IMIPRAMINE INDUCED COMPLETE REVERSAL OF CHLOROQUINE RESISTANCE IN Plasmodium falciparum INFECTIONS IN SUDAN

Mohamed E. Adami1, El Fatih I. A/Karim1, Abdelgader Y. Elkadaru2, Kamal E.E. Ibrahim1, Bradley J. Berger3, M. Wiese4, Hamza A. Babiker5

The effect of imipramine on reversal of chloroquine (CQ) resistance was examined in 40 Plasmodium falciparum patients in Sudan. Parasites were considered resistant if it was still present in peripheral blood on day 3 following treatment with a standard dose of CQ (25mg/Kg body weight), early treatment failure (ETF). Patients proven to be infected with CQ resistant parasites were then given imipramine HCl (100 mg, in two divided doses), on day 3 following chloroquine treatment, for 3 days. Imipramine reversed CQ resistance, within 3 days, in all patients (cure rate of 100%), with mild to moderate side effects. The plasma level of CQ three hours after the initial dose (10mg/Kg body weight), was higher in patients with sensitive parasites (0.649 ± 0.04 x 10^-6 mol/L) than in those with resistant parasites (0.585 ± 0.043 x 10^-6 mol/L). However, this situation was reversed following imipramine administration, the CQ plasma levels in patients with resistant parasites (0.645 ± 0.089 x 10^-6 mol/L) becoming significantly higher (P < 0.05) than in patients with sensitive parasites (0.397 ± 0.032 x 10^-6 mol/L). The CQ plasma levels

1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, P O. Box 1996, Khartoum-Sudan.
2Omdurman Hospital for Tropical Diseases, Omdurman- Sudan.
3Institute of Biochemistry, University of Dundee, United Kingdom.
4Institute for Pharmazeutische Chemie, Fachbereich Pharmazie, Martin - Luther Universitat Halle - Wittenberg, Germany.
5Institute of Cell, Animals and Population Biology, University of Edinburgh, Kings Buildings, Edinburgh, United Kingdom.

*To whom correspondence should be addressed.
remained higher among the group infected with resistant *P. falciparum* on day 7 compared to the chloroquine sensitive group. The treatment regimen used was well tolerated, and the plasma concentration of imipramine and desipramine in treated patients accorded with that reported for *in vitro* reversal of CQ resistance.

**Keywords:** Chloroquine, imipramine, resistance, reversal, HPLC

**Introduction**

Resistance to chloroquine (CQ), the drug of choice for treatment and prophylaxis of *Plasmodium falciparum* malaria, has hindered control measures in tropical Africa and thus malaria has become a leading health problem. There are several views on the mode of action of chloroquine in *Plasmodium falciparum* (1-4). It has been demonstrated that resistant malaria parasites accumulate less CQ than susceptible forms (4,5). The reduced accumulation of CQ has been attributed to a rapid efflux of the drug by the resistant parasite. Verapamil, calcium channel blockers and other unrelated compounds e.g. antihistamines inhibit this process and reverse CQ resistance in *P. falciparum in vitro and in vivo* (5-8). Some tricyclic psychotrophic drugs have been found to have intrinsic antimalarial activity (9). This property has prompted some workers to study the effect of some of these drugs in combination with CQ for treatment of chloroquine resistant malaria. Imipramine and desipramine (the major metabolite of imipramine) in combination with CQ were found to inhibit *in vitro* growth of chloroquine resistant *P. falciparum* clones FCR-3 and W-2. The mean concentration of imipramine and desipramine that produced 50% inhibition of these clones ranged between $0.09 \times 10^{-6}$ mol/L and $0.12 - 0.11 \times 10^{-6}$mol/L, respectively (10). It has thus been suggested that desipramine and other tricyclic antidepressant compounds could reverse CQ resistance at therapeutic plasma levels such as those seen in patients undergoing treatment for depression. Subsequently, *in vivo* studies *Plasmodium chabaudi* have shown that these drugs when administered alone had little or no effect. However, on administration with CQ, these drugs suppressed the parasite growth in a dose dependent manner (9). Accordingly the need for *in vivo* studies of the effect of a combination of CQ/imipramine or CQ/desipramine on *P. falciparum* is warranted (11).

