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Bivalent Cholera and Typhoid Vaccine

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Contents

Abstract

▲ The live attenuated vaccine strains *Vibrio cholerae* CVD 103-HgR and *Salmonella typhi* Ty21a can be combined into an oral bivalent vaccine without compromising the immunogenicity of the individual vaccine strains.

▲ Seroconversion rates of 87 to 94% for Inaba vibriocidal antibodies and 72 to 91% for anti-*S. typhi* lipopolysaccharide antibodies (IgG or IgA) were reported in healthy European volunteers receiving a bivalent CVD 103-HgR/Ty21a vaccine-based schedule (bivalent vaccine on day 1 and monovalent Ty21a vaccine on days 3 and 5).

▲ The immunogenicity of bivalent CVD 103-HgR/Ty21a vaccine is not adversely affected by concomitant administration of mefloquine, yellow fever vaccine or oral polio vaccine. Chloroquine may reduce the immunogenicity of the CVD 103-HgR component and proguanil may reduce the immunogenicity of the Ty21a component. Bivalent CVD 103-HgR/Ty21a vaccine does not adversely affect the immunogenicity of the yellow fever YF 17D vaccine.

▲ The type, incidence and severity of adverse events seen in individuals receiving bivalent CVD 103-HgR/Ty21a vaccine-based schedules are similar to those that occur with the monovalent vaccines.

Features and properties of bivalent cholera and typhoid vaccine (CVD 103-HgR/Ty21a)	
Indication	
Prevention of cholera and typhoid fever	
Mechanism of action	
Bivalent live oral attenuated vaccine	
Dosage and administration	
Vaccine content (per dose)	2×10^8 to 6×10^8 colony-forming units (CFU) of CVD 103-HgR + 2×10^9 to 6×10^9 CFU of Ty21a
Route of administration	Oral
Vaccination schedule	Bivalent vaccine on day 1, monovalent Ty21a vaccine on days 3 and day 5
Adverse events	
Most frequent	Diarrhoea, nausea, abdominal discomfort

Cholera and typhoid fever remain endemic in many parts of the world, particularly in developing countries. Furthermore, typhoid and paratyphoid organisms can cause aggressive opportunistic infections in HIV-infected patients. Multidrug-resistant isolates of both *Vibrio cholerae* and *Salmonella typhi* are now emerging, making treatment of such infections problematic.[1,2]

Live oral attenuated vaccines for both cholera and typhoid have been developed and are currently in use, including the *V. cholerae* CVD 103-HgR vaccine and the *S. typhi* Ty21a vaccine. Used individually in liquid formulations, these vaccines have shown good tolerability, immunogenicity and protective efficacy.[1,3,4] The vaccines induce strong mucosal and systemic immunity after oral ingestion, and long term local immunological memory is elicited.[5] Ty21a, but not CVD 103-HgR, induces cell-mediated immunity.[5]

Because cholera and typhoid are often coendemic, combination of these vaccines in a bivalent formulation is now being investigated.

1. Vaccine Production

- CVD 103-HgR is a live attenuated vaccine prepared from *V. cholerae* O1 Classical Inaba strain 569B. Recombinant DNA technology was used to delete 94% of the gene encoding the enzymatically active A subunit toxin of the wild-type strain.[3]

- Ty21a is a live attenuated vaccine derived from the *S. typhi* Ty strain. It has a mutation within the *galE* gene and other genes. Consequently, the vaccine strain lacks activity of the gene product uridine diphosphate galactose-4-epimerase and synthesises incomplete lipopolysaccharide.[1,4]

- For use as a bivalent vaccine, the CVD 103-HgR and Ty21a vaccine strains are lyophilised and then mixed.[2] The bivalent vaccine is reconstituted to a liquid solution with water and buffer (2.5g of sodium bicarbonate and 1.65g of ascorbic acid) immediately before ingestion.

