In Vitro Activity of Cycloguanil against African Isolates of *Plasmodium falciparum*

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Received 23 June 1992/Accepted 5 February 1993

The in vitro activity of cycloguanil was assessed against 86 African isolates of *Plasmodium falciparum* by a semi-micro assay system. A bimodal distribution of susceptibility patterns was observed, with 44% of the isolates being cycloguanil susceptible. Cycloguanil alone retains a high activity against the intraerythrocytic forms of some isolates and, together with its activity against the hepatic stages, may be useful for chemoprophylaxis when combined with chloroquine.

With the spread of chloroquine resistance in most of the regions where falciparum malaria is present, chloroquine alone cannot be relied upon for the chemoprophylaxis of nonimmune travelers. One of the recommended chemoprophylactic regimens is the combination of chloroquine and proguanil (12). Proguanil, a dihydrofolate reductase inhibitor, is a prodrug which exerts an antimalarial action against both liver and intraerythrocytic stages through its biologically active metabolite, cycloguanil (11). Soon after its introduction in the early 1950s for therapeutic use, resistance to proguanil was recognized in many parts of the world (7), but its prophylactic efficacy, which is partly attributable to its marked inhibitory action against the hepatic stages, was apparently not affected. These observations have curtailed the use of proguanil exclusively for chemoprophylaxis. Although the combination of chloroquine and proguanil is one of the recommended regimens for travelers visiting Central and West Africa (12), the current extent of resistance to cycloguanil is not well defined in Africa. Because of the concern for a rapid selection of cycloguanil-resistant parasites, we have evaluated the in vitro activity of cycloguanil against fresh clinical isolates of *Plasmodium falciparum*.

Eighty-six isolates of *P. falciparum* were obtained before treatment from malaria-infected travelers returning to France from the francophone countries in Central or West Africa between January 1991 and April 1992. The recommended French prophylactic regimen is chloroquine (100 mg/day) plus proguanil (200 mg/day) or mefloquine (250 mg weekly) for these regions of Africa. Six patients presented with malaria despite full compliance with chloroquine plus proguanil. Seven additional travelers were taking chloroquine alone (100 mg/day) correctly.

Cycloguanil base was provided by ICI Pharmaceuticals (Macclesfield, United Kingdom). Chloroquine sulfate was obtained from Specia (Paris, France). Stock solutions were prepared in sterile distilled water. Twofold dilutions with final concentrations ranging from 12.5 to 1600 nmol/liter were distributed in 24-well plates in triplicate.

After being washed with RPMI 1640 medium thrice, the infected erythrocytes were suspended in folate- and p-aminobenzoic acid-free RPMI 1640 medium supplemented with 10% human serum and buffered with 25 mmol of HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid) per liter and 25 mmol of NaHCO3 per liter to obtain a hematocrit of 2.5% and an initial parasite density of 0.1 to 1.0%. A similar suspension was prepared with the standard RPMI 1640 medium for the determination of chloroquine susceptibility. The in vitro drug susceptibility test of Le Bras and Deloron (6) was used in this study. The 50% inhibitory concentration (IC50) was defined as the drug concentration corresponding to 50% of the incorporation of the isotop by the parasites in the drug-free control wells.

The levels of response to cycloguanil were defined as susceptible (IC50 < 100 nmol/liter), intermediate (IC50 = 100 to 1,600 nmol/liter), and resistant (IC50 > 1,600 nmol/liter) (Table 1). According to these definitions, 38 of 86 (44%) isolates were susceptible to cycloguanil, with a geometric mean IC50 of 22.5 nmol/liter (95% confidence intervals, 17.7 to 28.6 nmol/liter). Our results seem to indicate a bimodal distribution of response. A similar distribution of IC50 was observed in the previous investigations (8, 10, 11). The bimodal distribution may reflect the genetic mechanism of cycloguanil resistance involving single point mutations in the dihydrofolate reductase-thymidylate synthase gene (3, 9), producing an all-or-none phenomenon in the response pattern. The intermediate group, for which the IC50 were between 100 and 1,600 nmol/liter, may represent mixed populations of susceptible and resistant parasites.

Of the 86 isolates, 57 were resistant to chloroquine in vitro (IC50 > 100 nmol/liter). Nineteen chloroquine-resistant isolates were susceptible to cycloguanil, while 27 chloroquine-resistant parasites were also resistant to cycloguanil. The isolates from five of six prophylactic failures with chloroquine and proguanil and the parasites from five of seven failures with chloroquine alone were resistant in vitro to both chloroquine and cycloguanil. Since the rest of the patients were not on chemoprophylaxis or were noncompliant with the recommended regimens (with noncompliance defined as underdosage, correct but early abandonment of drug intake, or use of inappropriate drugs), a meaningful correlation of the in vitro responses and clinical efficacy cannot be analyzed in the present study.

The results presented in this report should not be interpreted as fully representative of the responses of the naturally occurring isolates of *P. falciparum* or predictive of the
prophylactic efficacy of proguanil. Our recruitment procedure favors an inclusion of parasites from resistant or severe cases of malaria seen at specialized centers. Moreover, our study was limited to the susceptibility patterns of the intraerythrocytic parasites. There are no published laboratory data on the inhibitory effect of cycloguanil against the liver stages of *P. falciparum*. Nevertheless, studies using rodent and simian malaria models have shown the high activity of cycloguanil against the hepatic stages at low nanomolar concentrations (1, 2). In these models, a parasite strain which is resistant to cycloguanil during the intraerythrocytic stages may be susceptible to the compound during the liver stage (5). The results of the present study are consistent with the clinical efficacy of chloroquine and proguanil in nonimmune residents in Africa (4).

The high in vitro activity against some intraerythrocytic parasites, evidence of a marked effect against the hepatic stages, good tolerance and safety for infants and pregnant women, and lack of well-tolerated alternative prophylactic drugs seem to provide a sound basis for the continued use of the combination of chloroquine and proguanil for chemoprophylaxis in most regions of Central and West Africa. In view of the presence of chloroquine- and/or cycloguanil-resistant isolates, however, a continuous surveillance of the in vitro activity and the clinical efficacy of chloroquine and proguanil is needed for further adjustments in the recommendations of malaria chemoprophylaxis.

REFERENCES


**TABLE 1.** In vitro activity of cycloguanil against 86 African isolates of *P. falciparum*

<table>
<thead>
<tr>
<th>Response to chloroquine</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; of cycloguanil (range) [nM]</th>
<th>Intermediate [17]</th>
<th>Resistant [31]</th>
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<tr>
<td>Resistant</td>
<td>22.6 (6.6-97.6) [19]</td>
<td>585 (195–1,030) [11]</td>
<td>&gt;1,600 [27]</td>
</tr>
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</table>

* Geometric means and ranges are expressed as nanomoles per liter. The response to cycloguanil was defined arbitrarily as susceptible (IC<sub>50</sub> < 100 nmol/liter), intermediate (IC<sub>50</sub> = 100 to 1600 nmol/liter), and resistant IC<sub>50</sub> > 1,600 nmol/liter). The threshold IC<sub>50</sub> for chloroquine resistance is 100 nmol/liter, as correlated with clinical response.