

Immunogenicity and Safety of Vi Capsular Polysaccharide Typhoid Vaccine in Healthy Persons in Korea

LIM, SANG-MIN, HAHN-SUN JUNG, MIN JA KIM¹, DAE-WON PARK¹, WOO-JOO KIM¹,
HEE-JIN CHEONG¹, SEUNG-CHUL PARK¹, KWANG-CHUL LEE², YOUNG-KYOO SHIN²,
HYUN KWANG TAN, SANG-LIN KIM, AND JANG WOOK SOHN^{1*}

Central Research Institute, Boryung Pharm. Co. Ltd., Ansan 425-120, Korea

¹Department of Internal Medicine and ²Department of Pediatrics, Korea University, College of Medicine, Seoul 136-705, Korea

Received: September 20, 2006

Accepted: December 29, 2006

Abstract The purpose of this study was to evaluate the immunogenicity and safety of *Salmonella* Typhi Vi capsular polysaccharide vaccine (Vi vaccine) in Korea. The immunogenicity of a single dose of Vi vaccine was evaluated in 157 subjects (75 children and 82 adults) before and at 1, 6, and 12 months after vaccination. Immunogenicity was measured with a passive hemagglutination assay (PHA), quantified as geometric mean titers (GMTs) and seroconversion rates. The safety of the vaccine was investigated by determining adverse reactions occurring within 4 h, 3 days, and 1 month after injection. The seroconversion rate for children and adults 1 month after vaccination was 96.92% and 89.02%, respectively. In the case of children, the GMTs of Vi antibodies before vaccination were 5.87±1.34 and 142.59±2.39 at one month after vaccination. For adults, the GMTs before and one month after vaccination were 5.58±1.28 and 58.56±3.67, respectively. Vi antibodies persisted for as long as 6 and 12 months after vaccination. All adverse reactions in adults and children were minor and did not require treatment. The Vi CPS vaccine was safe and immunogenic in adults and children older than 5 years.

Keywords: Vi capsular polysaccharide typhoid vaccine, immunogenicity, safety

Typhoid fever is a systemic bacterial infectious disease caused by *Salmonella enterica* serotype Typhi. Globally, 17 million people are annually infected by the bacterium and more than 600,000 people die from it. Prevention is especially important in Asia, the Middle East, and Latin America, where the resistance to multiple antibiotics is steadily rising [2, 15, 17].

Three kinds of typhoid vaccine have been developed: Whole-cell killed vaccine (TAB vaccine), live oral attenuated Ty21a vaccine (Ty21a vaccine), and parenteral Vi capsular polysaccharide vaccine (Vi vaccine). Currently, just two types of Ty21a vaccine and the Vi vaccine are used in Korea. Vi vaccine showed 55% (30% to 71%) efficacy after one-time vaccination of adults and children over the age of five. Immunogenicity is maintained for more than 3 years, and antibodies persist for 10 years in more than 50% of subjects [9, 11]. Even though a minimum of three doses are used, the Ty21a vaccine displays a low efficacy of 51% (35% to 63%) in school-aged children and adults, and has no proven efficacy in very young children (≤ 24 months old) [7, 18]. The TAB vaccine has yielded the highest protective efficacy, but also the largest number of adverse effects [4, 7].

Typhoid fever is rarely epidemic in Korea, with about 300 people infected annually. The Korea Center for Disease Control and Prevention recommends restricted vaccination of high-risk infectious groups, which include travelers to endemic areas, persons with intimate exposure to an *S. Typhi* carrier, employees of mess halls and sanitary facilities, and those in microbiology laboratories who work frequently with *S. Typhi* [12]. Typhoid fever has a well-documented ability to cause large single-source outbreaks [16, 19], and the Center for Disease Control and Prevention (CDC) has classified *Salmonella* species together with other food safety threats as the second highest priority for potential bioterrorism agents (category B) [6, 27].

The investigational Vi vaccine was produced in Korea. After *S. Typhi* was first mass-cultured in a fermentor, the Vi capsular polysaccharide (CPS) was highly purified from the culture broth. The purpose of this study was to identify the immunogenicity and safety of the Vi vaccine in adults and children in Korea.

