–17.2]</td>
</tr>
<tr>
<td>High-grade fever, temperature ≥39°C</td>
<td>0 (0) [0.0–11.9]</td>
<td>0 (0) [0.0–11.6]</td>
<td>0 (0) [0.0–11.6]</td>
</tr>
<tr>
<td>Chills</td>
<td>1 (3.4) [0.1–17.8]</td>
<td>1 (3.3) [0.1–17.2]</td>
<td>2 (6.7) [0.8–22.1]</td>
</tr>
</tbody>
</table>

*See Subjects and Methods for a description of the scoring system.

**NOTE.** Data are no. of subjects (%) [95% confidence interval].

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moderate severity. The only adverse event reported was a urinary tract infection. No serious adverse events occurred. There were no significant changes in laboratory values (complete blood count, blood chemistry analyses, liver function tests, and urinalysis) from day 0 to days 4 and 28.

Immunogenicity. Prevacination immunity to each vaccine serogroup, measured by the proportion of subjects with SBA titers ≥8, the SBA GMT, and the anticapsular IgG GMC, was not statistically different among any of the dose groups (table 2). For each serogroup and in each dose group, the SBA GMT and the GMC of IgG antibodies increased after immunization. As analyzed by serogroup and dose group, 65%–100% of subjects had a 4-fold increase in SBA titer after immunization; for each serogroup other than Y, this proportion increased with increasing doses. Before vaccination, 79 (97.5%) of 81 participants had an SBA titer against serogroup A ≥8; after vaccination, all achieved an SBA titer of 10 against serogroup A. There was a dose-dependent, statistically significant difference among groups at day 28 in antibody levels against serogroup A, both with (P < .001 for both GMT and GMC, ANCOVA) and without (P < .001 for GMT and P = .021 for GMC, ANOVA) adjustment for baseline levels.

Only 31 (38.3%) of 81 participants had prevaccination SBA titers ≥8 against serogroup C. At 28 days after vaccination, 23 (88.5%) of 26 recipients of the 1-µg dose and all recipients of the 2 higher-dose groups had SBA titers ≥8. The differences among dose groups are statistically significant (P = .03, Fisher’s exact test) and demonstrate a significant trend toward increasing SBA titers with increasing dose (P = .027, Cochran-Armitage test for trend). The differences among dose groups in SBA GMT and GMC of IgG against the serogroup C capsule were significant before (P = .041 for GMT and P = .002 for GMC, ANCOVA) and after (P = .022 for GMT and P = .003 for GMC, ANCOVA) adjustment for baseline antibody levels.

Fifty-one percent (41/81) of the volunteers had preexisting SBA titers ≥8 against serogroup Y. Overall, 71 (87.7%) of 81 subjects had postvaccination SBA titers ≥8 against serogroup Y, and there was no significant difference among the dose groups in the proportion of volunteers with titers above that threshold. Unlike the dose-dependent responses to the A, C, and W-135 capsular antigen components, there was no statistically significant difference in antibody levels among the 3 dose groups for serogroup Y.

For serogroup W-135, 74 (91.4%) of 81 subjects had postvaccination SBA titers ≥8, with no statistical difference in baseline titers among the dose groups. SBA GMTs and anticapsular IgG GMCs against W-135 after vaccination were found to be significantly different among the groups, both with (P = .022 for GMT and P = .001 for GMC, ANCOVA) and without (P = .041 for GMT and P = .026 for GMC, ANOVA) correction for baseline levels. Although all analyses were performed using specimens from the 81 evaluable subjects, intent-to-treat analysis led to similar conclusions (data not shown).

Discussion

The A, C, Y, and W-135 N. meningitidis–diphtheria toxoid conjugate vaccine, when given to healthy adults as a single intramuscular injection of 1, 4, or 10 µg of each serogroup, is acceptably tolerated and immunogenic. Although recipients reported some transient mild or moderate vaccine-related local and systemic reactions, only 1 of 89 volunteers reported any reaction graded as severe. In the 4- and 10-µg-dose groups, all subjects had SBA titers ≥8 against serogroups A and C, whereas 89% and 93% had titers higher than this threshold against serogroups Y and W-135, respectively. For serogroups A, C, and W-135, there appeared to be a dose-related effect on

Table 2. Serogroup and dose-specific immune responses to conjugate tetravalent meningococcal vaccine at days 0 and 28.

