Safety and Immunogenicity of Live Oral Cholera and Typhoid Vaccines Administered Alone or in Combination with Antimalarial Drugs, Oral Polio Vaccine, or Yellow Fever Vaccine

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The effects of concomitant administration of antimalarial drugs, oral polio vaccine, or yellow fever vaccine on the immune response elicited by the *Vibrio cholerae* CVD103-HgR and *Salmonella typhi* Ty21a live oral vaccines were investigated. Healthy adults were immunized with CVD103-HgR alone or combined with Ty21a. Subjects were randomized to simultaneously receive mefloquine, chloroquine or proguanil, or oral polio or yellow fever vaccine. The vibriocidal antibody seroconversion rate was significantly reduced ($P = .008$) only in the group that received chloroquine with the CVD103-HgR. The geometric mean vibriocidal antibody titer was significantly decreased in the groups that received chloroquine ($P = .001$) or mefloquine ($P = .02$) compared with titers in groups that received CVD103-HgR alone. However, similar immunosuppressive effects were not observed in the groups immunized with Ty21a and CVD103-HgR. Only the concomitant administration of proguanil effected a significant ($P = .013$) decline in the anti–*S. typhi* lipopolysaccharide antibody response. These results indicate that chloroquine and proguanil should not be simultaneously administered with the CVD103-HgR and Ty21a vaccine strains, respectively.

Live oral attenuated vaccines against polio, typhoid fever, and cholera are licensed for human use [1–3]. These vaccines have the distinct advantage of stimulating local intestinal immunity, thereby blocking infection at the earliest possible stage. In addition, their ease of administration greatly facilitates mass vaccination campaigns. Adverse reactions associated with the use of the *Salmonella typhi* Ty21a and *Vibrio cholerae* CVD103-HgR vaccine strains are infrequent, mild, and transient [4, 5]. These products have served as models for the future development of vaccines against a wide variety of bacterial and viral enteric pathogens.

In order to induce a protective immune response, live vaccines must undergo a limited degree of replication in vivo. Interference with this process would be expected to diminish the level of immunity provided. For example, the concomitant use of antibiotics or antimalarial drugs with activity against live bacterial vaccine strains could markedly suppress their multiplication, hence lowering the level of protection conferred [5–8]. Another potential concern is that the coadministration of $\geqslant 2$ live oral vaccines could interfere with the immune response to $\geqslant 1$ of the vaccine strains. Finally, the simultaneous administration of multiple oral vaccines could increase the frequency and severity of adverse reactions compared with those when vaccines are given individually.

We have recently demonstrated that the Ty21a and CVD103-HgR vaccine strains could be simultaneously administered without compromising safety or immunogenicity of either strain [9, 10]. This finding paves the way for the development of additional multivalent live oral vaccine formulations.

The effects of simultaneous administration of live vaccines or the concomitant use of antimalarial drugs with antibacterial activity on the immune response to immunization have not been studied in great detail. Chloroquine has been shown to significantly decrease the immune response to parenterally administered human diploid cell–derived rabies vaccine [11]. When administered together, parenteral cholera and yellow fever vaccines have an immunosuppressive effect on each other [12]. The fact that many travelers are often prescribed $\geqslant 1$ of the above-mentioned vaccines together with an antimalarial drug makes these issues of significant practical importance. We therefore conducted a study among Austrian travelers to determine the effect of simultaneously administering various vaccines and antimalarial drugs on the immune response to the Ty21a and CVD103-HgR vaccine strains.

Materials and Methods

Vaccines. The CVD103-HgR and Ty21a vaccines were manufactured by the Swiss Serum and Vaccine Institute as previously described [9]. The vaccines were presented in a double-chambered aluminum foil sachet. One chamber contained $\sim 5 \times 10^8$ cfu of CVD103-HgR alone or in combination with $\sim 5 \times 10^9$ cfu of

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The study protocol was approved by the Ethical Committee, University of Vienna Medical School, Vienna, Austria. Written informed consent was obtained from each volunteer.

