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In Vitro Activity of Chloroquine and Quinine in Combination With Desferrioxamine Against *Plasmodium falciparum*

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The activity of chloroquine and quinine, alone and in combination with desferrioxamine (7 μmol/liter), was evaluated in vitro against susceptible and resistant clones of *Plasmodium falciparum* by a semimicroassay system. The addition of desferrioxamine had no effect on the activity of chloroquine against both clones. Desferrioxamine had no effect on the activity of quinine against the susceptible clone but had slightly enhanced quinine action against the resistant clone. Further development of desferrioxamine as an antimalarial drug may be of limited interest. © 1993 Wiley-Liss, Inc.

Key words: malaria, antimalarial drugs, quinoline compounds

INTRODUCTION

Desferrioxamine, an iron chelator, is a potential candidate for the treatment of malaria. In vitro studies have demonstrated that it can inhibit the growth of both erythrocytic and hepatic stages of *Plasmodium falciparum* at concentrations between 5 and 20 μmol/liter [1–3]. Although these concentrations are attainable in humans treated for iron overload, desferrioxamine is at least 20 times less active than the standard antimalarial drugs. Its in vitro activity is within the same range as the antibiotics used in malaria treatment. The moderate in vitro activity of desferrioxamine is in agreement with animal studies showing suppression of parasite growth, but not cure, against *P. vinckei* in mice [4] and *P. falciparum* in experimentally infected owl monkeys [5].

These observations may imply that desferrioxamine alone is not sufficient to eliminate the malaria parasites completely. To be of clinical use, it probably must be administered with other antimalarial drugs. Among the currently available drugs, chloroquine and quinine seem to be the best candidates for a combination therapy with desferrioxamine because of their wide availability in the endemic areas and the increasing spread of resistance to these drugs. Furthermore, although the mechanism of action of quinoline compounds is still much debated, a hypothesis concerning the inhibition of iron release from heme during the process of hemoglobin degradation in the parasite's vacuole was recently advanced [6]. We hypothesized that, if this mechanism of action of chloroquine and quinine holds true, a combination of chloroquine or quinine and desferrioxamine may enhance the drug action of each other, since iron is required for parasite development. To test this hypothesis, the in vitro drug interaction between chloroquine or quinine and desferrioxamine was evaluated against susceptible and resistant clones of *P. falciparum*.

MATERIALS AND METHODS

The chloroquine- and quinine-resistant FCM 29/Cameroon clone and the chloroquine- and quinine-susceptible L-3/Côte d'Ivoire clone were originally obtained from travelers returning from Africa, maintained in continuous culture under standard conditions, and cloned by a limiting dilution method. Desferrioxamine mesylate was obtained from Sigma (St. Louis, MO). Chloroquine sulfate was obtained from Specia (Paris, France). Pure quinine base was prepared by Prof. R. Farinotti (Depart-

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ment of Clinical Pharmacy, Hôpital Bichat-Claude Bernard, Paris, France). Stock solutions of chloroquine and quinine were prepared in distilled water and 50% methanol, respectively. Twofold serial dilutions were prepared in distilled water and distributed in triplicate in flat-bottom 24-well plates. Desferrioxamine was dissolved in distilled water. A final concentration of 7 μmol/liter was added to the chloroquine and quinine plates. This concentration corresponds to the mean steady-state concentration of the drug in adult volunteers [7]. The antimalarial activity of desferrioxamine alone was determined over the concentration range between 1.2 and 160 μmol/liter in triplicate.

The in vitro drug susceptibility test described by Le Bras and Deloron [8] was used in this study. When the majority (> 75%) of asynchronous parasites were in the young ring and trophozoite stages at a parasitemia of 2–5%, infected erythrocytes were diluted with uninfected erythrocytes and resuspended in RPMI 1640 medium supplemented with 10% human serum and buffered with 25 mmol/liter HEPES and 25 mmol/liter $NaHCO_3$ to obtain an initial parasitemia of 0.1–0.5% (hematocrit 2.5%). The suspension (700 μl/well) was distributed in the plates and incubated at 37°C in 5% O_2, 5% CO_2, and 90% N_2 for 42 hr. [G-^{3}H]hypoxanthine was added to assess parasite maturation. The amount of radioactivity incorporated by the parasites was measured by a liquid scintillation counter. Linear regression analysis of the concentration-response data was used to calculate and compare the 50% inhibitory concentrations (IC_{50}). The experiments were replicated four times for each clone.

