INFECTION AND IMMUNITY, Oct. 1992, p. 4430–4432
0019-9567/92/104430-03$02.00/0

Vol. 60, No. 10

Safety and Immunogenicity in North Americans of a Single Dose of Live Oral Cholera Vaccine CVD 103-HgR: Results of a Randomized, Placebo-Controlled, Double-Blind Crossover Trial

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Received 4 May 1992/Accepted 7 July 1992

We conducted a double-blind, placebo-controlled, randomized crossover study to evaluate the safety and immunogenicity of a single 5 × 10^8-CFU dose of live oral recombinant cholera vaccine CVD 103-HgR in 94 North American adults. The vaccine was well tolerated without associated adverse reactions. Despite minimal fecal excretion of vaccine, 97% of subjects exhibited serum vibriocidal antibody and 72% had antitoxin responses.

CVD 103-HgR is a live oral cholera vaccine strain constructed by recombinant DNA methods from classical Inaba strain 569B by deletion of the genes encoding the A subunit of cholera toxin and introduction of a gene encoding resistance to Hg^+ (as a marker) into the *hlyA* locus of the bacterial chromosome (5, 6, 9). Heretofore, this vaccine has been extensively tested in randomized, placebo-controlled trials in adults and children in less-developed countries, where it has been well tolerated and has elicited high rates of seroconversion of vibriocidal antibody (11, 13, 15, 16). The goal in such populations is to evaluate CVD 103-HgR as a possible tool in the control of endemic and epidemic cholera.

Since CVD 103-HgR also holds promise as a one-dose vaccine to prevent cholera in travelers from industrialized countries who visit less-developed areas (4), we conducted a randomized, double-blind, placebo-controlled study to assess the safety and immunogenicity of CVD 103-HgR and the frequency and duration of vaccine excretion in Maryland adults.

After obtaining informed consent in accordance with the guidelines of the Department of Health and Human Services and the University of Maryland, 94 healthy college students aged 18 to 40 years (mean age, 24.4 years) were enrolled; those who had lived in an area in which cholera was endemic or had received antibiotics within the preceding 2 weeks were excluded (8, 9). This sample size was chosen to permit detection of a 6% difference between recipients of vaccine and placebo in symptoms that were estimated to occur in 1% of unvaccinated subjects (alpha = 0.05; beta = 0.2; two tailed). Fifty-three subjects (56.4%) were male. The racial-ethnic distribution included 81 whites (86.2%), 7 blacks (7.4%), 4 Hispanics (4.3%), and 2 Asians (2.1%).

In a double-blind fashion, subjects were randomly allocated to receive a single dose of 5 × 10^8 CFU of either lyophilized vaccine (49 subjects) or heat-killed *Escherichia coli* K-12 placebo (45 subjects) (2). Sachets of vaccine and placebo also contained 25 mg of aspartame as sweetener. Immediately before vaccination, a packet of buffer containing $NaHCO_3$ (2.5 g) and ascorbic acid (1.65 g) was added to 100 ml of distilled water in a cup. One dose of vaccine or placebo was added to the buffer to prepare a suspension, which the subject ingested. Subjects fasted 90 min before and after ingesting the vaccine. Eight days after primary inoculation, the placebo and vaccine groups crossed over while maintaining the blind, so that vaccine recipients received a dose of placebo and placebo recipients were inoculated with vaccine.

For 7 days following each administration of vaccine or placebo, subjects were monitored daily for occurrence of adverse reactions. Subjects recorded the character of every stool passed (formed or loose) and their evening oral temperatures. Diarrhea was defined as four or more loose or watery stools during 24 h (3), fever was defined as an oral temperature of 100.0°F (37.8°C) or greater, and vomiting was defined as one or more episodes of emesis. McNemar's matched-pair statistics were used to compare the frequencies of adverse reactions after vaccination and placebo. The blind was maintained through analysis of data.

No adverse reactions attributable to vaccination were noted (Table 1). Symptoms which occurred after vaccine was ingested but not after placebo was ingested, such as headache (six subjects), malaise (one subject), fever (two subjects), anorexia (three subjects), cramps (eight subjects), vomiting (two subjects), and diarrhea (one subject), were seen in a comparable proportion of subjects after placebo was ingested.

Among volunteers who experienced symptoms, the complaints were mild. All episodes of fever were low grade (≤100.8°F [ca. 38.2°C]) and occurred in otherwise asymptomatic individuals. No subject exceeded the minimum definition of diarrhea (four stools within 24 h) or vomiting (one episode of emesis) or met these criteria for more than 1 day. Even when a less-severe definition of diarrhea was used, i.e., three stools within 24 h, no significant association with vaccination was seen (P = 0.08) (Table 1).