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Ty21a. The other chamber contained a buffer composed of 2.5 g of sodium bicarbonate and 1.65 g of ascorbic acid.

Trivalent oral polio vaccine (Polio Sabine Oral; SmithKline Beecham, Riksensart, Belgium) was administered as a single 0.5-mL dose.

Yellow fever vaccine (Arilvax, lot no. 1/350; Burroughs Wellcome, Beckenham, UK) contained the attenuated 17D strain of yellow fever virus. A single 0.5-mL subcutaneous injection was administered in the deltoid area.

Antimalarial drugs. Mefloquine (Lariam F; Hoffmann-La-Roche, Basel, Switzerland) was administered orally in tablet form (250 mg) at weekly intervals. Chloroquine (Resochin; Bayer, Leverkusen, Germany) was administered in tablet form (2 tablets corresponding to 250 mg of chloroquine diphosphate) at weekly intervals. Proguanil (Paludrine; Zeneca, Macclesfield, UK) was administered orally (2 100-mg tablets) daily for 7 days.

MIC determinations. The in vitro MICs of mefloquine, chloroquine, and proguanil for the Ty21a and CVD103-HgR strains were determined by a standard tube dilution method. The MIC was defined as the lowest concentration of drug that resulted in no visible growth after incubation at 37°C for 24 h.

Study design. Healthy men and women ⩾18 years of age were recruited from the student body of the University of Vienna. Exclusion criteria included acute febrile illness, history of allergies, immunization against either cholera or typhoid fever within the past 3 years, immunization against polio within the past 5 years, previous vaccination against yellow fever, use of antibiotics within 7 days of initiating the study, treatment with immunosuppressive drugs, underlying immunodeficiency state, epilepsy, clinical depression, receipt of an experimental drug within the past 3 months, receipt of blood products within the preceding 3 months, use of β-blockers, acute or chronic gastrointestinal illness, pregnancy or lactation, or current participation in another trial.

After being screened, the volunteers were randomized into groups that received the following treatment regimens: Groups A–F received a single dose of CVD103-HgR on day 1. Groups G–L received a single oral dose of the combined Ty21a and CVD103-HgR vaccines on day 1, with monovalent Ty21a given on days 3 and 5. Groups B and H also received a single dose of mefloquine on days 1 and 8. Groups C and I also received 2 chloroquine tablets on days 1 and 8. Groups D and J also received 2 tablets of proguanil on days 1–7. Groups E and K were immunized with yellow fever vaccine on day 1. Groups F and L were immunized against polio on day 1. There were 45 subjects in groups A and G; the remaining groups contained 30 persons. The subjects were instructed to fast for 1 h before and after vaccination. The Ty21a and CVD103-HgR vaccines were suspended in 100 mL of water, mixed with a spoon to yield a homogeneous suspension, and immediately ingested. Other vaccines or antimalarial drugs were administered just after ingestion of the first dose of Ty21a or CVD103-HgR (or both) vaccines. Venous blood samples were obtained at immunization and 14 days later. Serum was collected, placed into labeled, dated tubes, and stored at −20°C. Subjects were instructed to record all adverse events on a report form for 7 days following the initial immunization. Specifically, subjects were asked to note the following symptoms: headache, malaise, nausea, vomiting, abdominal discomfort, diarrhea, cutaneous reactions such as rash or pruritus, fever, and any other clinically relevant event potentially related to the study drugs.

Serologic analysis. Antibody titrations were done in a blinded manner. Vibriocidal antibody titers were determined using a microtiter plate assay as previously described, using *V. cholerae* 569B (classical, Inaba) as the test strain [13]. A significant response was defined as a ⩾4-fold rise in titer over baseline. Anti–*S. typhi* IgG and IgA lipopolysaccharide antibodies were quantitated using an ELISA as described elsewhere [14]. An increase of ⩾0.15 optical density units over baseline was considered significant.

Statistical analysis. Statistical differences in vibriocidal and anti–*S. typhi* IgG response rates between groups were determined by χ^2 analysis. Differences in the geometric mean antivibriocidal titers between groups were determined using a one-tailed *t* test. The rate of adverse reactions between groups was analyzed using χ^2 analysis with Yates's correction.

