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Research article

Safety and immunogenicity of three doses of an eleven-valent diphtheria toxoid and tetanus protein – conjugated pneumococcal vaccine in Filipino infants

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Abstract

Background: An 11-valent pneumococcal conjugate vaccine could provide significantly larger reduction in pneumococcal disease burden than the currently available 7-valent vaccine formulation in many countries.

Methods: In total, 50 infants were enrolled to this open, uncontrolled study, which evaluated the safety and immunogenicity of an aluminium adjuvanted 11-valent mixed-carrier diphtheria toxoid or tetanus protein-conjugated vaccine (11-PncTD) when administered in three doses at 6, 10 and 14 weeks of age simultaneously with DTwP//PRP-T and OPV vaccines in Filipino infants.

Results: The rates of local reactions between the two injection sites, those associated with the 11-PncTD vaccine and those with the DTwP//PRP-T were almost of equal frequency for all three vaccine doses except for induration, which was significantly more common in the DTP//PRP-T injection site. Fever was present in 39%, 22% and 21% of infants following each of the three doses. Antibody responses were determined by an enzyme immunoassay method before the first vaccination and after the three doses. The vaccine elicited a significant anti-pneumococcal polysaccharide antibody response against all serotypes included in the vaccine, except for type 14, for which the pre-vaccination geometric mean antibody concentration (GMC) was high (1.61 μg/ml). The GMCs one month after the vaccination series ranged from 1.1 micrograms/ml for type 6B to 23.4 μg/ml for type 4.

Conclusion: The 11-PncTD vaccine is safe, well-tolerated and immunogenic. The effectiveness of the non-adjuvanted formulation of the vaccine in preventing pneumonia is currently being evaluated in the Philippines.

Background

Infections caused by *Streptococcus pneumoniae* (pneumococcus) are responsible for over 1 million deaths among children less than 5 years of age in developing countries

[1]. This estimate of pneumococcal disease burden may be an underestimate as the aetiology of pneumonia, the most common of severe infections is difficult to assess [2].

Two studies, which reported 97.4% (95% CI 82.7, 99.9%) [3] and 76.8% (95% CI -9.4, 95.1%) [O'Brien, personal communication] efficacy against serotypes included in the vaccine, and the post-licensure surveillance [4] have shown that the seven-valent pneumococcal conjugate vaccine (Prevenar®) prevents majority of invasive pneumococcal disease in children. The vaccine also prevented 20.5% (95% CI 4.4, 34.0%) of radiologically confirmed pneumonia [5] and 57 % (95%CI 44, 67%) of acute otitis media episodes caused by the vaccine serotypes [6]. The vaccine is primarily developed for the U.S. and European epidemiological situation, and it has only a limited coverage of serotypes causing serious pneumococcal infections in most developing countries. In Asia, for example, the coverage of serotypes causing invasive pneumococcal infections among young children is less than 50% [7]. The vaccine formulations with capsular polysaccharides of 11 pneumococcal serotypes could provide nearly 80% serotype coverage in most countries being thus justified for global use [7].

This report describes a safety and immunogenicity study of an aluminium adjuvanted eleven-valent mixed carrier diphtheria toxoid and tetanus protein-conjugated pneumococcal vaccine in Filipino infants. The study preceded a large-scale study, started in July 2000 in the Philippines, in which the effectiveness of the non-adjuvanted formulation of the vaccine is evaluated in prevention of radiologically confirmed pneumonia in children.

Methods

Study subjects

From June 1998 to August 1999, all infants born at full term of pregnancy (≥ 37 weeks) and aged 6 to 9 weeks, who were to start their national vaccination program, were offered enrolment to the open, uncontrolled, descriptive study at the village health centre in Cabuyao, a semi-urban municipality of Laguna, Island of Luzon, the Philippines. Written informed consent was obtained from the guardians of the study children. The study was approved by the Ethical and Institutional Review Board of the Research Institute for Tropical Medicine (RITM), Manila, the Philippines. The study was conducted in compliance with the Helsinki Declaration.

