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Brief report

Safety and preliminary immunogenicity of Cuban pneumococcal conjugate vaccine candidate in healthy children: A randomized phase I clinical trial

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ABSTRACT

A new heptavalent conjugate vaccine (PCV7-TT) is under development in Cuba. PCV7-TT contains 2 μg of serotypes 1, 5, 14, 18C, 19F, 23F and 4 μg of 6B, each one conjugated to tetanus toxoid (TT). This vaccine was designed with the serotypes that cause most invasive pneumococcal diseases (IPD) worldwide. In the present study, we investigated the safety and explored the immunogenicity of PCV7-TT during a controlled, randomized and double blind clinical trial phase I in 4–5-year-old children. PCV7-TT was well tolerated and as safe as Synflorix used as control vaccine. Following a single-dose vaccination, all individual serotypes included in PCV7-TT induced statistically significant increase of IgG GMC and OPA GMT. These are the first clinical results of PCV7-TT in children and they pave the way toward next clinical trials in children and infants. This clinical trial was published in the Cuban Public Register of Clinical Trials with code RPCEC00000173.

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1. Introduction

The introduction of Prevnar in the USA in 2000 marked a breakthrough in the fight against pneumococci due to its high efficacy in preventing IPD [1]. Also, a reduction of child mortality was reported in children below 2 years and a substantial decrease in IPD in other non-immunized age groups was documented [2,3]. After more than a decade of Prevnar use, the currently available pneumococcal conjugate vaccines (PCV7, PCV-10 and PCV-13) have been showed to be safe and effective for reducing pneumonia, meningitis and deaths caused by *Streptococcus pneumoniae* [4]. Thanks to different actions, these very complex and expensive vaccines have been introduced in some low income countries [5], but several middle income countries are not able to access them because of the high prices of these vaccines [6].

The idea of Prevnar (PCV-7) was that a conjugate vaccine would have a tremendous impact on health care with only seven serotypes if they were accurately selected as those causing the majority of diseases. The original selection of the serotypes in Prevnar 7 was based on serotypes causing pneumococcal diseases in the USA [7], although the seven most important serotypes worldwide were not the same [8]. A systematic worldwide evaluation of serotypes causing invasive pneumococcal disease among children under five showed that seven serotypes (1, 5, 6A, 6B, 14, 19F, 23F) were the most common globally and, based on year 2000 incidence and mortality estimates, these seven serotypes accounted for 300,000 deaths in Africa and 200,000 deaths in Asia [8]. This idea of the seven most important serotypes was boosted in WHO recommendations on the Target Product Profile (TPP) for the Advance Market Commitment (AMC). The TPP recognizes that a combination of a limited number of serotypes (1, 5, 6A/6B, 14, 19F, and 23F) accounts for at least 60% of diseases both globally and in every region of the world, and a vaccine with this coverage would be expected to have a considerable public health impact and would be favorably assessed by cost-effectiveness [8,9].

Cuba has been developing a new heptavalent conjugate vaccine (PCV7-TT) during the last 8 years, and our strategy is different to the current trends of making PCV more complex by adding more serotypes. We developed a PCV containing the seven most prevalent serotypes in children below 6 years: 1, 5, 6B, 14, 18C, 19F and 23F, which account for ~70% of isolated serotypes in the America and other regions [8,10]. PCV7-TT contains 2 μg of serotypes 1, 5, 14, 18C, 19F and 23F and 4 μg of 6B, each conjugated to tetanus toxoid (TT); and aluminum phosphate is used as adjuvant.

A year ago, the safety of this PCV7-TT vaccine was initially demonstrated in 40 healthy young adults during a clinical trial phase I, controlled, randomized and double blind, using polysaccharide vaccine Pneumo-23 as control (Sanofi-Pasteur) (data submitted for publication). Clinical trials in children and infants are the next stages in the clinical evaluation of this vaccine to get the license.

In the present study, we investigated the safety and preliminary immunogenicity of PCV7-TT during a randomized phase I clinical trial in 4–5-year-old. This clinical trial was designed as a bridge recommended by the Cuban National Regulatory Agency (CNRA) to assess the safety in children around 5-year-old previous to authorize clinical trials in infants, for this reason the sample of children is small.

2. Materials and methods

A phase I, controlled, randomized (ratio 1:2) and double blind clinical trial was designed to assess the safety and to explore the immunogenicity of PCV7-TT in 4–5-year-old children. The protocol was reviewed by the Ethics Committee of Children University Hospital "Juan Manuel Márquez". The clinical trial was authorized by the CNRA, published in the Cuban Public Register of Clinical Trials with code RPCEC00000173 and named "Evaluation of the safety and immunogenicity of the Heptavalent Pneumococcal Conjugate Vaccine in healthy children. Phase I". It was conducted in accordance with the code of Ethic of the World Medical Association for experiments in human beings [11].

