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# Live Oral Cholera Vaccine: Evaluation of the Clinical Effectiveness of Two Strains in Humans

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El Tor Ogawa C14-S5 and EW-6, two live vaccine candidate strains, were given to volunteers in varying doses with and without bicarbonate. Vibrios were found in the stool of one of 32 men given the vaccine strain, and only three men developed a significant titer rise (fourfold or greater) at 2 weeks of vibriocidal or antitoxic antibody. Five men who had previously received $10^9$ organisms of the C14-S5 strain were challenged subsequently with virulent Ogawa 395 *Vibrio cholerae*. The rate of clinical infection in these men was no different than in unvaccinated controls. It was demonstrated that the live oral cholera vaccines did not remain viable in the intestine long enough to act antigenically.

Limitations of the present parenteral cholera vaccines have been demonstrated in field trials (1, 8); the protection was 40 to 80% against clinical illness for 6 to 12 months after vaccination when high potency bivalent or monovalent vaccines were used. In recent years interest has been renewed in the development of both live and killed oral vaccine preparations. Two live vaccine candidate strains that have been well characterized are El Tor Ogawa C14-S5 and El Tor Ogawa EW-6. Since 1969 the Division of Infectious Diseases, University of Maryland School of Medicine, has studied induced cholera in volunteers in order to evaluate various types of cholera prophylaxis and determine the immunological response to illness (5, 10; R. A. Cash, S. I. Music, J. P. Libonati, J. P. Craig, N. F. Pierce, and R. B. Hornick, J. Infect. Dis., in press). A natural extension of our studies of immunogenic agents was the evaluation of these two live vaccine strains, C14-S5 and EW-6, in volunteers.

## MATERIALS AND METHODS

Studies in induced human cholera were carried out at the Maryland House of Correction, Jessup, Md. Approval for these studies was obtained from the University of Maryland Committee for Volunteer Research and the Cholera Advisory Committee of the National Institutes of Health, Bethesda, Md.

Inmate volunteers were asked to participate after the nature of the study had been explained to them. No coercion was used; each man was informed of his right to withdraw at any time. No volunteer had a history of cholera or cholera vaccination for 2 years prior to the study.

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Volunteers were accepted for study if medical history, physical examination, chest X-ray, electrocardiogram, urinalysis, blood chemistry, and hematology identified them as healthy. All volunteers were hospitalized and under constant surveillance by nurses and physicians. Stool volume and fluid intake were recorded every 8 h from the day of challenge.

The El Tor Ogawa vibrio C14-S5 was obtained from K. Bhaskaran of the Central Drug Institute, Lucknow, India. C14-S5 is an attenuated, streptomycin-resistant dwarf vibrio (2). Nonpathogenic El Tor Ogawa EW-6 was supplied by S. C. Sanyal of the Vibrio Reference Center, Calcutta, India. Isolated from a water source in Calcutta in 1958, this vibrio strain has been tested extensively in laboratory animals and man (3, 4, 9, 11).

The virulent strain, classical Ogawa 395, was provided by C. C. J. Carpenter of the Johns Hopkins Medical School, Baltimore, Md. The strain has been given to volunteers and produces cholera similar to that seen in natural infection (5).

Stock cultures of a *Vibrio cholerae* were kept in skimmed milk at −70 C prior to culture on brain heart infusion agar (BBL) at 37 C overnight. Identity was tested with group- and type-specific antisera, and 20 to 30 colonies were suspended in brain heart infusion (BBL). Preincubated brain heart infusion agar plates were inoculated with brain heart infusion suspension. After 5 to 6 h of incubation, each plate was harvested with 5 ml of sterile saline buffered to pH 7.2 ± 0.1 (one part 0.067 M Sorensen phosphate buffer to three parts saline). The harvested organisms were centrifuged in the cold at 750 × *g* for 10 min, resuspended, and washed twice in four times the original volume. This suspension was standardized spectrophotometrically and diluted to approximate the number of organisms required for each challenge.

Subjects were given nothing by mouth, and smoking was prohibited 2 h before and 2 h after challenge. The vibrios were suspended in 1.0 ml of buffered saline (pH 7.2 ± 0.1) and were given either with or without sodium bicarbonate. If vibrios were given with

base, 2 g of sodium bicarbonate was dissolved in 60 ml of distilled water; half of this basic solution was drunk before and half after the vibrio suspension was given. Plate counts of viable vibrios were made before and after challenge to assure dosage accuracy. A normal diet was allowed beginning 2 h postchallenge.

A total of 27 men were given an inoculum of C14-S5 vibrio with and without bicarbonate in doses ranging from $10^2$ to $10^9$ organisms (Table 1). Five men received $10^8$ EW-6 vibrio with bicarbonate. A dose of $10^6$ organisms of the virulent strain Ogawa 395 (with bicarbonate) was subsequently given to five men who had received $10^9$ C14-S5 5 weeks previously. Eight unvaccinated controls were challenged simultaneously.

Cholera infections were graded on clinical and bacteriological grounds. The grading system was as follows: grade 0, all stool cultures negative for *V. cholerae* and no diarrhea; grade 1, at least one stool culture positive for *V. cholerae* and no diarrhea; grade 2, at least one stool culture positive and at least one diarrheal stool, that is, a liquid or nonformed stool; and grade 3, *V. cholerae*-positive stool and diarrhea severe enough to require intravenous fluids. Tetracycline administration was begun simultaneously with intravenous fluid in those with grade 3 illness. Before discharge all volunteers received a 5-day course of tetracycline (250 mg four times a day).

The method of stool culture has been described (5). If the volunteer did not have a daily bowel movement or if the stool was lost to study, a rectal swab was taken and placed into NGP broth (3.0% gelatin [BBL], 1.0% NaCl, and 0.5% $K_2HPO_4$) as transport media. The broth was cultured on arrival at the laboratory and again the following day.

