Free Rad. Res. Comms., Vol. 15, No. 1, pp. 1–10
Reprints available directly from the publisher
Photocopying permitted by license only

GROWTH INHIBITION OF *PLASMODIUM FALCIPARUM* INVOLVING CARBON CENTERED IRON-CHELATE RADICAL ($L^{\cdot}$, X^{-})-Fe(III) BASED ON PYRIDOXAL-BETAINE. A NOVEL TYPE OF ANTIMALARIALS ACTIVE AGAINST CHLOROQUINE-RESISTANT PARASITES

EUGENE N. IHEANACHO,† SHALOM SAREL,‡ AMRAM SAMUNI,§ SCHELLY AVRAMOVICI-GRISARU‡ and DAN T. SPIRA†

†The Kuvin Centre for the Study of Infectious and Tropical Diseases, §Department of Molecular biology, Hebrew University – Hadassah Medical School, ‡Department of Pharmaceutical Chemistry, Hebrew University School of Pharmacy, Jerusalem, Israel

(Received March 27th, 1990; in revised form April 18th, 1990)

Malaria parasites have been shown to be more susceptible to oxidative stress than their host erythrocytes. In the present work, a chloroquine resistant malaria parasite, *Plasmodium falciparum* (FCR-3) was found to be susceptible in vitro to a pyridoxal based iron chelator – (1-[*N*-ethoxycarbonylmethyl-pyridoxlidenium]-2-[2′-pyridyl] hydrazine bromide – (code named L2-9). 2h exposure to 20 μM L2-9 was sufficient to irreversibly inhibit parasite growth. Desferrioxamine blocked the drug effect, indicating the requirement for iron. Oxygen however, was not essential. Spectrophotometric analysis showed that under anoxic conditions, L2-9-Fe(II) chelate undergoes an intramolecular redox reaction which presumably involves a one electron transfer and is expected to result in the formation of free radical. Spin trapping coupled to electron spin resonance (ESR) studies of L2-9-iron chelate showed that L2-9-Fe(II) produced free radicals both in the presence and absence of cells, while L2-9-Fe(III) produced free radicals only in the presence of actively metabolising cells.

KEY WORDS: red blood cells, *Plasmodium falciparum*, iron chelates, free radicals, electron spin resonance.

ABBREVIATIONS: RBC, red blood cells; DFO, desferrioxamine; ESR, electron spin resonance.

INTRODUCTION

The wide spread of multidrug resistant strains of the malarial parasite – *P. falciparum* that are now resistant to the quinine-related antimalarials calls for fundamental change in the design of novel chemotherapeutical agents. Clinical observations have shown that iron metabolism and malarial infection are closely interrelated,[1,2] and several studies have been designed to explore the effect of iron chelators on the growth of *P. falciparum*.[3–6] The binding of iron to redox-active sequestering agents could give

Correspondence address: Professor Shalom Sarel, Department of Pharmaceutical Chemistry, Hebrew University School of Pharmacy, P.O. Box 12065, Jerusalem 91120, Israel.

This article was due to appear in 1990, vol. **11**, issue 4–5, pp. 213–222.

rise to opposite biological effects, namely, either to enhancement of an oxidative (free radical) damage to the cell,[7] or inversely to an inhibition of such an effect. Mechanistically, chelators can operate by several distinctly different modes. One mode may involve mediation in transferring of the metal–ion to a cell receptor, invoking a damaging process to the latter. Another mode may invoke modification of the electrochemical gradient of the metal–ion. Alternatively, the chelator may act as a catalyst for converting essential cell metabolites into harmful free radicals by promoting a single-electron-transfer (SET) process.

In vivo and *in vitro* observations suggest that oxidative stress plays a dominant role in the defense against parasitic infections. Malarial parasites have been shown to be more susceptible to oxidative stress than their host erythrocytes.[8–11] Injection of malaria infected mice with alloxan which is known to increase in vivo the production of highly reactive oxygen species such as $O_2^{\cdot}$, H_2O_2, and $^{\cdot}OH$ markedly reduced the parasitemia.[12,13] There is also evidence that activated macrophages contribute through reactive oxygen intermediates (ROI) and other secretory products to rodent immunity against malaria.[14] The concept of free radical induced damage and the greater susceptibility of parasitized erythrocytes to oxidant damage provides a number of possibilities for malaria control.

