Am. J. Trop. Med. Hyg., 69(5), 2003, pp. 542–547

TRANSMISSION-BLOCKING ACTIVITY OF TAFENOQUINE (WR-238605) AND ARTELINIC ACID AGAINST NATURALLY CIRCULATING STRAINS OF *PLASMODIUM VIVAX* IN THAILAND

NARONG PONSA, JETSUMON SATTABONGKOT, PATTAMAPORN KITTAYAPONG, NANTANA EIKARAT, AND RUSSELL E. COLEMAN

Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Center of Vectors and Vector-Borne Diseases and Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

Abstract. The sporontocidal activity of tafenoquine (WR-238605) and artelinic acid was determined against naturally circulating isolates of *Plasmodium vivax* in western Thailand. Primaquine was used as a negative control and a dihydroacridine-dione (WR-250547) was used as a positive control. Laboratory-reared *Anopheles dirus* mosquitoes were infected with *P. vivax* by allowing mosquitoes to feed on blood (placed in an artificial-membrane feeding apparatus) collected from gametocytemic volunteers reporting to local malaria clinics in Tak province, Thailand. Four days post-infection, mosquitoes were refed on uninfected mice treated 90 minutes earlier with a given drug. Drug activity was determined by assessing oocyst and sporozoite development. Neither primaquine nor artelinic acid affected oocyst or sporozoite development at a dose of 100 mg of base drug/kg of mouse body weight. In contrast, tafenoquine and WR-250547 affected sporogonic development at doses as low as 25.0 and 0.39 mg/kg, respectively. The potential role of these compounds in the prevention of malaria transmission is discussed, as are alternative strategies for the use of transmission-blocking antimalarial drugs.

INTRODUCTION

The rapid emergence of resistance to antimalarial drugs from diverse chemical classes is a major factor affecting the treatment and control of malaria.[1–3] The use of compounds capable of interrupting the transmission of malaria has been advocated as a way of preventing the development of drug resistance and limiting the spread of resistant parasites.[4–8] Transmission-blocking antimalarial drugs can affect the sexual stages of the parasite in the human host (gametocytocidal activity) or development of the parasite in the mosquito (sporontocidal activity). It has been suggested that all existing antimalarial drugs should be evaluated for gametocytocidal and/or sporontocidal action;[9] however, relatively few studies have systematically characterized the gametocytocidal or sporontocidal property of antimalarials.

In this study, we evaluated the sporontocidal activity of tafenoquine (WR-238605) and artelinic acid against naturally circulating isolates of *Plasmodium vivax*. Primaquine was used as a negative control[5,6] and the dihydroacridine-dione WR-250547[4–6] was used as a positive control. Criteria used to assess sporontocidal activity included 1) percentage of mosquitoes with oocysts, 2) mean number of oocysts per infected mosquito, 3) average oocyst diameter, and 4) percentage of mosquitoes with sporozoites in their salivary glands.[4]

MATERIALS AND METHODS

Human and animal use. All human subjects research conducted in these studies was reviewed and approved by the Institutional Ethics Committee of the Thai Ministry of Public Health and the Human Subjects Research Review Board (HSRRB) of the United States Army. Research was conducted under the auspices of HSRRB Protocol A-9488 entitled *Production of Plasmodium Sporozoites for Use in the Development of Malaria Vaccines and Drugs*.

All research using animals was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. This study was reviewed by the Institutional Animal Care and Use Committee of the Armed Forces Research Institute of Medical Sciences and conducted under the auspices of Protocol 99-01 entitled *Evaluation of Transmission-Blocking Antimalarials for the Control of Malaria.*

Mosquitoes. *Anopheles dirus* A has been maintained at the Armed Forces Research Institute of Medical Sciences in Bangkok, Thailand for more than 25 years. Mosquitoes were reared at 26°C and a relative humidity of 70–80% and provided with 10% sucrose and water *ad libitum*. Groups of 100 mosquitoes were transported from Bangkok to the field sites in pint cartons placed inside a cooler. At the field sites, 6–8-day-old mosquitoes were provided with water only for 12 hours prior to feeding.

Study site and patients. The study was conducted in Tak Province in western Thailand. *Plasmodium vivax* and *P. falciparum* are the predominant parasite species in the region, followed by *P. malariae* and *P. ovale*.[10] Individuals who participated in the study were volunteers $\geq$ 15 years old and older seeking treatment at the Mae Sod and Mae Kasa malaria clinics. Thick and thin blood smears were prepared for each individual and stained with 10% Giemsa by the malaria clinic staff. Gametocyte and trophozoite densities were determined for all *P. vivax*-positive patients by counting the number of parasites per 500 leukocytes using oil immersion microscopy, and converting raw counts to parasites/microliter by assuming a count of 7,000 leukocytes/μL. If gametocytes were present, the patient was asked to enroll in the study. After the patients were briefed on the project and completed consent forms, lithium-heparinized Vacutainer tubes were filled with 10 mL of blood drawn by venipuncture from each patient.

