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Safety and immunogenicity of two octavalent pneumococcal conjugate vaccines in American Indian infants

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Abstract

We evaluated the safety and immunogenicity of two octavalent pneumococcal polysaccharide vaccines (serotypes 3, 4, 6B, 9V, 14, 18C, 19F, and 23F) conjugated to either diphtheria toxoid (PncD) or tetanus protein (PncT) among White Mountain Apache and Gila River Indian Community infants. This was a prospective, non-randomized, open label, comparative pilot study of PncD and PncT. Since this was a pilot study, a small sample size of 60 infants was enrolled. Enrolled healthy infants received either PncD or PncT at 2, 4, and 6 months of age. Antibody concentrations were measured by enzyme-immunoassay (EIA) prior to each dose and 1 month after the last dose. Local reaction rates were similar between PncD and PncT groups. The geometric mean concentrations (GMCs) were significantly higher for PncD than PncT for serotype 3 whereas GMCs were significantly higher for PncT for serotype 4. For this pilot study, both vaccines appeared to be safe and immunogenic in this group of American Indian infants.

Keywords: Pneumococcal conjugate vaccine; Immunogenicity; American Indian

1. Introduction

Streptococcus pneumoniae is responsible for both invasive and non-invasive pneumococcal infections, including bacteremia, pneumonia, bacterial meningitis, and acute otitis media. It is a leading cause of pneumococcal pneumonia, causing over 1 million deaths per year worldwide. Children under 2 years of age, the elderly, and those with certain underlying medical conditions are at highest risk for pneumococcal infection. In addition, some American Indians and Alaska Natives have considerably higher rates of invasive pneumococcal disease than those of the general US population. From 1983 to 1990, the annual incidence of invasive pneumococcal disease among White Mountain Apache children under 2 years of age was 1820 per 100,000 compared to approximately 100 per 100,000 in the general US population [1].

Pneumococcal conjugate vaccines, containing 4–11 serotypes, have been developed and found to be safe, immunogenic, and able to induce immunologic memory [2–16]. Prevnar®, Pnc-CRM_{197} (Wyeth Lederle Vaccines, Pearl River, New York), the heptavalent pneumococcal CRM_{197} conjugate vaccine licensed for use in the United States and other parts of the world, has been found to be safe and immunogenic in children below 2 years of age [17,18]. This vaccine was also shown to be highly efficacious (97.4%, CI 82.7–99.9%) in preventing invasive vaccine serotype pneumococcal disease in children below 3 years of age [19]. Although the serotypes contained in the Pnc-CRM_{197} vaccine represent over 85% of serotypes causing invasive disease in the US, only 60% of the serotypes causing otitis media in children in the US are represented and less than 60% of the serotypes causing invasive disease in developing countries and Apache/Navajo Indian populations are contained in the vaccine [20–22]. Therefore, there is a continued need to evaluate conjugate vaccines that contain additional serotypes. Serological correlates of protection, i.e. specific serologic threshold concentration associated with protection from disease, of $\geq$0.2 μg/ml and $\geq$0.35 μg/ml have been suggested criteria for comparing vaccine formulations [23,24].

Prior to the licensure of Pnc-CRM_{197}, this study was designed to investigate two octavalent pneumococcal conjugate vaccines manufactured by Aventis Pasteur, previously known as Pasteur Mérieux Connaught. The study was conducted as a pilot, open label, non-randomized trial to assess the safety and immunogenicity of two octavalent pneumococcal conjugate vaccines. Each vaccine differed only by their carrier, either diphtheria toxoid (PncD) or tetanus

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protein (PncT). The major objective of the study was to compare the serotype specific immunogenicity between the two different carriers among the study populations.

