Citalopram Enhances the Activity of Chloroquine in Resistant Plasmodium in Vitro and in Vivo

S. G. EVANS, N. BUTKOW, C. STILWELL, M. BERK, N. KIRCHMANN and I. HAVLIK

Department of Experimental and Clinical Pharmacology (S.G.E., N.B., C.S., N.K., I.H.) and Department of Psychiatry (M.B.), University of the Witwatersrand, Parktown, South Africa

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ABSTRACT

Citalopram is an extremely potent inhibitor of neuronal serotonin reuptake. It is structurally unrelated to other antidepressants, but it contains the chemical features associated with reversal of drug resistance and exhibits minimal cardiotoxic side effects and fewer of the anticholinergic and adrenolytic side effects associated with other psychotropic agents. Sensitivity tests to citalopram alone and in combination with chloroquine were performed against chloroquine-resistant and chloroquine-sensitive strains of Plasmodium falciparum and Plasmodium chabaudi. Citalopram alone showed intrinsic activity against the chloroquine-resistant strains of P. falciparum (IC_{50} = 1.51 ± 0.6 μM) but only limited activity against the chloroquine-sensitive strain (IC_{50} = 33.27 ± 5.87 μM) and no activity in vivo. The interaction of chloroquine and citalopram in vitro resulted in a synergistic response in the chloroquine-resistant strain but there was no interaction between the drugs in the chloroquine-sensitive strain—a pattern found with other reversal agents. Citalopram enhanced chloroquine susceptibility in both strains of P. chabaudi, however, the potentiating effect was seen at lower doses in the chloroquine-resistant strain. The results of this study suggest that citalopram may have potential as a chemosensitizer in Plasmodium infections on the basis of the low toxicity of citalopram at concentrations potentiating chloroquine activity both in vitro and in vivo.

Malaria is a significant source of global morbidity and mortality. Despite the development of new antimalarial agents such as mefloquine, halofantrine and the artemisins, chloroquine remains the drug of choice for the treatment of uncomplicated Plasmodium falciparum malaria infections, due to its low cost, rapid onset of action and its low toxicity. However, the efficacy of chloroquine has diminished due to the emergence and prevalence of chloroquine-resistant strains of P. falciparum (Wensdorfer and Payne, 1991).

The rapid development and spread of resistance to chloroquine and other antimalarials, and the tremendous cost of drug development has emphasized the necessity to optimize the use of existing antimalarial agents (Schuster and Milhous, 1993). A chemotherapeutic strategy that strives to augment the efficacy of chloroquine with adjunct agents that reverse chloroquine resistance has been developed.

A number of adjunct drugs have been identified from a wide variety of chemical classes including calcium-channel blockers (Kyle et al., 1990; Martin et al., 1987), antihistamines (Basco et al., 1981) tricyclic antidepressants (Bitonti et al., 1988) and most recently selective serotonin reuptake inhibitors (Gerena et al., 1992). However, the clinical usefulness of these agents has been limited due to high protein binding and toxicity at the concentrations required to reverse resistance (Ford and Hait, 1993).

Citalopram, 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3dihydroisobenzofuran-5-carbonite, is an extremely potent inhibitor of neuronal serotonin reuptake (Christensen et al., 1977; Pawlowski et al., 1981). The high specificity results in minimal effects on other neurotransmitter receptors and uptake. Thus citalopram shows fewer of the anticholinergic or adrenolytic side effects associated with other psychotropic agents and it has minimal cardiotoxic side effects. Citalopram is structurally unrelated to other antidepressants, but it contains the chemical features associated with reversal of drug resistance (Zamora et al., 1988).

The low toxicity coupled with the chemical similarity to chemosensitizers (resistance reversal agents) prompted us to investigate the chemosensitizing effect of citalopram in Plasmodium. In this study, we screened citalopram for chloroquine potentiating activity in chloroquine-resistant and chloroquine sensitive-parasites; both in vitro against P. falciparum and in a rodent malaria model (Plasmodium chabaudi).

Methods

Effect of Citalopram in Vitro

Parasites. Two well-characterized isolates of P. falciparum were used for the drug assays. The chloroquine-resistant FCR-3 strain (IC_{50} = 150 nM) (donated by J. Freese, Research in diseases of the Tropical Environment, Durban, South Africa) and the chloroquine-
sensitive 3D7a strain (IC$_{50}$ ~ 20 nM) (donated by D. Walliker, WHO Standard Registry of malaria strains, University of Edinburgh, Edinburgh, Scotland). Parasites were cultured according to the method of Freese et al. (1988).

**Drug assays.** The susceptibilities of each clone to citalopram hydrobromide (donated by Lundbeck A/S Copenhagen, Denmark) alone and in combination with chloroquine diphosphate (Sigma Chemical Co., St. Louis, MO) were evaluated using the hypoxanthine uptake method as an index of parasite growth (Desjardens et al., 1979). Briefly, synchronous (Lambros and Vanderberg, 1979) ring stage parasites (1% hematocrit and 0.5% parasitemia) in RPMI medium supplemented with 25 mM HEPES, 32 mM NaHCO$_3$, and 10% human plasma were added to 96-well microtiter plate containing fixed ratios of drugs (Evans and Havlik, 1994; Martin et al., 1987). After 24 hr of incubation of the plates in an anaerobic environment at 37°C, $^3$H-hypoxanthine (1.85 μCi) (Amersham Life Sciences, Buckinghamshire, UK) was added to each well. The plates were incubated a further 18 hr before harvesting. Incorporation of radiolabel was determined by scintillation spectrophotometry. The IC$_{50}$ values, representing the molar concentration resulting in a 50% decrease in $^3$H-hypoxanthine incorporation compared to drug-free and uninfected erythrocyte controls, were calculated from computer generated dose response curves analyzed by nonlinear regression.

