

## Evaluation of Artemisone Combinations in *Aotus* Monkeys Infected with *Plasmodium falciparum*<sup>†</sup>

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Artemisone (single oral dose, 10 mg/kg of body weight) cured nonimmune *Aotus* monkeys of their *Plasmodium falciparum* infections when combined with mefloquine (single oral dose, 5 and 10 mg/kg but not 2.5 mg/kg). In combination with amodiaquine (20 mg/kg/day), artemisone (10 mg/kg/day) given orally for 3 days cured all infected monkeys. Three days of treatment with artemisone (30 mg/kg/day) and clindamycin (100 mg/kg/day) was also curative.

Increasing parasite resistance to standard antimalarial drugs is encouraging more widespread use of artemisinin and some of its derivatives—artesunate, artemether, and dihydroartemisinin—for the treatment of malaria. Although they act more rapidly than other antimalarials, 3-day monotherapy courses are associated with a high parasite recrudescence rate, presumably due to the short pharmacological half-lives of the artemisinins. Patient compliance with longer, curative treatment courses is poor, especially in malarious areas with limited health infrastructures. Shorter 3-day courses are curative only when combined with other less-effective but longer-acting drugs. Artemisinin-based combination therapy, administered for 3 days, has now become the latest tool for curing multidrug-resistant malaria infections and retarding the development of drug resistance (1, 18).

Artemisone (BAY 44-9585) (ASO), a 10-alkylaminoartemisinin, is a new artemisinin derivative that is being developed according to international drug regulatory standards (5). In contrast to some artemisinins (2, 22), ASO displays low lipophilicity and negligible neuro- and cytotoxicity in *in vitro* and *in vivo* assays (6, 12). Compared to artesunate, the most widely used artemisinin derivative, ASO shows greater activity (5, 16, 21) in the Peters 4-day test rodent-*Plasmodium berghei* model (15) and against multidrug-resistant isolates of *Plasmodium falciparum* both *in vitro* (4) and in the *ex vivo* bioassay *Saimiri/Aotus* monkey model (8). Preliminary studies at the Army Malaria Institute (AMI) Australia also showed that malaria-infected *Aotus* monkeys clear parasites faster after a 3-day course of ASO (total dosage, 30 mg/kg of body weight) than after the same course of artesunate and, although only one of four monkeys was cured, parasite recrudescences tended to occur later in the ASO-treated monkeys (6).

These encouraging results prompted AMI to determine whether the addition of mefloquine (MQ) to ASO might cure

infected monkeys. Since many patients are not cured of their malaria infections because they fail to complete their treatment course, studies were initiated with short 1-day courses of treatment. After approval by the AMI Animal Ethics Committee (approval no. 03/2000), naive *Aotus* monkeys, weighing between 0.68 and 1.29 kg, were inoculated intravenously with  $1 \times 10^6$  to  $6 \times 10^6$  parasites of the FVO strain of *P. falciparum*, which is resistant to chloroquine and quinine but susceptible to pyrimethamine and MQ. Three to four days after the onset of patency, parasite counts had reached between  $29.2 \times 10^3$  and  $325.0$  (median, 60.0)  $\times 10^3/\mu\text{l}$  blood. Monkeys then received various 1-day regimens of ASO alone or ASO in combination with MQ by being allowed to swallow drugs suspended in orange juice by slow expulsion from a syringe. After treatment, thick blood smears were examined daily by counting parasites against 500 leukocytes. When no parasites were detected in 200 microscopic fields, follow-up blood smears were examined twice a week for at least 60 days after treatment. Body weights, hematocrit values, and leukocyte and platelet counts were monitored carefully after treatment, and any monkeys not responding appropriately to treatment were cured of their infections with MQ (20 mg/kg). The results from AMI are summarized in Table 1.

One-day treatment of two monkeys with ASO (10 mg/kg) every 2 h on two or three occasions (total dosage, 20 or 30 mg/kg) produced a 300- to 2,000-fold reduction in parasite counts but did not clear parasitemia, indicating that multiple doses on 1 day are not as effective as the same total dosage administered over 3 days (6). When a single dose of MQ (2.5, 5.0, or 12.5 mg/kg) was added to ASO (10 mg/kg), all four monkeys cleared parasitemia by day one. Except for the monkey receiving the lowest MQ dosage, all monkeys were cured. It is noteworthy that the dosage of 5 mg/kg is equivalent to 100 mg MQ administered to a 60-kg human adult, using a 3:1 ratio to convert the body surface area from monkey to human (5). Since this is far below the curative MQ dose, this drug combination may prove useful in areas with low malaria transmission, with or without MQ resistance (3, 13, 23). It is likely to be less useful in areas with high levels of malaria transmission

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TABLE 1. Responses of six malaria-infected monkeys to 1-day treatment with multiple doses of ASO alone or single doses of ASO combined with MQ

Drug(s) <sup>a</sup>	Dosage (mg/kg)	No. of monkeys		Parasite recrudescence (days)
		Treated	Cured	
ASO	20	1	0	No clearance
ASO	30	1	0	No clearance
ASO + MQ	10, 2.5	1	0	24
ASO + MQ	10, 5	2	2	None
ASO + MQ	10, 12.5	1	1	None

<sup>a</sup> All drugs given orally at AMI.