2. Materials and methods

2.1. Population and study design

The study protocol was approved by the Johns Hopkins University School of Medicine Joint Committee on Clinical Investigations, the Phoenix Indian Health Service Institutional Review Board, and the tribal councils and health boards of the respective tribes. Healthy infants aged 6–12 weeks from the White Mountain Apache reservation and Gila River Indian Community in Arizona were enrolled in the study. Eligible infants were identified from Indian Health Service (IHS) facility birth logs. Written informed consent was obtained by the parent/guardian of each infant prior to participation in the study. Sixty consented infants were enrolled in the study between November 1995 and July 1996. The first 30 enrolled infants were assigned to the PncD group and the next 30 enrolled infants were assigned to the PncT group. Infants received three doses of the appropriate study vaccine at 2, 4, and 6 months of age. Infants concurrently received *H. influenzae* type b, reconstituted with diphtheria, tetanus toxoid and whole cell pertussis (DTP//ActHIB, Connaught Laboratories (Aventis Pasteur), Swiftwater, Pennsylvania) and oral polio (OPV, Wyeth Lederle) at 2, 4, and 6 months of age and hepatitis B vaccine (HBV, Merck, Sharpe and Dohme or Smith Kline Beecham) at 2 and 6 months of age. Infants were closely monitored for adverse events for 15 min following each vaccination. Parents/guardians were given a diary card to record the infant's morning daily rectal temperature, reactions at the injection site including erythema (none, <1, or ≥ 1 in.), swelling/hardness, tenderness upon touch, and pain/soreness, and systemic reactions including irritability, drowsiness, anorexia, diarrhea, vomiting, rash, adenopathy, and unusual or persistent crying. All reactions were recorded for 3 days following each vaccination. Parents/guardians were contacted by home visit or phone 4 days after each vaccination to review the information recorded on the diary card. Study participants returned to the clinic for their immunizations at 4 and 6 months of age and for a follow up visit at 7 months of age. At these visits a physical exam, rectal temperature, standardized questionnaire, and chart review were conducted to further evaluate safety.

During each visit, serum samples (5 ml of whole venous blood) were collected from each infant prior to vaccination and 1 month after the third dose. Blood samples were centrifuged within 4 h of collection and separated into two aliquots. Aliquots containing sera were frozen at $-20\,^{\circ}C$ at the clinic within 4 h of separation from the cells. Serum samples were assayed by enzyme-immunoassay (EIA) at Pasteur Mérieux, France (Aventis Pasteur) for antibodies to pneumococcal types 3, 4, 6B, 9V, 14, 18C, 19F, 23F capsular polysaccharides, as described previously [2]. The lower limit of detection for this assay was 0.025 μg/ml for serotype 3, 0.05 μg/ml for serotypes 4, 6B, 9V, 18C, 19F, and 23F, and 0.1 μg/ml for serotype 14.

2.2. Vaccines

PncD and PncT contained pneumococcal capsular polysaccharides (PS) of serotypes 3, 4, 6B, 9V, 14, 18C, 19F, and 23F. Each 0.5 ml dose of PncD consisted of 3 μg of each pneumococcal PS covalently conjugated to diphtheria toxoid. Each 0.5 ml dose of PncT consisted of 1 μg of each pneumococcal PS covalently conjugated to tetanus protein.

Vaccines were injected intramuscularly into the upper part of the right anterolateral thigh. Routine pediatric vaccines (DTwP, Hib, HBV) administered concurrently with the study vaccine were injected into the left thigh.

2.3. Statistical analysis

For each infant, all available data until the infant withdrew or completed the study were used in the analyses. For immunogenicity analysis, the *N* may vary by serotype due to insufficient sera collected. Since this was a pilot study, the study was not adequately powered. To account for this, precision calculations were performed for percentage calculations for each vaccine arm by serotype and ranged from ± 0 to 19% depending on the vaccine and serotype. To account for the multiple statistical testing between vaccine and serotype groups, a *P*-value of 0.01 was considered significant. Analyses were performed using Stata 7.0 [25].

Erythema was categorized as none or yes if a <1 or ≥ 1 in. response was given. The percent of infants with local reactions at the injection site of study vaccines versus routine pediatric vaccines were performed using a sign test. Pearson's chi-square test was used to compare the percent of local reactions of the PncD group to those of the PncT group. Geometric mean concentrations (GMCs) and the percentage of infants reaching antibody levels of ≥ 0.2 and ≥ 0.35 μg/ml for each serotype were calculated and compared by vaccine arm using Fisher's exact test.

3. Results

Of the 60 infants enrolled in the study, 55 completed immunization with three doses of either PncD or PncT. Four infants from the PncT group and one infant from the PncD group did not receive all three doses. Reasons for missed doses or withdrawal from the study were adverse events (2), protocol deviation (2), and parental request (1). Characteristics of the study subjects were similar between the two vaccine groups (data not shown).

