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Preliminary Assessment of the Safety and Immunogenicity of Live Oral Cholera Vaccine Strain CVD 103-HgR in Healthy Thai Adults

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A single dose (5×10^8 organisms) of attenuated $A^- B^+$ *Vibrio cholerae* classical Inaba recombinant vaccine strain CVD 103-HgR or placebo was administered to 24 healthy young Thai adults in a randomized, placebo-controlled, double-blind trial of safety and immunogenicity. None of the volunteers experienced untoward reactions. The vaccine strain was recovered from 2 of 12 vaccinees. The vibriocidal antibody response (the best immunological correlate of protection) was good: 11 of 12 vaccinees (92%) manifested significant serotype-homologous Inaba antibody rises with a peak reciprocal geometric mean titer (RGMT) postvaccination of 3,417; 9 of 12 exhibited significant serotype-heterologous Ogawa antibody rises (prevaccination RGMT, 180; peak RGMT, 2,874). Nine of 12 vaccinees had significant rises in serum antitoxin. None of the controls exhibited rises in vibriocidal or antitoxic antibody. This preliminary study further confirms the safety and immunogenicity of CVD 103-HgR live oral cholera vaccine and paves the way for larger community studies of this candidate cholera vaccine.

The modern approach to development of new cholera vaccines has focused on the oral route of immunization to elicit protective immunity against this enteric infection (1, 9–11, 18). The candidate vaccines under evaluation consist of either nonliving antigens (1, 9, 18) or attenuated strains (10, 11). A live oral cholera vaccine can confer protection after administration of just a single dose (10, 11). CVD 103 (10) is an attenuated strain of *Vibrio cholerae* O1 in which the genes encoding the A (enzymatically active) subunit of cholera toxin were deleted by recombinant DNA techniques (7, 10). CVD 103-HgR is a further derivative of CVD 103, in which a gene encoding resistance to Hg^{2+} was recombined into the vibrio chromosome in the *hlyA* locus to provide a marker to differentiate the vaccine strain from wild-type *V. cholerae* O1 (7, 10). CVD 103 and CVD 103-HgR have been extensively evaluated as candidate live oral cholera vaccines in healthy young adult North American volunteers (10). These genetically engineered, attenuated *V. cholerae* O1 vaccine strains were shown to be well tolerated and highly immunogenic; moreover, after only a single dose, these live oral vaccines provided a high level of protection against experimental challenge with pathogenic *V. cholerae* O1 of either biotype (classical or El Tor) or serotype (Inaba or Ogawa) (10). Based on these highly encouraging results in adults in a nonendemic area, a series of clinical studies were planned to evaluate the safety and immunogenicity of CVD 103-HgR in adults in Bangkok, Thailand, where foci of endemic cholera persist. This report describes the first of these studies, consisting of a randomized, placebo-controlled, double-blind trial among healthy young adults confined to a research isolation ward.

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MATERIALS AND METHODS

Volunteers. The volunteers consisted of 24 healthy Thai adults 20 to 30 years of age with normal medical histories and physical examinations. Informed, witnessed consent was obtained from all participants. The volunteers were from middle-class and lower-middle-class backgrounds and included university students and graduates of a vocational school. To ensure the informed nature of the consent process, the volunteers had to pass a written examination consisting of approximately 20 true-or-false questions on all aspects of the study (8, 14). The 24 volunteers were randomized to receive a preparation coded A, B, C, or D, two of which were vaccine and the other two placebo. The study was carried out in the 24-bed research isolation ward maintained by the Vaccine Trial Centre in the Faculty of Tropical Medicine. The sewage system that drains the isolation ward is self contained. Meals for the volunteers came from the central kitchen of the Tropical Diseases Hospital. Volunteers were not allowed to prepare food on the ward.

Vaccine and placebo. Pathogenic *V. cholerae* O1 classical Inaba strain 569B is a potent producer of cholera toxin but does not express the Shiga-like toxin of *V. cholerae* O1 (16); the pathogenicity of 569B has been clearly established in volunteer studies (2, 10, 12). Vaccine candidate strain CVD 103 was derived from 569B by deletion of the genes encoding the A subunit of cholera toxin by recombinant DNA techniques (6, 10). The introduction of a gene encoding resistance to Hg^{2+} (7) into the *hlyA* locus of the chromosome of CVD 103 led to further derivative CVD 103-HgR. The formulation of the vaccine consisted of two sachets, one containing 5×10^8 lyophilized vaccine organisms and 25 mg of aspartame and the other containing a buffer to neutralize gastric contents (2.5 g of $NaHCO_3$ and 1.65 g of ascorbic acid).

