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In Vitro Activities of Novel Catecholate Siderophores against *Plasmodium falciparum*

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The activities of novel iron chelators, alone and in combination with chloroquine, quinine, or artemether, were evaluated in vitro against susceptible and resistant clones of *Plasmodium falciparum* with a semimicroassay system. N^4-nonyl,N^1,N^8-bis(2,3-dihydroxybenzoyl) spermidine hydrobromide (compound 7) demonstrated the highest level of activity: 170 nM against a chloroquine-susceptible clone and 1 μM against a chloroquine-resistant clone (50% inhibitory concentrations). Compounds 6, 8, and 10 showed antimalarial activity with 50% inhibitory concentrations of about 1 μM. Compound 7 had no effect on the activities of chloroquine, quinine, and artemether against either clone, and compound 8 did not enhance the schizontocidal action of either chloroquine or quinine against the chloroquine-resistant clone. The incubation of compound 7 with $FeCl_3$ suppressed or decreased the in vitro antimalarial activity of compound 7, while no effect was observed with incubation of compound 7 with $CuSO_4$ and $ZnSO_4$. These results suggest that iron deprivation may be the main mechanism of action of compound 7 against the malarial parasites. Chelator compounds 7 and 8 primarily affected trophozoite stages, probably by influencing the activity of ribonucleotide reductase, and thus inhibiting DNA synthesis.

Malaria is one of the major parasitic diseases in the subtropical and tropical regions of the world. One reason for the stagnant or rising global incidence of malaria over the past three decades has been the development and spread of drug-resistant *Plasmodium falciparum* (32).

Iron has a critical role in host-parasite interactions (5). It is essential for the growth of bacteria (18) and fungi, protozoan parasites, and mammalian cells (30). Desferrioxamine, a parenteral iron chelator used for the treatment of iron overload, has been shown to be active against *P. falciparum* both in vitro (1, 14, 21, 31) and in experimentally infected *Aotus* monkeys (19). In vitro studies have demonstrated that it can inhibit the growth of both erythrocytic and hepatic stages of *P. falciparum* at concentrations between 5 and 20 μM (21, 25, 31). Preliminary clinical trials with desferrioxamine, alone or in combination with other antimalarial agents, have demonstrated its good tolerance by the study subjects and its moderate efficiency (7, 8, 27). These studies suggest that iron deprivation may be a useful approach to the treatment of malaria. Conversely, iron supplementation in anemic children and pregnant women in areas where malaria is endemic may favor malarial infections (17).

The mechanism of action of iron chelators is still debated. Several hypotheses have been suggested: iron binding; the inhibition of iron-dependent enzymes (such as ribonucleotide reductase [23], phosphoenol pyruvate carboxykinase [8], dihydroorotate dehydrogenase, cytochrome *c* oxidase [22], or superoxide dismutase [2]); and the formation of free radicals [29]. In this report we present the antimalarial activities of several novel siderophores, alone and in combination with reference antimalarial drugs, and evaluate whether iron binding is the basis of their antimalarial activities.

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MATERIALS AND METHODS

Parasites. Two clones of *P. falciparum*, the chloroquine-susceptible D6/Sierra Leone strain (D6 clone) and the chloroquine-resistant W2/Indochina strain (W2 clone), were maintained in a continuous culture in type O^+ erythrocytes in complete RPMI 1640 medium supplemented with 10% human serum and buffered with 25 mM $NaHCO_3$ and 25 mM *N*-(2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (HEPES) (26).

Drugs. Desferrioxamine mesylate, quinine hydrochloride, ferric chloride, copper sulfate, and zinc sulfate were purchased from Sigma Chemical Co. (St. Louis, Mo.). Chloroquine sulfate was obtained from Specia (Paris, France), and artemether was obtained from Rhône-Poulenc-Rorer (Paris, France). The catecholate siderophores were synthesized by several of the authors (F. R., L. B., and G. K.). The catechol derivatives 2,3- and 3,4-dihydroxybenzoic acid were attached to putrescine (compounds 1 and 2), cysteamide (compounds 3 and 4), spermidine (compounds 5, 6, 7, and 8), or Tris(dihydroxybenzoyl)triaminoethylaminoethylamine (TRENCAM) (compounds 9 and 10). Compounds 7 and 8 were obtained by treatment of the corresponding α- and ω-diacylated spermidines, respectively, with 1-bromomanane in dry acetone in the presence of anhydrous potassium carbonate and then deprotection of the methoxy groups with boron tribromide. The chemical structures of these catechols are presented in Fig. 1.