2. Immunogenicity

In clinical trials, each dose of bivalent vaccine contained approximately 2×10^8 to 6×10^8 colony-forming units (CFU) of CVD 103-HgR and 2×10^9 to 6×10^9 CFU of Ty21a.[2,6,7] Heat-killed *Escherichia coli* K-12 (5×10^8 CFU) was used as a placebo. Vaccination was conducted over a 5-day period with doses on days 1, 3 and 5. As vaccination against cholera requires only 1 dose of CVD 103-HgR but vaccination against typhoid requires 3 doses of Ty21a, the bivalent vaccine was given on 1 day and monovalent Ty21a vaccine was administered on the remaining 2 vaccination days.

All studies involved healthy adult volunteers aged ≥16 or ≥18 years residing in Austria.[2,6,7] Thus, study participants were unlikely to have been primed by prior natural exposure to cholera or typhoid pathogens.

Seroresponse to the cholera CVD 103-HgR component of the vaccine was measured by Inaba vibriocidal antibody titres using a microtitre plate assay; a ≥4-fold rise in antibody titre was considered to indicate seroconversion. Seroresponse to the typhoid Ty21a component of the vaccine was measured by anti-*S. typhi* lipopolysaccharide antibody titres using an enzyme-linked immunosorbent assay; an increase of ≥0.15 optical density units was considered to indicate seroconversion. Antibody responses were measured over 14 or 28 days.

- A vaccination schedule of bivalent CVD 103-HgR/Ty21a vaccine on day 1 and monovalent Ty21a vaccine on days 3 and 5 was compared with *E. coli* placebo vaccination in 325 evaluable volunteers (n = 260 and 65, respectively) in a randomised double-blind trial.[6] The seroconversion rate for Inaba vibriocidal antibodies was 94% in the bivalent vaccine group and 6% in the placebo vaccine group; individuals with a baseline antibody titre ≥80 were less likely to seroconvert than those with a lower baseline antibody level ($p < 0.05$, no further details reported). For anti-*S. typhi* antibodies (IgG or IgA), 91% of bivalent vaccine recipients seroconverted, whereas no placebo recipients seroconverted. Geometric mean antibody titres (GMTs) for bivalent vaccine recipients are shown in figure 1.