Fifteen healthy 4–5-year-old children resident in Havana, who had not received prior pneumococcal vaccination, were enrolled in the study once their parents gave written informed consent for their participation. They were recruited by their doctors in the primary health attention system after detailed information to the parents. The children were randomized to receive a single intramuscular dose in their right arm of either Synflorix ($n = 5$) or PCV7-TT ($n = 10$).

PCV7-TT (Batch Neu-13.02, Manufacturer: Center for Bimolecular Chemistry) contains 2 μg of serotypes 1, 5, 14, 18C, 19F, 23F and 4 μg of serotype 6B, each one conjugated to TT for 24.5 μg of total TT in the formulation, 125 μg of aluminum phosphate and 0.058 mg of thiomersal. The vaccine is a suspension for injection and is presented in single dose vial; one dose is contained in 0.5 ml. Its routine storage is between 2 and 8 °C.

Synflorix vaccine (Batch ASPNA305AA, Manufacturer: GSK) was used as control. It is composed of the capsular polysaccharides purified from 10 serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F each one conjugated to a carrier protein, either protein D (PD), tetanus toxoid (TT) or diphtheria toxoid (DT). Synflorix is a preservative-free liquid suspension, adjuvanted with aluminum phosphate and presented as pre-filled glass syringes ready for intramuscular injection [12].

In order to assure the double-blinding because the presentation of both vaccines is different, we used a barrier system where the nurse can access to the arm of the children but she cannot see who is the child.

The primary outcome of this study was safety but immunogenicity was also explored. Post-vaccination, children were followed for 30 days to assess adverse events. Serum was obtained before and 30 days after vaccination for assessment of immunogenicity.

Each subject was closely followed for 3 h post-vaccination for immediate adverse effects. During the next 30 days the child's parents recorded any adverse events in a diary, and they received medical visits on 1, 2, 3, 7, 21 and 30 days after immunization.

Local injection-site adverse events (local pain, tenderness, redness, induration, swelling, local temperature increase, functional impotence, abscess or necrosis) and systemic symptoms (fever, anaphylactic shock, drowsiness, nausea, vomits and headache) were recorded during the first 7 days after vaccination. These were the expected adverse events declared in the protocol. Any other events occurring with the child were recorded by the parents during 30 days and registered as unexpected adverse event.

The immunological evaluations were performed at the WHO Reference Laboratory, UCL Institute of Child Health in London. Three hundred microlittermilliliters of each serum samples were taken to London ensuring the cold chain. Pneumococcal serotype-specific anti-capsular polysaccharide antibodies for the seven serotypes in PCV7-TT and 19A and 6A were measured by ELISA using double absorption with C and 22F polysaccharides, following the WHO recommended protocol [13]. Additionally, the antibody response to carrier protein TT was evaluated, using indirect ELISA assay for quantification of tetanus antitoxin in human serum, with the use of a previously calibrated standard [14].

Functional opsonophagocytic antibodies were measured by a viable counting MOPA method using differentiated HL60 cells as effector cells. The OPA titer was expressed as the reciprocal of the serum dilution that caused a 50% reduction of the colony-forming units compared to a control that contained no human serum, following the WHO recommended protocol [15].

2.1. Statistics

Sample size was determined based on practical considerations, taking into account that this clinical trial was only a bridge between trials in adults and infants, to show the safety of the vaccine previous to immunize infants, as recommended by the CNRA.

Random list was generated using R program and randomization 1:2 was used to assign 10 subjects to the group PCV7-TT and 5 subjects to the group Synflorix. Subjects were included in the safety analysis if they received the required dose and all evaluation visits were made. All researchers and participants were blinded until the end of the study.

A non-parametric test (Fisher's exact test for count data, with error $\alpha = 0.05$ or 95% of safety) was used to compare incidence for each adverse event. Frequency of occurrence for adverse events was reported in both groups. This trial had the power in the range from 65% to 90% (depended on the adverse event) to detect significance differences between proportions of appearance in both groups (at least 10% of difference).

Immunogenicity data were reported as antipolysaccharide IgG antibody geometric mean concentrations with 2-sided 95% confidence intervals. Functional antibody activity (OPA) was reported as a geometric mean titer with 2-sided 95% confidence intervals. Non-parametric tests were used to compare pre-vaccination and post-vaccination states between groups for each serotype and TT (Mann Whitney *U* test and Sign test with error $\alpha = 0.05$). Numbers of subjects with OPA titer $\geq$ 1:8 and IgG concentration $\geq$ 0.35 μg/ml were calculated for each serotype in both groups.

