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Safety, Immunogenicity, and Excretion Pattern of Single-Dose Live Oral Cholera Vaccine CVD 103-HgR in Peruvian Adults of High and Low Socioeconomic Levels

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Groups of 122 Peruvian adults of low socioeconomic level (SEL) and 125 of high SEL received a randomly allocated 5×10^9- or 5×10^8-CFU dose of CVD 103-HgR live oral cholera vaccine or a placebo. The vaccine was well tolerated. Vibriocidal seroconversions occurred in 78% of high-SEL and 72% of low-SEL subjects who ingested the high dose and in 78 and 49%, respectively, of those who received the low dose.

Beginning in January 1991, in several cities along the coast, Peru was struck by an explosive epidemic of cholera (17, 20) constituting a public health problem of enormous dimensions accompanied by social and economic upheaval. This experience, in a country where large segments of the population are not served by potable water and sanitation, led to consideration of the possible use of cholera vaccines in controlling epidemic cholera in newly affected populations. The inactivated whole-cell parenteral cholera vaccines available for the past 90 years were deemed of little practical use in the control of cholera in Peru, since they confer only partial protection that lasts for only a few months (11). Indeed, on-site field experience from the Peruvian epidemic confirmed our belief that in order for a cholera vaccine to offer practical promise as a public health tool it should be a single-dose oral vaccine that confers at least a moderate level of long-lived protection and that initiates its protective effect within a few days of vaccination.

Genetically engineered attenuated *Vibrio cholerae* O1 strain CVD 103-HgR (6, 9, 10) has shown considerable promise as a single-dose live oral vaccine in phase 1 and 2 studies in adult and pediatric populations in cholera-endemic areas (8, 14, 18, 19) and in adults from industrialized countries (3, 4, 7, 9, 10). For the latter group, a single dose of CVD 103-HgR has conferred significant protection against experimental cholera, with protection commencing as early as 8 days and continuing for at least 6 months after vaccination (21).

Earlier studies with CVD 103-HgR in Thailand (18) and Indonesia (19) suggested that in less developed countries, a single dose containing 5×10^9 CFU is more immunogenic than a dose containing 5×10^8 CFU. The studies in Asia also hinted that in some way socioeconomic level (SEL) affects the rate of seroconversion (18, 19). Accordingly, this evaluation of the reactogenicity, immunogenicity, and excretion pattern of CVD 103-HgR in Peruvian adults included a comparison of doses of 5×10^9 and 5×10^8 CFU in a placebo-controlled trial among subjects of low and high SELs.

Adults of low SEL lived in Canto Grande, a periurban slum community without potable water or sanitation (13); medical students and physicians of the Facultad de Medicina, Universidad Peruana Cayetano Heredia, constituted the high-SEL subjects. Specifically excluded were pregnant women, individuals who had received antibiotics or who had diarrhea within the previous 72 h, and subjects who had previously received cholera vaccine. Eligible adults 18 to 38 years of age were administered coded preparations sequentially labelled A, B, or C, two of which contained the vaccine and the other a placebo. Informed consent was obtained from the study subjects, and human experimentation guidelines of the U.S. Department of Health and Human Services, as well as those of the local institutions, were followed.

Packets of vaccine contained 5×10^9 or 5×10^8 CFU of lyophilized, filtered CVD 103-HgR and 18.8 mg of aspartame (as a sweetener) (7, 18, 19). The placebo (5×10^8 cells of inactivated *Escherichia coli* K-12) and buffer were as previously described (7, 10, 14, 18). The buffer powder was introduced into a cup containing 100 ml of water and the mixture was stirred, after which the vaccine (or placebo) packet was emptied into the buffer solution and the mixture was stirred again and immediately given to the subject. An individual was considered vaccinated if he or she imbibed 70% or more of the suspension containing the vaccine or placebo.

The clinical protocol was reviewed by ethics committees at the Universidad Peruana Cayetano Heredia, the Instituto de Investigacion Nutricional (this committee is registered with the Office of Protection of Research Risks of the National Institutes of Health, Bethesda, Md.), the Ministry of Health of Peru, and the University of Maryland at Baltimore. It also received technical review by the Food and Drug Administration, Rockville, Md.

