ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1990, p. 1981–1985
0066-4804/90/101981-05$02.00/0

Vol. 34, No. 10

Potentiation of Chloroquine Activity against *Plasmodium falciparum* by the Peroxidase-Hydrogen Peroxide System

KHUSHBEER MALHOTRA,†* DOMINIQUE SALMON, JACQUES LE BRAS, AND JEAN-LOUIS VILDE

Institut National de la Santé et de la Recherche Médicale U 13 et Département de Biologie Parasitaire, Hôpital Claude Bernard, 75019 Paris, France

Received 1 November 1989/Accepted 23 July 1990

In this study, we examined the potential interactions between antimalarial (chloroquine, quinine, and mefloquine) and oxidant reagents. The data indicate that their effects enhance those of one another in vitro. The viability of *Plasmodium falciparum* in culture was assessed by [^{3}H]hypoxanthine incorporation during 24 h of incubation in the presence of lactoperoxidase, glucose-glucose oxidase, hydrogen peroxide, chloroquine, quinine, and mefloquine, either alone or in combination. At subinhibitory concentrations, a significant inhibition was produced by the following combinations: lactoperoxidase plus hydrogen peroxide, lactoperoxidase plus glucose-glucose oxidase, lactoperoxidase plus hydrogen peroxide or glucose-glucose oxidase plus chloroquine or quinine but not with mefloquine. Deletion of any component from the system markedly decreased the toxic effect on *P. falciparum*. This toxic effect was not inhibited by catalase. These results indicate that the peroxidase-hydrogen peroxide system and antimalarial drugs can potentiate each other to inhibit the growth of *P. falciparum*.

The peroxidase-hydrogen peroxide (H_2O_2) and halide microbicidal system is lethal to many microorganisms (2) and to some protozoa (9, 13, 16). Past studies have indicated that cells capable of generating a vigorous respiratory burst, e.g., neutrophils and monocytes, possess substantial activity against many microorganisms (13, 14). The asexual stages of *Plasmodium falciparum* have been shown to be susceptible in vitro to oxygen radicals, such as H_2O_2 either alone or generated by the glucose-glucose oxidase (G-GO) system (7, 18, 23), and also in vivo to the administration of free oxygen radicals such as *tert*-butyl hydroperoxide and alloxan (4, 5). Moreover, monocyte and neutrophil secretory products are also able to kill the intraerythrocytic parasites in vitro by the liberation of oxygen radicals (15, 17, 23). It has also been demonstrated in vivo that splenic macrophages damage intraerythrocytic parasites by the liberation of oxygen radicals. It is now suggested that these radicals may participate in the host defense mechanism against severe and acute forms of malaria (14, 18).

Recently, the components of the microbicidal system of phagocytes, peroxidase combined with H_2O_2 and halide or thiocyanate, have been shown to potentiate the inhibition of the growth of *P. falciparum* in vitro (15). We now report that the combination of a peroxidase-H_2O_2-mediated system and certain antimalarial drugs also potentiates the inhibition of growth of *P. falciparum* in vitro.

(This work was presented in part at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, Calif., 23 to 26 October 1988.)

MATERIALS AND METHODS

Parasite strain. The strain used to perform these studies is a chloroquine-resistant clone from Cameroon (FCM 29). The parasites were grown in erythrocyte (O^+) suspension in vitro according to the technique described by Trager and Jensen (21) in RPMI 1640 medium containing a 0.2% glucose, 25 mM $NaHCO_3$, and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer supplemented with 10% pooled human serum (culture medium). Incubation was performed under an atmosphere of 5% CO_2–5% O_2–90% N_2 at 37°C (model no. EC, O_2/CO_2 incubator; Heraeus, Les Ulis, France).

Synchronization of cultures. The schizonts were concentrated to 70 to 80% purity by Percoll gradient centrifugation (20), placed in culture with fresh uninfected erythrocytes, and incubated at 37°C for 6 h. The culture was then treated with 5% sorbitol for 5 min to select the ring stage (12) and further incubated for 18 h. Observation was performed on thin blood smears stained by 3% Giemsa.

