Research

Investigations into the safety and immunogenicity of a killed oral cholera vaccine developed in Viet Nam

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Objective To evaluate a killed oral cholera vaccine produced in Viet Nam, and to compare the Vietnamese vaccine with one that is licensed internationally.

Method Two-dose regimens of a locally produced, bivalent, anti-O1, anti-O139 killed oral whole-cell cholera vaccine (biv-WC) and of a commercially available, monovalent (anti-O1) oral recombinant B subunit-killed whole-cell cholera vaccine (rBS-WC) were compared in two trials in Viet Nam. In the first trial, 144 adults were randomized to biv-WC with or without buffer, rBS-WC with buffer, or placebo without buffer. In the second, 103 children aged 1–12 years were randomized to biv-WC without buffer, rBS-WC with buffer, or placebo without buffer.

Findings No regimen was associated with significant side-effects. In adults, ca 60% of recipients of either vaccine exhibited at least fourfold serum anti-O1 vibriocidal antibody responses and ca 40% of recipients of biv-WC demonstrated anti-O139 vibriocidal responses. Both anti-O1 (ca 90% in each vaccine groupand anti-O139 (68% in the biv-WC group) vibriocidal responses occurred more frequently in children. The responses to biv-WC were unaffected by the receipt of buffer.

Conclusion It was concluded that biv-WC was safe and immunogenic, that it could be administered without buffer, and that it could elicit robust immune responses even in children, for whom the risk of endemic cholera is highest.

Keywords Cholera vaccines/immunology/adverse effects; Vaccines, Inactivated/immunology/adverse effects; Clinical trials; Comparative study; Viet Nam (*source: MeSH, NLM*).

Mots clés Vaccins anticholériques/immunologie/effets indésirables; Vaccin inactivé/immunologie/effets indésirables; Essai clinique; Etude comparative; Viet Nam (*source: MeSH, INSERM*).

Palabras clave Vacunas contra el cólera/inmunología/efectos adversos; Vacunas inactivadas/inmunología/efectos adversos; Ensayos clínicos; Estudio comparativo; Viet Nam (*fuente: DeCS, BIREME*).

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Introduction

Work began in Viet Nam during the mid-1980s on the production of a killed oral cholera whole-cell vaccine that could be used in the country's public health programmes (*1*). A two-dose regimen of a first-generation vaccine against *Vibrio cholerae* O1, produced at US$ 0.10 per dose, conferred over 60% protection against El Tor cholera in both children and adults in a large-scale field trial (*2*). Because of the emergence of the new form of epidemic cholera caused by the O139 serogroup (*3*, *4*), this killed oral whole-cell vaccine has been augmented with killed O139 whole cells to create a bivalent killed whole-cell vaccine (biv-WC).

We report the findings of two trials of this vaccine in Vietnamese adults and children. Its safety and immunogenicity were compared with those of a Swedish monovalent (anti-O1), recombinant B subunit-killed whole-cell (rBS-WC) oral cholera vaccine that has been licensed for use in several European countries.

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Methods

Two randomized trials of biv-WC vaccine were conducted, one in adults and the other in children. The protocols for the trials were approved by review boards of the National Institute of Hygiene and Epidemiology in Viet Nam, the World Health Organization, the University of Gothenburg, and the National Institute of Child Health and Human Development in the USA. Written informed consent was obtained from subjects or their parents or guardians before participation, in accordance with the human experimentation guidelines of the United States Department of Health and Human Services.

Vaccines and placebo

Each dose of locally produced biv-WC vaccine consisted of the following: 2.5 x 10^{10} heat-killed *V. cholerae* O1 Inaba, classical biotype cells (strain Cairo 48); 2.5 x 10^{10} heat-killed *V. cholerae* O1 Ogawa, classical biotype cells (strain Cairo 50); 5 x 10^{10} formalin-killed *V. cholerae* 01 Inaba, El Tor biotype cells (strain Phil 6973); 2.5 x 10^{10} formalin-killed *V. cholerae* O1 Inaba, classical biotype cells (strain 569B); and 5 x 10^{10} formalin-killed *V. cholerae* O139 (strain AI4456). The locally produced placebo consisted of a heat-killed *Escherichia coli* K12 strain and had the same optical density as the vaccine.