*Corresponding author

Phone: 82-2-920-5018; Fax: 82-2-920-5616;

E-mail: jwsohn@kumc.or.kr

MATERIALS AND METHODS

Study Design

The study protocol was approved by the Korea Food and Drug Administration and by the institutional review boards of Korea University Anam and Guro Hospitals in Seoul. Informed consents were obtained from adults and from parents of vaccinees younger than 15 years of age.

This study was conducted from October 2000 to March 2002. Subjects had to be healthy, over the age of 5 years, and were not previously vaccinated with Vi polysaccharide. Additionally, the subjects could not have a history of typhoid fever. We also excluded those who were given immunosuppressive medicine or blood derivatives within 6 months of the screening time point and those with an anti-Vi titer higher than 1:20 at the screening time point. Individuals with a history of allergic or immunologic disorders, pregnant or nursing women, alcoholics, and drug abusers were also excluded from the study.

Subjects were screened at a baseline visit according to medical history, physical examination, laboratory safety parameters (complete blood count, liver function tests, and renal function tests), urine pregnancy test, and anti-Vi antibody assay (passive hemagglutination assay, PHA) within two weeks prior to injection.

Qualified subjects were checked (prior to injection) for fever to ensure that they presented a normal body temperature ($<37.5^{\circ}\text{C}$). If they were found to have an abnormal body temperature, they were excluded from the study. The healthy subjects were then injected once in the deltoid muscle with 0.5 ml of Vi vaccine (Zerotyph II inj., Lot No. 0550001) and observed for 20 min after injection. The subjects themselves recorded in their subject diary variations of body temperature and other adverse reactions for 4 h and three days. For subjects younger than 15 years of age, the subject diary was recorded by the parent. Thirty days after vaccination, the subjects received a medical reexamination to confirm adverse effects.

Vaccine

The subjects were injected with Zerotyph II inj. (Boryung Pharm. Co. Ltd., Seoul, Korea), which contains 25 μg /0.5 ml/dose of Vi capsular polysaccharide as the main ingredient. The strain used to produce the vaccine was *Salmonella* Typhi (ATCC 9992V), allocated to us by the Korean National Institutes of Health.

The vaccine strain was cultured for 18 h at 37°C in a fermentor, and then the bacteria were removed by centrifugation ($6,000 \times g$). NaCl was added to the supernatant at a final concentration of 5%, followed by chilled ethanol. The sediment was dissolved in tris (hydroxymethyl) aminomethane-chloride buffer to a final concentration of 0.05 N and then incubated overnight with benzonase nuclease (Novagen, WI, U.S.A.) and protease (Sigma, MO, U.S.A.) at 37°C .

Next, the mixture was treated with hexadecyltrimethyl ammonium bromide (Fluka, Buchs, Switzerland) and centrifuged ($6,000 \times g$) for 20 min. The pellet was dissolved in 1 M CaCl_2 (KP, Siheung, Korea), diluted with ethanol, and centrifuged ($6,000 \times g$) for 20 min. After the pellet was dissolved in pyrogen-free water (PFW), the solution was diafiltered, concentrated, and lyophilized. The lyophilized powder was dissolved in phenol-phosphate buffer to a final concentration of 50 $\mu\text{g}/\text{ml}$ and filtered at $0.22 \mu\text{m}$ [8, 28].

Immunogenicity and Persistence of Vaccine-Induced Vi Antibodies

In order to measure the seroconversion rate, blood samples were collected from the subjects once before injection and again 30 days after injection. To evaluate the persistence of vaccine-induced Vi antibodies, blood samples were also taken at 6 and 12 months after injection. Anti-Vi capsular polysaccharide antibodies were measured by PHA. In a V-bottom microplate, subject serum was diluted twofold from a ratio of 1:2.5 to 1:320, so that each well contained 25 μl . Purified Vi antigen-sensitized sheep blood cells were added in a volume of 25 μl to each well. Following at least one hour of incubation at room temperature, the assay was analyzed [14, 25]. The result of the antibody titer was expressed as the inverse of the final dilution fold of serum. Seroconversion was defined as antibody levels more than 4 times the amount before injection of the vaccine, and the cutoff point was in the ratio of 1:20 [26].

