In vitro response of chloroquine-resistant *Plasmodium falciparum* to mefloquine

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Abstract

The present study was conducted to evaluate the application of the in vitro microtechnique system in determining the response of chloroquine-resistant Plasmodium falciparum to mefloquine.

Using isolates of *P. falciparum* from Boa Vista, Brazil, and Villavicencio, Colombia, mefloquine was more than 7.7, 7.1, 7.1, and 6.4 times more effective than chloroquine in vitro at the *ED\(_{80}\)*, *ED\(_{95}\)*, *ED\(_{99}\)*, and *ED\(_{99.5}\)* levels, respectively.

Clinical chloroquine resistance of *Plasmodium falciparum* was first observed in Colombia in 1960 (1) and subsequently reported from various parts of South America, especially Brazil and Colombia (2, 3, 4, 5). The standard in vitro test for drug susceptibility described by Ricekman et al. in 1968 (6) has been used to study the geographical distribution of chloroquine-resistant *P. falciparum* (7, 8).

Clinical chloroquine resistance is classified by degrees: R-I, recrudescence between days 7 and 28 after complete disappearance of the parasite from the peripheral blood; R-II, failure of the parasite to disappear from the peripheral blood, but reduction below 25% of the pretreatment level of parasitaemia during the first 6 days; and R-III, only slight reduction, maintenance, or increase of parasitaemia during treatment.

In the standard in vitro test, complete inhibition of parasite growth at 1.0 nmol of chloroquine per ml of defibrinated blood indicates full sensitivity to the drug (9)\(^a\) while growth at 1.25 nmol/ml is a sign of resistance.\(^b\)

Chloroquine resistance of *P. falciparum* is of considerable clinical and epidemiological importance. Patients with resistant strains may continue to exhibit patent parasitaemia and symptoms, or after apparent cure may remain exposed to a potentially fatal infection. In resistant falciparum malaria, the parasite reservoir is maintained thus serving as a source of further transmission and promoting the spread of resistant strains.

The combination of sulfadoxine and pyrimethamine is generally used for the treatment of uncomplicated chloroquine-resistant malaria. This combination is more expensive than chloroquine and fails to cure vivax malaria. The development of operationally useful alternative drugs is essential, since it is likely that resistance to the sulfadoxine/pyrimethamine combination will ultimately occur. One of the most promising drugs developed recently at the Walter Reed Army Institute of Research, Washington, DC, is mefloquine, a well-tolerated compound that is effective against *P. falciparum* both for treatment and for prophylaxis (10, 11, 12). Mefloquine is also active against *P. vivax*.

In the Americas, a system of monitoring drug response, using both in vivo and in vitro test methods, is being developed by the Gorgas Memorial Laboratory in collaboration with the Pan Ameri-


Table 1. Response of chloroquine-resistant *P. falciparum* to mefloquine in vitro (micromethod)

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Number of parasites per μl blood before incubation</th>
<th>Schizonts in control samples (%)</th>
<th>Effective doses (ED) in pmol/5 μl blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloroquine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ED$_{90}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D_0</td>
</tr>
<tr>
<td>BVRB 008</td>
<td>28244</td>
<td>88</td>
<td>19.6</td>
</tr>
<tr>
<td>BVRB 009</td>
<td>18720</td>
<td>100</td>
<td>17.0</td>
</tr>
<tr>
<td>BVRB 010</td>
<td>5280</td>
<td>37</td>
<td>5.0</td>
</tr>
<tr>
<td>VMC 009A</td>
<td>3900</td>
<td>70</td>
<td>&gt;32</td>
</tr>
<tr>
<td>VMC 019</td>
<td>4620</td>
<td>90</td>
<td>13.2</td>
</tr>
<tr>
<td>VMC 023</td>
<td>500</td>
<td>76</td>
<td>15.3</td>
</tr>
</tbody>
</table>

* The ED$_{99.9}$ is considered to be the first concentration at which complete inhibition is observed.

can Health Organization and the World Health Organization. The present study was conducted to determine the susceptibility of chloroquine-resistant strains of *P. falciparum* to mefloquine in the *in vitro* test system. These trials were carried out in 1978 in Boa Vista, Brazil, and Villavicencio, Colombia.

