Potentiation of antimalarial drug action by chlorpheniramine against multidrug-resistant *Plasmodium falciparum* in vitro

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Abstract

Chlorpheniramine, a histamine H1 receptor antagonist, was assayed for in vitro antimalarial activity against multidrug-resistant *P. falciparum* K1 strain and chloroquine-resistant *P. falciparum* T9/94 clone, by measuring the ³H-hypoxanthine incorporation. Chlorpheniramine inhibited *P. falciparum* K1 and T9/94 growth with IC₅₀ values of 136.0±40.2μM and 102.0±22.6μM respectively. A combination of antimalarial drug and chlorpheniramine was tested against resistant *P. falciparum* in vitro. Isobologram analysis showed that chlorpheniramine exerts marked synergistic action on chloroquine against *P. falciparum* K1 and T9/94. Chlorpheniramine also potentiated antimalarial action of mefloquine, quinine or pyronaridine against both of the resistant strains of *P. falciparum*. However, chlorpheniramine antagonism with artesunate was obtained in both *P. falciparum* K1 and T9/94. The results in this study indicate that antihistaminic drugs may be promising candidates for potentiating antimalarial drug action against drug resistant malarial parasites.

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1. Introduction

Multidrug resistance in *Plasmodium falciparum* malaria now occurs in most endemic areas. Combination chemotherapy is a rational approach to the containment of drug resistance in malaria [1]. Pyrimethamine plus sulfadoxine was combined with mefloquine. The Chinese drug pyronaridine has also been occasionally given in conjunction with pyrimethamine and sulfadoxine [2]. Quinine is most often combined with tetracycline or doxycycline or clindamycin [3]. Atovaclone is effective in combination with proguanil for the treatment of malaria [4], and in some areas of Southeast Asia, artesunate is used in combination with mefloquine or with tetracycline [5–7].

The emergence of chloroquine resistance has led to the investigation of drugs that modulate chloroquine resistance. Chloroquine resistance in malaria parasite can be reversed by a combination of chloroquine and calcium channel blockers, verapamil or chlorpromazine [8,9]. The tricyclic antidepressant drug, desipramine, restores the blood schizontocidal action of chloroquine against chloroquine-resistant strains in vitro [10]. Antihistamine compounds, cyproheptadine, ketotifen, pizotyline and azatadine produce marked reversal of chloroquine resistance in *P. yoelii* mice and in *P. falciparum* in vitro [11,12]. Chlorpheniramine, a histamine H1 receptor antagonist, has been shown to reverse chloroquine resistance in vitro in the African isolate of *P. falciparum* [13,14]. Chlorpheniramine enhances the efficacy of chloroquine in treating acute uncomplicated *P. falciparum* in children from an endemic area of Nigeria [15]. In further clinical trials, Sowunmi et al. [16,17] found that a higher dosage of chlorpheniramine produced a more significant and beneficial action. Antihistamines are widely available at low cost in malaria endemic zones. They have been prescribed often in children above 2 years of ages [18]. Chlorpheniramine produces drowsiness as a side effect [15]. The relative safety of these drugs prompted us to study the in vitro activity of standard antimalarial drugs (Fig. 1) in combination with chlorpheniramine against multidrug-resistant or chloroquine-resistant *P. falciparum*. The results from this study demonstrate that chlorpheniramine showed synergistic effect with chloroquine, mefloquine, quinine or pyronaridine against both *P. falciparum*...
K1 and T9/94. In contrast, chlorpheniramine combined with artesunate showed antagonistic effect.

2. Materials and methods

Multidrug-resistant *P. falciparum* K1 strain was obtained from an infected individual in Kanchanaburi province, Thailand. They can be routinely maintained in human group O red blood cells in a medium containing RPMI 1640 supplemented with 10% human serum using the candle jar method [19]. Isolate T9 of chloroquine-resistant *P. falciparum* was obtained from a patient at Tak province, Thailand and T9/94 clone was prepared by limiting dilution. The isolation and characterization of *P. falciparum* T9/94 clone have been previously described [20]. Antimalarial drugs (Fig. 1) in combination with antihistaminic drug were tested by using 3H-hypoxanthine incorporation [21]. An aliquot of 25 μl of drug solution dissolved in culture medium and 200 μl of the parasitized erythrocyte suspension, in which red blood cells were suspended at 3% hematocrit concentration, were placed in 96 well plates in triplicate. At the start of the experiment, 0.5% red blood cells were infected with K1 or T9/94 strain. After incubation at 37°C for 48h, 25 μl of 3H-hypoxanthine was added to a final concentration 1μCi/ml, followed by further incubation at 37°C for 48h. Red blood cells were harvested using an automated sample harvester and counted for radioactivity incorporated into the parasites with a Beckman liquid scintillation counter, model LS-1801 (Beckman Instruments, Inc.). 3H-hypoxanthine uptake was assayed in the presence of both a single drug and a combination of antimalarial drug with antihistaminic drug, and was expressed as percentage of control. The 50% inhibitory concentration (IC50) values, defined as the drug concentration required for 50% reduction of the 3H uptake by parasites as compared to control (without the drugs), were determined from the dose–response curve. The antimalarial drug IC50 for various antihistamine concentrations