Stock solutions of chloroquine and quinine were prepared in distilled water and 50% methanol–50% water, respectively. The stock solution of artemether was prepared in methanol. Desferrioxamine was dissolved in distilled water. Stock solutions of the catechols were prepared in methanol. Twofold serial dilutions in distilled water were distributed into flat-bottom 24-well plates in triplicate and dried.

In vitro susceptibility test. The in vitro susceptibility test described by Le Bras and Deloron (13) was used in the present study. When the majority (>90%) of asynchronous parasites were in the young ring and trophozoite stages, parasitized erythrocytes were diluted with fresh, uninfected erythrocytes and were suspended in a complete RPMI 1640 medium to obtain a hematocrit of 1.5% and an initial parasitemia of 0.5%. The suspension (700 μl per well) was distributed into 24-well plates, and the plates were incubated at 37°C in 5% O_2–5% CO_2–90% N_2 at 95% humidity for 42 h. [G-^{3}H]hypoxanthine (40 μCi/ml; 25 μl per

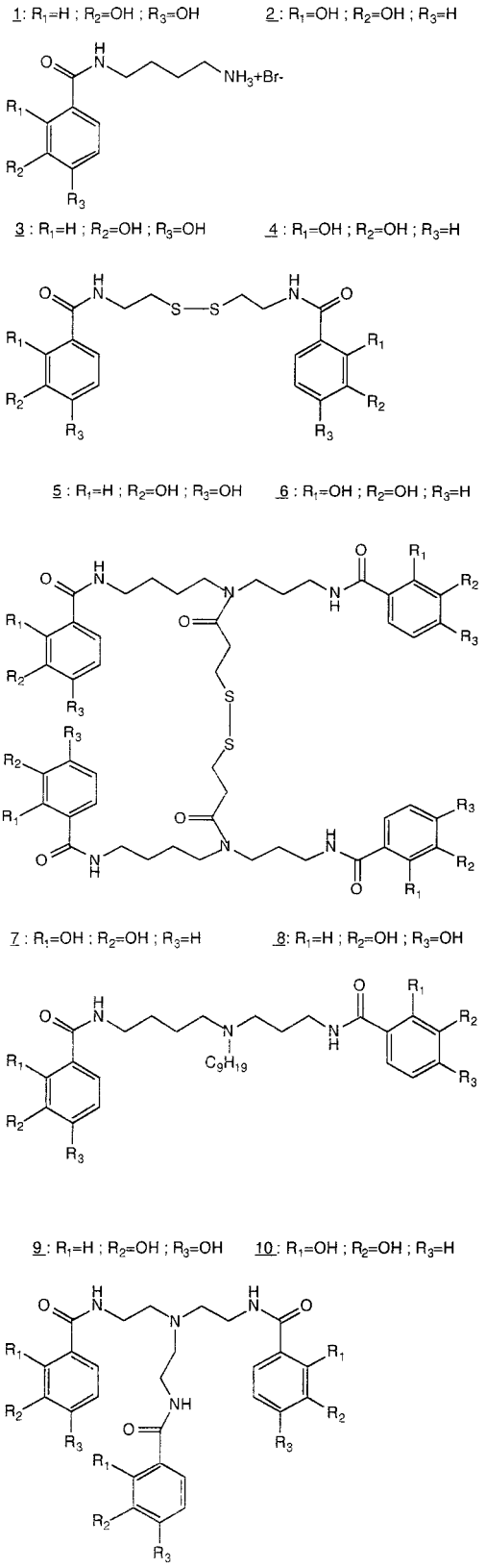

FIG. 1. Chemical structures of catecholate siderophores.

well; Amersham, Buckinghamshire, United Kingdom) was added 18 h later to assess parasite growth. At the end of the incubation period, the plates were frozen and then thawed to lyse the erythrocytes. The contents of each well were collected and washed with a cell harvester (Semiautomatic Cell Harvester; Skatron, Lier, Norway). The amount of radioactivity incorporated by the parasite was measured with a liquid scintillation counter (Wallac 1410; Pharmacia, Uppsala, Sweden).