Statistical Analysis

Antibody levels were expressed as geometric mean titers (GMTs). Comparisons of geometric means were performed with a paired *t*-test or repeated measured ANOVA. *P* values <0.05 were considered statistically significant.

RESULTS

A total of 157 subjects participated in the study, including 75 children under 15 years of age and 82 adults. Thirty days after vaccination, immunogenicity was evaluated in 147 subjects. The reason for exclusion of ten children was that eight children did not visit the hospital after vaccination and two children were included in subject exclusion criteria because of other vaccinations one month prior to vaccination. Mean ages (\pm standard deviation) of the children group and the adult group were 8.65 ± 2.43 (range, 5–14) and 38 ± 14.81 (range, 19–72) years old, respectively. The ratio of men to women was 1:1.1 in the children group, and 1:3.8 in the adult group. In the pre-vaccination screening process, serum of all subjects was negative for Vi antibody.

The seroconversion rate one month after injection was 96.92% in children and 89.02% in adults. The seroconversion of all subjects combined was 92.5% at one month after

Table 1. Seroconversion rates 1 month after vaccination and Vi antibody levels before and 1 month after vaccination.

Group	n	Serum Vi antibody levels ^a		Seroconversion rate (90% CI ^c)
		Before injection (\pm SD)	1 mo after injection (\pm SD)	
Children ^b	65	5.87 \pm 1.34	142.59 \pm 2.39	96.92% (93.40–100.45%)
Adults ^b	82	5.58 \pm 1.28	58.56 \pm 3.67	89.02% (83.34–94.70%)

^aAntibody levels are given as geometric mean titers.

^bAntibody titers increased significantly after injection, based on a paired *t*-test ($P=0.0001$).

^c90% confidence interval.

injection. At one month before and after injection, respectively, GMTs of Vi antibody were 5.87 \pm 1.34 and 142.59 \pm 2.39 for children and 5.58 \pm 1.28 and 58.56 \pm 3.67 for adults (Table 1). Statistically, these increases were significant at one month after injection ($p=0.0001$).

For persistency of the antibody titer, only 37 children and 28 adults were available for follow-up to 12 months. Blood was taken before vaccination and 1, 6, and 12 months later (Table 2). After injection, the antibody titers of both children and adults were significantly greater for up to 12 months than those before injection ($p=0.0001$).

All adverse reactions in adults and children resulting from immunization are shown in Table 3. Local and systemic adverse reactions were mild and did not require treatment in either age group. No fevers of $>38^{\circ}\text{C}$ were recorded up to 4 h and 3 days after injection of the vaccine. Laboratory parameters for safety assessment one month post-vaccination were within the normal range, compared with pre-vaccination levels (data not shown). In children, all systemic and localized reactions disappeared within 24 h following injection. Every systemic and localized reaction disappeared by 3 days after injection in adults, although most reactions were gone within 24 h. There were no adverse reactions after 3 days post-injection.

DISCUSSION

Based on PHA (cutoff value 1:20) evaluation, the Vi vaccine was highly immunogenic, especially in children. GMT levels in children were the highest 1 month after injection, and then decreased as time passed. In adults, the highest GMT was at 6 months after injection, followed by a decrease. Of 2 children without anti-Vi antibodies, one

(antibody titer 20, 1 month post-injection) did have measurable antibodies at 6 and 12 months after injection, with seroconversion at 6 months following injection. The titer was held at 160 at 6 and 12 months. In adults (28 total), 9 subjects were negative for antibody at 1 month post-injection, but 3 of these had detectable anti-Vi antibodies at 6 months after injection. Their antibody titer was less than 5 at 1 month post-injection and increased by 40 at 6 months post-injection. Seroconversion could have occurred at any point between 1 and 6 months post-injection.