Results

Adverse reactions associated with immunization or the administration of antimalarial drugs (or both) are shown in table 1. The overall rate of adverse events reported was comparatively high, with such nonspecific events as malaise and headache being reported by >50% of subjects in several groups. This can most likely be attributed to the fact that the volunteers were highly motivated to report any and all symptoms that could be related to vaccination. The vast majority of reactions were transient, mild, and resolved without medical intervention. No concomitant treatment resulted in a statistically significant higher rate for any type of adverse event.

The effect of concomitant administration of antimalarial drugs or vaccines on the vibriocidal antibody response engendered by CVD103-HgR alone or in combination with Ty21a is shown in table 2. The MICs for mefloquine, chloroquine, and proguanil against CVD103-HgR were 25 μg/mL, >500 μg/mL, and >50 μg/mL, respectively. For subjects receiving CVD103-HgR alone, only the coadministration of chloroquine resulted in a significant ($P = .008$) reduction in the percent who responded with a significant (⩾4-fold) rise in vibriocidal antibody titers. Analysis of postimmunization geometric mean titers (GMTs) of vibriocidal antibody showed that both mefloquine and chloroquine exerted suppressive effects. Of interest, the highest response rate, as gauged by both seroconversion rate and GMT, was seen in the group that also received yellow fever vaccine. In contrast to the above, no concomitant treatment was found to exert a deleterious effect upon either the vibriocidal seroconversion rate or postimmunization GMTs of subjects who received the combined CVD103-HgR plus Ty21a vaccine formulation. In fact, the highest seroconversion rates and GMTs were achieved in the groups that received mefloquine or oral polio vaccine.

To determine whether the negative effects of mefloquine and chloroquine on the vibriocidal antibody response in the group that received only the CVD103-HgR vaccine were related to the relatively small sample size, the following analysis was performed. To enlarge the sample size, values from groups with the same concomitant treatment, such as B and H, were

Table 1. Adverse events following immunization with various combinations of vaccines and antimalarial drugs.

Vaccine, group (n)	Concomitant treatment	% reporting adverse reaction						
		Diarrhea	Nausea	Vomiting	Abdominal discomfort	Headache	Malaise	Cutaneous
CVD 103-HgR								
A (45)	None	22.2	20	0	22.2	51.1	44.4	6.7
B (30)	Mefloquine	33.3	13.3	0	16.7	36.7	36.7	10
C (30)	Chloroquine	10	13.3	3.3	28.3	36.7	33.3	3.3
D (30)	Proguanil	30	36.7	6.7	23.3	56.7	50	6.7
E (30)	Yellow fever vaccine	10	10	0	3.3	46.7	63.3	20
F (30)	Oral polio vaccine	10	10	0	6.7	30	46.7	3.3
CVD-HgR/Ty21a								
G (45)	None	31	15.6	2.2	13.3	40	51.1	11.1
H (30)	Mefloquine	40	20	3.3	16.7	50	53.3	3.3
I (30)	Chloroquine	20	23.3	0	23.3	63.3	30	0
J (30)	Proguanil	30	33.3	0	10	40	53.3	10
K (30)	Yellow fever vaccine	30	6.7	0	10	33.3	43.3	13.3
L (30)	Oral polio vaccine	30	3.3	0	13.3	30	40	6.7

pooled and compared with the pooled values for the 2 control groups (A and G). Chloroquine was still found to significantly ($P = .014$) lower the seroconversion rate and postimmunization GMT ($P = .001$). Similarly, mefloquine treatment resulted in a slightly decreased vibriocidal GMT ($P = .032$), although the seroconversion rates were virtually identical (88% vs. 87.5%) between the pooled groups. Concurrent immunization with oral polio vaccine or yellow fever vaccine did not impact the immune response evoked by CVD103-HgR given alone or in combination with Ty21a.