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TABLE 1. Clinical response in subjects inoculated with live oral cholera vaccine or placebo

Symptom[a]	No. (%) with symptom after:		
	Vaccine and placebo[b]	Vaccine but not placebo	Placebo but not vaccine
Headache	2 (2.1)	6 (6.3)	8 (8.5)
Malaise	0	1 (1.1)	3 (3.2)
Fever	0	2 (2.1)	2 (2.1)
Anorexia	0	3 (3.2)	1 (1.1)
Borborygmi	6 (6.4)	43 (45.7)	45 (47.9)
Cramps	1 (1.1)	8 (8.5)	10 (10.6)
Vomiting	0	2 (2.1)	0
Loose stools			
3/24 h	0	6 (6.4)	2 (2.1)
4/24 h	1 (1.1)	1 (1.1)	0

[a] Using McNemar's test, no significant differences were seen among the matched pairs for the criteria listed.
[b] Vaccine was 5×10^8 CFU of CVD 103-HgR, and placebo was *E. coli* K-12. Ninety-four subjects participated in the trial.

Subjects swabbed their perianal areas after defecation on days 1, 3, and 7 following each dose of vaccine or placebo to detect vaccine excretion. A rectal swab was collected on site from subjects who were unable to produce a postdefecation specimen. Swabs were immediately inoculated into Cary-Blair transport medium and maintained at room temperature for up to 24 h until cultured for *Vibrio cholerae* O1 as described elsewhere (10, 14). Vaccine was excreted on at least 1 day by 18 subjects (19.1%). Shedding was detected on day 1 after vaccination in 9 subjects (9.6%), at 3 days in 11 subjects (11.7%), and at 7 days in 5 subjects (5.3%). No excretion was detected 8, 11, and 15 days after vaccination in the 49 subjects who received vaccine in the primary inoculation.

Serum was collected before and on days 8, 15, 21, and 28 following each dose of vaccine or placebo and tested for Inaba vibriocidal antibody (1) and immunoglobulin G antitoxin (12). A fourfold or greater rise in vibriocidal antibody occurred in 97% of subjects after vaccination, with a reciprocal geometric mean peak titer of 2,656; 67% reached titers of 1:2,560 or higher (Table 2). An antitoxin response was seen in 72% of vaccinated subjects, with a mean peak titer of 0.91 net optical density units.

We conclude from this placebo-controlled trial that a single 5×10^8-CFU dose of CVD 103-HgR is well tolerated by healthy young Maryland adults. We did not detect a significantly greater rate of diarrhea or other adverse reactions following ingestion of vaccine versus placebo, even when a highly sensitive definition of diarrhea (three or more loose stools within 24 h) was used.

As in many previous (albeit much smaller) cohorts who have received CVD 103-HgR in the United States (7, 9, 11, 17) and Switzerland (2), a single 5×10^8-CFU dose of vaccine elicits significant rises in serum vibriocidal antibody (the best correlate of protection) in approximately 90% of vaccinated subjects. Of interest, while this vaccine is highly immunogenic, the rate and extent of vaccine excretion are minimal. This latter characteristic should minimize concerns raised by some environmentalists about the release into the environment of recombinant vaccine strains.

CVD 103-HgR has significantly protected volunteers against experimental challenge with virulent *V. cholerae* O1 of either biotype and either serotype (7, 9, 17). These observations, in conjunction with the excellent clinical tolerance of CVD 103-HgR as documented in this study, its impressive immunogenicity following administration of a single dose (Table 2), and its minimal excretion, make CVD 103-HgR highly attractive as a future cholera vaccine for travelers to areas where cholera is endemic or epidemic.

TABLE 2. Immune response to vaccination of subjects with a single dose of live oral cholera vaccine CVD 103-HgR

Parameter[a]	Response	95% confidence limits
Vibriocidal antibody		
% with ≥4-fold rise in titer	97	94–100
% with titer of ≥1:2,560	67	57–77
Geometric mean titer[b]		
Preimmunization	20	15–25
Postimmunization peak	2,656	1,831–3,853
Antitoxin antibody		
% with ≥4-fold rise in titer	72	63–81
Geometric mean titer[c]		
Preimmunization	0.15	0.10–0.20
Postimmunization peak	0.91	0.69–1.13

[a] Ninety-four subjects participated in the trial. The dose of vaccine was 5×10^8 CFU.
[b] Back-transformed from logs of reciprocal titer.
[c] Expressed as net optical density units.

This study was supported in part by funding from the Swiss Serum and Vaccine Institute.

We gratefully acknowledge the volunteers who participated in this trial. We thank William Willis, Jr., Donald Haines, Joanna Becker, Catherine Black, and Ronald Grochowski for their invaluable contributions to subject recruitment and data collection and Yu Leung Lim, Linda Guers, and Mardi Reyman for assistance in performing laboratory assays.

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