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3-Halo Chloroquine Derivatives Overcome *Plasmodium falciparum* Chloroquine Resistance Transporter-Mediated Drug Resistance in *P. falciparum*

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Polymorphism in the *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 627,000 deaths annually (1). The use of chloroquine (CQ), a once highly effective and inexpensive antimalarial drug, has been discontinued due to the rise and spread of CQ resistance in most regions of endemicity (2). Chloroquine has been shown to accumulate in the digestive vacuole (DV), whereby it binds to hemin and interferes with hemozoin crystal formation (3). Chloroquine-resistant parasites have been shown to encode a mutant form of the *Plasmodium 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 activities 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 diphosphate or triphosphate white solids and characterized by elemental analysis, nuclear magnetic resonance (NMR), and infrared (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 the 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 50% inhibitory concentrations (IC_{50}s) of 367 to 747 nM against 3D7 and 623 nM to 1,163 nM against Dd2. These IC_{50}s 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 IC_{50}s versus an ~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 (Fig. 1). Hence, modification of the 3rd position on the 4-aminoquinoline has the potential to bypass Dd2 resistance mechanisms (e.g., mutations in PfCRT and PfMDR1) but reduces the 3-halo-CQ antiproliferative activity against the parasite. However, it is presently unclear if the observed reduction (~10-fold) in the IC_{50}s seen

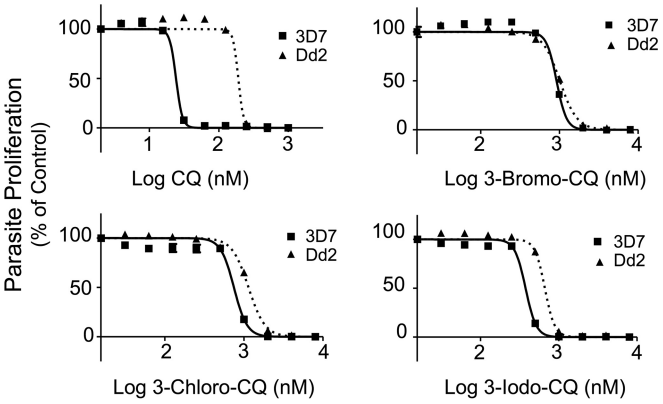

FIG 1 Effects of 3-halo chloroquine derivatives on the proliferation of CQ-susceptible and -resistant strains of *P. falciparum*. Chloroquine-susceptible (3D7) and -resistant (Dd2) *P. falciparum* strains were cultured in the presence of increasing concentrations of CQ or each of the 3-halo derivatives.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

with the three halo derivatives is due to modifications of this 3rd position of the quinoline ring, 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 IC_{50} toward 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

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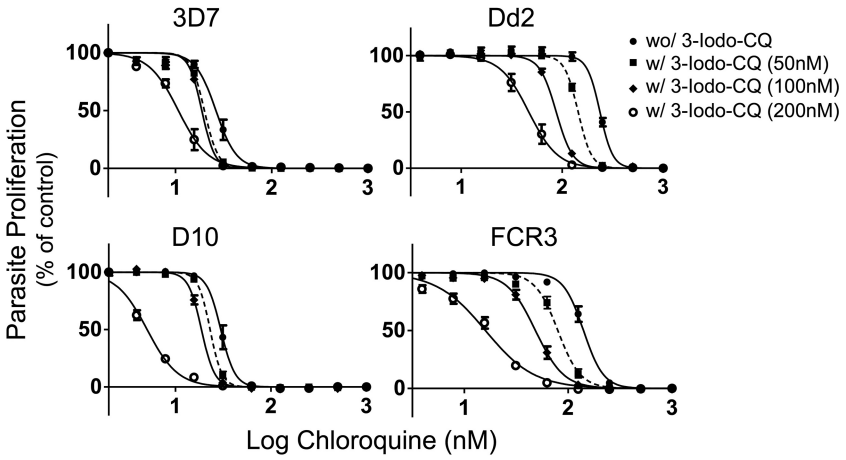

FIG 2 3-Iodo CQ potentiates CQ growth-inhibitory effects. Chloroquine-susceptible (3D7, D10) and -resistant (FCR3, Dd2) strains of *P. falciparum* were cultured in the presence of increasing concentrations of CQ alone or in the presence of fixed molar concentrations (e.g., 50, 100, and 200 nM) of 3-iodo-CQ. The graphs show the means ± SD from three independent experiments done in triplicate. "wo/" and "w/" indicate without and with 3-iodo-CQ, respectively, at the concentration given in parentheses.

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 Fig. 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 IC_{50} of both drugs in combination is lower than the IC_{50} 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 potentiation of the toxicity of CQ sensitivity in *P. falciparum* by micromolar concentrations of primaquine is mediated primarily through the drug interaction with mutant PfCRT that allows the transport of protonated CQ (CQH_2^{++}) from the parasite's digestive vacuole (12). Although it remains to be determined if 3-iodo-CQ plus CQ is a better drug combination than primaquine plus CQ (12), given the associated side effects of primaquine (13), our results show that 10 μM concentrations of 3-iodo-CQ and CQ are equally tolerated by two mammalian cell lines (HeLa and CCRF-CEM [data not shown]). Moreover, a lower concentration of 3-iodo-CQ was required to potentiate the growth-inhibitory activity of CQ toward CQ-resistant *P. falciparum* through primaquine or quinine dimers (12, 14). Drug combinations of verapamil (VP) plus CQ have been previously used to reverse CQ resistance *in vivo* (15). 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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