

Effects of Piperaquine, Chloroquine, and Amodiaquine on Drug Uptake and of These in Combination with Dihydroartemisinin against Drug-Sensitive and -Resistant *Plasmodium falciparum* Strains[∇]

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Piperaquine 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 piperaquine, chloroquine, and amodiaquine were indifferent tending toward antagonism. Competitive uptake of radiolabeled chloroquine and dihydroartemisinin in combination with other antimalarials was observed.

Bis-4-aminoquinoline piperaquine (PPQ) and its analogues have been shown to be potent against chloroquine (CQ)-sensitive (CQS) and CQ-resistant (CQR) parasites in vitro (1, 5) and in the field (18). Artemisinin derivatives are being evaluated as combination regimens (ACTs) to treat malaria and in particular to combat multidrug-resistant *Plasmodium 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 (20). 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), finding 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 various degrees of drug resistance and to compare these results with DHA combined with the common 4-aminoquinolines CQ and amodiaquine (AQ). In addition, we examined the effect of a range of antimalarial drugs on the in vitro uptake of radiolabeled DHA and CQ.

Dose-response assays to obtain 50% inhibitory concentration (IC₅₀) values of individual drugs and fixed ratio combination assays were carried out as previously published (10) at 1% parasitemia and 1% hematocrit. Uptake of [³H]DHA (Moravek Biochemicals) at 1.4 Ci/mmol and [³H]CQ (DuPont NEN) at 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 unlabeled antimalarial and the radiolabeled drug (12).

Samples were then centrifuged through silicon oil (AnalaR; BDH) and processed (12), and the radioactivity was determined on a Beckman liquid scintillation spectrometer. Uptake was represented as a percentage of control parasitized erythrocytes minus the uptake of drug in uninfected erythrocytes (\pm the relative standard deviation). 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 line 106/1 was moderately CQR. CQ IC₅₀ values correlated well with CQ uptake values since 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 than did other CQS lines ($P \leq 0.024$), 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 ^{lys76} mutation seen in all resistant isolates or the ^{lys76} PfCRT mutation in 34-1/E (8). The marked increase in CQ IC₅₀ with the single mutation in *pfcr*t 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,

TABLE 1. In vitro sensitivities of eight parasite lines to the antimalarial drugs CQ, AQ, and PPQ^a

Parasite	Mean in vitro sensitivity (IC ₅₀ [nmol/liter]) \pm SEM		
	CQ	AQ	PPQ
CQS			
FC27	16.48 \pm 0.74	6.68 \pm 0.38	29.61 \pm 3.75
T996	23.37 \pm 0.21	14.78 \pm 0.21	19.93 \pm 2.01
3D7	22.76 \pm 0.51	18.36 \pm 0.52	36.90 \pm 2.16
106/1	48.52 \pm 5.12	17.48 \pm 0.55	22.22 \pm 1.78
CQR			
K1	266.58 \pm 15.06	22.38 \pm 0.73	49.03 \pm 1.79
RSA11	220.36 \pm 6.63	26.66 \pm 1.89	51.38 \pm 1.68
7G8-mdr ^{7G8}	290.05 \pm 12.09	32.71 \pm 4.03	49.71 \pm 1.33
34-1/E	299.45 \pm 1.60	17.24 \pm 0.51	16.38 \pm 1.20

^a CQR lines are regarded as an IC₅₀ of >100 nM.

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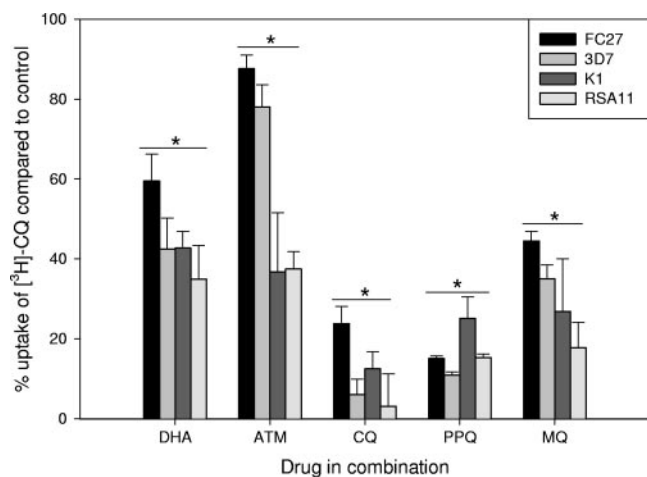