<table>
<thead>
<tr>
<th>Serogroup, dose</th>
<th>No. of subjects</th>
<th>Proportion of subjects with SBA titer ≥8</th>
<th>Proportion of subjects with 4-fold SBA titer increase at day 28</th>
<th>SBA GMT (95% CI)</th>
<th>ELISA GMC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 28</td>
<td>Day 0</td>
<td>Day 28</td>
<td>Day 0</td>
</tr>
<tr>
<td>A</td>
<td>1 µg</td>
<td>26</td>
<td>0.96</td>
<td>1</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>4 µg</td>
<td>28</td>
<td>0.93</td>
<td>1</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>10 µg</td>
<td>27</td>
<td>1</td>
<td>1</td>
<td>0.96</td>
</tr>
<tr>
<td>C</td>
<td>1 µg</td>
<td>26</td>
<td>0.42</td>
<td>0.89</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>4 µg</td>
<td>28</td>
<td>0.36</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>10 µg</td>
<td>27</td>
<td>0.37</td>
<td>1</td>
<td>0.96</td>
</tr>
<tr>
<td>Y</td>
<td>1 µg</td>
<td>26</td>
<td>0.42</td>
<td>0.85</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>4 µg</td>
<td>28</td>
<td>0.46</td>
<td>0.89</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>10 µg</td>
<td>27</td>
<td>0.63</td>
<td>0.89</td>
<td>0.67</td>
</tr>
<tr>
<td>W-135</td>
<td>1 µg</td>
<td>26</td>
<td>0.31</td>
<td>0.89</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>4 µg</td>
<td>28</td>
<td>0.54</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>10 µg</td>
<td>27</td>
<td>0.3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

NOTE: CI, confidence interval; GMC, geometric mean concentration; GMT, geometric mean titer; SBA, serum bactericidal antibody.
antibody response, with maximum proportions of seroconversions and maximum antibody levels elicited by the 10-μg dose.

SBA titers are considered to be the best serologic correlates of protection against invasive meningococcal disease [3]. In one study, 93% of adults with SBA titers ≥8, as measured with an assay using rabbit complement, were found to have an SBA titer ≥4 when titers were measured with an assay using human complement (the reference-standard protective level) [12]. In the present study, one month after receipt of a single dose of tetravalent conjugate vaccine, volunteers given either 4 or 10 μg of vaccine had high inverse GMTs of SBA (386–10,865); no immunologic follow-up beyond 1 month was performed to evaluate the durability of the serologic response.

Other meningococcal conjugate vaccines against 1–4 serogroups have been evaluated for immunogenicity and safety. These vaccines are highly immunogenic and acceptably tolerated. In November 1999, the United Kingdom introduced, in a phased fashion, single-dose monovalent meningococcal C-CRM197 conjugate into its routine vaccination program. During the first 9 months, the vaccine had 92% effectiveness and led to a 76% decrease in the number of cases of meningitis among teenagers and a 34% decrease in the number of cases of meningitis among toddlers [8]. During the same time period, there was a slight increase in cases of meningitis caused by serogroups B and C among adults beyond the target age group for immunization. Introduction in the United States of a conjugate vaccine that includes serogroups C and Y in a strategy targeting meningitis among toddlers [8]. During the same time period, there was a slight increase in cases of meningitis caused by serogroups B and C among adults beyond the target age group for immunization. Introduction in the United States of a conjugate vaccine that includes serogroups C and Y in a strategy targeting meningitis among toddlers [8]. During the same time period, there was a slight increase in cases of meningitis caused by serogroups B and C among adults beyond the target age group for immunization. Introduction in the United States of a conjugate vaccine that includes serogroups C and Y in a strategy targeting meningitis among toddlers [8].

Because the vaccine has been shown in the present study to be both safe and immunogenic, continued evaluation of tetravalent meningococcal conjugate vaccines is warranted for children, immunocompromised individuals, travelers, and those living in the developing world, particularly sub-Saharan Africa. The recent epidemics in West Africa of meningococcal meningitis due to serogroup W-135 highlight the need for a multivalent vaccine. The effect of the concomitant administration of tetravalent vaccine on immune responses to other childhood and adult vaccines must be studied further. Immune hyporesponsiveness, as is seen after second doses of meningococcal polysaccharide vaccine, has not been shown to occur with the conjugates [15, 16], but future studies must continue to evaluate this phenomenon. Finally, research on the use of combinations of single conjugate vaccines directed against multiple bacteria, such as S. pneumoniae, H. influenzae type b, and N. meningitidis, should proceed so that the addition of more injections into the infant vaccine schedule can be avoided, when possible.

Acknowledgments

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References