Pharmacological Research 121 (2017) 194–201

Contents lists available at ScienceDirect

Pharmacological Research

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

Outer membrane vesicles extracted from *Neisseria meningitidis* serogroup X for prevention of meningococcal disease in Africa

Reinaldo Acevedo[a,*,1], Caridad Zayas[a,1], Gunnstein Norheim[b], Sonsire Fernández[a], Barbara Cedré[a], Yisabel Aranguren[a], Maribel Cuello[d], Yaimara Rodriguez[a], Humberto González[a], Aleida Mandiarote[e], Marylin Pérez[f], Maritza Hernández[a], Mabel Hernández-Cedeño[a], Domingo González[a], Sverre-Henning Brorson[c], Einar Rosenqvist[b], Lisbeth Naess[b], Gro Tunheim[b], Daniel Cardoso[a], Luis García[a]

[a] *Finlay Institute, P.O. Box 16000, La Lisa, Havana, Cuba*
[b] *Norwegian Institute of Public Health, Oslo, Norway*
[c] *Oslo University Hospital, Norway*
[d] *Faculty of Engineering and Technology, Techinal University "Luis Vargas Torres", Emeralds, Ecuador*
[e] *Quality Control Direction, Center of Molecular Inmunology, Cuba*
[f] *Pharmaceutical Laboratories AICA, Havana, Cuba*

ARTICLE INFO

Article history:
Received 22 September 2016
Received in revised form 13 April 2017
Accepted 29 April 2017
Available online 8 May 2017

Keywords:
Outer membrane vesicle (OMV)
Neisseria meningitidis
Serogroup x
Vaccine

ABSTRACT

Meningococcal disease is caused mainly by serogroups A, B, C, Y, W of *N. meningitidis*. However, numerous cases of meningitis caused by serogroup X *N. meningitidis* (MenX) have recently been reported in several African countries. Currently, there are no licensed vaccines against this pathogen and most of the MenX cases have been caused by meningococci from clonal complex (c.c) 181. Detergent extracted meningococcal outer membrane vesicle (dOMV) vaccines have previously shown to be safe and effective against epidemics of serogroup B meningococcal disease in all age groups. The aim of this work is therefore to obtain, characterize and evaluate the vaccine potential of dOMVs derived from a MenX strain (OMVx). Three experimental lots of OMVx were prepared by deoxycholate extraction from the MenX strain BF 2/97. Size and morphology of the vesicles was determined by Dynamic Light Scattering and electron microscopy, whereas the antigenic composition was characterized by gel electrophoresis and immunoblotting. OMVx were thereafter adsorbed to aluminium hydroxide (OMVx/AL) and two doses of OMVx were administered s.c. to groups of Balb/c mice three weeks apart. The immunogenicity and functional antibody activities in sera were evaluated by ELISA (anti-OMVx specific IgG responses) and serum bactericidal activity (SBA) assay. The size range of OMVx was shown to be between 90 and 120 nm, whereas some of the antigens detected were the outer membrane proteins PorA, OpcA and RmpM. The OMVx/AL elicited high anti-OMVx antibody responses with bactericidal activity and no bactericidal activity was observed in the control group of no immunised mice. The results demonstrate that OMVx are immunogenic and could form part of a future vaccine to prevent the majority of meningococcal disease in the African meningitis belt.

1. Introduction

Neisseria meningitidis is a leading cause of meningitis and septicemia and is estimated to cause more than 1.2 million cases of invasive meningococcal disease and 135,000 deaths each year across the globe [1]. *N. meningitidis* serogroups A, B, C, Y and W are responsible for about 90% of all reported cases [2]. In the African meningitis belt, an area stretching from Senegal to Ethiopia, most cases are caused by serogroup A and W meningococci [3,4]. Most of these belong to a very limited set of sequence types (STs) [5]. The monovalent serogroup A conjugate vaccine (MenAfriVac) constitutes a major step forward in elimination of meningococcal disease in the meningitis belt [6]. Currently licensed tetravalent conjugate vaccines (Menactra™/Sanofis, Menveo™/Novartis and Nimenrix™/GSK) [7–9] represent important tools for reducing the

* Corresponding author.
E-mail address: racevedo@finlay.edu.cu (R. Acevedo).
[1] Equal Contribution

http://dx.doi.org/10.1016/j.phrs.2017.04.030
1043-6618/

disease burden of meningococcal disease worldwide. However, the current high price of these multivalent conjugate vaccines will severely limit their use in the African meningitis belt; the area with the world's highest incidence of meningococcal disease.

Disease caused by *N. meningitidis* serogroup X (MenX) were infrequently reported in the past; however recent reports have demonstrated an apparent increase in case numbers and outbreaks of MenX disease in the African countries Burkina Faso, Kenya, Niger, Togo and Uganda [10,11]. Most of the MenX strains causing these outbreaks typically belong to sequence types (ST) ST 181 and ST-751, which represents the clonal complex (c.c) 181 [10]. A recent study found that post-immunsation sera from individuals vaccinated with the multicomponent protein vaccine 4CMenB (Bexsero®) were shown to exert bactericidal activity against MenX strains [12]. However, there is currently no vaccine against MenX available for the African Meningitis Belt.

