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RESEARCH PAPER

Immunogenicity and safety of a meningococcal serogroups A and C tetanus toxoid conjugate vaccine (MenAC-TT): two immune schedules in toddles aged 12–23 months in China

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ABSTRACT

Background: This exploratory study aimed to assess the immunogenicity and safety of 1 and 2 doses of meningococcal serogroups A and C tetanus toxoid-conjugate vaccine (MenAC-TT) in toddles.

Methods: Healthy participants aged 12–23 months were randomized into two groups to receive 1 or 2 doses of the tested vaccine. The interval was 28 days between two doses. Blood samples were collected at day 0 before the immunization and day 28 post-each dose. Safety observation was conducted during 28 days after each vaccination. Serious adverse event (SAE) was conducted throughout 6 month observation period.

Results: Overall 301 toddles were vaccinated. Twenty-eight days post full-course vaccination, ≥97.20% toddles had titers ≥1:8 and ≥81.48% had titers ≥1:128 for MenA and MenC in the two schedules groups. There were no significant differences between the two schedule groups for each titer thresholds and serogroups. Up to month 12 post the first dose, titers ≥1:8 and 1:128 were declined to 71.32–80.83% and 26.67–57.85% for each meningococcal serogroups. Most adverse reactions (ARs) were mild or moderate, and the incidence of grade 3 ARs was below 3.33%. The incidence of redness was significantly higher in the two doses group than that in the one dose group, in terms of grade 1 and grade 2 were higher. No SAEs were considered causally related to vaccination.

Conclusion: The MenAC-TT showed similarly safety and immunogenicity profile in toddles with two schedules. It will be more important to provide the data for formulating appropriate immunization strategies in different age groups in China.

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Meningococcal conjugate vaccine; immunogenicity; safety; clinical trial

Introduction

Invasive meningococcal disease (IMD), such as meningitis and meningococcemia, is caused by *Neisseria meningitidis* and has their highest incidence in infants. The annual number of cases is estimated to be at least 1.2 million with 135,000 deaths over the worldwide. Approximately 10–20% of patients suffer from significant clinical sequelae such as limb loss, deafness, seizures or psychomotor retardation.[1–5] The most important disease-causing serogroups of *Neisseria meningitidis* are MenA, MenB, MenC, MenW and MenY. Their prevalence varies geographically, MenA and MenC are more prominent in Asia, such as China.[6] To combat IMD, an increasing number of countries have included vaccines against *N. meningitidis* in their routine immunization programs.

The specific vaccine use in each country depends on the factors mainly include predominant serogroups, cost and availability. Polysaccharide vaccines were used and in routine immunization in China.[7] Polysaccharide meningococcal vaccines have been used widely in high-risk individuals and for the control of outbreaks. These vaccines elicit a largely T-cell-independent response and are poorly immunogenic in young children, and cannot confer long-lasting immunity,[8] as well as repeated administration of these vaccines may result in hyporesponsiveness.[9] Recommendations for vaccination in the Asia-Pacific region are highly variable.[6,10] The vaccination policy of conjugate vaccine in China is currently under review, despite the availability of conjugate vaccines.[11] In China, The routine IMD immunization schedule for children aged 6–18 months is two doses of the MenA polysaccharide vaccine at an interval of 3 months, and subsequently a single dose of the MenA plus MenC polysaccharide vaccine at ages 3 and 6 years.[11] Study showed that routine use of meningococcal conjugate vaccine in toddlers could expand protection against IMD.[12–] The introduction of conjugate vaccinations into the expanded program on immunization (EPI) schedule is being considered to increase the proportion of the population protected against MD, and new vaccination strategies may be required including the use of conjugate vaccines.[11] This clinical trial is planning to evaluate the immunogenicity and safety of bivalent meningococcal serogroups A and C tetanus toxoid conjugate vaccine in Chinese healthy toddles aged 12–23 months with two different immunization schedules.