In the present study, the chloroquine-resistant strain of *P. falciparum* is shown to be susceptible to 20 μM of an iron chelator (L2-9 = L^+, X^-) based on pyridoxal-betaine. Most significantly, the latter was observed to inhibit the growth of parasites after 2 hours of incubation.[3] This inhibition could be reversed by equimolar concentration of desferrioxamine (DFO). This result could be rationalized by viewing the chelator as functioning not only as a metal sequestrant capable of depriving the parasite of essential iron, but also as an inducer of free radical intermediates which lead to the death of the parasite. To substantiate this rational, we undertook an electron spin resonance (ESR) study (using spin traps such as PBN and DMPO) of L2-9. Most significantly, we observed that whereas the Fe(II)-chelate [(L^+, X^-)-Fe(II)] induces the production of carbon-centered free radicals both in the presence and in the absence of cells (parasites), the corresponding Fe(III)-chelate [(L^+, X^-)-Fe(III)] could do so only in the presence of living cells.

MATERIALS AND METHODS

Chemicals: The ligand, 1-[*N*-ethoxycarbonylmethyl-pyridoxylidenium]-2-[2′-pyridyl] hydrazine bromide (code named L2-9) was synthesized by exposing pyridoxal pyridyl hydrazone to the action of ethyl bromoacetate. Desferrioxamine B methanesulphonate (DFO) was obtained from CIBA, Horsham, Sussex-UK. α-phenyl-*N*-*tert*-butylnitrone (PBN) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) were purchased from Aldrich. DMPO was purified before use by distillation or by activated charcoal. The DMPO concentration in aqueous solutions was determined spectroscopically. $Fe_2(SO_4)_3$ and $FeSO_4$ were obtained from Fisher Scientific Co. (Silver Spring, MD); RPMI 1640 from GIBCO (Grand Island, NY USA); HEPES from Sigma Chem. Co. (USA). All other chemicals were of analytical grade.

Parasites: *P. falciparum* (FCR-3) was obtained from a stock of a continuous line maintained in human A^+ erythrocytes in RPMI 1640 medium containing 10% plasma.[15] Parasites were synchronized by either the sorbitol treatment,[16] gelatine sedimentation[17]

or a combination of both. Before use, parasites were washed twice with RPMI 1640 without plasma and once with HEPES/$NaHCO_3$ buffer (pH 7.2), and resuspended in the HEPES/$NaHCO_3$ buffer to a concentration of 10^9 cells/ml.

Influence of DFO on the inhibitory potential of L2-9. Parasites were synchronized by gelatin sedimentation and adjusted to 0.4% parasitemia and 4% haematocrit. This was dispensed equally into 15 ml centrifuge tubes. To these were added either 4 ml of RPMI 1640 normal growth medium, L2-9, DFO or 2 ml each of DFO and L2-9. For the L2-9/DFO mixture, parasites were first suspended in 2 ml of 80 μM DFO for 15 min, and then 2 ml of 80 μM L2-9 was added to give a final L2-9/DFO concentration of 40 μM. All four tubes were incubated at 37°C for 2 h. After the 2 h incubation, the cells were washed thrice with RPMI washing solution and cultured in normal growth medium. Parasitemia was determined daily by microscopical counts of number of parasites per 1000 cells.

ESR measurements: Spectra were recorded with a Varian E4 X-band spectrometer (9.5 GHz) with a field modulation frequency of 100 kHz, using non-saturating microwave power and modulation amplitude of 1 G. Measurements were made using a gas permeable teflon capillary which was inserted inside quartz tubing and placed in spectrometer.

Spectroscopic measurements: Measurements were made with a dual beam Kontron model Uvikon 860 spectrophotometer at room temperature in quartz curvettes with optical path length of 1 cm. In order to prevent hydrolysis, iron was initially dissolved in sulphuric acid at pH 2.0 and complexed to the chelator anaerobically at the acid pH, [L2-9]/[iron] = 1 : 1. The concentration was adjusted to 0.5 mM L2-9 with tripple distilled water. Just before use, the pH of the complex was adjusted to 7.2 using HEPES buffer to give a final L2-9-Fe concentration of 50–100 μM and 40 mM buffer concentration.

RESULTS

The exposure of the parasites to 20 μM L2-9 for 2 h resulted in inhibition of the *P. falciparum* growth. This inhibitory effect could be reversed by adding an equimolar concentration of DFO. This reversal action was observed only when the parasites were suspended first in DFO medium for 15 min followed by addition of the chelator (L2-9). If the parasites were added to a mixture containing DFO and the pyridoxal-based chelator, no effect of DFO was observed (Figure 1).