- The serological response to the heterologous

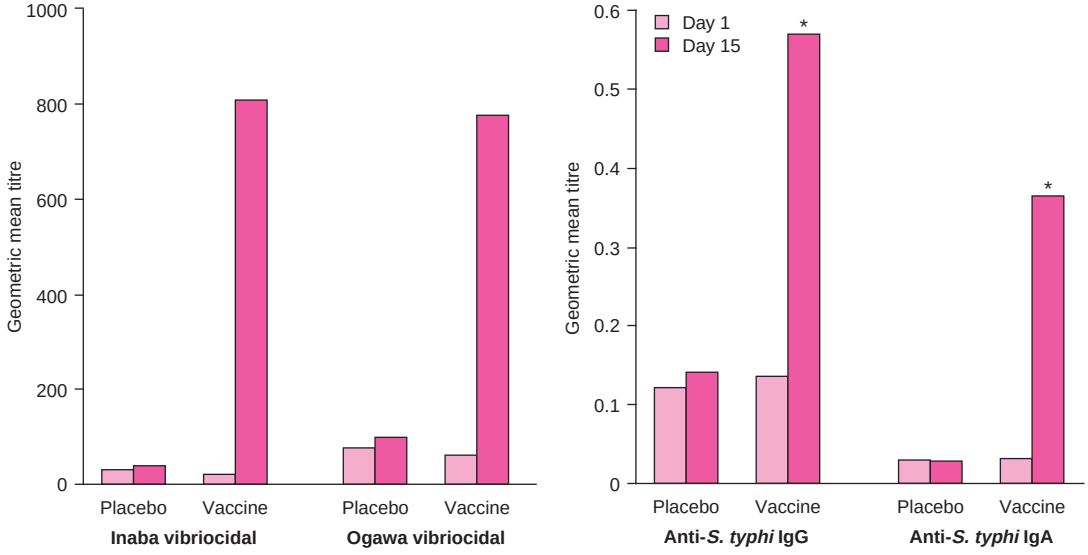

Fig. 1. Geometric mean antibody titre responses to oral bivalent cholera CVD 103-HgR **(left)** and typhoid Ty21a vaccine over time **(right)**.[6] 260 healthy volunteers received the bivalent vaccine on day 1 plus monovalent Ty21a vaccine on days 3 and 5. An additional 65 volunteers received 3 doses of placebo *Escherichia coli* vaccine. Serum Inaba vibriocidal antibody titres (homologous strain) and Ogawa vibriocidal antibody titres (heterologous strain) were measured by a microtitre plate assay. Anti-*Salmonella typhi* lipopolysaccharide antibody titres were measured by an enzyme-linked immunosorbent assay. * $p < 0.01$ *vs* baseline (statistical analysis of vibriocidal antibody data not provided).

V. cholerae strain Ogawa was also assessed in the above study.[6] The seroconversion rate was 80% in bivalent vaccine recipients versus 0% in placebo recipients. The relative increase from baseline in the Ogawa GMT was more modest than for the Inaba GMT (12-fold *vs* >40-fold at day 15), but this may have been affected by the higher baseline GMT for Ogawa (fig. 1).

• Bivalent CVD 103-HgR/Ty21a vaccine-based regimens produced similar immunological response rates to those for the corresponding monovalent vaccine regimens in 171 evaluable volunteers (fig. 2).[2] Volunteers were randomised to receive in a blinded fashion the bivalent vaccine on day 1 plus monovalent Ty21a vaccine on days 3 and 5 (first-dose bivalent regimen), monovalent Ty21a vaccine on days 1 and 3 and the bivalent vaccine on day 5 (last-dose bivalent regimen), monovalent CVD 103-HgR vaccine on day 1 plus *E. coli* placebo vaccine on days 3 and 5 or monovalent Ty21a vaccine on days 1, 3 and 5.

• The timing of the bivalent vaccine dose (i.e. as the first or the third dose) did not have a statistically significant effect on seroconversion rates in this study, although administration of the bivalent vaccine as the first dose appeared to produce the best Inaba vibriocidal antibody seroconversion rates (92.5 *vs* 78% with the last-dose bivalent regimen).[2] The seroconversion rate for *S. typhi* (IgG or IgA) was 72% with both regimens.

• Although seroconversion rates for Inaba vibriocidal antibodies were similar with the bivalent CVD 103-HgR/Ty21a and monovalent CVD 103-HgR vaccines in the latter study, the peak GMT was significantly higher in individuals who received the bivalent vaccine ($p < 0.005$).[2] Peak GMTs were 2549 with the first-dose bivalent regimen, 1389 with the last-dose bivalent regimen and

331 with monovalent CVD 103-HgR. The killed *E. coli* placebo may have reduced the magnitude of the immune response to CVD 103-HgR in the latter group; such an interaction has previously been reported.[8]

Effect of Concomitant Agents

- The effect of coadministration of antimalarial agents (chloroquine, mefloquine, proguanil) or oral polio or yellow fever vaccines on the immunogenicity of a bivalent CVD 103-HgR/Ty21a-based vaccine schedule (CVD 103-HgR/Ty21a on day 1, Ty21a on days 3 and 5) or monovalent CVD 103-HgR vaccine was investigated in 390 healthy volunteers in a randomised trial.[7]

- The Inaba vibriocidal antibody seroconversion rates when concomitant treatments were administered with the bivalent CVD 103-HgR/Ty21a-based regimen (77 to 97%) were not significantly different than after vaccination with the bivalent vaccine regimen alone (87%) [fig. 3].[7] However, the seroconversion rate was lowest when chloroquine (250mg on day 1 and 8) was administered (77%), and a more marked detrimental effect of chloroquine was evident with monovalent CVD 103-HgR vaccination (67%; p = 0.0008 *vs* monovalent CVD 103-HgR alone). It may be prudent to delay chloroquine administration for at least 8 days after vaccination, or use mefloquine instead, in individuals receiving CVD 103-HgR in a bivalent, as well as in a monovalent, vaccine.

