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Perspective

Oxidants, Oxidant Drugs, and Malaria

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I. Introduction

Complex adaptations are necessary for the survival of malaria parasites. Not only must plasmodia survive temporarily as free living organisms (sporozoites or merozoites) but they also must be able to recognize an appropriate host red cell, penetrate, and replicate within it. In the intraerythrocytic phase, successful replication of the parasite entails having as little adverse effect upon the red cell as possible. In the event the host cell is damaged so that it cannot survive the necessary 48–72 h, the parasite is doomed because immature blood-form plasmodia cannot survive.

There is good reason to believe that a tenuous balance exists between the requirements for successful replication of the malaria parasite and maintenance of an intact host erythrocyte. This is most evident in the oxidant damage which may be done to the host cell by the parasite. As reviewed below, the parasite causes measurable oxidation of the host red cell, doing damage which may come close to destroying the erythrocyte before the parasite is able to mature. In fact, natural selection has taken advantage of this circumstance; positive selection has led to relatively high frequencies of genetically variant erythrocytes, deficient in the enzyme glucose-6-phosphate dehydrogenase (G-6-PD), which are particularly oxidant sensitive and less able to survive parasite-induced oxidation. It is probably not a coincidence that numerous structurally diverse oxidant drugs have substantial antimalarial effects. In the discovery of these drugs, we may unwittingly have exploited the same principle discovered by natural selection—that malaria-infected red cells are selectively damaged by oxidants.

II. Nature and Origins of Malaria-Induced Red Cell Oxidant Stress

The oxidant sensitivity of malaria-infected erythrocytes may arise both from precedent damage by parasite-generated oxidants and from a weakening of the oxidant defense mechanisms of the erythrocyte. Direct oxidation of red cell components has been documented in several studies. Red cells from mice infected with *Plasmodium berghei* have elevated levels of methemoglobin, and the extent of elevation correlates closely with the severity of infection in individual animals.[1] One proximate cause of elevated methemoglobin may be an increased intracellular flux of hydrogen peroxide[1] as detected by the rate of 3-amino-1,2,4-triazole-mediated irreversible inhibition of red cell catalase (an inhibition which absolutely requires H_2O_2).[2] In whole blood from *P. falciparum* infected humans, spontaneous generation of "radical oxygen species" was found to be elevated 7-fold compared to blood from normal controls.[3] Although some of this increased generation of activated oxygen species may reflect the heightened activity of phagocytes in the infected blood, a certain fraction may also derive from the infected red cells.

The enhanced oxidation caused by the malaria parasite is not restricted to the hemoglobin of infected erythrocytes. Red cells parasitized with *P. falciparum*,[4] *P. vinckei*,[5] and *P. berghei*[6,7] exhibit increased lipid peroxidation. The *P. vinckei* parasite has both increased amounts of readily oxidizable polyunsaturated fatty acids[8] and plasma membranes that are more vulnerable to oxidative injury than those of the host erythrocyte.[8,9] Furthermore, the parasite is susceptible to several aldehydic products of lipid peroxidation.[10] A diet high in polyunsaturated fatty acids suppresses *P. berghei* infections in mice; vitamin E completely reverses this inhibition.[11] Mice deficient in vitamin

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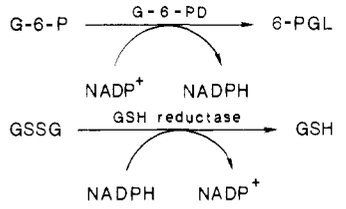
E (a deficiency which causes red cells to be especially sensitive to lipid peroxidation and peroxide hemolysis) survive *P. berghei* infections much longer than do their normal counterparts. Not only do deficient animals have lower parasitemias, the parasites are also located preferentially in the younger and more oxidant resistant reticulocytes.[12] It is reasonable to suppose that the resistance conferred by vitamin E deficiency is due to premature hemolysis of infected erythrocytes caused in part by oxidants of parasite origin.

