IN VITRO REVERSAL OF CHLOROQUINE RESISTANCE WITH CHLORPHENIRAMINE AGAINST AFRICAN ISOLATES OF PLASMODIUM FALCIPARUM

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SUMMARY: The in vitro chloroquine potentiating action of racemic chlorpheniramine was assessed by a semi-microtest against chloroquine-resistant African isolates of Plasmodium falciparum. At the chlorpheniramine concentrations of 312 and 625 nM, chloroquine resistance was reversed in 4 and 11 of 14 isolates, respectively, with a diminution of their 50% inhibitory concentration (IC50) values for chloroquine to below 100 nM. No effect was observed against the chloroquine-susceptible clone. The results of the study suggest that chlorpheniramine may be a promising chloroquine resistance modulator.

The spread of chloroquine-resistant Plasmodium falciparum poses a serious challenge to the treatment of malaria. In various experimental models of malaria, chloroquine resistance can be reversed by calcium channel blockers, tricyclic antidepressants, antihistaminics, and neuroleptic phenothiazines (1-3). One of the foreseeable problems in the eventual clinical application of the reversal of drug resistance is side-effects of these resistance modifiers. Antihistaminics, including cyproheptadine, azatadine, and ketotifen, are relatively safe drugs. Recently, chlorpheniramine was described to be an in vitro resistance modifier at micromolar concentrations (4). In the present study, we investigated the in vitro...
chloroquine potentiating action of chlorpheniramine against chloroquine-resistant African isolates of *P. falciparum*.

The chloroquine-susceptible L-3/Côte d'Ivoire and the chloroquine-resistant FCM 29/Cameroon reference clones were used. The complete data on their drug susceptibility patterns were published previously (5,6). Fresh clinical isolates of *P. falciparum* originating from sub-Saharan Africa were obtained from malaria-infected travelers returning to France. The isolates were cultured under standard conditions for two to three asexual intraerythrocytic life cycles (7). During the first life cycle, drug susceptibility patterns of the isolates were determined. The isolates were used when the in vitro response indicated that they were chloroquine-resistant.

Racemic chlorpheniramine maleate and its (S)-(+)-enantiomer were obtained from Sigma Chemical Co., St. Louis, MO, USA. Chloroquine sulfate was supplied by Specia, Paris, France. A stock solution of chloroquine was prepared in sterile distilled water. A stock solution of chlorpheniramine maleate was prepared in 75% ethanol. Further dilutions were prepared in sterile distilled water. The final concentrations of chloroquine ranged from 12.5 to 1,600 nM. Each concentration was tested in triplicate. Two fixed subinhibitory concentrations of racemic chlorpheniramine (312 and 625 nM) were added to the chloroquine plate.

A semi-micro in vitro assay described by Le Bras and Deloron was used (8). Briefly, a suspension (700 μl/well) of parasitized erythrocytes (1.5% hematocrit, 0.2-0.5% initial parasitemia) in RPMI 1640 medium supplemented with 10% human serum and buffered with 25 mM of HEPES and 25 mM of NaHCO₃ was distributed in the predosed 24-well plates. The plates were incubated for 42 hr at 37 C in 5% O₂, 5% CO₂, and 90% N₂. [G-3H]Hypoxanthine was added 18 hr later to assess parasite growth. The contents of each well were collected and washed with a cell harvester. The radioactivity incorporated by the parasites was measured with a liquid scintillation counter. The 50% inhibitory concentration (IC₅₀) values were determined by non-linear regression analysis of log-dose/response curves. Chloroquine resistance was defined as IC₅₀ > 100 nM, as correlated with the clinical response of malaria-infected patients (9). Reversal of chloroquine resistance was defined as IC₅₀ < 100 nM in the presence of chlorpheniramine.