The 50% inhibitory concentrations (IC_{50}s), defined as the drug concentrations corresponding to 50% of the uptake of [G-^{3}H]hypoxanthine by the parasites in drug-free control wells, were determined by nonlinear regression analysis of log dose-response curves. To study the effects of drug combinations, fixed concentrations of compounds 7 and 8, ranging from 0 to 70% of the respective IC_{50}s, were added to various concentrations of chloroquine, quinine, and artemether. Student's *t* test was used to compare the differences between the mean IC_{50}s.

The mechanisms of action of the siderophores were evaluated by combining a candidate siderophore and various metallic salts. Twofold serial dilutions of $FeCl_3$, $CuSO_4$, and $ZnSO_4$ (final concentrations, 125 to 2,000 nM) were added to the iron chelator compound 7 (final concentration, 1 μM), and the mixtures were incubated for 1 h at room temperature and distributed in triplicate into 24-well plates.

In further experiments, stage-specific drug susceptibility was determined as described previously (33). The parasites were synchronized twice by sorbitol lysis at 48-h intervals when the percentage of rings was greater than 90% (12). Parasites were exposed to compounds 7 and 8 for 6 h at 0 (early rings), 6, 12, 18, 24, 30, 36, 42, and 48 h. The parasites were washed three times with drug-free medium. The cell pellet was resuspended in 700 μl of complete drug-free medium, and the suspension was distributed into 24-well plates. [G-^{3}H]hypoxanthine (40 μCi/ml; 25 μl per well) was added, and the plates were incubated for 36 h.

RESULTS

The in vitro antimalarial activities of the compounds investigated are summarized in Table 1. Data are expressed as the mean ± standard deviation IC_{50}s of three to six independent experiments for each clone. Compounds 1 and 2 were inactive (IC_{50}s, >250 μM). Compounds 3 and 4 were less active than desferrioxamine. Compound 5 was as active as desferrioxamine against the D6 clone and two times less active against the W2 clone. Compound 9 was twice as active as desferrioxamine against the D6 clone, but its level of activity was similar to that of desferrioxamine against the W2 clone. Compounds 6, 8, and 10 were about 5 to 13 times more active than desferrioxamine against both clones. Compound 7 turned out to be 7 and 60 times more active than desferrioxamine against the W2 (IC_{50}, 1.02 μM) and D6 (IC_{50}, 170 nM) clones, respectively.

The IC_{50}s of chloroquine, quinine, and artemether alone and in combination with compound 7 were not significantly different for either clone ($P > 0.05$) (data not shown). The IC_{50}s of chloroquine and quinine alone and in combination

TABLE 1. In vitro activities of catecholate siderophores against the chloroquine-susceptible D6 clone and the chloroquine-resistant W2 clone of *P. falciparum*

Iron chelator	IC_{50} (μM)[a]	
	D6 clone	W2 clone
1	>250	>250
2	>250	>250
3	16.22 ± 6.46	21.47 ± 1.54
4	20.34 ± 3.72	34.70 ± 0.75
5	9.95 ± 4.50	15.95 ± 7.26
6	1.38 ± 0.45	1.41 ± 0.52
7	0.17 ± 0.04	1.02 ± 0.14
8	0.82 ± 0.13	0.96 ± 0.34
9	5.50 ± 0.06	6.91 ± 0.83
10	1.01 ± 0.07	1.18 ± 0.33
Desferrioxamine	10.75 ± 1.67	7.14 ± 0.70

[a] Values are means ± standard deviations of three to six independent experiments.

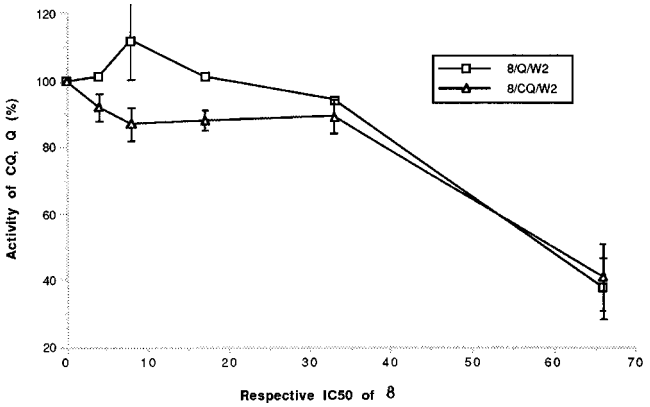

FIG. 2. In vitro activities of chloroquine (CQ) and quinine (Q) in combination with siderophore compound 8 against the chloroquine-resistant W2 clone of *P. falciparum* (*y* axis, percentage of IC_{50} of the drugs alone; *x* axis, various concentrations of compound 8 expressed as the percentage of IC_{50} of the siderophore alone).

