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Failure of Doxycycline as a Causal Prophylactic Agent against *Plasmodium falciparum* Malaria in Healthy Nonimmune Volunteers

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■ *Objective:* To determine whether doxycycline, 100 mg administered as a single daily oral dose, is effective as a causal prophylactic agent, an agent active against the pre-erythrocytic liver stage of *Plasmodium falciparum* malaria parasites, in healthy nonimmune persons. If effective, the recommendation by the Centers for Disease Control and Prevention (CDC) that doxycycline be continued for 4 weeks after returning from malaria-endemic areas could be shortened to 1 week.

■ *Design:* Randomized, double-blind, placebo-controlled trial.

■ *Setting:* Medical ward at the U.S. Army Research Institute of Infectious Diseases, Fort Detrick, Maryland.

■ *Participants:* 18 nonimmune, healthy, adult male volunteers, age 21.7 ± 2.9 (SD) years, were enrolled in two groups, one of 8 persons and one of 10 persons. Six participants in the first group and 7 in the second group received doxycycline. The remaining participants received placebo. Two volunteers were dropped from the study, leaving 16 participants for analysis.

■ *Intervention:* Each participant received doxycycline, 100 mg, or placebo in a single daily oral dose starting 3 days before exposure to *P. falciparum*–infected mosquitoes and ending 6 days after exposure.

■ *Measurements:* Monitoring for parasitemia, plasma doxycycline concentrations, and mosquitoes' salivary-gland sporozoite grade.

■ *Results:* 6 of 6 (100% [95% CI, 54% to 100%]) participants on doxycycline in the first group and 2 of 6 (33% [CI, 4% to 78%]) in the second group were protected from malaria. No differences were found between protected and nonprotected participants in the doxycycline elimination half-life ($T_{1/2}$) (20.8 ± 5.0 h compared with 21.9 ± 5.2 h), the steady-state average plasma concentration (1626 ± 469 ng/mL compared with 1698 ± 651 ng/mL), or other pharmacokinetic parameter estimates. The mean mosquito salivary-gland sporozoite grade was significantly higher (P = 0.02) in protected (3.5 ± 0.3) than in nonprotected persons (3.1 ± 0.1). Overall, 8 of 12 persons on doxycycline were protected from malaria, yielding a causal prophylactic efficacy rate of 67% (CI, 35% to 90%).

■ *Conclusions:* A dosing regimen of doxycycline, 100 mg once daily, administered as a causal prophylactic agent against *P. falciparum* malaria in healthy, nonimmune volunteers, had an unacceptably high failure rate. Therefore, the CDC recommendation that doxycycline should be taken daily starting 1 to 2 days before travel, during travel, and for 4 weeks after travel should still be followed.

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With the growth of international travel, increasing numbers of nonimmune travelers may be at risk for exposure to *Plasmodium falciparum* malaria (1–3). Since the emergence and spread of multidrug-resistant *P. falciparum* parasites (4, 5), the choice of drug to prevent malarial infections has become problematic (6, 7). In most areas endemic for malaria, chloroquine is no longer the drug of choice. Chemoprophylaxis with Fansidar (Roche Laboratories; Nutley, New Jersey) has been discouraged in recent years after reports of severe life-threatening side effects (8) and the emergence of parasite resistance to this combination drug (9, 10). The Centers for Disease Control and Prevention (CDC) list of alternative preventive agents for travelers to areas with chloroquine-resistant *P. falciparum* malaria includes mefloquine, doxycycline, or the combination of proguanil plus chloroquine (11). Mefloquine is currently the drug of choice. However, reports of increasing parasite resistance to this drug (12–14) and possible mefloquine-associated neuropsychiatric side effects (15–17) may limit its usefulness as a prophylactic agent. Proguanil, although ineffective alone, is widely used overseas in combination with chloroquine. It is not, however, commercially available in the United States.