Assessment of growth of *P. falciparum*. Maturation of *P. falciparum* trophozoites to schizonts was measured by [^{3}H]hypoxanthine incorporation. Tests were performed in 24-well culture plates (Falcon 3047; Beckton Dickinson Labware, Oxnard, Calif.) with 2.5% hematocrit and parasitemia ranging from 0.2 to 2%. Briefly, in each well, 700 μl of suspension in culture medium of erythrocytes containing parasites at the trophozoite stage was mixed with 25 μl of [^{3}H]hypoxanthine (1 μCi/0.7 ml) (Amersham International Ltd., Les Ulis, France). Different reagents of the oxygen-dependent microbicidal system and antimalarial drugs, such as quinine, chloroquine, and mefloquine, were added at the same time.

For the antibiotics, incubation was performed up to 48 h and [^{3}H]hypoxanthine was added after 24 h. Plates were incubated for 24 h and then frozen at −80°C for 2 h and thawed. The content of each well was harvested on a Whatman fiberglass paper (Cell harvester 500; Titertek, London, United Kingdom), washed with distilled water, and dried. The radioactivity of filters containing *P. falciparum* nucleoproteins was determined in a liquid scintillation spectrophotometer (Pharmacia-LKB, Paris, France).

Reagents. Lactoperoxidase (LPO) (EC 1.11.17; lyophilized bovine milk), glucose oxidase (GO) (EC 1.1.3.4; fungal type II, *Aspergillus niger*), and catalase (EC 1.11.1.6; bovine liver powder) were obtained from Sigma Chemical Co., St.

* Corresponding author.
† Present address: Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

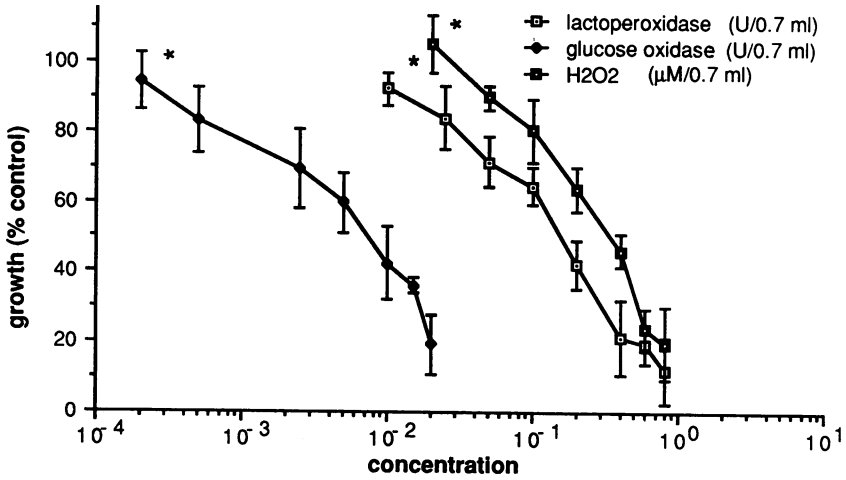

FIG. 1. Inhibition of *P. falciparum* growth by lactoperoxidase, glucose-glucose oxidase, and hydrogen peroxide. Each point represents the mean value ± the standard deviation of GI of three experiments, each one in triplicate. *, Subinhibitory concentration.

Louis, Mo. Chloroquine sulfate (CQ) was obtained from Specia, Paris, France. Purified quinine hydrochloride (Q) was a gift from R. Farinotti, Pharmacology Laboratory, University of Paris XI. Mefloquine hydrochloride (MF) was obtained from Roche, Neuilly-sur-Seine, France; spiramycin hydrochloride and tetracycline hydrochloride were obtained from Specia; ciprofloxacin was obtained from Bayer Pharma, Puteaux, France; and rifampin dihydrochloride was obtained from Merrell, Paris, France. Stock solutions were aliquoted and stored at −20°C.