The nature and origins of the activated oxygen species responsible for enhanced oxidation of host erythrocyte components are presently unknown. One possibility is that some of the oxidants are produced secondary to catabolism of host cell hemoglobin by the parasite. As the parasite matures, it consumes hemoglobin by a process resembling phagocytosis. While in the food vacuole and being digested, hemoglobin may spontaneously autoxidize, a process known to give rise to superoxide.[13,14] The superoxide, in turn, can mediate membrane lysis[15] and further oxidation of hemoglobin,[16] perhaps leading to the accumulation of oxidatively denatured hemoglobin even in sites outside the food vacuole. More importantly, the accumulated ferruginous waste arising from parasite catabolism of hemoglobin is almost certainly a major source of oxidative stress. Thus, for example, free heme spontaneously lost from hemoglobin may be a major cause of red cell membrane damage in sickle cell disease.[17,18] Finally, the metabolism of the malaria parasite may produce a certain amount of activated oxygen. Plasmodia have enzymes such as dihydroorotate dehydrogenase[19] which, like other flavoproteins, will reduce oxygen to superoxide[20] and hydrogen peroxide.

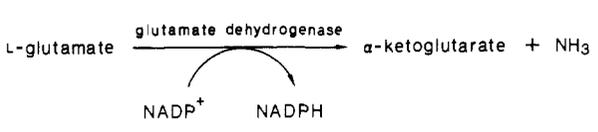
To an extent, the increased oxidation of host erythrocyte components may be due to impairment, by the parasite, of normal erythrocyte oxidant defense and repair pathways. For example, *P. berghei* infected murine erythrocytes tend to become quantitatively deficient in the activity of superoxide dismutase (SOD),[6,21,22] the enzyme responsible for catalyzing the dismutation of superoxide to hydrogen peroxide. This deficiency arises, in part, through "adoption" of host cell SOD by the malaria parasite, which internalizes the mammalian enzyme and may use it in its own oxidant defense.[21] An increase in parasite load is also accompanied by decreased activities of the red cell enzymes catalase,[7,9,22-24] glutathione peroxidase,[6,7,9] NADPH:cytochrome *c* reductase,[25] and NADH-methemoglobin reductase.[9,25]

The enzyme glucose-6-phophosphate dehydrogenase (G-6-PD) is a crucial arm of the oxidant defense system of the red cell. This enzyme is needed for the reduction of NADP to NADPH, which, in turn, is required for the maintenance of glutathione in the reduced form (GSH):



A most important contributor to the enhanced oxidant sensitivity of infected red cells may be the fact that many species of plasmodia (*P. berghei*, *P. lophurae*, and *P. vinckei*) entirely lack G-6-PD activity of their own,[23,26-28] while *P. falciparum* has only very low levels of activity.[29-31] Conflicting reports have noted increased,[27] unchanged,[32] or decreased[33] total G-6-PD activity in *P. knowlesi* infected erythrocytes.

Although a 25-fold increase in hexokinase activity (which catalyzes the biosynthesis of G-6-P, the substrate for G-6-PD) was observed in *P. falciparum* infected erythrocytes,[34] it is likely that *P. falciparum* and other plasmodia spp. rely heavily upon host cell G-6-PD for reduction of NADP to NADPH via G-6-P and, perhaps, for crucial metabolic intermediates such as ribosyl pyrophosphate for nucleic acid metabolism.[35] In many or all cases, plasmodia may rely completely upon NADPH generated by the host cell for reduction of intra-parasitic oxidized glutathione (GSSG) to reduced glutathione (GSH)[9,36] although a parasite-specific NADP-dependent glutamic acid dehydrogenase is present in *P. berghei*,[37] *P. lophurae*,[38] *P. chabaudi*,[39] and *P. falciparum*.[30,35]



This "parasitism" of host cell NADPH is probably facilitated by the fact that plasmodial glutathione reductase

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has a higher affinity for NADPH than does the host enzyme.[40] At present, it is unclear whether this reliance of the parasite upon the host cell for maintenance of glutathione in the reduced form has an adverse effect upon the overall levels of GSH within infected erythrocytes. Thus, Roth et al.[35] and Sherman[28] find no significant decrement in the GSH content of *P. falciparum* and *P. lophurae* infected erythrocytes, while *P. vivax* and *P. knowlesi* infected erythrocytes are reported to have lower GSH than normal erythrocytes.[41-43] Conversely, both *P. berghei* and *P. vinckei* have increased GSH levels,[23,36] the additional GSH probably being within the parasite.