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Results

A total of 301 participants were enrolled and randomized (150 in the one dose group and 151 in the two doses group). All the participants were analyzed for safety. Seventeen participants (4 in one dose group and 13 in two doses group) did not complete the study because of voluntary withdrawal not due to an adverse reaction (AR). (Figure 1) For MenA, the modified per-protocol set (mPPS) included 144 in the one dose group and 136 in the two doses group. For MenC, the mPPS included 143 in the one dose group and 135 in the two doses group (Figure 1). The demographic characteristics (age, sex and body mass index

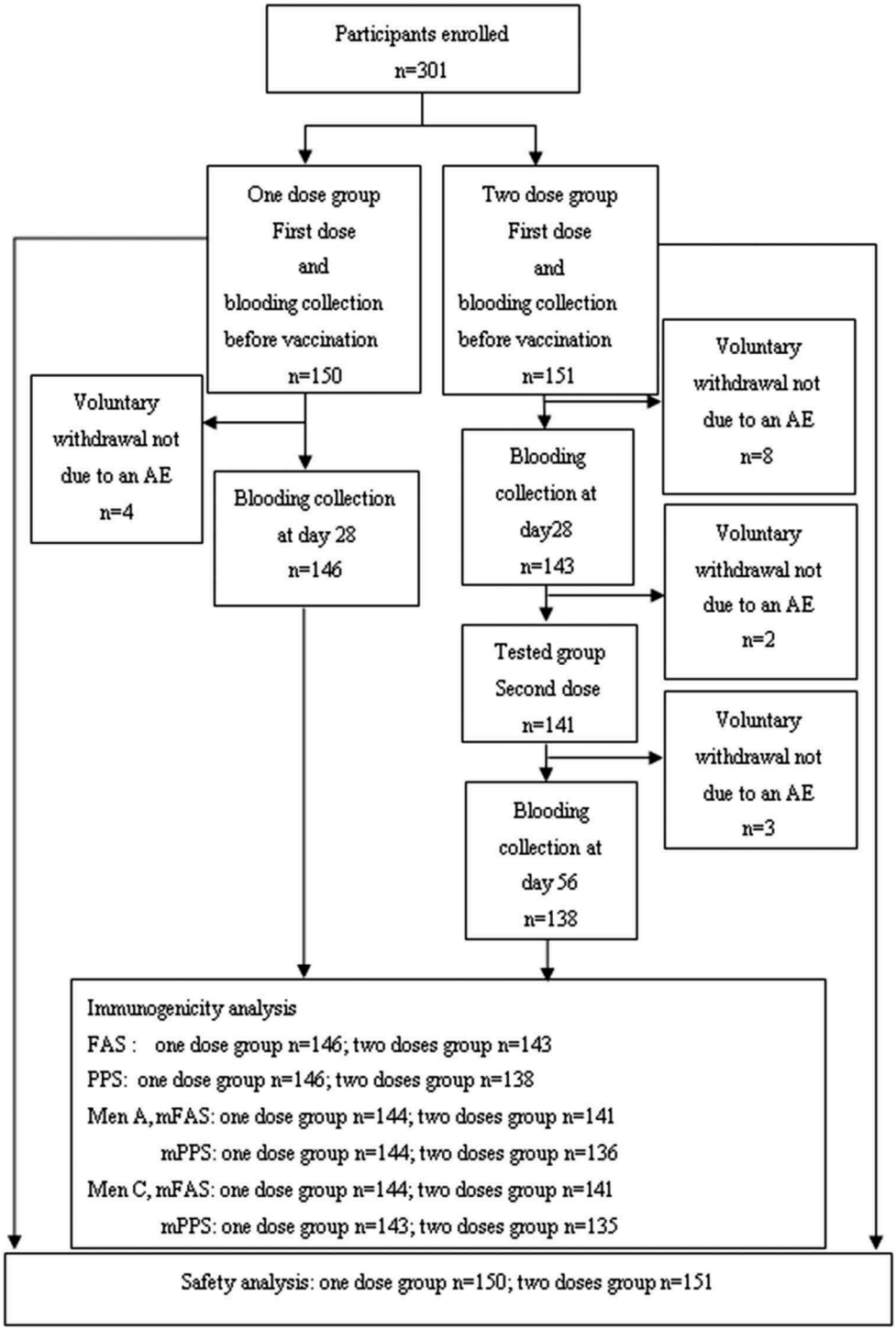

Figure 1. Flow chart for the recruitment of volunteers in the study. Titers for antibody to MenA≥1:8 pre-vaccination were two participants in the one and two doses group, respectively. Titers for antibody to MenC ≥1:8 pre-vaccination were three participants in the one and two doses group, respectively. They were removed from the analysis set (mPPS) for immunogenicity.

Table 1. Baseline characteristics of participants (total vaccinated cohort).

Variable		One dose group N = 150	Two doses group N = 151
Mean age at first vaccination (SD), month		18.44 (3.49)	18.59 (3.17)
Sex	Male, n (%)	88 (58.67%)	80 (52.98%)
	Female, (%)	62 (41.33%)	71 (47.02%)
BMI (95% CI)		17.05 (16.78–17.32)	16.84 (16.59–17.09)

One dose group, participants who received 1 dose at day 0; two doses group, participants who received 2 dose at day 0 and 28; N, number of participants with available results; SD, standard deviation; n (%), number (percentage) of participants in a given category; 95% CI, 95% confidence interval.

[BMI]) were similar without significant difference between the two study groups. (Showed in Table 1, all $P > .05$.)

Immunogenicity

The antibodies to MenA and MenC of participants in mPPS at baseline had titers <1:8, with the geometric mean titer (GMT) of 1.0 in both two schedule groups. At day 28 post completion of the single dose in one dose group and day 28 post completion of the second dose in two doses group, a higher proportion of participants had detectable serum bactericidal activity