COMPARISON OF β-ARTEMETHER AND β-ARTEETHER AGAINST MALARIA PARASITES IN VITRO AND IN VIVO

MOSHE J. SHMUKLARSKY, DANIEL L. KLAYMAN, WILBUR K. MILHOUS,
DENNIS E. KYLE, RICHARD N. ROSSAN, ARBA L. AGER JR, DOUGLAS B.
TANG, MELVIN H. HEIFFER, CRAIG J. CANFIELD, AND BRIAN G. SCHUSTER

*Division of Experimental Therapeutics, and Division of Biometrics, Walter Reed Army Institute
of Research, Washington, DC; Gorgas Memorial Laboratory, Panama, Panama; Center for Tropical
Parasitic Diseases, University of Miami School of Medicine, Miami, Florida; Pharmaceutical
Systems, Inc., Gaithersburg, Maryland*

Abstract. The antimalarial activity of β-artemether and β-arteether was compared in three test systems: in vitro against chloroquine-resistant and chloroquine-sensitive *Plasmodium falciparum* parasites, in mice infected with *P. berghei*, and in *Aotus* monkeys infected with chloroquine-resistant *P. falciparum*. In vitro, the mean 50% inhibitory concentration (IC_{50}) for β-artemether was 1.74 nM (range 1.34–1.81 nM), and this value for β-arteether was 1.61 nM (range 1.57–1.92 nM). They were approximately 2.5-fold more potent than artemisinin, which had a mean IC_{50} of 4.11 nM (range 3.36–4.60 nM). In the mouse model, the 50% curative doses (CD_{50}) of β-artemether and β-arteether had a mean value of 55 mg/kg (32–78 mg/kg). The 50% effective curative doses (ED_{50}) in the *Aotus* monkey were 7.1 mg/kg (95% confidence interval [CI] = 3.7–13.5) for β-artemether and 11.8 mg/kg (95% CI = 6.5–21.3) for β-arteether. Overall, the activities of the two drugs were comparable.

Plasmodium falciparum malaria is a leading cause of morbidity and mortality in developing countries.[1] Because of the widespread resistance of malaria parasites to currently available drugs,[2, 3] there is an urgent need for new ones. Over the past decade, a novel class of compounds based on artemisinin (qinghaosu) (Figure 1a), a naturally-occurring, 15-carbon lactone endoperoxide, has been found to be effective against the erythrocytic stage of chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*.[4] When artemether, the methyl ether derivative of dihydroartemisinin (DHA) (Figure 1b) is synthesized, it yields two crystalline anomers, i.e., α- and β-artemether (Figure 1c and d, respectively). The latter anomer predominates and is marginally more active against *P. berghei*.[5] β-Artemether has been demonstrated by Chinese investigators to be effective against chloroquine-resistant *P. falciparum* malaria in humans,[6] and it is currently available for clinical use in China and a few other countries in Southeast Asia.

Early in 1985, the World Health Organization selected another derivative of DHA for development outside China. This compound was the β-ethyl ether anomer of DHA (Figure 1f), which had been originally synthesized in China and showed good activity against *P. berghei* infections in mice.[5] The choice by the World Health Organization of arteether over artemether was based on the consideration that arteether would be more lipophilic, a possible advantage for its accumulation in brain tissues in patients with cerebral malaria. It was also considered to be potentially less toxic because one of its putative metabolites would be ethanol rather than methanol.[7] In addition, the β anomer of arteether, which is a crystalline solid, was chosen because it is the predominant anomer upon synthesis and is relatively easy to separate from the α anomer, which is a liquid (Figure 1e), a potential advantage for large-scale production. The combination of the two anomers cannot be readily developed as a drug in the United States because of regulatory considerations as long as their potencies, toxicities, and pharmacokinetic properties have not been shown to be comparable. Preclinical studies have been performed jointly by the Walter Reed Army Institute of Research and the World Health Organization.

