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In Vitro Activities of Benflumetol against 158 Senegalese Isolates of Plasmodium falciparum in Comparison with Those of Standard Antimalarial Drugs

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The 50% inhibitory concentration (IC$_{50}$) of benflumetol (range, 12.5 to 240 nM; mean, 55.1 nM) for 158 Senegalese isolates were evaluated. Ten isolates (6%) showed decreased susceptibility to benflumetol. Benflumetol was slightly more potent against chloroquine-resistant isolates ($P < 0.025$). No correlation or weak correlations in the responses to benflumetol and pyrimethamine, chloroquine, amodiaquine, artemether, quinine, and pyronaridine were observed, and these correlations are insufficient to suggest cross-resistance.

Despite considerable efforts to eradicate or control malaria, the disease continues to be a major cause of human morbidity and mortality in the tropics. Malaria is a major cause of death in children (20), usually within the first 24 h of treatment in a hospital (6). Although quinine is effective in most patients in Africa, its onset of action is slow and it has been postulated that a faster-acting drug would be more effective for the sickest children. In addition, clinical failures with quinine have been observed in Africa (5, 7). This has led to a search for an effective alternative antimalarial drug with minimal side effects.

Benflumetol is a racemic fluorene derivative with the chemical name 2-dibutylamino-1-[2,7-dichloro-9-(4-chlorobenzyl)-fluoren-4-yl]-ethanol. It conforms structurally and in mode of action to the structure and mode of action of the aryl amino alcohol group of antimalarial drugs, including quinine, mefloquine, and halofantrine. Investigations confirm the accepted view that benflumetol exerts its antimalarial effect as a consequence of its interaction with heme, a degradation product of hemoglobin metabolism (17). A recent approach has been to combine an artemisinin-derived antimalarial agent with proven efficacy, artemether, with benflumetol. This approach is being jointly developed by the Chinese Academy of Military Medical Sciences and Novartis (Basel, Switzerland).

This combination offered a rapid and highly effective treatment for acute uncomplicated falciparum malaria in China (21), Thailand (14), and Gambia (4, 16). No evidence of substantial differences in activity between enantiomers of benflumetol and racemic benflumetol were observed in vitro (18). Benflumetol was also shown to be highly active against 61 Cameroonian isolates (1).

The aim of this study was to assess the in vitro activity of benflumetol against 158 Senegalese Plasmodium falciparum field isolates and to compare its activity with those of chloroquine, quinine, amodiaquine, pyronaridine, artemether, and pyrimethamine.

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**In vitro assay.** The isotopic, micro drug susceptibility test used in this study was described previously (11). The 50% inhibitory concentration (IC$_{50}$), i.e., the drug concentration...
resulting in 50% of the uptake of [3H]hypoxanthine by the parasites in drug-free control wells, was determined by nonlinear regression analysis of log dose-response curves. Data were expressed as the geometric mean \( IC_{50} \) and 95% confidence intervals (CIs) were calculated. The unpaired \( t \) test was used to compare the \( IC_{50} \) for chloroquine-susceptible and chloroquine-resistant isolates. Assessment of the cross-resistance of benflumetol with the other antimalarial agents was estimated with the Pearson correlation coefficient \( r \) and the coefficient of determination \( r^2 \). A positive correlation in the response to two drugs may be interpreted as resistance to the first drug that facilitated resistance to the other drug (19). The cutoff for reduced susceptibility to benflumetol in vitro has not yet been determined. Isolates were considered chloroquine-resistant if the \( IC_{50} \) was greater than 100 nM. Cutoff values for resistance to quinine, amodiaquine, pyrimethamine, artemether, and pyrimethamine were 500, 80, 15 (11), 10.5 (10), and 2,000 nM, respectively. The in vitro threshold value for resistance to antimalarial agents has been defined statistically (>2 standard deviations above the mean). Only in vitro resistance to chloroquine, evaluated statistically, has been confirmed by correlation with therapeutic effectiveness in vivo (13).