The rBS-WC oral cholera vaccine, produced by SBL Vaccine AB and licensed in several European countries, was included as a comparison agent in these studies because it had been reported as free of side-effects and as inducing substantial protection against cholera (*5–9*). Each dose contained 1 mg recombinant cholera toxin B subunit, together with the same O1 serogroup cellular constituents as biv-WC vaccine except that there were half as many formalin-killed *V. cholerae* 01 Inaba, El Tor biotype, strain Phil 6973 cells (2.5 x 10^{10}), and 2.5 x 10^{10} formalin-killed *V. cholerae* O1 Ogawa, classical biotype strain Cairo 50 cells instead of 2.5 x 10^{10} formalin-killed *V. cholerae* O1 Inaba, classical biotype strain 569B cells. The latter change had been made to ensure expression of the fimbrial colonization factor antigen, TCP, which was undetectable in the SBL vaccine (Jan Holmgren, unpublished data).

The two vaccines and the placebo were packaged as liquid formulations in identical vials containing five 1.5-ml doses. Each agent was stored at 4–8 °C before administration, and was given in two doses separated by an interval of two weeks. The same dose of each agent was given to all age groups.

Study on adults

For the study on adults we recruited Hanoi residents aged 17–25 years. We excluded subjects from the first dose if they had had diarrhoea during the preceding week, chronic or recurrent abdominal pain, or chronic or recurrent diarrhoea; if they were pregnant; if they were taking steroid or other immunosuppressive medications; if they were taking antibiotics; or if they had AIDS or another immunosuppressive condition. No symptoms observed after the first dose were judged sufficient to preclude the receipt of a second dose.

Consenting eligible subjects in blocks of eight were randomly allocated to the following groups, which were of equal size: 1) biv-WC without buffer; 2) biv-WC with buffer; 3) rBS-WC with buffer; or 4) placebo without buffer. The vials with the agent for each group were labelled with one of two code letters, i.e. there were eight code letters for the four groups. Groups 2 and 3 received 1.5 ml vaccine mixed with 150 ml buffer (2.66g sodium bicarbonate per 100 ml. water: Samarin, Cedaerroths Nordic AB, Upplands, Vasby, Sweden). Groups 1 and 4 were given 1.5 ml vaccine by oral syringe, followed by water ad lib. The codes were kept secret from all persons involved in the study until freezing of the data set. The study was thus double-blind with respect to the allocation of rBS-WC vs. biv-WC with buffer and biv-WC vs. placebo without buffer but not with respect to the allocation of buffer.

Of the 206 subjects asked to participate, 33 refused and 30 were judged ineligible, leaving 143 subjects who received a first dose. One subject refused a second dose.

Study on children

The adult study having revealed no important side-effects, we initiated a trial in children aged 1–12 years who attended an elementary school or a day care centre in Hanoi. The eligibility criteria were identical to those applied in the study on adults. No clinical event observed after the first dose was judged sufficient to preclude the receipt of a second dose.

Because pre-enrolment screening revealed problems in recruiting potential subjects, we decided to study only three of the four cell types included in the adult trial: 1) biv-WC without buffer; 2) rBS-WC with buffer; and 3) placebo without buffer. Groups 1 and 3 were given 1.5 ml biv-WC and placebo respectively by oral syringe, followed by water ad lib. Group 2 received 1.5 ml rBS-WC mixed with 40 ml of the buffer solution used in the adult study for children under 60 months of age or with 80 ml for children aged 60 months or over.

Consenting eligible participants were randomly allocated to three groups of equal size in blocks of six individuals. Separate blocking schemes were used in the day care centre and in the school. The vials with the agent for each group were labelled with one of two code letters, i.e. there were six codes for the three groups. The codes differed from those used in the adult study. The codes were not broken until freezing of the data set. The study was thus double-blind with respect to the allocation of biv-WC vs. placebo without buffer but not with respect to the allocation of rBS-WC. Among the 165 children whose parents or guardians were approached, 107 agreed to participate in the trial and the 103 who were eligible received a first dose. Ninety-seven of these children received a second dose but one of them was erroneously given an agent different from that assigned and was therefore excluded from the analyses of side-effects after the second dose and of immune responses.

