PASTEUR ORAL CHOLERA VACCINE: STUDIES OF REACTOGENICITY, CLINICAL ACCEPTABILITY AND IMMUNOGENICITY IN HUMAN VOLUNTEERS

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Abstract. Pasteur cholera vaccine consists of isolated antigenic fractions from *V. cholerae* El Tor Ogawa and Inaba. Enteric coated microgranules were prepared from antigen lyophilisate. Three doses of this vaccine were administered orally to 19 healthy young Thai adults at one week intervals. None of the volunteers experienced untowards reactions. The vibriocidal antibody responses manifested a significant antibody rise (≥ 4 fold) to serovar Inaba in 8 vaccinees (42.1%) and Ogawa in 4 (21.1%). Five and 6 vaccinees (26.3% and 31.6%) showed a ≥ 4 fold rise of IgG and IgA anti-LPS, respectively.

INTRODUCTION

Vibrio cholerae is a non-invasive pathogen that does not penetrate the intestinal mucosa and some evidence suggests that the immunity is mediated by intestinal secretory IgA (sIgA) antibodies (Svennerholm *et al,* 1978; Pierce, 1978). Oral administration of an antigen is the most efficient method for eliciting intestinal secretory antibodies, so most current research involves the use of oral vaccine with either non-living antigen or live attenuated vaccine strains.

The major attraction of non-living oral vaccines is their safety but multiple doses are required. Until now two oral cholera vaccines consisting of killed *V. cholerae* 01 whole cells of both biovars and serovars, either with or without the B subunit of cholera toxin, have been studied (Svennerholm *et al,* 1982; 1984 a, b; Black *et al,* 1987; Migasena *et al,* 1989; Clemens *et al,* 1988). Three doses of 1×10^{11} organisms of killed whole cells (WC) plus 1 mg of B subunit (BS) given at two week intervals elicited 33% and 42% significant rise of vibriocidal antibodies to Inaba and Ogawa, respectively (Migasena *et al,* 1989). A field trial in Bangladesh recently showed that three doses of 1×10^{11} organisms of killed WC and 1 mg of BS given at four week intervals provided 85%, 62% protection during the first six months and one year of follow up respectively (Clemens *et al,* 1988).

Another candidate oral cholera vaccine is the Pasteur vaccine. It consists of isolated antigenic fractions from *V. cholerae* El Tor Ogawa and Inaba (Dodin and Wiart, 1975). It was shown that extracts of *Vibrio cholerae* given to rabbits by the oral route could induce a strong antibody response in both serum and intestinal fluid (Dodin and Wiart, 1975). This vaccine was tried during an epidemic of cholera in Zaire and was claimed to have 96% efficacy (Dodin *et al,* 1984), but the study design was not quite optimal. Other studies were carried out suggesting that the vaccine induced both serum vibriocidal titers and intestinal production of antigen specific IgA (Langevin-Perriat *et al,* 1988). With these promising findings, it was considered appropriate to evaluate its safety and immunogenicity in volunteers in Thailand where cholera is endemic.

MATERIALS AND METHODS

Volunteers

Participants in this study were healthy young Thai adults aged 20-40 years from the Bangkok area who passed the medical and psychological screening and gave informed, signed consent. No volunteers had a history of cholera vaccination for the past five years.

Vaccines

Pasteur oral cholera vaccine consisted of isolated antigenic fractions from *V. cholerae* El Tor Ogawa and Inaba. The component includes a complex of

smooth type lipopolysaccharide and outer membrane proteins. Enteric coated microgranules were prepared from antigen lyophilisate. Each capsule contains 370 mg of enteric coated microgranules which comprises 30 mg of lyophilisate corresponding to 3 mg of the antigenic fraction.

Immunization schedule

All volunteers received three oral doses at one week intervals. Volunteers fasted for two hours and 90 minutes prior to and post vaccination.

Immune response

Sera were collected from volunteers before and 7, 14 and 21 days after the first dose of vaccination. Jejunal fluid was collected by aspiration before and 21 days after the first dose of vaccine.