In this report, we compare the activity of β-artemether and β-arteether in three test systems that are used for preclinical evaluation of potential antimalarial compounds at the Walter Reed Army Institute of Research: the *P. falcip-*
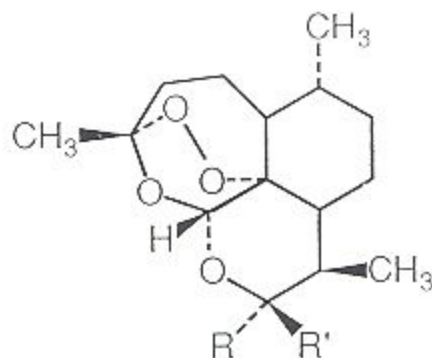
Figure 1

a   R, R' = O $=$ artemisinin

b   R = H, R' = OH
                           dihydroartemisinin
    R = OH, R' = H

c   R = OCH$_3$, R' = H   α-artemether

d   R = H, R' = OCH$_3$ β-artemether

e   R = OC$_2$H$_5$, R' = H   α-arteether

f   R = H, R' = OC$_2$H$_5$ β-arteether

FIGURE 1.   Structure of artemisinin and derivative compounds.

arum in vitro test system,[8] the *P. berghei*-mouse test system,[9] and the *P. falciparum-Aotus* monkey system.[10, 11]

MATERIALS AND METHODS

Plasmodium falciparum *in vitro test system*

The antimalarial activities of artemisinin, α and β anomers of artemether and arteether, and DHA were assessed in vitro relative to the activities of chloroquine, mefloquine, and quinine in the semiautomated microdilution method of Desjardins and others,[8] as modified by Milhous and others.[12] In this assay, the activity of a candidate compound is tested for its ability to in-

hibit radiolabeled ^{3}H-hypoxanthine uptake by chloroquine-resistant and chloroquine-sensitive clones of *P. falciparum* parasites. The drugs were tested against the Sierra Leone (D-6) clone of *P. falciparum*, which is resistant to mefloquine but sensitive to chloroquine, quinine, sulfadoxine, and pyrimethamine, and against the Indochina (W-2) and the Vietnam Smith/RE clones, which are resistant to chloroquine, quinine, sulfadoxine, and pyrimethamine but sensitive to mefloquine. Bulk drugs of 99% or greater purity, prepared by Starks Associates (Buffalo, NY), were dissolved in dimethylsulfoxide or 70% ethanol and diluted in RPMI 1640 culture medium containing 10% human plasma.

Microtiter plates, prepared with two-fold serial dilutions of the drugs over a concentration range of 0.062–256 ng/ml, and the parasite suspension (at a 0.5% parasitemia and a 1% hematocrit) were incubated at 37°C in an air-tight plexiglass box that was flushed with 5% oxygen, 5% carbon dioxide, and 90% nitrogen. After incubation for 24 hr, the cultures were labeled with ^{3}H-hypoxanthine and incubated for an additional 18–20 hr. Particulate matter was then harvested from each well by using an automated cell harvester (MACH II; Tomtec, Orange, CT). ^{3}H-Hypoxanthine that was incorporated by the parasites in each well was then measured by scintillation spectrophotometry (LKB 1205 Betaplate; Wallac, Inc., Gaithersburg, MD). All tested drugs, as well as positive and negative controls were run in duplicate.

Plasmodium berghei-*mouse test system*

The *P. berghei*-mouse test system provides a measure of the antimalarial efficacy of candidate drugs administered subcutaneously in graded doses by the response of *P. berghei* KBG 173 malaria parasites in young CD-1 Swiss mice.[9] The activity of a compound is measured by its ability to increase survival time or cure infected mice. The drugs were prepared by dissolving them in sesame oil (USP), the formulation of β-arteether intended for clinical use, or peanut oil (USP), the formulation of β-artemether in clinical use and the usual solvent used in the mouse test system. Starting at a dose of 640 mg/kg, each drug was administered in three graded doses diluted four-fold to groups of five or 10 mice per dose level. Once a drug showed activity on initial screening, it was fur-

ther tested at additional dose levels obtained by two-fold dilution. The tests were performed at successively lower doses until the lower limit of activity was reached. Young, untreated, control mice injected with a standard inoculum of *P. berghei* have a survival time of 6.8 ± 0.5 days (mean $\pm$ SD).