Spectrophotometric studies showed that under anoxic conditions and at pH 5.0–5.6, the reaction between the ligand and Fe(II) gives rise to a green coloured complex characterised by an absorption band $\varepsilon^{507\,nm}_{L2\text{-}9\text{-}Fe(II)} = 5.25\,mM^{-1}\,cm^{-1}$. Within 30–60 min and even in the absence of oxygen, the green L2-9-Fe(II) chelate gradually changes to a red L2-9-Fe(III) chelate having an extinction coefficient $\varepsilon^{474\,nm}_{L2\text{-}9\text{-}Fe(III)} = 19.05\,mM^{-1}\,cm^{-1}$ (Figure 2). The L2-9-Fe(III) generated from L2-9-Fe(II) under anoxic conditions was spectrometrically similar, though not equal to that formed by the direct chelating of L2-9 to Fe(III) ion, having $\varepsilon^{44\,nm}_{L2\text{-}9\text{-}Fe(III)} = 60.8\,mM^{-1}\,cm^{-1}$. The anoxic conversion of L2-9-Fe(II) to L2-9-Fe(III) indicates some intramolecular redox process which presumably involves a one electron transfer and is therefore expected to yield some

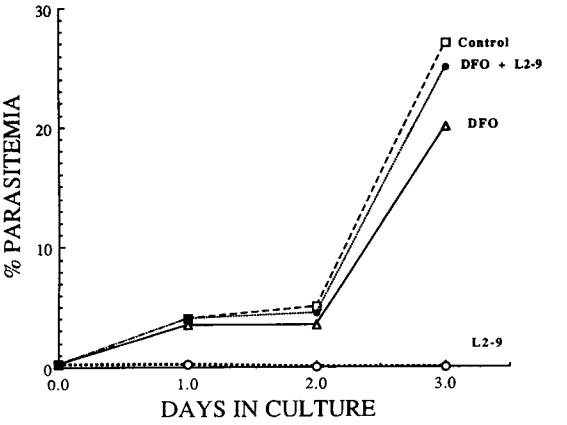


FIGURE 1 Effect of Desferrioxamine (DFO) on the antimalarial activity of L2-9. Gelatin synchronized parasites *P. falciparum* culture was dispensed in aliquots of 5 ml of either normal medium (control), L2-9, DFO or L2-9 + DFO. All concentrations were 40 μM at final hematocrit of 4% and a parasitemia of 0.2%. They were incubated at 37°C for 2 h. In the case of L2-9 + DFO, DFO was first added and left to stand for 15 min before addition of L2-9. After incubating for 2 h, parasites were washed thrice with RPMI 1640 washing solution and cultured in RPMI complete medium. Parasitemia was determined daily by microscope counts of number of parasites per 5 × 10^3 erythrocytes. Each value represents an average of 3 counts.

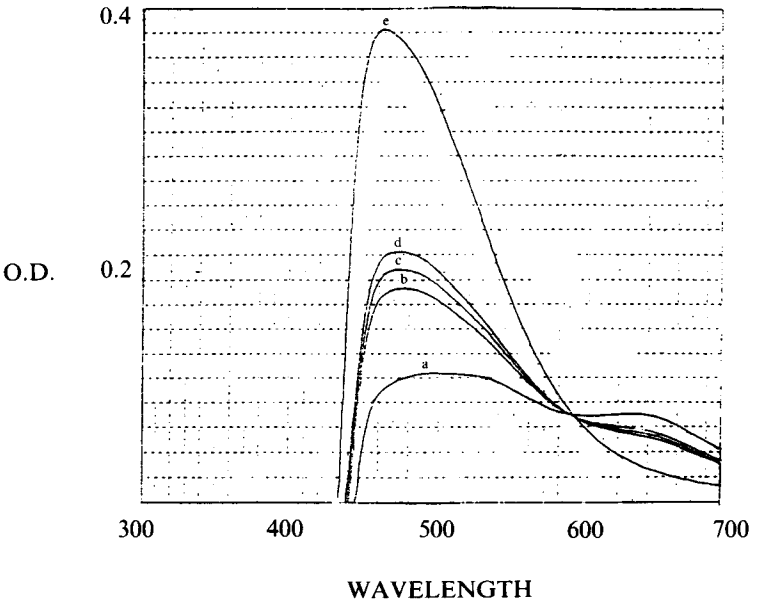


FIGURE 2 Absorption spectra of the anaerobic oxidation of Fe(II) to Fe(III) by L2-9. 1 mM $Fe(NH_4)_2(SO_4)_2$ in 10 mM sulphuric acid at pH 2.0, was complexed to L2-9 anaerobically in a ratio of [L2-9]/[iron] = 1 : 1. The concentration was adjusted to give 50 μM (L2-9)-Fe(II) in 40 mM HEPES buffer pH 7.4. Change in spectra with time after complexing were (a) 20 min; (b) 1 h; (c) 2 h; (d) 3 h; and (e) 20 h. The assay was performed anaerobically at room temperature using a dual beam Kontron model Uvikon 860 spectrophotometer.