Table 1
Geometric mean concentrations of each serotype by vaccine type and dose

Serotype	Geometric mean concentration (μg/ml) (95% CI)							
	Prevaccination		Post-dose 1		Post-dose 2		Post-dose 3	
	PncD	PncT	PncD	PncT	PncD	PncT	PncD	PncT
N	30[a]	28	27[b]	27	26	24	26	24[c]
3	0.20 (0.14–0.28)	0.32 (0.19–0.53)	5.64 (4.20–7.56)	1.89 (1.37–2.61)	4.47 (3.28–6.11)	1.12 (0.80–1.57)	4.23 (3.37–5.32)	1.84 (1.24–2.74)
4	0.16 (0.12–0.23)	0.15 (0.10–0.23)	0.24 (0.17–0.35)	1.78 (1.15–2.75)	1.11 (0.75–1.65)	2.04 (1.24–3.38)	1.27 (0.92–1.75)	3.36 (1.94–5.80)
6B	0.20 (0.14–0.30)	0.37 (0.21–0.66)	0.10 (0.06–0.17)	0.25 (0.17–0.38)	0.65 (0.40–1.04)	0.39 (0.20–0.75)	1.92 (1.13–3.24)	1.32 (0.72–2.40)
9V	0.22 (0.16–0.30)	0.31 (0.19–0.50)	0.32 (0.22–0.49)	0.24 (0.16–0.36)	1.60 (0.91–2.81)	0.47 (0.31–0.71)	2.38 (1.81–3.15)	1.33 (0.80–2.22)
14	0.84 (0.53–1.33)	0.97 (0.55–1.73)	0.72(0.47–1.10)	0.79 (0.50–1.26)	2.64 (1.57–4.43)	1.24 (0.82–1.90)	5.61 (3.60–8.75)	2.61 (1.50–4.54)
18C	0.18 (0.12–0.27)	0.22 (0.15–0.34)	0.55 (0.33–0.91)	0.19 (0.13–0.28)	1.50 (0.93–2.42)	0.40 (0.24–0.66)	2.15 (1.59–2.92)	1.43 (0.88–2.34)
19F	0.73 (0.48–1.10)	0.74 (0.45–1.23)	1.02 (0.66–1.60)	1.06 (0.77–1.47)	5.16 (3.24–8.22)	2.48 (1.62–3.80)	7.04 (4.75–10.43)	7.54 (4.80–11.84)
23F	0.24 (0.16–0.36)	0.37 (0.24–0.57)	0.25 (0.16–0.40)	0.24 (0.18–0.31)	0.93 (0.55–1.54)	0.32 (0.18–0.54)	1.34 (0.81–2.20)	1.34 (0.69–2.64)

[a] $N = 29$ for serotype 6B and 18C.
[b] $N = 26$ for serotype 3, 18C, 19F, and 23F.
[c] $N = 23$ for serotype 9V and 19F.

3.1. Safety

For within subject comparisons, the percent of infants with local reactions within 72 h following each dose of PncD or PncT were similar or lower compared to routine pediatric vaccinations. The percent of infants with local reactions after PncD compared to PncT was not statistically different for each reaction after each dose (data not shown).

3.2. Immunogenicity

Pre-immunization GMCs of pneumococcal antibodies at 2 months of age ranged from 0.16 μg/ml (serotype 4) to 0.84 μg/ml (serotype 14) for the PncD group and from 0.15 μg/ml (serotype 4) to 0.97 μg/ml (serotype 14) for the PncT group (Table 1). After three doses of either PncD or PncT, antibody titers increased more than two-fold from the pre-immunization titers for all serotypes. Post-dose 3 GMCs varied by serotype and vaccine formulation. After three doses of PncD, GMCs ranged from 1.27 μg/ml (serotype 4) to 7.04 μg/ml (serotype 19F). After three doses of PncT, GMCs ranged from 1.32 μg/ml (serotype 6B) to 7.54 μg/ml (serotype 19F) (Table 1). Serotype 3 had a significantly higher post-dose 3 GMC following PncD than PncT ($P = 0.0004$) (Table 2). However, PncT induced a significantly higher post-dose 3 GMC to serotype 4 than PncD ($P = 0.002$). For serotypes 6B, 9V, 14, 18C, 19F, and 23F post-dose 3 GMCs were not statistically significantly different between PncD and PncT.

