Vaccine 30 (2012) 712–721

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Phase I study of a *Neisseria meningitidis* liposomal vaccine containing purified outer membrane proteins and detoxified lipooligosaccharide☆

Wendell D. Zollinger*,[2], Janiine G. Babcock[1], Elizabeth E. Moran, Brenda L. Brandt[1], Gary R. Matyas, Nabila M. Wassef[1], Carl R. Alving

The Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD 20910, United States

ARTICLE INFO

Article history:
Received 22 October 2011
Received in revised form
16 November 2011
Accepted 21 November 2011
Available online 3 December 2011

Keywords:
Neisseria meningitidis
Vaccine
Liposome
Lipooligosaccharide
Human

ABSTRACT

Purified outer membrane proteins and purified deacylated lipooligosaccharide (dLOS) were formulated for use as a vaccine in three formulations for clinical use. The three vaccine formulations included (1) purified outer membrane proteins (OMPs) and L8-5 dLOS adsorbed to aluminum hydroxide; (2) purified OMPs and L8-5 dLOS incorporated into liposomes; and (3) purified OMPs and L7 dLOS incorporated into proteoliposomes. The vaccines were compared for immunogenicity and safety in a phase 1clinical study. Ten adult volunteers were vaccinated with each of the three vaccine formulations. Two 50 μg doses were given six weeks apart, and serum samples were obtained at 0, 2, 6, 8 and 14 weeks. Volunteers were evaluated for reactogenicity 30 min after vaccination and at days 1, 2, and 14 after each vaccination, and laboratory safety tests were done at 0, 2 and 6 weeks. Overall, the vaccines were well tolerated. Bactericidal assays against a homologous strain showed a four-fold or greater increase in titer in 6 of 7 volunteers in group one, 9 of 10 volunteers in group two, and 5 of 10 volunteers in group three. A quantitative enzyme linked immunosorbant assay showed increases in antibody against both OMPs and LOS antigens. The liposome formulation appeared to be particularly effective in presenting the dLOS as an antigen.

1. Introduction

A variety of different strategies have been employed over a period of more than four decades in attempts to develop an effective vaccine for group B *Neisseria meningitidis*. Most recently, purified recombinant protein antigens discovered using genomic approaches have shown considerable promise for being effective as a vaccine against a broad range of group B strains [1,2]. Vaccines based on this approach may soon be licensed. However, strain to strain variation in sequence and/or expression of these relatively conserved surface proteins may render them less effective in infants than initially hoped [3].

Outer membrane vesicles extracted with deoxycholate have been shown in several controlled field trials to be capable of providing protection against homologous strains in the context of clonal group B epidemics [4–7]. The predominant protective antigen in these vaccines has been shown to be PorA, the subtype antigen, which is known to exhibit considerable antigenic variation from strain to strain within group B. To overcome this problem, experimental vaccines have been developed that contain six to nine different PorA proteins [8,9]. Since some subtypes are more prevalent than others, this approach could in principle protect against a majority of strains. The deoxycholate-extracted vesicles have been made safe for human vaccination by removal of most of the LOS from the outer membrane vesicles by the detergent. The residual wild type LOS in these vesicle vaccines, which likely provides some adjuvant activity, has generally been immunotype L3,7. Detergent extraction of the vesicles would also be expected to remove most of the surface-associated lipoproteins such as factor H binding protein and transferrin binding protein B. Potentially, the LOS could be a useful protective antigen [10], but its low concentration in these vesicles limits the extent of its contribution to the bactericidal antibody response. The use of native outer membrane vesicles (NOMV) prepared, without exposure to detergent, from strains with genetically detoxified LOS is one way to avoid loss of LOS and lipoproteins from the vesicles [11–13]. Over the past several years we have

☆ *Disclaimer*: The opinions and findings contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the WRAIR, the U.S. Department of the Army or the Department of Defense.

