

## Safety and Immunogenicity of *Salmonella typhi* Ty21a Vaccine in Young Thai Children

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***Salmonella typhi* Ty21a vaccine in a liquid formulation was evaluated in 634 Thai children 2 to 6 years of age. The seroconversion rate was 69% for those who received vaccine versus 14% for those who received placebo ( $P < 0.005$ ). The immune responses among vaccine recipients ranged from 60% in 3-year-olds to 81% for 6-year-olds.**

Typhoid fever is a significant public health problem in underdeveloped areas of the world (3, 4, 13). The appearance of multiresistant *Salmonella typhi* strains has greatly complicated treatment (1, 5). Parenteral typhoid vaccine is not effective as a public health tool because of its high rate of adverse reactions (7, 8, 12). Therefore, compliance with immunization programs has been poor, especially among school-aged children, who are at greatest risk.

The *S. typhi* Ty21a live oral vaccine confers long-lasting protection against typhoid fever in Chilean schoolchildren and young adults (6). This vaccine delivered in a buffer solution was more efficacious than an enteric-coated capsule version in two recent field trials (8, 12). Because the majority of the study populations were  $\geq 6$  years old, little is known concerning the safety and immunogenicity of this vaccine in pre-school-aged children (11).

The relatively high incidence of typhoid fever among 2- to 5-year-olds residing in certain regions where typhoid is endemic indicates that vaccination should take place at the earliest age possible in order to maximize the public health benefit (12, 13). The Ty21a liquid formulation can be administered in as little as 50 ml, making it amenable for use in young children, who often have difficulties swallowing capsules. We therefore conducted a day-care center-based, randomized, double-blind, placebo-controlled trial to evaluate the safety and immunogenicity of the liquid Ty21a vaccine formulation in young Thai children.

The vaccine ( $3 \times 10^9$  *S. typhi* Ty21a organisms) and placebo ( $2 \times 10^9$  heat-killed *Escherichia coli* K-12 organisms) were packaged in identically appearing aluminum foil sachets. Both formulations contained 25 mg of aspartame as a sweetening agent. A packet of buffer also contained 1.3 g of sodium bicarbonate and 0.8 g of ascorbic acid.

The study protocol was reviewed and approved by the Ministry of University Affairs, Mahidol University, Bangkok, Thailand, and the Ministry of Public Health, Chachoengsao, Thailand. Healthy children 2 to 6 years of age with no history of typhoid fever were enrolled from day-care centers in Chachoengsao Province and from the Bangkok metropolitan

area. Written informed consent was obtained from the parent or adult guardian of each child.

Children were randomized to receive a packet bearing a given letter code. The buffer was reconstituted in 50 ml of water. The contents of the lettered packet were then added, and the suspension was gently mixed and immediately ingested. The children did not consume any food for at least 1 h before and after immunization. Fluid intake was not restricted. The vaccine was administered on alternate days for a total of three doses. Each child was observed for 1 h after immunization. Adverse reactions were recorded on a report sheet by the parents. In addition, the parents and children were interviewed approximately 21 days after the first dose of vaccine to ascertain vaccine acceptability. A venous blood sample was taken at the time of immunization and 21 days later, and serum was collected. Serum tubes were labeled with the subject's trial number, the letter code, and the date and were frozen at  $-20^\circ\text{C}$ .

Anti-*S. typhi* lipopolysaccharide (LPS) antibody was measured by the use of an enzyme-linked immunosorbent assay (9, 11). Paired serum samples were tested in parallel on the same plate in a blinded fashion. The samples were first tested at a 1:100 final dilution. Those baseline samples which yielded a high optical density ( $\geq 0.75$ ) were serially diluted (twofold) and reassayed. A significant rise in antibody was defined as a net increase of  $\geq 0.15$  optical density units above baseline values (9).

Significance in the immune response rates between groups was determined by chi-square analysis.

A total of 634 children were enrolled. Of these, 190 were from Chachoengsao Province, and 444 were from the Bangkok metropolitan area. Most children who participated were 3 to 5 years old because this age-group constituted the majority of day-care center attendees.

The vaccine was equally well tolerated among children in both trial sites. In the placebo group, a total of eight children presented with an adverse reaction which included vomiting (five children), malaise (one child), headache (one child), fever (two children), and rash (two children). Five children in the vaccine group noted an adverse event such as vomiting (three children), diarrhea (one child), and fever (one child). All reactions were mild and did not appear to be age

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TABLE 1. Serum anti-*S. typhi* LPS antibody response after oral ingestion of placebo or *S. typhi* Ty21a

| Subject age (yr) | No. of significant <sup>a</sup> anti- <i>S. typhi</i> LPS antibody responses/no. of subjects studied (%) |              |
|------------------|--|--------------|
|                  | Placebo  | Vaccine      |
| 2                | 0/4 (0)  | 2/2 (100)    |
| 3                | 6/54 (11)  | 48/80 (60)   |
| 4                | 11/75 (15)   | 66/98 (67)   |
| 5                | 11/58 (19)   | 42/53 (79)   |
| 6                | 2/22 (9)   | 20/22 (91)   |
| All subjects     | 30/213 (14)  | 178/255 (69) |

<sup>a</sup>  $P < 0.005$  for placebo versus vaccine.

dependent. A total of 20 children (3%; 16 placebo, 4 vaccine) reported difficulty in ingesting the preparations because of the taste and/or smell of the bacterium-buffer suspension. However, all 20 did consume the entire amount.