In this study, we have examined the efficacy of a combination of CQ/imipramine in treatment of patients with chloroquine resistant *P. falciparum*. The plasma levels of CQ, imipramine and desipramine in these patients were determined.

**Materials and methods**

**i- In vivo response of *P. falciparum* to chloroquine or chloroquine plus imipramine:**

This study was carried out in the Omdurman Hospital for Tropical Diseases, Sudan. *P. falciparum* patients who failed CQ or other antimalarials treatment were referred to this hospital for further management. In October 1997, 277 patients were referred during the peak of malaria transmission. Patients who were severely ill, with cardiovascular diseases, hyperthyroidism, impaired renal function, and history of epilepsy, glaucoma or urinary retention were excluded from the study.

Patients included in the study were informed of the overall as well as the specific aims of the study, and provided informed consent. The proposal was given ethical clearance by the Ethical Committee of the Ministry of Health, Sudan.

Patients who fulfilled the inclusion criteria were given a standard dose of CQ (15 mg kg$^{-1}$ on day 1 and 5 mg kg$^{-1}$ on day 2 and 3). The cases selected for this study were cases of early treatment failure (ETF) only. A patient was considered to harbour resistant *P. falciparum* infection if parasitaemia was still evident on day 3, following treatment, early treatment failure (ATF). A total of 40 patients with CQ resistant *P. falciparum* were identified in this way, 22 males and 18 females aged 16-50 years. They consented to take part in this study and were then admitted to the hospital for one week. On diagnosis of chloroquine resistant *P. falciparum* infection patients selected for imipramine treatment were given a daily dose of 100 mg (in two divided doses, 50 mg each), for 3 days. Patients abstained from taking any other drugs, acidic food or drinks 2 hours before and after CQ and imipramine therapy. All patients were followed up clinically for seven days. Blood smears for microscopical examination of malaria parasites were collected daily for the first 7 days and then weekly for 3 further weeks.

A venous blood sample (3ml) was collected, in lithium heparin tube, before CQ treatment and then 3 hours, 3 days and 7 days after treatment. Similar blood samples were collected before imipramine treatment and 3 hours (day one) and three days post...
treatment. The plasma was separated immediately and stored frozen (-20°C) until analysis.

(ii) Chromatographic measurement of chloroquine, imipramine, and desipramine -

Samples were analyzed for CQ, imipramine and desipramine by a reversed-phase HPLC. The method was based on previously described methods (12-16).

The HPLC consisting of model 126 pumps, model 166 photodiode array detector, model 507e autosampler, and System Gold operating software (Beckman; High Wycombe, UK), and octadecyl column (250 x 4.6 mm, 5 um particle size) for the separations (Alltech, UK).

The measurement of CQ was made at 343 nm (max. for CQ). The mobile phase used consisted of phosphate buffer (0.1M, pH 3): acetonitrile (88:12) and ethylamine (1% w/v) as base modifier. The flow rate was 1 ml min⁻¹. All measurements were made at ambient temperature and all solutions were protected from light.

The calibration curve for CQ was constructed using CQ free plasma in the concentration range 0.1-0.5 µg ml⁻¹ using quinidine (0.09 µg ml⁻¹) as internal standard. The plasma CQ was measured similarly using 0.5 ml of the plasma and quinidine as internal standard. Imipramine and desipramine were measured at 200 nm (max. for both drugs). The mobile phase consisted of phosphate buffer (0.1M, pH 3): acetonitrile (75:25) and nonylamine (0.06%v/v) as base modifier. The flow rate was 1 ml min⁻¹. A calibration curve for imipramine and desipramine was constructed using imipramine and desipramine-free plasma in a concentration range of 0.02 - 0.4 µg ml⁻¹ of each drug and nortriptyline (0.3) µg ml⁻¹ as internal standard. Plasma concentrations of both drugs were measured using 0.5 ml plasma from the patients and nortriptyline as internal standard.