Surveillance for adverse events

Subjects in both studies were observed for 30 minutes after each dose and were visited daily for three consecutive days to ascertain symptoms, using a structured symptom questionnaire administered by physicians who were unaware of the code letter of the agent given. Interviews were also conducted two weeks after each dose in order to obtain information about any intervening illnesses requiring medical care.

The axillary temperature was measured in subjects complaining of subjective fever during the preceding 24 hours. Objective fever was defined as existing if the temperature was 37 °C or above; diarrhoea was defined as occurring if there were three or more loose or liquid motions in a 24-hour period;

dysentery was defined as occurring if there was at least one loose or liquid motion with blood in a 24-hour period; and a severe symptom was defined as one requiring bed rest for at least one hour or prompting a request for medical treatment.

Serological surveillance

Blood samples were obtained by venipuncture on the day of the first dose and two weeks after the second dose. Of the subjects who received two doses, 141 adults and 91 children consented to give a second sample. The blood samples were kept on ice until arrival in the laboratory within four hours of collection, where the sera were separated and stored at –20 °C until testing was performed. Paired sera from different subjects were tested immunologically in random order by technicians at the University of Gothenburg who were unaware of the identities of the agents received by the subjects.

Serum vibriocidal antibodies to *V. cholerae* O1 (El Tor Inaba; strain T19479) were evaluated by a microtitre assay (*10*). In order to measure vibriocidal antibodies to *V. cholerae* O139, O139 vibrios of the partly encapsulated vaccine strain 4260B and the capsule-deficient mutant strain M010-T4, provided by the Virus Research Institute, Cambridge, Massachusetts, were used in a final concentration of 2.5 x 10^5 bacteria ml^{-1}. Fresh guinea pig complement was added in a fourfold less diluted concentration (1:7.5) than in the O1 vibriocidal assay. The vibriocidal titre was defined as the highest dilution causing complete inhibition of bacterial growth. Twofold serial dilutions of prevaccination and postvaccination specimens were tested side-by-side in duplicates. The titres were adjusted in relation to a reference serum specimen included in each test to compensate for variations between analyses on different occasions. The antibody titre ascribed to each sample was the mean of the duplicated determinations, which were not allowed to vary by more than one twofold dilution for either the reference or the test sera. When larger variations occurred the tests were repeated. A fourfold or greater increase in titre between prevaccination and postvaccination sera was taken as indicating seroconversion.

Sample size

In order to anticipate a placebo-controlled effectiveness trial of biv-WC vaccine, sample sizes for each study were calculated to meet two criteria. Firstly, we wanted to be able to evaluate whether the occurrence of diarrhoeal side-effects in the biv-WC and placebo groups was equivalent to that for the rBS-WC group, since rBS-WC had been reported as non-reactogenic (*5*, *8*). Using formulae for non-inferiority studies (*11*), we assumed that the background three-day risk of diarrhoea in this population was 5% after each dose. In order to exclude the possibility, with $\geqslant 0.8$ power and $P < 0.05$ (1-tailed), that the biv-WC vaccine or the placebo imparted a true risk of diarrhoea $\geqslant 15\%$ higher than that for the rBS-WC group, 27 analysable subjects per group were needed.

Secondly, we wished to exclude the possibility that serum anti-O1 vibriocidal responses to the biv-WC vaccine were unacceptably low in relation to those to the rBS-WC vaccine. We assumed that 50% of two-dose recipients of rBS-WC would respond with $\geqslant$4-fold rises in antibody titres. In order to exclude a seroconversion rate in the biv-WC group that was $\geqslant 30\%$ lower than that for the rBS-WC group, at $P < 0.05$ (1-tailed) with $\geqslant 0.8$ power, 30 analysable subjects per group were needed.

In order to meet both sample size objectives, therefore, a minimum of 30 analysable subjects per group had to be assembled.

