Statins as Potential Antimalarial Drugs: Low Relative Potency and Lack of Synergy with Conventional Antimalarial Drugs

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The in vitro sensitivity of Plasmodium falciparum to atorvastatin and rosuvastatin was assessed using chloroquine-sensitive and chloroquine-resistant strains. Although atorvastatin was more potent, it had weak activity (mean 50% inhibitory concentration of ≥17 μM) and an indifferent interaction with chloroquine and dihydroartemisinin. Bioassay of plasma from an atorvastatin-treated subject showed similar results.

Statins are well tolerated and widely used drugs that reduce cardiovascular morbidity and mortality (24, 28). They inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis, and consequently lower serum low-density lipoprotein–cholesterol concentrations. There has been recent interest in potential alternative applications based on their ability to inhibit the growth of bacteria (6), yeasts (22), and protozoa (1, 7, 18, 26). The first study of their antimalarial effects found that lovastatin and simvastatin inhibited in vitro intraerythrocytic development of Plasmodium falciparum (12). More recently, Pradines et al. examined the in vitro susceptibilities of P. falciparum to six statins and demonstrated that atorvastatin had the greatest activity (21). Despite these encouraging findings, neither simvastatin nor atorvastatin in high doses improved the outcome in Plasmodium berghei-infected mice (3, 14) and there was no effect on parasitemia (3). However, no study to date has included the most potent statin in clinical use, rosuvastatin (23).

In addition, despite the demonstration of in vitro synergy between mevastatin and the glycoprotein inhibitor tunicamycin against P. falciparum (19), the interaction between statins and conventional antimalarial drugs has not been evaluated. We have, therefore, investigated the in vitro antimalarial activity of atorvastatin and rosuvastatin against P. falciparum and the in vitro interactions between these statins and both chloroquine (CQ) and dihydroartemisinin (DHA).

The laboratory-adapted P. falciparum strains 3D7 (Africa; CQ sensitive), E8B (Brazil; CQ resistant), and Dd2 (Indochina; CQ resistant) were maintained in continuous culture using a modified candle jar method (25). Synchronous cultures were prepared by sorbitol lysis (16). Dilutions from stock solutions of atorvastatin and rosuvastatin (Waterstonetech, Carmel, IN) dissolved in dimethyl sulfoxide were prepared in RPMI and added in triplicate to 96-well plates to final concentrations of 0.3 to 200 μM (atorvastatin) and 0.6 to 400 μM (rosuvastatin). Parasite suspensions were standardized to 1.0% parasitemia and 1.5% hematocrit, and the mixture was incubated for 48 h. Parasite growth was measured by colorimetric detection of plasmodium lactate dehydrogenase (17). The final drug concentrations that inhibited parasite growth by 50% (IC50) and 90% (IC90) were determined by nonlinear regression analysis. A modified fixed-ratio isobologram method was used to assess drug interactions (11). Inhibition assays using 3H-hypoxanthine incorporation were first performed to determine individual IC50 values for statins, CQ, and DHA (9). These were used to establish test concentration ranges in the combination assays. Fractional inhibitory concentrations (FICs) of each drug in each combination determined from dose-response curves were used to construct isobolograms from which the sum of each FIC (2FIC) was calculated (2).

Because of the possibility that active metabolites of atorvastatin or other in vivo factors might contribute to enhanced antimalarial activity, such as has been described for atovaquone (5, 10), a bioassay study was performed. A pretreatment venous blood sample was taken from a healthy volunteer who was then given atorvastatin at 80 mg (Lipitor; Pfizer, NY) once daily for 4 consecutive days. A second venous blood sample was drawn three hours after the last dose at the time of the predicted maximal plasma concentration at steady state (8). Approval for these procedures was obtained from the South Metropolitan Area Health Service Human Research Ethics Committee. The blood samples were centrifuged promptly, and aliquots of separated plasma were stored at −20°C. Pre- and posttreatment plasma samples were assayed for atorvastatin by using high-performance liquid chromatography (20). A modified microdilution isotopic method (9, 15) was used to determine the antimalarial activities of pre- and posttreatment plasma spiked with CQ (concentration range, 3.9 to 250 nM), DHA (0.9 to 60 nM), or an equivalent volume of drug-free RPMI. In triplicate experiments, aliquots of 100 μl of spiked plasma were added to 90 μl of parasite suspension (1.0% parasitemia, 1.5% hematocrit) and 10 μl of 3H-hypoxanthine (0.5 μCi) in 96-well plates, and the mixture was incubated, harvested, and counted.

