Comparison of β-Artemether and β-Arteether Against Malaria Parasites In Vitro and In Vivo


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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 (IC50) 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 has a mean IC50 of 4.11 nM (range 3.36-4.60 nM). In the mouse model, the 50% curative dose (CD50) of β-artemether and β-arteether had a mean value of 55 mg/kg (32-78 mg/kg). The 50% effective doses (ED50) 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.
COMPARISON OF β-ARTEMETHER AND β-ARTEETHER AGAINST MALARIA PARASITES IN VITRO AND IN VIVO

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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 (IC₅₀) 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 IC₅₀ of 4.11 nM (range 3.36–4.60 nM).

In the mouse model, the 50% curative doses (CD₅₀) of β-artemether and β-arteether had a mean value of 55 mg/kg (32–78 mg/kg). The 50% effective curative doses (ED₅₀) 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. Because of the widespread resistance of malaria parasites to currently available drugs, there is an urgent need for new ones. Over the past decade, a novel class of compounds based on artemisinin (qinghaosu) (Figure Ia), 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. When artemether, the methyl ether derivative of dihydroartemisinin (DHA) (Figure Ib) is synthesized, it yields two crystalline anomers, i.e., α- and β-artemether (Figure Ic and d, respectively). The latter anomer predominates and is marginally more active against P. berghei. β-Artether has been demonstrated by Chinese investigators to be effective against chloroquine-resistant P. falciparum malaria in humans, 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 If), which had been originally synthesized in China and showed good activity against P. berghei infections in mice. 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. In addition, the β anomer of artemether, 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 Ic), 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-
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 1.5% 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.

**Figure 1.** Structure of artemisinin and derivative compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
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<tbody>
<tr>
<td>a</td>
<td>R, R' = O === artesinin</td>
</tr>
<tr>
<td>b</td>
<td>R = H, R' = OH dihydroartemisinin</td>
</tr>
<tr>
<td></td>
<td>R = OH, R' = H</td>
</tr>
<tr>
<td>c</td>
<td>R = OCH$_3$, R' = H α-artemether</td>
</tr>
<tr>
<td>d</td>
<td>R = H, R' = OCH$_3$ β-artemether</td>
</tr>
<tr>
<td>e</td>
<td>R = OC$_2$H$_5$, R' = H α-arteether</td>
</tr>
<tr>
<td>f</td>
<td>R = H, R' = OC$_2$H$_5$ β-arteether</td>
</tr>
</tbody>
</table>

Plasmodydia 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* K362 malaria parasites in young CD-1 Swiss mice. 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 0-artemether intended for clinical use, and peanut oil (USP), the formulation of 1-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 $6.8 \pm 0.5$ days
(mean ± SD).

The curative antimalarial activity of β-artem-
ether and β-arteether was measured in either
male or female mice weighing 18–22 g that were
injected intraperitoneally with 0.02 ml of hepa-
rinized blood containing with $6 \times 10^5$ eryth-
rocytes 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 experi-
ment, 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 sus-
cceptibility of the host, or procedural errors. Sur-
viving 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 Panaman-
ian 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. Brief-
ly, 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. falci-
parum, using 2.5% sodium citrate as an antico-
agulant, and diluted with 0.85% saline solution
to form a suspension containing $5 \times 10^6$ tro-
phozoites/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 exam-
ined 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 appropri-
ate, to yield the assigned doses. For each ex-
perimental 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 be-
came moribund.

Parasite counts were determined by the Earle-
Perez method. 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 ex-
amined daily. Treated monkeys, whose parasite
counts continued to increase for several days
after treatment, and monkeys whose parasit-
emia recrudesced were treated with mefloquine.
After blood films showed no evidence of para-
sites for 100 days, the infection was considered
cured. Response to treatment was categorized
as clearance and cure, clearance and recrudes-
cence, 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 recrudes-
cence was the first of three consecutive days of
positive thick blood films after a period of clear-
ance. Suppression was defined as a transient de-
crease 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, addi-
tional 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 con-
centration of each in vitro assay. The model
includes a parameter, the 50% inhibitory con-
centration (IC$_{50}$), corresponding to the drug
concentration yielding an expected count of ra-
diolabeled $^3$H-hypoxanthine midway between
the upper asymptote (as the drug concentration
approaches zero) and lower asymptote (expect-
Parameter estimates and 95% confidence intervals (CI) were obtained using iterative least square (PONLINE, 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\textsubscript{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\textsubscript{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\textsubscript{50}) in mice and a 50% efficacy rate (ED\textsubscript{50}) in monkeys, and corresponding 95% CI.\textsuperscript{14} 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 \(\beta\) anomers of artemether and arteether showed comparable activity against both drug-sensitive and drug-resistant \textit{P. falciparum} clones in the in vitro test system (Table 1). The mean IC\textsubscript{50} of \(\beta\)-artemether was 1.74 nM (range 1.34–1.81 nM), and the corresponding value for \(\beta\)-arteether was 1.61 nM (range 1.57–1.92 nM). They were approximately 2.5-fold more active than artemisinin, whose mean IC\textsubscript{50} was 4.11 nM (range 3.4–4.6 nM). The activities of the \(\alpha\) and \(\beta\) anomers of artemether and arteether were also comparable, although the data suggest that the
ANTIMALARIAL ACTIVITY OF β-ARTEMETHER AND β-ARTEETHER

TABLE 2
Fifty percent curative doses (CD50) of β-artemether and β-arteether given subcutaneously in a single dose to mice infected with Plasmodium berghei*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formulation</th>
<th>No. of mice</th>
<th>Sex</th>
<th>CD50 (mg/kg)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Artemether</td>
<td>Sesame oil</td>
<td>70</td>
<td>F</td>
<td>49</td>
<td>33–72</td>
</tr>
<tr>
<td>β-Artemether</td>
<td>Sesame oil</td>
<td>70</td>
<td>M</td>
<td>73</td>
<td>50–107</td>
</tr>
<tr>
<td>β-Arteether</td>
<td>Peanut oil</td>
<td>60</td>
<td>F</td>
<td>51</td>
<td>33–80</td>
</tr>
<tr>
<td>β-Arteether</td>
<td>Peanut oil</td>
<td>30</td>
<td>M</td>
<td>78</td>
<td>43–141</td>
</tr>
<tr>
<td>β-Arteether</td>
<td>Sesame oil</td>
<td>70</td>
<td>F</td>
<td>46</td>
<td>31–68</td>
</tr>
<tr>
<td>β-Arteether</td>
<td>Sesame oil</td>
<td>70</td>
<td>M</td>
<td>32</td>
<td>21–48</td>
</tr>
</tbody>
</table>

*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 IC50 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 CD50 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 parasitemias 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-artemether 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 ED50 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 ED50 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 ED50 values are expressed in
SHMUKLARSKY AND OTHERS

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*  

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

* 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. 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 arteether and the β anomer of arteether showed overall comparable activity. The two in vivo efficacy models also showed that the β anomer of arteether 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, 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 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). 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\textsuperscript{60} and in vivo\textsuperscript{61} in our laboratory. The optimal dosing regimen of \(\beta\)-artemether in humans, as determined from the cumulative clinical experience with that drug, could be an excellent guide in choosing the dosing regimen for \(\beta\)-artether in early efficacy studies in humans.

Acknowledgments: We express our gratitude to Craig Morrissette (Department of Biostatistics, Walter Reed Army Institute of Research [WRAIR]) for assistance in the statistical analysis, and to Specialist Glenn T. Bass (Department of Parasitology, WRAIR) for performing the in vitro assay.


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DTIC QUALITY INSPECTED ✔