The curative antimalarial activity of β-artemether and β-arteether was measured in either male or female mice weighing 18–22 g that were injected intraperitoneally with 0.02 ml of heparinized blood containing with 6×10^5 erythrocytes parasitized with *P. berghei*. The drugs were administered subcutaneously 72 hr after inoculation. By that time, the infection is well established and presents candidate compounds with a standardized challenge. In each experiment, there were 15 untreated control mice and 10 pyrimethamine-treated (120 mg/kg) control mice. The control mice were included to check for infectivity of *P. berghei*, change in the susceptibility of the host, or procedural errors. Surviving mice were monitored daily for 60 days at which time they were considered cured.

Plasmodium falciparum-Aotus *monkey test system*

In this model, the activity of a compound is measured by its ability to cure adult Panamanian owl monkeys (*Aotus lemurinus lemurinus*) of both sexes infected with the Vietnam Smith/RE strain of *P. falciparum*. Fifty-eight monkeys weighing 750–950 g were randomly assigned to receive β-artemether or β-arteether dissolved in sesame oil (USP). Infections were induced in accordance with published procedures.[10] Briefly, 0.5–1.0 ml of blood was drawn from the femoral artery of an untreated monkey infected with the Vietnam Smith/RE strain of *P. falciparum*, using 2.5% sodium citrate as an anticoagulant, and diluted with 0.85% saline solution to form a suspension containing 5×10^6 trophozoites/ml. One milliliter of the suspension was then injected into the saphenous vein of the experimental and control monkeys. Beginning with the first postinoculation day, Giemsa-stained blood films were prepared and examined daily; by the fifth postinoculation day the parasitemia was well established. The drug was administered intramuscularly in three equal doses at 12-hr intervals starting on the fifth day postinoculation. These injections were admin-

istered at alternating sites along the left and right thigh muscles. The initial concentration of the drugs was 100 mg/ml; the drugs were diluted with additional sesame oil (USP), as appropriate, to yield the assigned doses. For each experimental group, several untreated monkeys were followed to verify the course of untreated infections. They were, however, treated with mefloquine (30 mg/kg of base) when they became moribund.

Parasite counts were determined by the Earle-Perez method.[13] Parasitemia was monitored daily until the thick blood films were negative for at least seven consecutive days; thereafter, blood films were monitored twice a week. If recrudescence occurred, blood films were examined daily. Treated monkeys, whose parasite counts continued to increase for several days after treatment, and monkeys whose parasitemia recrudesced were treated with mefloquine. After blood films showed no evidence of parasites for 100 days, the infection was considered cured. Response to treatment was categorized as clearance and cure, clearance and recrudescence, or suppression without clearance. The day of clearance was defined as the first of three consecutive days in which the thick blood films were parasite negative. The day of recrudescence was the first of three consecutive days of positive thick blood films after a period of clearance. Suppression was defined as a transient decrease in the parasite count post-treatment without clearance.

After a preliminary toxicity and tolerance study confirmed that a total dose of 192 mg/kg given in three divided dosages 12 hr apart was well tolerated in noninfected monkeys, additional monkeys were treated at this same dose and at lower dose levels until a dose was reached in which suppression or clearance was no longer accompanied by cure.

Data analysis

A four parameter logistic model was fitted to the radioactivity count versus the log drug concentration of each in vitro assay. The model includes a parameter, the 50% inhibitory concentration (IC_{50}), corresponding to the drug concentration yielding an expected count of radiolabeled ^{3}H-hypoxanthine midway between the upper asymptote (as the drug concentration approaches zero) and lower asymptote (expect-

ed minimum count). Parameter estimates and 95% confidence intervals (CI) were obtained using iterative least square (PCONLINE, Version 3.0, SCI Software; Statistical Consultants, Inc.. Lexington, KY). Both nonweighted (constant weight) and reweighted (weight = 1/expected count) estimates were obtained. Nonweighted results are reported. Use of the reciprocal count as weight gave almost identical parameter estimates for the IC_{50}, with a somewhat smaller standard error. Expressions of potency of one compound relative to another were based on the ratio of the corresponding estimated IC_{50} values. The potency of artemisinin was used as an index, i.e., potency of 1.0, in terms of which all other potencies were expressed.

