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

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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 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 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 1.2×10^5 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^+ erythrocytes from one healthy donor, added to 96-well test plates at a hematocrit of 1.5% in parasite culture medium (RPMI 1640, 25 mM HEPES, 2 mM L-glutamine, 50 μg/ml gentamicin, and 0.5% albumax), and incubated for 72 h at 37°C in a candle jar. The first 22 samples were tested in triplicate, and the remaining ones ($n = 59$) were tested in duplicate because interwell variance was very low. A thick blood smear of one control well (untreated) was done after 26 h, and simultaneously, a similar sample was frozen to calculate background histidine-rich protein 2 production. Parasite culture was judged successful when at least 20% of the parasites matured to schizonts at the 26-h time point. Parasite growth, calculated from the levels of histidine-rich protein 2, was measured with a commercial assay (MalariaAg CELISA, Cellabs, Australia) as recommended. In accordance with WHO protocols, chloroquine and mefloquine resistance was calculated from the theoretical blood volume that was inoculated (1 and 5 μmol/liter, respectively) since the drugs accumulate in erythrocytes (14). Individual inhibitory concentrations were determined by nonlinear regression anal-

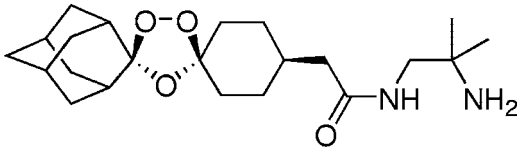

FIG. 1. Chemical structure of OZ277.

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TABLE 1. Median inhibitory concentrations of study drugs in field isolates from Gabon[a]

Drug (no. of isolates)	IC_{50}	IC_{90}	IC_{99}
OZ277 (38)	0.47 (0.13–2.23)	1.29 (0.26–5.00)	3.73 (0.37–6.84)
Artesunate (43)	0.96 (0.20–5.95)	2.47 (0.33–29.9)	5.76 (0.57–49.0)
Chloroquine (43)[b]	113 (12.4–332)	241 (20.2–737)	544 (40.2–967)
Mefloquine (44)	1.94 (0.24–21.2)	4.02 (0.27–48.8)	10.1 (0.30–124)

[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).

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 (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 mefloquine. 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 Pgh1 (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 Pgh1, 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.

TABLE 2. Pairwise correlations of IC_{50} values of study drugs

Drugs	No. of pairs	r^2	P
Mefloquine vs artesunate	43	0.53	<0.001
OZ277 vs artesunate	38	0.50	0.002
Chloroquine vs artesunate	42	0.28	0.08
Mefloquine vs chloroquine	42	0.24	0.12
OZ277 vs chloroquine	38	0.13	0.44
OZ277 vs mefloquine	38	−0.05	0.77

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