Inhibition of *P. falciparum* growth by the components of the microbicidal system and antimalarial drugs or antibiotics. The effect of each reagent either alone or in combination was studied at different concentrations. Reagents were added to the culture at the same time as [^{3}H]hypoxanthine and incubated for 24 h, except for antibiotics (known to be active after a long time in culture), which were added 24 h prior to the addition of [^{3}H]hypoxanthine. Controls without reagents and with reagents alone were tested in each experiment. Osmolarity and pH of the supernatant of the infected culture were also checked.

Morphological examination. Crisis forms, characterized by intraerythrocytic deterioration of the parasites (3), were counted on Giemsa-stained thin blood films at the end of the tests.

Expression of results. The growth index (GI) was calculated by comparing the incorporation of [^{3}H]hypoxanthine by parasitized erythrocytes with a given concentration of reagents to controls as follows: GI = mean cpm with reagent(s) or antimalarial agent/mean cpm without reagent(s) or antimalarial agent × 100, where cpm is counts per minute; each value represents the mean ± the standard deviation of three experiments, each one performed in triplicate.

Subinhibitory concentration was defined as the highest concentration of the drug or reagent which did not significantly decrease the GI as compared with controls.

Uptake of [^{3}H]CQ by parasitized and nonparasitized erythrocytes. Accumulation of [^{3}H]chloroquine ([^{3}H]CQ) was calculated from the residual radioactivity of the culture medium supernatant after centrifugation of the parasitized erythrocytic suspension (10). Briefly, the parasitized erythrocytes (hematocrit 10%; parasitemia 8 to 10%) in 700 μl of culture medium were distributed in 24-well culture plates. This suspension was exposed to LPO plus H_2O_2 and 400 nM of [^{3}H]CQ (0.28 Ci/mmol; Amersham) for 2 h. The parasitized erythrocytes were then pelleted by a brief pulse in a microcentrifuge. The radioactivity of 200 μl of the culture medium was counted in the liquid scintillation spectrophotometer (Pharmacia-LKB). Counts per minute were determined from the culture medium containing the various reagents and from the control ([^{3}H]CQ alone). The same experiment was also performed with nonparasitized erythrocytes. Steady state was reached after 2 h of incubation.

The method of calculation was as follows: A, counts per minute from culture medium (without erythrocytes); B, counts per minute from supernatants of suspension containing 10% parasitized erythrocytes; C, counts per minute from supernatants of suspension containing 10% parasitized erythrocytes and [^{3}H]CQ alone; D, counts per minute from supernatants of suspension containing 10% nonparasitized erythrocytes; A − C, accumulation of [^{3}H]CQ in parasitized erythrocytes; A − D, accumulation of [^{3}H]CQ in nonparasitized erythrocytes; and B − D, accumulation of [^{3}H]CQ in parasites.

Statistical analysis. Statistical differences were determined by Student's two-tailed *t* test for independent means (not significant for $P > 0.05$) and analysis of variance for multiple comparisons.

RESULTS

(i) Inhibition of [^{3}H]hypoxanthine incorporation by each reagent and drug alone. For each reagent, a subinhibitory concentration (SIC) was determined and defined as the maximum concentration that had no significant effect on growth of *P. falciparum* (GI ≥ 90%; *P* not significant versus control cultures performed without drug and reagents). SICs were 0.01 U, 0.02 μmol, and 0.2 mU per well for LPO, H_2O_2, and GO, respectively (Fig. 1). SICs for CQ, Q, and MF were 400, 200, and 10 nM, respectively, for the CQ-resistant strain used (Fig. 2). Antibiotics such as rifampin, ciprofloxacin, tetracycline, and spiramycin exhibited no inhibitory effect on the parasites at concentrations up to 0.025, 0.5, 5, and 0.5 μg per well, respectively (dose-response curves not shown).