Doxycycline is recommended for prevention of malaria in persons traveling for short periods to areas with chloroquine-resistant *P. falciparum* malaria who cannot tolerate mefloquine or for whom the drug is contraindicated (11). In addition, travelers to areas where mefloquine resistance has been identified, such as the Thai-Burmese border area, may be particularly good candidates for doxycycline prophylactic therapy. Current recommendations advise starting the drug 1 to 2 days before travel and taking it daily throughout the period of potential exposure to infected mosquitoes and for 4 weeks after returning from an endemic area. The terminal use of doxycycline for 4 weeks after possible exposure is intended to eliminate any erythrocytic parasites. This use is based on doxycycline's presumed activity as a suppressive prophylactic agent, that is, an agent effective against blood stages of the parasite. Continued daily drug ingestion for 4 weeks in the absence of symptoms, however, requires well-disciplined compliance and is expensive. It is important, therefore, to ensure that the use of doxycycline for 4 weeks after potential exposure has ceased is necessary.

A drug that is active against the pre-erythrocytic liver stage, thereby preventing erythrocytic malaria, is called

a causal prophylactic drug. If it is effective against *P. falciparum* parasites, such a drug prevents symptomatic malaria and needs to be taken only during the hepatic phase of infection, normally about 1 week from the time of mosquito bite. Studies in the early 1970s (18, 19) suggested that tetracycline compounds were effective as causal prophylactic agents. Minocycline, a semi-synthetic, long-acting tetracycline ($T_{1/2}$, 18 h ± 4 h) (mean ± SD), when administered as 100 mg daily for 7 days, starting 1 day before exposure to *P. falciparum* parasites, prevented parasitemia in four of four persons (18). Two other persons receiving 100 mg on the day of mosquito challenge and on day 3 after challenge were also protected. Doxycycline, another long-acting tetracycline analog ($T_{1/2}$, 16 h ± 6 h) (20), has been shown in field trials to have prophylactic activity (21, 22). Whether its mechanism of action was causal or suppressive, however, could not be determined.

We designed our study to determine whether oral doxycycline, 100 mg administered daily, is effective as a causal prophylactic agent against *P. falciparum* malaria. If so, it would justify shortening the duration of terminal prophylactic therapy from 4 weeks to 1 week.

Methods

Volunteers were recruited under a protocol approved by the Human Use Review Committee, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, and the Surgeon General's Human Subjects Research Review Board of the Department of the Army. Eighteen healthy men (age, 21.7 ± 2.9 (SD) years) were selected after giving written informed consent. The screening evaluation included a baseline medical history, physical examination, electrocardiogram, and chest roentgenogram. Exclusion criteria included a history of malaria; splenectomy; allergy to doxycycline, tetracycline, or chloroquine; abnormal results of a complete blood count or tests for aspartate aminotransferase, alanine aminotransferase, bilirubin, lactic dehydrogenase, alkaline phosphatase, blood urea nitrogen, or creatinine; hematuria or proteinuria; glucose-6-phosphate-dehydrogenase deficiency; antibody to hepatitis B surface antigen; or antibody to human immunodeficiency virus.

Informed Consent

Prospective volunteers received a briefing and a written information sheet describing the procedures and potential risks associated with the study. Those willing to participate signed a consent form. None of the volunteers received compensation. They were assured of their right to withdraw from the study at any time without penalties. The study was designed to minimize the number of volunteers that might develop malaria. The number enrolled was based on the presumption that doxycycline protection rate would exceed 90% when administered as a causal prophylactic agent. Such a level of efficacy would have justified, in our opinion, changing the current CDC recommendation for terminal prophylactic therapy. The number of volunteers assigned to receive placebo was the minimum needed to ensure infectivity of the mosquitoes. To minimize morbidity, all participants were closely monitored throughout the study. Monitoring was intensified during periods of greatest likelihood of emergence of parasitemia by hospitalizing the participants on the medical ward. Given the close monitoring and the early treatment when parasitemia was low, the risk to the participants was felt to be minimal.