The MICs for mefloquine, chloroquine, and proguanil against Ty21a were 10 μg/mL, >500 μg/mL, and >200 μg/mL, respectively. Only proguanil was found to exert a significant ($P = .013$) decrease upon the combined IgG or IgA serum anti–*S. typhi* lipopolysaccharide antibody response. Mefloquine, which had the highest level of in vitro activity against Ty21a, did not adversely affect the immune response engendered by Ty21a, as gauged by the combined IgG or IgA response rate. However, the IgG response was somewhat lower in the group that received mefloquine (table 3).

Discussion

The current study demonstrated that the simultaneous administration of certain antimalarial drugs commonly given to trav-

Table 2. Effect of concomitant treatment with antimalarial drugs or various vaccines on the immune response to *Vibrio cholerae* CVD103-HgR.

Vaccine, group	Concomitant treatment	Vibriocidal seroconversion rate, no./total (%)	*P**	Vibriocidal GMT		*P**
				Before	After	
CVD 103-HgR						
A	None	41/45 (91)	—	28.9	1516	—
B	Mefloquine	24/30 (80)	>.05	19.5	597	.02
C	Chloroquine	20/30 (67)	.008	36.8	359	<.001
D	Proguanil	25/30 (83)	>.05	20	1064	>.05
E	Yellow fever vaccine	29/30 (97)	>.05	21.4	4256	>.05
F	Oral polio vaccine	27/30 (90)	>.05	21.4	1309	>.05
CVD 103-HgR/Ty21a						
G	None	39/45 (87)	—	21.3	806	—
H	Mefloquine	29/30 (97)	>.05	25.8	1470	>.05
I	Chloroquine	23/30 (77)	>.05	23.5	640	>.05
J	Proguanil	25/30 (83)	>.05	24.6	752	>.05
K	Yellow fever vaccine	25/30 (83)	>.05	15.9	864	>.05
L	Oral polio vaccine	28/30 (93)	>.05	21.9	1539	>.05

NOTE. GMT = geometric mean titer.

* A vs. B–F or G vs. H–L for seroconversion rates and comparison of postimmunization antibody titers.

Table 3. Effect of concomitant treatment with antimalarial drugs or CVD-HgR/Ty21a vaccine on the immune response to *Salmonella typhi* Ty21a.

Group	Concomitant treatment	Anti–*S. typhi* LPS response (%)			*P**
		IgG	IgA	IgG or IgA	
G	None	71.1	48.9	77.8	—
H	Mefloquine	50	50	70	.73
I	Chloroquine	56.7	56.7	70	.73
J	Proguanil	50	36.7	50	.01
K	Yellow fever vaccine	66.7	56.7	76.7	.91
L	Oral polio vaccine	83.3	66.7	86.7	.86

NOTE. LPS = lipopolysaccharide.
* G vs. H–L for IgA or IgG antibody response rates.

elers may interfere with the immune response engendered by the Ty21a or CVD103-HgR live oral vaccine strains. Serum antibody response was used as a surrogate marker to predict a protective immune response. This approach is supported by studies demonstrating that serum anti–*S. typhi* antibodies and vibriocidal antibodies correlate with protection against typhoid fever and cholera, respectively, in field trials and in volunteer challenge studies [2, 6, 15–17]. Many antimalarial drugs, such as chloroquine, mefloquine, and proguanil, have in vitro antibacterial activity that could limit in vivo replication of the vaccine strain, thereby diminishing efficacy. An important finding in the present investigation was that in vitro activity of various antimalarial drugs was not predictive of their in vivo effect upon the immune response engendered by live oral vaccines.

Despite the fact that mefloquine is highly active against the Ty21a vaccine strain in vitro (MIC, 5–10 μg/mL), it did not exert a significant negative effect on the combined serum anti–*S. typhi* IgG or IgA antibody response. The fact that chloroquine also did not exert an immunosuppressive effect is not altogether surprising, given its high in vitro MIC ($>$250 μg/mL). However, the daily administration of proguanil did effect a marked reduction in the immune response engendered by Ty21a even though it had a MIC ($>$200 μg/mL) comparable to that of chloroquine.

