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Preclinical safety and immunogenicity evaluation of a nonavalent PorA native outer membrane vesicle vaccine against serogroup B meningococcal disease☆

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ABSTRACT

Background: An improved nonavalent PorA native outer membrane vesicle vaccine was developed with intrinsic adjuvating activity due to presence of less-toxic (*lpxL1*) LPS. In the present study, the safety and immunogenicity of this next-generation NonaMen vaccine were evaluated following repeated vaccination in rabbits and mice.
Methods: A repeated–dose toxicology study was performed in rabbits. Immunogenicity of next-generation NonaMen was evaluated by determining the serum bactericidal antibody (SBA) titers against meningococcal serogroup B strains containing several PorA subtypes. Release of the pro-inflammatory cytokine, interleukin-6 (IL-6), by the human monocytic cell line (MM6) was measured to estimate pyrogenic activity.
Results: No toxicologically relevant findings were noted in vaccinated rabbits receiving plain next-generation NonaMen. In agreement, next-generation NonaMen induced reduced amounts of the pro-inflammatory cytokine, IL-6, released by human monocyte cell line. In both rabbits and mice, next-generation NonaMen induced high SBA titers against all tested MenB strains regardless of whether or not aluminium phosphate adjuvant is used.
Conclusions: The data suggest that next-generation NonaMen is a safe vaccine with the potential to develop a broadly protective immune response and encourage the start of the first clinical studies.

1. Introduction

At present, no broadly protective vaccine against MenB strain infections is available. So far, only MenB vaccines based on OMV have been proven successful in controlling prevailing MenB epidemics [1–6]. OMV vaccines have been traditionally prepared with detergent extraction to remove reactogenic lipopolysaccharides (LPS) and to increase vesicle release. The LPS of *N. meningitidis* is highly toxic, but residual amounts are needed to maintain the outer membrane vesicle structure and to potentiate the immune response by its adjuvating activity [7]. The use of a detergent has major disadvantages. Along with LPS, detergents remove phospholipids and membrane lipoproteins that may contribute to immunogenicity [8,9]. In addition, the vesicle integrity can be affected by treatment with detergent resulting in aggregation of the OMV vaccine product [10,11]. A detergent-free OMV purification process retains all LPS, which results not only in a preserved native vesicle structure, but also in an OMV vaccine product that is too toxic for parenteral immunization [4]. Discovery of *lpxL1* mutant strains, at National Institute for Public Health and the Environment (RIVM) in the Netherlands, provides a solution for the undesired toxic effect of LPS [12,13]. Inactivation of the *lpxL1* gene attenuates LPS toxicity, while preserving the adjuvant activity needed to potentiate the immune response [9,12]. Human monocytes, macrophages or peripheral blood mononuclear cells exposed to *lpxL1*-LPS induced less pro-inflammatory cytokines compared to exposure to wild-type LPS, indicating that the reactogenicity of *lpxL1*-LPS in human is expected to be far less than that of wild-type LPS [8,9,11,14,15]. Furthermore, it was found that the poor immunogenicity of OMV from LPS-deficient strains could

Abbreviations: MenB, *Neisseria meningitidis* serogroup B; OMV, outer membrane vesicles; DOMV, detergent OMV; NOMV, native OMV; PorA, outer membrane porin A protein; LPS, lipopolysaccharide; MAT, monocyte activation test; SBA, serum bactericidal antibody; LAL, limulus amebocyte lysate; DTwcP-IPV, diphtheria toxoid, tetanus toxoid, whole cell pertussis, inactivated poliomyelitis virus type 1, 2, 3.

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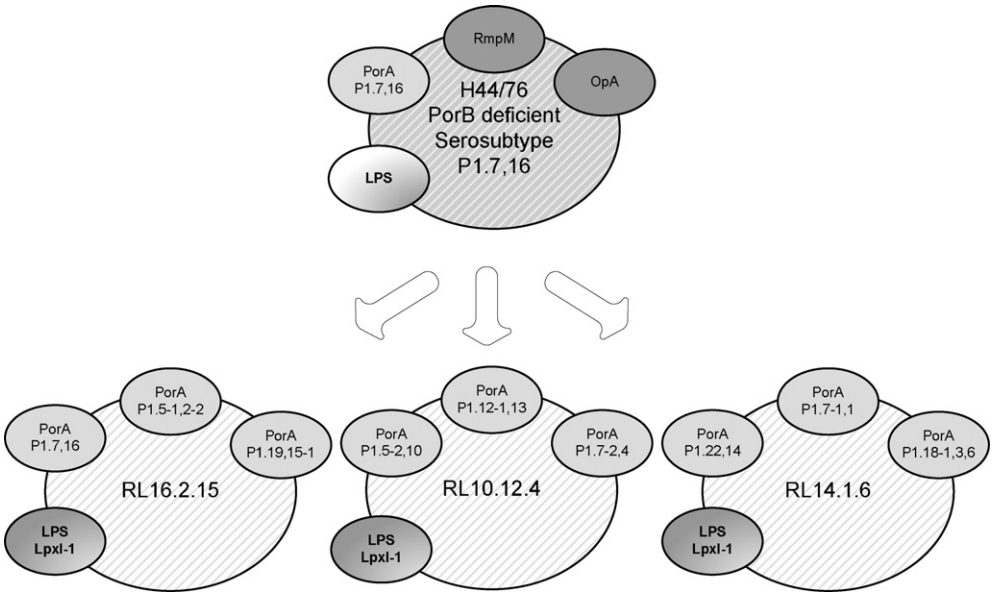