Serological response was followed by vibriocidal and antitoxin determination (6) and the formalinized erythrocytes were sensitized with Wyeth cholera toxin (lot no. 002) prepared by the aluminum hydroxide method of Spyrides and Feeley (12). Blood was collected prechallenge and every 2 weeks postchallenge for 8 weeks. Sera were stored at −20 C and all specimens from an individual were tested simultaneously.

The antitoxin reference sera used in the studies were NIH experimental cholera antitoxin lot no. 18 and SSVI-EC3 (A-2/67)-B; the vibriocidal reference antiserum was NIH reference G005-501-572. Our results with these reference sera have been reported (5; Cash et al., J. Infect. Dis., in press).

## RESULTS

A single man receiving $10^9$ C14-S5 with bicarbonate had one vibrio-positive stool, without diarrhea, within 24 h of ingesting the vaccine strain. No other individual receiving either vaccine strain had vibrio-positive stools or developed any illness.

Serological data are summarized in Table 1. A fourfold or greater rise in titer was considered significant. The 2-week serology of the 27 men who had received the C14-S5 demonstrated one man with a significant rise in vibriocidal Inaba titer, two men with a significant rise in vibriocidal Ogawa titer (one of these had the vibriocidal Inaba titer rise), and one man with a significant rise in antitoxin hemagglutination titer. No significant serological rises were seen after ingestion of the EW-6 strain. Of the five men who had previously received $10^9$ C14-S5 organisms, four developed clinical cholera after challenge with $10^6$ Ogawa 395 (Table 2). The individual who had C14-S5 in his stool developed grade 2 illness after challenge with Ogawa 395. One of the men who developed diarrhea had had significant vibriocidal Ogawa and vibriocidal Inaba titer rises after immunization with C14-S5.

## DISCUSSION

Our data gives evidence that there was little if any colonization of the intestine by these vaccine strains of vibrio: (i) stools were consistently negative for vibrio except for one stool of an

TABLE 1. *Significant titer rises[a] in vaccinees after ingestion of Live E1 Tor Ogawa C14-S5 or EW-6*

| Organism and dose | Sodium bicarbonate (2 g) | No. of volunteers | No. of patients with a significant rise in serum titers[b] | | |
|---|---|---|---|---|---|
| | | | VI | VO | HA |
| C14-S5 | | | | | |
| $10^2$ | No | 4 | | | |
| $10^4$ | No | 4 | | | |
| $10^6$ | No | 3 | | | |
| $10^8$ | No | 3 | | | 1 |
| $10^8$ | Yes | 5 | | | |
| $10^9$ | Yes | 8 | 1 | 2[c] | |
| EW-6 | | | | | |
| $10^8$ | Yes | 5 | | | |

[a] Fourfold or greater 2 weeks after ingestion of the organism.

[b] VI, Vibriocidal Inaba titer; VO, vibriocidal Ogawa titer; HA, hemagglutination (antitoxin) titer.

[c] One of these men also had the VI titer rise.

TABLE 2. *Effect of challenge with $10^6$ classical Ogawa 395 in controls and in individuals previously exposed to $10^9$ E1 Tor Ogawa C14-S5[a]*

| Group | No. of volunteers | Grade of cholera | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| Previous C14-S5[b] | 5 | 1 | 0 | 2 | 2 |
| Controls | 8 | 4 | 0 | 1 | 3 |

[a] No significant difference in diarrhea rate (grades 2 and 3) between controls and vaccinees.

[b] $10^6$ Ogawa 395 given 5 weeks after inoculum of C14-S5.

individual receiving $10^9$ C14-S5 with bicarbonate; and (ii) only two of these 18 patients (11%) given $10^8$ or $10^9$ organisms with bicarbonate had a significant rise in the vibriocidal titer at 2 weeks. (Overall, two of 32 men had a significant vibriocidal titer rise).

These results differ somewhat from those of Sanyal and Mukerjee (11). Although they found EW-6 vibrios in the stool of only four of 25 men given $10^9$ or $10^{10}$ organisms, they demonstrated a significant rise (fourfold or greater) in vibriocidal titers in six of 25 men (25%) 8 to 10 days after ingestion of the vibrio and in nine of 25 men at 3 months. They did give a larger dose of vibrio and followed with booster doses of vibrio on either one or two occasions. Additionally, 23 of their 35 volunteers gave a history of cholera vaccination. Thus, the previous exposure to vaccine (or the live vibrio, for the Calcutta area has experienced cholera epidemics) might cause an anamnestic response after exposure to the vaccine strain.

Patients rechallenged after illness with a homologous organism have been shown to be 100% protected against developing diarrhea (Cash et al., J. Infect. Dis., in press). Volunteers who did not show evidence of colonization after their first ingestion of virulent vibrio developed cholera on rechallenge. This high degree of protection after illness is indicative of the level of protection that is theoretically possible with oral immunization.

As Howard has noted, "A (live) vaccine is required which will continue to act antigenically for a prolonged period" (7). This idea must be kept in mind if further development of live oral cholera vaccines is to be productive.

Although there is not a direct correlation between an individual's serological titer and protection from subsequent challenge (Cash et al., J. Infect. Dis., in press), a vibriocidal or antitoxin titer rise does indicate contact between gut mucosa and cholera antigen. Our studies demonstrate that one dose of either living vaccine strain C14-S5 or EW-6 at the dosages given will not provide protection in humans against infection with virulent *V. cholerae*. Live vaccine strains are theoretically feasible but should not be considered for field testing, unless there is indication that in humans the organism consistently gives bacteriological or serological evidence of prolonged contact with the intestinal mucosa.

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