For both vaccines, 95% or more of the infants had antibody concentrations ≥0.2 μg/ml for each serotype, with the exception of 9V (PncT: 91.3%) and 23F (PncD: 92.3%, PncT: 87.5%) (Table 2). More than 90% of infants had antibody concentrations ≥0.35 μg/ml for each serotype and vaccine, with the exception of 6B for both PncD and PncT (88.5 and 87.5%, respectively) and 9V and 23F for PncT (87.0 and 79.2%, respectively) (Table 2). The percent of infants having antibody concentrations ≥0.2 and ≥0.35 μg/ml after three doses for each serotype was statistically similar between PncD and PncT.

4. Discussion

In this pilot study, three doses of either PncD or PncT appeared to be safe and immunogenic in White Mountain Apache and Gila River Indian Community infants. The incidence of local reactions for both of the study vaccines was similar or less than those seen after routine pediatric vaccinations. Since routine pediatric vaccinations were administered at the same time as the study vaccines, it is not possible to determine the percent of systemic reactions that are directly related to the study vaccines.

Three doses of either PncD or PncT were immunogenic in this group of infants. However, it appeared that antibody levels to serotype 3 were higher when induced by PncD, while antibody levels to serotype 4 were higher when induced by PncT. We found no statistically significant differences in GMCs between study vaccines for serotypes 6B, 9V, 14, 18C, 19F, and 23F after three doses. A similar pattern was also seen in Finnish and Icelandic trials of these two vaccines [14,15]. They reported that PncD induced higher responses for serotypes 3, 9V, and 18C while PncT induced a higher response in serotype 4. The findings from the these studies contributed to the development of the new 11-valent mixed carrier PncD/PncT vaccines [12–16]. Our findings provide further support for the selection of diphtheria toxoid and tetanus protein as carrier proteins for the 11-valent conjugate vaccine.

There are limitations to this study. Because it was designed as a pilot study, a small sample size was used to determine whether these vaccines could elicit a strong immune response in American Indian infants before expanding to a larger scale trial. Due to the small sample size and lack of statistical power, it is difficult to draw firm conclusions about the differences in the immunogenicity between the two vaccines and to predict the immunogenicity for these vaccines in a large population. Another limitation of the pilot study was the lack of a control group. Third, infants were not randomly assigned to a vaccine group. This could lead to a bias in selection of infants, causing infants in one vaccine group to be different than the other vaccine group. Lastly, if vaccine

Table 2
Comparison of GMCs and percent of subjects with defined pneumococcal antibody levels 30 days after the third dose of PncD or PncT

Serotype	PncD ($N = 26$)			PncT ($N = 24$[a])			*P*-value		
	A (GMC)	(≥0.2 μg/ml)	C (≥0.35 μg/ml)	E (GMC)	F (≥0.2 μg/ml)	G (≥0.35 μg/ml)	A vs. E[b]	B vs. F[c]	C vs. G[c]
3	4.23	100	100	1.84	100	95.8	<**0.001**	1.000	0.480
4	1.27	100	96.2	3.36	95.8	95.8	**0.002**	0.480	1.000
6B	1.92	96.2	88.5	1.32	95.8	87.5	0.335	1.000	1.000
9V	2.38	100	100	1.33	91.3	87	0.037	0.215	0.096
14	5.61	100	100	2.61	100	95.8	0.030	1.000	0.480
18C	2.15	100	100	1.43	95.8	91.7	0.145	0.480	0.225
19F	7.04	100	100	7.54	100	100	0.813	1.000	1.000
23F	1.34	92.3	92.3	1.34	87.5	79.2	0.988	0.661	0.409

[a] $N = 23$ for serotype 9V and 19F.
[b] *P*-values based on two-sided *t*-test.
[c] *P*-values based on Fisher's exact test.

groups had different natural exposures to pneumococcus during the trial then that could potentially bias the antibody responses. However, we found that children in each study group had statistically similar baseline characteristics and pre-immunization serotype specific antibody concentrations.

The addition and variation in serotypes used in pneumococcal conjugate vaccines is important for protection against a wider variety of pneumococcal strains and to provide protection in various populations and regions with different serotype prevalence. This study suggests that three doses of either PncD or PncT appear safe and immunogenic in White Mountain Apache and Gila River Indian Community infants. These findings are consistent with previous studies that found higher valent vaccines to be safe and immunogenic [8,11,14–16]. This study also provides further support regarding differences in response to PncD and PncT for certain serotypes and provides further foundation for using mixed carrier vaccines, such as those used in the 11-valent pneumococcal conjugate vaccine [12,13,16].

5. Disclaimer

The opinions expressed in this paper are those of the author and do not necessarily reflect the views of the IHS.

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