In sum, the presently available data support the idea that the malaria parasite may impair oxidant defense and repair functions of host erythrocytes in several ways. These adverse effects upon host cell antioxidant systems may stem from a variety of parasite-induced abnormalities of the infected erythrocyte. These include: (1) incorporation or destruction of host cell enzymes, e.g., during parasite catabolism of the cytosol of the infected cell; (2) consumption of host cell reducing equivalents such as NADPH; (3) direct production of activated oxygen species; and (4) accumulation of heme compounds ("hemozoin" or malaria pigment) and, perhaps, other reactive ferruginous materials.[44]

III. Protection against Malaria by Oxidant-Sensitive Host Red Cells

As reviewed above, the malaria parasite diminishes the ability of the host cell to prevent and/or repair oxidative damage through a number of mechanisms.[9,36,45-47] In fact, a number of inherited red cell "disorders" which are frequent in certain human populations may afford partial protection against the development of severe malaria infection by causing the erythrocyte to be abnormally oxidant sensitive.

The outstanding example of this are deficiencies of red cell G-6-PD.[1,36,48-52] These disorders are present at high frequency in several human populations. Because the gene for G-6-PD is x-linked, the deficiency is present mostly in males whereas females, when affected, tend to be heterozygous. G-6-PD is the first and rate-determining enzyme of the pentose phosphate pathway, and quantitative deficiency of this enzyme diminishes the maximal rate at which this pathway can generate NADPH.[53-55] This, in turn, dictates the ability of the erythrocyte to maintain glutathione in the reduced form, especially when the cell is stressed by exposure to oxidants.[54,56] For this reason, in the common variants of G-6-PD deficiency, hemolysis most often follows exposure to oxidant compounds. A well-known example of this is the hemolysis which follows exposure of Mediterranean G-6-PD deficients to the fava bean (a condition known as "favism").[57,58]

Support for the suppression of malaria by G-6-PD deficiency derives from several disparate observations. First, there is a clear geographic coincidence between the common deficiencies of G-6-PD and endemic malaria.[59,60] Second, examination of blood smears made from heterozygous females who are infected with *P. falciparum* reveals a preponderance of parasites within the G-6-PD normal erythrocytes.[61] Due to its x-linked nature, G-6-PD deficiency is expressed as a "mosaic" condition in heterozygous females, with roughly half the red cells deriving from deficient and half from G-6-PD normal progenitor cells in the marrow. Third, and perhaps most compelling, enzyme-deficient erythrocytes are inefficient at supporting parasite development during in vitro culture.[62,63] This protection afforded by G-6-PD deficiency probably depends, in part, on oxidants of unknown origin. Thus, the inhibition of parasite growth by G-6-PD deficiency increases in the presence of exogenous oxidant stress[49] or following the purposeful depletion of host cell GSH.[64] Additional oxidant stress exerted by the parasite may eventuate in the destruction of infected G-6-PD-deficient cells. Alternatively, it may be that the oxidant sensitivity of enzyme-deficient red cells per se leads to more effective destruction of infected erythrocytes by the reticuloendothelial system.

There are other inherited red cell disorders which protect against malaria, perhaps partially through sensitizing the host cell to oxidants. These include: (1) heterozygosity for hemoglobin S[65-68] (homozygosity for hemoglobin S is associated with spontaneous oxidation of various erythrocyte components);[18,69,70] (2) thalassemia;[40,50,71-78] (3) he-

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reditary persistence of fetal hemoglobin;[49,79] and (4) hemoglobin E.[80-82] In practically all the above conditions, the erythrocyte membranes are abnormally susceptible to spontaneous or drug-induced oxidation.[18,49,50,69,80,83-88] Furthermore, both hemoglobins S and F appear to autoxidize more readily than normal adult hemoglobin,[18,69,79] and hemoglobin S has decreased erythrocyte GSH peroxidase and catalase activities.[66] In addition, both hemoglobin S and thalassemic erythrocytes have increased membrane protein thiol oxidation[70,88-90] and decreased GSH[71,91] and α-tocopherol[85,92] levels.