Double-blind clinical follow-up was maintained for 7 days following vaccination. Diarrhea was defined as passage of at

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TABLE 1. Adverse reactions among low- and high-SEL Peruvian adults receiving a single 5×10^9- or 5×10^8-CFU dose of CVD 103-HgR or placebo[a]

Group and dose of CVD 103-HgR (CFU)	Mean age ± SD (yr)	No. of patients with reaction/total no. inoculated (%)			
		Diarrhea	Vomiting	Abdominal cramps	Fever
Low SEL					
5×10^9	25.4 ± 5.7[b]	4/41 (9.8)	0/41 (0.0)	15/41 (37.0)	1/41 (2.4)
5×10^8	26.7 ± 5.9[b]	2/41 (4.9)	3/41 (7.0)	17/41 (41.0)	3/41 (7.3)
None (placebo)	26.7 ± 5.9	3/40 (7.5)	1/40 (2.5)	16/40 (40.0)	4/40 (10.0)
High SEL					
5×10^9	25.0 ± 4.3[b]	1/40 (2.5)	0/40 (0.0)	1/40 (2.5)	0/40 (0.0)
5×10^8	26.7 ± 5.2[b]	0/41 (0.0)	0/41 (0.0)	3/41 (7.0)	1/41 (2.4)
None (placebo)	26.7 ± 5.0	2/44 (4.5)	0/44 (0.0)	3/44 (6.8)	2/44 (4.5)

[a] Reactions were monitored for 7 days after oral inoculation.
[b] Analysis of variance: low SEL, $P = 0.52$; high SEL, $P = 0.19$.

least three loose stools within 24 h. Vomiting was defined as one or more episodes of emesis. An oral temperature of ≥38°C signified fever. From September to December 1991, 247 eligible adult volunteers entered the study and completed the clinical follow-up, including 122 individuals of low SEL and 125 of high SEL. The occurrence of adverse reactions in the various groups is summarized in Table 1. No gastrointestinal adverse reactions were attributable to the vaccine.

Stool samples or rectal swabs obtained from the subjects on the first and seventh days after vaccination were transported in Cary-Blair medium and processed as described elsewhere (14, 18, 19). The vaccine strain was isolated on day 1 from 3 of the 40 high-SEL individuals who received vaccine containing 5×10^9 CFU (7.5%); the day 7 cultures were negative. The vaccine strain was not recovered from any other subjects.

Venous blood (10 ml) was collected on the day of vaccination and 7 and 28 days thereafter for measurement of Inaba vibriocidal antibody (1); a fourfold or greater rise was considered significant (i.e., seroconversion). These serum antibodies are markers for the elicitation of protective intestinal immune responses (11). Immunoglobulin G cholera antitoxin in serum diluted 1:50 was measured by enzyme-linked immunosorbent assay (12); in the few instances in which the net optical density of the prevaccination specimen was ≥1.00, serum specimens were tested at a 1:200 dilution. A ≥0.20 rise in the net optical density of the postvaccination specimen over that of the prevaccination specimen was considered significant (i.e., seroconversion) (12). Overall rates of seroconversion were compared by the chi-square test with Yates' correction or by Fisher's exact test when appropriate. Prior to their analysis, serum Inaba vibriocidal-antibody titers and net antitoxin optical density were transformed to logarithms to better approximate normality.

Pre- and postimmunization specimens were available from 237 subjects. Serum Inaba vibriocidal-antibody responses are summarized in Table 2. Several points are worth noting. The dose of 5×10^9 CFU of vaccine organisms was more immunogenic than the lower dose, particularly in the low-SEL population. An unexpected finding was that 16% of the low-SEL subjects and 20% of the high-SEL subjects who were given the placebo manifested fourfold or greater rises in vibriocidal-antibody titers. We have not observed this level of seroconversion among placebo recipients in other populations in the Americas, Europe, or Asia (4, 7–9, 14, 18, 19).