Analyses

Two-group contrasts of dichotomous outcomes were evaluated by means of the chi-square test or, where the data were sparse, by means of the Fisher exact test. Contrasts of dimensional outcomes were appraised by means of Student's t test or, when parametric assumptions were not fulfilled, with the Mann-Whitney U test. Because of skewed distributions of reciprocal titres of serum antibodies, as well as of fold-rises in these titres, we transformed the titres and fold-rises to logarithms before statistical analysis. Analysis of covariance was used to adjust intergroup contrasts of fold-rises in titres for imbalances in baseline titres.

The two principal objectives of these trials were to assess whether the risk of diarrhoea was similar in the biv-WC and placebo groups on the one hand and in the rBS-WC group on the other, and whether anti-O1 vibriocidal responses to biv-WC were similar to those after rBS-WC administration. We evaluated each of these two-group contrasts with $P < 0.05$ (one-tailed), since we were only interested in knowing whether the biv-WC and placebo agents were less safe than rBS-WC and whether biv-WC was less immunogenic than rBS-WC. All other contrasts were evaluated at $P < 0.05$ (two-tailed). The 95% confidence intervals for these differences, calculated with test-based methods (*12*) or, when data were sparse, with the profile likelihood method (*13*), were estimated

Table 1. **Demographic features of subjects who received dose 1 and dose 2**

Age group, dose and feature	Group[a]			
	biv-WC/H_2O	biv-WC/B	rBS-WC	Placebo
Adults				
Dose 1	***n* = 36**	***n* = 36**	***n* = 35**	***n* = 36**
Male	24 (67%)	25 (69%)	28 (80%)	28 (78%)
Mean age (years)	21.2 (±1.5)[b]	20.7 (±2.0)	20.9 (±1.5)	20.8 (±1.5)
Dose 2	***n* = 36**	***n* = 35**	***n* = 35**	***n* = 36**
Male	24 (67%)	25 (71%)	28 (80%)	28 (78%)
Mean age (years)	21.2 (±1.5)	20.8 (±2.0)	20.9 (±1.5)	20.8 (±1.5)
Children				
Dose 1	***n* = 33**	–	***n* = 36**	***n* = 34**
Male	17 (52%)	–	22 (61%)	13 (38%)
Mean age (years)	7.9 (±3.3)	–	7.9 (±3.1)	7.9 (±3.4)
Dose 2	***n* = 29**	–	***n* = 35**	***n* = 32[c]**
Male	17 (59%)	–	22 (63%)	13 (41%)
Mean age (years)	8.3 (±3.2)	–	8.0 (±3.0)	7.7 (±3.4)

[a] biv-WC/H_2O = locally produced, killed, bivalent whole-cell vaccine, administered with water; biv-WC/B = locally produced, killed, bivalent whole-cell vaccine, administered with buffer; Placebo = locally produced, killed *E. coli* K12 placebo administered with water; rBS-WC = recombinant B subunit-killed whole-cell vaccine, administered with buffer.

[b] (± standard deviation).

[c] One subject, who was assigned to the placebo group, took placebo for the first dose but erroneously took a second dose of a different agent (biv-WC/H_2O). This subject is excluded from the second-dose analysis.

in a one-tailed or two-tailed fashion according to these rules.

Results

The groups in both studies revealed no significant differences in age and sex distributions (Table 1). No adverse event occurred significantly more frequently in the biv-WC and placebo groups than in the rBS-WC group (Table 2). Moreover, 95% confidence intervals for the contrasts of the occurrence of diarrhoea in the biv-WC and placebo groups versus the rBS-WC group excluded a greater than 10% higher occurrence in the former. No severe adverse events were detected that might have been attributable to an administered agent.

In the adult study, geometric mean-fold (GMF) rises and fourfold or larger rises in reciprocal titres of anti-O1 serum vibriocidal antibodies (Table 3) were similar for recipients of biv-WC irrespective of whether buffer was coadministered (GMF rises of 5.2-fold and 5.6-fold, and ⩾4-fold rises in 57% and 54% with and without buffer, respectively). Similar responses were also seen in recipients of rBS-WC (GMF rise of 5.4-fold, and ⩾4-fold rise in 63% of the group). In the study on children, GMF and ⩾4-fold rises in anti-O1 titres were greater than those in adults, and were similar for the biv-WC and rBS-WC groups (GMF rises of 17.5-fold and 22.6-fold, and ⩾4-fold rises in 93% and 88% of the respective groups).