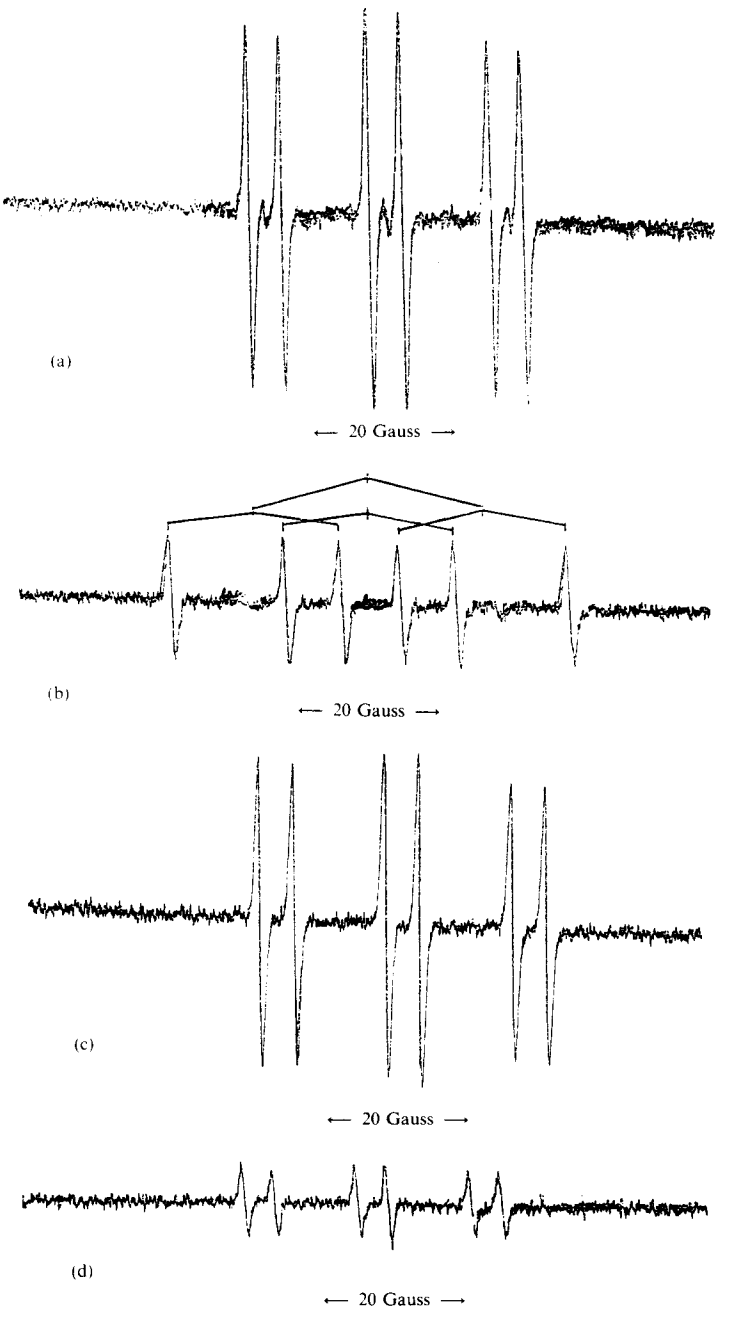


FIGURE 3 Electron spin resonance (ESR) spectra of L2-9-Iron complexes. L2-9-Fe(II) was prepared anoxically and immediately scanned for ESR signal in the presence and absence of cells, (a) 15 mM PBN; (b) 135 mM DMPO; (c) in the presence of 15 mM PBN and 10^9 *P. falciparum* schizonts/ml; (d) (L2-9)-Fe(III), 15 mM PBN and 10^9 *P. falciparum* schizonts/ml.

reduced intermediate of the ligand L2-9. A tentative explanation for this observation is that Fe(III)–($L^{\cdot}$, X^{-}) tends to enter into a demonstration reaction, as follows: 2Fe(III)–($L^{\cdot}$, X^{-}) → Fe(II)–(L^{+}, X^{-}) + Fe(III)–(L^{+}, X^{-}). To investigate this possibility, the reaction was studied using electron spin resonance (ESR) spectroscopy. When the ligand-iron II complex was anaerobically prepared and scanned for the presence of free radicals, no ESR signal was detected. Because the lack of any observable ESR signal could be due to instability of the active intermediates, the spin trapping technique was utilized. L2-9-Fe(II) was anaerobically incubated in the presence of spin traps such as PBN and DMPO. In the presence of either PBN or DMPO, six lines ESR signals were accumulated and persisted. Both ESR spectra were characteristic of a carbon centered spin adduct having $\mathbf{a}_N$ = 15.75 G and $\mathbf{a}_H$ = 4.25 G of PBN-$R^{\cdot}$ (Figure 3a) and $\mathbf{a}_N$ = 15.75 G and $\mathbf{a}_H$ = 23.5 G of DMPO-$R^{\cdot}$ (Figure 3b) respectively. The same ESR signals were observed when the experiments were repeated under aerobic conditions but no spin adducts of $^{\cdot}OH$ or $O_2^{\cdot-}$ were detected.