- Proguanil (200 mg/day for 7 days) significantly reduced the seroconversion rate for anti-*S. typhi* antibodies (IgG or IgA) for the bivalent CVD 103-HgR/Ty21a-based regimen (50 *vs* 78% for vaccination in the absence of proguanil; p = 0.01) [fig. 3].[7] In individuals receiving a CVD 103-HgR/Ty21a-based vaccine schedule, proguanil treatment should be delayed for at least 3 days after the last dose of Ty21a (the maximum period for which Ty21a persists in the human gut lumen before undergoing lysis[9]). Other concomitant treatments (chloroquine, mefloquine, oral polio vaccine or yellow fever vaccine) administered with the bivalent vaccine did not have a significant effect on the seroconversion rate for anti-*S. typhi* antibodies (IgG or IgA) [fig. 3].[7]

- Further analysis of patients in the latter study indicated that bivalent CVD 103-HgR/Ty21a vaccine does not interfere with the antibody response to yellow fever vaccine YF 17D.[10] All 29 individuals receiving the CVD 103-HgR/Ty21a-based schedule and yellow fever vaccine developed yellow fever neutralising antibodies (measured in

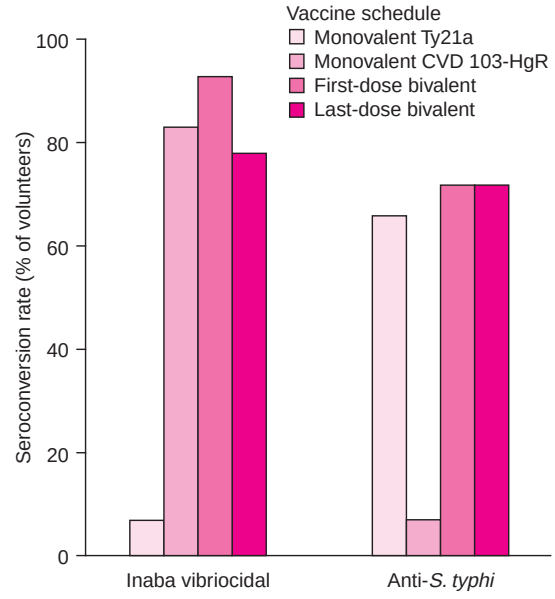

Fig. 2. Seroconversion rates after oral bivalent cholera CVD 103-HgR and typhoid Ty21a vaccine.[2] Healthy volunteers received bivalent CVD 103-HgR/Ty21a vaccine on day 1 plus monovalent Ty21a vaccine on days 3 and 5 (first-dose bivalent regimen; n = 53), monovalent Ty21a vaccine on days 1 and 3 and bivalent CVD 103-HgR/Ty21a vaccine on day 5 (last-dose bivalent regimen; n = 60), monovalent CVD 103-HgR vaccine on day 1 plus killed *Escherichia coli* placebo on days 3 and 5 (n = 29) or monovalent Ty21a vaccine on days 1, 3 and 5 (n = 29). The serum antibody response was measured over 28 days. Seroconversion was defined as a ≥4-fold rise in antibody titre for Inaba vibriocidal antibodies (microtitre plate assay) and as an increase of ≥0.15 optical density units for anti-*Salmonella typhi* lipopolysaccharide antibodies (IgG or IgA) [enzyme-linked immunosorbent assay].