The placebo also consisted of two sachets, one containing 5×10^8 heat-killed rough *Escherichia coli* K-12 organisms and the other containing the identical buffer as used with the vaccine. After reconstitution (vide infra), the placebo and vaccine suspensions appeared similar.

The vaccine and placebo were manufactured in Bern and hand carried to Bangkok maintaining a strict 4°C cold chain during the entire trip. In Bangkok, the sachets were stored at 4°C for several days until the day of inoculation.

Administration of vaccine and placebo. For each volunteer, the coded sachet containing lyophilized vaccine or placebo was mixed with the contents of the sachet containing buffer in a cup with 100 ml of distilled water; the suspension was then stirred and ingested by the volunteer. Volunteers fasted for 6 h before and 90 min after the single oral vaccination.

Clinical surveillance for adverse reactions. The study was carried out in double-blind fashion without the volunteers, the nursing staff, or the clinical investigators knowing the identity of the contents of the packets. A four-letter code for the packets was employed as an extra precaution to maintain double blindness. The volunteers were admitted to the isolation ward 2 days prior to vaccination for screening and acclimitization. For 5 consecutive days after receiving vaccine or placebo, the volunteers were interviewed and examined to elicit complaints of untoward effects including diarrhea, abdominal cramps or borborygmi, malaise, nausea, vomiting, fever, and headache. The results were recorded on a clinical flow sheet.

After the 5 days of clinical observation following oral inoculation with vaccine or placebo, the volunteers were given a 5-day course of tetracycline (500 mg every 6 h) to eradicate intestinal carriage of the vaccine strain. Coprocultures of all volunteers had to be negative for the vaccine strain for at least 3 consecutive days prior to discharge.

Stool collection. All stool specimens from all volunteers during the study were collected in specialized sterilizable plastic bedpans that fit on the commode (8, 14). Each stool was graded on a five-point scale as previously described (8, 14): grade 1, formed; grade 2, soft but formed; grade 3, thick liquid; grade 4, opaque watery; grade 5, clear rice water. Loose stools (i.e., grades 3 to 5) were weighed. Diarrhea was defined as passage of at least two loose stools within 48 h with a combined weight of 200 g or a single loose stool of 300 g or greater (8, 14). After extracting a small amount of stool for bacteriological studies, the remainder of the stool was treated with disinfectant and flushed into a self-contained system.

Bacteriology. Specimens of all stools were cultured. If no stool specimen was available in a 24-h period, a rectal swab was obtained. Stools and swabs were inoculated directly onto thiosulfate-citrate-bile salts-sucrose agar (BBL Microbiology Systems, Cockeysville, Md.) and inoculated into alkaline peptone water enrichment broth (17). After overnight incubation, the enrichment broths were subcultured onto thiosulfate-citrate-bile salts-sucrose agar. Suspicious colonies were tested for agglutination with Inaba and Ogawa typing sera.

Serology. Serum samples were collected from all volunteers before, and at 10, 21, and 28 days after oral inoculation. Vibriocidal antibody was measured against both classical *V. cholerae* O1 serotype Inaba (strain 89) and Ogawa (strain 79) by a microdilution technique as previously described (5, 11); fourfold-or-greater rises were considered significant. Immunoglobulin G cholera antitoxin was measured by enzyme-linked immunosorbent assay in sera diluted 1:50 (13); based on statistically derived criteria previously described (13), a rise in net optical density of 0.15 or greater was considered significant.

RESULTS

Clinical acceptability. In this double-blind study, no significant adverse reactions, including fever, diarrhea, vomiting, anorexia, or abdominal cramps were observed in any participant during the 7-day period of observation.

Bacteriology. Vaccine organisms were recovered from stool cultures of 2 of the 12 vaccinees (17%). None of the controls shed the vaccine strain.

Immune response to CVD 103-HgR. The serum Inaba and Ogawa vibriocidal and antitoxin responses are summarized in Table 1. Significant (fourfold-or-greater) rises in serotype-homologous Inaba vibriocidal antibody titer were observed in 11 of 12 vaccinees (92%); the peak postvaccination geometric mean reciprocal titer was 3,417. A significant rise in vibriocidal antibody against the heterologous serotype, *V. cholerae* Ogawa, was detected in 9 of the 12 vaccinees. The Ogawa vibriocidal reciprocal geometric mean titer rose from 180 prevaccination to a postvaccination peak of 2,874.