Vaccines and immunization schedule

The study vaccine (lot # S 3497; Aventis Pasteur, Lyon, France) included pneumococcal PS of serotypes 3, 6B, 14 and 18C coupled to diphtheria toxoid protein and pneumococcal PS of serotypes 1, 4, 5, 7F, 9V, 19F and 23F coupled to tetanus protein. The vaccine was formulated with aluminium hydroxide as an adjuvant. It contained 1 μg/dose of PS type 1, 4, 5, 7F, 9V, 19F and 23F, 3 μg/dose of PS type 3, 14 and 18C, and 10 μg/dose of type 6B.

The concomitant vaccines used included diphtheria, tetanus, whole-cell pertussis and *Haemophilus influenzae* type b (DTwP//PRP-T, Aventis Pasteur, France), oral poliovirus (OPV, Aventis Pasteur, France) and plasma-derived hepatitis B vaccine (Med Test, Korea).

The study vaccine for intramuscular injection was presented in a pre-filled (0.5 ml) ready-to-use glass syringe. The vaccine was administered by the deep intramuscular route into the anterolateral aspect of the right thigh. The DTwP//PRP-T vaccine was administered in the opposite thigh and the hepatitis B vaccine in the deltoid area of the left arm. The three doses of study vaccine were given simultaneously with the concomitant vaccines according to the Expanded Program on Immunisation (EPI) schedule at 6, 10 and 14 weeks of age.

Three ml of blood was collected by venipuncture before the vaccination at 6 weeks of age and after three doses at 18 weeks of age. Blood samples were allowed to clot from 30 minutes to 2 hours at room temperature and then centrifuged. Serum samples were stored at the RITM laboratory at -20°C until transported on dry ice to Helsinki, Finland for analysis.

Safety evaluation

Each infant was observed for 30 minutes after the injection for any immediate vaccine related reactions. A digital thermometer was supplied to parents to measure infants' rectal temperature. Local reactions such as induration, redness and swelling were measured by a plastic calliper. Fever was defined as rectal temperature ≥ 38°C whereas the local reactions were considered significant if measured > 2 cm. The parents or guardians were asked to record local and systemic reactions in the evening of the vaccination day (approximately 8 hours after injection) and in the morning of the first to fifth day post-vaccination. The safety surveillance data was then collected by study personnel and entered to database. A serious adverse event was defined as any untoward medical occurrence that at any dose resulted in death, was life-threatening, required inpatient hospitalisation or prolongation of existing hospitalisation, resulted in persistent or significant disability/incapacity, or was a congenital anomaly/birth defect. All such events were followed up and reported by the study personnel.

Immunogenicity of the vaccine

A standardised enzyme immunoassay (EIA) was used to quantify the serum IgG antibodies to capsular polysaccharides of each of the eleven serotypes [8]. The EIA results

were expressed as micrograms/ml calculated based on the assigned IgG values of the 89 – SF2 reference serum from Food and Drug Administration (Bethesda, MD, USA). The detection limits (μg/ml) for each serotypes were: serotypes 1(0.1), 3, 4,18C (0.07), 5(0.15), 6B and 23F (0.09), 7F, 9V (0.08), 14 (0.14) and 19F (0.7), respectively. The assay was performed at the Vaccine immunology laboratory of the National Public Health Institute (KTL), Helsinki, Finland. The detailed serotype specific antibody responses following each of the vaccine doses have been published earlier [9].

Statistical Methods

Immunogenicity results are given as geometric mean concentrations (GMCs) of antibodies, proportions of infants with over 2- and 4-fold increases between different series, and as proportion of children achieving a predefined cut off value of ≥1.0 μg/ml of antibodies concentration. Antibody values under the detection limit for each serotype were given an arbitrary value, half of the detection limit. Statistical comparisons to test the hypothesis of equal GMCs were made with paired t-test using log-transformed data. Fisher's exact test was used for comparison of local reactions associated with EPI and study vaccine injection sites. Chi square test was used to analyse the rates of systemic reactions after each vaccine dose. The level of significance was set at $p < 0.05$.