Results

a) Chloroquine treatment:

Two hundred (72.2%) out of 277 patients examined were found to be infected with *Plasmodium falciparum* and were given the standard dose of CQ. One hundred and sixty (80%) out of the above 200 patients responded well to CQ treatment, clearing their clinical symptoms and parasitaemia by day 3 (48 hours following chloroquine treatment), and were thus referred to as the chloroquine sensitive group. Forty (20%) out of the 200 patients failed to respond to CQ treatment; they remained parasitaemic with malaria symptoms, and classified as the chloroquine resistant group.

b) Chloroquine/imipramine treatment:

Table 1 shows results of parasites and clinical symptoms clearance time following co-administration of CQ and imipramine. 29 (72.5%) out of 40 patients who received the CQ/imipramine combination showed neither parasitaemia nor symptoms after administration of 100 mg imipramine HCl (day 1 of imipramine treatment). Following the administration of a second dose of 100 mg imipramine, 34 (85%) out of the 40 patients cleared their parasitaemia and malaria symptoms. Total clearance of parasitaemia and clinical symptoms were observed in all 40 patients (100%) by day 3, after the administration of a third dose of 100 mg imipramine. Thus the curative dose of imipramine HCl reached a total of 300 mg.

Twenty-one (52%) of 40 patients, who received imipramine, did not report any side effects. However, 14 (35%) patients reported mild side effects of anticholinergic nature (dry mouth, blurred vision, drowsiness and fatigue), and 5 (13%) patients reported moderate side effects manifested in weakness, fatigue, drowsiness and disorientation which all subsided 2 days after termination of imipramine treatment. No significant difference (p < 0.05) was observed in the total white blood cell count before and after treatment.

Following imipramine treatment, (36 %) patients experienced an increase in hemoglobin from 2-15 %, while (32 %) patients showed a decrease ranging between 2 – 8 %. In (32 %) patients, hemoglobin levels did not change.

c) Plasma levels of chloroquine and imipramine:

Measurement of CQ was accomplished using a modified reversed phase HPLC method where the ion-pairing agent was not used and diethylamine (1% w/v) was used as base modifier. The calibration curve for CQ was prepared using CQ free-plasma spiked with quinidine (0.09 mg ml⁻¹) as an internal standard and a concentration range of 0.1 - 0.5 mg ml⁻¹ for CQ. The recovery was found to be 88%, with a lower limit of detection of 10 ng. The results obtained showed a linear relationship over the concentration range used (y = 9.87x + 0.018; r = 0.998) There was no significant difference between determinations. The precision of repeated
determinations in 3 days was RSD = 2.1%.

CQ plasma levels among patients with sensitive and resistance response are shown in Table 2. The mean CQ plasma concentration for patients with sensitive and resistance response, before treatment (with chloroquine), was \(0.099 \pm 0.03 \times 10^{-6} \text{ mol/litre}\) and \(0.165 \pm 0.055 \times 10^{-6} \text{ mol/litre}\), respectively. Three hours after the first dose of CQ (10 mg kg\(^{-1}\)) the level of the drug in patients with sensitive response was, \(0.649 \pm 0.04 \times 10^{-6} \text{ mol/liter}\), similar to that of patients with resistance response, \(0.585 \pm 0.043 \times 10^{-6} \text{ mol/liter}\). However, 3 days later the plasma CQ concentration in the sensitive patients (0.3965 \pm 0.032 \times 10^{-6} \text{ mol/liter}) was significantly lower than in patients with resistant infection (0.645- \pm 0.089 \times 10^{-6} \text{ mol/liter}). This difference became more apparent seven days after treatment, the plasma CQ concentrations in patients with sensitive (no imipramine) and resistant (imipramine treated) infections were 0.21 \pm 0.019 \times 10^{-6} and 0.33 \pm 0.038 \times 10^{-6} \text{ mol/litre} respectively.

