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Functional immune responses to 11 non-PCV13 serotypes after immunization with a 23-valent pneumococcal polysaccharide vaccine in older adults

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ABSTRACT

Background: The 23-valent pneumococcal polysaccharide vaccine (PPSV23) has been recommended for adults aged ≥65 years. To evaluate functional immune response against the additional 11 serotypes that are included in PPSV23, but not the 13-valent pneumococcal conjugate vaccine (PCV13), serotype-specific anti-pneumococcal antibodies were examined using an opsonophagocytic assay (OPA).
Methods: Participants ≥65 years of age that were naïve to the pneumococcal vaccine were enrolled. They were divided into two groups according to their age: group 1 (N = 30; aged 65–74 years) and group 2 (N = 32; aged ≥75 years). The functional antibody response prior to and 4 weeks post-immunization with PPSV23 was determined, using a multiplexed OPA (MOPA) for 11 pneumococcal serotypes (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F, and 33F).
Results: Geometric mean OPA titers (GMTs) to 11 serotypes were significantly increased in both groups post-immunization compared to those prior to immunization. The GMTs for all serotypes were not significantly different between the two groups after immunization. The proportion of subjects with OPA titers post-immunization of ≥8 and ≥64 was 93–100% and 80–100% for the 11 serotypes, respectively, while subjects with a ≥4-fold increase in OPA titers ranged from 9 to 90% for the 11 serotypes.
Conclusions: This study revealed that PPSV23 vaccination induced significant functional immune responses to 11 non-PCV13 serotypes in older adults. The MOPA has been shown to be a useful tool for future application in evaluating new PCVs in older adults.
The clinical trial registration number is KCT 0001963 (CRIS, https://cris.nih.go.kr/cris/en/).

1. Introduction

Streptococcus pneumoniae is a significant cause of community-acquired pneumonia and invasive bacterial diseases, such as bacteremia and meningitis, in older adults [1]. The pneumococcal capsule is critical to bacterial survival during infection in humans, acting by evasion of phagocytosis [2–4]. Currently, over ninety serotypes of the pneumococcal capsule have been identified [5], which vary in their virulence and capacity to elicit a host immune response in different age groups or ethnic populations [6]. Immune protection against the pneumococcus is primarily based on serotype-specific opsonic antibodies [7].

To prevent pneumococcal infections, a pneumococcal polysaccharide vaccine was developed in the 1980s, the 23-valent pneumococcal polysaccharide vaccine (PPSV23). This included the capsules from 23 serotypes, which were the causal types in more than 85% of all pneumococcal diseases until the late 1990 s [8]. PPSV23 covers approximately 73% of invasive pneumococcal diseases (IPD), based on the 2007 American serotype distribution [9]. Many studies have examined the efficacy of PPSV23 against IPD, bacteremic pneumonia, and non-bacteremic pneumonia caused by vaccine-type pneumococci, all pneumococci, or all-causes. PPSV23 have been reported to have a protection rate of

Abbreviations: PPSV23, 23-valent pneumococcal polysaccharide vaccine; IPD, invasive pneumococcal diseases; PCV13, 13-valent pneumococcal conjugate vaccine; OPA, opsonophagocytic assay; MOPA, multiplexed OPA; OBB, opsonization buffer B; BRC, Baby rabbit complement; THY, Todd-Hewitt yeast; GMTs, Geometric mean OPA titers; CIs, confidence intervals; RCDCs, reverse distribution curves; ELISA, enzyme-linked immunosorbent assay.

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40–90% in many studies [10–16]. A recent Cochrane database review by Moberley et al. demonstrated the efficacy of PPSV23 to protect against IPD in adults, supporting the recommendation for immunization [17]. Protective efficacy was also shown against all-cause pneumonia in low-income but not in high-income countries, in both the general population and in adults with chronic illness.

Many countries recommend universal pneumococcal vaccination for the elderly, but there are differences in vaccination strategies across countries. In most European countries and the Republic of Korea, 13-valent pneumococcal conjugate vaccine (PCV13) is recommended only for high-risk groups [18,19]. After the CAPiTA trial, however, the US Advisory Committee on Immunization Practice recommended the sequential administration of both PCV13 and PPSV23 for all adults 65 years or older [20]. These differences are attributable to the inconsistent results of the efficacy, effectiveness, and cost-effectiveness of the two types of pneumococcal vaccines [21]. Following the universal introduction of a PPSV23 vaccination program in Korea, an assessment of the impact of this on the incidence of pneumococcal diseases is needed. However, the lack of a national dataset and surveillance system, use of the PCV13 in the same age group in the private sector, high coverage rate of pneumococcal conjugate vaccines in children <5 years old, and the absence of diagnostic tools for nonbacteremic pneumococcal pneumonia, make conducting such studies difficult.