(ii) Effect of antimalarial drugs combined with the LPO plus H_2O_2 system. LPO, H_2O_2, CQ, Q, and MF were used at their respective SICs in these experiments. LPO plus H_2O_2 in the presence of either CQ or Q displayed a significant potentiation (Table 1). The GI of LPO plus H_2O_2 was 71.5% ± 11.2%

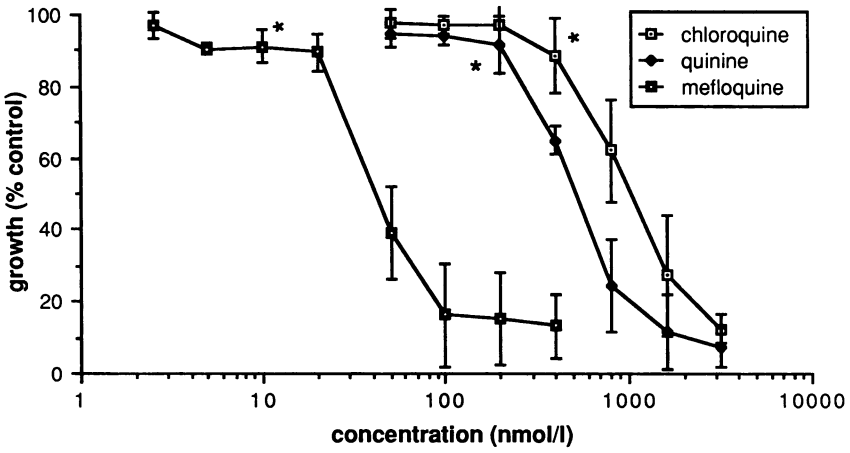

FIG. 2. Inhibition of *P. falciparum* growth by chloroquine, quinine, and mefloquine. Each point represents the mean value ± the standard deviation of GI of three experiments, each one in triplicate. *, Subinhibitory concentration.

and was lowered to 32.4% ± 11.0% with LPO plus H_2O_2 plus CQ and to 27.8% ± 10.8% with LPO plus H_2O_2 plus Q ($P < 0.001$ versus LPO plus H_2O_2). MF showed no significant potentiation.

Addition of CQ or Q to LPO plus G-GO at their respective SICs yielded a highly potentiating effect, as shown by the decrease of GI from 55.7% ± 2.0% in the presence of LPO plus G-GO to 27.3% ± 8.8% with LPO plus G-GO plus CQ and to 17.8% ± 3.0% with LPO plus G-GO plus Q ($P < 0.001$ versus LPO plus G-GO). However, the addition of MF to LPO plus the G-GO system exhibited no effect. With combination of CQ or Q plus LPO or H_2O_2 or G-GO, a lower but still significant potentiation was observed ($P < 0.001$) (Table 1).

To analyze this potentiation more precisely, further experiments were performed by using a concentration of antimalarial drugs lower than their respective SICs. No potentiation was observed at these concentrations (data not shown).

(iii) Effect of antibiotics combined with the LPO plus H_2O_2 system. In contrast to CQ and Q, no potentiation was demonstrated with LPO plus H_2O_2 combined with rifampin, spiramycin, ciprofloxacin, or tetracycline on the growth of *P. falciparum*. Therefore, these drugs were not tested with the LPO plus G-GO system (data not shown).

(iv) Morphological examination. At the end of the experiments, a large number of crisis forms of the parasites were observed on thin blood film by Giemsa staining. The percentage of crisis forms was compared with that obtained with controls (Table 2). No significant difference was observed between the percentage of crisis forms with each reagent at SIC versus controls. The GI was inversely correlated to the percentage of crisis forms ($r = -0.92$; df = 6; $P < 0.001$).

(v) Effect of catalase on the combination of components of the microbicidal system and antimalarial drugs. Addition of 286 U of catalase per ml partially reversed the inhibitory effect of LPO plus H_2O_2 on *P. falciparum* growth; GI increased from 68.5 ± 2.3% without catalase to 86.4 ± 3.7% with catalase. On the contrary, the addition of 286 U of catalase per ml did not prevent the inhibitory effect on *P. falciparum* growth caused by the combination of LPO, H_2O_2, and CQ or the combination of LPO, H_2O_2, and Q system (Table 3). The same results were obtained with twice the concentration of catalase (715 U/ml).