The effect of antimalarial drugs on the immune response engendered by CVD103-HgR administered alone or in combination with Ty21a was more difficult to assess. While chloroquine resulted in a significant decline in both vibriocidal seroconversion rate and GMT when given with CVD103-HgR, this same effect was not seen when CVD103-HgR was combined with Ty21a. Similarly, mefloquine effected a decrease in the vibriocidal GMT only when administered with CVD103-HgR alone. However, the seroconversion rate in either group, which appears to be the best predictor of immunity to experimentally induced cholera in volunteer studies [2, 16, 17], was not adversely affected. These findings may be partly attributed to an enhancement of the immune response elicited by CVD103-HgR when combined with Ty21a [10].

The disparate findings between in vitro and in vivo activity of the antimalarial drugs evaluated may, in large part, be related to their pharmacokinetic properties. For example, mefloquine is rapidly adsorbed into the bloodstream (absorption half-life of 0.36–2 h, manufacturer's information; Roche Laboratories, Nutley, NJ). Since neither Ty21a nor CVD103-HgR enters the circulatory system, the duration of contact with these drugs is therefore most likely limited. The MIC values of proguanil do not accurately reflect its in vivo activity since cycloguanil, a metabolite produced by the liver, is believed to be the active form of this drug (unpublished data, Zeneca). Chloroquine has been shown to suppress the immune response to even an inactivated rabies vaccine [11]. This immunosuppressive effect may be related to the fact that chloroquine is deposited in various tissues, including lymphocytes, and can attain levels 2–700 times higher than those attained in plasma (unpublished data, Sanofi Winthrop Pharmaceuticals, New York).

Clearly, the concomitant administration of yellow fever or oral polio vaccines did not affect the immune response to either the Ty21a or CVD103-HgR vaccine strain. The study design did not allow us to determine if there was any effect on the immunogenicity of the polio or yellow fever vaccines. There has been some concern that immunization with live oral attenuated vaccines may elevate local gut interferon levels, which could, in theory, diminish the potency of oral polio vaccine. However, without supporting data, the Centers for Disease Control and Prevention found no reason to contraindicate the simultaneous use of oral polio vaccine and Ty21a [18].

The above results illustrate that certain antimalarial drugs can exert a detrimental effect on the immunogenicity of the Ty21a and CVD103-HgR vaccine strains. Based on our current findings, a number of recommendations can be offered. Foremost among these are that proguanil not be administered together with Ty21a and that chloroquine not be given simultaneously with CVD 103-HgR. Proguanil chemoprophylaxis should be initiated no sooner than 10 days after the last dose of Ty21a vaccine. This recommendation is based on the kinetics of the immune response after immunization with Ty21a, when near-maximal serum antibody responses are observed ~10–14 days after completion of the vaccination regimen [9, 10]. The coadministration of mefloquine and Ty21a would most likely not compromise vaccine efficacy. Since protection against experimental cholera is conferred as soon as 8 days after immunization with CVD103-HgR, chloroquine prophylaxis should be initiated no sooner than 8 days after vaccination [18]. While mefloquine effected a decrease in the vibriocidal GMT, the seroconversion rate was not significantly reduced. A minimal protective titer has yet to be determined for CVD103-HgR. As noted above, the seroconversion rate was not adversely affected by ingestion of mefloquine. Therefore, the concomitant administration of mefloquine and CVD103-HgR would not be expected to compromise vaccine efficacy. Finally, if both Ty21a

and CVD 103-HgR are administered, only mefloquine should be used.

The present study indicates that certain antimalarial drugs have the potential to adversely effect the efficacy of live oral bacterial vaccines when concomitantly administered. Physicians, especially those specializing in travel medicine, must consider this when prescribing the Ty21a and CVD103-HgR vaccines, especially when malaria prophylaxis is indicated. While the Ty21a vaccine formulation used in this study differs from the enteric-coated version now marketed, there is no reason to believe that this would significantly affect the interactions noted. The fact that combining the 2 vaccine strains appeared to circumvent the detrimental effects of chloroquine on the CVD103-HgR strain is intriguing and is yet another argument for the development of combined live oral vaccines against enteric pathogens.

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