Finally, extracellular oxidants may also play a role in the protection afforded by oxidant-sensitive host erythrocytes. Malaria parasites are readily destroyed by reactive oxygen intermediates produced by activated macrophages.[93-96] Hydrogen peroxide produced by activated macrophages appears to be the critical agent in causing parasite destruction.[97] In fact, the inability of phagocytes in *P. berghei* infected mice to produce normal amounts of H_2O_2 may be one reason for the rapidly lethal course of this infection.[98] It has been suggested that there is a relationship between the extent of impairment of phagocyte function and the lethality of murine malarias; in nonlethal *P. chabaudi* and *P. yoelii* infections, macrophages can generate more activated oxygen than in lethal *P. berghei*.[99,100] Phagocytosis, however, often appears to be secondary to intra-erythrocytic death of parasites or extracellular lysis of infected cells.[45] Nonetheless, if the malaria parasites reside within oxidant-sensitive host erythrocytes, the ready destruction of these cells by phagocyte-generated oxidants would help limit the development of severe infection.

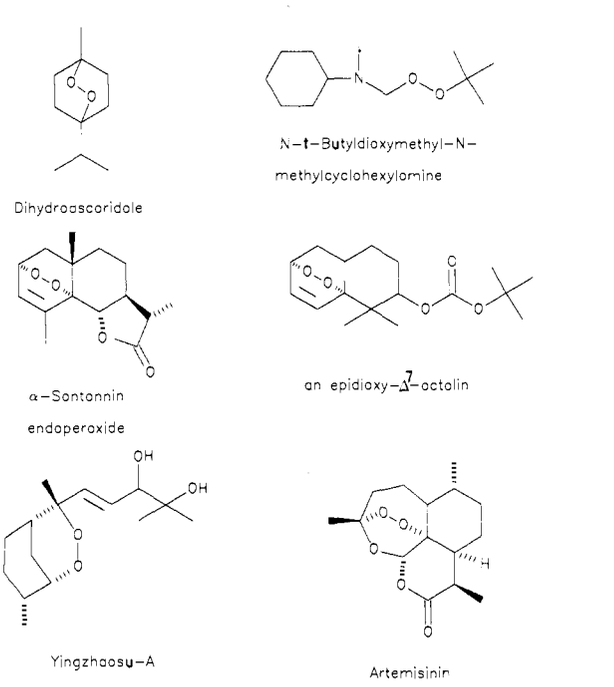

Figure 1. Selected antimalarial peroxides.

IV. Antimalarial Effects of Oxidants and Oxidant-Generating Drugs

A. Peroxides. In view of the foregoing, it is perhaps not surprising that many oxidant drugs selectively damage parasitized red cells. Even structurally simple peroxides, such as hydrogen peroxide and *tert*-butyl hydroperoxide, exert powerful oxidant effects on any cell and particularly upon malaria-infected erythrocytes. Thus, hydrogen peroxide, at micromolar concentrations, will kill (in vitro and in vivo) *P. yoelii* and (in vitro) *P. berghei* parasites.[101] *P. falciparum* is similarly sensitive to hydrogen peroxide.[102] The combination of hydrogen peroxide and superoxide which is generated by glucose/glucose oxidase is also lethal for *P. yoelii*[103] and *P. falciparum*.[104] In both cases, a significant protection to the parasite is afforded by the addition of catalase,[103,104] demonstrating that the active principle is hydrogen peroxide. Moreover, the low activity

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of glutathione peroxidase in both *P. berghei* and *P. vinckei* may contribute to the lethal effects of hydrogen peroxide.[105]

In vivo, *tert*-butyl hydroperoxide is curative in mice with *P. vinckei* infections[106,107] and has an IC_{50} of 41 μM against *P. falciparum* in vitro.[106] The peroxide functional group is essential for this antimalarial activity; *tert*-butyl alcohol is without effect.[107] *tert*-Butyl hydroperoxide may be particularly effective because of its relative lipophilicity and because the steric bulk of this compound prevents its reduction by catalase,[108] normally a very important route of catabolism of high concentrations of peroxide.

Although *tert*-butyl hydroperoxide causes hemolysis of infected erythrocytes at parasiticidal concentrations, this may not solely account for its antimalarial effect; degenerate parasites are found within intact erythrocytes exposed to the drug both in vivo and in vitro.[106,107] However, it is also true that doses of *tert*-butyl hydroperoxide which do not cause hemolysis in normal mice do so in parasitized animals.[107] The mechanisms responsible for peroxide-induced hemolysis of either normal or parasitized erythrocytes are not known but may include: (1) accelerated hemoglobin degradation;[109] (2) generation of cross-linked, high molecular weight material in the membrane;[110] (3) decreased deformability of the membrane;[110] (4) massive lipid peroxidation[109,111] which may lead to the secondary generation of toxic fragments of fatty acids; and (5) decreased glutathione levels[111] which, in some cell types, triggers cessation of protein synthesis.