**Evaluation of drug interactions.** To evaluate citalopram’s modulation of chloroquine-resistance, isobolograms were constructed for both parasite strains by plotting the fractional IC$_{50}$ of chloroquine alone against the fractional IC$_{50}$ of chloroquine plus citalopram. Points lying above the straight diagonal line (corresponding to the points where there is no interaction between the drugs) are antagonistic, points below the straight diagonal line are considered to be synergistic (Berenaus, 1978).

**Effect of Citalopram in Vivo**

**Infection.** Female BALB/c mice, 6 to 8 wk old, were infected with either a chloroquine-resistant or chloroquine-sensitive strain of *P. chabaudi* (donated by D. Walliker, WHO Standard Registry of Malaria strains, University of Edinburgh, Edinburgh). Briefly, blood was collected from infected mice and their erythrocytes were suspended in saline at a density of $5 \times 10^7$ parasitized red blood cells/ml. Mice were inoculated i.p. with 0.1 ml of parasite suspension. The parasitemia was monitored daily by counting the number of infected erythrocytes per 10,000 erythrocytes on tail blood smears stained with Giemsa. The results are expressed as the mean parasitemia ± S.D. and the difference was analyzed by Student’s t test.

**Administration of drugs.** Chloroquine was administered to the mice at a previously determined dose that failed to clear the parasites—2 mg/kg for the chloroquine-sensitive strain and 3 mg/kg for the chloroquine-resistant strain. Chloroquine and citalopram were dissolved to the desired concentrations in saline. The drug combinations of no more than 0.2 ml were injected s.c. daily for four consecutive days starting 60 min after infection.

**Results**

The mean IC$_{50}$ value for citalopram against the chloroquine-resistant strain is 1.51 ± 0.6 μM, which is significantly lower than the concentrations reported for other reversing agents that range from 5 to 20 μM (Basco et al., 1991; Bitonti et al., 1988; Gerena et al., 1992; Martin et al., 1987). In the chloroquine-sensitive strain the IC$_{50}$ was 33.27 ± 5.87 μM, more than 20 times that of the resistant strain. A decrease in sensitivity of chloroquine-sensitive strains to “reversal agents” is well characterized, however, the mechanism underlying the phenomenon is unknown. The intrinsic activity observed *in vitro* was not translated into an *in vivo* effect where a dose of 120 mg/kg citalopram had no effect on parasite survival (data not shown).

Citalopram showed a typical synergistic response in the chloroquine-resistant strain of *P. falciparum in vitro* (fig. 1) (Martin et al., 1987). Citalopram enhanced the susceptibility of chloroquine-resistant parasites to chloroquine in *in vitro* at concentrations >1 μM. The potentiating effect was not seen in the chloroquine-sensitive parasites except at high concentrations of citalopram.

Citalopram enhanced chloroquine susceptibility in both strains of *P. chabaudi*. Figure 2a illustrates the effect of citalopram on the growth of the chloroquine-resistant *P. chabaudi* on day 5 postinfection. Citalopram alone (120 mg/kg), did not affect the growth of the parasite. However, a combination of chloroquine with citalopram suppressed the parasite growth in a dose-dependent manner (the decrease in parasitemia was significant at 10, 50 and 120 mg/kg, P < .001). Because citalopram at these doses does not inhibit parasite development, the results indicate citalopram reverses chloroquine-resistance. The results of citalopram treatment on the course of infection in the chloroquine-sensitive strain of *P. chabaudi* (fig. 2b) are similar to those in the chloroquine-resistant strain. Citalopram when administered alone (120 mg/kg) did not affect the development of the parasitemia. Citalopram in combination with chloroquine in the chloroquine-sensitive strain also significantly suppressed parasitemia (at doses of 50 (P < .01) and 120 mg/kg (P < .001). A similar synergy pattern has been demonstrated for other “reversal” agents (Miki et al., 1992).

**Discussion**

These results indicate citalopram can be added to the growing list of agents that show chemosensitizing properties in malaria. Citalopram has not previously been identified as a P-glycoprotein inhibitor (Ford, 1995) but it shares the critical structural requirements identified for P-glycoprotein inhibition, namely two tricyclic rings with an alkyl bridge (Zamora et al., 1988).

In cancer cell lines, the resistance reversing action of chemosensitizing drugs has been fairly well characterized. The chemosensitizing agent is thought to compete with the anti-tumor drug for a limited number of translocation sites on the P-glycoprotein molecule (Ford, 1995). The resistance reversal mechanism in *P. falciparum* is not so well characterized although clearly chemosensitizing agents can cause resistant parasites to accumulate more chloroquine (Krogstad et al., 1998).
1987). Initially it was believed that the chemosensitizers inhibited P-glycoprotein in the parasite. A growing body of evidence suggests that P-glycoprotein represents only a part of a more complex multicomponent system that mediates drug resistance in *P. falciparum* (Wernsdorfer and Payne, 1991).

Numerous currently available drugs can modulate chloroquine-resistance in *P. falciparum in vitro*. Unfortunately the maximum tolerated dose of the clinically available modulators yield serum levels significantly below those required for resistance reversal. Citalopram shows a greater potency for tors yield serum levels significantly below those required for maximum tolerated dose of the clinically available modulator.

**References**


**Fig. 2.** Parasitemia on day-5 postinfection after treatment with chloroquine and different doses of citalopram. A, The chloroquine-resistant and B, the chloroquine-sensitive strain of *P. chabaudi* (*P* < .01, **P** < .001)