Results

In total, 47 of the 50 infants enrolled to the study completed the primary series of three vaccine doses. Serum sample was available from 49 infants before and from 47 after the three vaccine doses. The reasons for withdrawal included: parental request (1) and transfer of residence (1). One infant did not continue vaccination series due to a hypotonic hyporesponsive episode following immunization.

There were 28 boys (56%) and 23 girls (44%). The mean ages of infants at each vaccination visit were 7.1 weeks (range: 6–8 weeks) at dose 1; 11.2 weeks (range: 10–13 weeks) at dose 2; 15.6 weeks (range: 14–18 weeks) at dose 3 and 19.5 weeks (range 18–23 weeks) on the fourth visit when the post vaccination serum sample was collected.

The most common local reactions in the order of frequency were induration, pain, swelling and redness (Table 1). The rate of local reactions between the two injection sites, those associated with the study vaccine and the DTwP//PRP-T vaccine were almost of equal frequency following each of the three doses, except for induration. The rate of induration was significantly more common after all of the three doses in the DTwP//PRP-T vaccine than the study vaccine: following dose 1 (45.1 % vs. 8.0 %, $p < 0.001$); dose 2 (30.6 % vs. 10.2 %, $p < 0.01$) and dose 3 (19.1% vs. 4.2%, $p < 0.02$).

The clinical parameters included in the evaluation of systemic events after each dose included fever, vomiting, diarrhoea, inconsolable crying, unusual drowsiness, irritability, anorexia and insomnia (Table 2). The rate of fever (≥ 38°C) after each of the three doses were 39.2%, 22.4% and 21.3%. Fever of 38.7°C or more occurred at 8.0 %, 12.2% and 8.5% of the infants. All local and systemic reactions resolved without medical intervention. There was a trend in the frequency of both local and systemic reactions to decrease with successive doses of vaccine.

There were nine serious adverse events, which consisted of eight hospitalisations. The reasons included: one case of suspected meningitis, 2 cases of acute gastroenteritis, 3 cases of bronchopneumonia, one case of bronchitis and one urinary tract infection. The patient with suspected meningitis had received immunization 17 days before the onset of fever and convulsions, which led to death very soon after admission to hospital. The aetiology of infection remained unconfirmed. All these adverse events were reported as not related to the study vaccine by the investigators. There was one hypotensive hyporesponsive episode that occurred 20 minutes after administration of 11-PncTD and DTwP//PRP-T vaccines. The episode resolved within 15 minutes and was considered as probably study vaccine related. Another case of cyanosis of the lips and nails, fever and chills was reported the day of the third vaccination and was considered as probably related to the vaccination by investigators. At follow-up, this event was re-evaluated as being non-serious. The investigator decided however to withdraw the subject from the study.

The GMCs of antibodies before and after three vaccine doses of the 11-PncTD vaccine are summarised in Table 3. The pre-vaccination GMCs at 6 weeks of age varied between 0.27 μg/ml for serotype 4 and 1.61 μg/ml for type 14. After three vaccine doses, all GMCs of antibodies, except for serotype 14 ($p = .253$) were significantly higher than before the vaccination series. At 18 weeks, all infants had antibody concentration > 1.0 μg/ml against serotypes 1, 3, 4, 5, 7F, 9V, and 19F whereas 49, 83, 91.5 and 87.2 % of infants had concentration > 1.0 μg/ml against serotypes 6B, 14, 18C, and 23F, respectively.