(vi) Uptake of [^{3}H]CQ by a CQ-resistant strain of *P. falciparum* in the presence of the components of the microbicidal system. The uptake of [^{3}H]CQ by parasitized erythrocytes in the presence or absence of components of the microbicidal system is shown in Table 4. The accumulation of [^{3}H]CQ was approximately threefold in parasitized erythrocytes as compared with nonparasitized erythrocytes. No difference in [^{3}H]CQ accumulation was observed in the presence or absence of the components of the microbicidal system with parasitized erythrocytes.

TABLE 1. Effect of oxygen-dependent components and antimalarial drugs on *P. falciparum* growth[a]

AM/% parasite growth[b]	Parasite growth (%) with oxygen-dependent components				
	LPO	G-GO	H_2O_2	LPO + G-GO	LPO + H_2O_2
None	92.2 ± 4.6 (3)	94.1 ± 8.3 (8)	105 ± 8.0 (6)	55.7 ± 2.0[c] (4)	71.5 ± 11.2[c] (4)
CQ/89.2 ± 10.4 (8)	43.5 ± 7.3[c] (6)	74.7 ± 8.4[c] (4)	61.7 ± 9.5[c] (4)	27.3 ± 8.8[c,d,e,f] (3)	32.4 ± 11.0[c,d,e,f] (4)
Q/91.6 ± 7.9 (7)	48.7 ± 10.7[c] (7)	55.7 ± 7.9[c] (4)	60.7 ± 11.4[c] (7)	17.8 ± 3.0[c,d,e,f] (3)	27.8 ± 10.8[c,d,e,f] (7)
MF/91.7 ± 10.5 (9)	79.7 ± 10.5 (3)	81.5 ± 16.6 (3)	89.2 ± 7.4 (3)	64.3 ± 13.2[c] (3)	79.8 ± 9.4 (3)

[a] AM, Antimalarial drugs. Components were used at SICs of 0.01 U/0.7 ml (LPO), 0.02 μmol/0.7 ml (H_2O_2), 0.2 mU/0.7 ml (GO), 400 nM (CQ), 200 nM (Q), and 10 nM (MF).

[b] The number in parentheses indicates the number of experiments in triplicate.

[c] Value significantly different from that of LPO, G-GO or H_2O_2, Q, or CQ alone ($P < 0.001$).

[d] Value significantly different from that of LPO plus G-GO or H_2O_2 ($P < 0.001$).

[e] Value significantly different from that of G-GO or H_2O_2 plus Q or CQ ($P < 0.001$).

[f] Value significantly different from that of LPO plus Q or CQ ($P < 0.001$).

TABLE 2. Morphological examination after action of reagents on *P. falciparum* growth

Reagent[a]	% of parasite crisis forms[b]
Control[c]	5.5 ± 2.1
LPO	5.6 ± 2.5
G-GO	7.0 ± 3.6
H_2O_2	6.5 ± 2.1
LPO + H_2O_2	36.0 ± 5.2
LPO + G-GO	50.6 ± 9.0
LPO + H_2O_2 + CQ	57.0 ± 14.8
LPO + H_2O_2 + Q	65.7 ± 8.6
LPO + G-GO + CQ	73.1 ± 10.3
LPO + G-GO + Q	81.3 ± 13.6

[a] LPO, G-GO, H_2O_2, and CQ and Q were used at SICs indicated in Table 1, footnote *a*.
[b] Each value represents the mean ± the standard deviation of three experiments, each one in triplicate.
[c] Control indicates the mean ± the standard deviation of results obtained without reagents.

