Short Communication

Fluoxetine Potentiates Chloroquine and Mefloquine Effect on Multidrug-Resistant *Plasmodium falciparum* In Vitro

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**SUMMARY**: Fluoxetine (FLX), a P-glycoprotein inhibitor with antimalarial activity, is a promising candidate for reversing chloroquine/mefloquine (CQ/MQ) resistance. The Dd2 strain of CQ- and MQ-resistant *Plasmodium falciparum* was synchronized and assayed with various concentrations of CQ/MQ individually and in combination with FLX or verapamil (VPL). Our results indicated the 50% inhibitory concentration values of CQ and MQ were greatly lowered when FLX was used simultaneously. Isobolograms indicated that CQ-FLX combinations are more synergistic (mean fractional inhibitory concentration [FIC] index 0.55) than MQ-FLX (mean FIC index 0.64), and their synergistic effect was better than or at par with CQ-VPL (mean FIC index 0.88) and MQ-VPL (mean FIC index 0.60) combinations. We conclude that the FLX potentiates the CQ and MQ response on multidrug-resistant *P. falciparum* at clinically achievable concentrations.

The resistance of *Plasmodium falciparum* to chloroquine (CQ) has become a worldwide issue (1). Mefloquine (MQ) has been used to treat CQ-resistant malaria; however, MQ-resistant strains of *P. falciparum* have appeared in recent years, resulting in a need to find new treatment strategies against multidrug-resistant *P. falciparum* strains. P-glycoprotein (P-gp) inhibitors such as verapamil (VPL) reverse multidrug resistance (MDR) in cancer (2). A similar protein, P-gp homologue (Pgh1), is localized on the food vacuole of *P. falciparum*, and MQ resistance is associated with increased *P. falciparum* multidrug-resistant (*pfmdr1*) gene copy number (3). Therefore, it was postulated that Pgh1 in *P. falciparum* could serve as an efflux pump and reduce the concentration of CQ in the digestive vacuole, its site of action (4). Contrary to these earlier reports, it has also been found that overexpression of P-gp causes resistance to MQ through an unknown mechanism and increases sensitivity to CQ (5, 6). Specific mutations such as N86Y in *pfmdr1* appear to be more closely related to the modulation of CQ resistance, while other polymorphisms are also being tied to MQ resistance (7).

Fluoxetine (FLX), a selective serotonin reuptake inhibitor and a common antidepressant drug, has low antimalarial activity apart from being a known P-gp inhibitor and has the potential to enhance CQ sensitivity (8-10). However, there is no study yet discussing the effect of FLX on MQ-resistant strains. P-glycoprotein (P-gp) inhibitors such as verapamil (VPL) reverse multidrug resistance (MDR) in cancer (2). A similar protein, P-gp homologue (Pgh1), is localized on the food vacuole of *P. falciparum*, and MQ resistance is associated with increased *P. falciparum* multidrug-resistant (*pfmdr1*) gene copy number (3). Therefore, it was postulated that Pgh1 in *P. falciparum* could serve as an efflux pump and reduce the concentration of CQ in the digestive vacuole, its site of action (4). Contrary to these earlier reports, it has also been found that overexpression of P-gp causes resistance to MQ through an unknown mechanism and increases sensitivity to CQ (5, 6). Specific mutations such as N86Y in *pfmdr1* appear to be more closely related to the modulation of CQ resistance, while other polymorphisms are also being tied to MQ resistance (7).

Fluoxetine (FLX), a selective serotonin reuptake inhibitor and a common antidepressant drug, has low antimalarial activity apart from being a known P-gp inhibitor and has the potential to enhance CQ sensitivity (8-10). However, there is no study yet discussing the effect of FLX on MQ-resistant *P. falciparum*.

In this study, we planned to evaluate whether FLX is equally effective or superior in reversing MQ resistance in comparison to CQ resistance in *P. falciparum* and to compare its effect with that of a chemosensitizer, VPL.

ATCC (MRA-156), a cell line of CQ- and MQ-resistant *P. falciparum* strain Dd2, was used and maintained according to the methods of Trager and Jensen (11) in 10% pooled serum and 5% O2 human erythrocytes. Growth medium RPMI 1640 (GIBCO™; Invitrogen Corp., Auctland, NZ) was supplemented with gentamicin (1 mg/ml) and glucose (0.9%). When the parasitaemia reached 5 to 8% with a majority of ring forms, cultures were synchronized by using 5% D-sorbitol (Sigma, St. Louis, Mo., USA) and were cultured for another 48 h prior to drug assays. When more than 80% of parasites were at the ring stage of infection and more than 0.5% of infected erythrocytes were evident, the cultures were then used in drug assays.

CQ diphosphate and FLX hydrochloride (Sigma-Aldrich, Steinheim, Germany) were dissolved in sterile distilled water, and MQ hydrochloride (Mepha, Basel, Switzerland) was dissolved in 95% ethanol and diluted in RPMI media to make 1% ethanol solution. We evaluated the susceptibility of the Dd2 to the test drugs by using a modification of the semiautomated microdilution technique (12). The drug assays were repeated 2 times. CQ and MQ were diluted serially in an individual microtiter plate to yield a concentration range from 500 ng/ml to 1 ng/ml. A series of fixed doses of FLX were added to each plate containing 0.5 to 1% parasites, 1% erythrocytes, and 10% serum, and the microtiter plate was then incubated at 37°C in airtight desiccators in the presence of 5% O2, 5% CO2 and 90% N2. Twenty-four hours post incubation, the culture was labeled with [3H]-Hypoxanthine (0.5 μCi/ml/well; Amersham Life Sciences, Buckinghamshire, UK) and incubated for another 48 h prior to harvesting. The incorporation of tritiated hypoxantine in *P. falciparum* was determined by using a liquid scintillation counting detector (Plate CHAMELEON multilable counter 425 -104; Hidex Oy, Turku, Finland), and the 50% inhibitory concentration (IC50) values of CQ, MQ, FLX and various combinations of CQ + FLX and MQ + FLX were derived from probit methods according to Litchfield and Wilcoxon by using SPSS software.