A number of other peroxides and hydroperoxides have also been evaluated as antimalarials (Figure 1). We recently screened 23 structurally diverse endoperoxides, dialkyl peroxides, hydroperoxides, and peroxy ketals, carbonates, carbamates, and esters.[112] None is active in vivo against *P. berghei*, although several are quite potent against *P. falciparum* in vitro as exemplified by dihydroascaridole, a derivative of the naturally occurring endoperoxide ascaridole (Figure 1). This agent has an ED_{50} of 0.21 and 0.08 μM against chloroquine-resistant and chloroquine-sensitive strains, respectively. 1-Hydroxycyclohexyl, 1-hydroxycyclopentyl, and *tert*-amyl hydroperoxides were claimed to have potent antimalarial activity[113] although no data have been published. Incorporation of the *tert*-butyl hydroperoxy function into a series of amine peroxides increases the in vitro antimalarial effect of *tert*-butyl hydroperoxide by 1 order of magnitude.[114]

A number of mono- and bicyclic endoperoxides (Figure 1) have been tested in vitro against *P. falciparum*.[115] The most active of these compounds is an epidioxy-Δ^7-octalin with a potency comparable to quinine. This peroxide has an IC_{50} of 0.33 and 0.19 μM against chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum*, respectively.[115] An endoperoxide derived from α-santonin appears to be less active against in vitro *P. falciparum* (ED_{50} = 2.8 μM).[116] Finally, the plants *Artobotrys hexapetalus* and *uncinatus*, used in folk remedies for malaria in China, contain yingzhaosu A, an endoperoxide sesquiterpene diol[117] which may be respondible for its antimalarial properties.[117-119] Isolation of yingzhaosu A, however, was found to be dependent on prior storage of the raw plant material,[120] suggesting that it may be an artefact.

Artemisinin, a novel endoperoxide sesquiterpene lactone (Figure 1), was isolated from the plant *Artemisia annua*, also used historically as a Chinese folk remedy for malaria. This drug is exceptionally active in vitro against *P. falciparum* (IC_{50} = 0.004–0.02 μM) and is clinically useful against *P. falciparum*.[121-124] Although the stereochemical integrity of artemisinin is required for complete antimalarial potency,[124,125] its endoperoxide function is the one absolute essential for antimalarial activity,[126] suggesting an oxidant mode of action. A progressive increase in the potency of artemisinin with increasing oxygen tensions and a significant reduction in its potency by coadministration of dithiothreitol, α-tocopherol, or catalase[127] support this hypothesis. Furthermore, the in vitro antimalarial action of artemisinin is enhanced by the potential "catalytic" oxidant catechol flavones, casticin and artemitin.[128] Fi-

Table I. Selected Antimalarials That May Undergo Redox Cycling

naphthoquinones	
	menoctone,[a] lapinone[a]
	menadione[b]
	BW58C[c]
catechols	
	Quercetin[d]
	RC-12[e]
	gossypol[f]
pyrimidines	
	alloxan[g]
	divicine[h]
	isouramil[i]
nitro compounds[j,k]	
methylene blue[l]	

[a] Reference 137. [b] Reference 138, 139. [c] Reference 140. [d] Reference 141, 142. [e] Reference 143. [f] Reference 144. [g] Reference 145. [h] Reference 146, 147. [i] Reference 58. [j] Reference 148–150. [k] Figure 3. [l] Reference 151, 152.