DISCUSSION

Previous studies have shown that the oxygen-dependent components of the microbicidal system in vitro can inhibit the growth of *P. falciparum* and demonstrated the inhibitory effect of H_2O_2 or of an H_2O_2-generating system such as G-GO (7, 18, 23). Peroxidases such as LPO and H_2O_2 in combination with either halides or thiocyanate also potentiate the *P. falciparum* growth inhibition (15). In this report, we demonstrate that the oxygen-dependent components of the microbicidal system and some antimalarial drugs such as CQ and Q, but not MF, and the antibiotics tetracycline, rifampin, spiramycin, and ciprofloxacin potentiate the inhibition of the growth of *P. falciparum*. As no isobolograms were performed, we cannot ascertain the synergism. Combination of LPO with H_2O_2 or G-GO exerted a potentiation when each was added at its respective SIC. The LPO plus G-GO system was more effective, since G-GO constitutes an enzymatic system that is likely to liberate H_2O_2 extensively into the medium. The effect of LPO plus H_2O_2 or G-GO was due to the potentiation of the inhibitory effect of H_2O_2 by LPO (9). Addition of the Q and CQ at their respective SICs enhances the inhibitory effect of the LPO plus H_2O_2 or G-GO system. Q was more effective than CQ in our system. Deletion of any components from the complete system markedly decreased the inhibitory effect on parasite growth. Tetracycline, rifampin, spiramycin, and ciprofloxacin and MF did not show any potentiation in either system. It has already been shown, however, that elevation of oxygen levels enhances the antimalarial activity of some antibiotics such as imidazoles (6, 19). This potentiation suggests that the drugs themselves may participate in the mechanism of oxidant stress on the infected erythrocytes. With the antibiotics used in these experiments, no inhibitory effect was observed with LPO, H_2O_2, or G-GO. Moreover, increased oxygen tensions (particularly those greater than atmospheric) are deleterious to the parasites and potentiate the effects of some antimalarial drugs (6, 19).

TABLE 3. Effect of catalase on components of an oxygen-dependent microbicidal system and antimalarial drugs in the presence of *P. falciparum*[a]

Component	Parasite growth (%)[b]	Probability
Catalase alone	162 ± 7.2 (3)	
LPO + H_2O_2	68.5 ± 2.3 (2)	
Catalase added	86.4 ± 3.7 (2)	$P < 0.05$ vs LPO + H_2O_2
LPO + H_2O_2 + CQ	36.4 ± 13.0 (3)	$P < 0.001$ vs LPO + H_2O_2
Catalase added	36.2 ± 12.5 (3)	NS[c] vs LPO + H_2O_2 + CQ
LPO + H_2O_2 + Q	37.8 ± 10.8 (3)	$P < 0.001$ vs LPO + H_2O_2
Catalase added	37.6 ± 12.1 (3)	NS vs LPO + H_2O_2 + Q

[a] LPO, H_2O_2, Q, and CQ were used at SICs indicated in Table 1, footnote *a*, except catalase (286 U/ml).
[b] Number in parentheses indicates number of experiments in triplicate.
[c] NS, Not significant.

TABLE 4. Uptake of [^{3}H]CQ into parasitized erythrocytes from supernatants after 2-h exposure to oxygen-dependent components[a]

Component	Depletion from medium (cpm)[b]	Accumulation in pellets (cpm)
NPE + PE + CQ	11,337 ± 627	6,167 ± 592
NPE + PE + LPO + CQ	11,007 ± 949	6,189 ± 646
NPE + PE + H_2O_2 + CQ	11,217 ± 525	6,950 ± 382
NPE + PE + LPO + H_2O_2 + CQ	11,374 ± 809	6,297 ± 457
NPE + CQ	3,383 ± 318	2,117 ± 264

[a] PE, Parasitized erythrocytes; NPE, nonparasitized erythrocytes. LPO, H_2O_2, and CQ were used at SICs indicated in Table 1, footnote *a*. Two experiments were performed in duplicate for every determination.
[b] Counts per minute of CQ in erythrocytes equals counts per minute of CQ in supernatant without erythrocytes minus counts per minute of CQ in total suspension (culture medium + CQ = 32,265 ± 870).

The efflux of CQ from CQ-resistant parasites, previously demonstrated by Krogstad et al. (10), shows that CQ-resistant *P. falciparum* accumulate significantly less CQ than susceptible parasites do. To determine the mechanism of potentiation, we performed the experiments with [^{3}H]CQ in combination with LPO plus H_2O_2. These results indicate that the oxygen-dependent components do not increase the accumulation of [^{3}H]CQ in parasitized erythrocytes.