In a previous study [22], we reported functional immune responses to 12 serotypes, which are included in both the PCV13 and PPSV23, in older adults after PPSV23 immunization. To our knowledge, however, there is no report regarding functional immune responses to the 11 additional serotypes contained only in the PPSV23 in any age groups or population.

This study was performed to evaluate functional immune responses by measuring opsonophagocytic assay (OPA) titers against the 11 additional serotypes included only in the PPSV23 in older adults before and after immunization with PPSV23.

2. Methods

2.1. Participants and study design

A total of 62 subjects who have never received the pneumococcal vaccine were enrolled in 2013–2014 into two age groups; group 1 (N = 30; aged 65–74 years) and group 2 (N = 32; aged ≥75 years). Eligibility and exclusion criteria have been described in a previous study [22]. A single dose of PPSV23 (Prodiax23®, Merck & Co. Inc., Whitehouse Station, NJ, USA) was administrated by intramuscular injection. Blood was collected before and approximately 4 weeks (mean: 28.1 days, range: 26–35 days) post-immunization. Sera were stored at −80 °C until use.

2.2. Multiplexed OPA (MOPA) for immunogenicity assessment

The functional opsonic antibodies against 11 serotypes of pneumococcus (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F, and 33F), present in PPSV23 but additional to those in PCV13, were evaluated in 124 sera collected from 62 subjects by MOPA as previously described [23]. Briefly, serum samples were serially diluted (3-fold) in opsonization assay buffer B (OBB; Hanks' balanced salt solution [with magnesium and calcium] with 0.1% gelatin and 5% defined fetal bovine serum) with 20 μL of serum tested in duplicate in 96-well round-bottom plates. Frozen working stocks of each of the four target strains were thawed and washed once with OBB by centrifugation (13,000 rpm for 2 min), and a bacterial mixture was prepared in OBB that contained approximately 5×10^4 CFU/mL of each of the four strains. Ten microliters of the bacteria mixture was added to each well, and plates were incubated at room temperature for 30 min with shaking. After incubation, 10 μL of baby rabbit complement (BRC; Pel-Freeze Biologicals, Rogers, AR) collected from 3- to 4-week-old rabbits was added to all wells except control A wells, which received 10 μL of heat-inactivated BRC (heated at 56 °C for 30 min). Forty microliters of differentiated HL60 cells (containing 4×10^5 cells) were added to all wells, and the plates were incubated for 45 min in a 37 °C/5% CO_2 incubator with shaking. Afterwards, a 10 μL aliquot of the final reaction product from each well was spotted onto Todd-Hewitt yeast (THY) agar (1.5%) plates. Overlay agar (THY with 0.75% agar) containing one of the four antibiotics and 2,3,5-triphenyltetrazolium chloride (TTC; Sigma) was added, and the plates were incubated overnight (37 °C/5% CO_2). The number of surviving colonies was enumerated, and the OPA titers were calculated using linear interpolation. OPA titer is defined as the reciprocal of the interpolated dilution of serum that kills 50% of bacteria. For experiments involving the single-serotype format, the same protocol was followed except that, after being washed, the bacteria were diluted to approximately 1×10^5 CFU/mL. After the assay, 5 μL of the final reaction product from each well was spotted onto THY agar (1.5%) plates. A detailed protocol can be found at www.vaccine.uab.edu.

2.3. Statistical analysis

Geometric mean OPA titers (GMTs) and two-sided 95% confidence intervals (CIs) to each pneumococcal serotype were calculated for both groups. Differences in GMTs between pre- and post-immunization 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 was applied to adjust *P* value for multiple comparisons. Reverse cumulative distribution curves (RCDCs) were used to represent the percentage of subjects that achieved different OPA titers to each of the pneumococcal serotypes. *P* values < 0.05 were considered significant. Statistical analyses were performed using IBM SPSS, version 23.0 (IBM Software, Armonk, NY, US).

2.4. Ethical considerations

This study protocol was reviewed and approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital (EUMC 2016–06-030). This 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 subjects following a detailed explanation of the study. This clinical trial was registered with the Clinical Research Information Service (CRIS, https://cris.nih.go.kr/cris/en/), number KCT 0001963.

3. Results

A total of 62 subjects were analyzed: 30 in group 1 and 32 in group 2. The demographic characteristics of the two groups are the same as described in our previous study [22].

The GMTs and 95% CIs pre- and post-immunization are shown in Table 1. The pre-immunization GMTs for serotype 15B were significantly higher in group 1 than in group 2, and the GMT for serotypes 9N and 11A were significantly higher in group 2 than group 1 ($P < 0.05$). Following immunization, GMTs for all 11 serotypes were significantly increased in both age groups (Fig. 1). The fold increase in OPA titers for serotypes 9N was significantly higher in group 1 compared to group 2 ($P < 0.05$). The number of subjects

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