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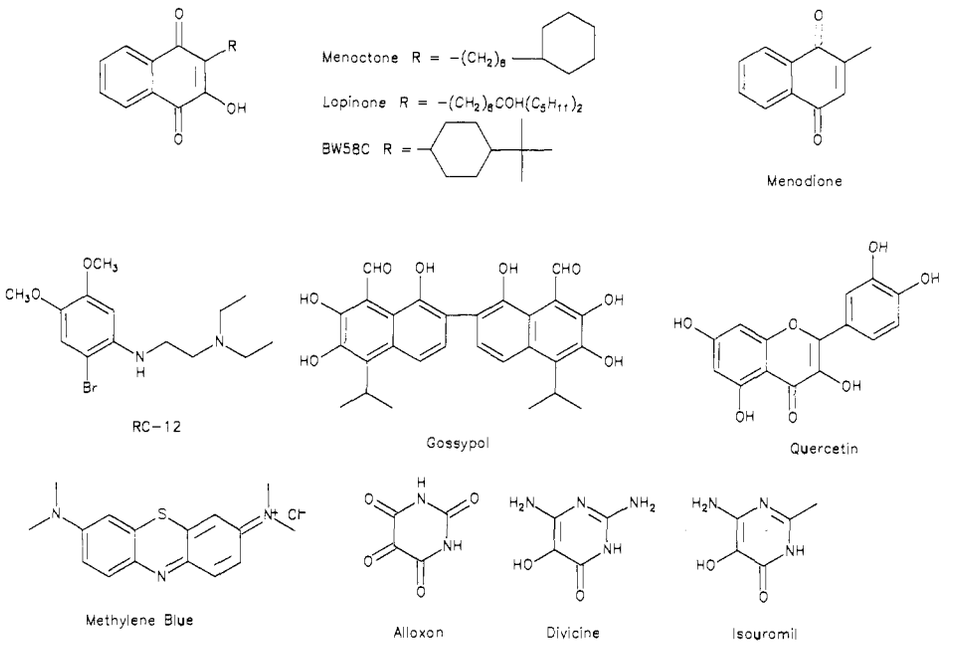

Figure 2. Potential catalytic oxidant antimalarials.

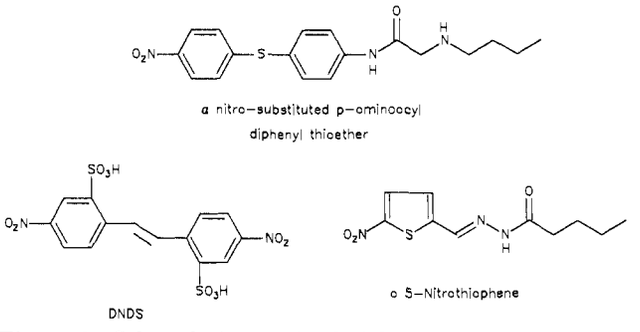

Figure 3. Selected nitro antimalarials.

nally, *P. berghei* parasites in the more oxidant-resistant reticulocytes are less sensitive to artemether (the methyl ether of dihydroartemisinin) than those in the more oxidant-sensitive mature erythrocytes.[129]

The mode of action of these endoperoxide sesquiterpenes is not known at present. However, artemisinin and its analogues have been reported to exert several toxic effects including: (1) damaging parasite membranes;[130] (2) inhibiting inosine monophosphate dehydrogenase and succinic acid dehydrogenase;[131] (3) causing mitochondrial swelling;[132] (4) decreasing parasite cytochrome oxidase activity;[133] and (5) triggering rapid decreases in parasite protein synthesis.[134] Uninfected erythrocytes concentrate dihydroartemisinin less than 2-fold, while infected erythrocytes (*P. falciparum*) concentrate the drug over 300-fold in a reversible and saturable uptake process.[135] Regardless

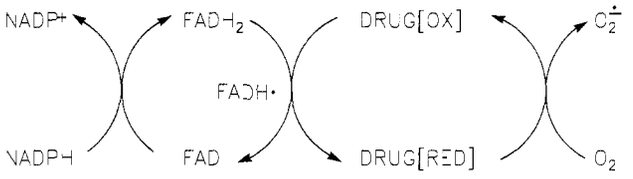

Figure 4. Redox cycling.

of the precise mechanism of action, this unique endoperoxide sesquiterpene lactone and its derivatives hold great promise for the future effective therapy of malaria, especially drug-resistance forms; only a slight cross-resistance was observed between artemisinin and its analogues.[136]

B. Quinones and Other Redox Cycling Drugs. Many oxidant antimalarials[58,137-152] (Table I, Figures 2 and

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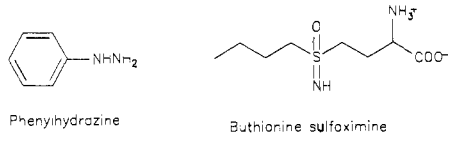

Figure 5. Miscellaneous oxidant antimalarials.