3. Results and discussion

A total of 26 subjects were evaluated for inclusion and 15 were included. They were randomized in two groups: 10 subjects received one dose of PCV7-TT and 5 received one dose of Synflorix as control vaccine. Demographic characteristic of the two groups were similar for gender, ethnicity and age. Subjects were followed-up during 30 days after vaccination. Data from all included subjects were analyzed for safety and immunogenicity outcomes. No losses or exclusions were reported.

Table 1
Adverse events reported after immunization with one dose of PCV7-TT or Synflorix in children.

Adverse events		Synflorix ($N = 5$) *n*	PCV7-TT ($N = 10$) *n*
Local adverse events (expected)	Local pain	2	7
	Redness	0	1
	Indurations	2	3
	Local temperature increase	1	1
	Swelling	2	3
Systemic adverse events (expected)	Fever	1	1
	Vomits	1	0

N: number of children by group; *n*: number of reported adverse events.

Local adverse events were reported in 7 of 10 subjects immunized with PCV7-TT and 2 of 5 with Synflorix. Local pain was the most frequent event, reported in two children with Synflorix and seven with PCV7-TT (Table 1). Also, indurations were reported in two of the children immunized with Synflorix and three with PCV7-TT. Only two systemic adverse events were reported: fever and vomits, and only the fever was common for both vaccines (one subject in each group). Vomits were reported for one subject immunized with Synflorix (Table 1). In all cases, these local and systemic events appeared and disappeared during the first 72 h after vaccination, and all of them were related with the vaccination. No local or systemic event was reported as severe. No statistical difference ($p \geq 0.05$) was found between groups for incidence of local and systemic adverse events.

Some unexpected adverse events were reported in the subjects during 30 days after immunization: 3 unrelated events were reported in children immunized with Synflorix while 11 unexpected events were reported for PCV7-TT. One subject immunized with PCV7-TT reported 8 of the 11 unexpected events, which appeared between 7 and 21 days after vaccination and all of them were symptoms associated with a diagnostic of pharyngoamigdalitis lymphonodular: (fever, headache, rash, skin lesions, vomits, cough, papules and sore throat).

No serious adverse events were reported using PCV7-TT. In general, both vaccines were well tolerated, and local pain was the more

Table 2
IgG antibody response against individual pneumococcal serotypes and TT.

Serotype	Blood sampling time point	IgG GMC (μg/ml) with 95% CI		# Subjects ELISA GMC $\geq$ 0.35 μg/ml	
		Synflorix ($N = 5$)	PCV7-TT ($N = 10$)	Synflorix ($N = 5$)	PCV7-TT ($N = 10$)
1	T-0	0.164 (0.11, 0.24)	0.161 (0.13, 0.19)	1	0
	T-30 days	1.009 (0.088, 11.5)	1.658 (0.34, 8.08)	4	10
5	T-0	0.191 (0.073, 0.5)	0.29 (0.1, 0.83)	1	7
	T-30 days	0.843 (0.174, 4.09)	0.624 (0.1, 4.18)	5	8
6B	T-0	1.976 (0.298, 13.1)	0.52 (0.63, 4.31)	5	6
	T-30 days	3.452 (0.88, 13.55)	5.775 (0.25, 134.2)	5	9
14	T-0	0.449 (0.04, 4.602)	0.433 (0.03, 6.26)	3	6
	T-30 days	2.762 (0.827, 9.2)	6.075 (0.37, 99.84)	5	10
18C	T-0	0.226 (0.075, 0.68)	0.302 (0.019, 4.87)	2	2
	T-30 days	6.301 (1.122, 35.5)	3.402 (1.09, 10.67)	5	10
19F	T-0	0.347 (0.08, 1.59)	0.424 (0.04, 4.2)	3	6
	T-30 days	3.127 (0.33, 29.63)	7.212 (0.82, 63.34)	5	10
23F	T-0	0.462 (0.036, 6)	0.217 (0.06, 0.82)	3	3
	T-30 days	2.246 (0.26, 19.82)	3.018 (0.31, 29.48)	5	10
Serotypes not included in PCV7-TT and Synflorix					
6A	T-0	0.437 (0.053, 3.59)	0.563 (0.13, 2.41)	3	7
	T-30 days	0.681 (0.076, 6.1)	1.364 (0.05, 34.47)	5	8
19A	T-0	0.692 (0.038, 12.6)	1.016 (0.04, 25.7)	4	8
	T-30 days	1.362 (0.251, 7.4)	2.713 (0.27, 27.2)	5	10
		IgG GMC (UI/ml) with 95% CI			
Carrier TT	T-0	0.97 (0.23, 4.21)	1.34 (0.62, 2.67)		
	T-30 days	14.8 (5.26, 18.67)	34.04 (11.23, 36.12)		

N: number of children by group.

