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A phase 1 study of a meningococcal native outer membrane vesicle vaccine made from a group B strain with deleted *lpxL1* and *synX*, over-expressed factor H binding protein, two PorAs and stabilized OpcA expression☆

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ABSTRACT

This phase I clinical trial assessed the safety and immunogenicity of a native outer membrane vesicle (NOMV) vaccine prepared from an *lpxL1*(−) *synX*(−) mutant of strain 8570(B:4:P1.19,15:L8-5) of *Neisseria meningitidis*. Additional mutations enhance the expression of factor H binding protein variant 1 (fHbp v.1), stabilize expression of OpcA and introduce a second PorA (P1.22,14). Thirty-six volunteers were assigned to one of four dose groups (10, 25, 50 and 75 mcg, based on protein content) to receive three intramuscular injections at six week intervals with aluminum hydroxide adjuvant. Specific local and systemic adverse events were solicited by diary and at visits on days 2, 7, and 14 after each vaccination. Blood chemistries, complete blood count, and coagulation studies were measured on each vaccination day and again 2 and 14 days later. Blood for ELISA and serum bactericidal assays was drawn two and six weeks after each vaccination.

The proportion of volunteers who developed a fourfold or greater increase in bactericidal activity to the wild type parent of the vaccine strain at two weeks after the third dose was 27 out of 34 (0.79, 95% C.I. 0.65–0.93). Against four other group B strains the response rate ranged from 41% to 82% indicating a good cross reactive antibody response. Depletion assays show contributions to bactericidal activity from antibodies to lipooligosaccharide (LOS), fHbp v.1 and OpcA.

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1. Introduction

The development of a vaccine against group B meningococcus has been hampered by the poor immunogenicity of the capsular polysaccharide [1] and the antigenic and phase variability of subcapsular antigens [2]. One approach that has been successfully used in the context of group B meningococcal epidemics has been to immunize with outer membrane vesicles from the epidemic strain. After detoxifying these OMV with deoxycholate, the resulting immunity, particularly in infants, is largely specific to the PorA type of the parent strain [3]. Such vaccines are therefore considered inadequate for prevention of disease by other serosubtypes.

To overcome this limitation, vaccines that include multiple subcapsular antigens are under development. OMV from PorA-negative strains have been shown to induce a serosubtype independent bactericidal response in mice [4]. Another strategy is to present multiple recombinant subcapsular antigens [5,6].

In an earlier study we showed the feasibility of using genetically detoxified (Δ*lpxL2*) native outer membrane vesicles (NOMV) to induce bactericidal activity in humans against a specific outer membrane antigen (OpcA) [7]. Here we report the results of a phase I trial of an NOMV vaccine in which the expression of multiple outer membrane antigens has been stabilized or enhanced and the LOS has been genetically detoxified (Δ*lpxL1*). We envision this vaccine as one component of a multivalent NOMV vaccine that will be effective against the majority of meningococci for which no commercially available vaccine currently exists.

2. Materials and methods

2.1. Vaccine

The vaccine in this trial consists of outer membrane vesicles from a pathogenic strain of group B *Neisseria meningitidis*, 8570(B:P4:P1.19,15:P5.C:L8-5), that has been genetically modified to decrease toxicity and increase immune responses to specific

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antigens conserved among group B meningococci, as described previously [8]. The mutations include (1) inactivation of *lpxL1* also known as *htrB*, resulting in a lipid A molecule with only five acyl chains which has reduced endotoxicity compared with the hexaacylated wild type [9]; (2) stabilization of high expression of OpcA, by replacing a 12 bp poly-C sequence in the promoter of *opcA* with a new sequence of the same length containing both C and G nucleotides; (3) insertion of a second *porA* (P1.22,14) by transformation with a plasmid construct containing *opaD* interrupted by the sequence for the second *porA* and its promoter, to extend protection to this serosubtype; (4) a deletion in *synX* to prevent capsule formation and sialylation of the LOS; (5) and insertion of a second copy of GNA1870 (factor H binding protein variant 1) (fHbp v.1) from strain 44/76 to increase levels of this antigen. These mutations and the resulting vaccine are referred to collectively by the resulting acronym, HOPS-G. This vaccine is of immunotype L8-5, which indicates the oligosaccharide branch I structure of L8 (lactose linked with the KDO-bound heptose I [10]) and the oligosaccharide branch II structure of L5 (glucose rather than phosphoethanolamine linked at the C3 position of heptose II [11]). The vaccine strain was cultured and NOMV were prepared as described previously [8] with the NOMV suspended in 0.9% sterile saline. Doses of vaccine were based on protein concentration using the Lowry method [12]. Vaccines were combined with aluminum hydroxide (Rehydragel HPA, Reheis Inc., Berkeley Heights, NJ) on the day of administration at 300 mcg of aluminum per dose