against MenA and MenC in each group. In the one dose group, seroconversion rates (titers ≥1:8), seroprotection rates (titers ≥1:128) and GMT were 98.61%, 90.97% and 325.7 for MenA, and 97.2%, 84.62% and 227.9 for MenC. Those indicators in the two doses group were 100.00%, 86.76% and 269.4 for MenA, and 98.52%, 81.48% and 177.6 for MenC. The seroconversion and seroprotection rates and GMTs showed no significant differences between the two schedule groups for each titer thresholds and serogroups. (See in Table 2.)

To the month 12 post the first dose, the seroconversion rate, seroprotection rate and GMT were declined to 71.32%, 57.36% and 53.9 for MenA, and 78.13%, 29.69% and 29.2 for MenC in the dose one group. Those indicators in the two doses group were 79.34%, 57.85% and 67.4 for MenA, and 80.83%, 26.67% and 26.1 for MenC. The immunogenicity variables were no significant difference between the two schedule groups.

Safety

From the first dose to day 28 post completing full-course vaccination, two schedule groups showed good safety profiles. The intensities of most AR were mild or moderate (≤grade 2), the AR of grade 3 were at a low reported rate below 3.33% in both two study groups. The total incidence of solicited AR was 58.28% in the two doses schedule group, which was significantly higher than that found (44.00%) in the one dose group ($P = .0132$). The incidence of moderate AR (grade 2) was significantly higher in the two doses group than that in the one dose group ($P = .0016$). The most common symptoms of local solicited ARs were redness and swelling with the incidence of 10.67% and 10.00% in the one dose group compared to 22.52% and 11.92% in the two doses group. The most common symptoms of systemic solicited ARs were fever with the incidence 29.33% *vs.* 39.07% in the one and two dose groups. The incidences of other ARs were lower below 5.00%. Almost all the symptoms were similar in the two groups, excepting redness was reported significantly in the two doses group ($P = .0057$) (see Table 3). Only one participant in the two doses group occurred bronchopneumonia at 7 days later post the first dose. This SAE was assessed without relation to vaccination (data were not shown).