with compound 8 were not significantly different for the chloroquine-resistant W2 clone (they were not tested in combination with compound 8 against the chloroquine-susceptible D6 clone). The diminution of the chloroquine and quinine IC_{50}s by 35 to 40% in combination with 65% of the IC_{50} of compound 8 is probably due to the intrinsic antimalarial activity of compound 8 (Fig. 2).

The in vitro antimalarial activities of compound 7 (1 μM) in combination with ferric chloride against the W2 clone are presented in Fig. 3. Six independent experiments against the chloroquine-resistant W2 clone were performed. The in vitro antimalarial activity of compound 7 was inhibited by the presence of ferric chloride. There was no statistically significant diminution of the IC_{50} of compound 7 plus copper sulfate or zinc sulfate compared with that of compound 7 alone (data not shown). At equimolar concentrations of ferric chloride (1 μM), parasite growth was 94% of that in the drug-free wells, whereas for compound 7 alone it was 52% of that in the drug-free wells.

By using synchronous cultures of *P. falciparum*, considerable variation in the susceptibility to the siderophore compounds 7 and 8 was demonstrated, depending on the different parasite stages (Fig. 4). When compound 7 or 8 was added to synchronized cultures containing early rings, the parasites developed normally, without growth inhibition. Profound growth inhibition occurred when the trophozoites were exposed to these chelators. Little or no effect on schizonts was found. Erythrocyte invasion by merozoites proceeded in the presence of chelators.

DISCUSSION

The in vitro antimalarial activities of some of the new catechols have been enhanced by increasing their lipophilicities. Catechols with high molecular weights (27, 30, or 48 carbon atoms; compounds 5 to 10) were more active than the smaller siderophores containing 11 or 18 carbon atoms (compounds 1 to 4). In addition, catechol compound 7 (C_{30}) was more active than the siderophore compound 6 (C_{48}). This result may be due to the relatively high molecular weight (1,008) of compound 6. A high molecular weight and therefore a greater size may reduce the level of penetration of this siderophore into the erythrocytes. Chelators may enter through the erythrocyte and parasite membranes and be concentrated in the parasites. Pouvelle et al. (20) suggested that a duct may connect the extracellular compartment and the parasitophorous vacuole. Molecules of high molecular weight may enter the parasite through this duct. Our experiments do not provide arguments against this hypothesis, especially since physicochemical studies undertaken in our laboratory have indicated that compounds 7 and 8 form micellar aggregates in aqueous solution at physiological pH (unpublished data).

The IC_{50}s of chloroquine, quinine, and artemether alone and in combination with compound 7 were not significantly different for either clone. Compound 7 did not enhance the schizontocidal action of either chloroquine, quinine, and artemether against the chloroquine-susceptible clone or the chloroquine-resistant clone. Compound 8 did not enhance the schizontocidal action of either chloroquine or quinine against the chloroquine-resistant clone. The mechanisms of action of chloroquine, artemether, and siderophores are still unknown. Meshnick et al. (16) demonstrated that chelators antagonized arteether; this antagonism was assessed with isobolograms. The construction of isoboles should apply to combinations of different agents if they had similar modes of action and similar dose-response curves. However, as in our study, Kamchonwongpaisan et al. (10) showed that the presence of iron chelators associated with artemisinin did not potentiate or antagonize its action and that various derivatives of artemisinin covalently linked to iron chelators retained activities comparable to that of artemisinin. In the presence of Fe^{3+}, artemisinin, the parent compound of artemether, may release free radicals by Fenton reactions (11). Furthermore, the inhibition of superoxide dismutase by iron chelators may decrease the catabolism of free radicals. Consequently, there may be a suppression of these two antagonistic effects when artemether is combined with an iron chelator.