Discussion

A new vaccine should have a protective immunogenicity and an acceptable safety profile similar to present childhood vaccines. The results of our study suggests that immunisation with three doses of 11-PncTD vaccine is safe, well-tolerated and immunogenic in infants. This is in agreement with other studies with the 11-PncTD vaccine

Table 1: Local reactions (%) within the first 5 days after vaccination in infants receiving three doses of DTwP//PRP-T, OPV, hepatitis B and the 11PncTD conjugate vaccines

Reaction	Dose 1		Dose 2		Dose 3	
	DTP	11-PncTD	DTP	11-PncTD	DTP	11-PncTD
Local reactions	64.7	41.4	42.9	26.5	34.0	23.4
Induration	45.1	7.8	30.6	10.2	19.1	4.3
Pain	45.1	27.5	30.6	22.4	23.4	21.3
Redness	7.8	0	2.0	0	2.1	0
Swelling	15.7	7.8	6.1	4.1	0	0

Vaccine administered at 6 (N = 50), 10 (N= 49) and 14 (N= 47) weeks of age

Table 2: Systemic reactions (%) within the first 5 days after vaccination in infants receiving three doses of DTwP//PRP-T, OPV, hepatitis B and the 11PncTD conjugate vaccines

Reaction	Dose 1	Dose 2	Dose 3
All systemic	80.4	53.1	46.8
Fever	40.0	22.3	21.3
Vomiting	4.0	2.0	4.3
Diarrhoea	10.0	6.2	10.6
Inconsolable crying	34.0	28.6	14.9
Unusual drowsiness	16.0	2.0	2.1
Irritability	44.0	24.5	10.6
Anorexia	14.0	8.2	6.4
Insomnia	36.0	12.2	10.6

Vaccine administered at 6 (N = 50), 10 (N= 49) and 14 (N= 47) weeks of age

Table 3: Geometric mean concentration (GMC, μg /ml) and 95 % confidence intervals (CI) of antibodies against pneumococcal serotypes included in the 11-valent pneumococcal vaccine given at 6, 10 and 14 weeks of age in Filipino infants

Age	1	3	4	5	6B	7F	9V	14	18C	19F	23F
6 wk (n = 49)	0.48 (0.34–0.68)	0.40 (0.30–0.54)	0.27 (0.19–0.38)	0.53 (0.37–0.77)	0.33 (0.24–0.46)	0.80 (0.58–1.12)	0.53 (0.39–0.77)	1.54 (1.03–2.53)	0.38 (0.26–0.52)	0.96 (0.64–1.45	0.49 (0.35–0.73)
18 wk (n = 47)	15.23 (12.75–18.19)	4.87 (3.91–6.08)	23.41 (18.95–28.92)	12.49 (10.77–14.48)	1.12 (0.77–1.61)	8.87 (6.82–11.53)	7.69 (6.27–9.43)	2.18 (1.65–2.87)	4.50 (3.18–6.38)	16.11 (12.81–20.24)	3.89 (2.83–5.34)

Serum sample withdrawn before vaccination at 6 weeks of age and 4 weeks after the third dose at 18 weeks of age

formulations [10,11] [In Iceland Sigurdardottir, personal communication and in Chile Lagos, personal communication] and with other pneumococcal conjugate vaccines [3,6,12,13].

No significant safety problems have been reported in safety and immunogenicity studies, in which over 20 000 subjects received various number of doses of different pneumococcal vaccine candidates [14,15]. Although the lack of standard definitions for different clinical signs and symptoms may bias the comparison of safety profiles of different pneumococcal vaccines, the local reactions such as redness, swelling, induration and pain at the site of injection have been the most commonly reported adverse effects. The rate of local reactions has varied between 6 and 38% in different studies, but, as observed also in this study, has been lower at the pneumococcal than at the DTP-Hib vaccine site [3,6,11,16].

The 11-PncTD vaccine formulation used in this study contained aluminium adjuvant, which has been associated with increased local reactions [17]. In Israel and Finland the non-adjuvanted 11-PncTD formulation was less frequently associated with local reactions than the adjuvan-

ted vaccine formulation [10,11]. This, together with the general development towards aluminium-free vaccines despite the acceptable safety of aluminium adjuvants over past six decades [18], led to the decision of selecting the non-adjuvanted 11-PncTD for the on-going effectiveness study.