* Corresponding author at: Division of Bacterial and Rickettsial Diseases, Walter Reed Army Institute of Research, 503 Robert Grant Ave., Silver Spring, MD 20910-7500, United States. Tel.: +1 801 701 0486/301 319 9764; fax: +1 301 319 9801.
E-mail addresses: wendell.zollinger@us.army.mil, wendell.zollinger@gmail.com (W.D. Zollinger).
[1] These authors have left the WRAIR since their participation in the study.
[2] Correspondence address (after 31 December 2011): 146 W Siena Drive, Pleasant Grove, UT 84062, United States. Tel.: +1 801 701 0486.

0264-410X/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2011.11.084

evaluated two approaches for using NOMV as a vaccine. These were (1) the use of NOMV containing wild type LOS as an intranasal vaccine [11,14], and (2) the use NOMV from a strain with genetically detoxified LOS as a parenteral vaccine [13,15]. In these vaccines the LOS has been shown to be an important protective antigen.

Another vaccine approach that could allow higher vaccine concentrations of LOS is the isolation of the outer membrane proteins and the LOS and their reconstitution in an artificial liposomal membrane, which is the subject of the present study.

The importance of vaccinating with OMPs that were embedded in a membrane was emphasized by the results of an efficacy trial conducted in Chile using a vaccine consisting of purified OMPs noncovalently complexed to group C capsular polysaccharide [16]. The results of the study showed about 70% protection in older children but no protection in children under the age of five years. The clinical outcome correlated with bactericidal antibody titers but not with overall antibody response to OMPs by ELISA. ELISA results showed that the young children actually had a stronger overall antibody response than the older children, but there was low associated bactericidal activity. We interpreted these results as suggesting that the young children who had had less exposure to the outer membrane proteins on intact organisms (via carriage) preferentially responded to vaccine OMP epitopes that were not accessible on the intact organism. Whereas the older children who were more frequent carriers of meningococci had been primed to epitopes exposed on the surface of the intact organisms and consequently responded preferentially to those surface-exposed epitopes resulting in the production of antibodies that recognized the intact organisms and were bactericidal. These results demonstrated the need to present the integral OMPs in a native conformation and membrane environment.

We decided to compare the vaccine potential of three different vaccine approaches that allow presentation of the OMPs in a membrane and include LOS as a major antigen in the vaccine. Results of phase 1 studies with NOMV given intranasally [11,14] or parenterally [13,15] have been published. In this paper we report results of an earlier phase 1 study of vaccines consisting of purified OMPs and dLOS in liposomes.

2. Materials and methods

2.1. Bacterial strains

Strains of *N. meningitidis* used for vaccine production were from the culture collection of the Walter Reed Army Institute of Research. Strain 9162 ΔsynX (–:15:P1.7-2,3:L7-u), was derived from a case isolate from Chile and was used for production of the purified OMPs and the purified L7-u LOS. This strain reacts with the L3,7,9 immunotyping monoclonal antibody, 9-2-L379, but it appears from other data (Zollinger, unpublished observations) that its LOS core structure is not identical with the published L3,7 structure [17]. Thus it is specified as L7-u, indicating it has the L7 alpha chain but unknown core structure. Similarly, L8-u and L3-u refer to LOS with the L8 or L3 alpha chain but undetermined core structure. Strain 8532 (B:15:P1.7-2,3:L8-5), also a case isolate from Chile, was used for the production of the purified L8-5 LOS. The designation L8-5 is used to specify LOS that has a L8 alpha chain (lactose) and L5 core structure. L8 refers to the traditional L8 with the L3 core structure.

Strains used as test strains in the bactericidal assays included two phase variants of 9162 (B:15:P1.7-2,3) expressing L3-u,7-u or L8-u LOS, 8532 (B:15:P1.7-2,3:L8-5), two phase variants of 44/76 (B:15:P1.7-2,16) expressing L3,7 or L8 LOS, and three phase variants of 8570 (B:4:P1.19,15) expressing L3,7; L8-5; or L3-5,7-5 LOS. All strains used were ET-5/ST-31 sequence types.