Analyses of anti-O1 responses were unaffected by analytical adjustment for intergroup imbalances in baseline titres. The 95% confidence intervals for comparisons of ⩾4-fold responses in the biv-WC versus the rBS-WC groups excluded more than a 29% lower response rate in the biv-WC group of the adult study and more than a 10% lower response rate in the biv-WC group of the child study.

In adults, serum GMF anti-O139 responses in the two biv-WC groups ranged from 1.4-fold to 2.4-fold for the two test organisms and were not affected by the coadministration of buffer. Between 17% and 31% of subjects receiving this vaccine gave ⩾4-fold responses. Analysis of the maximum of responses to either test organism for each subject revealed somewhat higher values (3.1–3.4 GMF rises and 40–43% ⩾4-fold responses).

Like anti-O1 responses, anti-O139 responses to the biv-WC vaccine were greater in children than in adults (3.0–5.2 GMF rises and 40–56% ⩾4-fold rises to the two test organisms, and 7.8-fold GMF rise and 68% ⩾4-fold rise when analyses were restricted to the maximum of the responses to either test organism in each subject).

The analyses of anti-O139 responses in adults and children remained similar after adjustment for intergroup imbalances in baseline titres.

Discussion

A two-dose regimen of the biv-WC was well tolerated in adults and children, and induced appreciable serum anti-O1 vibriocidal responses in both age groups. These responses were not affected by the coadministration of buffer and were similar to those seen after receipt of the licensed rBS-WC vaccine. Serum anti-O139 responses to biv-WC were smaller.

Table 2. **Occurrence of symptoms after receipt of first and second doses in studies on adults and children**

Dose and symptom	Adults study[a]				Children study[a]		
	biv-WC/H_2O	biv-WC/B	rBS-WC	Placebo	biv-WC/H_2O	rBS-WC	Placebo
Dose 1[b]	***n* = 36**	***n* = 36**	***n* = 35**	***n* = 36**	***n* = 33**	***n* = 36**	***n* = 34**
Subjective fever	2 (6%)[c]	1 (3%)	0	0	0	0	0
Confirmed fever	0	0	0	0	0	0	0
Diarrhoea	0 [8%][d]	0 [8%]	0	0 [8%]	0 [7%]	1 (3%)	0 [7%]
Abdominal pain	3 (8%)	1 (3%)	3 (9%)	3 (8%)	0	1 (3%)	2 (6%)
Loss of appetite	2 (6%)	1 (3%)	3 (9%)	4 (11%)	0	0	0
Nausea	3 (8%)	1 (3%)	1 (3%)	0	–	–	–
Vomiting	0	0	0	0	0	0	0
Any severe symptom	0	0	0	0	0	0	0
Dose 2[b]	***n* = 36**	***n* = 35**	***n* = 35**	***n* = 36**	***n* = 29**	***n* = 35**	***n* = 32[e]**
Subjective fever	0	0	0	0	0	0	0
Confirmed fever	0	0	0	0	0	0	0
Diarrhoea	0 [8%]	0 [8%]	0	0 [9%]	0 [10%]	0	0 [9%]
Abdominal pain	0	3 (9%)	3 (9%)	2 (6%)	0	0	0
Loss of appetite	0	0	3 (9%)	0	0	0	0
Nausea	0	0	0	1 (3%)	–	–	–
Vomiting	0	0	0	0	0	0	0
Any severe symptom	0	0	0	0	1 (3%)[f]	1 (3%)[f]	0

[a] biv-WC/H_2O = locally produced, bivalent whole-cell vaccine, administered with water; biv-WC/B = locally produced, killed, bivalent whole-cell vaccine, administered with buffer; Placebo = locally produced, killed *E. coli* K12 placebo administered with water; rBS-WC = recombinant B subunit-killed whole-cell vaccine, administered with buffer.

[b] Nausea was not assessed in the children study.

[c] Values in parentheses represent percentage of group total.

[d] Values in brackets represent the upper boundaries of the one-tailed, 95% confidence intervals for contrast of the cited group with the group receiving rBS-WC.