In this study, fever was the most commonly noted systemic adverse effect following immunization. The rate between 39% and 21%, depending on the number of the dose, is similar to other studies, where fever above 38.5 C has been reported in up to 52% of children, 22 % in toddlers and 4% in adults [14]. As this study did not include a control group with no pneumococcal vaccination given it is difficult to estimate if the 11PncTD increased the rate of systemic reactions when compared to infants receiving only EPI vaccines. Whether the one hypotensive hyporesponsive episode noted in this study was due to 11-PncTD vaccine or other concomitantly administered vaccines is difficult to determine. The other serious adverse events reported were unlikely to have a causal relationship with the 11-PncTD vaccine.

The 11PncTD vaccine elicited high antibody responses in Filipino infants. The antibodies at 6 weeks of age were most likely passively transferred maternal antibodies. The GMCs of antibodies against several serotypes such as 7F, 14 and 19F were considerably high at 6 weeks, but the detailed analysis of antibody responses to each vaccine dose did not support the hypothesis of interference in immune response due to pre-existing antibodies [9].

The GMCs following three doses of vaccination against most pneumococcal serotypes with the exception of types 6B and 14 were higher than described in infants receiving other pneumococcal conjugate vaccines in other populations [3,6,19] or in Finnish and Israeli infants [10] or toddlers [11] receiving the same vaccine. The causes for this variation among populations are unknown yet, and may include reasons such as in-utero priming due to maternal vaccination with tetanus toxoid, boosting effect of early pneumococcal carriage common in developing countries, age at immunization, genetic factors and other concomitant vaccines. This study did not evaluate the immunogenicity of other concomitantly administered vaccines. However, another safety and immunogenicity study conducted in the Philippines with the same vaccine did not demonstrate significant interference between different vaccine antigens [Biltoft, personal communication].

Both quality and quantity of serotype specific antibodies are important characteristics of immunogenicity of pneumococcal conjugate vaccines. In this study, majority of infants had antibody concentration ≥ 1.0 μg/ml against the eleven serotypes included in the vaccine. Without established serologic correlates of protection against pneumococcal diseases, the actual impact of the 11-PncDT vaccine in reducing pneumococcal specific disease burden is difficult to estimate [19]. When the vaccine is administered simultaneously with the whole-cell pertussis containing DTP-vaccine, the similar or higher post-immunisation GMCs of antibodies compared to those achieved in the U.S. with the 7-valent vaccine suggest, however that the efficacy could be at least similar. Furthermore, pneumococcal conjugate vaccines, like the Hib conjugates evoke a T-cell dependent immune response, which leads to formation of immunologic memory [20,21]. This, combined with the herd immunity effect achieved through reduced circulation of pneumococci due to lower carriage acquisition of vaccine serotypes [22], may further increase the effectiveness of the pneumococcal conjugate vaccines.

Conclusions

The 11-PncTD vaccine is safe, well-tolerated and immunogenic in infants. The effectiveness of the non-adjuvanted formulation of the vaccine in prevention of pneumonia is currently being evaluated in the Philippines. The availability and routine use of pneumococcal conjugate vaccines might have a very significant public health impact in developing countries where the pneumococcal infections are a major cause for childhood mortality.

Competing interests

H Käyhty has provided consultancies on advisory boards for Aventis Pasteur and GlaxoSmithKline; has had travel paid for by Aventis Pasteur, GlaxoSmithKline, Spectrum Medical Sciences and Wyeth Lederle Vaccines as an invited speaker or expert at symposia; and has received honoraria from Aventis Pasteur, GlaxoSmithKline, and Wyeth Lederle Vaccines. The other authors declare no competing interests.

Authors' contributions

MRZC analysed the safety and TP immunogenicity data. CPG was the study physician responsible for enrolment and evaluation of study subjects. HK supervised the immunogenicity analysis and participated in the planning of study design together with MGL, who was the principal investigator and HN, who was the study coordinator. All authors contributed to the writing of the manuscript and approved the final version.

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