Infection of mosquitoes. Whole blood (1 mL) was added to a glass feeder (5 cm diameter) closed with a Baudruche membrane. To prevent exflagellation of microgametocytes, a constant temperature of 37°C was maintained using a water jacket circulation system. The mosquitoes were allowed to

feed for 30 minutes, after which the glass membrane feeder was removed from the top of the carton and all unengorged mosquitoes were removed. Engorged mosquitoes were returned to Bangkok and maintained on a 5% sugar solution at 25–27°C and a relative humidity of 70–80%. After mosquito feeding was completed, volunteers were released from the study and received antimalarial treatment from the malaria clinic staff. The complete process (entry into the malaria clinic for initial diagnosis until receiving malaria treatment) took approximately 90 minutes.

Antimalarial drugs tested in this study. All compounds examined in this study were provided by the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (Silver Spring, MD). The four compounds used were tafenoquine (WR-238605): 8-[(4-amino-1-methyl butyl) amino]-2,6-dimethoxy-4-methyl-5-(3-tri-fluoromethylphenoxy) quinoline succinate; artelinic acid: dihydroartemisinin 4-carboxybenzyl ether; primaquine: 6-methoxy-8-(4′-amino-1′-methylbutyl amino) quinoline; and WR-250547: (R)-7-chloro-3-(2',4'-dichlorophenyl)-1,2,3,4-tetrahydro-1-[(3-(dimethylamino)propyl)imino]-9-acridinol.

Drug preparation. All drugs were diluted in a 1:3 solution of 70% ethanol:phosphate-buffered saline (diluent).[4] Infected mosquitoes received the drugs by feeding on mice that had received an intraperitoneal inoculation of a given drug 90 minutes earlier. Drugs were diluted so that each dose was delivered in 0.25–0.30 mL of diluent, with control animals receiving diluent only.

Initial assessment of sporontocidal activity of antimalarials. Three days after feeding on gametocytemic malaria patients, mosquitoes were randomly separated into groups of 100 each and provided with water only. Twenty-four hours later (four days after the infectious feed), each group was allowed to feed for 30 minutes on three anesthetized mice that had received an intraperitoneal inoculation of a given drug (100 mg/kg of mouse body weight) 90 minutes earlier. Unengorged mosquitoes were removed from the cages, and mosquitoes were maintained as previously described until assessed for sporogonic development.

Determination of minimum effective doses. Minimum effective doses were determined for compounds that demonstrated sporontocidal activity at a dose of 100 mg/kg of mouse body weight. Procedures for establishing the minimum effective doses were identical to those previously described, except that serial dilutions of each drug were tested until no significant sporontocidal activity was detected at a given dose.

Determination of the stage of sporontocidal development in which drugs were active. This experiment was intended to determine the period of sporogonic development in which selected drugs could prevent transmission of *P. vivax*. Procedures followed those previously described; however, infected mosquitoes were refed on drug-treated mice (100 mg of drug/kg of body weight) 4, 8, 11, or 16 days post-infection.

Assay for sporogonic development. Procedures used to determine oocyst and sporozoite production were modified from those described by Coleman and others.[4–7] In each experiment, mosquitoes were sampled for oocysts on day 10 post-infection and for sporozoites on day 21 post-infection. Midguts were stained with mercurochrome and oocysts were counted by phase-contrast microscopy (200× and 400×). Oocyst development was quantified by measuring oocyst diameter using an ocular micrometer. Measurements were recorded for the five largest oocysts present in mosquitoes with at least five oocysts, and for all oocysts in mosquitoes with fewer than five oocysts. Salivary glands were removed on day 21 post-infection and examined for the presence or absence of sporozoites by phase-contrast microscopy (400×).

Statistical analysis. Three replicates were conducted for each trial and the results were pooled. Due to a limited number of *P. vivax*-infected mosquitoes, only two replicates were performed to determine minimum inhibitory concentrations. Chi-square analysis was used to determine if the percentage of drug-treated mosquitoes with oocysts or sporozoites was different from the percentage of control mosquitoes. Student's *t*-test was used to determine if the number of oocysts per infected mosquito differed significantly, or if mean oocyst diameter differed significantly between treated and control groups.