Detergent extracted meningococcal outer membrane vesicle (dOMV) vaccines derived from epidemic strains are relatively simple and inexpensive to manufacture and could present an affordable alternative to conjugate vaccines. Licensed dOMV vaccines against *N. meningitidis* serogroup B, like VA-MENGOC BC®, have demonstrated to be safe and induce protection in humans in clonal outbreaks [13]. Preclinical results indicate that a bivalent A + W dOMV vaccine is able to induce protective antibodies in animals [32], and this could present also an approach suitable for prevention of disease caused by MenX. The aim of this work was therefore to prepare and characterize dOMVs derived from an epidemic MenX strain (OMVx) and perform a preclinical evaluation. The BF 2/97 strain (c.c 181) was selected, based on the similarities with the genotype and phenotype of different MenX isolates in the region [5,6,11]

2. Materials and methods

2.1. Bacterial isolates

MenX disease isolates were from Burkina Faso and received from the WHO Collaborating Centre for Reference and Research on Meningococci, Oslo, Norway. The isolate were designated BF 2/97 and BF 7/07, and determined by MLST to be ST-751 and ST-181, respectively. Both isolates belongs to c.c 181 and expresses PorA of serosubtype P1.5-1,10-1. [5,6].

2.2. Cultivation conditions, OMVx extraction and purification

For preparation of whole cells for antigen analysis, strain BF 7/07 was cultivated for 4 h on blood agar plates and bacteria thereafter resuspended in phosphate buffer and inactivated at 56 °C for 30 min. For preparation of dOMVs, strain BF 2/97 was cultured in 20 L Fermentor (Chemap, Switzerland using Frantz modified medium for 12 h with rotational agitation [14]. Three lots of OMVx (Lots 102, 103 and 104) were thereafter obtained by gentle extraction with sodium deoxycholate (Merck, Germany). Bacterial debris was removed by centrifugation and pellets were homogenized in Tris-EDTA buffer (Merck, Germany). The detergent extract was purified by differential centrifugation and size exclusion chromatography (SEC) on Sephacryl S-300 (HPLC Knauer, Germany) [14], and the first SEC peak was collected. Total protein was determined by Lowry assay [15]. Purified OMVx were diafiltrated using a 100 000 kDa membrane (Sartocon Slice 200, Sartorius, Germany) against a buffer of Sucrose 3% and phosphate (0.8 mM). OMVx in sucrose were adsorbed to aluminium hydroxide ($Al(OH)_3$) 3 mg/mL (Superfos, Denmark). The degree of adsorption was measured by centrifugation (3500 rpm × 30 min) of the adsorbed vaccine product and quantification of the total protein concentration in the supernatant.

2.3. Vesicle composition

The protein content of the OMVx preparations was determined by modified Lowry technique and 12% SDS-PAGE gels with Coomassie brilliant blue staining [16]. Proteins were identified with monoclonal antibodies (MAb) in immunoblotting, as described in the next section. The lipopolysaccharides (LPS) were visualized by separation in 15% SDS/PAGE gels and quantified by silver staining [17] digital densitometric analysis, using an standard curve (10–100 ng) from *N. meningitidis* B purified LPS L 3,7,9 (Finlay Institute). LPS was also identified using anti-LPS L3,7,9 (NIPH, Norway) by immunoblot as described in next section.

2.4. Immunoblot

A panel of anti- *N. meningitidis* MAb was used to identify some of the major antigenic components in OMVx and the whole cell lysate prepared from the MenX strain BF 7/07. These were: anti-OpcA (279/5c) [18], anti- RmpM (185, H-8) [19], and anti-P1.5 PorA (NIBSC 03/226, UK). In addition, polyclonal sera from mice immunised with OMVx was used to identify potential antigens in preparations from MenX strains BF 2/97 and BF 7/07. Samples were run in SDS-PAGE (acrylamide 12.5%), transferred to a nitrocellulose membrane and incubated with the MAb as previously described [16]. The Molecular weight standard was revealed with Red Ponceau which is a non-selective dye. An anti-mouse peroxidase conjugated IgG (Sigma Chemical Co., St. Louis, Mo.) and diaminobenzidine as the substrate were used to detect the bound antibodies.

2.5. Vesicle size

The morphology of OMVx in sucrose was characterized with a transmission electron microscope (Tecnai 12, FEI, Oregon USA) and negative staining with 2% of aqueous uranyl acetate (University of Oslo, Norway). Particle size and polydispersion of the OMVx samples in sodium deoxycholate buffer (before diafiltration) and sucrose buffer (after diafiltration) were measured using a dynamic light scattering particle sizer (Beckman Coulter Delsa Nano C, Germany) at room temperature [20].

2.6. OMVx formulation and quality controls

OMVx suspended in sucrose were adsorbed to $Al(OH)_3$ 2–3 mg/mL (Superfos, Denmark). OMVx were gently added to ($Al(OH)_3$ up to 0.1 mg/mL of protein and stirred for 1 h (ratio of Protein: ($Al(OH)_3$ was approximately 1:30). Thiomersal was added as preservative to a final concentration of 0.01%.

Quality controls to formulation were carried out to evaluate the amount protein by Lowry assay [15], aluminium by a complexometric method [21], thiomersal by a mercury-specific spectrophotometric method [22] and sucrose by HPLC [23]. Description of formulations was carried out by visual inspection and pH was determined by potentiometric method [23]. Sterility test was carried out as recommended by USP [23]. The endotoxic potential of formulations was evaluated by LAL assay [21] and identity was carried out by SDS/PAGE and immunoblot as described above. For the identity test, desorption of OMVx was performed using a solution of SDS 2% and phosphate buffer (0.5 M).