The antimalarial activities of our most active siderophore and desferrioxamine were inhibited by the presence of ferric chloride. Thus, compound 7 seems to exert its antimalarial action by sequestering iron, which is essential for parasite growth, since preincubation of the chelator with exogenous iron considerably decreases its antimalarial activity, while copper and zinc privation had no influence on the schizontocidal action of compound 7. However, the zinc-desferrioxamine complex may penetrate parasitized erythrocytes better than free desferrioxamine (4). The former may enter the cell and exchange its bound zinc with ferric ions, thus rendering the iron unavailable for vital parasite functions. The results presented above indicate that the zinc-desferrioxamine complex is

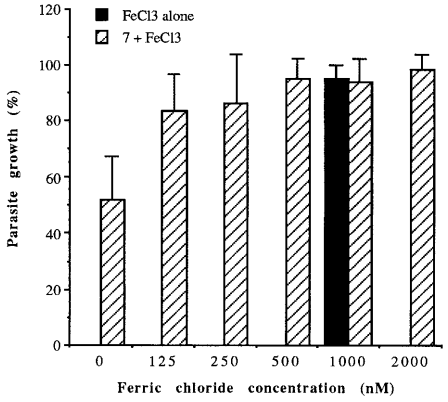

FIG. 3. In vitro activity of compound 7 (1 μM) in combination with metallic salts against the chloroquine-resistant W2 clone of *P. falciparum.*

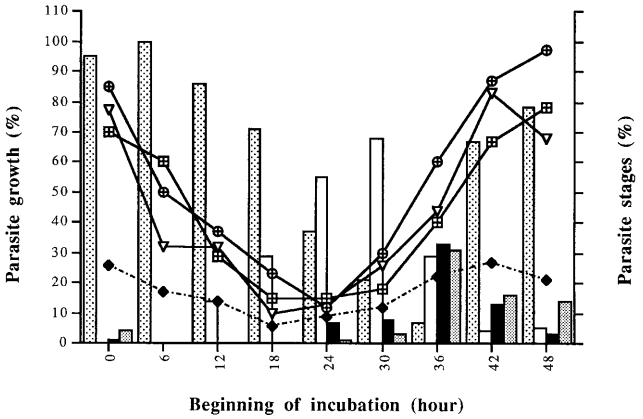

FIG. 4. In vitro stage-dependent effects of chelator compounds 7 and 8 on clone D6 of *P. falciparum*. ▨, percent rings; □, percent trophozoites; ■, percent early schizonts; ▤, percent mature schizonts; ⊞, percent parasite growth with 2.5 μM compound 7; ◆, percent parasite growth with 5.0 μM compound 7; ⊕, percent parasite growth with 5.0 μM compound 8; ▽, percent parasite growth with 10.0 μM compound 8.

more active than desferrioxamine at concentrations below 20 μM. However, in the present study, we demonstrated that zinc sulfate at >10 μM had toxic effects against parasitized erythrocytes and that the combination of desferrioxamine (22 μM) with zinc sulfate (concentrations ranging from 1 to 44 μM) retained activity comparable to that of desferrioxamine alone.

By using synchronous cultures of *P. falciparum*, considerable variation in susceptibility to siderophore compounds 7 and 8 was demonstrated at different parasite stages. These chelators and desferrioxamine (3, 31) primarily affected trophozoite stages. Iron chelators probably act by affecting the activity of iron-dependent ribonucleotide reductase (1, 15, 23) leading to an inhibition of DNA synthesis.

Among the siderophores examined in the present study, three (compounds 6, 8, and 10) were active in vitro in the micromolar range. Other known iron chelators, such as desferrithiocin, desferricrocin, and α-ketohydroxypyridines, are also active within a similar range of concentrations against the intraerythrocytic stages of *P. falciparum* (6, 9). Further chemical syntheses of new chelators, especially hydroxamate-based agents, are in progress (24, 28). Combinations with iron chelators might be of therapeutic value. Further structure-activity relationship studies and chemical analysis should provide more information on the role of lipophilicity, permeability, and iron-binding capacity. Several of the iron-binding compounds tested in the present study should also be tested with various mammalian cell lines in culture to identify possible toxic effects as well as with in vivo models of malaria to evaluate their potential clinical usefulness; at present, no acute toxic effect was seen in mice given compounds 7 and 8 at 50 mg per kg of body weight four times by the intraperitoneal route.