L2-9-Fe(III), unlike L2-9-Fe(II), did not elicit any detectable spin adduct. It was therefore anticipated that in the presence of metabolising cells, which are capable of reducing Fe(III) to Fe(II), the generation of free radicals by both chelates would be potentiated. When the experiments were repeated using *Plasmodium falciparum* (10^9 schizonts/ml), the chelates of both Fe(II) and Fe(III) gave rise to ESR signals identical to those observed in cell-free systems (Fig. 3c and 3d). However, the spin adduct signal generated by L2-9-Fe(II) was bigger than that formed by L2-9-Fe(III).

DISCUSSION

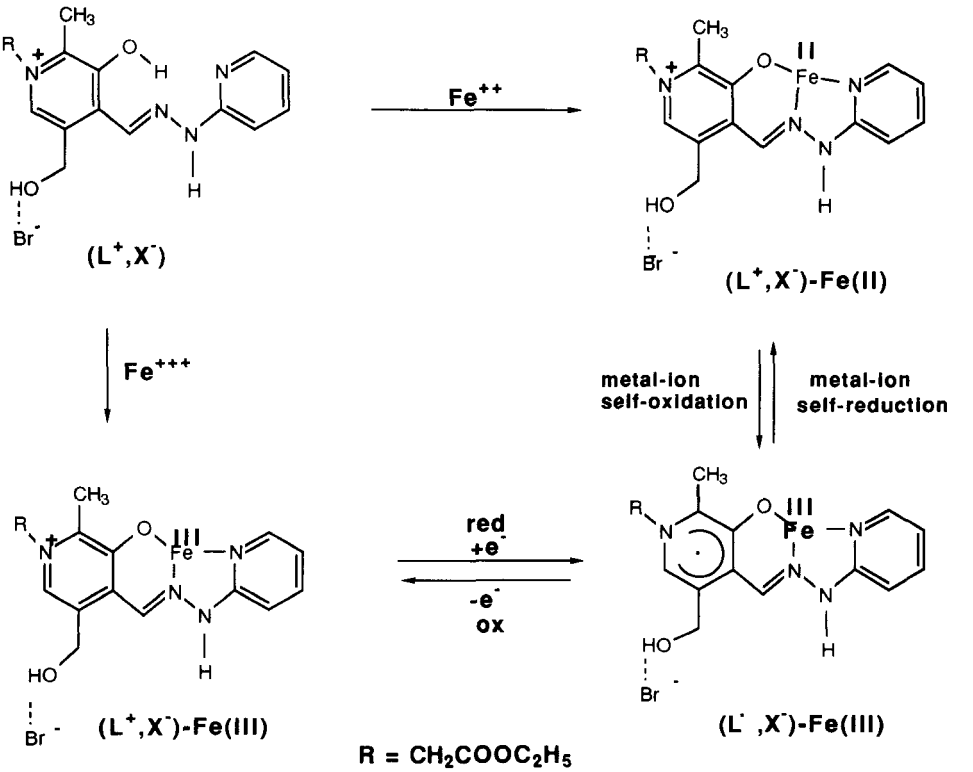
In this study we have shown that the chelator L2-9 (L^{+}, X^{-} in chart) induces the production of free radicals in the presence of Fe(II). In the presence of metabolising cells or reducing agents, free radicals were produced by both Fe(II) and Fe(III). It thus appears that L2-9 inhibits the growth of parasites not just by the simple chelating process of iron withholding, but by the generation of free radicals. The death of parasites after two hours of incubation with L2-9 is attributable to some active intermediates induced by the chelator in the presence of Fe(II) rather than a simple nutritional deprivation of iron.