[e] One subject who was assigned to the placebo group and took placebo for the first dose, but erroneously took a second dose of a different agent, is excluded from the second-dose analysis.

[f] Subjects had symptoms judged compatible with bacterial infections (one pharyngitis, one conjunctivitis), unrelated to vaccination.

Table 3. **Serum vibriocidal antibody titres to 01 serogroup at baseline and two weeks after second dose among subjects with paired blood specimens**[a]

	Adults study[b]				Children study[b]		
	biv-WC/H_2O *n* = 35	biv-WC/B *n* = 35	rBS-WC *n* = 35	Placebo *n* = 36	biv-WC/H_2O *n* = 27	rBS-WC *n* = 33	Placebo *n* = 31
GMT[c]							
Bleed 1	11.2	13.1	12.0	11.8	10.3	6.6[d]	14.2
Bleed 2	62.6[e]	69.1[e]	64.7[e]	11.1	179.5[e]	149.9[e]	12.2
GMF-rise[f]	5.6[e]	5.2[e]	5.4[e]	0.9	17.5[e]	22.6[e]	0.9
⩾4-fold rise[g]	19[e] (54%)	20[e] (57%)	22[e] (63%)	0	25[e] (93%)	29[e] (88%)	1 (3%)
95% CI lower boundary[h]	–29%	–26%	–	–	–10%	–	–

[a] Numbers of subjects cited in this table are slightly lower than those noted as having received a second dose in Table 1 because of refusals to accept second bleed.
[b] biv-WC/H_2O = locally produced, killed, bivalent whole-cell vaccine, administered with water; biv-WC/B = locally produced, killed, bivalent whole-cell vaccine, administered with buffer; Placebo = locally produced, killed *E. coli* K12 placebo administered with water; rBS-WC = recombinant B subunit-killed whole-cell vaccine, administered with buffer.
[c] Geometric mean reciprocal titre for cited bleed.
[d] $P < 0.05$ (2-tailed) for contrast of cited group with placebo group.
[e] $P < 0.001$ (2-tailed) for contrast of cited group with placebo group.
[f] Geometric mean-fold rise in titre between baseline and second bleed.
[g] Number and (% of group) achieving a ⩾4-fold rise in titre between baseline and second bleed.
[h] Lower boundaries of the one-tailed, 95% confidence intervals for the percentage achieving a ⩾4-fold rise in titre in cited group minus that for group receiving rBS-WC. See text for explanation.

Table 4. **Serum vibriocidal antibody titres to 0139 serogroup at baseline and two weeks after second dose among subjects with paired blood specimens**[a]

Test organism, bleed, titre	Adults study[b]				Children study[b]		
	biv-WC/H_2O *n* = 35	biv-WC/B *n* = 35	rBS-WC *n* = 34	Placebo *n* = 36	biv-WC/H_2O *n* = 25	rBS-WC *n* = 32	Placebo *n* = 30
4260B[c]							
GMT[d]							
Bleed 1	12.0	11.9	15.0	14.7	11.1	8.8	12.3
Bleed 2	23.0	17.0	15.0	16.5	58.6[e]	11.9	16.3
GMF-rise[f]	1.9[g]	1.4	1.0	1.1	5.2[e]	1.4	1.3
⩾4-fold rise[h]	11[e] (31%)	6 (17%)	1 (3%)	2 (6%)	14[i] (56%)	4 (13%)	3 (10%)
M010-T4							
GMT[d]							
Bleed 1	77.8	35.7	79.1	68.8	66.1	48.0	43.7
Bleed 2	164.1	84.1	84.7	75.6	198.0[e]	66.5	53.5
GMF-rise[f]	2.1[e]	2.4[e]	1.1	1.1	3.0[e]	1.4	1.2
⩾4-fold rise[h]	7 (20%)	10[g] (29%)	0	2 (6%)	10[e] (40%)	4 (13%)	3 (10%)
Maximum of 4260B and M010-T4[j]							
GMF-rise[f]	3.4[i]	3.1[i]	1.2	1.4	7.8[i]	1.8	1.9
⩾4-fold	15[e] (43%)	14[e] (40%)	1 (3%)	4 (11%)	17[i] (68%)	6 (19%)	4 (13%)

