Synergistic In Vitro Antimalarial Activity of Omeprazole and Quinine

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Previous studies have shown that the proton pump inhibitor omeprazole has antimalarial activity in vitro. The interactions of omeprazole with commonly used antimalarial drugs were assessed in vitro. Omeprazole and quinine combinations were synergistic; however, chloroquine and omeprazole combinations were antagonistic. Artemisinin drugs had additive antimalarial activities with omeprazole.

We recently identified the proton pump inhibitor omeprazole as a potential novel antimalarial agent (19). The precise mode of action of omeprazole against Plasmodium falciparum is unknown, but it may have mechanisms distinct from those of conventional drugs (19). Although omeprazole can be given parenterally (12), its terminal elimination half-life is short (40 to 60 min [10]), and relatively high concentrations in plasma may be required for clinically useful activity (19). Nevertheless, as in the case of the gastric parietal cell (22), the binding of omeprazole or its sulfenamide metabolite to a P. falciparum ATPase may prolong its antimalarial effect.

Although these considerations suggest that omeprazole may be unsuitable as antimalarial monotherapy, it could form part of combination treatment with conventional agents in order to improve cure rates and reduce the likelihood of parasite resistance (13). We have therefore examined the in vitro antimalarial activity of omeprazole in combination with chloroquine, quinine, and several commonly used artemisinin drugs.

Parasites were maintained in modified candle jars (21). Stock 1-mmol/liter solutions of artemisinin (Sigma, St. Louis, Mo.), dihydroartemisinin (Cotec Pharmaceutical Company, Guangxi, China), arteether (Brian Schuster, Walter Reed Army Institute of Research, Washington, D.C.), artesunic acid (Gulinh Pharmaceuticals, Guangxi, China), omeprazole (Astra, Södertälje, Sweden), quinine (Sigma), and chloroquine (Sigma) were prepared in plasma-free RPMI and stored at −80°C. Dimethyl sulfoxide (1%, vol/vol) was used as the vehicle in all cases. Drug stocks were diluted in plasma-supplemented RPMI when required for assays.

Antimalarial drug combinations were assessed against clone 3D7 by isobolographic analysis (1). Assays were performed in 96-well microtiter plates containing 100 μl of 3D7 culture at 0.5% parasitemia and 2% hematocrit and 100 μl of the appropriate drug or drug combination. Parasite growth was determined by tritiated hypoxanthine incorporation (6). Media and vehicle controls were included on each plate. Experiments were repeated at least twice. Fifty percent effective concentration(EC50) (Table 1) were determined by linear interpolation (11). Isobolograms were constructed from normalized fractional-inhibitory concentration (FIC) values in each experiment, and isoboles were fitted with a standard hyperbolic function defined by the parameter I (2, 3). The significance of the difference of I from zero was assessed with Student’s t test. Dose factor potentiations were also estimated (16).

The interactions of omeprazole with quinine, chloroquine, and the artemisinin drugs ranged from significant synergism to mild antagonism. Combinations of omeprazole with quinine were significantly synergistic, but omeprazole and chloroquine were weakly antagonistic (Table 2; Fig. 1). Omeprazole and individual artemisinin drugs exhibited additivity in each case (Table 2). The combination of quinine and chloroquine was antagonistic (Table 2). Quinine remains a first-line treatment for severe falciparum malaria, but there are concerns regarding declining efficacy in some countries (15, 17). Our in vitro data demonstrate that omeprazole given to a severely ill patient may increase the effects of quinine and improve clinical outcome. The results of recent research suggest that omeprazole inhibits CYP3A-catalyzed 3-hydroxylation of quinine by human liver microsomes (23), an effect that may increase serum quinine concentrations in vivo but at the risk of increased toxicity. Since omeprazole is currently used for gastric acid suppression and as a part of the therapy for Helicobacter pylori infection, field studies of the quinine-omeprazole combination could be carried out to examine these issues.

The in vitro additive antimalarial activity of omeprazole plus an artemisinin derivative may be influenced by pharmacokinetic factors in vivo. Nevertheless, parasite clearance in vivo is already relatively rapid with the artemisinin derivatives, and it seems unlikely that the additive activity demonstrated in our in vitro study would be beneficial in the acute phase of infection. Nevertheless, the different actions of these drugs may both reduce recrudescence and prevent the development of parasite resistance.

In addition to possible clinical implications, our data may shed light on the mechanisms of action of some conventional drugs.

### TABLE 1. EC50 of individual antimalarial drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC50 (mol/liter)*</th>
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<tbody>
<tr>
<td>Omeprazole</td>
<td>1.4 × 10⁻⁵–3.6 × 10⁻⁵</td>
</tr>
<tr>
<td>Quinine</td>
<td>3.2 × 10⁻⁸–2.5 × 10⁻⁷</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>8.0 × 10⁻⁹–2.0 × 10⁻⁸</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>8.8 × 10⁻⁹–2.7 × 10⁻⁸</td>
</tr>
<tr>
<td>Arteether</td>
<td>2.3 × 10⁻⁹–5.7 × 10⁻⁹</td>
</tr>
<tr>
<td>Dihydroartemisinin</td>
<td>1.6 × 10⁻⁹–3.4 × 10⁻⁹</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>3.3 × 10⁻⁹–2.7 × 10⁻⁸</td>
</tr>
</tbody>
</table>

* EC50 are presented as ranges determined from all isobologram experiments.
antimalarial drugs. Our data confirm that chloroquine and quinine are antagonistic (20). Consistent with the observation that these drugs have similar stage-specific activities against pigment-producing parasites (18), both interfere with hemozoin formation (7). However, this probably occurs indirectly, before any ferrirrotoporphyrin binding, with chloroquine increasing the amount of a negative regulator of hemozoin formation (8). Although the final target of quinine is unknown, it may reverse chloroquine-induced inhibition of ferrirrotoporphyrin sequestration (8). The contrasting interactions of omeprazole with chloroquine and quinine in the present study provide additional indirect evidence that these quinoline drugs have different actions against the parasite.

Factors relating to the cellular effects of omeprazole might explain why omeprazole and quinine behave synergistically while chloroquine-omeprazole combinations are antagonistic.

Omeprazole may inhibit growth of *P. falciparum* by inhibiting parasite ATPase activity, which, in turn, increases the pH of the acidic food vacuole. Since both omeprazole and chloroquine are weak bases which accumulate in acidic environments (14), a higher pH and/or competition with omeprazole would also inhibit chloroquine at its site of action. In addition, chloroquine accumulation may be driven by a P-type ATPase (4), which may also be affected by omeprazole. Unlike chloroquine, quinine is more lipophilic and is not concentrated exclusively in food vacuoles (9). Indeed, there is evidence that quinine inhibits membrane ATPases in the same way as omeprazole (5).

Our data provide important preliminary evidence of interactions between the novel antimalarial agent Omeprazole and conventional drugs that may have clinical implications. At present, parasite resistance limits therapeutic options for malaria, and the artemisinin derivatives are not yet available in many Western countries. Omeprazole may prove to be a valuable adjunctive antimalarial agent in this context.

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**REFERENCES**


