Effects of piperaquine, chloroquine and amodiaquine on drug uptake and in combination with dihydroartemisinin against drug sensitive and resistant *Plasmodium falciparum* strains

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Abstract

Piperquine is being developed as a long-acting component in artemisinin combination therapies. It was highly active in vitro and drug interaction studies showed that dihydroartemisinin combinations with piperquine, chloroquine, and amodiaquine were indifferent tending towards antagonism. Competitive uptake of radiolabelled chloroquine and dihydroartemisinin in combination with other antimalarials was observed.
The bis-4-aminoquinoline piperaquine (PPQ) and its analogues have been shown to be potent against chloroquine-sensitive (CQS) and -resistant (CQR) parasites in vitro (1, 5) and in the field (18). Artemisinin derivatives are being evaluated as combination regimens (ACT) to treat malaria and in particular to combat multidrug resistant *P. falciparum*. It is hoped that combination chemotherapy will delay or at best prevent the onset of resistance to new agents and avoid cross-resistance to existing ones (21). PPQ has been used successfully for mass prophylaxis and treatment in China (18) and is increasingly being developed as a long-acting component in ACTs (7). One recent study assessed the in vitro interaction between PPQ and dihydroartemisinin (DHA) concluding antagonism for K1 (CQR) and no interaction for 3D7 (CQS) strains of *P. falciparum* (7). An antagonistic drug combination may compromise efficacy, and possibly increase the chances of resistance developing and spreading (10); and there may be situations, such as when treatment is incomplete, in which an antagonistic interaction could become significant (7). Our aim was to assess the in vitro effects of DHA in combination with PPQ against a range of *P. falciparum* strains with varying degree of drug resistance and to compare these results with DHA combined with common 4-aminoquinolines chloroquine (CQ) and amodiaquine (AQ). In addition, we examined the effect of a range of antimalarial drugs on the in vitro uptake of radiolabelled DHA and CQ.

Dose-response assays to obtain IC\textsubscript{50} values of individual drugs and fixed ratio combination assays were followed as previously published (10) at 1% parasitemia and 1% hematocrit. Uptake of \[^{3}\text{H}\]-DHA (Moravek Biochemicals, USA; 1.4 Ci/mmol) and \[^{3}\text{H}\]-CQ (DuPont NEN, USA; 50.4 Ci/mmol) was done at 37 °C for 90 min. The experiment was initiated with addition of trophozoite parasites (5% parasitemia, 1.5% hematocrit) to microtubes containing both the
unlabelled antimalarial and radiolabelled drug (12). Samples were then centrifuged through silicon oil (AnalaR, BDH), processed (12) and radioactivity was determined on a Beckman liquid scintillation spectrometer. Uptake was represented as a percentage of control parasitized erythrocytes minus uptake of drug in uninfected erythrocytes (± relative SD). All experiments were repeated at least twice in triplicate.

The sensitivities of eight laboratory parasite lines were assessed to a range of antimalarial drugs (Table 1). Overall, three of the parasite lines were CQS, four were CQR and 106/1, was moderately CQR. CQ IC₅₀ values correlated well with CQ uptake values as all CQR parasite lines accumulated ~4-fold less [³H]-CQ (P < 0.001; data not shown) in support of previous observations (9, 19). Parasite line 106/1 had a significantly higher CQ IC₅₀ compared to the other CQS lines (P ≤ 0.024) and a significant decrease in CQ susceptibility was seen with 34-1/E. The CQS 106/1 clone contains all of the ‘ancillary’ PfCRT mutations associated with CQ resistance but lacks the crucial lys⁷⁶thr mutation seen in all resistant isolates or the lys⁷⁶ile PfCRT mutation in 34-1/E (8). The marked increase in CQ IC₅₀ with the single mutation in pfcrt confirms previous observations (8, 16). This IC₅₀ difference could be explained, in part, by the decrease in [³H]-CQ uptake demonstrated in 34-1/E (data not shown). PPQ was active in all parasite lines; although some cross-resistance with CQ in CQR lines K1, RSA11 and 7G8 was observed. [³H]-CQ uptake (Figure 1) was significantly reduced in all four lines by DHA (P < 0.001) and ATM (P ≤ 0.008). However, in CQS FC27 and 3D7, ATM had a significantly weaker ability to reduce [³H]-CQ uptake compared to DHA (P < 0.001). In all lines, both unlabelled CQ and PPQ strongly reduced [³H]-CQ uptake (P < 0.001); but MQ had a lesser effect (P ≤ 0.003). The study of [³H]-DHA uptake in the four lines showed no significant difference in rate of uptake or final amount of drug...
accumulated (data not shown). In all lines, artemisinins DHA and ATM blocked majority of
$[^3]H$-DHA uptake (Figure 2), whilst quinolines CQ, AQ, PPQ and MQ all significantly ($P \leq
0.001$) reduced uptake. The fractional inhibitory concentration (FIC) values in Table 2 indicate
that the interactions between DHA and PPQ, CQ or AQ in the six lines tested were indifferent
tending towards antagonism. Previous findings of antagonism between artemisinin derivatives
and CQ support the above results (4, 11, 17).

