

Neuronal Monoamine Reuptake Inhibitors Enhance In Vitro Susceptibility to Chloroquine in Resistant *Plasmodium falciparum*

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Chloroquine resistance in *Plasmodium falciparum* was reversed in vitro by the neuronal monoamine reuptake inhibitors and antidepressants desipramine, sertraline, fluoxetine, and norfluoxetine but not by carbamazepine, an antiseizure and mood-stabilizing tricyclic drug resembling desipramine which only weakly inhibits neuronal monoamine reuptake. These findings have important clinical implications for drug combination therapy.

The goal of this work was to investigate the potential use of clinically relevant antidepressants as combination therapy with antimalarial drugs in drug-resistant malarial infections. Previous studies have shown that antidepressants such as desipramine, imipramine, protriptyline, nortriptyline, doxepin, and fluoxetine modify chloroquine resistance in *Plasmodium falciparum* in vitro (1, 4, 11). These tricyclic antidepressants and fluoxetine block the sodium-dependent neuronal reuptake of either norepinephrine or serotonin, and tricyclic antidepressants such as imipramine also block the H⁺-ATPase-regulated monoamine uptake by monoamine storage granules (5). More recently, it has been shown that the monoamine transport proteins in the neuronal plasma cell membrane (10, 18) and neuronal monoamine storage vesicles (10) belong to a superfamily of transport proteins with 12 transmembrane domains which also include the malaria parasite protein *mdr1* (7, 19) and the P glycoprotein in tumor cells (9). In this article, we report that the two antidepressants and neuronal monoamine reuptake inhibitors sertraline and norfluoxetine modify antimalarial resistance to chloroquine in vitro. Taken together with previous studies (1, 4, 11), these findings suggest that antidepressants which act as neuronal monoamine reuptake inhibitors may also enhance the antimalarial activity of chloroquine against drug-resistant forms of *P. falciparum*.

The organisms used in these studies were (i) the chloroquine-resistant clone W2 of *P. falciparum* from Indochina and (ii) the chloroquine-sensitive clone HB3 of *P. falciparum* from Honduras. The drugs tested were obtained from Lilly (fluoxetine HCl [molecular weight, 346] and norfluoxetine HCl [molecular weight, 331]), Pfizer (sertraline HCl [molecular weight, 343]), USV (desipramine HCl [molecular weight, 303]), and Sigma (carbamazepine [molecular weight, 236] and chloroquine diphosphate [molecular weight, 516]). Sertraline, fluoxetine, and norfluoxetine block the neuronal reuptake of serotonin, but the chemical structure of sertraline is different from the structures of fluoxetine and norfluoxetine.

The concentrations of desipramine, fluoxetine, and norfluoxetine were verified by high-performance liquid chromatog-

raphy analysis performed by the Psychiatric Chemistry Laboratory, a division of the New England Deaconess Hospital, Boston, Mass. Sertraline, fluoxetine, norfluoxetine, desipramine, and carbamazepine were dissolved in absolute ethanol and diluted approximately 1,000-fold in aqueous media prior to addition to cell culture. Drug sensitivity testing was done by using the incorporation of [³H]hypoxanthine according to a modification of the method of Desjardins et al. (8). Serial dilutions of the drug being tested were done in triplicate for each assay. The [³H]hypoxanthine incorporation data were analyzed by nonlinear regression to obtain the 50% inhibitory concentration (IC₅₀).

We examined the reversal of chloroquine resistance in the W2 clone of *P. falciparum* by several neuronal monoamine reuptake inhibitors, namely, desipramine, fluoxetine, norfluoxetine, and sertraline. The monoamine reuptake inhibitors had intrinsic antimalarial activities only at very high concentrations. The IC₅₀s obtained for the W2 clone for each drug are as follows: chloroquine, 65 ng/ml; desipramine, 2,770 ng/ml; fluoxetine, 2,455 ng/ml; norfluoxetine, 1,045 ng/ml; and sertraline, 1,458 ng/ml. The drug IC₅₀s determined for the chloroquine-sensitive clone HB3 are as follows: chloroquine, 10 ng/ml; desipramine, 4,000 ng/ml; and sertraline, 1,590 ng/ml.

In Table 1, we present the response modification index (RMI) for each monoamine reuptake inhibitor, which reflects modulation of susceptibility to chloroquine in the presence of one of the monoamine reuptake inhibitors at predetermined concentrations. The RMI for each monoamine reuptake inhibitor decreased as the concentration of psychotropic drug was increased, showing that each drug potentiated the antimalarial activity of chloroquine in the clone W2 (1, 3, 4, 11). The isobolograms reflecting a drug-drug interaction between chloroquine and each monoamine reuptake inhibitor are shown in Fig. 1. For each of the monoamine reuptake inhibitors, we obtained a concave-shaped isobologram, indicating that desipramine, fluoxetine, norfluoxetine, and sertraline each acts synergistically with chloroquine to suppress the growth of the chloroquine-resistant clone W2 (6).