In analyzing the differences in geometric mean titers (GMT) in relation to the dose of vaccine organisms, the SEL, and the interaction of the two, an analysis of covariance was carried out (covariate = day 0 vibriocidal-antibody titer). Evaluation of the serological data by analysis of covariance indicated a significant effect of the day 0 (i.e., baseline) titer upon the peak vibriocidal-antibody titer ($P <$

TABLE 2. Serum Inaba vibriocidal-antibody and immunoglobulin G (IgG) responses following ingestion of a single dose of CVD 103-HgR live oral cholera vaccine or placebo by adult Peruvians of low or high SEL

Group and dose of CVD 103-HgR (CFU)	Vibriocidal antibody				IgG antitoxin		
	Seroconversion rate[a]	Reciprocal GMT (log GMT ± SD)		Rise in GMT	Seroconversion rate	Optical density[b]	
		Prevaccination	Peak			Prevaccination	Peak
Low SEL							
5×10^9	28/39 (72)	16 (2.7 ± 1.0)	202 (5.3 ± 2.0)	13	21/39 (54)[c]	0.35	0.69
5×10^8	19/39 (49)	21 (3.0 ± 1.3)	106 (4.7 ± 2.0)	5	10/39 (26)[c]	0.26	0.51
None (placebo)	6/38 (16)	13 (2.6 ± 0.7)	21 (3.1 ± 1.2)	1.6	5/38 (13)	0.12	0.23
High SEL							
5×10^9	31/40 (78)	14 (2.7 ± 1.0)	374 (5.9 ± 2.0)	26	29/40 (73)	0.19	0.59
5×10^8	31/40 (78)	15 (2.7 ± 0.9)	274 (5.6 ± 2.0)	19	28/40 (70)	0.18	0.66
None (placebo)	8/41 (20)	13 (2.5 ± 0.8)	26 (3.2 ± 1.5)	2.1	6/41 (15)	0.21	0.21

[a] Number of vaccinees with fourfold or greater rises in titer/total number vaccinated. Percentages are given in parentheses.
[b] Geometric mean of net optical density.
[c] $P = 0.013$.

TABLE 3. Relationship between baseline reciprocal Inaba vibriocidal titer and propensity to seroconvert among all recipients of the high (5×10^9 CFU) dose of CVD 103-HgR live oral cholera vaccine[a]

Baseline reciprocal vibriocidal-antibody titer	No. of subjects seroconverted/ no. of subjects vaccinated (%)[b]
<20	48/61 (79)
20	7/9 (78)
40	2/4 (50)
80	1/4 (25)
≥160	0/6 (0)

[a] The relationship between baseline reciprocal vibriocidal titer and rate of seroconversion was significant for trend ($P = 0.05$ by runs test).
[b] Numbers of high- and low-SEL vaccinees are combined.

0.001), a significant effect of vaccine group (placebo or 10^8 or 10^9 CFU of vaccine) ($P < 0.001$), a significant difference between SEL groups ($P = 0.004$), and a nonsignificant interaction between the vaccine group and the SEL group ($P = 0.32$).

Among all vaccinees, the propensity to seroconvert was clearly influenced by the baseline titer of vibriocidal antibody. Among high-SEL vaccinees, 61 of 76 (80%) with baseline titers of ≤1:40 but only 1 of 5 (20%) with titers of >1:40 exhibited significant rises in titer ($P = 0.01$). In the low-SEL group of vaccinees, significant rises in the vibriocidal-antibody titer were observed in 44 of 66 with prevaccination titers of ≤1:40 (67%) and in 4 of 12 with titers of >1:40 (33%) ($P = 0.05$). Since the rates of seroconversion for the two SEL groups were similar following ingestion of the 5×10^9-CFU dose of vaccine and since this is the dose intended for use in less developed countries, the relationship between baseline vibriocidal-antibody titer and the propensity to seroconvert was analyzed for all recipients of this higher dose of vaccine (Table 3). The rate of seroconversion decreased with increasing baseline titers ($P = 0.05$; runs test for trend).

The pattern of serum immunoglobulin G antitoxin response, summarized in Table 2, resembles that of the serum vibriocidal-antibody response. Overall, the rate of seroconversion was greater in the high-SEL vaccinees (57 of 80; 71%) than in the low-SEL vaccinees (31 of 80; 39%) ($P < 0.001$). Among the high-SEL vaccinees, there was little difference in the seroconversion rates of the recipients of doses of 5×10^9 and 5×10^8 CFU (73 and 70%, respectively). In contrast, among the low-SEL vaccinees, the rate of antitoxin seroconversion was significantly higher in recipients of 5×10^9 CFU (54%) than in those who received 5×10^8 CFU (24%) ($P = 0.013$).