Our preliminary study with the highly chloroquine-resistant FCM 29/Cameroon clone showed that the chloroquine-potentiating action of (+)- and racemic chlorpheniramine was similar (Table I). Neither the racemate nor the (+)-enantiomer of chlorpheniramine, at concentrations of 625 and 1,250 nM, affected the chloroquine activity against the chloroquine-susceptible L-3/Côte d'Ivoire
Table I. In vitro responses of *Plasmodium falciparum* clones to chloroquine alone and in combination with chlorpheniramine

<table>
<thead>
<tr>
<th>Drug combination*</th>
<th>Chloroquine IC(_{50}) (nM)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>L-3</td>
<td>FCM 29</td>
</tr>
<tr>
<td>CQ alone</td>
<td>18.1</td>
<td>920</td>
</tr>
<tr>
<td>CQ + (+)-chlorpheniramine 625 nM</td>
<td>18.8</td>
<td>362</td>
</tr>
<tr>
<td>CQ + (±)-chlorpheniramine 625 nM</td>
<td>19.0</td>
<td>336</td>
</tr>
<tr>
<td>CQ + (+)-chlorpheniramine 1250 nM</td>
<td>17.6</td>
<td>233</td>
</tr>
<tr>
<td>CQ + (±)-chlorpheniramine 1250 nM</td>
<td>17.9</td>
<td>219</td>
</tr>
</tbody>
</table>

*The IC\(_{50}\) values of (+)-chlorpheniramine alone were 95.9 \(\mu M\) and 102 \(\mu M\) against the chloroquine-susceptible L-3 and the chloroquine-resistant FCM 29 clones, respectively, and the IC\(_{50}\) values of (±)-chlorpheniramine alone were 94.1 \(\mu M\) and 125 \(\mu M\), respectively. CQ: Chloroquine.*

clone. Either racemic chlorpheniramine or (+)-chlorpheniramine alone was inactive (IC\(_{50}>90 \mu M\)) against the chloroquine-susceptible or the chloroquine-resistant clones. Since the racemic chlorpheniramine is associated with less side-effects due to antihistaminic activity than its dextrorotatory isomer, only the racemate was tested against the chloroquine-resistant isolates.

The in vitro responses to chloroquine alone and in combination with racemic chlorpheniramine of 14 *P. falciparum* isolates are shown in Fig. 1. At the chlorpheniramine concentration of 312 nM, chloroquine resistance was reversed (IC\(_{50}<100 \text{ nM}\)) in only 4 of 14 isolates. At the concentration of 625 nM, 11 of 14 isolates had IC\(_{50}\) values below 100 nM. Three isolates whose IC\(_{50}\) values remained >100 nM in the presence of 625 nM of chlorpheniramine were relatively more resistant to chloroquine than the other isolates. This observation is consistent with our previous in vitro findings suggesting that higher concentrations of resistance modifiers may be required to reverse resistance against highly chloroquine-resistant isolates (10).

Chloroquine resistance is associated with a rapid efflux of the drug from the resistant parasites (11). It has been hypothesized that P-glycoprotein encoded by the multidrug-resistant (MDR) gene may expel chloroquine from the parasites.
Fig. 1. In vitro responses of 14 chloroquine-resistant *Plasmodium falciparum* isolates to chloroquine (CQ) alone and in combination with racemic chlorpheniramine (CP). At chlorpheniramine concentration of 625 nM, chloroquine resistance (defined as IC$_{50}$ values $>$ 100 nM) was not reversed in three isolates (black squares).

and that various resistance modulators inhibit this efflux process by binding to P-glycoprotein (12). Synergistic action between chloroquine and chlorpheniramine is unlikely since chlorpheniramine alone is inactive against the malaria parasites. The present study suggests that 625 nM of chlorpheniramine may be effective in reversing in vitro chloroquine resistance against most isolates. Clinical studies have shown that peak plasma concentrations ranging from 16-131 ng/ml (58-477 nM) can be achieved after multiple oral doses of chlorpheniramine (13). Although our in vitro results can not be directly extrapolated to in vivo conditions, the data obtained in the present study are encouraging and call for a further evaluation of chlorpheniramine as a resistance modifier for chloroquine-resistant *P. falciparum*.
REFERENCES