![Fig. 1. Structure of antimalarial and antihistaminic drugs.](image1)

![Fig. 2. (a) Isobologram of chlorpheniramine in combination with chloroquine against *P. falciparum* K1 strain. (b) Isobologram of chlorpheniramine in combination with chloroquine against *P. falciparum* T9/94 clone.](image2)
and the antihistamine IC₅₀ for various antimalarial drug concentrations were determined and used to construct the isobologram [22].

3. Results

Chlorpheniramine was assayed for in vitro antimalarial activity against multidrug-resistant *P. falciparum* K1 strain and chloroquine-resistant *P. falciparum* T9/94 clone. *P. falciparum* K1 and T9/94 growth were inhibited by chlorpheniramine with IC₅₀ values of 136.0±40.2 µM and 102.0±22.6 µM, respectively. Combinations of antimalarial drugs with chlorpheniramine were tested against *P. falciparum* K1 strain and T9/94 clone. The isobologram of an additive combination of two agents lies on straight line. The isobologram of synergistic agents is concave. With antagonistic agents, the isobologram is convex. Chlorpheniramine showed synergistic effects with chloroquine both in *P. falciparum* K1 and T9/94 (Fig. 2a,b). Chlorpheniramine also potentiated antimalarial action of mefloquine, quinine or pyronaridine against both resistant strains of *P. falciparum* (Figs. 3a,b, 4a,b, and 5a,b). Our results demonstrate that chlorpheniramine potentiates in vitro antimalarial action of chloroquine, mefloquine, quinine or pyronaridine which are quinoline-containing drugs (Fig. 1). However, chlorpheniramine showed antagonism with artesunate for both *P. falciparum* K1 and T9/94 (Fig. 6a,b).

4. Discussion

In a previous study, Martin et al. [8] found that the blood schizontocidal action of chloroquine against chloroquine-resistant strain of *P. falciparum* could be reversed in vitro by exposing the parasitized red cells to a combination of chloroquine and verapamil. The resistance-reversing activity of verapamil is associated with the increased accumulation of chloroquine by resistant parasite [23]. The mechanism of reversal by calcium channel blockers is independent of the calcium channel [24]. The use of combination therapy with chloroquine and calcium antagonists is probably unsuitable for human malaria because of the high doses of antagonists needed to reverse chloroquine resistance. Other agents that reverse chloroquine resistance are chlorpromazine, fluoxetine and cyproheptadine [25–27]. Sowunmi and Oduola [18] reported that a chloroquine and chlorpheniramine combination produced a significantly higher cure rate than chloroquine alone in African children with acute symptomatic uncomplicated falciparum malaria. Chlorpheniramine plasma concentration collected 4 h after the oral dose of 10 mg. chlorpheniramine to a normal subject is 15.4 ng/ml [28]. In this study, we demonstrated the marked reversal effect of chlorpheniramine on chloroquine or multidrug-resistant *P. falciparum*. At the present time, the antimalarial activity of chlorpheniramine is still unknown, as is the way in which the chloroquine resistance of parasites is
reversed. The original hypothesis that they act as calcium channel blockers is no longer accepted [29]. Scheibel et al. [30] have indicated the possibility that such action is linked to the inhibition of parasite calmodulin functions rather than to a more direct action on calcium transport.

When a combination of chlorpheniramine and mefloquine was tested, chlorpheniramine could potentiate mefloquine action. Mefloquine resistance in *P. falciparum* from Nigeria can also be reversed by the neuroleptic drug, penfluridol, in vitro [31]. Synergism between chlorpheniramine and quinine was also observed in our study. A chlorpheniramine and quinine combination may offer an advantage over the current drug combination, tetracycline and quinine, in that chlorpheniramine can be prescribed in children above 2 years of age [18], while tetracycline should not be given to pregnant women and children [32]. Synergism between chlorpheniramine and pyronaridine indicates that this may be a promising combination against drug resistant malaria parasites. Pyronaridine has good antimalarial activity with little evidence for cross-resistance with other widely used drug [33]. Field tests in China reported that pyronaridine was well tolerated with few major adverse effects. Antagonism between chlorpheniramine and artesunate indicated that these two drugs cannot be used together. Our results demonstrate that chlorpheniramine potentiates in vitro antimalarial action of quinoline-containing antimalarial compounds. Because chlorpheniramine is a safe therapeutic drug, the clinical use of chlorpheniramine as a cheap and highly effective combination with quinoline-containing antimalarial drugs holds great promise against multidrug-resistant falciparum malaria.

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**References**