The serum antitoxin response was more modest: 9 of 12 vaccinees manifested significant rises (75%). There was not a correlation between the heights of rises of vibriocidal and antitoxic antibodies. A brisk response of one antibody was not necessarily accompanied by a comparable response of the other. None of the 12 control volunteers had significant rises in Inaba or Ogawa vibriocidal or antitoxin antibody.

DISCUSSION

To our knowledge, this study represents the first time that a live bacterial vaccine candidate against a human disease prepared by recombinant DNA technology has been evaluated in a developing country. For this reason, this initial study was carried out under a high level of physical containment. The primary questions addressed in this study include the following. (i) Would the vaccine strain be as well tolerated by young adult Thais as it was by the North Americans who previously received the vaccine? (ii) Would CVD 103-HgR elicit a good antibody response, particularly with respect to vibriocidal antibody, the best correlate of vaccine- or infection-derived protective immunity (15)? (iii) What would be the pattern of vaccine strain excretion, and would there be evidence of person-to-person transmission of the vaccine strain to the control volunteers? This study provides preliminary answers to each of these questions.

The first and second generation of cholera vaccine strains prepared by recombinant DNA methodology (6, 7, 11), while markedly attenuated in comparison with the virulent parent strains from which they were derived and while unable to cause severe diarrhea (11), nevertheless caused adverse reactions at such a high rate that they were deemed unacceptable. Thus, the third generation of attenuated *V. cholerae* O1 recombinant strains, CVD 103 and CVD 103-HgR, generated considerable enthusiasm because they were so well tolerated in North American volunteers (10). In this small study involving healthy young Thai adults, CVD 103-HgR was similarly well tolerated.

Beyond safety, the major question to be addressed was the immune response that a single dose of the live vaccine strain would elicit, particularly with respect to vibriocidal antibody. The prevaccination reciprocal geometric mean titer (RGMT) of Inaba vibriocidal antibody in the 12 Thai adults was 10-fold higher than the prevaccination RGMT in North

TABLE 1. Serological response of healthy Thai adults after ingestion of a single oral dose of 5×10^8 organisms of live oral cholera vaccine recombinant strain CVD 103-HgR

Volunteers	Titer of Inaba vibriocidal antibody		Presence (+) or absence (−) of significant rise	Titer of Ogawa vibriocidal antibody		Presence (+) or absence (−) of significant rise	Titer of immunoglobulin G antitoxin		Presence (+) or absence (−) of significant rise
	Prevaccination	Peak postvaccination		Prevaccination	Peak postvaccination		Prevaccination	Peak postvaccination	
Vaccinees									
8[a]	320	1,280	+	640	20,480	+	0.74	0.72	−
32	80	160	−	640	1,280	−	0.64	1.02	+
9	160	20,480	+	160	20,480	+	0.31	1.07	+
23	40	20,480	+	80	2,560	+	0.42	0.61	−
30	2,560	20,480	+	640	10,240	+	0.37	0.14	−
39	<20	320	+	<20	320	+	0.23	1.63	+
10	<20	5,120	+	80	20,480	+	0.39	0.99	+
31	10,240	40,950	+	1,280	20,480	+	0.43	0.68	+
43	160	640	+	40	160	+	0.34	0.84	+
12	160	1,280	+	160	320	−	0.49	1.29	+
44	160	640	+	80	160	+	0.05	0.33	+
41	2,560	40,960	+	640	20,480	+	0.91	1.25	+
Mean[b]	201	3,417		180	2,874		0.44	0.85	
Controls									
25	80	160	−	160	320	−	0.48	0.54	−
36	1,280	1,280	−	2,560	2,560	−	0.49	0.15	−
11	160	320	−	640	1,280	−	0.49	0.66	−
40	160	160	−	40	80	−	0.29	0.27	−
46	2,560	320	−	80	160	−	0.66	0.74	−
15	320	640	−	640	1,280	−	0.38	0.23	−
47	160	320	−	160	320	−	0.69	0.75	−
14	640	1,280	−	1,280	2,560	−	0.52	0.48	−
24	40	80	−	80	160	−	0.29	0.35	−
13	160	320	−	160	160	−	0.31	0.41	−
20	1,280	640	−	1,280	1,280	−	1.44	1.14	−
19	160	320	−	160	160	−	0.27	0.36	−
Mean[b]	285	359		285	453		0.52	0.51	−

[a] Identification number of volunteer.
[b] Means of Inaba and Ogawa vibriocidal antibody titers are geometric; means of immunoglobulin G antitoxin titers are arithmetic.