Competition for uptake of CQ and DHA at the same site (12) or competition for
ferriprotoporphyrin (FPIX), a by-product of hemoglobin breakdown, may lead to the observed
antagonism. As it was shown that CQ, AQ and PPQ reduce DHA accumulation, and vice versa
for CQ, it may be possible that this could be a contributing factor towards antagonism seen here.
CQ binds to FPIX extremely avidly (6) and this saturable CQ uptake into the digestive vacuole
has been proposed as the cause of intracellular accumulation of drug by the parasite (3).
Artemisinin reacts with FPIX to form an adduct (13, 14) and molecular modelling studies have
shown a stable docked configuration of artemisinin and FPIX could exist (15). Bound DHA
might sterically protect the CQ from interacting with FPIX, which could cause a decrease in CQ
accumulation. Drugs which bind to FPIX have been shown to competitively inhibit CQ uptake
(2). On the other hand, bound CQ would sterically protect FPIX from DHA interaction which
may protect FPIX from the free-radical producing reaction with artemisinins (22) leading to
antagonism. PPQ interacts in a similar fashion with FPIX in vitro as it has been shown to prevent
$\beta$-hematin formation (20) and could therefore antagonise similarly.

It is important to understand the effect of drug combinations at the parasite level in vitro as there
is concern that if drug combinations are antagonistic in vivo, the efficacy of such regimens might be compromised. It is difficult to predict in vivo drug interactions in humans based on in vitro findings, and the significance of an indifferent toward antagonistic interaction at typical therapeutic doses may be less apparent. However, further studies on the biochemical mechanisms behind antagonism or synergy are necessary to enhance our understanding.
We thank Dr. Piero Olliaro (WHO) for supplying PPQ. Prof. David Fidock (Albert Einstein College of Medicine, New York, USA) for 106/1 and 34-1/E. Prof. Pete Smith (Dept. Pharmacology, University of Cape Town, South Africa) for RSA11. Dr. Pat Bray and Prof. Steve Ward (Liverpool School of Tropical Medicine, UK) for purifying and supplying $[^3]H$-DHA and for 7G8-mdr. Originally from Prof. Alan Cowman (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). Quinton Fivelman was supported by the Association of Commonwealth Universities, Ipmia Adagu was supported by Romark Research laboratory and David Warhurst thanks UK PHLS for financial support.
References


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Table 1. In vitro sensitivities (nmol/L) of eight parasite lines to antimalarial drugs chloroquine (CQ), amodiaquine (AQ) and piperaquine (PPQ). Parasite lines in the top section of the table are chloroquine-sensitive and lower section chloroquine-resistant, respectively \(^a\).