These data constitute the first published report of the use of CVD 103-HgR (or any oral cholera vaccine) in a South American population exposed to epidemic El Tor cholera. The results fit well with those obtained for Asian populations in which cholera is endemic. CVD 103-HgR was well tolerated by Peruvian adults, irrespective of SEL. The main measure of immune response, vibriocidal-antibody titer, correlates with the elicitation of protective antibacterial immunity (2, 5, 11, 15, 16). Seroconversion of vibriocidal antibody is recognized as particularly useful in cholera-naive individuals but is less helpful in persons who already have demonstrable titers at baseline (18). In this study in Peru, the baseline reciprocal GMT of Inaba vibriocidal antibody was not significantly higher among the low-SEL adults (GMT = 16.3) than among the high-SEL adults (GMT = 13.7) ($P = 0.19$).

The rate of seroconversion of vibriocidal antibody recorded for the Peruvian adults is high, considering that only a single oral dose was administered. Most of the nonresponders had elevated baseline titers of vibriocidal antibody. The reciprocal GMT achieved closely resembles that seen for Thai adults (18). We have previously hypothesized that differences in the proximal small intestinal floras of persons of low SEL who live in more unsanitary conditions than persons of high SEL can influence the response to CVD 103-HgR when this oral vaccine is given as a dose of 5×10^8 CFU (18, 19). One would expect a higher proportion of low-SEL persons to have their proximal small intestines colonized with coliform and anaerobic bacteria, colonic floras which may inhibit the attenuated *V. cholerae* O1 vaccine strain. The results from this study support this hypothesis. The effect of vaccine dosage on the rate of seroconversion was more notable for persons of low SEL.

A characteristic of CVD 103-HgR that has been repeatedly documented in previous studies is that this live vaccine is highly immunogenic, while it is only minimally excreted (7–10, 14, 18, 19). Nevertheless, it is of interest that the only isolations of the vaccine strain in the current study occurred among high-SEL adults who received the higher dose of vaccine, further supporting the hypothesis discussed above.

The one finding in this clinical trial that contrasts with all earlier studies is the 16 to 20% seroconversion rate in placebo recipients (Table 2). Significant rises in vibriocidal-antibody titers among placebo recipients were observed for 0% of the Thai adults (14, 18) and for only 2 of 72 (2.8%) 5- to 9-year-old children living in a highly cholera-endemic area of Jakarta, Indonesia (19). In Santiago, Chile, where there had been only a small outbreak of approximately three dozen cases of cholera prior to a clinical trial, vibriocidal seroconversions were observed in only 2 of 41 adults who received a placebo (4.9%), in contrast to 34 of 40 vaccinees (85%) (8). Among 5- to 9-year-old Chilean children, none of 168 placebo recipients seroconverted, while 127 of 171 recipients of a single dose of vaccine had significant rises (74%). Thus, the 16 to 20% rate of seroconversion among the Peruvian adults may indicate an extraordinarily high degree of exposure to *V. cholerae* O1 antigen, albeit via inocula too small to cause overt clinical illness. Indeed, this study was carried out at the beginning of the second cholera season in Lima, which ultimately resulted in almost as many cases as in the first year of the epidemic. The lack of potable water and sanitation in Canto Grande makes it a highly permissive environment for the transmission of cholera. The presumed high level of contact with *V. cholerae* O1 antigen of the high-SEL controls is most likely explained by the fact that these subjects consisted of house officer physicians and medical students who may have had antigenic exposure stemming from the clinical care of cholera patients.

These data for Peruvian adults, which document that CVD 103-HgR is safe, immunogenic, and minimally excreted following administration of a single dose, pave the way for safety and immunogenicity studies in school-age and preschool-age children. It would be of interest to evaluate in a randomized, double-blind, placebo-controlled trial the efficacy of a single dose of CVD 103-HgR in preventing cholera in Peru.

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