Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Functional immune responses to twelve serotypes after immunization with a 23-valent pneumococcal polysaccharide vaccine in older adults

Jong Gyun Ahn[a], Han Wool Kim[a], Hee Jung Choi[b], Jin Hwa Lee[c], Kyung-Hyo Kim[a,*]

[a] Department of Pediatrics and Center for Vaccine Evaluation and Study, Medical Research Institute, Ewha Womans University School of Medicine, Seoul, Republic of Korea
[b] Division of Infectious Diseases, Department of Internal Medicine, Ewha Womans University School of Medicine, Seoul, Republic of Korea
[c] Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Ewha Womans University School of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 7 March 2015
Received in revised form 23 June 2015
Accepted 2 August 2015
Available online xxx

Keywords:
Pneumococcal polysaccharide vaccine
Immunogenicity
Opsonin
Phagocytosis
Elderly

ABSTRACT

Background: The 23-valent pneumococcal polysaccharide vaccine (PPSV23) was introduced as part of the national immunization program for the elderly (≥65 years of age) in Korea on 2013. To evaluate immune responses in this population, serotype-specific anti-pneumococcal antibodies were studied with opsonophagocytic assay (OPA).
Methods: Pneumococcal vaccine-naïve participants ≥65 years of age were enrolled. They were divided into two groups according to their age: 30 in (65–74 years) and 32 in group (≥75 years). The functional antibody response was determined by multiplexed OPA (MOPA) for 12 serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) before and 4 weeks after vaccination with PPSV23.
Results: Geometric mean titers (GMTs) to all tested serotypes significantly increased in both groups after vaccination compared to those before vaccination. There were no significant differences in either the fold rise (post-vaccination to pre-vaccination) or the percentage of participants with a ≥4-fold increase in OPA titers between two groups for any of the 12 serotypes. Following vaccination, GMT for serotype 9V was higher in group 1 than in group 2 ($P = 0.011$).
Conclusions: PPSV23 induces functional immune response for 12 vaccine serotypes in both age groups. Further analysis is needed for the remaining 11 serotypes in the PPSV23, in order to develop a better understanding of the immune responses induced by PPV23 in older adults.

1. Introduction

Older adults are at high risk of invasive pneumococcal disease (IPD) [1]. Prevention through vaccination has been used as an effective way to reduce disease burden. Currently, two pneumococcal vaccines are available for elderly people: the 23-valent pneumococcal polysaccharide vaccine (PPSV23) and the 13-valent pneumococcal conjugate vaccine (PCV13). PPSV23 was licensed in 1983 and is commonly recommended for prevention of IPD in high-risk adults and the elderly [2]. PCV13 was licensed in 2010 as a replacement for PCV7 in infants and young children and was recently approved in some countries including Korea for use in preventing pneumonia and IPD in adults aged ≥50 years [3–6].

Although PCV13 may be more effective than PPSV23, the strains contained in the PCV13 are likely to be greatly reduced in the population due to successful infant immunization schedules and, thus, uncommon isolates. Moreover, PPSV23 will provide protection against 10 additional serotypes and is less expensive than PCV13. For these reasons, to date, in most countries, PPSV23 is preferred as part of routine vaccination in the elderly [7].

In Korea, a national immunization program (NIP) for PPSV23 was introduced for all individuals aged ≥65 years in May 2013. To evaluate the impact of the universal PPSV23 program in the target population, a vaccine effectiveness (VE) study is needed using the serotype-specific incidence of pneumococcal disease before and after vaccine introduction, which is difficult to perform in Korea. Given this limitation, the immunogenicity of PPSV23 was studied alternatively by measuring functional antibodies with opsonophagocytic assay (OPA), which is a better predictor of protection than antibody titer evaluated with ELISA, especially in older adults [8,9].

In this study, we aimed to evaluate functional immune responses by measuring OPA titers for the 12 serotypes included in both PCV13 and PPSV23, in the elderly vaccinated with PPSV23.

* Corresponding author. Tel.: +82 2 2650 5700; fax: +82 2 2650 2817.
E-mail address: kaykim@ewha.ac.kr (K.-H. Kim).

http://dx.doi.org/10.1016/j.vaccine.2015.08.002
0264-410X/

2. Subjects and methods

2.1. Participants and study design

A total of 62 participants were enrolled into two age groups in 2013–2014: 30 in group 1 (65–74 years) and 32 in group 2 (≥75 years). Eligible individuals were ambulatory adults aged ≥65 years who never received the pneumococcal vaccine and in whom underlying chronic illnesses such as hypertension and diabetes mellitus were stable. Exclusion criteria were immune compromising conditions such as HIV infection, leukemia, lymphoma, Hodgkin's disease, multiple myeloma, generalized malignancy, chronic renal failure or nephrotic syndromes, congenital or acquired immunodeficiencies, diseases requiring treatment using immunosuppressive drugs, including long-term systemic corticosteroids or radiation therapy, solid organ transplantation, functional or anatomic asplenia, CSF leaks or cochlear implants, a history of hypersensitivity to vaccine or IPD, any coagulation disorder, and a history of antibiotic use within one week. All participants received PPSV23 (Prodiax-23®, Merck & Co. Inc., Whitehouse Station, NJ, USA) into the deltoid muscle. Blood samples were collected before and approximately 4 weeks (mean: 28.1 days, range: 26–35 days) after vaccination.