<table>
<thead>
<tr>
<th>Parasite</th>
<th>CQ</th>
<th>AQ</th>
<th>PPQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC(_{50}) ± SEM</td>
<td>IC(_{50}) ± SEM</td>
<td>IC(_{50}) ± SEM</td>
</tr>
<tr>
<td>FC27</td>
<td>16.48 ± 0.74</td>
<td>6.68 ± 0.38</td>
<td>29.61 ± 3.75</td>
</tr>
<tr>
<td>T996</td>
<td>23.37 ± 0.21</td>
<td>14.78 ± 0.21</td>
<td>19.93 ± 2.01</td>
</tr>
<tr>
<td>3D7</td>
<td>22.76 ± 0.51</td>
<td>18.36 ± 0.52</td>
<td>36.90 ± 2.16</td>
</tr>
<tr>
<td>106/1</td>
<td>48.52 ± 5.12</td>
<td>17.48 ± 0.55</td>
<td>22.22 ± 1.78</td>
</tr>
<tr>
<td>K1</td>
<td>266.58 ± 15.06</td>
<td>22.38 ± 0.73</td>
<td>49.03 ± 1.79</td>
</tr>
<tr>
<td>RSA11</td>
<td>220.36 ± 6.63</td>
<td>26.66 ± 1.89</td>
<td>51.38 ± 1.68</td>
</tr>
<tr>
<td>7G8-mdr(^{7G8})</td>
<td>290.05 ± 12.09</td>
<td>32.71 ± 4.03</td>
<td>49.71 ± 1.33</td>
</tr>
<tr>
<td>34-1/E</td>
<td>299.45 ± 1.60</td>
<td>17.24 ± 0.51</td>
<td>16.38 ± 1.20</td>
</tr>
</tbody>
</table>

\(^a\) CQ resistant lines regarded as IC\(_{50}\) > 100 nM.
Table 2. The Mean FIC of the interactions between dihydroartemisinin (DHA) and PPQ, CQ or AQ. Strains in lower section of the table are chloroquine-resistant \(^a\).

<table>
<thead>
<tr>
<th>Strain</th>
<th>PPQ + DHA</th>
<th>CQ + DHA</th>
<th>AQ + DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC27</td>
<td>1.66 ± 0.08</td>
<td>1.37 ± 0.09</td>
<td>1.34 ± 0.10</td>
</tr>
<tr>
<td>3D7</td>
<td>1.52 ± 0.16</td>
<td>1.55 ± 0.08</td>
<td>1.38 ± 0.09</td>
</tr>
<tr>
<td>T996</td>
<td>1.37 ± 0.11</td>
<td>1.60 ± 0.10</td>
<td>1.35 ± 0.07</td>
</tr>
<tr>
<td>K1</td>
<td>1.37 ± 0.08</td>
<td>1.36 ± 0.06</td>
<td>1.48 ± 0.09</td>
</tr>
<tr>
<td>RSA11</td>
<td>1.61 ± 0.15</td>
<td>1.48 ± 0.07</td>
<td>1.56 ± 0.07</td>
</tr>
<tr>
<td>7G8</td>
<td>1.47 ± 0.13</td>
<td>1.49 ± 0.09</td>
<td>1.39 ± 0.06</td>
</tr>
</tbody>
</table>

\(^a\) Mean FIC using IC\(_{50}\) values with SEM. CQ resistant lines regarded as IC\(_{50}\) > 100 nM.
Fig 1.

![Graph showing the percentage uptake of $[^{3}$H$]$-CQ compared to control for different drugs in combination.](image_url)
Fig. 1 (Fivelman et al.)

Effects of antimalarials on the uptake of 2.5 nM [3H]-CQ after 90 min in erythrocytes infected with *P. falciparum* a

a Each bar represents the average of at least 2 experiments. An asterisk signifies a statistically significant decrease (*P* < 0.05) in all four parasite lines compared to drug uptake alone.
Fig. 2.

[Graph showing the percentage uptake of [3H]-DHA compared to control for different drug combinations and strains.]

- FC27
- 3D7
- K1
- RSA11

Drug in combination:
- DHA
- ATM
- CQ
- AQ
- PPQ
- MQ

% uptake of [3H]-DHA compared to control
Fig. 2 (Fivelman et al.)

Effects of antimalarials on the uptake of 3 nM $[^3H]$-DHA after 90 min in erythrocytes infected with *P. falciparum* $^a$

$^a$ As for figure 1.
Drug in combination

% uptake of [3H]-CQ compared to control

FC27 3D7 K1 RSA11

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Drug in combination

% uptake of $[^3H]$-DHA compared to control

Drug in combination

DHA  ATM  CQ  AQ  PPQ  MQ

FC27  3D7  K1  RSA11

*