Proveblue (Methylene Blue) as an Antimalarial Agent: In Vitro Synergy with Dihydroartemisinin and Atorvastatin

Proveblue (international patent PCT/FR/2007/001193), which is a methylene blue preparation that complies with the European Pharmacopoeia and contains limited organic impurities and heavy metals of recognized toxicity, has previously been demonstrated to possess in vitro antimalarial activity (at a geometric mean 50% inhibitory concentration [IC50] of 3.62 nM) against 23 Plasmodium falciparum strains that are resistant to various other antimalarials (11). No significant association was found between Proveblue IC50s and polymorphisms in the genes that are involved in quinoline resistance, such as pfcr, pfmdr1, pfmdr2, pfnr1, and pfhni-1; furthermore, there was no significant association between Proveblue IC50 and the copy numbers of pfmdr1 and pfmdr2 (11).

In the present study, we tested the effects of Proveblue in combination with the standard antimalarial drugs chloroquine (CQ), monodesethylamodiaquine (MDAQ; the active metabolite of amodiaquine), quinine (QN), mefloquine (MQ), and dihydroartemisinin (DHA) and with atorvastatin (AVA), a potential antimalarial drug (9, 12).

The methodology of the in vitro potentiating test was previously described (7). We used nine well-established Plasmodium falciparum strains that had different phenotypic profiles: 3D7, W2, Palo Alto, FCR3, FCM29, ImtVol, ImtK2, ImtL1, and falciparum (8). We used nine well-established Plasmodium falciparum strains that are resistant to various other antimalarials (11). No significant association was found between Proveblue IC50s and polymorphisms in the genes that are involved in quinoline resistance, such as pfcr, pfmdr1, pfmdr2, pfnr1, and pfhni-1; furthermore, there was no significant association between Proveblue IC50 and the copy numbers of pfmdr1 and pfmdr2 (11).

While Proveblue was shown to have antagonistic effects in combination with CQ and additive effects in combination with MDAQ against the nine P. falciparum strains (Fig. 1), Proveblue exhibited noticeable synergistic effects in combination with MQ and QN (2). More interestingly, the combination of Neph MB with artemisinin, artesunate, or artemether was found to act synergistically on the K1 strain (2).

TABLE 1 Reduction of the in vitro IC50s of CQ, MDAQ, QN, MQ, DHA, and AVA in combination with Provebluea

<table>
<thead>
<tr>
<th>Antimalarial</th>
<th>0.04 nMb</th>
<th>0.08 nMc</th>
<th>0.16 nMd</th>
<th>0.31 nMe</th>
<th>0.63 nMf</th>
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<tbody>
<tr>
<td>CQ</td>
<td>4.3 [0.9–7.7] (0.250)</td>
<td>4.1 [0.6–7.6] (0.441)</td>
<td>8.8 [2.9–14.7] (0.130)</td>
<td>9.2 [1.1–17.4] (0.054)</td>
<td>11.8 [2.2–21.3] (0.054)</td>
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<tr>
<td>MDAQ</td>
<td>6.2 [0–12.6] (0.859)</td>
<td>15.1 [6.1–24.0] (0.075)</td>
<td>15.4 [7.4–23.2] (0.044)</td>
<td>19.3 [8.3–30.3] (0.039)</td>
<td>17.4 [4.3–30.6] (0.008)</td>
</tr>
<tr>
<td>MQ</td>
<td>3.0 [0–6.3] (0.820)</td>
<td>7.5 [6.1–17.4] (0.383)</td>
<td>8.3 [1.8–15.7] (0.074)</td>
<td>15.3 [5.6–24.9] (0.004)</td>
<td>20.6 [12.1–29.0] (0.009)</td>
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<tr>
<td>DHA</td>
<td>12.6 [5.0–20.1] (0.027)</td>
<td>15.1 [5.3–25.0] (0.020)</td>
<td>20.9 [8.4–33.5] (0.004)</td>
<td>25.6 [14.0–37.3] (0.004)</td>
<td>31.5 [22.7–40.3] (0.004)</td>
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<tr>
<td>AVA</td>
<td>18.9 [8.3–29.4] (0.012)</td>
<td>23.7 [11.8–35.5] (0.008)</td>
<td>33.0 [19.3–46.7] (0.008)</td>
<td>41.2 [27.9–54.5] (0.004)</td>
<td>48.0 [32.6–63.3] (0.009)</td>
</tr>
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<td></td>
<td>24.6 [13.8–35.4] (0.020)</td>
<td>37.0 [18.1–56.0] (0.020)</td>
<td>43.6 [28.9–58.2] (0.020)</td>
<td>56.3 [40.8–71.8] (0.020)</td>
<td>63.1 [51.7–74.4] (0.020)</td>
</tr>
</tbody>
</table>

a CI confidence interval. P values (for antimalarial plus Proveblue versus antimalarial alone) were determined by the Wilcoxon signed rank test. Significant P values (<0.05) are in bold.

b Mean IC50/140.

c Mean IC50/70.

d Mean IC50/35.

e Mean IC50/18.

f Mean IC50/9.
0.04 to 0.63 nM. Like Proveblue, AVA improved the in vitro activity of MQ (14), QN (10), or DHA (13) and the IC50s of AVA were unrelated to the mutations that occurred in the transport protein genes that are involved in quinoline resistance (9). The synergistic effect of AVA on MQ was significantly associated with the pfmdr1 copy number (14). However, there was no association between Proveblue activity and the pfmdr1 copy number (11). Even if we cannot explain the synergy between Proveblue and AVA, this observation supports the calls for in vivo evaluations in the murine malaria model.

These results confirm the therapeutic potential of Proveblue, which is a new methylene blue that contains limited organic im-
purities and heavy metals of recognized toxicity and could be integrated into new, low-cost antimalarial combination therapies.

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REFERENCES