Vaccine xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Adjuvant system AS02$_V$ enhances humoral and cellular immune responses to pneumococcal protein PhtD vaccine in healthy young and older adults: Randomised, controlled trials

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ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form 10 October 2013
Accepted 16 October 2013
Available online xxx

Keywords:
Streptococcus pneumoniae
Pneumonia
PhtD
Immunosenescence
Adjuvant
Vaccine

ABSTRACT

Background: The protection elicited by polysaccharide pneumococcal vaccines against community-acquired pneumonia in older adults remains debatable. Alternative vaccine targets include well-conserved pneumococcal protein antigens, such as pneumococcal histidine triad protein D (PhtD).
Objective: To evaluate humoral and cellular immune responses and safety/reactogenicity following immunisation with PhtD vaccine with or without adjuvant (alum or AS02$_V$) in older (≥65 years) and young (18–45 years) healthy adults.
Methods: Two phase I/II, single-blind, parallel-group studies were conducted in 150 older and 147 young adults. Participants were randomised to receive 2 doses (months 0 and 2) of PhtD 30 μg, PhtD 10 μg plus alum, PhtD 30 μg plus alum, PhtD 10 μg plus AS02$_V$ or PhtD 30 μg plus AS02$_V$, or the 23-valent polysaccharide pneumococcal vaccine (23PPV) at month 0 with placebo (saline solution) at month 2. Safety/reactogenicity was assessed. PhtD-specific antibody, T cell and memory B cell responses were evaluated.
Results: Solicited adverse events were more common in young participants and with adjuvanted vaccines. No vaccine-related serious adverse events were reported. Although anti-PhtD geometric mean antibody concentrations (GMCs) were consistently lower in the older adult cohort than in young adults, GMCs in the older cohort following PhtD 30 μg plus AS02$_V$ were comparable to those induced by plain PhtD or PhtD 30 μg plus alum in the young cohort. Compared with alum adjuvant, AS02$_V$ adjuvant system was associated with an increased frequency of PhtD-specific CD4 cells in both cohorts and a significantly higher specific memory B cell response in the older cohort, similar to responses obtained in the young cohort.
Conclusion: The improved immune response to PhtD vaccine containing the AS02$_V$ adjuvant system in comparison to alum suggests that the reduced immune response to vaccines in older adults can be partially restored to the response level observed in young adults. ClinicalTrials.gov identifiers: NCT00307528/NCT01767402.

Abbreviations: AE, adverse event; AS, adjuvant system; ATP, according-to-protocol; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean antibody concentration; GM-CSF, granulocyte-macrophage colony-stimulating factor; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; MPL, 3-*O*-desacyl-4′monophosphoryl lipid A; PCV, pneumococcal conjugate vaccine; PhtD, pneumococcal histidine triad protein D; 23PPV, 23-valent polysaccharide pneumococcal vaccine; QS21, *Quillaja saponaria* Molina fraction 21; TNF, tumour necrosis factor.

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http://dx.doi.org/10.1016/j.vaccine.2013.10.052

1. Introduction

It is widely accepted that 'immunosenescence', the deterioration of immune function with advancing age, also contributes to the increased incidence of morbidity and mortality from infectious diseases among older adults [1] and reduced responses to vaccination [2–4]. The 23-valent polysaccharide pneumococcal vaccine (23PPV) is recommended for use in older adults in most countries but, while there is evidence for a significant reduction in the risk of pneumococcal bacteraemia, protection against non-bacteraemic pneumonia in this population has been a matter of debate [5–9]. Being T cell independent, polysaccharide antigens also fail to induce adequate immunological memory and improved responses upon revaccination [10]. Polysaccharide pneumococcal conjugate vaccines (PCVs) elicit a better priming response in adults [11] but their impact on clinical respiratory outcomes in this population remains to be established. Also, the introduction of PCVs into paediatric vaccination programmes led to a decrease in vaccine serotype pneumococcal disease in adults, which may limit the benefit of PCVs with same serotype composition in older age groups [9,12].

Common pneumococcal proteins are interesting candidate antigens for new pneumococcal vaccines, since they offer the potential for serotype-independent coverage through either antibody or cellular responses [13–18]. The pneumococcal histidine triad protein D (PhtD) is one such conserved pneumococcal protein antigen that has been shown to elicit functional antibodies [19–21] and provide protection against pneumonia in animal models [20,22]. The need to improve immune responses in older adults has also led to the search for vaccine adjuvants that enhance the magnitude and quality of antigen-specific immune response [23]. $AS02_V$ is an adjuvant system that consists of an oil-in-water emulsion combined with two potent immunostimulants, 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL) and the saponin QS21 (*Quillaja saponaria* Molina, fraction 21; Antigenics Inc., a wholly owned subsidiary of Agenus Inc., Lexington, MA, USA) [24].

