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Immunogenicity of a new *Salmonella* Typhi Vi polysaccharide vaccine—vax-TyVi®—in Cuban school children and teenagers

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Abstract

A randomized, controlled, double blind study was carried out in Cuban children and teenagers aged 9–13 years to evaluate the immunogenicity of vax-TyVi®—*Salmonella* Typhi Vi polysaccharide vaccine—with respect control vaccines. Serum samples were taken before and 21 days after the immunization, and ELISA was used for the determination of antibodies to Vi polysaccharide. Subjects who received vax-TyVi® and TYPHIM Vi™ (Pasteur-Mérieux) showed seroconversion rates of 85.61 and 78.36%, respectively. The geometric mean titer (GMT) values for Vi antibodies induced after vaccination were 6.27 μg/ml (5.40–7.38 μg/ml) and 5.97 μg/ml (5.01–7.10 μg/ml), respectively. In contrast, subjects receiving the tetanus toxoid vaccine showed 0% seroconversion.

Keywords: Typhoid vaccine; Typhoid fever; Vi polysaccharide

1. Introduction

Typhoid fever remains a serious public health problem, especially in developing countries, with an estimated over million cases and more than 600,000 deaths every year. Control of the disease requires uncontaminated food and water and good sanitary disposal, prompt diagnosis and treatment of patients and asymptomatic carriers [1–6]. Typhoid fever has not been controlled by vaccination because of limitations of whole-cell killed vaccines [1–9].

The history of vaccination against typhoid fever goes back more than 100 years [2,7]. Various improvements of inactivated vaccine have been made but local and systemic reactions remained a major problem [2,7,9]. The tolerance of the live oral vaccine (Ty21a) is good, however, it requires several doses, and a variable efficacy is reported [2,8,9].

The Vi polysaccharide vaccine of *Salmonella* Typhi has several advantages. Side effects are infrequent and mild [1,2,4,5,7,9], a single dose yield consistent immunogenicity and efficacy [2,7,8]. Vi polysaccharide may be reliably standardized by physicochemical methods verified for other polysaccharide vaccines [10,11], Vi is stable at room temperature [9–11] and it may be administered simultaneously with other vaccines without affecting immunogenicity and tolerability [9].

Finlay Institute has developed a new typhoid Vi polysaccharide vaccine named vax-TyVi®. A clinical trial was carried out to evaluate the immunogenicity of this vaccine, as a first step to replace the former Cuban whole-cell killed vaccine.

2. Materials and methods

2.1. Tested vaccine

Composition per dose of 0.5 ml: *Salmonella* Typhi Vi polysaccharide 25 μg, NaCl 4.150 mg, $Na_2HPO_4{\cdot}2H_2O$ 0.065 mg, $NaH_2PO_4{\cdot}2H_2O$ 0.023 mg, Phenol 1 mg, and injection water, pH 6.5–7.5.

Quality specifications of the Salmonella Typhi *Vi polysaccharide*: O-acetyl groups >2 μmol/mg, proteins <10 μg/mg, nucleic acids <20 μg/mg, and endotoxins <150 EU/μg.

2.2. Methodology

A randomized controlled double blind study was carried out in children and teenagers aged 9–13 years during 2002.

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Ethical approval was obtained from the Ethical Committee for the Study of Human Subjects, Higher Medical Science Institute, Havana City. Signed, informed consent was obtained from all subjects before their assignment to the groups [12,13]. History of typhoid fever, any acute or chronic disease, immunological treatment, temperature >37.5 °C, pregnancy, breast feeding, or any typhoid vaccine used 3 years before, were exclusion criteria.

Three hundred and forty-six selected children and teenagers were randomly distributed in three groups. They were immunized with vax-TyVi® (Lot L111, Finlay Institute, single dose of 25 μg intramuscularly), TYPHIM Vi™ (Lot T1120, Pasteur-Mérieux, single dose of 25 μg intramuscularly) and vax-TET® (Lot L0079, Finlay Institute, single dose of 10 lf), respectively.

Blood samples were taken before (T0) and 21 days (T1) after immunization, and processed at the laboratory; the serum samples were stored at −20 °C until testing.

2.3. Study of the immunogenicity

High-binding ELISA plates (Costar, Cambridge, MA) were pre-coated by incubation with 100 μl of poly-L-lysine per well (3 μg/ml) (Sigma, St. Louis, MO) in 0.15 M phosphate buffered saline (PBS) pH 7.4 for 30 min at 20–25 °C. After the plates were washed three times with 300 μl of PBS per well, 100 μl of Vi polysaccharide (6 μg/ml) in PBS were added in each well. The plates were incubated overnight at 2–8 °C and then washed three times with PBS containing 0.05% Tween 20 (PBS-T). The standard curve was constructed by performing six two-fold serial dilutions of the Finlay standard serum with 4.8 μg/ml (starting dilution 1:20) in PBS-T containing 3% skim milk (PBS-TM) (Merck, Germany). The test sera were diluted among 1:20 and 1:800 in PBS-TM. Of each solution, 100 μl were added in duplicate to the wells for 1 h at 37 °C. After washing four times with 300 μl of PBS-T, 100 μl of conjugate solution (goat anti-human Igs:alkaline phosphatase) (Sigma, St. Louis, MO) in PBS-TM were added to each well and the plates were incubated for 1 h at 37 °C. The plates were washed again, and 100 μl of *p*-nitrophenylphosphate (1 mg/ml) (Sigma, St. Louis, MO) in 0.92 M diethanolamine buffer pH 9.8 were added to each well. The plates were left at 20–25 °C for 30 min and then the absorbances were determined at 405 nm (Anthos reader 2001, Labtec Instruments, Germany) [14,15]. Validation, quantitative determination and print out were performed using the ELISA software package from the Centers for Disease Control (CDC), Atlanta (GA).