RESULTS

Initial assessment of sporontocidal activity. At a dose of 100 mg/kg, both tafenoquine (WR-238605) and WR-250547 significantly affected both oocyst and sporozoite production (Table 1). However, neither primaquine nor artelinic acid affected oocyst production. Although treatment with artelinic acid significantly reduced the size of oocysts, this drug did not significantly affect the percentage of mosquitoes with sporozoites (Table 1). Primaquine had no effect on sporozoite production. Since primaquine and artelinic acid had no significant affect on sporogonic development, their activity was not assessed in subsequent experiments.

TABLE 1

Effect of selected antimalarials on the sporogonic development of *Plasmodium vivax* in *Anopheles dirus* mosquitoes*

Treatment	Percent of mosquitoes with oocysts (n)	Mean no. of oocysts per mosquito (± SEM)	Mean oocyst diameter (μm) (± SEM)	Percent of mosquitoes with sporozoites (n)
Control	77.8 (70/90)	15.26 (1.83)	61.29 (1.30)	70.1 (61/67)
Artelinic acid	73.3 (66/90)	10.44 (1.39)	53.00 (1.42)†	55.1 (49/89)
Control	93.3 (84.90)	8.38 (1.03)	76.3 (0.91)	91.1 (82/90)
Primaquine	88.9 (80/90)	11.94 (1.64)	75.00 (0.97)	88.9 (80/90)
Control	66.1 (41/62)	81.00 (16.34)	85.74 (0.94)	60.0 (42/70)
Tafenoquine	62.9 (39/62)	60.13 (11.14)	35.88 (1.21)†	7.1 (5/70)‡
Control	68.9 (62/90)	27.15 (5.00)	63.92 (1.83)	53.8 (43/80)
WR-250547	13.3 (12/90)‡	11.00 (2.88)†	14.11 (0.86)†	0.0 (0/75)‡

* Previously infected mosquitoes were refed 4 days later on mice treated 90 minutes earlier with a given drug. Drugs were provided at a dose of 100 mg of base drug/kg of mouse body weight.
† Significantly different from control ($P < 0.001$) using the *t*-test.
‡ Significantly different from controls ($P < 0.001$) using the chi-square test.

Determination of the stage of sporontocidal development in which drugs were active. Tafenoquine and WR-250547 were administered at 4, 8, 11, and 16 days post-infection at a dose of 100 mg of base drug/kg of mouse body weight. Administration of tafenoquine 8, 11, and 16 days post-infection had no impact on sporozoite invasion of the salivary glands (Table 2). As shown previously, administration of tafenoquine four days after the infectious feed inhibited sporozoite invasion of the salivary glands. In contrast, WR-250547 significantly inhibited sporozoite production when administered four or eight days post-infection (Table 2). Administration of this drug 11 days post-infection resulted in fewer mosquitoes developing salivary gland infections; however, this reduction was not significant. Administration of WR-250547 16 days post-infection had no effect on the percentage of mosquitoes with salivary gland sporozoites.

Determination of minimum effective doses. Doses of 6.25–100 mg of tafenoquine/kg of mouse body weight did not affect either the percentage of mosquitoes with oocysts or the number of oocysts per infected mosquito; however, all doses tested significantly affected oocyst development (Table 3). Doses of 25–100 mg/kg resulted in a significant reduction in the percentage of mosquitoes that developed salivary gland infections. In contrast, doses of 3.125–100 mg of WR-250547/kg significantly affected the percentage of mosquitoes with oocysts; however, no reduction in the number of oocysts per mosquito was observed. Doses of 0.39–100 mg/kg significantly inhibited both oocyst development and sporozoite invasion of salivary glands (Table 4), but a lower dose (0.195 mg/kg) had no effect on any stage of sporogonic development.

DISCUSSION

The use of compounds capable of interrupting the transmission of malaria has been advocated as a means of preventing the development of drug resistance and limiting the spread of resistant parasites.[4–8] Transmission of *Plasmodium* parasites by mosquitoes can be prevented using either gametocytocidal or sporontocidal compounds; however, the particular developmental stage affected depends on the drug used. The mechanism by which a gametocytocidal and/or sporontocidal agent acts is distinct from the mechanism by which schizontocidal compounds acts.[1] In drugs that exhibit both schizontocidal and gametocytocidal/sporontocidal properties, resistance to the schizontocidal properties is not necessarily correlated with resistance to the gametocytocidal/sporontocidal properties of the compound. In other words, even if schizontocidal resistance were to emerge against a particular drug (e.g., tafenoquine), the gametocytocidal/sporontocidal activity of the drug might prevent transmission of the resistant parasites. Hypothetically, this could significantly delay the emergence and spread of resistance to these compounds.[5]