Two studies were conducted to examine the immunogenicity and reactogenicity of two doses of PhtD vaccine administered with or without $AS02_V$ or alum adjuvant in healthy adults. The studies were of identical design, apart from the age of participants: one study included adults aged 65 years or older, while the other included younger adults.

2. Materials and methods

2.1. Study design and participants

In the phase I/II, single-blind, parallel-group studies, older (≥65 years) and young (18–45 years) adults in generally good health were eligible for inclusion. Major exclusion criteria included previous vaccination against *Streptococcus pneumoniae* or with a MPL/QS21-containing vaccine, administration of immune-modifying drugs within the last 6 months, and bacterial pneumonia within 3 years of the first dose. A full list of inclusion/exclusion criteria is provided in Appendices A and B.

Using a centralised randomisation system on the internet, participants were randomised (1:1:1:1:1:1:1) by the investigator at each site to receive vaccination at 0 and 2 months with PhtD 30 μg, PhtD 10 μg plus alum (aluminium phosphate, $AlPO_4$), PhtD 30 μg plus alum, PhtD 10 μg plus $AS02_V$ or PhtD 30 μg plus $AS02_V$, or 23PPV (*Pneumovax*™; Sanofi Pasteur MSD, Lyon, France) at month 0 followed by placebo (saline solution) at month 2 (control group). The studies were single-blind, meaning that participants were unaware of the vaccination assignment.

Co-primary objectives of the studies were to evaluate the safety, reactogenicity and immunogenicity of plain or adjuvanted PhtD, with evaluation of the persistence and quality of the antibody response, and cellular immune response as secondary objectives. The young cohort study was conducted at the Catholic University of Louvain, Brussels, Belgium, between October 2003 and November 2004 and the older cohort study was conducted at the Centre for Vaccinology, Ghent University Hospital, Belgium, between January 2004 and March 2005 followed by additional 2-year follow-up; 1-year results are presented for both cohorts. All participants gave written informed consent to the studies, which were approved by local ethics committees and conducted in accordance with the Declaration of Helsinki.

2.2. Vaccines

The PhtD vaccine was supplied as freeze-dried pellets in monodose vials to be reconstituted with phosphate buffered saline or with adjuvant formulation $AS02_V$ liquid in pre-filled syringes, or was supplied adsorbed on alum as a liquid in monodose vials. 23PPV was supplied in pre-filled syringes and placebo saline in monodose vials. Influenza vaccine (*Fluarix*™; GlaxoSmithKline, Rixensart, Belgium) was offered free of charge to the older cohort during the study. All study vaccines were administered by intramuscular injection (0.5 ml) into the deltoid region of the upper right arm. Participants were observed closely for at least 30 min after vaccination.

2.3. Reactogenicity and safety assessment

Solicited local (injection site pain, redness and swelling) and general (fatigue, fever, gastrointestinal symptoms, headache, malaise, myalgia and sweating) adverse events (AEs) were recorded by the participants on diary cards during the 7-day follow-up after each vaccination. Unsolicited AEs were recorded within 30 days after each vaccination. Serious AEs were reported throughout the study. Duration, causality and outcome of AEs were recorded. All solicited local reactions were considered causally related to vaccination; the relationship of other AEs was classified as possible or not causally related. AE intensity was scored on a scale from 1 to 3. Grade 3 AEs were defined as preventing normal daily activity, apart from grade 3 solicited fever, which was defined as oral/axillary temperature >39.0 °C, and grade 3 solicited swelling or redness, defined as diameter >50 mm. Grade 2 pain was defined as painful when the limb was moved.

2.4. Immunogenicity assays

In both cohorts, blood samples were taken within 14 days before the first vaccination and at post-vaccination intervals up to 10 months after the second vaccine dose. All blood samples were processed and stored appropriately until analysed using in-house methods at the laboratories of GlaxoSmithKline, Rixensart, Belgium.

Anti-PhtD antibody concentrations were measured at months 0 (before the first vaccine dose), 1, 2 (before the second dose), 3 and 12 using an enzyme-linked immunosorbent assay (ELISA; assay cut-off 0.04 μg/ml), as described previously [22]. A passive transfer mouse model assay was used to evaluate the protection provided by anti-PhtD antibodies in pooled sera collected at months 0 and 3 from participants who received PhtD 30 μg, PhtD 30 μg plus alum or PhtD 30 μg plus $AS02_V$. Sera from each vaccine group were administered intraperitoneally into OF1 mice 1 h before intranasal challenge with a lethal dose (10^5 cfu) of *S. pneumoniae* serotype 3. Mortality induced by infection was monitored for 10 days. A rabbit anti-PhtD antiserum, generated in-house using recombinant PhtD as immunogen, was the positive control in each experiment.

Please cite this article in press as: Leroux-Roels I, et al. Adjuvant system $AS02_V$ enhances humoral and cellular immune responses to pneumococcal protein PhtD vaccine in healthy young and older adults: Randomised, controlled trials. Vaccine (2014), http://dx.doi.org/10.1016/j.vaccine.2013.10.052