Calibration curves for imipramine and desipramine were prepared using plasma free of both drugs and spiked with nortriptyline (0.03µg ml\(^{-1}\)) as internal standard with concentration ranges of 0.05 - 0.4 \mu g ml\(^{-1}\) for both drugs. The recovery for both drugs was found to be 84% and linear relationships over the concentrations used was obtained, (for imipramine \(y = 4.27x+ 0.02197, r = 0.999\); for desipramine \(y = 4.469x+ 0.02197; r = 0.999\)). In the day to day variation RSDs were 2.5% and 1.9% for imipramine and desipramine, respectively.

Three hours after treatment, the plasma concentration of imipramine was in the range 0.0 - 0.32 \times 10^{-6} \text{ mol/litre} and that of desipramine was in the range 0.0 - 0.13 \times 10^{-6} \text{ mol/L}. After two days the plasma concentrations of imipramine and desipramine were 0.0 - 0.5 \times 10^{-6} \text{ mol/L} and 0.0 - 0.49 \times 10^{-6} \text{ mol/L} respectively. The corresponding levels of the two drugs, after 3 days, were 0.0 - 1.068 \times 10^{-6} \text{ mol/litre} and 0.0 - 0.68 \times 10^{-6} \text{ mol/L}, respectively.

### Discussion

The tricyclic antidepressant imipramine and desipramine possess little intrinsic antimalarial activity, however they are well known as chloroquine resistance reversal compounds in \textit{in vitro} studies (17). Clinical trials have produced variable results. Studies in Nigeria showed a high imipramine efficacy in treatment of patients with chloroquine resistant \textit{P. falciparum}. On the other hand, desipramine did not show efficacy among Somalian patients (18).

In the present study, we examined the efficacy of imipramine/chloroquine combination and bioavailability of the drugs and imipramine metabolic product (desipramine) among Sudanese with chloroquine resistant \textit{P. falciparum} infection. Early treatment failure has been used as indicator of chloroquine resistance in this study, this is due to the fact that the examined patients had previously failed chloroquine treatment, and to avoid possible disease progression a short in vivo test has been considered. In this area the semi immune inhabitants (adults and children) are at risk of clinical malaria. Imipramine/chloroquine combination has shown great efficacy in management of chloroquine resistant \textit{P. falciparum} infection among the examined patients. All patients cleared their parasitaemias and clinical symptoms within 72 hours following administration of imipramine HCl (100 mg daily for 3 days in combination with CQ). The majority of them (72.5%) were cured upon administration of only 100 mg of imipramine. No sign of parasite recrudescence or malaria relapse was reported in any of the patients within 4 weeks. All the above patients did not respond to chloroquine treatment and had high level of plasma CQ before the starting imipramine treatment (Table 1).

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>Patients n (%)</th>
<th>Imipramine dose (mg)</th>
<th>Plasma levels (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>29 (72.5)</td>
<td>100</td>
<td>0.0 — 0.32</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>34 (85)</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>40 (100)</td>
<td>300</td>
</tr>
</tbody>
</table>

### Table 1. Cure rate and imipramine and desipramine plasma levels among chloroquine resistance \textit{falciparum malaria} patients.

<table>
<thead>
<tr>
<th>Time of CQ administration</th>
<th>Plasma CQ, mean ± SD(µ mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day zero (before treatment)</td>
<td>0.099 ± 0.031</td>
</tr>
<tr>
<td>3 Hours (after treatment)</td>
<td>0.694 ± 0.04</td>
</tr>
<tr>
<td>Three days after treatment</td>
<td>0.396 ± 0.032</td>
</tr>
<tr>
<td>Seven days (after treatment)</td>
<td>0.202 ± 0.019</td>
</tr>
</tbody>
</table>

### Table 2. The mean plasma chloroquine level (µ mol/L) in-patients infected with sensitive and resistant \textit{P. falciparum} parasites.
Imipramine is mainly used for treatment of depression, in a daily dose ranging between 75 -100 and 300 mg. The pharmacokinetics of the drug is variable among individuals. Administration of an average dose of 100 mg imipramine gives a steady state plasma concentration ranging between 10 - 100 ng ml⁻¹imipramine and 20 - 330 ng ml⁻¹ desipramine (13). The pharmacokinetic parameters of both CQ and imipramine are not significantly altered when the two drugs are administered together (19). Concomitant administration of CQ and imipramine in a dose of 100 mg daily for 3 days is expected to attain the required plasma concentration of the latter, 0.06 - 1.5 x10⁻⁶mol/litre (10).