RESULTS AND DISCUSSION

The mean IC_{50} values (± SD) of the resistant and susceptible clones for desferrioxamine alone were 11.2 ± 1.8 μmol/liter and 15.3 ± 1.9 μmol/liter, respectively (Fig. 1A). The IC_{50} values of chloroquine, alone (979 ± 79 nmol/liter) and in combination with desferrioxamine (979 ± 114 nmol/liter), were not significantly different for the resistant clone (Fig. 1B). There was a slight but statistically significant diminution of IC_{50} value of quinine plus desferrioxamine (493 ± 48 nmol/liter) as compared with quinine alone (566 ± 46 nmol/liter). This additive effect was not sufficient to reverse quinine resistance against the resistant clone (threshold for quinine resistance > 450 nmol/liter). There was no significant difference between the IC_{50} values of chloroquine alone (31.9 ± 5.8 nmol/liter) and chloroquine plus desferrioxamine (31.6 ± 2.4 nmol/liter) or between the IC_{50} values of quinine alone (236 ± 36 nmol/liter) and quinine plus desferrioxamine (219 ± 29 nmol/liter) against the susceptible clone (Fig. 1C).

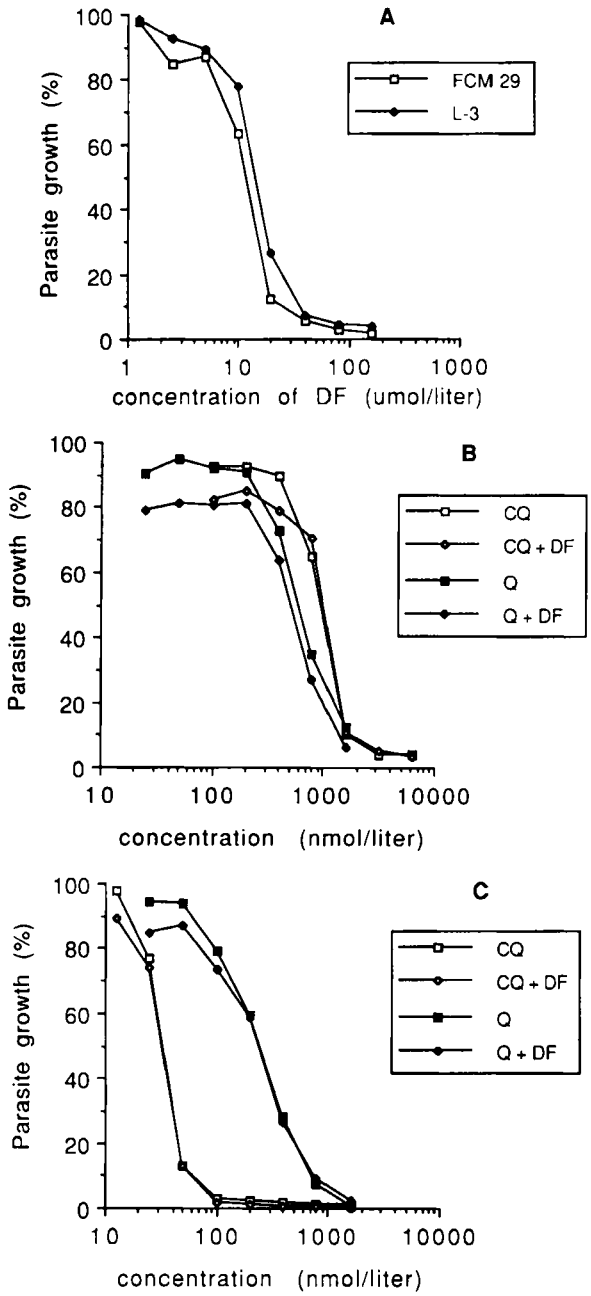

Fig. 1. In vitro response to desferrioxamine (DF) alone (A) and to the combination of chloroquine (CQ) or quinine (Q) and desferrioxamine (7 μmol/liter) against the resistant FCM 29 clone (B) and the susceptible L-3 clone (C). Each point represents the mean of four experiments. The threshold IC_{50} values for resistance are > 100 nmol/liter for chloroquine and > 450 nmol/liter for quinine.

The results of recent clinical trials with desferrioxamine in African adults were inconclusive. In the study conducted by Traore et al. [9], standard oral doses of chloroquine were administered with desferrioxamine,

and a small number of patients were enrolled, with all patients but one were infected by a chloroquine-susceptible *P. falciparum*. In the study of Gordeuk et al. [7], only asymptomatic patients with very low parasitemia were included. Both clinical studies were conducted in semi-immune African adults, and the enhancement of drug action by acquired immunity probably played an important role in the apparently encouraging results of the studies. Further clinical trials with desferrioxamine alone in nonimmune malaria-infected patients are needed to confirm its efficacy against malaria parasites.

The results of the present study did not fully confirm our initial hypothesis. At a clinically attainable concentration, desferrioxamine did not enhance the schizontocidal action of chloroquine against susceptible or resistant malaria parasites. A slight but inadequate enhancement of quinine activity was observed against the resistant clone. The activity of quinine remained unchanged in the presence of desferrioxamine against the susceptible clone. The moderate antimalarial activity of desferrioxamine, as well as its high cost, its short action, the requirement for parenteral administration, and the lack of sufficient potentiating effect on chloroquine and quinine may limit interest in further development of the drug for the treatment of malaria. In view of novel modes of action against malaria parasites [10], however, other iron chelators under development may be potentially useful for the treatment of malaria.

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