FIG. 1. Effects of antimalarials on the uptake of 2.5 nM [^3H]CQ after 90 min in erythrocytes infected with *P. falciparum*. Each bar represents the average of at least two experiments. An asterisk signifies a statistically significant decrease ($P < 0.05$) in all four parasite lines compared to drug uptake alone.

although some cross-resistance with CQ in CQR lines K1, RSA11, and 7G8-*mdr*^{7G8} was observed. [^3H]CQ uptake (Fig. 1) was significantly reduced in all four lines by DHA ($P < 0.001$) and artemether (ATM; $P \leq 0.008$). 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 unlabeled CQ and PPQ strongly reduced [^3H]CQ uptake ($P < 0.001$), but mefloquine (MQ) had a lesser effect ($P \leq 0.003$). The study of [^3H]DHA uptake in the four lines showed no significant difference in the rate of uptake or the final amount of drug accumulated (data not shown). In all lines, artemisinins DHA and ATM blocked majority of [^3H]DHA uptake (Fig. 2), while the quinolines CQ, AQ, PPQ, and MQ all significantly ($P \leq 0.001$) reduced uptake. The fractional inhibitory concentration (FIC) values in Table 2

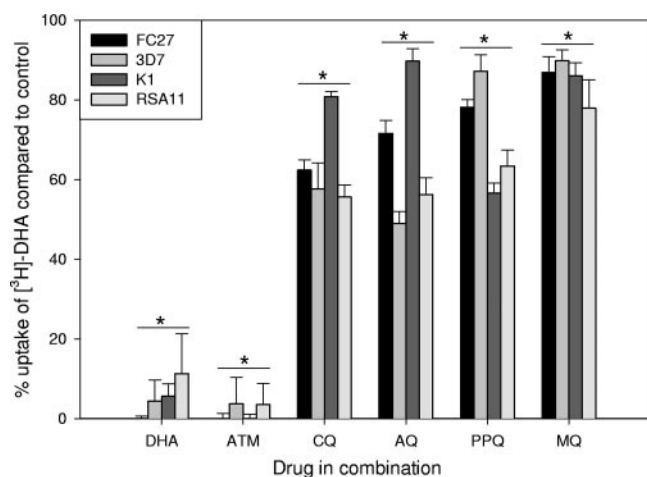


FIG. 2. Effects of antimalarials on the uptake of 3 nM [^3H]DHA after 90 min in erythrocytes infected with *P. falciparum*. Each bar represents the average of at least two experiments. An asterisk signifies a statistically significant decrease ($P < 0.05$) in all four parasite lines compared to drug uptake alone.

TABLE 2. Mean FICs of the interactions between DHA and either PPQ, CQ, or AQ^a

Strain	Mean FIC \pm SEM		
	PPQ + DHA	CQ + DHA	AQ + DHA
CQS			
FC27	1.66 \pm 0.08	1.37 \pm 0.09	1.34 \pm 0.10
3D7	1.52 \pm 0.16	1.55 \pm 0.08	1.38 \pm 0.09
T996	1.37 \pm 0.11	1.60 \pm 0.10	1.35 \pm 0.07
CQR			
K1	1.37 \pm 0.08	1.36 \pm 0.06	1.48 \pm 0.09
RSA11	1.61 \pm 0.15	1.48 \pm 0.07	1.56 \pm 0.07
7G8- <i>mdr</i> ^{7G8}	1.47 \pm 0.13	1.49 \pm 0.09	1.39 \pm 0.06

^a The mean FIC was determined using IC₅₀ values. CQR lines regarded as an IC₅₀ of >100 nM.

indicate that the interactions between DHA and PPQ, CQ, or AQ in the six lines tested were indifferent, tending toward antagonism. Previous findings of antagonism between artemisinin derivatives and CQ support these 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. Since 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 toward the 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 modeling studies have shown that 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 that 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 (21), leading to antagonism. PPQ interacts in a similar fashion with FPIX in vitro since it has been shown to prevent β -hematin formation (19a) and could therefore antagonize similarly.

It is important to understand the effect of drug combinations at the parasite level in vitro since 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 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.

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