Discussion

In this study, the conjugate MenAC-TT vaccine showed the profile of sturdy immunogenicity at day 28 post primary schedule in healthy toddlers aged 12–23 months in China. The immune response at the terms of seroconversion rates (rSBA titres ≥1:8) for MenA and MenC, was over 97% with one or two doses immune schedules. Seroprotection rates of titers ≥1:128 was high and approximate 80–90%. It was worth noting that GMT at day 28 post the vaccination in the one dose group was little higher than that at day 28 post the second dose in two dose group, even no significant difference was shown. Compared with the first dose in the two doses group, the second dose scarcely increased the immunogenicity which seemed was elicited mostly at the first dose (see in the Table 2). Cutland et al. showed in 2017, 2 month interval of two dose MenACWY-TT just a little bit increased at month 3 compared to month 1.[13] In addition, study showed that the immunological hyporesponsiveness may was induced to the participant who had history of polysaccharide vaccine vaccination, especially to younger children.[14,15] According to the national immunization program in China, the infants should complete two dose meningococcal polysaccharide vaccine before age of 12 months, the participants in the group aged 12–23 months all had history of two dose of meningococcal polysaccharide A vaccine. It showed the

Table 2. Percentage of participants with rSBA titers≥1:8 and ≥1:128 and geometric mean titers against the serogroups MenA and MenC (mPPS cohort for immunogenicity).

	One dose group				Two doses group			
	N	titer≥1:8, %(95%CI)	titer≥1:128, %(95%CI)	GMT(95%CI)	N	titer≥1:8, %(95%CI)	titer≥1:128, %(95%CI)	GMT(95%CI)
MenA								
Day 0	14	0.00	0.00	1.0	136	0.00	0.00	1.0
Day 28	144	98.61 (95.07–99.83)	90.97 (85.06–95.11)	325.7 (268.6–394.8)	135	100.00 (97.30–100.00)	85.19 (78.05–90.71)	311.2 (255.8–378.5)
Day 56	–	–	–	–	136	100.00 (97.32–100.00)	86.76% (79.89–91.96)	269.4 (228.8–317.2)
Month 12	129	71.32 (62.70–78.93)	57.36 (48.36–66.03)	53.9 (34.87–83.29)	121	79.34 (71.03–86.16)	57.85 (48.54–66.77)	67.4 (45.1–100.6)
MenC								
Day 0	143	0.00	0.00	1.0	135	0.00	0.00	1.0
Day28	143	97.20 (92.99–99.23)	84.62 (77.64–90.10)	227.9 (180.3–288.1)	134	99.25 (95.91–99.98)	81.34 (73.70–87.55)	206.0 (166.1–255.6)
Day56	–	–	–	–	135	98.52 (94.75–99.82)	81.48 (73.89–87.64)	177.6 (147.2–214.3)
Month12	128	78.13 (69.96–84.95)	29.69 (21.94–38.40)	29.2 (19.8–42.9)	120	80.83 (72.64–87.44)	26.67 (19.01–35.51)	26.14 (18.7–36.6)

One dose group, participants who received 1 dose at day 0; two doses group, participants who received 2 dose at day 0 and 28; N, number of participants with available results; day 0, the day of first dose; day 28, 28 days post the first dose; day 56, 56 days post the first dose; month 12, 12 month post the first dose; 95% CI, 95% confidence interval.

Table 3. Common symptoms analysis of solicited local and systemic ARs occurring after vaccination.