For quantal response data (in vivo assays), maximum likelihood (iterative probit analysis) was used to estimate the dose (mg/kg) required to produce a 50% cure rate (CD_{50}) in mice and a 50% efficacy rate (ED_{50}) in monkeys, and corresponding 95% CI.[14, 15] Within each in vivo test system, the overall parallelism of dose response curves (percent cure versus log dose) was tested. If no significant departure from parallelism was detected, reported potencies and 95% CI were based on the estimated common slope. Data on time to clearance and time to recrudescence are reported as means and standard deviations.

These investigations were conducted in compliance with the Animal Welfare Act and Federal statutes and regulations relating to animals and experiments involving animals, and adhered to principles stated in the 1985 *Guide for the Care and Use of Laboratory Animals*, NIH Publication 85-23.

RESULTS

Plasmodium falciparum *in vitro test system*

The β anomers of artemether and arteether showed comparable activity against both drug-sensitive and drug-resistant *P. falciparum* clones in the in vitro test system (Table 1). The mean IC_{50} of β-artemether was 1.74 nM (range 1.34–1.81 nM), and the corresponding value for β-arteether was 1.61 nM (range 1.57–1.92 nM). They were approximately 2.5-fold more active than artemisinin, whose mean IC_{50} was 4.11 nM (range 3.4–4.6 nM). The activities of the α and β anomers of artemether and arteether were also comparable, although the data suggest that the

TABLE 1

In vitro 50% inhibitory concentration (IC_{50}) of artemisinin, selected derivatives, and other antimalarial drugs against chloroquine-resistant and chloroquine-sensitive Plasmodium falciparum parasites*

Compound	Mol. wt.	W-2 Indochina clone			D-6 Sierra Leone clone			Vietnam Smith/RE clone		
		IC_{50} (nM)	IC_{50} (95% CI)	Relative potency	IC_{50} (nM)	IC_{50} (95% CI)	Relative potency	IC_{50} (nM)	IC_{50} (95% CI)	Relative potency
Artemisinin	282	3.36	1.36–5.37	1.0	4.60	4.29–4.92	1.0	4.36	4.09–4.63	1.0
α-Artemether	298	1.21	1.11–1.31	2.8	1.84	1.62–2.07	2.5	1.53	1.48–1.58	2.9
β-Artemether	298	1.34	1.15–1.53	2.5	1.68	1.43–1.92	2.7	1.81	1.74–1.88	2.4
α-Arteether	312	1.63	1.38–1.89	2.1	2.98	2.69–3.26	1.5	2.24	2.10–2.38	1.9
β-Arteether	312	1.57	1.16–1.97	2.1	1.73	1.61–1.84	2.7	1.92	1.81–2.03	2.3
Dihydroartemisinin	284	0.88	0.33–1.43	3.8	0.88	0.83–0.93	5.2	1.02	0.79–1.25	4.3
Chloroquine diphosphate	516	141	126–157	0.02	4.34	3.25–5.43	1.1	142	129–155	0.03
Mefloquine HCl	415	7.23	5.94–8.53	0.47	19.05	17.02–21.07	0.24	16.73	13.5–20.1	0.26
Quinine citrate	516	232	200–264	0.01	19.15	15.62–22.68	0.24	250	158–342	0.02

* CI = confidence interval. The relative potency is the ratio of the IC_{50} of artemisinin divided by the IC_{50} of the alternate compound.

TABLE 2

Fifty percent curative doses (CD$_{50}$) of β-artemether and β-arteether given subcutaneously in a single dose to mice infected with Plasmodium berghei*

Compound	Formulation	No. of mice	Sex	CD$_{50}$ (mg/kg)	95% CI
β-Artemether	Sesame oil	70	F	49	33–72
β-Artemether	Sesame oil	70	M	73	50–107
β-Arteether	Peanut oil	60	F	51	33–80
β-Arteether	Peanut oil	30	M	78	43–141
β-Arteether	Sesame oil	70	F	46	31–68
β-Arteether	Sesame oil	70	M	32	21–48

* CI = confidence interval.