Fig. 1. Schematic design of the three trivalent vaccine strains derived from the H44/76 parental (PorB deficient) strain that were used for preparing next-generation NonaMen.

be restored by adding external *lpxL1*-LPS [16,17]. PorA protein (class 1 protein) has been identified as major protective antigen of *N. meningitidis*. However, many PorA protein variants exist among circulating strains which complicates development of a broadly protective vaccine. To circumvent this, three meningococcal serogroup B vaccine strains obtained from the H44/76 parental strain were genetically engineered to express three different PorA subtypes [18,19]. OMV from these three recombinant strains were pooled to obtain the nonavalent PorA vaccine (NonaMen). NonaMen has the potential to prevent the majority of serogroup B infections in Europe, i.e. 80% of cases [18,20,21]. Based on the most recent national surveillance data available, our NonaMen vaccine formulation is expected to protect in 2007, 2008, 2009, 2010 and 2011 against respectively 89.0%, 89.3%, 94.0%, 91.7% and 85.5% of the occurring MenB serosubtypes in the Netherlands. Previously, detergent-extracted NonaMen was shown to be safe and immunogenic in rabbits and mice and well-tolerated in healthy adults [18,19]. Unfortunately, the need of a detergent for removal of toxic LPS in the production process of this NonaMen resulted in an OMV product with lower quality, i.e. partially disintegrated and aggregated vesicles. Inactivation of the *lpxL1* gene has solved this problem and facilitated generation of a stable OMV vaccine product without the need for detergent extraction to remove toxic LPS. To address the safety and immunogenicity of this next-generation NonaMen vaccine, a repeated dose study was conducted in both rabbits and mice.

2. Materials and methods

2.1. Vaccine preparations

Three trivalent PorA vaccine strains, which were genetically modified by inactivation of the *lpxL1* gene, were developed from a *rmpM* deletion mutant of the H44/76 MenB parental strain (Fig. 1). [11,12]. The subsequent three trivalent bulk OMV products were pooled to generate the next-generation nonavalent vaccine (NonaMen). Vaccine doses were based on total PorA concentration. Plain NonaMen 7.5 or 15 (contained respectively 7.5 μg or 15 μg PorA per subtype per dose of 0.5 mL) were formulated in Tris-sucrose buffer, adjuvated NonaMen 15 (15 μg PorA per subtype per dose of 0.5 mL) was formulated in Tris-sucrose buffer containing aluminium phosphate (3 mg/mL). A dose of 15 μg/PorA type in 0.5 mL represents the highest intended human dose. Placebo consisted of Tris-sucrose buffer containing aluminium phosphate (3 mg/mL) without MenB component.

2.2. GLP toxicology study in rabbits

A fully GLP compliant repeated-dose toxicology study (including local tolerance) was performed by an external contract research laboratory. Four groups of New Zealand White rabbits (2–3 kg), each consisting of 5 main animals per sex, and 3 recovery animals per sex, were immunized by 5 intramuscular injections, on days 1, 15, 29, 43 and 57, in alternating hind limbs. At day 60, main animals were bled under anesthesia. Recovery of initial pathological effects was investigated in the remaining recovery animals that were sacrificed and bled at day 71. The four groups received respectively, placebo, plain NonaMen 7.5, plain NonaMen 15 or adjuvated NonaMen 15. Rectal body temperature was measured 4 h after vaccination based on results of a previous study in which body temperature was measured continuously by using implanted temperature data showing a peak temperature 4 h after vaccination with NonaMen [22]. At the end of the study, macroscopic examination of organs, including organ weights, and histopathology on selected tissues were performed by a veterinary pathologist. Blood samples were taken at days 4, 57, 60, and 71 to determine relevant hematology and biochemical safety parameters.

2.3. Monocyte activation test

The pyrogenicity of the new-generation NonaMen was assessed by an in vitro monocyte activation test (MAT) as previously described [11]. Apart from the adjuvated and plain next-generation NonaMen 15, a Diphtheria toxoid (30 IU), Tetanus toxoid (60 IU), whole cell Pertussis (4 IU), Inactivated Poliomyelitis Virus (type 1 (40 D-antigen units (DU)); type 2 (4 DU); type 3 (7.5 IU)) (DTwcP-IPV) vaccine (supplied by Netherlands Vaccine Institute) and a previously clinically tested deoxycholate-extracted OMV (DOMV) NonaMen (lot005; containing GalE-LPS; 15 μg PorA per subtype per human dose of 0.5 mL) [19], were included in MAT. Briefly, human