3) are not direct oxidants such as the peroxide derivatives discussed above, but may catalyze the production of oxidants within the infected erythrocyte. Table I presents a list of such "catalytic" oxidant agents, all of which are capable of undergoing cyclic one-electron oxidation–reduction reactions. This futile "redox cycling" leads to the catalytic reduction of oxygen to superoxide[153] at the expense of reducing equivalents such as NAD(P)H[154–156] (Figure 4). An alternative oxidant mode of action by some antimalarials of this class (hydroquinones, quinones, quinone imines), as well as miscellaneous antimalarials such as dapsone, metal complexes of thiosemicarbazones, and tetracyclines, was recently proposed by Ames and co-workers,[157] and involves the generation of reactive oxygen species through charge-transfer interactions.

In some cases, it is clear that the drugs listed in Table I and Figure 2 do generate oxidants through cyclic redox reactions. For example, menadione does produce hydrogen peroxide within intact erythrocytes as judged by the peroxide-dependent irreversible inhibition of catalase by 3-amino-1,2,4-triazole.[139] Methylene blue is very rapidly reduced by an NADPH-dependent erythrocyte enzyme[54,152,158,159] in a reaction which causes $NADP^+$ to accumulate within the cell, thereby stimulating the pentose phosphate pathway[56,160,161] and producing an analogue of G-6-PD deficiency. Divicine and isouramil are the pyrimidine aglycons of the fava bean glucosides vicine and convicine, respectively. Both divicine and isouramil are capable of redox cycling, form hydrogen peroxide stoichiometrically,[146] and cause depletion of red cell GSH.[57,162] The structurally related, redox-active alloxan (5-oxobarbituric acid) can be reduced nonenzymatically by NADPH or NADH to dialuric acid;[163] autoxidation of dialuric acid produces hydrogen peroxide, superoxide, and hydroxyl radical.[164] Alloxan was particularly effective against *P. berghei* parasites in mature erythrocytes, whereas it had little detectable effect on parasites inside reticulocytes.[165,166] In other cases, while oxidant generation

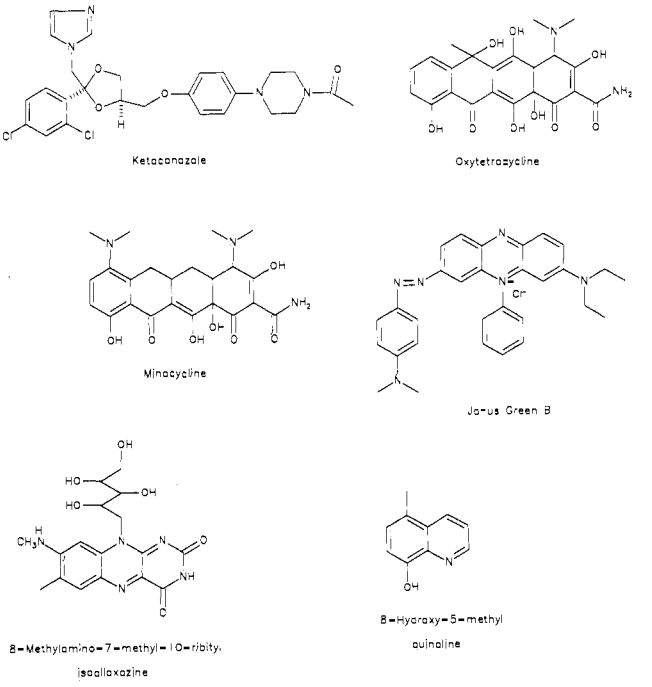

Figure 6. Selected antimalarial drugs synergistic with oxygen.

may be crucial to action of the drug, other effects have been noted. Thus, the hydroxynaphthoquinones lapinone, menoctone, and BW58C reduce parasite respiration,[137,140] perhaps through inhibition of parasite dihydroorotate dehydrogenase.[167,168]

It seems likely that antimalarial aromatic nitro compounds (Figure 3) are reduced in vivo to the nitro anion radical which undergoes a rapid air oxidation coupled with the catalytic generation of superoxide.[169–171] The antimalarial properties of 4,4′-dinitro-2,2′-stilbenedisulfonic acid (DNDS), an inhibitor of red cell anion transport, requires the presence of its nitro functional groups; reduction to the bis-aniline abolishes antimalarial activity.[149] Similarly, the antimalarial activity of several *p*-(aminoacyl)diphenyl thioethers is optimal when the drugs have a *p*-nitro substituent; negligible activity is observed when other substituents of similar electronegativity such as cyano and (trifluoromethyl)sulfonyl are substituted for the nitro group.[148] This requirement for the nitro functional group in these antimalarials indicates that a part of their activity probably derives from the nitro-catalyzed production of superoxide.