The production of oxygen-derived free radicals via the iron catalyzed Haber–Weiss reaction is well documented. However, considerable controversy exists as to whether the natural sequestered forms of iron or possibly other transition metal ions are effective in catalyzing this reaction.[18–23] The activity of iron is dependent upon the ligand to which the metal is complexed, since various chelators alter the redox potential and the reactivity of the bound metal ion to varying extents.[24,25] Iron chelators such as EDTA and DTPA inhibit or increase lipid peroxidation stimulated by chelatable iron depending on the ratio of chelator to iron, whereas DFO inhibits at all concentrations tested.[26–28] Clark et al.[29] have reported the in vivo and in vitro killing of *Plasmodium vinckei vinckei* and *Plasmodium falciparum* by *t*-butyl hydroperoxide (*t*-BHP) which generates oxygen derived free radicals. It was also shown that alloxan, a generator of reactive oxygen intermediates, cleared mice of lethal infection with *Plasmodium vinckei*.[13] In both experiments, pre-treatment with DFO for 15 min reportedly abolished the action of *t*-butyl hydroperoxide and alloxan. The present results suggest that free radical intermediates are instrumental in L2-9 toxicity

towards the parasites. The failure, however, to detect DMPO-OH spin adduct in the present study and the anoxic toxicity of L2-9 do not support the hypothesis that oxygen-derived radicals such as $^{\cdot}OH$ or superoxide, play any role in the drug's effect.

Oxidant generating drugs have been previously shown to have anti-malarial properties,[30] but the precise mechanism by which the human malarial parasite is killed by a variety of radical generating systems still remain unclear. The data produced here permits to suggest that the death of the parasite is likely caused by a sort of damaging process arising from the break down of an essential component of the cell, such as the DNA macromolecule. Evidences indicating damage to DNA by L2-9-Fe(II) is forthcoming.[31] This is substantiated by the appearance of ESR signals only when living cells are present in a mixture of the chelate L2-9-Fe(III) with spin traps such as PBN or DMPO, indicating that living cells are involved in a type of single electron-transfer [SET] as expressed by Eq. 3. The tendency of the DNA macromolecule to function as an electron-transfer oxidant (an electron acceptor) in redox reactions, is well documented.[32,33] It is plausible therefore to suggest that the iron chelate assumes most likely the role of an electron-transfer-reductant (an electron donor) in the latter process.[34,35] Accordingly we attribute reductive properties to the carbon-centered free radical of structure (L, X^-)-Fe(III) (see chart). The latter most likely arises from a reversible intramolecular SET shift (Eq. (2)), involving an initially formed (L^+, $X^{\cdot}$)-Fe(II) intermediate (Eq. (1)). The $(DNA)^{\cdot-}$ anion radical (eq. (3)) is known to be highly labile, tending to fragment and thus leading to the death of

CHART



the cell.

$$[H_2L^+, X^-] + Fe^{2+} \longrightarrow 2H^+ + [L^+, X^-]\text{-Fe(II)} \quad (1)$$

$$[L^+X^-]\text{-Fe(II)} \xrightarrow[\text{electron transfer}]{\text{intramolecular}} [L^{\cdot}, X^-]\text{-Fe(III)} \quad (2)$$

$$[L^{\cdot}, X]\text{-Fe(III)} + \text{DNA (parasite)} \xrightarrow{\text{SET}} [L^+, X^-]\text{-Fe(III)} + [\text{DNA}]^{\cdot-}\ \text{parasite} \quad (3)$$

$$\text{Parasite-}[\text{DNA}]^{\cdot-} \longrightarrow \text{Decomposition products} \quad (4)$$

Because of the low concentration of the ligand (20 μM) for effective antimalarial activity, its mode of action could not be explained in terms of mass-action alone. It could however be understood in terms of steady-state theory, by which the truly active species, namely, the carbon-centered free radical ($L^{\cdot}$, X^-)-Fe(III) is regenerated in the presence of living cells as soon as consumed. The free radical regeneration could be envisioned to result from a cascade of redox events involving ubiquitous cell reductants by (red-(I)-H_2), (red-(II)-H_2). etc., with matching electrochemical gradient as expressed by Eqs. (5) and (6).

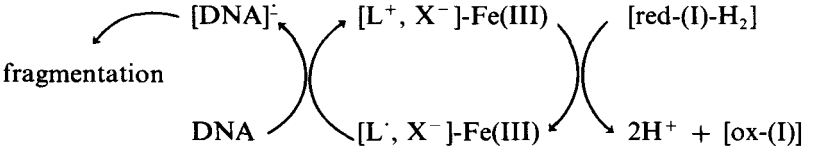
$$2[L^+, X^-]\text{-Fe(III)} + [\text{red-(I)-}H_2] \longrightarrow 2H^+ + [L^{\cdot}, X^-]\text{-Fe(III)} \quad (5)$$

$$[\text{ox-(I)}] + [\text{red-(II)-}H_2] \longrightarrow [\text{red-(I)-}H_2] + [\text{ox-(II)}] \quad (6)$$