Six months post-immunization, the seroconversion rate was 98.5% (64/65) in children, 92.7% (76/83) in adults, and 95.2% (140/147) in general. Antibody titers were not measured at 6 months post-injection in the 7 subjects who did not seroconvert at 1 month. These results indicate that Zerotyph II inj. is highly immunogenic, concordant with previous clinical studies carried out in Korea [21, 24].

In other studies carried out in other countries using the Vi vaccine, seroconversion rates at 4 weeks post-injection were 75% in Nepal [1], 67% in Malaysia [20], 81–92% in China [29], 71.6% in India [23], and 93–98% in the U.S.A. [10]. Zerotyph II inj. had a higher seroconversion rate than others. According to a recent report, the protectiveness of Vi vaccination is related with serological protection in endemic areas [11], demonstrating that Zerotyph II inj. offers good protection. However, the subjects have not been followed to clearly establish protective efficacy, which is a limitation of this particular study.

Immunogenicity data of Vi vaccine consist of anti-Vi antibody measurements obtained either by the PHA or RIA (radioimmunoassay) [22]. These two test results correlate well, although numerically higher results are obtained with PHA. The protective level of antibody is unknown. Thus, the results were evaluated for four-fold titer rises and

Table 2. Persistence of serum Vi antibodies in children and adult vaccinees.

Group	n	Serum Vi antibody levels ^a			
		Before injection (\pm SD)	1 mo after injection (\pm SD)	6 mo after injection (\pm SD)	12 mo after injection (\pm SD)
Children ^b	37	6.03 \pm 1.37	151.26 \pm 2.28	109.99 \pm 1.91	100.16 \pm 1.67
Adults ^b	28	5.66 \pm 1.31	64.03 \pm 2.81	105.03 \pm 2.29	84.06 \pm 2.12

^aAntibody levels are given as geometric mean titers.

^bAntibody titers increased significantly from the pre-vaccination baseline within 12 months after the injection, based on repeated measured ANOVA ($P=0.0001$).

Table 3. Adverse local and systemic reactions observed within 72 h of Vi vaccination.

Variable	Child (N=75)	Adult (N=82)
	No. (%)	No. (%)
Localized reactions		
Pain	27 (34.21)	36 (43.9)
Redness	2 (2.67)	8 (9.76)
Systemic reactions		
Fatigue	2 (2.67)	15 (18.29)
Malaise	2 (2.67)	3 (3.66)
Headache	1 (1.33)	8 (9.75)
Dizziness	1 (1.33)	5 (6.09)
Myalgia	1 (1.33)	9 (10.98)
Diarrhea	0	2 (2.44)
Others ^a		3 (3.66) ^a

^aArthralgia, rash, and rigor were each reported once.

GMTs [22]. Using the PHA, the cutoff point of Vi antibody was in the ratio of 1:20 [24–26].

Ty21a vaccine containing live bacteria cannot be used in immune-compromised patients including those with HIV infections [5]. Vi vaccine-induced Vi antibody levels in immune-compromised patients and HIV-infected patients with low CD4⁺ T-lymphocyte counts are significantly lower than those found in HIV-infected patients with high CD4⁺ T-lymphocyte counts and healthy controls [13].

A report of post-marketing safety surveillance of typhoid vaccines from 1990 to 2002 shows mild cases and severe adverse reactions [3], and each of the most severe adverse reactions, including Guillain-Barre syndrome, anaphylaxis, and perforated jejunum have been reported only once. In the present study, Zerotyp II inj. was found to be safe and showed no serious adverse reaction. Most adverse events were mild and required no specific treatment.

The above results led us to conclude that Zerotyp II inj. demonstrated excellent immunogenicity and safety for adults and children over the age of 5 years, when injected once in the muscle with a volume of 0.5 ml (25 µg as Vi capsular polysaccharide). Further investigation is needed to determine the extent and duration of antibody induced by the Vi vaccine.

Acknowledgments

This research was carried out as a part of the development of the Vi Polysaccharide Typhoid Vaccine in 1998 (HMP-98-D-0033), with the support of the Ministry of Health and Welfare, Korea.

REFERENCES

- Acharya, I. L., C. U. Lowe, R. Thapa, V. L. Gurubacharya, M. B. Shrestha, M. Cadoz, D. Schulz, J. Armand, D. A.