Modulation of IC50 values of CQ/MQ (drug A) in the presence of FLX (drug B) was expressed as a fractional inhibitory concentration 50 (FIC50), and the FIC50 and FIC index were calculated using the following formulae:

\[
\text{FIC}_{\text{A/B}} = \frac{\text{FIC}_{\text{A}}}{\text{FIC}_{\text{B}}}
\]

Where FIC50 A is the FIC index of drug A in the presence of drug B and FIC50 B is the FIC index of drug B in the presence of drug A.

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**REFERENCES:**


7. Azahri Noor A'shikin, Mohd Nor Norazmi, Manickam Ravichandran and Suraparaju Sivachandra Raju. Modulation of IC50 values of CQ/MQ (drug A) in the presence of FLX (drug B) was expressed as a fractional inhibitory concentration 50 (FIC50), and the FIC50 and FIC index were calculated using the following formulae:

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\]

Where FIC50 A is the FIC index of drug A in the presence of drug B and FIC50 B is the FIC index of drug B in the presence of drug A.
FIC\textsubscript{50}A = \frac{IC_{50} \text{ of drug A at fixed concentration of drug B}}{IC_{50} \text{ of drug A alone}}

FIC\textsubscript{50}B = \frac{IC_{50} \text{ of drug B at fixed concentration of drug A}}{IC_{50} \text{ of drug B alone}}

FIC index = FIC\textsubscript{50}A + FIC\textsubscript{50}B

From the plotted isobologram, synergism was defined as occurring when the derived FIC index was less than 1.0 (FIC index < 1.0), antagonism was defined as occurring when it was more than 1.0 (FIC index > 1.0) and an additive effect was defined as occurring when it was equivalent to 1.0 (FIC index = 1.0).

The mean IC\textsubscript{50} values of CQ, MQ and FLX were 60.0 ± 5.0 ng/mL, 16.0 ± 3.0 ng/mL and 2.0 ± 0.2 µg/mL, respectively. The IC\textsubscript{50} and FIC\textsubscript{50} values of CQ and MQ were lowered substantially in the presence of various fixed concentrations of FLX. As the concentration of FLX increased, the growth inhibitory effects of CQ and MQ increased and the growth curves shifted to the left (Fig. 1A and 1B), clearly demonstrating the synergistic interaction between FLX and CQ/MQ on the Dd2 strain of \textit{P. falciparum}.

The best method for analyzing the effect of drug combination is constructing isobolograms. As the isobolograms (Fig. 2) indicated the points on concave isobols, the response of CQ + FLX and MQ + FLX combinations was synergistic. However, CQ + FLX (mean FIC index 0.55) was more synergistic than MQ + FLX (mean FIC index 0.64). When compared to VPL, FLX was more synergistic with CQ (mean FIC index of CQ + VPL 0.88) and slightly less synergistic with MQ (mean FIC index of MQ + VPL 0.60) against the Dd2 strain of \textit{P. falciparum}.

The emergence and spread of multidrug-resistant \textit{P. falciparum} has severely limited the therapeutic options for the treatment of malaria. With ever-increasing failure rates associated with CQ or MQ treatment, attention has turned to the few alternatives, which include combination therapy with chemosensitizers. Several drugs, including FLX, have been reported to have a chemosensitizing effect on CQ-resistant malaria but not on MQ-resistant malaria. Only a few drugs, including ketoconazole (13), are known to have a chemosensitizing effect on MQ-resistant malaria. In fact, there is a great need to find suitable chemosensitizers for the treatment of multidrug-resistant malaria.

We found that FLX could inhibit plasmodium growth at a very high concentration, similar to that reported in earlier studies (9). FLX enhanced both CQ and MQ sensitivity, and its potentiating effect on CQ corroborates the findings reported previously (9,10). However, the potentiating effect of FLX on MQ is a new finding, and its mechanism is yet to be demonstrated. Cowman et al. (14) reported that P-gp inhibitors decrease CQ sensitivity and increase MQ sensitivity. According to their observation, FLX, a known P-gp inhibitor, should have decreased the CQ sensitivity and increased the MQ sensitivity. On the other hand, our results indicate that FLX reverses both CQ and MQ resistance and potentiates CQ more than MQ.

The IC\textsubscript{50} of FLX (2,167 ng/mL) is not achievable clinically (the peak concentration with the therapeutic dose is 531 ng/mL, and 94% of it is protein bound). However, the concentrations (15 ng/mL to 31 ng/mL) at which FLX has potentiated the effect of CQ and MQ are achievable. FLX is quite safe to use even during pregnancy and in elderly individuals (15). Unlike FLX, the potentiating effect of VPL was observed only at concentrations that are too high to be safe for use in clinical practice. Interestingly, we observed that the synergic effect of FLX-CQ/FLX-MQ combinations were
better than or at par with VPL-CQ/VPL-MQ combinations. From these results, we conclude that FLX potentiates the effects of both CQ and MQ on multidrug-resistant *Plasmodium falciparum* in vitro at clinically achievable concentrations.

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**REFERENCES**