TABLE 2

Effect of tafenoquine (WR-238605) and WR-250547 on the sporogonic development of *Plasmodium vivax* in *Anopheles dirus* mosquitoes*

Treatment	Percent of mosquitoes with sporozoites (number infected/number tested)
Day 4 drug feeds	
Control	73.2 (60/82)
Tafenoquine	11.4 (9/79)†
Control	71.3 (62/87)
WR-250547	2.2 (2/90)†
Day 8 drug feeds	
Control	65.6 (59/90)
Tafenoquine	53.3 (48/90)
Control	93.1 (81/87)
WR-250547	1.1 (1/90)†
Day 11 drug feeds	
Control	75.6 (68/90)
Tafenoquine	68.9 (62.90)
Control	92.2 (83/90)
WR-250547	78.9 (71/90)
Day 16 drug feeds	
Control	65.6 (59/90)
Tafenoquine	68.9 (62/90)
Control	92.0 (65/70)
WR-250547	94.6 (53/56)

* Previously infected mosquitoes were refed 4, 8, 11, or 16 days later on mice treated 90 minutes earlier with 100 mg/kg of tafenoquine or WR-250547.

† Significantly different from controls ($P < 0.001$) using the chi-square test.

Gametocytocidal compounds interact directly with gametocytes, whereas sporontocidal compounds inhibit parasite development in infected mosquitoes.[4,8] The gametocytocidal and sporontocidal modes of action can be differentiated by exposing the parasite to a particular drug during different stages of development. Determination of gametocytocidal activity is achieved by administering a particular drug with the infectious blood meal and evaluating subsequent mosquito infection rates, whereas sporontocidal activity is assessed by exposing previously infected mosquitoes to a drug. Methods of exposing previously infected mosquitoes to a drug include dilution of the drug in the sugar solution used to maintain adult mosquitoes[11] or administration of the drug to an uninfected mouse that is then exposed to infected mosquitoes.[4–7] The advantage of the latter method is that the drug may be metabolized prior to ingestion by the mosquito.

Tafenoquine and artelinic acid are relatively new antimalarials that are currently undergoing testing in humans and non-human primates. Tafenoquine was originally developed as a replacement for primaquine because it is less toxic and more effective than primaquine.[12,13] This drug is unique among antimalarials in that it affects all stages of parasites,[13,14] including exo-erythrocytic (liver)[15–17] and erythrocytic asexual forms,[17–20] sexual (gametocytes) stages,[14,20] and sporogonic development in the mosquito.[5–7] Artelinic acid is a water-soluble, semi-synthetic derivative of artemisinin (or Qinghaosu), which is the parent compound of a completely new class of antimalarials.[21] The artemisinin compounds are fast-acting blood shizontocides with potent gametocytocidal activity.[21–24] Although they show excellent efficacy in both severe and uncomplicated malaria, dosage regimens still need to be optimized and pharmacokinetic profiles defined. In the treatment of uncomplicated malaria, the artemisinin drugs are used in combination with a long-acting antimalarial to protect both drugs against the emergence of resistance.[24,25] Artelinic acid is currently undergoing pre-clinical testing in non-human primates.

Primaquine is commonly used for the eradication of hepatic stage *P. vivax,*[11,26] while in some areas (e.g., Thailand) it may be used as a gametocytocidal agent against *P. falciparum*.[26,27] In addition, primaquine is currently being evaluated as a potential prophylactic drug.[28–30] Although primaquine does not affect the sporogonic development of *P. berghei* or *P. falciparum*,[5,6] it is not known whether it has sporontocidal activity against *P. vivax*.

TABLE 3

Effect of various doses of tafenoquine (WR-238605) on the sporogonic development of *Plasmodium vivax* in *Anopheles dirus* mosquitoes*