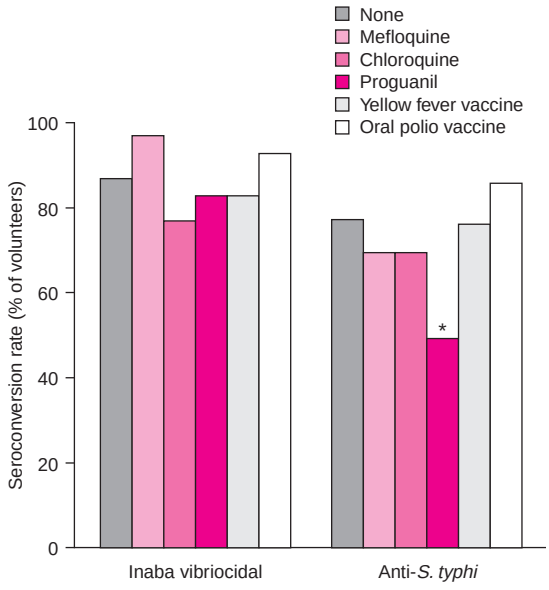

Fig. 3. Seroconversion rates with oral bivalent cholera CVD 103-HgR and typhoid Ty21a vaccine in the presence of concomitant agents.[7] Healthy volunteers received bivalent CVD 103-HgR/Ty21a vaccine on day 1 plus monovalent Ty21a vaccine on days 3 and 5 either alone (n = 45) or with oral mefloquine 250mg on day 1 and 8, oral chloroquine 250mg on day 1 and 8, oral proguanil 200 mg/day for 7 days, subcutaneous yellow fever vaccine 0.5ml on day 1 or oral trivalent polio vaccine 0.5ml on day 1 (n = 30 per group) [an additional 195 volunteers received monovalent CVD 103-HgR vaccine with or without concomitant agents; data not shown]. The serum antibody response was measured over 14 days. Seroconversion was defined as a ≥4-fold rise in antibody titre for Inaba vibriocidal antibodies (microtitre plate assay) and as an increase of ≥0.15 optical density units for anti-*Salmonella typhi* lipopolysaccharide antibodies (IgG or IgA) [enzyme-linked immunosorbent assay]. * p = 0.01 *vs* CVD 103-HgR/Ty21a-based regimen

plaque neutralisation tests). The GMT for yellow fever neutralising antibodies was 213; the study did not evaluate the administration of yellow fever vaccine alone so the relevant GMT in the absence of concomitant bivalent CVD 103-HgR/Ty21a vaccine was not available.

3. Tolerability

• The type, incidence and severity of adverse events seen in individuals receiving a vaccination schedule of bivalent CVD 103-HgR/Ty21a vaccine plus monovalent Ty21a vaccine were generally similar to those in individuals receiving monovalent Ty21a vaccine alone or monovalent CVD 103-HgR vaccine alone or with *E. coli* placebo vaccine.[2,7]

• The most common adverse events in study participants receiving a bivalent CVD 103-HgR/Ty21a-based vaccine schedule (1 dose CVD 103-HgR/Ty21a plus 2 doses Ty21a) included diarrhoea (in 10 to 31% of volunteers), nausea (5 to 16%) and abdominal discomfort (5 to 16%).[2,6,7] These adverse events did not occur significantly more often than with *E. coli* placebo vaccine, although the incidence of nausea and abdominal cramps tended to be higher in bivalent vaccine recipients (15 and 16% *vs* 9 and 9%, respectively).[6] Other commonly reported adverse events (e.g. headache and fatigue) in bivalent vaccine recipients generally occurred at least as commonly in volunteers receiving *E. coli* placebo or other vaccines (yellow fever or oral polio).[6,7] Rash, fever and vomiting occurred in ≤3% of bivalent vaccine recipients.[2,6,7]

• Most adverse reactions were mild and resolved spontaneously.[2,6,7] Gastrointestinal events may have been partly attributable to the sodium bicarbonate of the vaccine buffer.

4. Bivalent Cholera and Typhoid Vaccine: Current Status

A combined vaccine against cholera and typhoid that contains the live attenuated cholera CVD 103-HgR and typhoid Ty21a strains is under late-phase clinical investigation. Combination of these vaccines does not appear to significantly alter the immunogenicity and tolerability observed when the vaccines are administered separately.

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