- Bryla, B. Trollfors, T. Cramton, R. Schneerson, and J. B. Robbins. 1987. Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of *Salmonella typhi*: A preliminary report. *N. Engl. J. Med.* **317**: 1101–1104.
- Ackers, M. L., N. D. Puh, R. V. Tauxe, and E. D. Mintz. 2000. Laboratory-based surveillance of *Salmonella* serotype Typhi infections in the United States: Antimicrobial resistance on the rise. *JAMA* **283**: 2668.
- Beiger, E. M., D. R. Burwen, P. Haber, and R. Ball. 2004. Postmarketing safety surveillance for typhoid fever vaccines from the vaccine adverse event reporting system, July 1990 through June 2002. *Clin. Infect. Dis.* **38**: 771–779.
- Bodhidatta, L., D. N. Taylor, U. Thisyakorn, and P. Echeverria. 1987. Control of typhoid fever in Bangkok, Thailand, by annual immunization of school children with parenteral typhoid vaccine. *Rev. Infect. Dis.* **9**: 841–845.
- Center for Disease Control and Prevention. 1994. Typhoid immunization recommendation of the Advisory Committee on Immunization Practices (ACIP). *MMWR* **43(RR-14)**: 1–6.
- Centers for Disease Control and Prevention. High priority potential bioterrorism agents list. Available at <http://www.bt.cdc.gov/Agent/agentlist.asp>. Accessed 12 January 2004.
- Engels, E. A., M. E. Falagas, J. Lau, and M. L. Bennish. 1998. Typhoid fever vaccines: A meta-analysis of studies on efficacy and toxicity. *BMJ* **316**: 110–116.
- Joo, J. H. and J. W. Yun. 2005. Structural and molecular characterization of extracellular polysaccharides produced by a new fungal strain, *Trichoderma erinaceum* DG-312. *J. Microbiol. Biotechnol.* **15**: 1250–1257.
- Keddy, K. H., K. P. Klugman, C. F. Hansford, C. Blonde, and N. B. Nancy. 1999. Persistence of antibodies to the *Salmonella typhi*, Vi capsular polysaccharide vaccine in South African school children 10 years after immunization. *Vaccine* **17**: 110–113.
- Keitel, W. A., N. L. Bond, J. M. Zahradnik, T. A. Cramton, and J. B. Robbins. 1994. Clinical and serological responses following primary and booster immunization with *Salmonella typhi* Vi capsular polysaccharide vaccines. *Vaccine* **12**: 195–199.
- Klugman, K. P., H. J. Koornhof, J. B. Robbins, and N. N. Le Cam. 1996. Immunogenicity, efficacy and serological correlate of protection of *Salmonella typhi* Vi capsular polysaccharide vaccine three years after immunization. *Vaccine* **14**: 435–438.
- Korea Center for Disease Control and Prevention. Communicable disease information. 2000. Available at <http://dis.mohw.go.kr/information/info.asp>.
- Kroon, F. P., J. T. V. Dissel, E. Ravensbergen, P. H. Nibbering, and R. V. Furth. 1999. Impaired antibody response after immunization of HIV-infected individuals with the polysaccharide vaccine against *Salmonella typhi* (Typhim-Vi®). *Vaccine* **17**: 2941–2945.
- Landy, M. and E. Lamb. 1953. Estimation of Vi antibody employing erythrocytes treated with purified Vi antigen. *Proc. Soc. Exp. Biol. Med.* **82**: 593–598.
- Maskalyk, J. 2003. Typhoid fever. *CMAJ* **169**: 132.