For sera, vibriocidal antibody was measured by a microtechnique (Beneson *et al,* 1968). Antibody to *Vibrio cholerae* LPS was assayed by ELISA (Svennerholm *el al,* 1984 a, b).

For jejunal fluid, specific sIgA antibodies to LPS of *Vibrio cholerae* were measured by ELISA (Svennerholm *et al,* 1984 a, b; Young *et al,* 1980).

RESULTS

Nineteen volunteers, 15 males and 4 females with mean age of 24.5 years (ranged from 20 to 34 years) were studied. The mean weight was 56 kg, with a range of 50-62.5 kg. There were no adverse effects observed after any dose of vaccine.

Table 1 showed that the serum antibody levels before vaccination were low and the responses to vaccination were only modest. The detected rise is sIgA in jejunal fluid was due to a response in one volunteer.

The distribution of antibody responses in serum and in jejunal fluid are shown in Table 2. After three oral doses of Pasteur vaccine, 8 (42.1%) and 4 (21.1%) developed $\geq$ four-fold rise of vibriocidal antibody to Inaba and Ogawa, respectively. With respect to anti-LPS antibody, 5 (26.3%) and 6 (31.5%) developed $\geq$ four fold rise of serum IgG and IgA antibodies.

The antibody responses obtained were comparable with those after oral killed whole cell/B subunit vaccine reported from our previous studies (Migasena *et al,* 1989) (Table 3).

DISCUSSION

This study confirmed that Pasteur vaccine was safe, easily administered and caused no side effects in Thai adults. Similar results were obtained from the other ethnic population (Langevin-Perriat *et al,* 1988). The seroversion in terms of vibriocidal antibody was comparable between whole cell/B subunit vaccine and Pasteur vaccine (Migasena *et al,* 1989). However the peak GMT of anti-Inaba vibriocidal antibody with Pasteur vaccine in this study was higher than that with the WC-BS vaccine; on the other hand, with the anti-Ogawa antibody, the killed whole cell-B subunit was better. Even it is not certain that protection against cholera is solely due to anti-LPS antibody; however, a strong epidemiological correlation has been found between them in the field (Mosley *et al,* 1968). Since Pasteur vaccine is safe and easily administered, this may offer an alternative tool for preventing cholera and justifying examination in a challenge study.

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Table 1

Antibody responses after three oral doses of Pasteur vaccine (n = 19).

Antibody assay	Baseline titer GMT (range)	Peak titer GMT (range)
Serum: Vibriocidal antibody		
to Inaba	46 (20-80)	143 (40-640)
to Ogawa	35 (10-80)	69 (40-160)
Anti-LPS		
IgG	23 (20-40)	42.2 (20-1,280)
IgA	6 (2.5-20)	16.7 (2.5-320)
Jejunal fluid:		
Anti-LPS (sIgA)	2 (2)	8 (8)

Table 2

Rise of antibody responses after three oral doses of Pasteur vaccine (N = 19).

Type of antibodies	No. (%) with ≥ fold rise	No. (%) with ≥ 2 fold rise
In serum		
Vibriocidal antibody		
to Inaba	8 (42.1%)	17 (89.5%)
to Ogawa	4 (21.1%)	13 (68.4%)
Anti-LPS		
IgG	5 (26.3%)	8 (42.1%)
IgA	6 (31.5%)	14 (73.7%)
In jejunal fluid		
sIgA anti-LPS	1 (5.3%)	1 (5.3%)
Any responses of the above	13 (68.42%)	19 (100%)

Table 3

Comparison of serum vibriocidal antibody responses after three doses of Pasteur vaccine or killed whole cell-B subunit vaccine.

Vaccine	No. of volunteers	Percent of volunteers with fold rise					
		Inaba			Ogawa		
		≥4	≥8	≥16	≥4	≥8	≥16
Pasteur	19	42	21	5	21	11	0
WC (1×10^{11}) + 1 mg B-subunit	12	33	8	0	42	17	17
WC (2×10^{11}) + 5 mg B-subunit	11	36	9	0	91	64	18

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