			Total (%)			Grade1			Grade2			Grade3		
Symptom	Group	N	%	χ^2	*P*	%	χ^2	*P*	%	χ^2	*P*	%	χ^2	*P*
Adverse action	One dose group	150	44.00	6.1396	0.0132	29.33	0.1146	0.7349	10.67	9.9304	0.0016		[a]	0.7497
	Two dose group	151	58.28			31.13			24.50			3.33		
Pain	One dose group	150	2.67	[a]	1.0000	0.67	[a]	0.3708	0.67	[a]	0.4983	2.65	[a]	0.2475
	Two dose group	151	2.65			2.65			0.00			1.33		
Induration	One dose group	150	4.00	0.0001	0.9906	1.33	[a]	1.0000	0.67	[a]	0.6225	0.00	[a]	0.6225
	Two dose group	151	3.97			1.33			1.99			1.33		
Redness	One dose group	150	10.67	7.6282	0.0057	6.00	4.5368	0.0332	3.33	3.7254	0.0536	0.66	[a]	0.6225
	Two dose group	151	22.52			13.25			8.61			1.33		
Swelling	One dose group	150	10.00	0.2843	0.5939	6.67	0.4024	0.5259	0.67	[a]	0.3708	0.66	[a]	0.2141
	Two dose group	151	11.92			8.61			2.65			2.67		
Pruritus	One dose group	150	2.67	[a]	1.0000	1.33	[a]	1.0000	0.67	[a]	1.0000	0.67	[a]	0.4983
	Two dose group	151	2.65			1.99			0.66			0.00		
Fever	One dose group	150	29.33	3.1711	0.0750	19.33	0.1609	0.6883	8.00	4.4533	0.0348	2.00	[a]	1.0000
	Two dose group	151	39.07			21.19			15.89			1.99		
Allergic reaction	One dose group	150	0.00	[a]	1.0000	0.00	–	–	0.00	[a]	1.0000	0.00	–	–
	Two dose group	151	0.66			0.00			0.66			0.00		
Dysphoria	One dose group	150	0.00	[a]	1.0000	0.00	[a]	0.2475	0.00	[a]	1.0000	0.00	–	–
	Two dose group	151	2.65			1.99			0.66			0.00		
Diarrhea	One dose group	150	3.33	0.0876	0.7673	2.67	[a]	0.7497	0.67	[a]	0.4983	0.00	–	–
	Two dose group	151	3.97			3.97			0.00			0.00		
Fatigue	One dose group	150	0.67	[a]	0.6225	0.67	[a]	1.0000	0.00	[a]	1.0000	0.00	–	–
	Two dose group	151	1.99			1.33			0.66			0.00		
Loss of appetite	One dose group	150	2.00	[a]	1.0000	1.33	[a]	1.0000	0.67	[a]	1.0000	0.00	_	_
	Two dose group	151	1.99			1.33			0.66			0.00		

[a]Fisher's exact test; –: Not applicable; One dose group, participants who received 1 dose at day 0; two doses group, participants who received 2 dose at day 0 and 28; N, number of participants with available results; the common symptoms were compared at day 28 in one dose group and day 28 post-dose 2 in the two dose group.

hyporesponsiveness in term of lower GMT and or seroconversion rates after two dose vaccination compared to the first dose, even compared to other studies with one dose of meningococcal polysaccharide diphtheria toxoid conjugate vaccine (MenACYW–D) and MenACWY-TT.[8,13] Nevertheless, it speculated that the vaccines may be given too closely together at 4 weeks. The intervals in other studies were about 3–6 months with an increased immunogenicity post dose 2.[12,16]

Studies of conjugated meningococcal vaccine in toddlers aged 12–23 months showed that one dose of vaccination elicited highly immunogenic. A large phase III study in Finland, in the terms of proportions of toddlers with titers of rSBA ≥1:8 against MenA and MenC were 99.7% in the MenACWY-TT group on the day 42 after the one dose, and the titers of MenC-rSBA ≥1:8 was 97.5% in the MenC group.[17] Result from another phase III study demonstrated that a single dose of MenACWY-TT in toddlers was highly immunogenic, and the proportion of rSBA titres ≥1:8 for all serogroups were ≥97.3%.[18] Furthermore, one phase III study showed that one or two doses MenACWY-TT lead to similar immune response in toddlers aged 12–14 months, as assessed by the rSBA assay with proportions of rSBA titres ≥1:8 about 92.8–97.8%.[19] Although the interpretation of historical comparisons is difficult because of changes in population exposure and laboratory differences in SBA procedures, our results in this study showed similar immunogenicity with those studies at day 28 post the primary vaccination.