Treatment	Drug dose (mg/kg)	Molarity Moles/L)	Percent of mosquitoes with oocytes (n)	Mean no. of oocytes per mosquito (± SEM)	Mean oocyst diameter (± SEM)	Percent of mosquitoes with sporozoites (n)
Control	0.00	0.00000	97.8 (88/90)	55.64 (4.21)	95.25 (0.77)	97.8 (88/90)
Tafenoquine	6.25	0.00107	93.3 (84/90)	62.50 (5.51)	87.11 (1.07)†	90.0 (81/90)
Control	0.00	0.00000	84.4 (76/90)	48.07 (6.26)	83.86 (1.06)	73.2 (60/82)
Tafenoquine	12.50	0.00214	84.4 (76/90)	44.88 (4.57)	58.00 (0.98)†	54.5 (49/90)
	25.00	0.00429	80.0 (72/90)	48.69 (5.48)	41.13 (0.95)†	9.4 (8/85)‡
	50.00	0.00859	78.9 (71/90)	45.76 (4.12)	42.83 (1.02)†	16.7 (13/78)‡
	100.00	0.01710	80.0 (72/90)	64.72 (6.20)	41.82 (0.90)†	11.4 (9/70)‡

* Previously infected mosquitoes were refed 4 days later on mice treated 90 minutes earlier with various doses of the drug.
† Significantly different from controls ($P < 0.001$) using the *t*-test.
‡ Significantly different from controls ($P < 0.001$) using the chi-square test.

The dihydroacridine-dione WR-250547 has significant sporontocidal activity against *P. berghei*,[5,6] chloroquine-sensitive and multidrug-resistant *P. falciparum*,[5] and *P. vivax*.[4] Although WR-250547 was screened for antimalarial activity by the Walter Reed Army Institute of Research Antimalarial Screening Program, this compound is not currently under development as an antimalarial agent. In this study, we used WR-250547 as a positive control agent because it has documented sporontocidal activity against which the efficacy of tafenoquine and artelinic acid could be compared.

Transmission-blocking antimalarials may provide an elegant yet simple method of controlling malaria. Laboratory studies have clearly demonstrated that selected antimalarials can prevent the mosquito transmission of *P. berghei*, *P. vivax*, and drug- sensitive and multidrug-resistant strains of *P. falciparum*.[4–7] In studies conducted more than 40 years ago, mosquitoes were fed on *P. vivax*-infected patients and then maintained on sugar solutions spiked with an antimalarial agent.[11] However, use of spiked solutions does not simulate the normal route of parasite exposure to the drug because no metabolism of the drug occurs. In the model system used in this study, we expose mice to the drugs for 90 minutes prior to feeding mosquitoes on the animals, thereby allowing for absorption and metabolism of the compound by the mice. This mode of drug delivery is presumably more realistic than feeding mosquitoes on sugar pads impregnated with the drugs, although drug metabolism by mice may differ significantly from metabolism of the same drug by humans.

In contrast to most studies that have assessed sporontocidal drug activity using either rodent malaria models or gametocyte-producing *P. falciparum* cultures, we used naturally circulating isolates of *P. vivax* to evaluate drug activity. Although the heterogeneous parasite populations used in this system may result in greater variability in results, the use of naturally circulating parasite populations provides a more realistic understanding of how the drugs perform in nature. By using multiple criteria (oocyst size and numbers and sporozoite invasion of salivary glands) to assess sporontocidal activity, we get a more comprehensive view of drug activity, thereby allowing for differentiation of active and inactive compounds.

We used primaquine and the dihydroacridine-dione WR-250547 as negative and positive controls, respectively. Although Young[11] reported that primaquine exhibited sporontocidal activity against *P. vivax* when infected mosquitoes were fed on a primaquine-spiked sugar solution, previous studies in our laboratory found that primaquine had no sporontocidal activity against drug-sensitive or multidrug-resistant *P. falciparum*.[5] Data on the sporontocidal activity of primaquine in a *P. berghei* mouse model is less clear because sporontocidal activity was detected in one study[6] but not in another.[5] In this study, we found no evidence that primaquine (100 mg/kg of mouse body weight) had sporontocidal activity against *P. vivax* in *An. dirus* mosquitoes (Table 1).

Although WR-250547 is currently not under development as an antimalarial, several previous studies reported that WR-

TABLE 4

Effect of various doses of WR-250547 on the sporogonic development of *Plasmodium vivax* in *Anopheles dirus* mosquitoes*

