Evaluation of Artemisone Combinations in Aotus Monkeys Infected with Plasmodium falciparum


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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 chloroquine (100 mg/kg/day) was also curative.

Increasing parasite resistance to standard antimalarial drugs is encouraging more widespread use of artemisone and some of its derivatives—artesunate, arteether, 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 reduction rate, presumably due to the short pharmacokinetics of the artemisone. 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 become the latest tool for curing multidrug-resistant malaria infections and retarding the development of drug resistance (1, 2).

Artemisone (BAY 44-9583) (ASO), a 15-alkylaminosteroidal, is a new artemisinin derivative that is being developed according to international drug regulatory standards (3). In contrast to some artemisones (1, 2, 22), ASO displays low lipophilicity and good tolerability in mice and in vivo assays (3, 19). Compared to artesunate, the most widely used artemisinin derivative, ASO shows greater activity (3, 10, 21) in the Peters 4-day test and more Plasmodium berghei model (11) and against multidrug-resistant clones of Plasmodium falciparum both in vitro and in the two in vivo test systems Aotus monkey model (9).

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. 13/2009), some Aotus monkeys weighing between 0.88 and 1.28 kg, were inoculated intravenously with 1 x 10⁵ to 6 x 10⁵ 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 parasitemia, parasite counts had reached between 252 x 10⁵ and 925 x 10⁵/μl blood. Monkeys 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 infusion from a syringe. After treatment, thick blood smears were examined daily by counting parasites against 500 leukocytes. When no parasites were detected in 500 microscopic fields, follow-up blood smears were examined twice a week for at least 60 days after treatment. Body weights, hemoglobin 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 900- to 2000-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 6:1 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.
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.

<table>
<thead>
<tr>
<th>Drug(1)</th>
<th>Dose (mg/kg)</th>
<th>No. of monkeys</th>
<th>Paracetamol response</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASO</td>
<td>20</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ASO</td>
<td>50</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>ASO + M3</td>
<td>10, 25</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ASO + M2</td>
<td>10, 25</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ASO</td>
<td>12.5</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

(1) All drugs given orally at AMI.

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

<table>
<thead>
<tr>
<th>No. of days treated</th>
<th>Drug(1)</th>
<th>Dose (mg/kg)</th>
<th>No. of monkeys</th>
<th>Paracetamol response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ASO + AQ</td>
<td>30, 20</td>
<td>3</td>
<td>1, 1, 1</td>
</tr>
<tr>
<td>2</td>
<td>AQ</td>
<td>30</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>ASO + AQ</td>
<td>30, 30</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>AQ</td>
<td>60</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>AQ</td>
<td>60</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>AQ + CM</td>
<td>50, 100</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

(1) All drugs given orally at TMRC/CGES.

(2) MQ very long persistence in the body, (20) cure with MQ in the rapid development of MQ resistance in infected patients.

With this in mind, studies were approved by Corgas Memorial Institute for the Investigation and Prevention of Malaria, and the Tropical Medicine Research Center, and have been reviewed and approved by the Institution for Health Studies (TMRC/CGES), Panama, using a larger group of monkeys than was available at AMI. Although AQ is a 4-aminoquinoline drug, its resistance profile, and a lack of resistance to other antimalarial drugs, is not limited to chloroquine (14, 20, 24). Although AQ is more expensive, parenteral resistance to this drug is noted in some susceptible species, and in combination with an antimalarial agent, a lower dose of AQ can be used safely to treat children and pregnant women (9, 10, 11). Experimental conditions at TMRC/CGES were essentially similar to those at AMI, but drugs were administered by gastric intubation and treatment was initiated at lowest possible doses, ranging from 4.5 x 10^{-3} to 6.1 (median = 6.75) mg/kg. The results from TMRC/CGES are summarized in Table 2.

A 1-day treatment with a single non-curative dose of ASO (30 mg/kg) plus AQ (20 mg/kg) cleared parasites but failed to cure any of the three monkeys. Two-day treatment with ASO (30 mg/kg) plus AQ (30 mg/kg) cured one of the three monkeys. Three-day treatment combining ASO (30 mg/kg) with AQ (20 mg/kg) cured all three monkeys receiving this drug regimen, however, both drugs alone failed to cure any of the six monkeys. Further studies with ASO (30 mg/kg) and CM (100 mg/kg) showed that 3 days of treatment with this drug combination cured three of the monkeys whereas one of two monkeys was not cured using ASO (30 mg/kg) alone.

The results of our studies, using the Aedes aegypti model, indicate the need for investigations with humans to determine optimum drug regimens for treating malaria in Africa. A 1-day 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 in a single course in order to be most potent against Plasmodium falciparum. In view of ASO's efficacy and low toxicity (6), ASO combination therapy could become a very important addition to our armamentarium against drug-resistant malaria.

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REFERENCES