In 2006, Auckland et al. found that comparing to unvaccinated ones, subjects with vaccine failure response to the capsular polysaccharide of MenC by eliciting higher SBA titers in convalescent serum samples and IgG avidity in acute serum samples, but remain occurred MenC cases and not confer protection.[20] The booster response is not sufficiently rapid to prevent the invasion which occurred within a few days of colonization. Although evidence showed that meningococcal serogroup C conjugate vaccine (MCC)-induced immune memory, the high level of direct protection decline subsequently.[21,22] Immunological memory alone was insufficient to provide long-term disease protection. Persistence of antibodies may be a more appropriate correlate of protection, rather than the booster response which could not be relied upon to provide protection from meningococcal disease.[20] Few available studies evaluate antibody persistence after primary vaccination with Men-ACWY conjugate vaccines in toddlers. Vesikari et al. phase 2 follow-up study in 2012, the results showed that 98.8% and 90.8% of original Men-ACWY-TT-recipients aged 12–23 months had rSBA titers ≥1:8 against MenA and MenC up to 3 years after vaccination.[23] The result of Vesikari et al. phase 3 study in 2015 showed that 59.9–74.1% and 35.9–40.4% of toddlers retained rSBA titers ≥1:8 against MenA and MenC at year 3 and 4 after priming.[24] Although no direct evidence of efficacy of MCC vaccine, a comprehensive surveillance program was initiated in the UK in terms of herd immunity. The surveillance showed that a reduction of 67% in attack rate of those

unvaccinated occurred from 2001 to 2002, compared with cases reported in 1998–1999.[25] Up to month 12 in this study, 71.32% and 79.34% of the participants with the rSBA titers ≥1:8 against MenA, 78.13% and 80.83% of those against MenC. The seroprotective antibody levels declined markedly in both two schedule groups, especially those against MenC. This situation had been seen in the UK in the subjects between 12 and 15 months who had a boost of MCC.[26] The percentages of subjects with rSBA titers ≥1:8 was declined from 92% to 54% at month 12 after boost. Those data suggest that the immunization strategy (2 + 1 schedule) had not any substantial impact on sustaining seroprotecion. This supports a need for older childhood or adolescent booster vaccination to enhance population immunity.[27] In 2003, the UK had changed immunization schedule to one dose at 3 month of age and one dose at 12 month, meanwhile introduced an adolescent booster at age 13–14 years and a booster dose under the age of 25 years.[28] It will be more important to provide the data for formulating appropriate immunization strategies in different age groups in China.

The two study schedules showed the similar immunogenicity, even similar incidences of AR post the first dose (data were not shown) the incidences of common local and systemic symptoms included redness, swelling were higher in the two doses group post the second dose, related to vaccination times. The incidence of ARs in this study was approximate to other studies, and the safety can be acceptable.[29,30]

One limitation in this study was no control group. A Control is important both for reasons of safety comparator as well as to lend reassurance that there was non-specific rSBA titers were not generated.The aim of this exploratory study just to tentatively provided data for the schedule of experimental vaccine in toddles. More rigorous experimental design and larger sample size is need in the future research to supplied data for immunization strategies.

In conclusion, this study confirmed that two schedules of MenAC-TT induced the similar immune response with a high seroconversion rates of serogroups MenA and MenC over 97% at day 28 post full course vaccination, and declined up to month 12, especially for MenC. MenAC-TT had a good safety profile with little higher incidence of ARs in terms of the symptom of redness and severity of grade 2 in the two doses group caused by more vaccination times. It will be more important to provide the data for formulating appropriate immunization strategies in different age groups in China.