In 1972, Homewood and colleagues noted that CQ produced a marked swelling of the parasite food vacuole, suggesting that CQ may produce its antimalarial effect by raising the pH of the vacuole (8). They proposed that this effect may stop parasite growth by raising the vacuole pH above the optimal level for the acid proteases of the parasite, thus inhibiting the proteolysis of hemoglobin, which is essential for normal parasite growth. It is possible that the effects of our system are also mediated by a rise in vacuole pH. On the other hand, however, the presence of quinolines with LPO, H_2O_2, or G-GO inside the parasites may act to decrease cellular pH and thus potentiate the action of peroxidase, which is highly effective at low pH. The intracellular vesicle of *P. falciparum* is known to have a pH around 5.0 (11).

Some quinone antitumor drugs with hydroxyl substitutions (9-hydroxyellipticine) have been demonstrated to be oxidized and activated by the peroxidase-H_2O_2 system (1). The quinoline methanols (Q and MF) and 4-amino quinolines (CQ) do not possess a similar oxidizing capacity. It is unlikely that these drugs are directly activated by the oxygen-dependent microbicidal components. Indeed, the concentration of CQ obtained by high-performance liquid chromatography was not modified after incubation with the LPO plus H_2Q_2 system (data not shown).

Other possible mechanisms of potentiation may involve an increased intracellular drug concentration in relation with a transporter or receptor modification by the oxidative prod-

ucts. An increased activity of oxidative components within the parasitized erythrocytes in the presence of antimalarial drugs is an alternative hypothesis. However, this hypothesis was not supported by our experiments, since the uptake of [^{3}H]CQ was not modified by the oxidative system at the potentiating concentration.

On the other hand, quinolines, which are negatively charged weak bases, could act as a substitute for halide and be a cofactor of LPO in the microbicidal system. Therefore, additional studies of other drugs that act as oxidants, such as artemisinin, naphthoquinones, or substances that interact with membrane permeability, could be performed.

To investigate whether the parasite inhibition by components of the microbicidal system and antimalarial drugs was secondary to the initial damage of the erythrocytes, we have examined the supernatant from each well after the exposure to the components. No erythrocytic damage and no change in pH and osmolarity were observed after the addition of components in the culture medium (data not shown).

The susceptibility of the parasites to the combination of the oxygen-dependent microbicidal system and antimalarial agents was confirmed by the morphological observations at the end of the experiments. The presence of abnormal and intraerythrocytic deteriorated parasites, described as crisis forms, suggests that they were killed in situ. The percentage of crisis forms correlated with the GI. However, complete inhibition of [^{3}H]hypoxanthine incorporation was not achieved within 24 h for different reasons: (i) our experiments were not designed to obtain 100% killing but to assess the toxicity of the microbicidal system in short experiments of 24 h; (ii) the parasites were 70 to 80% synchronous at the trophozoite stage, and all the developmental stages are not equally susceptible to these reagents, schizonts being the most sensitive (23); (iii) the parasites may have incorporated the [^{3}H]hypoxanthine before the complete action of the microbicidal system.

Recently, a number of oxidant drugs have been found to have impressive antimalarial potency (22). These drugs include direct oxidants (i.e., peroxides) and agents which undergo oxidation-reduction cycling (i.e., quinones). Therefore, we suggest that future rational design of antimalarial drugs should include consideration of the oxidant sensitivity of infected erythrocytes and the effectiveness of oxidant drugs which act as antimalarial agents.

ACKNOWLEDGMENTS

We thank Robert A. Nicholas, Derk Schultz, and Léonardo K. Basco for careful review of the manuscript.