Study Design

The study was a randomized, double-blind, placebo-controlled trial. Doxycycline, 100 mg daily, was administered orally, starting 3 days before and ending 6 days after exposure to *P. falciparum*-infected mosquitoes. The study was implemented in two phases, 2 months apart. In phase 1, eight persons (group 1) were randomly assigned to receive doxycycline (six persons) or placebo (two persons). In phase 2, ten persons (group 2) were randomly assigned to receive either doxycycline (seven persons) or placebo (three persons). The drug used was doxycycline hyclate (Vibramycin, Pfizer Inc.; New York, New York), which was given in 100-mg capsules; identical placebo capsules were prepared by the University of Iowa Pharmaceutical Services Division.

After an overnight fast the participants were hospitalized on the medical ward for 24 hours. Within 30 minutes after breakfast, either a doxycycline or a placebo capsule was administered to each participant. Nine samples for plasma doxycycline level measurements were then collected from each person during the first 24 hours, with eight additional samples collected during the following 12 days.

On the afternoon of day 4 of drug administration, each participant was exposed to *Anopheles stephensi* mosquitoes infected with chloroquine-sensitive *P. falciparum* malaria. Giemsa-stained malaria smears were prepared daily from day 5 to day 15 after exposure and at least once weekly for 2 months. Thick and thin malaria smears were examined by two of the investigators. When present, parasitemia was quantified on a thick smear by the method of Earle and Perez (23). All volunteers were hospitalized during week 2 after exposure, the time of highest risk for developing malaria. Those who remained well were discharged on day 15 and followed as outpatients. Persons who became febrile during the outpatient period were rehospitalized and evaluated for malaria with twice-daily blood smears. If parasites were found, standard chloroquine therapy, 1500-mg base for 48 hours, was administered. The participants were discharged after their blood films were clear of parasites and their symptoms resolved for 3 days.

Induction of Malaria Infection

Anopheles stephensi mosquitoes were used to transmit NF54 strain *P. falciparum* parasites. Female mosquitoes, 4 to 7 days after emergence, were membrane-fed on a mixture of cultured gametocytes (24), defibrinated blood, and human serum negative for hepatitis B surface antigen and human immunodeficiency virus antibody. Thirty-five days and 21 days after membrane feeding, the mosquitoes inoculated the first and second group of participants, respectively. To increase the survival rate of the mosquitoes in the first colony to 35 days, the environmental temperature was decreased from room temperature to 20 °C in the last 2 weeks before human exposure. Mosquitoes used to induce infection in the second group were given a second blood meal when they were found to have low oocyst counts on day 6 after membrane feeding.

On day 4 of drug administration, 6 to 8 hours after the morning medication, a cage containing five *A. stephensi* mosquitoes was placed on the forearm of each volunteer for 5 minutes. Those mosquitoes ingesting a blood meal were dissected and the sporozoite density of the paired salivary glands was quantified on a log-based categorical scale of 0 to 4. Each volunteer was then exposed to additional mosquitoes, as necessary, until five mosquitoes with sporozoite densities of 2 or greater had successfully taken a blood meal. Previous experience in our laboratory using these procedures resulted in an infection rate of 100% (25–27).

Doxycycline Plasma Concentration

Seventeen plasma samples were collected for pharmacokinetic analysis: a sample before dosing and at 1, 2.5, 3, 4, 6, 9, 12, 24, 72, 75, 168, 215, 218, 240, 264, and 288 hours after the first dose. Three of the samples were collected at times corresponding to steady-state trough levels. The last three samples were collected at 24-hour intervals, starting 24 hours after the last dose, to characterize the terminal elimination half-life of doxycycline. Doxycycline was measured using reverse-phase high-performance liquid chromatography with a minimum detection limit of about 50 ng/mL (28).