16. Mermin, J. H., R. Villar, J. Carpenter, L. Roberts, A. Samariddin, L. Gasanova, S. Lomakina, C. Bopp, L. Hutwagner, P. Mead, B. Ross, and E. D. Mintz. 1999. A massive epidemic of multidrug-resistant typhoid fever in Tajikistan associated with consumption of municipal water. *J. Infect. Dis.* **179**: 1416–1422.
17. Murdoch, D. A., N. A. Banatvala, B. I. Shoismatulloev, L. R. Ward, and E. J. Threlfall. 1988. Epidemic ciprofloxacin-resistant *Salmonella typhi* in Tajikistan. *Lancet* **351**: 339.
18. Murphy, J. R., L. Grez, L. Schlesinger, C. Ferreccio, S. Baqar, C. Muñoz, S. S. Wasserman, G. Losonsky, J. G. Olson, and M. M. Levine. 1991. Immunogenicity of *Salmonella typhi* Ty21a vaccine for young children. *Infect. Immun.* **59**: 4291–4293.
19. Olsen, S. J., S. C. Bleasdale, A. R. Magnano, C. Landrigan, B. H. Holland, R. V. Tauxe, E. D. Mintz, and S. Luby. 2003. Outbreaks of typhoid fever in the United States, 1960–99. *Epidemiol. Infect.* **130**: 13–21.
20. Panchanathan, V., S. Kumar, W. Yeap, S. Devi, R. Ismail, S. Sarijan, S. M. Sam, Z. Jusoh, S. Nordin, D. Leboulleux, and T. Pang. 2001. Comparison of safety and immunogenicity of a Vi polysaccharide typhoid vaccine with a whole-cell killed vaccine in Malaysian Air Force recruits. *Bull. World Health Organ.* **79**: 811–817.
21. Park, J. W., W. S. Shin, M. W. Kang, D. K. Ko, and J. H. Kang. 1991. Safety and immunogenicity of the typhoid Vi capsular polysaccharide vaccine. *Kor. Soc. Infec. Dis.* **23**: 145–153.
22. Plotkin, S. A. and N. Bouveret-Le Cam. 1995. A new typhoid vaccine composed of the Vi capsular polysaccharide. *Arch. Intern. Med.* **155**: 2293–2299.
23. Sabitha, P., M. R. P. Adhikari, A. Chowdary, M. Prabhu, M. Soofi, M. Shetty, A. Kamath, S. S. Lokaranjan, and S. S. Bangera. 2004. Comparison of the immunogenicity and safety of two different brands of *Salmonella typhi* Vi capsular polysaccharide vaccine. *Indian J. Med. Sci.* **58**: 141–149.
24. Sohn, Y. M., J. H. Kim, and D. B. Gae. 1993. Immunogenicity and safety of the Typhoid Vi capsular polysaccharide vaccine (Typhovax) in children. *J. Korean Soc. Chemother.* **11**: 185–191.
25. Tacket, C. O., C. Ferreccio, J. B. Robbins, C. M. Tsai, D. Schulz, M. Cadoz, A. Goudeau, and M. M. Levine. 1986. Safety and immunogenicity of two *Salmonella typhi* Vi capsular polysaccharide vaccines. *J. Infect. Dis.* **154**: 342–345.
26. Tacket, C. O., M. L. Levin, and J. B. Robbins. 1988. Persistence of antibody titers three years after vaccination with Vi polysaccharide vaccine against typhoid fever. *Vaccine* **6**: 307–308.
27. Torok, T. J., R. V. Tauxe, R. P. Wise, J. R. Livengood, R. Sokolow, S. Mauvais, K. A. Birkness, M. R. Skeels, J. M. Horan, and L. R. Foster. 1997. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *JAMA* **278**: 389–395.
28. Wong, K. H., J. C. Feeley, R. S. Northrup, and M. E. Forlines. 1974. Vi antigen from *Salmonella typhosa* and immunity against typhoid fever. I. Isolation and immunologic properties in animals. *Infect. Immun.* **9**: 348–353.
29. Yang, H. H., C. G. Wu, G. Z. Xie, Q. W. Gu, B. R. Wang, L. Y. Wang, H. F. Wang, Z. S. Ding, Y. Yang, W. S. Tar, W. Y. Wang, X. C. Wang, M. Qin, J. H. Wang, H. A. Tang, X. M. Jiang, Y. H. Li, M. L. Wang, S. L. Zhang, and G. L. Li. 2001. Efficacy trial of Vi polysaccharide vaccine against typhoid fever in south-western China. *Bull. World Health Organ.* **79**: 625–631.