

Efficacy of Purified Vi Polysaccharide Typhoid Vaccine

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ABSTRACT

This experiment was conducted to assess the efficacy of typhoid vaccine newly produced by purifying Vi antigen of *Salmonella typhi*. With Karber method, LD₅₀ of challenging organism (*S. typhi* ty2) was determined as 6.31 CFU/mouse, and then the organism was used for the study. With Probits method, ED₅₀ of the vaccine was determined as 0.016 µg / 0.5 ml / mouse. The ELISA titer (0.5097 ± 0.0606) was 4 times in the group treated with high dose (0.25 µg/0.5ml) as in control (0.1113 ± 0.0110). Six major protein bands of 66, 55, 35, 33, 18, and 9 kd were detected in Western blot analysis with serum of a vaccine treated mouse, whereas only one weak band of about 35 kd was detected with serum of a control mouse. We concluded that typhoid vaccine produced by purifying Vi antigen of *S. typhi* very effectively prevent *S. typhi* infection in mice.

Key words : *Salmonella typhi*, Vi antigen, Karber method, Probits method, ELISA

Introduction

Typhoid fever, caused by infection with *Salmonella typhi*, remains an important health problem in many part of the world, with an estimated annual incidence of about 16 million cases and 600,000 deaths [15]. Typhoid fever can be occurred throughout the year in Korea, especially in summer, and is characterized by the clinical symptoms such as typhoid fever, abdominal pain, and diarrhea. Mortality rates for typhoid fever of up to 30% have been reported from some developing countries [1, 4, 9, 12].

In the past, chloramphenicol and ampicillin have been effectively used for many years, but treatment and control of typhoid fever has become increasingly difficult because strains of *S. typhi* resistant to these antibiotics have been

emerged worldwide [27]. Typhoid vaccines composed of inactivated cells of *S. typhi* were developed early in the 20th century based on principles articulated by Pasteur, but they were recently not used because the vaccine can be attacked with a undesirable disease such as high typhoid fever, whole-cell vaccines are only up to 70% effective, and the immunity does not persist for more than three to five years [2, 3, 14, 15].

Germanier et al have isolated a mutant *S. typhi* strain, Ty21a that has been used as an orally administered, attenuated vaccine. Strain Ty21a has lost an epimerase capable of converting glucose to galactose, a loss resulting in defective synthesis of the polysaccharide component of LPS. As a result Ty21a is not well adapted to survive and multiply in the intestinal tract [7]. In these days, parenteral vaccine, which made of purified Vi capsular polysaccharide have been widely used [6, 7]. Vi polysaccharide is a well-standardized antigen that is effective in a single parenteral dose, is safer than whole-cell vaccine [24]. Hessel et al showed that a vaccine composed of purified Vi capsular polysaccharide of *S. typhi*, given as a single intramuscular or deep subcutaneous injection, has consistent immunogenicity and efficacy, and side effects were infrequent and mild [11]. This experiment was conducted to identify the immunity of mice prevented with purified Vi polysaccharide, submitted from Greencross Company, based on Guideline for standard and test procedure of biological materials, recommended by Korean Food & Drug Administration [8].

Materials and Methods

1. Challenging organism

Salmonella typhi ty2 offered from Korean National Institute of Health was used as challenging organism. The bacterium was subcultured twice in tryptic soy agar (Difco, USA) and tryptic soy broth (Difco, USA), and used in the study after counted by plate count technique.

2. Animals

Four week-old SPF male BALB/c mice were provided from Samtako (Korea). The animals were kept in plastic cages (polycarbonate, 22x27x13 Cm) at 22 °C in negative rack (Three shine, Korea) with hepafilter. Animal food (CRF-1, Charles river, Japan) and water were provided *ad libitum*.

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After accommodation period of one week, the animals were used in the study.

3. Lethal dose 50 (LD50)

Based on Guideline for standard and test procedure of biological materials, recommended by Korean Food & Drug Administration [8], the challenging organism must be below 20 bacteria in LD50 value. In order to assess LD50 *S. typhi ty2* was diluted in 5% mucin with concentrations of 1000, 100, 10, 1 CFU/0.5ml. Ten mice of each group were intraperitoneally administered with the challenging organism, and then mortality was evaluated for three days. LD50 was calculated with Trimmed Spearman-Karber method [10].

4. Effective dose 50 (ED50)

Typhoid vaccine was diluted with sterilized phosphate buffered saline (PBS, pH 7.2) with concentration of 0.25, 0.05, 0.01, and 0.002 μ g/0.5ml and fifteen mice of each group were intraperitoneally administered with the diluted vaccine of each concentration. After 12 post-inoculation days, ten mice of each group were inoculated with challenging organisms of 1000 CFU/0.5ml, and mortality was evaluated for three days. ED50 was obtained with Probits methods (Quantal Dose-Response, Pharmacologic Calculation System-Version 4.1).