[a] Numbers of subjects cited in this table are slightly lower than those noted in Table 3 because thee were insufficient sera for 0139 assays.
[b] biv-WC/H_2O = locally produced, killed, bivalent whole-cell vaccine, administered with water; biv-WC/B locally produced, killed, bivalent whole-cell vaccine, administered with buffer; Placebo = locally produced, killed *E. coli* K12 placebo administered with water; rBS-WC = recombinant B subunit-killed whole-cell vaccine, administered with buffer.
[c] Test strain used (see text).
[d] Geometric mean reciprocal titre for cited bleed.
[e] $P < 0.01$ (2-tailed) for contrast of cited group with placebo group.
[f] Geometric mean-fold rise in titre between baseline and second bleed.
[g] $P < 0.05$ (2-tailed) for contrast of cited group with placebo group.
[h] Number and (% of group) achieving a ⩾4-fold rise in titre between baseline and second bleed.
[i] $p < 0.001$ (2-tailed) for contrast of cited group with placebo group.
[j] In analyses of maximum GMF-fold rises, the larger of fold-rises in titre to the 4260B or the M010-T4 test strains was taken as the value for each subject; in analyses of ⩾ 4-fold rises a subject was considered to have experienced such a rise if a ⩾4-fold increase in titre occurred to either test strain.

The adult trial was double-blind only in respect of the allocation of biv-WC versus rBS-WC with buffer and biv-WC versus placebo without buffer, while the trial on children was double-blind in respect of the allocation of biv-WC versus placebo but not with respect to that of rBS-WC. In view of the infrequency of dropouts after randomization, the blinding of interviewers to the codes of received agents when assessing side-effects, and the blinding of laboratory technicians to these codes when performing vibriocidal assays, it is unlikely that the absence of full double-blinding diminishes the validity of our conclusions. It is reassuring that in each study the occurrence of postdosing symptoms was similar not only for groups for which the comparison was double-blind but also for groups for which it was not fully blind (Table 2).

Serum anti-O1 and anti-O139 vibriocidal responses to the biv-WC vaccine were similar, regardless of whether buffer was coadministered with the vaccine. These results probably reflect the acid-insensitivity of *V. cholerae* lipopolysaccharide, which is thought to be responsible for eliciting vibriocidal antibodies (*14*), and suggest that the expense and logistical complexity of delivering biv-WC vaccine can be reduced by giving it without buffer.

The magnitude of anti-O1 vibriocidal responses was similar for groups receiving the bivalent biv-WC vaccine and the monovalent rBS-WC vaccine. Although the O1 whole-cell constituents of the biv-WC and rBS-WC vaccines were not completely identical, these findings support the notion that the addition of O139 killed cells to a killed oral anti-O1 whole-cell vaccine does not result in interference with anti-O1 immune responses (*15*).

It is noteworthy that the anti-O1 responses to the biv-WC and rBS-WC vaccines, ca fivefold in adults and ca twentyfold in children, were appreciably greater than the responses observed in past studies after the administration of rBS-WC vaccine (or BS-WC vaccine, its predecessor with non-recombinant BS but with identical whole cells) in developing countries, which ranged from twofold in Bangladesh to 2.6-fold in Peru (*5*, *16*). Greater serum vibriocidal responses have also been observed in studies of recent lots of rBS-WC in adult European volunteers (*15*) as compared with studies of older lots. Interestingly, the greater vibriocidal responses to more recent lots correlate with an almost twofold higher lipopolysaccharide content of these lots because of different methods of standardization (Jan Holmgren, unpublished data). Conversely, the relatively modest anti-O139 responses to the biv-WC vaccine may have resulted from a suboptimal number of 0139 cells in this vaccine.

Greater anti-O1 and anti-O139 vibriocidal vaccine responses were observed in children than in adults. Although the explanation for these age-related differences is not known, the robust responses seen in children may help to explain the high protection of young children by an earlier monovalent version of the Vietnamese oral vaccine (*2*).

