Antimalarial Activity of a Synthetic Endoperoxide (RBx-11160/OZ277) against *Plasmodium falciparum* Isolates from Gabon

Andrea Kreidenweiss,1,2† Benjamin Mordmüller,1,2† Sanjeev Krishna,1,3 and Peter G. Kremsner1,2

Medical Research Laboratory, Albert Schweitzer Hospital, Lambaréne, Gabon; Department of Parasitology, University of Tübingen, Germany; Division of Cellular and Molecular Medicine, Centre for Infection, St. George’s, University of London, Cranmer Terrace, London, SW17 0RE, Great Britain

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*OZ277* is a newly developed, fully synthetic endoperoxide antimalarial that we tested against field isolates from Gabon. A comparison of activities of OZ277 with artesunate, mefloquine, and chloroquine showed OZ277 to be highly active against all parasite isolates. Artesunate and mefloquine also showed potent antiparasitic activity, but all isolates were chloroquine resistant.

One of the most important obstacles to reducing mortality from malaria is the establishment of (multi)drug-resistant strains in areas of endemicity (5, 6). Artemisinins became a crucial part of most recommended regimens because they work against otherwise-resistant parasites. Recent signs of in vitro resistance to some artemisinins make the development of new treatments an even more urgent priority (4, 11). Also, supply may not match demand for artemisinins because they are synthesized from plants, which require time to cultivate. Vennerstrom et al. synthesized several synthetic trioxolane derivatives incorporating the critical endoperoxide pharmacophore of artemisinins (13). They obtained synthetic peroxides with similar kinetics compared with those of semisynthetic artemisinins (13). They obtained synthetic peroxides with similar or enhanced antimalarial properties and improved the pharmacokinetics compared with those of semisynthetic artemisinin derivatives. One compound, OZ277 (also known as trioxolane 7 and RBx-11160), is in a clinical development program. OZ277 is highly active against laboratory-adapted *Plasmodium falciparum* strains and rodent parasites in vivo (13). However, the testing of novel antimalarials against non-culture-adapted field isolates of *P. falciparum* is important for assessing the variability of drug activity in areas where parasites are resistant to other classes of antimalarials.

We tested activities of OZ277, artesunate, chloroquine, and mefloquine in *P. falciparum* isolates that were obtained from patients with malaria in Lambaréne, Gabon, between August and December 2004. Most parasites in this area have a high level of chloroquine resistance, whereas mefloquine and artesunate remain efficacious (2, 9). Informed consent and assent were always obtained from the legal representative and the participating child, respectively. Investigations were approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréne. Parasites were from patients, aged 1 to 15 years, with uncomplicated malaria who presented with *P. falciparum* monoinfection (between 103 and 1.2 × 106 parasites/μl blood; determined by thick blood smear) and no intake of antimalarial drugs for at least 1 month. Venous blood (0.5 ml) taken into tubes containing 16 units lithium heparin (Sarstedt) was processed immediately. OZ277 (molecular weight [MW], 565) (Fig. 1 displays the structure for OZ277), artesunate (MW, 384), chloroquine diphosphate (MW, 515), and mefloquine (MW, 415) were from Medicines for Malaria Venture and dissolved in dimethyl sulfoxide (OZ277), 70% ethanol (artesunate), or methanol (mefloquine) at a concentration of 10 mg/ml. Chloroquine was prepared in double-distilled water (5 mg/ml). Drugs were predosed in 96-well plates the day before use in seven twofold (OZ277) or threefold (all other drugs) serial dilutions. Drug sensitivities were assayed as published previously (7), with minor modifications. Parasitemia was adjusted to 0.05% with O

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* Corresponding author. Mailing address: Department of Parasitology, University of Tübingen, Wilhelmstr. 27, D-72074 Tübingen, Germany. Phone: 49 7071 2980240. Fax: 49 7071 295189. E-mail: benjamin.mordmueller@uni-tuebingen.de.
† These two authors contributed equally to this work.

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FIG. 1. Chemical structure of OZ277.
yasis of log concentration-response curves by using Table Curve 2D version 4 (SPSS, Inc.). Pairwise correlations of the study drugs were assessed with Pearson’s coefficient of logarithmically transformed 50% inhibitory concentration (IC$_{50}$) values (JMP version 5.0.1.2; SAS Institute).