5. Enzyme linked immunosorbent assay

To evaluate the antibody titer against Vi antigen, mice sera were collected through abdominal vein at 12 days post immunization. *Salmonella typhi ty2* cultured in tryptic soy broth were collected and washed three times with sterilized PBS and crushed with sonicator. Five μ g of total bacterial protein was loaded into each well of ELISA plate and incubated at 4 °C overnight. After washed three times with PBS, the plate was blocked with 1% bovine serum albumin at 4 °C for 2 hr and incubated with sera (1:50 diluents) of all mice treated and untreated with the vaccine at room temperature for 2 hr. Then it was washed and incubated with HRP-conjugated anti mouse IgG antibodies (Promega, USA) at RT for 1 hr. Finally it was washed and visualized with o-phenylenediamine dihydrochloride (Sigma, USA) and the absorbance was measured at 450 nm wavelength.

6. Western blot

The sonicated cells were suspended in 2% SDS and proteins were extracted by boiling for 10 min. Then the protein was mixed with sample buffer (0.1 M Tris-HCl, pH 6.8, 10% Glycerol, 2% SDS, 5% 2-mercaptoethanol, 0.05% bromophenol blue) and boiled for 5 min. It was separated on 12.5% polyacrylamide gel at 200 V (BioRad, USA) for 45 min and transferred to nitrocellulose membrane at 100 V for 1 hr. After transferred, the membrane was blocked with 5% skim milk at 4 °C for 2 hr and incubated with each serum of a mouse (1:100 diluents) administered with 0.25 μ g/0.5ml of typhoid vaccine and a control mouse at 4 °C overnight. After

washed, it was reacted with HRP-conjugated anti mouse IgG antibodies (Promega, USA) at room temperature for 1 hr and visualized with 3, 3'-diaminobenzidine (Vector, USA).

Results

1. LD50

When calculated with Trimmed Spearman-Karber method, LD50 of challenging organism was 6.31 CFU/mouse, and we considered that the bacterium was suitable for this study (Table 1).

Table 1. LD50 of challenging organism in mice

Microorganism (CFU/mouse)	Challenge dose (CFU/mouse)	Mortality	LD50 (CFU/mouse)
<i>S.typhi ty2</i>	1000	10/10	6.31
	100	10/10	
	10	7/10	
	1	0/10	

2. ED50

When calculated with Probits method, ED50 of typhoid vaccine was 0.016 μ g/mouse (Table 2).

3. ELISA

Sera of five mice of each group were used to evaluate the antibody level for Vi antigen. The results showed that antibody level was higher in mice treated with vaccine than in untreated mice. In mice group (0.5097 \pm 0.0606) treated with high dose (0.25 μ g/0.5ml) vaccine, antibody level to Vi antigen was 4 times as in control mice (0.1113 \pm 0.0110) (Fig 1). When titration was evaluated with serum of a mouse showing middle value in each group, a significant positive reaction was identified up to a diluted concentration of 1:320 in 0.25 μ g/0.5ml treated mouse, 1:160 in 0.05 μ g/0.5ml, 1:80 in 0.01 μ g/0.5ml, and 1:40 in 0.002 μ g/0.5ml (Fig 2).

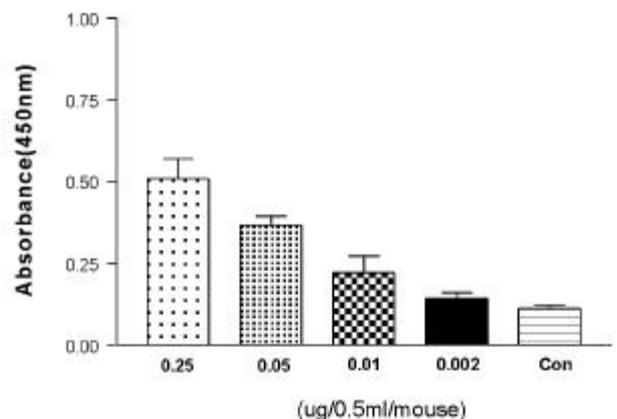
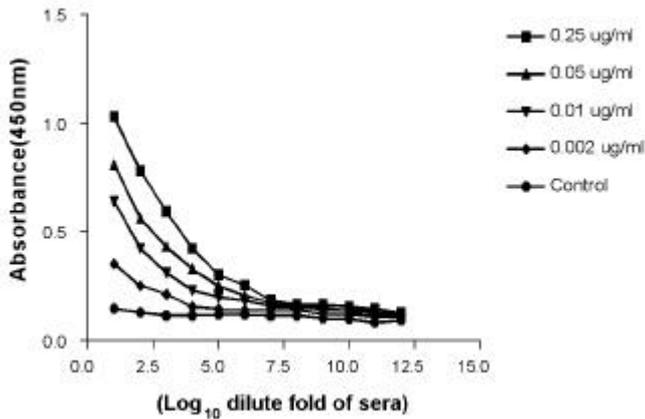


Fig 1. Mean value of antibody titers against *S. typhi* in mice administered with typhoid vaccine of various concentrations (0.25, 0.05, 0.01, 0.002 μ g/0.5ml) and PBS.