The safety and immunogenicity of the biv-WC vaccine, especially its ability to elicit robust anti-O1 serum vibriocidal responses, are encouraging, particularly because of its low cost of production (ca US$ 0.20 per dose) in Viet Nam. Further studies are needed in order to evaluate the clinical protection it confers and to explore its modification with a view to improving anti-O139 vibriocidal responses. ■

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Résumé

Recherches sur l'innocuité et l'immunogénicité d'un vaccin anticholérique oral tué développé au Viet Nam

Objectif Comparer un vaccin anticholérique de production locale avec un vaccin disponible dans le commerce.

Méthodes Lors de deux essais réalisés au Viet Nam, on a comparé un schéma en deux doses d'un vaccin anticholérique oral tué à germes entiers, bivalent anti-O1, anti-O139 de production locale (biv-WC) et d'un vaccin anticholérique oral contenant la sous-unité B recombinante et des germes entiers tués, monovalent anti-O1, disponible dans le commerce (rBS-WC). Dans le premier essai, 144 adultes ont reçu après tirage au sort le vaccin biv-WC avec ou sans tampon, le vaccin rBS-WC avec tampon, ou un placebo sans tampon. Dans le deuxième essai, 103 enfants de 1 à 12 ans ont reçu après tirage au sort le vaccin biv-WC sans tampon, le vaccin rBS-WC avec tampon, ou un placebo sans tampon.

Résultats Aucun schéma n'a été associé à des effets secondaires significatifs. Chez les adultes, environ 60 % des sujets vaccinés par l'un ou l'autre produit ont présenté au moins un quadruplement des anticorps vibriocides anti-O1 et environ 40 % des sujets ayant reçu le vaccin biv-WC ont présenté une réponse vibriocide anti-O139. La réponse au vaccin biv-WC n'était pas modifiée par la présence d'un tampon. Les réponses en anticorps vibriocides anti-O1 (environ 90 % dans chaque groupe) et anti-O139 (68 % dans le groupe biv-WC) étaient observées plus fréquemment chez les enfants que chez les adultes.

Conclusion Il a été conclu que le vaccin biv-WC était efficace et sans danger, qu'il pouvait être administré sans tampon et qu'il pouvait induire une forte réponse immunitaire même chez les enfants pour lesquels le risque de choléra endémique est le plus élevé.

Resumen

Investigación de la inocuidad e inmunogenicidad de una vacuna anticolérica oral inactivada desarrollada en Viet Nam

Objetivo Comparar una vacuna anticolérica producida a nivel local con otra vacuna comercial.

Métodos Mediante dos ensayos llevados a cabo en Viet Nam, se procedió a comparar los efectos de regímenes de dos dosis de una vacuna (local) anticolérica oral inactivada de células enteras de tipo bivalente contra las cepas O1 y O139 (biv-WC) con los de otra vacuna (comercial) anticolérica oral inactivada de células enteras de tipo monovalente basada en la subunidad B recombinante (anti-O1) (rBS-WC). En el primer ensayo se distribuyó aleatoriamente a 144 adultos para que recibieran ya fuese biv-WC con o sin tampón, rBS-WC con tampón, o placebo sin tampón. En el segundo se designó aleatoriamente a 103 niños de 1 a 12 años para que recibieran ya fuera biv-WC sin tampón, rBS-WC con tampón o placebo sin tampón.

Resultados Ninguno de los regímenes tuvo efectos secundarios importantes. En los adultos, aproximadamente un 60% de los receptores de cualquiera de las vacunas mostró como mínimo una cuadruplicación de la producción de anticuerpos vibriocidas anti-O1 en el suero, y un 40% de los receptores de la biv-WC mostró respuestas vibriocidas anti-O139. Las respuestas a la biv-WC no se vieron afectadas por la recepción de tampón. Las respuestas vibriocidas, tanto anti-O1 (un 90% en cada grupo) como anti-O139 (68% en el grupo tratado con biv-WC), fueron más frecuentes en los niños que en los adultos.

Conclusión La vacuna biv-WC es segura e inmunogénica, se puede administrar sin tampón, y puede inducir respuestas inmunitarias robustas incluso en los niños, que presentan un mayor riesgo de cólera endémico.

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