Treatment	Drug dose (mg/kg)	Molarity (moles/L)	Percent of mosquitoes with oocytes (n)	Mean no. of oocysts per mosquito (± SEM)	Mean oocyst diameter (± SEM)	Percent of mosquitoes with sporozoites (n)
Control	0.000	0.000	94.2 (49/52)	17.16 (3.19)	72.21 (1.16)	95.9 (47/49)
WR-250547	0.195	0.040	98.1 (53/54)	25.47 (3.39)	68.37 (1.12)	94.1 (48/51)
Control	0.000	0.000	93.2 (68/73)	14.38 (1.90)	81.45 (1.03)	75.9 (44/58)
WR-250547	0.390	0.081	83.3 (60/72)	17.34 (2.50)	42.58 (1.17)†	41.0 (25/61)‡
	0.781	0.163	76.6 (49/64)	25.80 (3.63)	41.41 (1.93)†	42.9 (27/63)‡
	1.562	0.327	73.8 (45/61)	7.89 (1.50)	23.90 (1.05)†	23.6 (3/55)‡
Control	0.000	0.000	89.3 (67/75)	43.96 (7.89)	79.59 (0.87)	79.4 (50.63)
WR-250547	3.125	0.655	62.5 (50/80)‡	30.44 (4.11)	20.93 (0.82)†	7.2 (6/83)‡
Control	0.00	0.00	80.8 (63/78)	45.17 (8.39)	81.26 (0.94)	64.2 (34/53)
WR-250547	6.25	1.31	49.3 (37/75)‡	52.30 (9.26)	22.17 (1.12)†	6.5 (5/77)‡
Control	0.00	0.00	77.8 (70/90)	10.09 (1.31)	74.66 (1.03)	71.3 (62/87)
WR-250547	12.50	2.62	38.9 (35/90)‡	18.14 (4.50)	16.30 (0.26)†	1.1 (1/90)‡
	25.00	5.24	28.9 (26/90)‡	21.96 (4.80)	16.67 (0.28)†	0.0 (0/90)‡
	50.00	10.4	24.4 (22/90)‡	11.32 (3.75)	15.59 (0.30)†	0.0 (0.90)‡
	100.00	20.9	20.0 (18/90)‡	10.33 (2.53)	17.83 (1.11)†	2.2 (2/90)‡

* Previously infected mosquitoes were refed 4 days later on mice treated 90 minutes earlier with various doses of the drug.
† Significantly different from controls ($P < 0.001$) using the *t*-test.
‡ Significantly different from controls ($P < 0.001$) using the chi-square test.

250547 had significant sporontocidal activity against *P. berghei*, drug-sensitive and drug-resistant *P. falciparum*, and *P. vivax*.[4–6] The minimum inhibitory dose (MID) of WR-250547 against *P. berghei* was 0.1563 mg of base drug/kg of mouse body weight.[6] In this study, the MID was 0.39 mg/kg against *P. vivax* (Table 4); however, the mode of administration was not identical to the *P. berghei* study. These data clearly demonstrate that WR-250547 has sporontocidal activity.

A recent study suggested that artelinic acid was an ideal antimalarial candidate because it had higher plasma concentrations, higher red blood cell binding capacity, longer half-life, and lower toxicity than equivalent doses of other artemisinin derivatives.[31] This drug was developed by the Walter Reed Army Institute of Research for treatment of uncomplicated multidrug-resistant *P. falciparum* malaria; however, it is also a potent gametocytocide.[21] We injected mice with artelinic acid 90 minutes prior to feeding mosquitoes on the animals. Since the elimination half-life of artelinic acid in rats is 1.35 hours,[31] we believe that our model should have detected any sporontocidal activity. Although we did observe a reduction in the size of oocysts treated with 100 mg of artelinic acid/kg of mouse body weight, this reduction was not significant and no other phases of sporogony were affected (Table 1). Although Tripathi and others[21] demonstrated that artelinic acid was a potent gametocytocide using a *P. cynomolgi*/rhesus monkey model, they detected no sporontocidal activity.

In contrast to primaquine, tafenoquine (a primaquine analog) exhibited significant sporontocidal activity against naturally circulating strains of *P. vivax* (Tables 1–3). This data supports our previous studies with *P. berghei* and *P. falciparum* and extends it to a new species. Tafenoquine has an elimination half-life of approximately 14 days, suggesting an increased probability that sporogonic-stage parasites might be exposed to the drug (when compared with artelinic acid). Although tafenoquine was less effective than WR-250547 on a mg/kg basis (MID = 25 mg of tafenoquine/kg versus 0.39 mg of WR-250547/kg), when molarity of the compound was considered, tafenoquine was actually active at lower doses than WR-250547 (MID = 0.004 moles of tafenoquine/L versus 0.08 moles of WR-250547/L).

Neither tafenoquine or WR-250547 completely eliminated oocysts; however, oocyst development was significantly reduced even when oocyst numbers were not affected (Tables 1, 3, and 4). Some of these oocysts eventually produced sporozoites, which succeeded in invading the salivary gland. Although the ideal drug would completely prevent sporozoite invasion of salivary glands, a delay in invasion could have a significant impact on transmission rates. The extrinsic incubation (EI) period is one of the key parameters that drives transmission of any vector-borne disease.[32] Although we did not evaluate the impact of the drugs on the EI period, the incomplete reduction in the percentage of mosquitoes with sporozoites in the salivary glands may have reflected a delay in the EI period. Since even minimal increases in the EI period can have significant impacts on disease transmission, both tafenoquine and WR-250547 may be even more effective than our data would suggest.