Data Analysis

Pharmacokinetics

Plasma doxycycline concentration versus time data were fitted to a multiple-dose pharmacokinetic model using nonlinear least-square regression analysis (29). The terminal elimination rate constant was used to calculate the elimination half-life. The minimum concentration at steady state was an average of the three measured trough plasma concentrations at steady state. The average steady-state doxycycline concentration was calculated from the area under the concentration-time curve. Plasma doxycycline concentration at the time of exposure to the mosquitoes (t = 78 h) was calculated from the best-fit triexponential regression model for each participant. Other pharmacokinetic variables, such as the area under the concentration-time curve, clearance, volume of distribution at steady state, and mean residence time, were derived from standard formulas using the linear parameters of the regression model and the rate constants of absorption, distribution, and elimination (30).

Statistical Analysis

Data are presented as mean ± SD. The two-tailed Wilcoxon rank-sum test was used to test for differences between the mean salivary-gland sporozoite grades in groups 1 and 2 and in protected and nonprotected participants. The unpaired two-tailed t-test was used to test for differences in the mean pharmacokinetic parameters in protected and nonprotected volunteers. Exact (two-sided) confidence intervals for infection rates were calculated based on the binomial distribution (31). A P value of 0.05 or less was considered statistically significant.

Results

Doxycycline Efficacy

In the first group, none of the six persons receiving doxycycline developed malaria. One placebo recipient developed parasitemia 12 days after mosquito exposure. The second placebo recipient was noted on routine laboratory tests to have rising plasma glucose levels associated with glycosuria. Nine days after exposure to the mosquitoes, he was withdrawn from the study and treated with a therapeutic regimen of chloroquine, although he was aparasitemic at the time of treatment. Subsequent evaluation confirmed that he had new-onset diabetes mellitus.

In the second group, parasitemia developed in four of six persons who received doxycycline and in all three placebo recipients. One doxycycline recipient was withdrawn from the study on day 25 of outpatient follow-up after he was treated by another physician for nonspecific urethritis with doxycycline, 200 mg daily, for 6 days. He remained aparasitemic during 2 months of follow-up. Because of the unscheduled treatment, he was not included in the analysis.

Overall, 8 of 12 doxycycline-treated persons were protected from malaria, yielding an efficacy rate of 66.7% (95% CI, 35% to 90%). Two of four placebo recipients had delayed patency; they developed parasitemia at 15 and 21 days after mosquito exposure. Similarly, all four doxycycline-treated persons who developed malaria had a delayed prepatent period, with parasitemia appearing at 15, 22, 23, and 24 days.

Mosquitoes' Infectivity

The salivary-gland sporozoite grade was significantly higher (P = 0.04) in the mosquitoes used in phase 1 (3.5 ± 0.26) compared with the mosquitoes used in phase 2 (3.3 ± 0.25) of the study. Similarly, the sporozoite grade of the mosquitoes (3.5 ± 0.26) used in persons who did not develop malaria on doxycycline was significantly greater (P = 0.02) than in those who developed malaria (3.1 ± 0.12). Persons in phase 2 were exposed to a greater number of mosquitoes (8.5 ± 2.6 compared with 6.5 ± 1.3 mosquitoes in phase 1); this was due, however, to a greater number of nonproductive bites—bites by noninfected mosquitoes or mosquitoes with a sporozoite grade less than 2.

Doxycycline Pharmacokinetics

The best model describing the concentration-time data for plasma doxycycline levels was a multiple-dose, two-compartment, open model with first-order input, first-order output, and a lag time. A typical concentration-time profile is shown in Figure 1. None of the pharmacokinetic parameters were significantly different in persons protected by doxycycline compared with those not protected. These parameters include the elimination half-life ($T_{1/2}$), trough concentration at steady state, steady-state average concentration, and concentration at the time of mosquito exposure (Table 1). Similarly, clearance (37.2 ± 10.4 compared with 32.8 ± 12.2 mL/h per kg body weight), steady-state volume of distribution (982 ± 316 compared with 946 ± 160 mL/kg), area under the concentration-time curves per dose (39.0 ± 11.2 compared with 40.7 ± 15.6 (μg*h/mL) and the mean resident time (the time needed for 63.2% of the administered dose to be eliminated; 26.8 ± 6.1 h compared with 30.7 ± 7.2 h) in protected compared with nonprotected persons, respectively, did not differ significantly.