(e.g., Africa) because MQ's very long persistence in the body (20) would favor the rapid development of MQ resistance in reinfecting patients.

With this in mind, studies were approved by Gorgas Memorial Institutional Animal Care and Use Committee (approval no. 2000/02) to assess two less-persistent, potential partner drugs, artemisinin (AQ) and clindamycin (CM), at the Tropical Medicine Research, Malaria Drug and Vaccine Evaluation Center/Gorgas Memorial Institute of Health Studies (TMR/ICGES) Panama, using a larger group of monkeys than was available at AMI. Although AQ is a 4-aminoquinoline drug, parasites are often less resistant to it than to chloroquine (14, 19, 24). It is less expensive than most other antimalarial drugs and is now being used increasingly in Africa in combination with either artesunate (7, 11) or sulfadoxine-pyrimethamine (11, 25). Although CM is more expensive, parasite resistance to this short-acting antibiotic is unknown, and in combination with artesunate, quinine, or chloroquine, it can be used safely to treat children and pregnant women (9, 10, 17). Experimental conditions at TMR/ICGES were essentially similar to those at AMI, but drugs were administered by gastric intubation and treatment was usually started at lower parasite counts, ranging from  $4.5 \times 10^3$  to  $71.1$  (median =  $21.6$ )  $\times 10^3/\mu\text{l}$  blood. The results from TMR/ICGES are summarized in Table 2.

One-day treatment with a single noncurative dose of ASO (30 mg/kg) plus AQ (20 mg/kg) cleared parasitemia but failed to cure any of the three monkeys. Two-day treatment with ASO (30 mg/kg/day) plus AQ (30 mg/kg/day) cured two of three monkeys. Three-day treatment combining ASO (10 mg/kg/day) with AQ (20 mg/kg/day) cured all three monkeys receiving this drug regimen; however, both drugs given alone for 3 days failed to cure six of six monkeys. Further studies with ASO (30 mg/kg/day) and CM (100 mg/kg/day) showed that 3 days of treatment with this drug combination cured three of three monkeys whereas one of two monkeys was not cured using ASO (30 mg/kg/day) alone.

Our results show that administering ASO plus AQ for 3 days was curative at an AQ dosage comparable to that employed for treating malaria patients (using a 3:1 conversion factor) and at a daily ASO dosage that was one-third of that used in the 1- or 2-day treatment course. Considering these results in conjunction with previous findings at AMI (6), it is clear that various total dosages (20 to 90 mg/kg) of ASO alone, administered over 1 to 3 days, are unable to cure nonimmune monkeys. However, monkeys can be cured when ASO is combined with

TABLE 2. Responses of 12 malaria-infected monkeys to 1-, 2-, or 3-day treatment with ASO in combination with AQ or CM; 11 other monkeys were treated with either AQ or ASO alone

No. of days treated	Drug(s) <sup>a</sup>	Dosage (mg/kg)		No. of monkeys		Parasite recrudescence (days)
		Daily	Total	Treated	Cured	
1	ASO + AQ	30, 20	30, 20	3	0	11, 11, 12
	AQ	20	20	3	0	No clearance
2	ASO + AQ	30, 30	60, 60	3	2	19
3	ASO + AQ	10, 20	30, 60	3	3	None
	AQ	20	60	3	0	9, 11, 16
	ASO	10	30	3	0	17, 17, 19
3	ASO + CM	30, 100	90, 300	3	3	None
	ASO	30	90	2	1	22

<sup>a</sup> All drugs given orally at TMR/ICGES.

a single, subcurative dose of MQ or with a 3-day treatment course of either AQ or CM.

The results of our studies, using the *Actus-P. falciparum* model, indicate the need for investigations with humans to determine optimum drug regimens for treating falciparum infections. A phase I clinical trial has shown ASO to be well tolerated and safe in healthy subjects (12). With the possible exception of MQ, it is likely that ASO combinations will have to be given for 3 days if a cure is to be achieved in most patients having little or no immunity to falciparum malaria. In view of ASO's efficacy and low toxicity (6), ASO combination therapy could become a very important addition to our sparse armamentarium against drug-resistant malaria.

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