The antiseizure and mood-stabilizing drug carbamazepine has a tricyclic structure similar to those of the tricyclic antidepressants imipramine and desipramine. However, unlike imipramine and desipramine, carbamazepine only weakly inhibits neuronal monoamine reuptake (16). In our studies of the reversal modulation of chloroquine resistance in W2 by car-

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TABLE 1. Modulation of chloroquine resistance in *P. falciparum* by neuronal monoamine reuptake inhibitors

Drug concn (ng/ml)	RMI ^a of:				
	Sertraline (n = 5) ^b	Fluoxetine (n = 5) ^b	Norfluoxetine (n = 2)	Desipramine (n = 1)	Carbamazepine ^c (n = 1)
1,000	0.23 ± 0.04	0.15 ± 0.04	0.004	0	0.91
500	0.15 ± 0.03			0.09	1.04
300	0.21 ± 0.10	0.24 ± 0.06			
250	0.30 ± 0.04		0.11	0.16	0.85
150	0.36 ± 0.16	0.27 ± 0.07			
125	0.40 ± 0.09	0.46 ± 0.16	0.16	0.21	0.91
75	0.65 ± 0.18	0.57 ± 0.11	0.25		
37	0.95 ± 0.08			0.24	
0	1.0	1.0	1.0	1.0	1.0

^a The RMI equals the IC₅₀(A,B) divided by the IC₅₀(A), where IC₅₀(A) is the chloroquine IC₅₀ and IC₅₀(A,B) represents the new chloroquine IC₅₀ obtained in the presence of the modulator B at a given concentration.

^b Values for sertraline and fluoxetine are means ± standard deviations.

^c Carbamazepine weakly inhibits neuronal monoamine reuptake (16).

bamazepine, carbamazepine had no intrinsic antimalarial activity at concentrations up to 10 µg/ml and failed to modulate chloroquine resistance in W2 at concentrations up to 10 µg/ml (Table 1). Carbamazepine has a tricyclic structure resembling that of the neuronal monoamine reuptake inhibitor desipramine and the antihistaminic drug cyproheptadine, both of which have been reported to reverse chloroquine resistance in vitro (1–4, 13). However, carbamazepine, which is a weak inhibitor of neuronal monoamine reuptake, did not reverse chloroquine resistance in the clone W2.

We also examined the capacity of low concentrations of desipramine (25 to 50 ng/ml), a neuronal reuptake inhibitor of norepinephrine (but not serotonin), to enhance the reversal

modulation of chloroquine resistance by sertraline and fluoxetine, two neuronal reuptake inhibitors of serotonin (but not norepinephrine). We found that low concentrations of desipramine (which themselves reverse chloroquine resistance [Table 1]) did not enhance the reversal by sertraline and fluoxetine (data not shown).

The RMI for sertraline (concentration range, 0 to 300 ng/ml) in the chloroquine-sensitive clone HB3 of *P. falciparum* was 1.05, showing that the resistance modulator sertraline had no meaningful potentiation of chloroquine sensitivity in clone HB3. Earlier studies have also reported that desipramine (1, 4) and fluoxetine (11) did not potentiate chloroquine sensitivity in chloroquine-sensitive clones of *P. falciparum*.

Fluoxetine and its biologically active metabolite norfluoxetine both have long drug half-lives in humans (fluoxetine, 60 h; norfluoxetine, 190 h), while desipramine and sertraline have drug half-lives of approximately 20 h. The concentrations of desipramine and sertraline needed to reverse antimalarial drug resistance in vitro are well within the ranges for blood levels of desipramine (12) or sertraline (14) during antidepressant treatment (Table 1). While desipramine should be used with caution in prepubertal children (15), low-cost, clinically relevant antidepressants such as desipramine (1, 3, 4, 17) and sertraline may restore the therapeutic efficacy of chloroquine against resistant parasites in vivo and thereby enhance the clinical utility of chloroquine against widespread drug-resistant *P. falciparum*.

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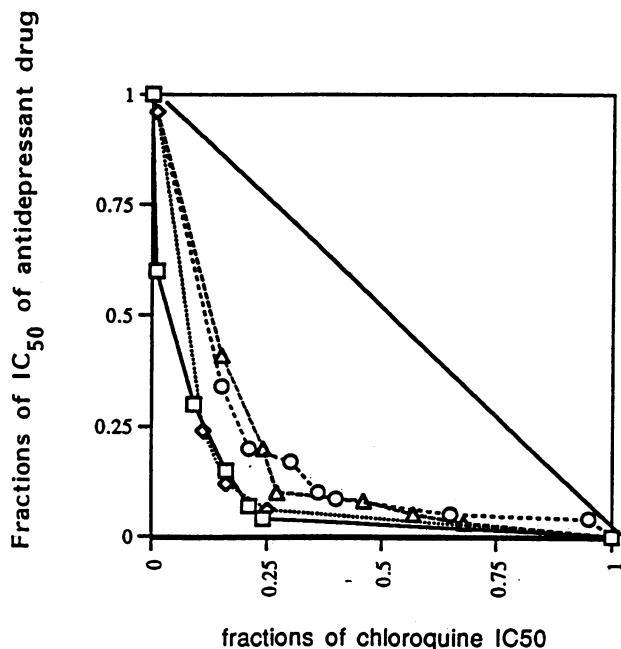


FIG. 1. Isobolograms showing synergistic antimalarial activity between chloroquine and desipramine (□), fluoxetine (△), norfluoxetine (◇), and sertraline (○). One unit of IC₅₀ refers to the normalized IC₅₀ for either chloroquine alone or antidepressant alone. In each drug combination, the resulting IC₅₀ was plotted as a fraction of the IC₅₀ for either chloroquine alone or antidepressant alone.

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