2.2. Multiplexed OPA (MOPA) for immunogenicity assessment

MOPA was performed for 12 serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) as previously described [10]. Briefly, HL-60 cells were differentiated into granulocytes by culturing in RPMI 1640 (Welgene, Daegu, Korea) with 10% fetal bovine serum and 0.8% dimethyl formamide for 5 days. After differentiation, HL-60 cells were diluted to 10^7 cells/mL in Hanks' buffer containing 0.1% gelatin and 5% fetal bovine serum. All serum samples were also diluted in the same buffer. Target bacteria with resistance to one of four antibiotics (optochin, streptomycin, spectinomycin, or trimethoprim), but susceptibility to the other three were prepared for the 12 serotypes. Equal volumes of the four bacterial suspensions selected to be analyzed were pooled. A serially diluted test serum (20 μL) was mixed with 10 μL of pneumococcal suspension containing 2000 CFU in each well of a 96-well microtiter plate. After 30 min of incubation at room temperature with shaking, 40 μL of HL-60 cell suspension (4×10^5 cells per well) and 10 μL of baby rabbit complement (Pel-Freez, Brown Deer, WI, USA) were added to each well. Plates were incubated in a tissue culture incubator (37 °C, 5% CO_2) with shaking for 45 min. An aliquot of the final reaction mixture (10 μL) was spotted onto four different Todd-Hewitt agar yeast extract plates. After the fluid was absorbed into the agar, each plate was overlaid with molten Todd-Hewitt agar (0.75%) containing yeast extract, one of the four antibiotics, and 100 mg/L of 2,3,5-triphenyltetrazolium chloride. After overnight incubation in a candle jar at 37 °C, the bacterial colonies on the agar plates were counted using colony counting software, NICE (NIST [National Institute of Standards and Technology, US]'s Integrated Colony Enumerator). OPA titer was defined as the serum dilution that killed 50% of bacteria, which was determined by linear interpolation.

2.3. Statistical analysis

Geometric mean titers (GMTs) were calculated and two-sided 95% confidence intervals (CIs) were determined in each pneumococcal serotype for both groups. Differences in GMTs between pre- and post-vaccine sera were compared using a two-sample, paired *t*-test after logarithmic transformation. Comparisons between both groups were evaluated by the Student's *t*-test for continuous variables and the Pearson χ^2 test or Fisher's exact test for categorical variables. Holm's multiple test procedure [11] was applied to adjust *P* values for multiple comparisons. Reverse cumulative distribution curves (RCDCs) were used to represent the percentage of participants that achieved different OPA titers to each of the pneumococcal serotypes. *P* values less than 0.05 were considered significant. Statistical analysis was performed using SPSS statistical software (version 18.0; SPSS Inc., Chicago, IL, USA).

Table 1
Demographic characteristics of the participants.

Characteristic	Group 1, aged 65–74 years (*N* = 30)	Group 2, aged ≥75 years (*N* = 32)	*P* value
Male, *n* (%)	15 (50.0)	18 (56.3)	NS*
Age, years			
Mean	71.6	78.5	<0.001
Median (range)	71.5 (68–74)	78.0 (75–85)	
Underlying conditions, *n* (%)			
Diabetes	8 (26.7)	3 (9.4)	NS
Cardiac disease	17 (56.7)	14 (43.8)	NS
Lung disease	1 (3.3)	0 (0)	NS
Cirrhosis	0 (0)	0 (0)	
Two or more†	6 (20.0)	3 (9.4)	NS

* NS, not significant.
† Participants with two or more of the following underlying conditions: diabetes, cardiac disease, lung disease, and cirrhosis.

2.4. Ethical considerations

The study protocol was reviewed and approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital (ECT 13-24B-21). The study was conducted in accordance with good clinical practices (national regulations and ICH E6) and the principles of the Helsinki Declaration. Written informed consent was obtained from all participants following a detailed explanation of the study.

3. Results

3.1. Baseline participant characteristics

A total of 62 participants were studied, 30 in group 1 and 32 in group 2. The demographic characteristics of the two groups are summarized in Table 1. The median age of group 1 and group 2 was 71.5 years (range: 68–74 years) and 78.0 years (range: 75–85 years), respectively. There were no significant differences in gender or the presence of underlying disease between the two groups.

3.2. Immunogenicity

The GMTs and 95% CIs for pre- and post-vaccination OPA titers are shown in Table 2. Following vaccination, both groups exhibited significant increases in GMTs for all 12 serotypes. There were no differences in either the fold increase (post-vaccination to pre-vaccination) or the proportion of participants with a 4-fold or greater increase in OPA titers between the two groups for any of the 12 serotypes.

There were no differences in the baseline GMTs between two groups, with the exception of serotype 9V, for which group 1 had a higher GMT than group 2 ($P < 0.001$). After vaccination, GMT for serotype 9V was also higher in group 1 compared to group 2 ($P = 0.011$) (Table 2, Fig. 1).

Table 3 presents the number and percentage of participants in both groups with OPA titers ≥8 and ≥64 before and after immunization. These titers were chosen as a point of reference and do not necessarily correspond to seroprotection, because such a correlate has not been established in adults. Before vaccination, the number of participants with an OPA titer ≥8 ranged from 11 (37%) for serotype 1 to 30 (100%) for serotypes 9V, 14, and 19A in group

Please cite this article in press as: Ahn JG, et al. Functional immune responses to twelve serotypes after immunization with a 23-valent pneumococcal polysaccharide vaccine in older adults. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.08.002