Table 2
Seroprotection rates by vaccine group

Group	Anti-Vi ≥1 μg/ml *N* (%)	
	T0	T1
vax-TyVi®	27 (19.42)	127 (91.37)
TYPHIM Vi™	20 (14.93)	123 (91.79)
vax-TET®	13 (20.97)	13 (20.97)

2.4. Statistical methods

Calculation of the sample size was based on a TYPHIM Vi™ vaccine seroconversion of 80%, and vax-TyVi® vaccine seroconversion among 70–80%, a statistical power of 80%, and an alpha significance level of 0.05. Enrollment was augmented to compensate for attrition. The geometric mean titers (GMT) and 95% confidence intervals (CI) were calculated for anti-Vi antibody titers distribution in T0 and T1. Seroconversion rates (≥4-fold increase of anti-Vi antibody titers over pre-immunization levels) were calculated and compared between the groups. 1 μg/ml was considered as the protective level of anti-Vi antibodies [2–9]. Variance analysis and Fisher's test were used for statistical analysis [16,17].

3. Results

The immunogenicity was studied in the useful paired-samples corresponding to 335 subjects (Table 1). The groups vaccinated with both polysaccharide vaccines had a homogeneous pre-vaccination immunity ($P = 0.5276$). Subjects who received vax-TyVi® and TYPHIM Vi™ got similar seroconversion rates ($P = 0.1185$), also both vaccines showed similar GMT values for Vi antibodies induced after vaccination: 6.27 and 5.97 μg/ml, respectively ($P = 0.1768$). In contrast, subjects receiving the tetanus toxoid vaccine showed 0% seroconversion ($P = 0.000$). No significant differences were seen between seroprotection rates in both Vi polysaccharide vaccines (Table 2).

Table 1
Geometric mean titers, 95% confidence intervals and seroconversion in children and teenagers vaccinated with vax-TyVi®, TYPHIM Vi™ and vax-TET®

Group	*n*	GMT (95% CI) μg/ml		Seroconversion % (95% CI)
		T0	T1	
vax-TyVi®	139	0.56 (0.45–0.69)	6.27 (5.40–7.38)	85.61 (78.66–90.98)
TYPHIM Vi™	134	0.60 (0.50–0.72)	5.97 (5.01–7.10)	78.36 (70.42–85.00)
vax-TET®	62	0.68 (0.53–0.87)	0.65 (0.51–0.83)	0.00 (0.00–5.83)

GMT: geometric mean titers of Vi polysaccharide antibodies; CI: confidence interval; T0: pre-immunisation sample; T1: post-vaccination sample.

4. Discussion

Until recently, the only available typhoid vaccine was inactivated whole-cell vaccine. Although this vaccine is effective, it has not been recommended for use in typhoid control programs because of unwanted side effects. Two effective vaccines against typhoid fever have been developed and licensed, the live oral vaccine (Ty21a), and the purified Vi polysaccharide, whose safety, immunogenicity and efficacy is firmly established [1,2,4–7,9]. Despite the clear advantages of this vaccine, the use of the whole-cell killed vaccine has remained popular because of its lower cost and the inherent resistance to change [2,7].

The Finlay Institute has developed a low-price *Salmonella* Typhi Vi polysaccharide vaccine—vax-TyVi®—to improve the Cuban Immunization Programs. Although this vaccine is structurally similar to other Vi polysaccharide vaccines [10,11], clinical trials were performed to evaluate its immunogenicity.

The present study of Vi antibodies performed using a simple and reproducible ELISA [14] has demonstrated that the immunogenicity of vax-TyVi® was not lower than TYPHIM Vi™.

With regard to other studies, a seroconversion rate among 62.5–96.3% has been obtained by using TYPHIM Vi™, therefore, seroconversion in our clinical trial agrees with previous reports [1–5,7,9].

The safety and reactogenicity evaluation is in course, however adverse events are infrequent and mild (unpublished data), and an efficacy trial will be conducted as part of the clinical evaluation. However, despite this trial has not been carried out yet, vax-TyVi® is a good vaccine candidate, and could be used in the Cuban Immunization Program, in other developing countries, and as a vaccine for travelers or others exposed to typhoid bacilli.

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