**Results.** The \( IC_{50} \) of benflumetol for the 158 Senegalese wild isolates ranged from 12.5 to 240 nM (mean \( IC_{50} \) 55.1 nM), and the 95% CI was 48.1 to 62.1 nM. Ten isolates (6%) showed decreased susceptibility to benflumetol in vitro (\( IC_{50} > 150 \) nM). This in vitro threshold value for reduced susceptibility to benflumetol was defined statistically (>2 standard deviations above the mean). On the basis of our criterion for resistance (\( IC_{50} > 100 \) nM), 78 of 158 fresh isolates of \( P. falciparum \) studied were considered to be chloroquine resistant (Table 1). Benflumetol was slightly more potent against chloroquine-resistant isolates than against chloroquine-susceptible parasites (\( P < 0.025 \)). There was a significant positive correlation between the responses to benflumetol and artemether (\( r = 0.40; P < 0.001 \)), benflumetol and quinine (\( r = 0.37; P < 0.001 \)), and benflumetol and pyrimethamine (\( r = 0.32; P < 0.001 \)) and a nonsignificant positive correlation between the responses to benflumetol and amodiaquine (\( r = 0.22; P < 0.05 \)) (Table 2). A negative nonsignificant correlation in the response to benflumetol and chloroquine (\( r = -0.11; P > 0.1 \)) was found. No correlation between the responses to benflumetol and pyrimethamine was observed (\( r = -0.04; P > 0.1 \)).

**Discussion.** Patients were treated with chloroquine (the first-line drug in Senegal) and sulfadoxine-pyrimethamine (the second-line drug). In a study carried out in the Fatick Region of Senegal in 1995, 29% of isolates exhibited resistance to chloroquine (9). That study revealed an increase in the prevalence (49%) of in vitro resistance to chloroquine in 1996. The in vitro activity of benflumetol was four- to sixfold greater than that of quinine, and benflumetol was even three times as effective against chloroquine-resistant isolates. As shown in Table 1, benflumetol was slightly more potent against chloroquine-resistant isolates than against chloroquine-susceptible isolates (\( P < 0.025 \)) whereas quinine (\( P < 0.001 \)), pyrimethamine (\( P < 0.002 \)), and amodiaquine (\( P < 0.025 \)) were less potent against chloroquine-resistant parasites. Artemether and pyrimethamine were equally effective against chloroquine-sensitve and chloroquine-resistant isolates. For 10 isolates (6%) benflumetol \( IC_{50} \)s were greater than 150 nM, suggesting reduced susceptibility in vitro. This finding could indicate that some \( P. falciparum \) isolates may be innately less susceptible to benflumetol. The negative in vitro correlation between the responses to chloroquine and benflumetol remained below the threshold of statistical significance (\( r = -0.11; P > 0.1 \)). These observations led us to believe that benflumetol may be an important alternative drug for the treatment of chloroquine-resistant malaria.

A positive correlation between the \( IC_{50} \)s of two antimalarial drugs may suggest in vitro cross-resistance, but the relationship between in vitro and in vivo resistance depends on the level of resistance and the coefficients of correlation \( r \) and determination \( r^2 \). The positive correlations between the responses to benflumetol and artemether, benflumetol and quinine, and benflumetol and pyrimethamine that we observed are insufficient to suggest cross-resistance: only 16% of the variations in responses to benflumetol are explained by variations in responses to artemether; these proportions are 14% for quinine and 11% for pyrimethamine. From these data we can only suggest that common mechanisms of action may explain the positive correlations between these drugs. In addition, previous study showed that the susceptibility of the parasites to artemether was enhanced by the presence of low levels of benflumetol in the medium (8). This result suggests that pharmacologically relevant concentrations of benflumetol do potentiate the antimalarial activity of artemether.

Since the mechanisms underlying the antimalarial actions of benflumetol, quinine, artemether, pyrimethamine, and amodiaquine are still unclear, one can only speculate about the positive correlations between responses to artemether and benflumetol. Benflumetol seems to exert its antimalarial effect as a consequence of its interaction with heme (17). It conforms structurally, physicochemically, and in mode of action to the aryl amino alcohol group of antimalarial agents, including quinine, mefloquine, and halofantrine. Previous in vitro studies in Cameroon have demonstrated a high correlation between benflumetol and mefloquine and between benflumetol and halofantrine (1). Several findings suggest that heme may be the common link between quinoline antimalarial agents (2, 3, 12).
Benflumetol may be an important alternative drug for the treatment of chloroquine-resistant malaria, despite the positive weak correlations between responses to benflumetol and artemether, quinine, and pyronaridine for P. falciparum isolates. However, positive correlations in vitro suggest common features in drug uptake and/or mode of action or resistance and reinforce the idea that a novel antimalarial agent should not be used for monotherapy. In addition, benflumetol and artemether have been reported to act in a synergistic manner (8). This combination may be an important alternative drug for the treatment of chloroquine-resistant malaria. Recent studies have confirmed that the combination of artemether-benflumetol (CGP 56 697; Novartis) is effective in vivo and is very well tolerated in the treatment of multidrug-resistant falciparum malaria (4, 14–16).

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