3-halo Chloroquine Derivatives Overcome PfCRT-mediated Drug Resistance in

*Plasmodium falciparum*

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Running Title: 3-iodo Chloroquine Reverses PfCRT-mediated Drug Resistance

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Polymorphism in *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) was shown to cause chloroquine resistance. In this report we examined the antimalarial potential of novel 3-halo chloroquine derivatives (3-chloro, 3-bromo and 3-iodo) against chloroquine-susceptible and -resistant *P. falciparum*. All three derivatives inhibited the proliferation of *P. falciparum*; with 3-iodo chloroquine being most effective. Moreover, 3-iodo chloroquine was highly effective at potentiating and reversing chloroquine toxicity of drug-susceptible and -resistant *P. falciparum*.

Malaria is a devastating infectious disease worldwide, with 135 million to 287 million cases in 2014 and an estimated 627000 deaths annually (1). The use of chloroquine (CQ), a once highly effective and inexpensive anti-malarial drug, has been discontinued due to the rise and spread of CQ resistance in most endemic regions (2). Chloroquine has been shown to accumulate in the digestive vacuolar (DV) whereby it binds to hemin and interferes with the hemozoin crystal formation (3). Chloroquine-resistant parasites have been shown to encode mutant form of *P. falciparum* chloroquine resistance transporter (PfCRT) (4). However, several novel drug candidates based on CQ structure, with modifications of both the side chain and the quinoline ring, have been shown to bypass PfCRT-mediated resistance (5-8). In this report we evaluated the antimalarial activity of three novel halo-chloroquine derivatives, with halogen groups (iodine, bromine or chlorine) at the 3rd position of 4-aminoquinoline. The 3-halo derivatives of...
chloroquine were isolated as di-phosphate or tri-phosphate white solids, characterized by elemental analysis, NMR, and IR spectroscopy (detailed elsewhere). The antimalarial activity of the three halo-derivatives against CQ-susceptible (3D7) and -resistant (Dd2) strains of *P. falciparum* was evaluated *in vitro*. Figure 1 shows the proliferation of 3D7 and Dd2 strains of *P. falciparum* in the presence of increasing molar concentrations of CQ or 3-halo-CQ derivatives. The three derivatives inhibited the proliferation of 3D7 and Dd2 *P. falciparum* to different extents with IC₅₀ values of 367 nM – 747 nM against 3D7, and 623 nM to 1163 nM for Dd2. These IC₅₀ values were higher than those seen with CQ for the two different strains (e.g., 21 nM and 178 nM for 3D7 and Dd2, respectively). However, unlike CQ, the 3-halo-CQ derivatives were equally effective against CQ-susceptible and –resistant parasites with ~2 fold differences in IC₅₀ values versus ~8.5 fold difference for CQ. These results suggest that, unlike CQ, the resistance mechanisms in Dd2 are less effective against the 3-halo derivatives (figure 1). Hence, modifying the 3rd position on the 4-aminoquinoline has the potential to bypasses Dd2 resistance mechanisms (e.g., mutations in PfCRT and PfMDR1), but reduces the 3-halo CQ anti-proliferative activity against the parasite. However, it is presently unclear if the observed reduction (~10-fold) in the IC₅₀ values seen with the three halo-derivatives is due to modifications of this 3rd position of the quinoline ring or due to the addition of polar groups and consequently reduced drug accumulation or both. Interestingly, among the three derivatives, 3-iodo-CQ showed the lowest IC₅₀ towards 3D7 and Dd2 (e.g., 367 nM and 623 nM, respectively). Taken together, our results are consistent with earlier findings whereby modification of the 7-chloro-4-aminoquinoline ring of CQ reduced their antimalarial activity (9, 10), while changes to the CQ side chain enhanced their activities (11). Given these results for 3-
halo CQ, with respect to bypassing PfCRT-mediated resistance, it was of interest to determine if 3-iodo-CQ potentiates CQ antimalarial activity against CQ-susceptible and –resistant *P. falciparum*. The results in figure 2 show that increasing doses of 3-iodo-CQ (50, 100 and 200 nM) render 3D7, D10, FCR3 and Dd2 more sensitive to CQ. The fact that the IC₅₀ value of both drugs combination is lower than the IC₅₀ value of each drug alone suggests a chemosensitization or reversal effect. Taken together, the above results suggest that 3-iodo-CQ may be a clinically useful compound, in combination with CQ, as antimalarial therapy against CQ-resistant *P. falciparum*. Of special interest are the findings of PfCRT-dependent synergy between primaquine (an 8-aminoquinoline) and CQ, whereby the micromolar concentrations of primaquine potentiates the toxicity of CQ sensitivity in *P. falciparum* is mediated primarily through the drug interaction with mutant PfCRT that allows the transport of protonated CQ (CQH₂⁺⁺) from the parasite’s digestive vacuole (13). Although it remains to be determined if 3-iodo-CQ plus CQ is a better drug combination than primaquine plus CQ (13), given the associated side effects of primaquine (14), our results show that 10 µM of 3-iodo-CQ and CQ are equally tolerated by two mammalian cell lines (HeLa and CCR F-CEM; results not shown). Moreover, lower concentration of 3-iodo-CQ was required to potentiate the growth inhibitory activity of CQ towards CQ-resistant *P. falciparum*, primaquine or quinine dimers (13, 15). Verapamil plus CQ drug combinations have been previously used to reverse CQ resistance *in vivo* (16). Thus, it will be of interest to determine if 3-iodo-CQ/CQ is more effective *in vivo* than the CQ-VP combinations.
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Conflicts of interest. There are no conflicts of interest from any of the authors.

REFERENCES


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**FIGURE LEGENDS**

**Figure 1.** *Effects of 3-halo chloroquine derivatives on the proliferation of CQ-susceptible and – resistant strains of P. falciparum* - Chloroquine-susceptible (3D7) and -resistant (Dd2) *P. falciparum* strains were cultured in the presence of increasing concentrations of CQ or each of the 3-halo derivatives. The graphs show the mean ± SD of three independent experiments done in triplicates.

**Figure 2.** *3-iodo CQ potentiates CQ growth inhibitory effects* - Chloroquine-susceptible (3D7, D10) and -resistant (FCR3, Dd2) strains of *P. falciparum* were cultured in the presence of increasing concentrations of CQ alone or in the presence of fix molar concentrations (e.g., 50, 100 and 200 nM) of 3-iodo-CQ. The graphs show the mean ± SD of three independent experiments.
experiments done in triplicates. The notation in the figure as wo/3-iodo-CQ or w/3-iodo-CQ refer to without or with/3-iodo-CQ, respectively.
Parasite Proliferation
(\% of Control)

Log 3-Chloro-CQ (nM)

Log CQ (nM)

Log 3-Iodo-CQ (nM)

Log 3-Bromo-CQ (nM)