Discussion

Our results showed that doxycycline has some causal prophylactic activity. At the doses administered in this study, however, the failure rate was unacceptably high. To be acceptable as a causal prophylactic agent against *P. falciparum* malaria, a drug should have, in our opinion, an efficacy rate of at least 90%. The efficacy of doxycycline that we have found does not merit recommending it for use as a causal prophylactic agent. The remaining puzzle is why doxycycline failed to prevent infection in some participants and not in others. The pharmacokinetics of doxycycline in protected and nonprotected persons in our study was comparable. The number of sporozoites in the salivary glands of the mosquitoes also could not explain the failure of the doxycycline therapy. That all the placebo recipients developed malaria in our study and in all previous studies in our laboratory where the same procedures were used (25–27) supports our belief that the sporozoite challenge is reproducible and sufficient to produce malaria consistently in all nonimmune, untreated persons. That all the failures occurred in the second group of participants suggests that differences between phases 1 and 2 of the study may have resulted in lower drug efficacy.

The age of the mosquitoes (and sporozoites) at time

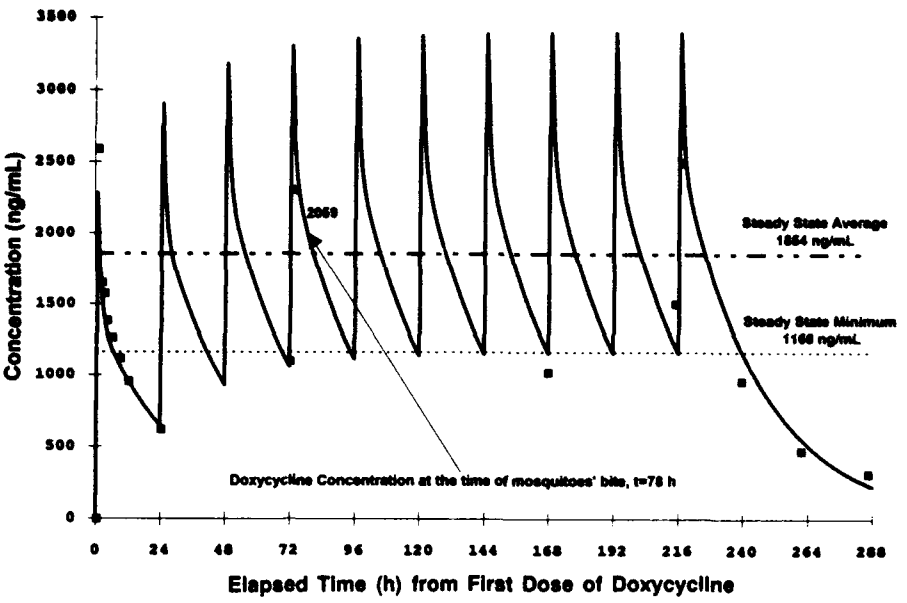

Figure 1. Typical plasma concentration-time profile of multiple-dose doxycycline. Volunteer 13. Dosing: 100 mg orally every 24 hours for 10 days. Steady-state minimum concentration is the mean of three measured doxycycline trough concentrations at steady state. Steady-state average concentration is a calculated average steady-state doxycycline concentration from the best-fit pharmacokinetic model. (■ = actual concentrations of doxycycline measured by a high-performance liquid chromatography method.)