The model system presented here and in our previous study[4] provides advantages over other models used to assess sporontocidal activity; however, this model is clearly not perfect since drug metabolism by mice may be different from that of humans. The results obtained in this study need to be confirmed with further testing in humans. Since tafenoquine is currently undergoing clinical testing in humans, it should be possible to more fully elucidate its role as a sporontocidal agent.

Received October 31, 2002. Accepted for publication January 20, 2003.

Acknowledgments: We are grateful to Drs. Gabby Zollner, James Jones, and R. Scott Miller for reviewing the manuscript and to the staff of the Mae Sod and Mae Kasa malaria clinics for their support of this study. The test compounds originated from the Chemical Synthesis Program of the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (Washington, DC).

Financial support: Funding for this project was provided by the Military Infectious Diseases Research Program of the U.S. Army Medical Research and Materiel Command, Fort Detrick (Frederick, MD).

Disclaimer: The opinions of assertions contained in this manuscript are the private ones of the authors and are not to be construed as the official or reflecting views of the Department of Defense or the Armed Forces Research Institute of Medical Sciences.

Authors' addresses: Narong Ponsa and Pattamaporn Kittayapong, Center of Vectors and Vector-Borne Diseases and Department of Biology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand. Jetsumon Sattabongkot, Nantana Eikarat, and Russell E. Coleman, United States Army Medical Component, Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand.

Reprint requests: Department of Entomology, Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand.

REFERENCES

1. World Health Organization, 1987. The Biology of Malaria Parasites. Report of a WHO Scientific Group. *World Health Organ Tech Rep Ser 743*. Geneva.
2. Gilles HM, Warrell DA, 1993. *Bruce-Chwatt's Essential Malariology.* Third edition. London: Edward Arnold, 164–195.
3. Oaks SC, Mitchell VS, Pearson GW, Carpenter CCJ, 1991. *Malaria: Obstacles and Opportunities*. Washington: National Academy Press.
4. Coleman RE, Ponsa N, Eikarat N, Kollars TM Jr, Sattabongkot J, 2001. Prevention of sporogony of *Plasmodium vivax* in *Anopheles dirus* mosquitoes by transmission-blocking antimalarials. *Am J Trop Med Hyg 65:* 214–218.
5. Coleman RE, Nath AK, Schneider I, Song G-H, Klein TA, Milhous WK, 1994. Transmission-blocking antimalarials prevent sporogony of *Plasmodium falciparum* and *P. berghei* in *Anopheles stephensi* mosquitoes. *Am J Trop Med Hyg 50:* 646–653.
6. Coleman RE, Clavin AM, Milhous WK, 1992. Gametocytocidal and sporontocidal activity of antimalarials against *Plasmodium berghei* ANKA in ICR mice and *Anopheles stephensi* mosquitoes. *Am J Trop Med Hyg 46:* 169–182.
7. Coleman RE, 1990. Sporontocidal activity of the antimalarial WR-238605 against *Plasmodium berghei* ANKA in *Anopheles stephensi. Am J Trop Med Hyg 42:* 196–205.
8. Butcher GA, 1997. Antimalarial drugs and the mosquito transmission of *Plasmodium. Int J Parasitol 27:* 975–987.
9. Teklehaimanot A, Nguyen-Dinh P, Collins WE, Barber AM, Campbell CC, 1985. Evaluation of sporontocidal compounds using *Plasmodium falciparum* gametocytes produced *in vitro. Am J Trop Med Hyg 34:* 429–434.
10. Coleman RE, Maneechai N, Yim-Amnuaychok N, Kumpitak C, Soyseng V, Miller RS, Thimasarn K, Sattabongkot J, 2002. Field evaluation of the ICT malaria Pf/Pv immunochromatographic test for the detection of asymptomatic malaria in a *Plasmodium falciparum/vivax* endemic area in Thailand. *Am J Trop Med Hyg 66:* 379–383.