The in vitro inhibitory effects of the two statins and CQ are summarized in Table 1. Both statins showed antimalarial activity, but atorvastatin was more potent than rosuvastatin. The IC50 and IC90 values for each statin did not differ between CQ-sensitive and CQ-resistant strains and were well above those for CQ, even against CQ-resistant strains. Isobolograms

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for atorvastatin with CQ and DHA are shown in Fig. 1. The interaction was indirect in each case (ΣFIC mean [95% confidence interval] of 1.01 [0.92 to 1.10] and 1.09 [1.01 to 1.17], respectively). Plasma atorvastatin concentrations in the healthy volunteer were undetectable at pretreatment and were 118 µg/liter (0.1 µM) posttreatment. Neither pre- nor post-treatment plasma inhibited growth of 3D7. The 3D7 IC₅₀ for CQ alone, CQ plus pretreatment plasma, and CQ plus post-treatment plasma were similar (20.5 nM, 20.5 nM, and 20.4 nM, respectively), as were those for DHA alone, DHA plus pretreatment plasma, and DHA plus posttreatment plasma (12.7 nM, 20.1 nM, and 19.2 nM, respectively).

The present data confirm that atorvastatin inhibits the growth of *P. falciparum* in vitro (12, 21). This activity is greater than that of rosuvastatin, but the atorvastatin IC₅₀ (15 to 25 µM; Table 1) is approximately 100 times above that achievable in plasma with repeated maximal doses (0.1 to 0.3 M; Table 1) is approximately 100 times above that achievable in plasma with repeated maximal doses (0.1 to 0.3 µM) (4). As might have been predicted from this observation, and consistent with the hypothesis that the metabolism of atorvastatin does not generate compounds with antimalarial activity, the bioassay showed that therapeutic plasma concentrations had no inhibitory effect against cultured *P. falciparum*. In addition, there was no synergy with conventional antimalarial drugs.

The ability of the malaria parasite to synthesize cholesterol de novo appears limited (27, 29), and the presence of an HMG-CoA homolog was not revealed by BLASTX analysis of the *P. falciparum* sequence with other protozoal HMG-CoA protein sequences (21). These observations and the greater antimalarial potency of atorvastatin versus rosuvastatin (the reverse of their ability to inhibit cholesterol synthesis in humans [13]) suggest an alternative, albeit low-potency, mechanism of antimalarial action to inhibition of HMG-CoA reductase. Our in vitro findings and outcome data from animal models of severe malaria (3, 14) do not support calls for clinical trials of statins as adjuvant antimalarial therapy (3).

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### REFERENCES


### TABLE 1. In vitro activities of statins against CQ-sensitive and CQ-resistant strains of *P. falciparum*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Atorvastatin IC₅₀ (µM)</th>
<th>Atorvastatin IC₉₀ (µM)</th>
<th>Rosuvastatin IC₅₀ (µM)</th>
<th>Rosuvastatin IC₉₀ (µM)</th>
<th>CQ [IC₅₀ (nM)]</th>
</tr>
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<tbody>
<tr>
<td>3D7</td>
<td>25 (16–39)</td>
<td>68 (38–121)</td>
<td>80 (47–137)</td>
<td>205 (113–373)</td>
<td>30 (23–39)</td>
</tr>
</tbody>
</table>

° The means (and 95% confidence intervals) shown are from between four and seven independent triplicate experiments. Data from CQ-resistant strains DD2 and E8B were similar and have been pooled.