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

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Received 11 November 2005/Returned for modification 28 January 2006/Accepted 9 February 2006

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 kinetics compared with those of semisynthetic artemisinins (13). However, the testing of novel antimalarials against non-culture-adapted strains and rodent parasites in vivo (13).

We tested activities of OZ277, artesunate, chloroquine, and mefloquine in P. falciparum isolates that were obtained from patients with malaria in Lambaréné, 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 Lambaréné. Parasites were from patients, aged 1 to 15 years, with uncomplicated malaria who presented with P. falciparum monoinfection (between 10^3 and 1.2 × 10^6 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 OZ277 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.

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ysis 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 (IC₅₀) 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 Lambarende to Tubingen. 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 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 (IC₅₀) values (JMP version 5.0.1.2; SAS Institute).

TABLE 1. Median inhibitory concentrations of study drugs in field isolates from Gabona

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC₅₀</th>
<th>IC₉₀</th>
<th>IC₉₉</th>
</tr>
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<tbody>
<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)</td>
<td>113 (12.4–332)</td>
<td>241 (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).

correlation between IC₅₀ 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 IC₅₀ values to both mefloquine and artesunate (8). Increased copy number for pfmdr1 has not been observed recently in Lambarende (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 Lambarende (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.

We thank participating children, their parents, and the staff of the Albert Schweitzer Hospital for their participation and support. The study was funded by the Medicines for Malaria Venture.

REFERENCES