Methods

Study design

This exploratory study was one part of a randomized phase 3 clinical trial (ClinicalTrials.gov: NCT03714737), which was conducted between March, 2016 and October, 2017 in Jiangsu province of China. Protocol was designed by the Jiangsu Provincial Center for Disease Control and Prevention (JSCDC) and OLYMVAX biopharmaceutical Co., LTD (the study sponsor and manufacture of the vaccine). It was approved by the institutional ethics committee of JSCDC. Parents or legal representatives provided written informed consent before enrollment. Healthy participants aged 12–23 months were randomized in a 1:1 ratio to receive one or two doses the tested vaccine. The interval in two doses group was 28 days. Blood samples were collected for immunogenicity assessment on day 0 and day 28 post-each dose. Safety date was collected from day 0 to day 28 post-each dose and serious adverse event was observed extend to 6 months after vaccination.

Participants

Eligible participants were healthy children aged 12–23 months and never received meningococcal conjugate vaccine or meningococcal polysaccharide vaccine over 3 months. Key exclusion criteria included a history (confirmed clinically, serologically or microbiologically) of meningococcal disease, acute disease/infection according to the investigator or fever (axillary temperature > 37.0°C on the day of vaccination), history of allergy or allergic to any ingredient of vaccine, bleeding disorders or history of known thrombocytopenia, immunodeficiency conditions, receipt of subunit or inactivated vaccine, or immunoglobulin in past 7 days, receipt of attenuated live vaccine in past 14 days, had acute illness or acute onset of chronic disease in last 3 days before vaccination.

Sample size

In the initial randomized phase 3 clinical trial, the expected seroconversion of bactericidal antibodies against group A and group C of meningococcal bacteria was over 80% in the control vaccine group after the primary vaccination,[31] and the rates in the experimental vaccine group was assumed no lower than those in the control vaccine group at the same time with the non-inferiority margin value −0.1. The minimum number of subjects in the experimental group and the control group was calculated to be 252, with about 15% shedding rate. The sample size of each group was adjusted to be 300. That is, 300 people in each age group, the experimental group and the control group. The age group 6–-23 months in initial phase 3 trial was divided into two subage group with a sample size of 150 in each. So the sample size of each groups in exploratory study was 150.

Vaccine

The study vaccine was a polysaccharide protein conjugate vaccine manufactured by OLYMVAX biopharmaceutical Co., LTD, prepared with serogroups A and C meningococcal polysaccharides, each covalently bound to tetanus toxoid (TT). polysaccharides of MenA and MenC were obtained by fermented, formalin-inactivated, purified. Each dose of MenAC-TT vaccine was 0.5 mL contained 10 μg each of MenA and MenC polysaccharides conjugated to TT (total TT content 23.6–58.3 μg), added 5–10 mg lactose as a stabilizer to freeze dry with 0.5 mL sodium phosphate buffer as the vaccine diluent.

Serologic evaluations

Functional meningococcal antibody activity against MenA and MenC antigens contained in MenAC-TT was measured using a serum bactericidal assay (SBA) with rabbit complement.[32] The strains of CMCC29019 (MenA) and CMCC29026 (MenC) for

rSBA was afforded by National Center for Medical Culture Collections (CMCC). The lower limit of quantitation of the rSBA was a titer of 2. Assays were performed by the National Institute for Food and Drug Control (NIFDC, Beijing, China). The analysis set of immunogenicity was evaluated in mPPS (the participant who was seronegative pre-vaccination (titers <1:8), met all eligibility criteria, and adhered to the protocol). The primary endpoint was the seroconversion (defined as titers ≥1:8 post vaccination in initially seronegative participants (pre-vaccination titers <1:8)) against MenA and MenC at day 28 post each dose. Secondary endpoints included the rates of seroprotecion (rSBA titers ≥1:128) at day 28 post each dose.[8] The more conservative threshold of rSBA titres ≥1:128 was been previously associated with seroprotection against MenC,[13,15,18,33] and were applied to MenA in this study.[8,29]

Safety evaluation

Safety endpoints included: (1) the occurrence and intensity of solicited injection site reactions and systemic reactions occurring from day 0 through day 7 after vaccination, (2) the occurrence of unsolicited adverse events within 28 days after vaccination and (3) the occurrence of SAE occurring at any time during the study.