α anomer of arteether may be somewhat less potent.

β-Arteether was approximately five-fold more potent than mefloquine and approximately 90-fold more potent than chloroquine against the chloroquine-resistant W-2 clone of *P. falciparum* parasites. β-Arteether was also more active than mefloquine and chloroquine against the the Vietnam Smith/RE clone of *P. falciparum* parasites by nine-fold and 77-fold, respectively. It was approximately 11-fold more potent than mefloquine and approximately 2.5-fold more potent than chloroquine against the chloroquine-sensitive D-6 clone.

Interestingly, DHA consistently showed the greatest activity among the compounds tested. It had a mean IC$_{50}$ of approximately 0.9 nM (range 0.88–1.02 nM), which exceeds the activities of artemisinin by approximately 4.5-fold and β-arteether by approximately two-fold.

Plasmodium berghei-*mouse test system*

β-Artemether and β-arteether showed comparable efficacy against *P. berghei* whether dissolved in peanut or sesame oil (β-arteether) or only in sesame oil (β-artemether) and whether administered to male or female mice. These compounds had a mean CD$_{50}$ value of 55 mg/kg (range 32–78 mg/kg) (Table 2).

Plasmodium falciparum-Aotus *monkey test system*

Untreated control monkeys typically experienced an increase in parasitemia with a maximum level 10–12 days after inoculation. Monkeys treated with curative doses of either β-artemether or β-arteether characteristically showed a precipitous decrease in the parasite count within one to two days after initiation of treatment. By one week after treatment, the parasites were usually barely detectable. Monkeys treated with subcurative doses showed a transient decrease in the parasite count that lasted several days after which the parasitemia resumed its upward trend. The typical rates of decrease of parasitemia for β-artemether and β-arteether were indistinguishable. The clearance times for β-artemether and β-arteether were 8.3 ± 1.7 and 7.5 ± 1.9 days (mean ± SD), respectively. Although parasitemia was suppressed in all monkeys receiving a total dose of 0.75 mg/kg of either β-artemether or β-arteether, none were cured (Table 3). In monkeys receiving 3 mg/kg, parasitemia cleared in four of four monkeys treated with β-arteether and in three of four treated with β-artemether; however, only one infection treated with β-arteether was cured. In all monkeys receiving 12 mg/kg, parasitemia cleared but recrudesced in two of eight monkeys treated with β-artemether and in four of eight treated with β-arteether. Parasitemia cleared in all monkeys treated with 24 mg/kg but recrudesced in two of four treated with β-arteether. At dosages of 48 mg/kg and 192 mg/kg, all infections were cured. The number of days to recrudescence for both drugs were comparable: 16.6 ± 5.5 (mean ± SD) for β-artemether and 16.2 ± 3.6 for β-arteether. The ED$_{50}$ for β-artemether was 7.1 mg/kg (95% CI = 3.7–13.5 mg/kg) and 11.8 mg/kg (95% CI = 6.5–21.3 mg/kg) for β-arteether. Although the potency of β-artemether (when the ED$_{50}$ values are expressed in mg/kg) was approximately 1.66-fold (95% CI = 0.71–3.92) greater than that of β-arteether, (or 1.74-fold [95% CI = 0.74–4.10] greater when the ED$_{50}$ values are expressed in

TABLE 3

Efficacy and 50% percent effective curative doses (ED$_{50}$) of β-arteether and β-artemether in Aotus *monkeys infected with the* Plasmodium falciparum *Vietnam Smith/RE strain**