Table 3
Opsonophagocytic response against individual pneumococcal serotypes.

Serotype	Blood sampling time point	OPA GMT (titer-1) with 95% CI		# Subjects OPA GMT ≥ 1:8	
		Synflorix ($N = 5$)	PCV7-TT ($N = 10$)	Synflorix ($N = 5$)	PCV7-TT ($N = 10$)
1	T-0	4(4,4)	4(4,4)	0	0
	T-30 days	15.8 (0.4, 627.1)	71.4(1.2, 4242.7)	2	7
5	T-0	4(4,4)	4(4,4)	0	0
	T-30 days	37.8 (1.3, 1135)	38.8 (0.9, 1621.1)	3	6
6B	T-0	228.6 (1.1, 47,298.2)	16.8 (0.1, 4568.5)	3	2
	T-30 days	1438.6 (437.3, 4732.8)	5439.7(1024, 8892)	5	10
14	T-0	436.1 (2.1, 90,773.6)	209.8 (1.6, 27,116.2)	4	6
	T-30 days	2639.3 (290.7, 23,961)	8518.8 (937, 77,434)	5	10
18C	T-0	4(4,4)	14.7 (0.1, 2464.2)	0	2
	T-30 days	11,234(2156, 8521.4)	6687.1 (1009, 4307)	5	10
19F	T-0	4(4,4)	12(0.2, 665.8)	0	2
	T-30 days	1780.8 (316.9, 10,007)	3033.8 (371, 24,808)	5	10
23F	T-0	32.9 (0.1, 10,936.3)	20.5 (0.1, 3682.1)	2	3
	T-30 days	1258.2 (375.5, 4216)	5684.8 (327, 98,692)	5	10
Serotypes not included in PCV7-TT and Synflorix					
6A	T-0	170.5 (0.3, 100,997.6)	15.5 (0.1, 3002.7)	2	2
	T-30 days	2486.5 (529.2, 1682)	5366.6 (704, 40,909)	5	9
19A	T-0	14(0.1, 1350.7)	22.4 (0.1, 4610.5)	2	4
	T-30 days	90.6 (0.3, 29,582.5)	222.2 (1.6, 30,820.7)	3	7

frequent adverse event for PCV7-TT. The safety profile showed by PCV7-TT is similar to other currently used PCV as PCV13, with local reactions as main adverse events in adults and infants [16,17].

Immunogenicity of PCV7-TT and Synflorix are summarized in Table 1, showing pre- and post-vaccination antibody concentration by ELISA. All individual serotypes included in PCV7-TT were able to induce statistically significant IgG GMC increases for all seven serotypes ($p \leq 0.05$). Following the single-dose vaccination, the 10 children vaccinated with PCV7-TT showed ELISA antibody concentration ≥ 0.35 μg/ml for serotypes 1, 14, 18C, 19F and 23F, 9 children for serotype 6B and 8 for serotype 5 (Table 2).

Statistical comparisons were performed for each treatment group in order to explore the immune response to TT for each subject included in the study, comparing the immunological results at baseline and 30 days after immunization (Sign test). Significant differences were found for GMC of anti-TT IgG in both groups ($p \leq 0.05$), but with a more rigorous test ($p \leq 0.01$), only the group with treatment PCV7-TT showed statistically significant differences in antibody response to TT after vaccination (Table 2). Synflorix has only 18C serotype conjugated with TT while PCV7-TT has the seven.

Functional antibody responses were measured 1 month after vaccination using an opsonophagocytic activity (OPA) assay. The number of subjects with pre-vaccination level of OPA ≥ 1:8 was low for most of the serotype, except for serotype 14. After vaccination with one dose of PCV7-TT or Synflorix, all children from both groups attained OPA ≥ 1:8 for serotypes 6B, 14, 18C, 19F, 23F, and lower (between 40 and 70%) for serotypes 1 and 5 (Table 3). For serotypes 6A and 19A, not included but related to the serotypes 6B and 19F included in both vaccines, most of the vaccinated children attained OPA ≥ 1:8, probably due to vaccine induced cross-protection (Table 3). Despite the small sample, the immunological results showed by PCV7-TT are in concordance with the immunogenicity reported for common serotypes with Synflorix in children aged over 2 years [18]. These results have the value to be the first one of this vaccine in children.

4. Conclusion

PCV7-TT was as safe as the control vaccine Synflorix in children aged 4–5 years. Despite the small sample of this study, the immunological results are considered very promising as the majority of children had OPA titer lower than 1:8 and became over 1:8 after vaccination for each serotype in PCV7-TT. A phase II–III clinical trial in children will be carried out in the coming months with the aim to demonstrate the immunogenicity of the PCV7-TT vaccine. Also, other clinical trials are planning in infants as target population of this vaccine.

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