of human bite, the environmental temperature of the mosquito colony, and the supplemental feeding that was provided to the mosquitoes differed between the two phases. It is likely that the increased age of the mosquitoes in phase 1 of the study and the temperature alteration that they endured resulted in diminished sporozoite virulence that was associated with increased susceptibility to doxycycline during their growth and maturation in the liver (32–37). The supplemental blood feeding that the mosquitoes received during phase 2 may have enhanced their sporozoite virulence. A decrease in the virulence of the sporozoites during phase 1 may have made them more susceptible to the plasma levels of doxycycline that were achieved with the dosing regimen used in this study, thus preventing malaria in all six treated persons. In contrast, for fully virulent sporozoites, in phase 2 of the study, a comparable drug concentration was apparently only partially effective, preventing malaria only in a few persons. Whether a dosing regimen of 100 mg twice daily (doubling the steady-state plasma concentration of doxycycline) would have been more effective can only be resolved by further studies.

Our findings of limited causal efficacy of doxycycline are consistent with the clinical observations by two of the coauthors (Pang and Boudreau), who noted a surge in the number of malaria cases within 3 weeks after discontinuing doxycycline therapy at the end of field studies in Thailand, in which doxycycline prophylactic efficacy was tested in children (21, 22). The surge in the number of cases soon after termination of the drug therapy suggested that the drug may have served primarily as a suppressive agent. Although it was not possible to rule out that some of the malaria cases observed represented newly acquired infections, it is more likely that many of the cases represented suppressed infections that became clinically apparent once doxycycline therapy was discontinued.

Table 1. Doxycycline: Estimates of Selected Pharmacokinetic Parameter in Protected and Nonprotected Participants

Participant	Weight	Dose	Half-life	Doxycycline Concentration			
				Trough after First Dose	Steady-state Minimum	Steady-state Average	At Time of Bite by Mosquitoes
	kg	*mg/kg*	*h*	← *ng/mL* →			
Protected participants							
1	85	1.18	26.7	392	612	879	1125
2	71	1.41	19.8	681	1050	1898	2189
4	75	1.33	30.1	737	1566	2251	2549
5	74	1.35	15.1	383	649	1299	1369
6	70	1.44	18.7	614	1372	1967	2100
7	84	1.19	18.2	375	851	1219	1352
14	63	1.58	19.8	653	910	1526	1867
17	75	1.33	18.2	729	1164	1966	2423
Mean ± SD	75 ± 7	1.35 ± 0.13	20.8 ± 5.0	571 ± 160	1022 ± 336	1626 ± 469	1872 ± 534
Nonprotected participants							
10	89	1.13	15.4	501	500	955	1186
11	87	1.15	23.9	604	828	1481	1613
12	83	1.21	27.7	628	1851	2503	2636
13	77	1.30	20.4	620	1160	1854	2059
Mean ± SD	84 ± 5	1.20 ± 0.08	21.9 ± 5.2	588 ± 59	1085 ± 577	1698 ± 651	1873 ± 621

Our findings may have limited applicability to the domain of natural exposure of nonimmune persons to infected mosquitoes. Whether we can generalize them depends on the extent to which our infective model emulates natural challenge. Our model was designed primarily to be highly reliable in inducing malaria infection and not necessarily to emulate natural exposure. Did our infective model pose an unusually severe challenge to doxycycline causal activity? Further, was the drug susceptibility profile of our parasite strain typical of strains found in indigenous areas? The answers to these questions are not known. We tested the causal prophylactic activity of doxycycline against a parasite sensitive to chloroquine. We do not know whether parasites resistant to chloroquine and mefloquine would have greater susceptibility to doxycycline causal prophylactic activity.

Considering our findings, we concur with the CDC recommendation that when doxycycline, in a 100-mg daily oral dose, is taken for the prevention of *P. falciparum* malaria, it should be started 1 to 2 days before departure and continued during the period of potential exposure to mosquitoes and for 4 weeks after returning from a malaria-endemic area. The 4 weeks of terminal prophylactic therapy is necessary to eliminate any malaria parasites as they emerge from the liver. Taking the drug for 1 week of terminal prophylaxis for its effect on tissue stages only is likely to result in an unacceptably high failure rate.

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