Adverse reactions were graded according to the scale, derived from the Division of Microbiology and Infectious Diseases pediatric toxicity table.[34] Injection site adverse reactions, such as erythema, indurate and swell, were categorized as grade 1 (<10mm), grade 2 (10–25mm), grade 3 (26–50mm) or grade 4 (>50mm). Pain was categorized as grade 1 (touching with a slight reaction), grade 2 (touching with a moderate reaction such as crying), grade 3 (touching with strong reaction such as scream and rejection) or grade 4 (hospitalization or emergency department visit). Itching was categorized as grade 1 (mild itching in injection site), grade 2 (moderate itching in injection site) or grade 3 (itching in the whole body). Systemic adverse reactions were graded for fever, allergic reaction, fatigue, vomiting, loss of appetite, fidgety (irritable, abnormal crying) and diarrhea. Fever was categorized as grade 1 (axillary temperature, 37.1~37.5°C), grade 2 (axillary temperature, 37.6~39.0°C), grade 3 (axillary temperature, >39.0°C). Fatigue was categorized as grade 1 (no interference with activity), grade 2 (some interference with activity), grade 3 (significantly prevent daily activity) or grade 4 (hospitalization or emergency department visit). Loss of appetite was categorized as grade 1 (intake less than normal), grade 2 (miss a meal or two), grade 3 (without eating for 24 h), grade 4 (without eating and administration of intravenous injection). Nausea, vomiting and diarrhea were categorized as grade 1 (once or twice per 24 h, no interference with activity), grade 2 (twice or five times per 24 h with limited activity), grade 3 (over six times in 24 h, administration of intravenous injection) or grade 4 (hypotension, hospitalization with nutritional supplements in other ways). Fidgety (irritable, abnormal crying) was categorized as grade 1 (sleep less than normal), grade 2 (difficult to fall asleep), grade 3 (persistent or prolonged crying).

Statistical analyses

Statistical comparisons were made using two-sided tests with an alpha value of 0.05. Geometric mean titers (GMT) were summarized with 95% confidence intervals (CIs) and compared by the Student's *t*-test or t' test, respectively. Comparison of seroconversion rate, rate of titers ≥1:128, baseline characteristics and adverse reactions/events rate were conducted by Chi-square test or Fisher's exact test. The statistical analyses were performed by an independent statistician using SAS (version 9.1, SAS Institute Inc., Cary, NC, USA).

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Authors' contributions

F-C Zhu, H-G Li, Y-M Hu, J-X Li and F-Y Meng contributed to conception and design of the study; K Chu and Q Liang coordinated the clinical aspects of the study and contributed to collecting data and managing participants; Li Luo analyzed the data; J-L Hu wrote the paper; All authors read and approved the final manuscript.

Trial Registration

ClinicalTrials.gov: NCT03714737

Abbreviations

IMD	invasive meningococcal disease
MenA	meningococcal serogroups A
MenC	meningococcal serogroups C
MenAC-TT	meningococcal Serogroups A and C tetanus toxoid conjugate vaccine
EPI	expanded program on immunization
MCC	meningococcal serogroup C conjugate vaccine
MenACYW–D	meningococcal polysaccharide diphtheria toxoid conjugate vaccine
AE	adverse event
AR	adverse reaction
GMT	geometric mean titer
SAE	serious adverse event
CI	confidence interval
mPPS	modified per-protocol set
IEC	independent ethics committee
CFDA	Chinese Food and Drug Administration
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
JSCDC	Jiangsu Provincial Center for Disease Control and Prevention

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