LITERATURE CITED

1. **Auclair, C., and C. Paoletti.** 1981. Bioactivation of the antitumor drugs 9-hydroxyellipticine and derivatives by a peroxidase-hydrogen peroxide system. J. Med. Chem. **24**:289–295.
2. **Babior, B. M.** 1978. Oxygen-dependent microbial killing by phagocytes. N. Engl. J. Med. **298**:659–668.
3. **Carlin, J. M., J. B. Jensen, and T. G. Geary.** 1985. Comparison of inducers of crisis forms in *Plasmodium falciparum* in vitro. Am. J. Trop. Med. Hyg. **34**:668–674.
4. **Clark, I. A., W. D. Cowden, and G. A. Butcher.** 1983. Free oxygen radical generators as antimalarial drugs. Lancet **ii**:234.
5. **Clark, I. A., and N. H. Hunt.** 1983. Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. Infect. Immun. **39**:1–6.
6. **Divo, A. A., T. G. Geary, and J. B. Jensen.** 1985. Oxygen- and time-dependent effect of antibiotics and selected mitochondrial inhibitors on *Plasmodium falciparum* in culture. Antimicrob. Agents Chemother. **27**:21–27.
7. **Dockrell, H. M., and J. H. L. Playfair.** 1983. Killing of blood-stage murine malaria parasites by hydrogen peroxide. Infect. Immun. **39**:456–459.
8. **Homewood, C. A., D. C. Warhurst, W. Peters, and V. C. Baggaley.** 1972. Lysosomes, pH and the antimalarial action of chloroquine. Nature (London) **235**:50–52.
9. **Jong, E. C., A. A. F. Mahmoud, and S. J. Klebanoff.** 1981. Peroxidase-mediated toxicity to schistosomula of *Schistosoma mansoni*. J. Immunol. **126**:468–471.
10. **Krogstad, D. J., I. Y. Gluzman, D. E. Kyle, A. M. J. Oduola, S. K. Martin, W. K. Milhous, and P. H. Schlesinger.** 1987. Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. Science **238**:1283–1285.
11. **Krogstad, D. J., and P. H. Schlesinger.** 1987. Acid-vesicle function, intracellular pathogens, and the action of chloroquine against *Plasmodium falciparum*. N. Engl. J. Med. **9**:542–549.
12. **Lambros, C., and J. P. Vanderberg.** 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. J. Parasitol. **65**:418–420.
13. **Locksley, R. M., and S. J. Klebanoff.** 1983. Oxygen-dependent microbicidal systems of phagocytes and host defense against intracellular protozoa. J. Cell. Biochem. **22**:173–185.
14. **Malhotra, K., D. Salmon, J. Le Bras, J. Savel, and J. L. Vilde.** 1988. Stimulation of chemiluminescence of human polymorphonuclear leukocytes by different intraerythrocytic stages of *Plasmodium falciparum*. C. R. Soc. Biol. **182**:270–278.
15. **Malhotra, K., D. Salmon, J. Le Bras, and J. L. Vilde.** 1988. Susceptibility of *Plasmodium falciparum* to a peroxidase-mediated oxygen-dependent microbicidal system. Infect. Immun. **56**:3305–3309.
16. **Murray, H. W., and Z. A. Cohn.** 1979. Macrophage oxygen-dependent antimicrobial activity. I. Susceptibility of *Toxoplasma gondii* to oxygen intermediates. J. Exp. Med. **150**:938–949.
17. **Nnalue, N. A., and M. J. Friedman.** 1988. Evidence for a neutrophil-mediated protective response in malaria. Parasite Immunol. **10**:47–58.
18. **Ockenhouse, C. F., and H. L. Shear.** 1984. Oxidative killing of the intraerythrocytic malaria parasites *Plasmodium yoelii* by activated macrophages. J. Immunol. **132**:424–431.
19. **Pfaller, M. A., and D. J. Krogstad.** 1983. Oxygen enhances the antimalarial activity of the imidazoles. Am. J. Trop. Med. Hyg. **32**:660–665.
20. **Rivadeneira, E. M., M. Wasserman, and C. T. Espinal.** 1983. Separation and concentration of schizonts of *Plasmodium falciparum* by Percoll gradients. J. Protozool. **30**:367–370.
21. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **193**:673–675.
22. **Vennerstrom, J. L., and J. W. Eaton.** 1988. Oxidants, oxidant drugs, and malaria. J. Med. Chem. **31**:1269–1277.
23. **Wozencraft, A. O., H. M. Dockrell, J. Taverne, G. A. T. Targett, and J. H. L. Playfair.** 1984. Killing of human malaria parasites by macrophage secretory products. Infect. Immun. **43**:664–669.