Americans (10), suggesting that the Thais had prior contact with *V. cholerae* O1 antigens. In such an immunologically primed population would the preexistent antivibrio immunity inhibit the interaction between the vaccine organisms and the intestinal immune system such that the vibriocidal response might be muted? Or would the vibriocidal responses in fact be greater than those seen in unprimed North Americans? In this study where we used a practical formulation of vaccine that was maintained in a strict cold chain, both the homologous (Inaba) and heterologous (Ogawa) vibriocidal responses were credible; 92% of the vaccinees manifested significant rises in Inaba vibriocidal antibody with a peak postvaccination RGMT of 3,417. The 3,417 peak postvaccination RGMT is significantly higher than the peak postvaccination RGMT of 1,810 recorded in North Americans who received CVD 103-HgR in the same dosage (10). Of note, it is also orders of magnitude greater than the peak postvaccination Inaba RGMT seen in Thai adults who ingested two different dosages of killed whole vibrio oral vaccine in combination with purified B subunit (peak RGMT, 42 and 62) (14).

The antitoxin response was much less impressive in the Thai adults, in contrast to the good antitoxin responses observed in North Americans (10). The Thais, of course, had significantly higher levels of antitoxin prevaccination. It may be that the background antibacterial immunity in this apparently primed population modified the behavior of the live oral vaccine strain in vivo by inhibiting proliferation of the strain in the small intestine, thereby limiting production of B subunit. This makes certain pediatric studies planned for the near future even more interesting and important to undertake. It is possible that young children in endemic areas, who are at the highest risk for cholera and who do not have the cumulative immunological experience of indigenous adults, may more closely resemble North Americans in their response to CVD 103-HgR.

Under any circumstances, we consider the antibacterial (vibriocidal) immune response to be the more critical to assure. This conclusion is derived from two main observations. First, immunization with a single oral dose of recombinant A^-B^- vaccine candidate JBK 70, which stimulated prominent vibriocidal antibody responses but no antitoxin, conferred 89% protection in volunteers challenged with fully toxinogenic *V. cholerae* El Tor Inaba strain N16961 (11). Second, results of the ongoing field trial of two inactivated cholera vaccines in Bangladesh (3, 4) indicate that the increased protective role played by the combination of antitoxin and antibacterial immunity over antibacterial immunity alone is short lived and that of the two types of immunity antibacterial is more critical. During the first 4 to 6

months of surveillance in this field trial, the combination of killed whole cell-B subunit oral vaccine provided significantly greater protection (85% vaccine efficacy) than did the oral killed whole vaccine alone (58% vaccine efficacy) (3, 4). However, after 3 years of surveillance, the protective efficacies conferred by the two oral vaccines are virtually equal (circa 50%) (4). These field trial data corroborate data generated earlier in the volunteer model of experimental cholera that led investigators to stress the critical role of antibacterial immunity in protecting against cholera (11, 12).

One of the potential advantages of the CVD 103-HgR strain in comparison with its immediate parent strain, CVD 103, is that with the former, fecal excretion is significantly decreased; CVD 103-HgR was recovered from only 28% of North American volunteers (10) and from only 17% of the Thai adults. A live recombinant vaccine that is diminished in excretion minimizes the chance of environmental spread of the vaccine strain, a subject of concern and debate in some quarters.

None of the controls excreted the vaccine strain or manifested seroconversion. Thus, under the controlled conditions of this study, there was no evidence of vaccine spread from vaccinees to controls. One must be cautious not to draw sweeping conclusions on transmissibility of the vaccine from this limited experience. To put this in perspective, conditions on the research ward are not compatible with food-borne transmission but personal hygiene practices of individual volunteers might theoretically permit transmission of enteric bacteria by direct contact or fomite fecal or oral spread. To definitively answer the question of the transmissibility of the vaccine strain, we will have to await results of more ambitious studies to be conducted in communities where conditions of sanitation and water supply and personal hygiene practices favor transmission of enteric organisms.

Vaccine development, including the clinical and immunological evaluation of candidate vaccines, is a stepwise process. In this regard, the results of this inpatient volunteer study are encouraging and pave the way for expanded randomized, double-blind, placebo-controlled studies of the safety, immunogenicity, and transmissibility of CVD 103-HgR to proceed in communities.

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