Eighty-one *P. falciparum* isolates were obtained with 50 (62%) fulfilling the criteria for successful culture. Finally, the susceptibility of 38 strains to OZ277, 43 strains to artesunate or chloroquine, and 44 strains to mefloquine was tested. Missing values were due to the loss of one sample container during transfer from Lambaréné to Tübingen. Minimizing experimental variation and stringent quality control is crucial for the testing of antimalarials (2) especially for drugs that partition within the parasite compartment, such as chloroquine (3) and OZ277 (13). The omission of human serum from the culture, similar starting parasitemias, and freshly prepared test plates maintained constant drug activity during the study (correlation of date of admission and drug activity, $r^2 = 0.003; P = 0.42$). The median coefficient of the determination of curve fittings was 0.99 (interquartile range, 0.98 to 1.0). All isolates were chloroquine resistant (Table 1). OZ277 showed the highest molar activity, with a range of activities in individual samples that was remarkably narrow (ratios of highest to lowest IC$_{50}$ value, 17.2 for OZ277, 28.0 for artesunate, 26.8 for chloroquine, and 88.3 for mefloquine). This is important because even a few outlying high IC$_{50}$ values can indicate a potential for resistance. Cross-sensitivity was measured by pairwise correlation of log-transformed IC$_{50}$ values (Table 2) between OZ277 activity and other drugs. It was highest with artesunate, the comparator endoperoxide. The highest correlation coefficient was found between artesunate and OZ277. In contrast to artesunate, OZ277 was not positively correlated with mefloquine. Correlations between artesunate and OZ277 suggest a shared mechanism of action and warrant further studies to test this hypothesis, particularly if the in vitro observations of resistance to artemether become clinically important. The lack of correlation between IC$_{50}$ values for OZ277 with mefloquine also suggest a difference with the way that artesunate is handled by parasites. In Southeast Asia, increased *pfmdr1* copy number is associated with increased IC$_{50}$ values to both mefloquine and artesunate (8). Increased copy number for *pfmdr1* has not been observed recently in Lambaréné (12), but amino acid polymorphisms in the C-terminal region of Pfgh1 (the gene product of *pfmdr1*), which modulates sensitivity to artemisinins (10), are frequent in Lambaréné (1). While artesunate and mefloquine may share variable capacities to act as substrates for transport by Pfgh1, this does not appear to be a feature of OZ277. These results demonstrate that OZ277 has excellent activity against fresh, chloroquine-resistant *P. falciparum* field isolates. It reinforces data from lab isolates and animal models (13) and encourages the clinical development of OZ277.

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### REFERENCES


### TABLE 1. Median inhibitory concentrations of study drugs in field isolates from Gabon$^a$

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC$_{50}$</th>
<th>IC$_{50}$</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no. of isolates)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OZ277 (38)</td>
<td>0.47 (0.13–2.23)</td>
<td>1.29 (0.26–5.00)</td>
<td>3.73 (0.37–6.84)</td>
</tr>
<tr>
<td>Artesunate (43)</td>
<td>0.96 (0.20–5.95)</td>
<td>2.47 (0.33–29.9)</td>
<td>5.76 (0.57–49.0)</td>
</tr>
<tr>
<td>Chloroquine (43)$^b$</td>
<td>113 (12.4–332)</td>
<td>244 (20.2–737)</td>
<td>544 (40.2–967)</td>
</tr>
<tr>
<td>Mefloquine (44)</td>
<td>1.94 (0.24–21.2)</td>
<td>4.02 (0.27–48.8)</td>
<td>10.1 (0.30–124)</td>
</tr>
</tbody>
</table>

$^a$ Results are shown in nanomolar (median [range]).
$^b$ All isolates are beyond the threshold level of resistance (1 μmol/liter corresponds to 30 nM in our assay conditions).

### TABLE 2. Pairwise correlations of IC$_{50}$ values of study drugs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>No. of pairs</th>
<th>$r^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefloquine vs artesunate</td>
<td>43</td>
<td>0.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OZ277 vs artesunate</td>
<td>38</td>
<td>0.50</td>
<td>0.002</td>
</tr>
<tr>
<td>Chloroquine vs artesunate</td>
<td>42</td>
<td>0.28</td>
<td>0.08</td>
</tr>
<tr>
<td>Mefloquine vs chloroquine</td>
<td>42</td>
<td>0.24</td>
<td>0.12</td>
</tr>
<tr>
<td>OZ277 vs chloroquine</td>
<td>38</td>
<td>0.13</td>
<td>0.44</td>
</tr>
<tr>
<td>OZ277 vs mefloquine</td>
<td>38</td>
<td>-0.05</td>
<td>0.77</td>
</tr>
</tbody>
</table>
single amino acid residue can determine the sensitivity of SERCAs to artemi-