2.2. Ethics and monitoring

This clinical study was conducted under a protocol reviewed and approved by the Institutional Review Board of the WRAIR and by the Human Subjects Research and Review Board of the Surgeon General of the U.S. Army at Fort Detrick, Maryland. The study protocol was submitted to the U.S. Food and Drug Administration for review as part of Investigational New Drug application BB-IND #13648. The study was monitored for regulatory compliance and data quality assurance by the United States Army Medical Materiel and Development Activity, Fort Detrick, Maryland. After obtaining written informed consent, volunteers were screened by history, physical examination and laboratory testing to determine their eligibility for enrollment.

2.3. Protocol

The study was an open-label, staggered-start, dose-escalating phase 1 trial intended to determine the safety and immunogenicity of three intramuscularly administered doses of the HOPS-G vaccine conducted at the WRAIR Clinical Trials Center. Volunteers were sequentially assigned to one of four dosage groups, each to receive three injections of 10, 25, 50 or 75 mcg of vaccine (based on protein content) at six week intervals.

2.4. Participants

Participants were adult males and females 18–45 years of age. Exclusion criteria included history of significant organ/system disease, known or suspected immunosuppression, use of systemic steroids, recent or planned receipt of any other investigational drug or vaccine, receipt of immunoglobulin or any blood product transfusion within 3 months of study start, abnormalities on screening laboratories (complete blood count, serum chemistry profile, plasma fibrinogen level, prothrombin time and activated partial thromboplastin time, urinalysis, urine pregnancy test for women), serologic evidence of previous hepatitis B or C infection, antibody to HIV, nasopharyngeal carriage of *N. meningitidis* at screening, or baseline bactericidal antibodies >1:16 against the parent strain (8570) of *N. meningitidis*. A total of 36 individuals were enrolled, 9 for each dose group.

2.5. Adverse events

Adverse events were solicited in the form of a diary that was kept from prior to vaccination (one week prior to the first, two days prior to the second and third) to one week following each vaccination. Volunteers were asked to record their temperature, measure any injection site erythema/redness or swelling/induration/nodule, and score the following symptoms daily: injection site pain, injection site erythema/redness or swelling/induration/nodule, headache, fatigue, malaise, body soreness/aching, nausea/vomiting or other. The scale used for scoring these was: 0 = none (no appreciable reaction), 1 = mild (does not interfere with routine activities), 2 = moderate (interferes with routine activities), 3 = severe (unable to perform routine activities).

Clinical laboratory tests were checked at screening, immediately prior to each vaccination, and at two and 14 days after each vaccination. Laboratory tests included serum chemistry, liver associated enzymes, complete blood count, plasma fibrinogen, coagulation times and urinalysis.

2.6. Immunogenicity

A fourfold or greater increase in serum bactericidal activity at day 98 (two weeks after the third dose) was used as the definition of an immunologic response. Sera with less than 50% killing at a dilution of 1:2 were assigned a titer of 1.

2.6.1. Serum bactericidal activity

Bactericidal assays were done as previously described [13] using normal human serum as a source of complement. After 1 h incubation with serum and complement at 37 °C with shaking, cells were plated on GC agar with defined supplement using the drip method. Colonies were counted after overnight incubation at 37 °C. Bactericidal titers are reported as the highest dilution of serum in which 50% or more of the test strain is killed.

2.6.2. Depletion assays

The bactericidal depletion assay was performed as previously described [13] by coating wells of a 96-well plate with serial dilutions of the antigens to be tested for the capacity to deplete bactericidal antibodies followed by blocking and washing. Serum was diluted to the 50% kill endpoint and incubated in the coated wells for 4 h, followed by transfer to a fresh plate before completing the bactericidal assay as described above.

2.6.3. Characterization of strains

In order to determine which antigens play a role in cross-protection from other strains of group B meningococcus, sera were assessed for bactericidal activity against two LOS variants of the parent strain (8570), and four other strains (44/76, 298I, 99M and 9162). These strains were characterized with respect to the mutations introduced into the vaccine strain, namely expression of fHbp v.1, OpcA, serosubtype (PorA) and immunotype (LOS) (Table 1).

2.6.4. Measurements of antibody levels to individual antigens

Levels of antibodies to recombinant fHbp v.1, purified OpcA, L3,7 LOS and L8-5 LOS as well as total IgG and IgM antibody to the vaccine NOMV were determined by quantitative enzyme-linked immunosorbent assay (ELISA) [14,15].