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REFERENCES

1. **Atkinson, C. T., M. T. Bayne, V. R. Gordeuk, G. M. Brittenham, and M. Aikawa.** 1991. Specific ultrastructural effects of desferrioxamine on *Plasmodium falciparum* in vitro. Am. J. Trop. Med. Hyg. **45:**593–601.
2. **Becuwe, P., S. Gratepanche, M. N. Fourmaux, J. Van Beeumen, B. Samyn, O. Mercereau-Puijalon, J. P. Touzel, C. Slomianny, D. Camus, and D. Dive.** 1996. Characterization of iron-dependent endogenous superoxide dismutase of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **76:**125–134.
3. **Cabantchik, Z. I.** 1995. Iron chelators as antimalarials: the biochemical basis of selective cytotoxicity. Parasitol. Today **11:**74–78.
4. **Chevion, M., L. Chuang, and J. Golenser.** 1995. Effects of zinc-desferrioxamine on *Plasmodium falciparum* in culture. Antimicrob. Agents Chemother. **39:**1902–1905.
5. **Finkelstein, R. A., C. V. Sciortino, and M. A. McIntosh.** 1983. Role of iron in microbe-host interactions. Rev. Infect. Dis. **5:**759–776.
6. **Fritsch, G., G. Sawatzki, J. Treumer, A. Jung, and D. T. Spira.** 1987. *Plasmodium falciparum*: inhibition in vitro with lactoferrin, desferrithiocin, and desferricrocin. Exp. Parasitol. **63:**1–9.
7. **Gordeuk, V., P. Thuma, G. Brittenham, C. McLaren, D. Parry, A. Backentose, G. Biemba, R. Msiska, L. Holmes, E. McKinley, L. Vargas, R. Gilkeson, and A. A. Poltera.** 1992. Effect of iron chelation therapy on recovery from deep coma in children with cerebral malaria. N. Engl. J. Med. **327:**1473–1477.
8. **Gordeuk, V., P. Thuma, G. Brittenham, S. Zulu, G. Simwanza, A. M'Hango, G. Flesch, and D. Parry.** 1992. Iron chelation with desferrioxamine B in adults with asymptomatic *Plasmodium falciparum* parasitemia. Blood **79:** 308–312.
9. **Heppner, D. G., P. E. Hallaway, G. J. Kontoghiorghes, and J. W. Eaton.** 1988. Antimalarial properties of orally active iron chelators. Blood **72:**358–361.
10. **Kamchonwongpaisan, S., S. Paitayatat, Y. Thebtaranonth, P. Wilairat, and Y. Yuthavong.** 1995. Mechanism-based development of new antimalarials: synthesis of derivatives of artemisinin attached to iron chelators. J. Med. Chem. **38:**2311–2316.
11. **Kamchonwongpaisan, S., N. Vanitchareon, and Y. Yuthavong.** 1992. The mechanism of antimalarial action of artemisinin, p. 363–372. *In* A. S. H. Ong and L. Packer (ed.) Lipid-soluble antioxidants: biochemistry and clinical applications. Birkhauser Verlag, Basel.
12. **Lambros, C., and J. P. Vanderberg.** 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. J. Parasitol. **65:**418–420.
13. **Le Bras, J., and P. Deloron.** 1983. In vitro study of drug sensitivity of *Plasmodium falciparum*: evaluation of a new semi-micro test. Am. J. Trop. Med. Hyg. **32:**447–451.
14. **Lytton, S. D., B. Mester, I. Dayan, H. Glickstein, J. Libman, A. Shanzer, and I. Cabantchik.** 1993. Mode of action of iron (III) chelators as antimalarials: membrane permeation properties and cytotoxic activity. Blood **81:**214–221.
15. **Lytton, S. D., B. Mester, J. Libman, A. Shanzer, and Z. I. Cabantchik.** 1994. Mode of action of iron (III) chelators as antimalarials. II. Evidence for differential effects on parasite iron-dependent nucleic acid synthesis. Blood **84:**910–915.
16. **Meshnick, S. R., Y. Z. Yang, V. Lima, F. Kuypers, S. Kamchonwongpaisan, and Y. Yuthavong.** 1993. Iron-dependent free radical generation from the antimalarial agent artemisinin (qinghaosu). Antimicrob. Agents Chemother. **37:**1108–1114.
17. **Oppenheimer, S. J., S. B. J. MacFarlane, J. B. Moody, O. Bunari, and R. G. Hendrickse.** 1986. Effect of iron on morbidity due to infectious disease: report on clinical studies in Papua New Guinea. Trans. R. Soc. Trop. Med. Hyg. **80:**596–602.
18. **Payne, S. M., and R. A. Finkelstein.** 1978. The critical role of iron in host-bacterial interactions. J. Clin. Invest. **61:**1428–1440.
19. **Pollack, S., R. N. Rossan, D. E. Davidson, and A. Escajadillo.** 1987. Desferrioxamine suppresses *Plasmodium falciparum* in *Aotus* monkeys. Proc. Soc. Exp. Biol. Med. **184:**162–164.
20. **Pouvelle, B., J. A. Gormley, and T. F. Taraschi.** 1994. Characterization of trafficking pathways and membrane genesis in malaria-infected erythrocytes. Mol. Biochem. Parasitol. **66:**83–96.
21. **Raventos-Suarez, C., S. Pollack, and R. L. Nagel.** 1982. *Plasmodium falciparum*: inhibition of in vitro growth by desferrioxamine. Am. J. Trop. Med. Hyg. **31:**919–922.
22. **Scheibel, L. W., and A. Adler.** 1980. Antimalarial activity of selected aromatic chelators. Mol. Pharmacol. **18:**320–325.
23. **Scheibel, L. W., and S. Rodriguez.** 1989. Antimalarial activity of selected aromatic chelators V. Localization of Fe^{59} in *Plasmodium falciparum* in presence of oxines, p. 119–149. *In* J. W. Eaton, S. R. Meshnick, and G. J. Brewer (ed.), Malaria and the red cells: 2. Alan R. Liss, Inc., New York.
24. **Scheibel, L. W., and G. G. Stanton.** 1986. Antimalarial activity of selected aromatic chelators. IV. Cation uptake by *Plasmodium falciparum* in the presence of oxines and siderochromes. Mol. Pharmacol. **30:**364–369.
25. **Stahel, E., D. Mazier, A. Guillouzo, F. Miltgen, I. Landau, S. Mellouk, R. L. Beaudoin, P. Langlois, and M. Gentilini.** 1988. Iron chelators: in vitro inhibitory effect on the liver stage of rodent and human malaria. Am. J. Trop. Med. Hyg. **39:**236–240.
26. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **193:**673–675.
27. **Traore, O., P. Carnevale, L. Kaptue-Noche, J. M'Bede, M. Desfontaine, J. Elion, D. Labie, and R. R. Nagel.** 1991. Preliminary report on the use of