**Materials and methods**

In these investigations, the *in vitro* microtechnique of Rieckmann et al. (13) was used with some modifications.

Flat-bottomed 8 cm × 12 cm tissue culture plates were dosed with 0.1 mg of the disodium salt of ethylenediamine tetraacetic acid (EDTA) in well 1; wells 2 and 3 were left untreated for the controls; wells 4–12 were dosed with either 1–32 pmol of chloroquine or 1–16 pmol of mefloquine. The plates were dried at 37 °C. Before use, wells 2–12 were charged with 50 μl/well of freshly prepared, sterile growth medium containing 10.4 g of RPMI 1640, 2 g of sodium bicarbonate, 6 g of HEPES buffer powder, and 4 mg of gentamicin in 1 litre of double-distilled water. The plates were gently agitated in order to dissolve the drugs. Parasitized blood was taken from a finger-prick by means of a 100-μl sterile, calibrated capillary tube and ejected into well 1. After brief stirring, the blood was transferred aseptically in 5-μl aliquots to wells 2–12 by means of an Eppendorf pipette. The plate was covered with a sterile lid, again gently agitated, and placed on a rack in a static water bath, and a lighted candle made of pure paraffin was used to produce the correct CO$_2$-air mixture. Finally, the slanting lid was put firmly in place on the water bath and sealed with plastic and silicone grease. The unit remained sealed at 38 °C for the incubation period of 24–30 hours.

After incubation the plates were taken from the water bath and the supernatant culture medium/plasma mixture removed from the individual wells by means of capillary tubes. Thick blood films were prepared from the sediment, dried for 2–24 hours, and stained for 10 minutes using a modified Romanowsky stain (14). The number of schizonts per 200 asexual parasites was determined in the samples from control and drug wells. The average of the two control readings was used as a basis for the calculation of proportional growth in the drug wells according to the standard procedure.

**Results**

Comparative tests were carried out with mefloquine in the blood samples of 6 patients with chloroquine-resistant *P. falciparum*. Relatively high schizont counts in the controls were obtained in all cases. The values of ED$_{90}$, ED$_{95}$, ED$_{99}$, and ED$_{99.9}$, i.e., the doses effecting a 90%, 95%, 99%, and 99.9% inhibition of schizont formation, are given in Table 1.

On the basis of the geometric mean values (pmol doses), mefloquine was more than 7.7, 7.1, 7.1, and 6.4 times as effective as chloroquine at the ED$_{90}$, ED$_{95}$, ED$_{99}$, and ED$_{99.9}$ levels, respectively. In case BVRB 009, with a highly chloroquine-resistant parasite population, the ED$_{90}$–ED$_{99}$ values of chloroquine were, consistently, more than 10 times as high as those of mefloquine. In cases BVRB 010 and VMC 019, which were less resistant to chloroquine, the difference was less marked, as was to be expected.

Discussion

The in vitro microtechnique, modified as described, can easily be performed by well trained technicians. It requires only a small quantity of blood, which can be drawn from a finger-prick. In contrast to the standard macromethod it can be performed with blood containing more than 100,000 parasites per microlitre and its results are less dependent on the availability of large rings.

Difficulties with blood inoculation into the wells are overcome by the use of EDTA as an anticoagulant. Earlier observations showed that it does not significantly interfere with parasite growth, adherence of blood to the microscope slides, or the staining properties.

The use of a sealed water bath for incubation proved to be highly effective and convenient, since the correct level of the medium/plasma mixture was maintained throughout incubation. Previous work with the conventional candle jar had resulted in drying of the wells, especially when operating in areas with low relative humidity.

The results obtained in vitro indicate that mefloquine is fully effective against chloroquine-resistant P. falciparum from Boa Vista, Brazil, and Villavicencio, Colombia.

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REFERENCES