Essentially it suggests that the antimalarial free radical species ($L^{\cdot}$, X^-]-Fe(III) plays a pivotal role in catalyzing the generation of electrons from ubiquitous cell reductants, and in mediating the transfer of the electrons to the parasite-DNA, by a multi-stage ET process, as outlined below:

$$[\text{red-(I)-H2}] + 2\text{DNA-parasite} \xrightarrow{\text{catalyst}} [\text{ox-(I)}] + 2H^+ + 2[\text{DNA-}]^{\cdot-}\ \text{parasite.} \quad (7)$$

The novel type of lipophilic-hydrophilic chelator based on pyridoxal-betaine (L2-9) described here is a representative of a family of new antimalarials operating by a hitherto unknown mechanism involving the generation and transfer of electrons from cell reductants to the parasite DNA (Eqs. (3–7)). The death of the parasite is believed to follow from the breakdown of the resulting DNA anion radical ($[DNA]^{\cdot-}$) (eqn. 4).[36,37]



The greater susceptibility of parasitized erythrocytes to oxidant damage,[14,38] and the relative abundance of iron in parasitized cells provide a number of possibilities for the design of new anti-malarial drugs based on intracellular iron dependent radical inducers.

References

1. P.W.J. Harvey, P.F. Heywood, M.C. Nesheim, K. Galme, M. Zegans, J-P. Habicht, L.S. Stephenson, K.L. Radimer, B. Brabin, K. Forsyth and M.P. Alpers (1989) The effect of iron therapy on malarial infection in Papua New Guinean school children. *American Journal of Tropical Medicine and Hygiene*, **40**, 12–18.

2. S.J. Oppenheimer, F.D. Gibson, S.B. Macfarlene, J.B. Moody, C. Harrison, A. Spencer and O. Bunari (1986) Iron supplementation increases prevalence and effects of malaria: Clinical studies in Papua New Guinea. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **80**, 603–612.
3. E.N. Iheanacho, A. Samuni, S. Avramovici-Grisaru, S. Sarel and D.T. Spira (1989) *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**, 213–216.
4. D.G. Heppner, P.E. Hallaway, G.J. Kontoghiorghes and J.W. Eaton (1988) Antimalarial properties of orally active iron chelators. *Blood*, **72**, 358–361.
5. E. Stahel, D. Mazier, A. Guillouzo, F. Miltgen, I. Landau, S. Mellouk, R.L. Beaudoin, P. Langbois and M. Gentilini (1988) Iron chelators: In vitro effect on the liver stage of rodent and human malaria. *American Journal of Tropical Medicine and Hygiene*, **39**, 236–240.
6. A.M. Yinnon, E.N. Iheanacho, R.W. Grady, D.T. Spira and C. Hershko (1989) Antimalarial effect of HBED and other phenolic and catecholic iron chelators. *Blood*, **74**, 2166–2171.
7. P. Saltman (1989). Oxidative stress: A radical view. *Seminars in Hematology*, **26**, 249–256.
8. A.U. Orji, H.S. Banyal, R. Chevli and C.D. Fitch (1981) Hemin lyses malaria parasites. *Science*, **214**, 667–669.
9. H.M. Dockrell and J.H.L. Playfair (1983) Killing of blood stage murine malaria parasites by hydrogen peroxide. *Infection and Immunity*, **39**, 456–459.
10. J. Miller, J. Golenzer, D.T. Spira and N.S. Kosower (1984) *Plasmodium falciparum*: Thiol status and growth in normal and G6PD deficient human erythrocytes. *Experimental Parasitology*, **57**, 239–247.
11. Y. Zhang, E. Hempelmann and R.H. Schirmer (1988) Glutathione reductase inhibitors as potential antimalarial drugs. Effects of Nitrosoureas on *Plasmodium falciparum* in vitro. *Biochemical Pharmacology*, **37**, 855–860.
12. K. Grankvist, S.L. Marklund and I.B. Taljedal (1981) CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. *Biochemical Journal*, **199**, 393–398.
13. I.A. Clark and N.H. Hunt (1983) Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. *Infection and Immunity*, **39**, 1–6.
14. R.H. Schirmer T. Schollhammer G. Eisenbrand and R.L. Krauth-Siegel (1987). Oxidative stress as a defense mechanism parasitic infections. *Free Radical Research Communications*, **3**, 3–12.
15. J. Golenser, D. Casuto and Y. Pollack (1981) *Plasmodium falciparum*: *In vitro* induction of resistance to aminoprotein. *Experimental Parasitology*, **52**, 371–377.
16. C. Lambros and J.P. Vanderberg (1979) Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *Journal of Parasitology*, **65**, 418–420.
17. J.B. Jensen (1978). Concentration from continuous culture of erythrocytes infected with trophozoites and schizonts of *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene*, **27**, 1274–1276.
18. D.R. Ambruso and R.B. Johnston (1981) Lactoferrin enhances hydroxyl radical production by human neutrophils, neutrophil particulate fractions and an enzymatic generating system. *Journal of Clinical Investigations*, **67**, 352–360.
19. J.M.C. Gutteridge, S.K. Paterson, A.W. Segal and B. Halliwell (1981) Inhibition of lipid peroxidation by the iron binding protein lactoferrin. *Biochemical Journal*, **199**, 259–261.
20. J.M.C. Gutteridge, D.A. Rowley and B. Halliwell, (1982) Superoxide dependent formation of hydroxyl radicals and lipid peroxidation in the presence of iron salts. *Biochemical Journal*, **206**, 604–609.
21. R.J. Youngman (1984) Oxygen activation: Is the hydroxyl radical always relevant? *Trends in Biochemical Sciences*, **9**, 280–283.
22. G.M. Vercellotti, B.S. VanAsbeck and H.S. Jacob (1985) Oxygen radical induced erythrocyte hemolysis by neutrophils – critical role of iron and lactoferrin. *Journal of Clinical Investigation*, **76**, 956–962.
23. G. Fritsch, G. Sawatzki, J. Treumer, A. Jung and D.T. Spira (1987) *Plasmodium falciparum*: Inhibition in vitro with lactoferrin, desferroferrithiocin, and desferricrocin. *Experimental Parasitology*, **63**, 1–9.
24. B. Halliwell and J.M.C. Gutteridge (1985) The importance of free radicals and catalytic metal ions in human diseases. *Molecular Aspects of Medicine*, **8**, 89–193.
25. R.A. Floyd (1983) Direct demonstration that ferrous ion complexes of di- and triphosphate nucleotides catalyze hydroxyl free radical formation from hydrogen peroxide. *Archieves of Biochemistry and Biophysics*, **225**, 263–270.
26. E.D. Wills (1969) Lipid peroxide formation in microsomes: The role of non-haem iron. *Biochemical Journal*, **113**, 325–332.
27. J.M.C. Gutteridge, R. Richmond and B. Halliwell (1982) Superoxide dependent formation of hydroxyl radicals from superoxide and of lipid peroxidation by Desferrioxamine. *Biochemical Journal*, **184**, 469–472.