Of the 634 children who completed the three-dose immunization schedule, paired serum samples were obtained from 468 (74%). Of these, 180 were from children residing in Chachoengsao Province and 287 were from Bangkok. The serum anti-*S. typhi* immunoglobulin G antibody response is shown in Table 1. The seroconversion rate for vaccine recipients of all ages was 69% versus 14% for those in the placebo group ( $P < 0.005$ ). The seroconversion rate for children aged 3 to 6 years who received placebo ranged from 9% (6-year-olds) to 19% (5-year-olds). The seroconversion rate among recipients of the Ty21a vaccine ranged from 60% in 3-year-olds to 91% in 6-year-olds, which was significantly ( $P < 0.005$ ) higher than that for age-matched placebo recipients. There was a trend towards higher seroconversion rates with increasing age for children who received the vaccine, which was not observed among the placebo group. Therefore, the response rate for 6-year-olds was significantly ( $P < 0.05$ ) higher than that for 3- and 4-year-olds, but not for 5-year-olds. Too few 2-year-olds were recruited to allow accurate evaluation of immunogenicity in this age-group. It is interesting to note, however, that both 2-year-olds who received vaccine seroconverted, while none of the four placebo recipients did so.

Fully 22% of the children possessed baseline anti-*S. typhi* LPS immunoglobulin G antibody levels (optical density,  $\geq 0.75$ ) indicating prior exposure to *S. typhi* or possibly another group D *Salmonella* sp. (9). Elevated baseline titers were more prevalent among those children residing in rural Chachoengsao Province (37%) than in Bangkok (13%) ( $P < 0.005$ ). There was no significant difference in the seroconversion rates between children with elevated baseline antibody levels and those with nonelevated baseline antibody levels ( $P > 0.05$ ).

Immunization of 2- to 6-year-old Thai children with the Ty21a liquid formulation was accomplished without an excess of adverse reactions compared with the placebo group. Compliance was also good, with only 3% of the children being somewhat reluctant to ingest the vaccine suspension because they disliked either the taste or the smell.

In Chilean field trials with the Ty21a vaccine, a rise in serum anti-*S. typhi* LPS antibody levels was correlated with protection (7). Therefore, seroconversion may act both as a marker for vaccine "take" and as a predictor of immunity.

Three doses of the Ty21a vaccine elicited a significant rise in antibody levels in approximately 70% of the Thai children

3 to 6 years of age, although there was a tendency for a higher seroconversion rate among older children. This may be attributable to the fact that older children may have been primed by previous exposure to *S. typhi* or another group D *Salmonella* sp.

The seroconversion rate among placebo recipients (14%) was higher than expected. We have been unable to identify the precise reason for this observation, although several possibilities exist. Subjects assigned to the placebo group could have inadvertently received one or more doses of vaccine. However, only one such case was found, and the subject was excluded from the study. At the dose administered, Ty21a is not excreted in the feces (11), thereby making the spread of the vaccine strain to placebo recipients an unlikely reason for seroconversion. Another possible explanation is that there was a substantial amount of exposure to *S. typhi* or another group D *Salmonella* sp. during the interval between vaccination and blood sampling. This finding could not be attributed to the assay system, because increasing or decreasing the cutoff value for seroconversion resulted in a nearly proportional change in both groups.

The rate of seroconversion attained with the Ty21a vaccine was substantially higher than previously reported (2, 10, 11). Black et al. found that only 40% of 6- to 9-year-old and ~20% of 2- to 3.5-year-old Chilean children mounted a significant antibody response (2). Neither a humoral nor a cell-mediated response could be detected in infants or toddlers (11). These differences may be attributable to several factors, foremost among them being vaccine formulation. In the Chilean studies, the Ty21a vaccine was administered by emptying the capsule contents into a buffer composed of sodium bicarbonate and milk. We have found that reconstitution of Ty21a vaccine in milk results in pronounced bacterial clumping, which could adversely affect gut transit time and the ability of the bacteria to interact with intestinal tissue. In contrast, the current formulation yields a homogeneous suspension with gentle mixing. Other factors which may have contributed to a higher seroconversion rate among the Thai children include an increased level of prior exposure to *S. typhi* and genetic differences between the study populations.

The current findings indicate that the Ty21a vaccine, presented as a liquid formulation, may prove useful for the immunization of young children against typhoid fever. Vaccination can therefore be performed at an early age so that the maximum public health benefit can be obtained.

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