Total dose (mg/kg)†	No. of monkeys	Response to treatment				
		No. suppressed	No. cleared	Days to clearance (mean ± SD)	No. cured	No. recrudesced
β-Arteether						
0.75	4	4	0	NA	0	0
3	4	0	4	7.25 ± 0.50	1	3
12	8	0	8	7.75 ± 1.75	4	4
24	4	0	4	5.00 ± 0.00	2	2
48	4	0	4	8.75 ± 2.06	4	0
192	5	0	5	8.40 ± 2.19	5	0
Total	29	4	25	7.5 ± 1.9	16	9‡
β-Artemether						
0.75	4	4	0	NA	0	0
3	4	1	3	8.75 ± 1.26	0	3
12	8	0	8	7.27 ± 0.46	6	2
24	4	0	4	8.75 ± 1.26	4	0
48	4	0	4	8.25 ± 2.50	4	0
192	5	0	5	9.60 ± 1.95	5	0
Total	29	5	25	8.3 ± 1.7	19	5§

* For β-arteether, ED$_{50}$ = 11.8 mg/kg (confidence interval [CI] = 6.5–21.3) or 39.5 μmol/kg (CI = 21.9–71, 4); for β-artemether, ED$_{50}$ = 7.1 mg/kg (CI = 3.7–13.5) or 22.7 μmol/kg (CI = 11.9–43.2). NA = not applicable.
† Administered intramuscularly in sesame oil in three equal doses at 12-hr intervals.
‡ Day of recrudescence = 16.2 ± 3.6 days from last day of drug dosing.
§ Day of recrudescence = 16.6 ± 5.5 days from last day of drug dosing.

μmol/kg), the differences between the two drugs were not statistically significant. The monkeys tolerated the drugs well at all dose levels.

DISCUSSION

Tripathi and others recently reported on the comparative efficacy of the blood schizontocidal activity of α, β, and α/β-arteether (a 30:70 mixture of anomers) against *P. cynomolgi* B infection in the rhesus monkey.[16] They concluded that β- and α/β-arteether have comparable activity and are curative at a total dose of 15 mg/kg given in three equally divided doses for three days; they also concluded that α-arteether was slightly less active. Our findings are generally consistent with theirs. In vitro, the α anomer of arteether had somewhat lower activity than the β anomer. Otherwise, the α and β anomers of artemether and the β anomer of arteether showed overall comparable activity. The two in vivo efficacy models also showed that the β anomers of artemether and arteether have comparable activity against malaria parasites.

These findings are important for further clinical development of β-arteether. First, since the Aotus monkey model is considered to be the best primate model for experimental treatment of blood-induced infections of human *P. falciparum*,[17] it provides good predictability for the potential efficacy of β-arteether as an antimalarial agent in humans. Second, the fact that β-artemether has already been shown to be highly effective in patients with malaria[4,6] provides further support for our belief that the in vivo results can be generalized to the activity of these compounds in human malaria. It is also interesting to note that the ED$_{50}$ of β-artemether of 7.1 mg/kg in the *Aotus* monkey is comparable to the clinically effective curative dose of β-artemether in humans of 8.6–12 mg/kg (in a 50-kg human receiving a dose of 480–600 mg intramuscularly in divided dose for several days).[6,18,19] Considering that the two compounds are closely related chemically and that their activities in our test systems are comparable, it is reasonable to conclude that they are likely to be equipotent in humans. This conclusion is consistent with the high likelihood that a major contributor to the activity of both drugs in vivo may be a common metabolite such as DHA. That DHA is a major metabolite of β-ar-

teether has already been demonstrated in vitro[20] and in vivo[21] in our laboratory. The optimal dosing regimen of β-artemether in humans, as determined from the cumulative clinical experience with that drug, could be an excellent guide in choosing the dosing regimen for β-arteether in early efficacy studies in humans.

Authors' addresses: Moshe J. Shmuklarsky, Daniel L. Klayman (deceased), Wilbur K. Milhous, Melvin H. Heiffer, and Brian G. Schuster, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC 20307-5100. Dennis E. Kyle, Department of Immunology and Parasitology, AFRIMS, APO AP 96546. Richard N. Rossan, PSC #02, Box 2209, APO AA 34002. Arba L. Ager Jr, Center for Tropical Parasitic Diseases, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33177. Douglas B. Tang, Division of Biometrics, Walter Reed Army Institute of Research, Washington DC 20307-5100. Craig J. Canfield, Pharmaceutical Systems, Inc. 927-B North Russell Avenue, Gaithersburg, MD 20879.