28. B. Halliwell and J.M.C. Gutteridge (1984) Oxygen toxicity, oxygen radicals transition metals and disease. *Biochemical Journal*, **229**, 1–14.
29. I.A. Clark, W.B. Cowden and G.A. Buther (1983) Free oxygen radical generators as antimalarial drugs. *Lancet*, **i**, 234.
30. J.L. Vennerstrom and J.W. Eaton (1988) Oxidants, Oxidant drugs, and Malaria. *Journal of Medicinal Chemistry*, **31**, 1269–1277.
31. E.N. Iheanacho, S. Sarel, A. Samuni, S. Avramovici-Grisaru and D.T. Spira (1990) Growth inhibition of *P. falciparum* by iron-chelate radical involving damage to DNA. *Free Rad Res. Comm.*, **11**(6), 307–315.
32. R.A. Floyd (1981) DNA-ferrous catalyzed hydroxyl free radical formation from hydrogen peroxide. *Biochemical and Biophysical Research Communications*, **99**, 1209–1215.
33. H.C. Birnboim and M. Kanabus-Kaminska (1985) The production of DNA strand breaks in human leukocytes by superoxide anion may involve a metabolic process. *Proceedings of the National Academcy of Sciences*, **82**, 6820–6824.
34. D. Brault and P. Neta (1981) Reactions of iron porphyrins with methyl radicals. *Journal of American Chemical Society*, **103**, 2705–2710.
35. Y. Sorek, H. Cohen and D. Meyerstein (1985) Reactions of Iron(II) Protophyrin with strongly reducing free radicals in aqueous solution. *Journal of the Chemical Society, Faraday Transactions 1*, **81**, 233–239.
36. C.G. Cochrane, I.U. Schraufstatter, P. Hyslop and J. Jackson (1988) Cellular and Biochemical events in oxidative injury. In B. Halliwell ed. Oxygen radicals and tissue injury symposium (Proceedings). Bethesda: *Federation of American Societies for Experimental Biology*. pp. 49–54.
37. A.C. Mello Filho and R. Meneghini (1985) Protection of mammalian cells by o-phenanthroline from lethal and DNA-damaging effects produced by active oxygen species. *Biochimica et Biophysica Acta*, **847**, 92–89.
38. A.O. Wozencraft (1986) Damage to malaria infected erythrocytes following exposure to oxidant generating systems. *Parasitology*, **92**, 559–567.

Accepted by Prof. G. Czapski