IN VITRO SUSCEPTIBILITY OF P. FALCIPARUM POPULATIONS FROM COLOMBIA AND TANZANIA TO A NEW SYNTHETIC PEROXIDE (OZ277)

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Abstract. Sensitivity of Plasmodium falciparum populations from Colombia (N = 38) and Tanzania (N = 45) to the newly developed, fully synthetic peroxide OZ277 was investigated using a standard isotopic microtest. OZ277 showed excellent activity against chloroquine-resistant isolates in Colombia with median IC50 [range] values of 2.5 ng/mL [0.34–8] (4.4 nM [0.6–14]) and Tanzania with 1.5 ng/mL [0.22–10] (2.65 nM [0.4–17.7]). The potency of OZ277 was similar to artesunate, showing median IC50 values of 1.5 ng/mL [0.42–8.6] (3.8 nM [1.1–22.3]) and 1.8 ng/mL [0.2–10] (4.7 nM [0.5–26.04]) in Colombia and Tanzania, respectively. These results support the development of this new antimalarial compound.

Resistance of Plasmodium falciparum to antimalarials makes chemotherapeutic choices increasingly difficult.1 Combinations with artesinin derivatives are becoming progressively more used in countries in and outside Africa because these compounds produce a very rapid therapeutic response (reduction of the parasite biomass and resolution of symptoms), are active against multidrug-resistant P. falciparum, are well tolerated by the patients, and reduce gametocyte carriage; thus, they have the potential to reduce transmission of malaria.2 However, it is difficult to comply rapidly with the worldwide demand for artesmimins because the parent compound is extracted from plants (Artemisia annua), which require time to cultivate. One way to overcome this problem would be to produce synthetic peroxide antimalarials for which OZ277 is the lead candidate.3 OZ277 has shown in vitro activity against laboratory-adapted isolates of P. falciparum [IC50 values for the chloroquine-sensitive NF54: 0.91 ± 0.12 ng/mL for OZ277, compared with 1.6 ± 0.1 ng/mL for artesunate (AS), 5.1 ± 0.8 ng/mL for chloroquine (CQ), and 5.8 ± 0.2 ng/mL for mefloquine (MO)] and rodent malaria parasites in vivo.4 To assess the distribution of inhibitory concentrations in the heterogeneous, natural parasite populations, we compared the in vitro activity of OZ277 with that of chloroquine, mefloquine, artesunate, and mexiteline blue (a known highly potent antimalarial) in fresh P. falciparum isolates from Colombia and Tanzania.

Clinical isolates of P. falciparum were collected with ≥ 5000/µL parasitemias (~ 120 asexual parasites per 200 leukocytes) from patients visiting malaria clinics in the municipalities of Buenaventura (B), Cali (C), Tumaco (TC), and Quibdo (QU) in Colombia and ≥ 2000/µL (50 parasites per 200 leukocytes) in Ifakara in Tanzania. Patients older than 7 years (in Colombia) or from 0.5 to 5 years of age (in Tanzania) who had a negative urine Dill-Glazco test for CQ4 (in Colombia) and denied the use of any other antimalarial drug were included in the study. Signed written consent was obtained from all patients and parents/guardians in the case of children. The study was approved by the Institutional Review Board of CIDEIM and the Ethics Committee of the Ifakara Health Research & Development Center. Samples in Tanzania were collected from February to June 2004, and those in Colombia were collected from June 2004 to April 2005. Giemsa-stained thick and thin blood smears were examined to determine parasite densities and to confirm P. falciparum monoinfection.

Venous blood (5 mL) was collected into EDTA Vacutainers (Becton Dickinson, Cockeysville, MD) before patient treatment and processed either within 24 h of collection at CIDEIM or immediately at the Ifakara Health Research & Development Center. In vitro susceptibility to OZ277 was determined against P. falciparum (NF54) with 0.4–17.7). The potency of OZ277 was similar to artesunate, showing median IC50 values of 1.5 ng/mL [0.42–8.6] (3.8 nM [1.1–22.3]) and 1.8 ng/mL [0.2–10] (4.7 nM [0.5–26.04]) in Colombia and Tanzania, respectively. These results support the development of this new antimalarial compound.
was < 4× the background (uninfected red blood cells) were not included in the analysis.

The median IC50 values and the corresponding ranges were calculated for each drug separately for Colombian and Tanzanian samples that were divided into two groups according to initial parasitemia (< 0.5% and ≥ 0.5%).

<table>
<thead>
<tr>
<th>Compound</th>
<th>&lt; 0.5%</th>
<th>≥ 0.5%</th>
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<tbody>
<tr>
<td></td>
<td>IC50 (ng/mL)</td>
<td>IC50 (ng/mL)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>IC50 (range)</td>
</tr>
<tr>
<td>OZ277</td>
<td>29</td>
<td>1.5 (0.22–10)</td>
</tr>
<tr>
<td>Artesunate</td>
<td>29</td>
<td>1.5 (0.20–10)</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>29</td>
<td>9 (1.6–77)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>29</td>
<td>64 (4.3–100)</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>–</td>
<td>13</td>
</tr>
</tbody>
</table>

OZ277 showed IC50 values comparable to those of AS. OZ277 has excellent activity against freshly obtained, CQ-resistant P. falciparum in vitro but, nevertheless, a high therapeutic efficacy when used in combination. This may indicate previously existing resistance, particularly in southeast Asia. On the other hand, the clinical significance of this correlation could be low because AS and MQ have also shown a positive correlation in vitro but, nevertheless, a high therapeutic efficacy when used in combination.

We conclude that our in vitro results demonstrate that OZ277 has excellent activity against freshly obtained, CQ-resistant P. falciparum field isolates. This reinforces in vitro but, nevertheless, a high therapeutic efficacy when used in combination.

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