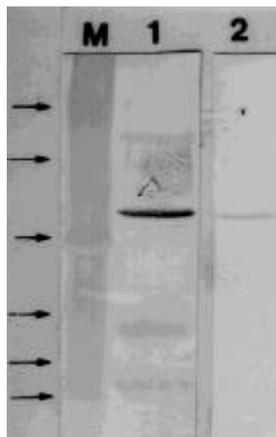
Table 2. ED50 of typhoid vaccine following intraperitoneal administration in mice

Dose (g/0.5ml/mouse)	Challenge dose (CFU/mouse)	Survival rate	ED50 (g/0.5ml/mouse)
0.25	1000	10/10	0.016
0.05	1000	9/10	
0.01	1000	6/10	
0.002	1000	1/10	

**Fig 2.** Titration of serum of the mouse that showed median value of antibody level against *S. typhi* in each group administered with typhoid vaccine.

4. Western blot analysis

Six major protein bands of about 66, 55, 35, 33, 18, and 9 kd were detected using a mouse serum treated with 0.25 $\mu\text{g}/0.5\text{ml}$ of the vaccine, whereas only one weak band of about 35 kd in a control mouse was detected. The protein band of 35kd was considered as nonspecific reaction (Fig 3).

**Fig 3.** Western blot analysis. M; molecular marker (66, 45, 29, 20, 14.2, and 6.5 kd from the top), lane 1; Major protein bands of about 66, 55, 35, 33, 18, and 9 kd were reacted with serum of a mouse administered with typhoid vaccine (0.25 $\mu\text{g}/0.5\text{ml}$), lane 2; The protein band of 35kd was reacted with serum of a control mouse.

Discussion

Salmonella typhi is gram-negative bacterium that belongs to the Enterobacteriaceae family. The bacterium is encapsulated by a polysaccharide layer and has following three major proteins:) the capsular polysaccharide or Vi antigen;) the O or somatic antigen (cell-wall lipopolysaccharide), which corresponds to the endotoxin; and) the H or flagellar antigen, which is a protein. *S. typhi* is distinctive among the *Salmonella* spp. in possessing the Vi antigen, although it is found in lower quantities in some strains of *S. paratyphi* C and *S. dublin* [15]. Vaccines that contain purified capsular polysaccharide antigens elicit serum antibodies that provide type-specific protection against invasive infections. The whole organism is not required to elicit this protective immune response. Vaccination with the Vi antigen alone elicits a much greater and more consistent Vi antigen antibody response than whole-cell vaccination [20, 21, 25].

Since the Vi antigen of *S. typhi* was first identified by Felix and Pitt [5], many studies about typhoid vaccine have been conducted [18, 19, 26, 28]. The Vi antigen physically prevents antibodies binding to the O antigen and is also associated with inhibition of complement activation as well as with resistance to complement-mediated lysis and phagocytosis [22, 26]. Thus, the Vi antigen allows *S. typhi* to survive in the blood, leading to septicemia. Specific serum antibody to Vi antigen is necessary to activate complement against *S. typhi*.

The Vi polysaccharide vaccine has been used in many part of world, and is administered in one dose of 25 $\mu\text{g}/0.5\text{ml}$ as an intramuscular or deep subcutaneous infection, with revaccination after 3 years recommended for individuals who remain at risk of infection [11].

In potency test using mice, mixture of challenging organism and mucin solution has been administered, which is to increase toxicity of the organism [23, 25]. By using the same method, this study could be effectively conducted.

Many studies about safety and immunogenicity of Vi vaccine have been reported in adults or children. In South Africa, Keddy et al. revealed that Vi vaccination has led to ongoing antibody production in greater than 50% of Vi vaccinated children in an endemic area for a period of 10 years [16]. According to Kim et al. of 137 vaccinees, 116 (84.7%) maintained a persistent rise in Vi antibody titer 12 months after vaccination, and 55 out of 100 (55.0%) had a

4-fold or greater rise at 36 months [17]. In our study, only one dose of 0.25µg/0.5ml of Vi polysaccharide vaccine, submitted from Greencross co., successfully could prevent lethal event in mice. To evaluate more precise effect in humans of Vi vaccine, clinical trial is to be conducted.

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