desferrioxamine in the treatment of *Plasmodium falciparum* malaria. Am. J. Hematol. **37:**206–208.
28. **Tsafack, A., J. Golenser, J. Libman, A. Shanzer, and Z. I. Cabantchik.** 1995. Mode of action of iron (III) chelators as antimalarials. III. Overadditive effects in the combined action of hydroxamate-based agents on in vitro growth of *Plasmodium falciparum*. Mol. Pharmacol. **47:**403–409.
29. **Vercellotti, G. M., B. S. VanAsbeck, and H. S. Jacob.** 1985. Oxygen radical-induced erythrocyte hemolysis by neutrophils. Critical role of iron and lactoferrin. J. Clin. Invest. **76:**956–962.
30. **Weinberg, E. D.** 1990. Cellular iron metabolism in health and disease. Drug. Metab. Rev. **22:**531–579.
31. **Whitehead, S., and T. E. A. Peto.** 1990. Stage-dependent effect of desferrioxamine on growth of *Plasmodium falciparum* in vitro. Blood **76:**1250–1255.
32. **World Health Organization.** 1990. Practical chemotherapy of malaria. Technical Report Series 805. World Health Organization, Geneva.
33. **Yayon, A., J. A. Vande Waa, M. Yayon, T. G. Geary, and J. B. Jensen.** 1984. Stage-dependent effects of chloroquine on *Plasmodium falciparum* in vitro. J. Protozool. **30:**642–647.