LOS phase variants were obtained by colony blotting with LOS-specific monoclonal antibodies [18].

2.2. Vaccines

Three vaccine formulations were prepared under cGMP for intramuscular injection and consisted of purified outer membrane proteins combined in three different ways with purified, alkaline detoxified LOS. The three formulations of the vaccine included (1) noncovalent complexes adsorbed to aluminum hydroxide (28.9:1 aluminum hydroxide to protein or 10:1 aluminum to protein) (OMP/dLOS), (2) noncovalent complexes incorporated into liposomes (OMP/dLOS liposome), and (3) the two components combined with a much smaller amount of lipid by a method that was designed to attempt to incorporate the vaccine antigens into the liposomal membrane (OMP–dLOS–lipid) (see Table 1).

Outer membrane proteins for use in the vaccine formulations were purified from NOMV from strain 9162 Δ*synX*(–:15:P1.7-2,3:L7-u) which was grown under iron limiting conditions (no iron added to the fermentation medium) to induce expression of iron uptake proteins. The NOMV were prepared as described [12,19]. The purified vesicles were dissolved in TEEN buffer (0.05 M Tris–HCl, 1% Empigen BB (a zwitterionic detergent, Allbright and Wilson, Whitehaven, UK) 0.15 M NaCl, 0.01 M EDTA, pH 8.0) at a concentration of about 3 mg protein/ml with the aid of a bath sonicator. LOS and phospholipids were separated from the OMPs by three rounds of precipitation with ammonium sulfate. While stirring the solution of dissolved NOMV in TEEN buffer, solid ammonium sulfate was slowly added to a concentration of 500 g/l of dissolved NOMV. After 30–60 min of stirring, the mixture was centrifuged at about 20,000 × *g* for 15 min and the precipitated proteins, which moved to the top of the centrifuge bottle, were recovered. The precipitated proteins were dissolved in TEEN buffer with the aid of bath sonication and the process repeated twice more using 600 g ammonium sulfate/l of protein solution. The final precipitated proteins were dissolved in TEEN buffer and the residual ammonium sulfate removed by ultrafiltration against TEEN buffer containing 0.1% Empigen BB.

The LOS used in the vaccine formulations was purified either from strain 8532 (L8-5), formulations 1 and 2, or strain 9162 Δ*synX* (L7-u), formulation 3, by the phenol–water method [20] and detoxified by treatment with 0.1 M NaOH for 3.5 h at 60 °C. Following treatment the solution was neutralized with 1 M acetic acid and the dLOS recovered by precipitation with four volumes of cold ethanol.

Lyophilized liposomes were prepared as described [21] and were composed of dimyristoyl phosphatidylglycerol, dimyristoyl phosphatidylcholine, cholesterol, and a mixture of monophosphoryl lipid A (MPLA) molecular species, with different numbers of fatty acids, purified from *Salmonella Minnesota* R595 (Avanti Polar Lipids, Inc., Alabaster, Alabama) at molar ratios of 5:45:38:1.

For formulations 1 and 2, non-covalent complexes of purified OMP and detoxified L8-5 LOS were formed by combining the OMP and LOS in TEEN buffer with 0.5% Empigen BB and then removing the detergent by ultrafiltration against water for injection using a UFP-3-C-6 column (A/G Technology, Inc. Corp., Needam, MA, USA). The resulting soluble complexes were sterilized by filtration through a 0.22 μm membrane filter. For formulation1, the complexes were bottled, stored frozen, and at the time of vaccine administration combined with aluminum hydroxide. For vaccine formulation 2, noncovalent complexes of OMP and dLOS (bulk product) were incorporated into liposomes by rehydrating lyophilized liposomes with the noncovalent complexes of purified OMPs and dLOS in saline. Free antigen not associated with liposomes was removed by centrifugation of the liposomes and resuspension in phosphate buffered saline.