11. Young MD, 1959. The effect of small doses of primaquine upon malaria infections. *Indian J Malariol 13:* 69–74.
12. Brueckner RP, Lasseter KC, Lin ET, Schuster BG, 1998. First-time-in-humans safety and pharmacokinetics of WR 238605, a new antimalarial. *Am J Trop Med Hyg 58:* 645–649.
13. Milhous WK, 2001. Development of new drugs for chemoprophylaxis of malaria. *Med Trop (Mars) 61:* 48–50.
14. Peters W, 1999. The evolution of tafenoquine—antimalarial for a new millennium? *J R Soc Med 92:* 345–352.
15. Brueckner RP, Coster T, Wesche DL, Shmuklarsky M, Schuster BG, 1998. Prophylaxis of *Plasmodium falciparum* infection in a human challenge model with WR 238605, a new 8-aminoquinoline antimalarial. *Antimicrob Agents Chemother 42:* 1293–1294.
16. Lell B, Faucher JF, Missinou MA, Borrmann S, Dangelmaier O, Horton J, Kremsner PG, 2000. Malaria chemoprophylaxis with tafenoquine: a randomised study. *Lancet 355:* 2041–2045.
17. Edstein MD, Walsh DS, Eamsila C, Sasiprapha T, Nasveld PE, Kitchener S, Rieckmann KH, 2001. Malaria prophylaxis/radical cure: recent experiences of the Australian Defence Force. *Med Trop (Mars) 61:* 56–58.
18. Cooper RD, Milhous WK, Rieckmann KH, 1994. The efficacy of WR 238605 against the blood stages of a chloroquine resistant strain of *Plasmodium vivax. Trans R Soc Trop Med Hyg 88:* 691–692.
19. Peters W, Robinson BL, Milhous WK, 1993. The chemotherapy of rodent malaria. LI. Studies on a new 8-aminoquinoline, WR 238,605. *Ann Trop Med Parasitol 87:* 547–552.
20. Obaldia N III, Rossan RN, Cooper RD, Kyle DE, Nuzum EO, Rieckmann KH, Shanks GD, 1997. WR 238605, chloroquine, and their combinations as blood schizonticides against a chloroquine-resistant strain of *Plasmodium vivax* in *Aotus* monkeys. *Am J Trop Med Hyg 56:* 508–510.
21. Tripathi R, Puri SK, Dutta GP, 1996. Sodium β-artelinate—A new potential gametocytocide. *Exp Parasitol 82:* 251–254.
22. Mehra N, Bhasin VK, 1993. *In vitro* gametocytocidal activity of artemesinin and its derivatives on *Plasmodium falciparum. Jpn J Med Sci Biol 46:* 37–43.
23. Chen PQ, Li GQ, Guo XB, He KR, Fu YX, Fu LC, Song YZ, 1994. The infectivity of gametocytes of *Plasmodium falciparum* from patients treated with artemisinin. *Chin Med J 107:* 709–711.
24. Price RN, 2000. Artemisinin drugs: novel antimalarial agents. *Expert Opin Investig Drugs 9:* 1815–1827.
25. Labbe AC, Loutfy MR, Kain KC, 2001. Recent advances in the prophylaxis and treatment of Malaria. *Curr Infect Dis Rep 3:* 68–76.
26. Lopez-Antunano FJ, 1999. Is primaquine useful and safe as true exo-erythrocytic merontocidal, hypnozoitocidal and gametocidal antimalarial drug? *Salud Publica Mex 41:* 410–419.
27. Thimasarn K, 1992. Current measures of containment of multidrug resistant falciparum malaria in Thailand. *Southeast Asian J Trop Med Public Health 23 (Suppl 4):* 139–142.
28. Jong EC, Nothdurft HD, 2001. Current drugs for antimalarial chemoprophylaxis: a review of efficacy and safety. *J Travel Med 8 (Suppl 3):* S48–S56.
29. Shanks GD, Kain KC, Keystone JS, 2001. Malaria chemoprophylaxis in the age of drug resistance. II. Drugs that may be available in the future. *Clin Infect Dis 33:* 381–385.
30. Kain KC, Shanks GD, Keystone JS, 2001. Malaria chemoprophylaxis in the age of drug resistance. I. Currently recommended drug regimens. *Clin Infect Dis 33:* 226–234.
31. Li QG, Peggins JO, Lin AJ, Masonic KJ, Trotman KM, Brewer TG, 1998. Pharmacology and toxicology of artelinic acid: preclinical investigations on pharmacokinetics, metabolism, protein and red blood cell binding, and acute and anorectic toxicities. *Trans R Soc Trop Med Hyg 92:* 332–340.
32. Institute of Medicine, 1991. *Malaria: Obstacles and Opportunities*. Oaks SC, Mitchell VS, Pearson GW, Carpenter CCJ, eds. Washington, DC: National Academy Press.