Most subjects (52%) did not suffer any side effect from imipramine. However, 48% of the patients reported some side effects, of an anticholinergic nature (dry mouth, blurred vision, drowsiness and fatigue). It was interesting to note that 66 % of those who reported side effects were females. This could be due to higher bioavailability of imipramine in female patients since they showed higher plasma concentrations (0.527 ± 0.03 x10⁻⁶ mol/L) compared to males (0.321 ± 0.04 x10⁻⁶mol/liter). However, on day 3 and 7, imipramine plasma level in patients infected with sensitive P. falciparum (0.649 ± 0.031x10⁻⁶mol/liter) compared to patients with resistant parasites ( 0.584 ± 0.055 x10⁻⁶mol/liter). However, on day 3 and 7, CQ plasma level in patients with resistance response (0.645 ± 0.032 x10⁻⁶ mol/liter on day 3 and 0.393 ± 0.038 x10⁻⁶mol/liter on day 7) has become significantly higher than in individuals who responded to the drug (0.396 ± 0.04 x10⁻⁶mol/liter in day 3 and 0.202 ± 0.019X10⁻⁶mol/liter on day 7).

The CQ plasma levels attained in patients infected with resistant parasites (3 hours after the administration of a loading dose of 10mg/Kg body weight) was approximately 3 times greater than the therapeutic level (20). This clearly indicated CQ resistant P. falciparum parasites persisted despite the high plasma level of the drug.

Measurements of imipramine and desipramine in plasma showed concentration ranges between 0.0-1.068x10⁻⁶ mol/L and 0.0 - 0.68 x10⁻⁶ mol/L in the three consecutive days of treatment. 75% of the patients had a level of imipramine and desipramine ranging between 0.03 - 1.068 x10⁻⁶mol/liter. The levels attained, agree well with the reported concentration (0.06-1.5 x 10⁻⁶ mol/L) for in vitro reversal of resistance by desipramine. Our findings disagree with that of Warsame et al., who examined the effect of imipramine treatment on chloroquine resistant P. falciparum in Somalia. This difference can be attributed to the use of a higher dose of 100 mg imipramine/day in our study compared to 75 mg/day used by Warsame et al., (18). The 100-mg imipramine/day in the present study resulted in attainment of plasma levels for imipramine and desipramine, which are adequate for reversal of resistance. High concentration of chemosensitizer, including desipramine, has been found to be important for reversal of P. falciparum chloroquine resistance (5). Unfortunately, no information was given on imipramine plasma level among falciparum patients in Somalia (18), and it was only presumed that the drugs, in the doses used, have reached plasma levels needed for reversal of resistance.

The lack of in vivo efficacy of some chloroquine resistance modulators including imipramine has been attributed to protein bindings and pharmacokinetic (21). Plasma proteins inhibit desipramine chloroquine resistance reversing effect, and this effect is more noticeable with desipramine than other chemosensitizers (22). Desipramine has an extensive plasma protein binding characteristic, in particular to (1 acid glycoprotein (22). The level of this protein can be attributed to the use of a higher dose of 100 mg imipramine/day in our study compared to 75 mg imipramine/day used by Warsame et al., (18). The 100-mg imipramine/day in the present study resulted in attainment of plasma levels for imipramine and desipramine, which are adequate for reversal of resistance. High concentration of chemosensitizer, including desipramine, has been found to be important for reversal of P. falciparum chloroquine resistance (5). Unfortunately, no information was given on imipramine plasma level among falciparum patients in Somalia (18), and it was only presumed that the drugs, in the doses used, have reached plasma levels needed for reversal of resistance.

This study has shown that imipramine/chloroquine combination can reverse response of CQ resistant P. falciparum parasite. The use of chloroquine resistance modifiers, such as imipramine and chlorpheniramine, may offer feasible strategy that can restore the efficacy of chloroquine in endemic countries and enable better management and malaria control.
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