Reprint requests: Moshe J. Shmuklarsky, Department of Pharmacology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC 20307-5100.

REFERENCES

1. Wyler DJ, 1983. Malaria-resurgence, resistance and research. N Engl J Med 308: 875–878, 934–940.
2. Peters W, 1982. Antimalarial drug resistance: an increasing problem. Br Med Bull 38: 187–192.
3. Wernsdorfer WH, Kouznetsov RL, 1980. Drug-resistant malaria-occurrence, control, and surveillance. Bull World Health Organ 58: 341–352.
4. Klayman DL, 1985. Qinghaosu (artemisinin): an antimalarial drug from China. Science 228: 1049–1055.
5. China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials, 1982. The chemistry and synthesis of qinghaosu derivatives. J Trad Chin Med 2: 9–16.
6. China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials, 1982. Clinical studies on the treatment of malaria with qinghaosu and its derivatives. J Trad Chin Med 2: 45–50.
7. Brossi A, Venugopalan B, Dominguez Gerpe L, Yeh HJC, Flippen-Anderson JL, Buchs P, Luo XD, Milhous W, Peters W, 1988. Arteether, a new antimalarial drug: synthesis and antimalarial properties. J Med Chem 31: 645–650.
8. Desjardins RE, Canfield CJ, Haynes DE, Chulay JD, 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob Agents Chemother 16: 710–718.
9. Osdene TS, Russell PB, Rane L, 1967. 2,4,7-Triamino-6-ortho-substituted arylpteridines. A new series of potent antimalarial agents. J Med Chem 10: 431–434.
10. Schmidt LH, 1973. Infections with Plasmodium falciparum and Plasmodium vivax in the owl monkey-model systems for basic biological and chemotherapeutic studies. Trans R Soc Trop Med Hyg 67: 446–474.
11. Schmidt LH, 1978. Plasmodium falciparum and Plasmodium vivax infections in the owl monkey (Aotus trivirgatus). III. Methods employed in the search for new blood schizonticidal drugs. Am J Trop Med Hyg 27: 718–737.
12. Milhous WK, Weatherley NF, Bowdre JH, Desjardins RE, 1985. In vitro activities of and mechanisms of resistance to antifol antimalarial drugs. Antimicrob Agents Chemother 27: 525–530.
13. Earl WC, Perez M, 1932. Enumeration of parasites in the blood of malaria patients. J Lab Clin Med 17: 1124–1130.
14. Finney DJ, 1978. Statistical Methods in Biological Assay. New York: Macmillan.
15. SAS Institute Inc., 1989. SAS/STAT User's Guide, Version 6. Fourth Edition, Volume 2. Gary, NC: SAS Institute, Inc.
16. Tripathi R, Dutta GP, Vishwakarma RA, 1991. Comparison of antimalarial efficacy of α, β, and α/β arteether against Plasmodium cynomolgi B infection in monkeys. Am J Trop Med Hyg 44: 560–563.
17. Rossan RN, Harper JS, Davidson DE, Escajadillo A, Christensen HA, 1985. Comparison of Plasmodium falciparum infections in Panamanian and Colombian owl monkeys. Am J Trop Med Hyg 34: 1034–1047.
18. Pe Than Myint, Tin Shwe, 1986. The efficacy of artemether (qinghaosu) in Plasmodium falciparum and P. vivax in Burma. Southeast Asian J Trop Med Public Health 17: 19–22.
19. Pe Than Myint, Tin Shwe, 1987. A controlled clinical trial of artemether (qinghaosu derivative) versus quinine in complicated and severe falciparum malaria. Trans R Soc Trop Med Hyg 81: 559–561.
20. Leskovac V, Theoharides AD, 1991. Hepatic metabolism of artemisinin drugs. I. Drug metabolism in rat liver microsomes. Comp Pharmacol Toxicol 99: 383–390.

21. Melendez V, Peggins JO, Brewer TG, Theoharides AD, 1991. Determination of the antimalarial arteether and its deethylated metabolite dihydroartemisinin in plasma by high-performance liquid chromatography with reductive electrochemical detection. *J Pharm Sci 80:* 132–138.