C. Additional Oxidant Antimalarials. Miscellaneous agents such as phenylhydrazine and buthionine sulfoximine may also be examples of oxidant antimalarials (Figure 5). Paradoxically, phenylhydrazine, a strong reducing agent and potent hemolytic drug, will suppress the acute phase of the simian malaria *P. knowlesi*[172] and has

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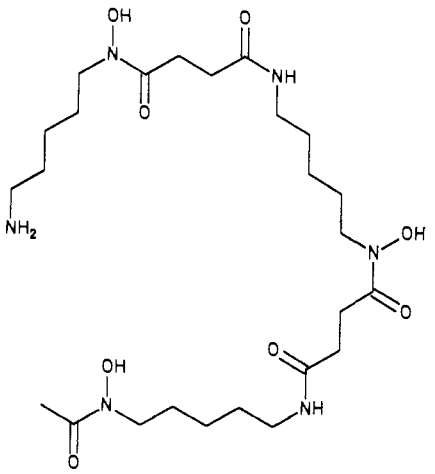

Figure 7. Structure of desferrioxamine.

antimalarial activity against *P. vinckei*[145] and *P. berghei*,[173] despite the hemolysis and reticulocytosis that it produces. This may be attributable, in part, to the fact that phenylhydrazine produces activated oxygen species through reactions with hemoglobin and other ferruginous compounds.[139,174] Buthionine sulfoximine inhibits the synthesis of GSH and exhibits antimalarial activity.[175] Presumably, if erythrocyte GSH is diminished sufficiently,[42,176] the increased oxidant burden presented by the parasite will be intolerable and the host cell may be destroyed. Alternatively, the drug may impair synthesis of GSH by the parasite itself.

Finally, the antimalarial effects of two proteins which may act by generation of oxidants also merit attention. A combination of polyamine oxidase and various polyamines[177-181] as well as the amino aldehyde products[182] of this enzymatic reaction were shown to have antimalarial properties. Catalase did not prevent this effect[180] even though polyamine oxidase also produces hydrogen peroxide.[181] Human lactoferrin, which may catalyze oxygen radical formation and damage red cell and parasite membranes[183] inhibits *P. falciparum* growth in vitro.[184]

D. Synergism and Antagonism of Oxidant Antimalarials with Other Factors. *Plasmodia* spp. are microaerophilic and grow best under reduced oxygen pressure, i.e. 20 mmHg or 15% of atmospheric oxygen pressure.[185] Increased oxygen tensions, particularly those greater than atmospheric, are poisonous to the parasite and act synergistically with some antimalarial drugs[49,175,185,187]

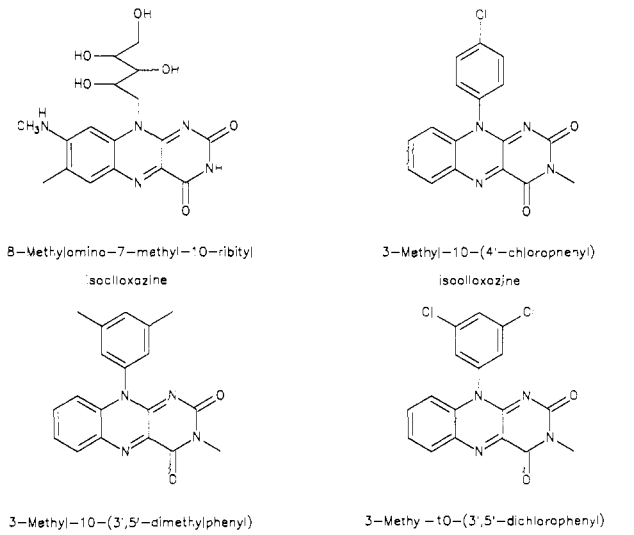

Figure 8. Antimalarial riboflavin antagonists.

(Figure 6). Elevation of oxygen levels from 0.3–1% to 15–18% enhances the antimalarial activity of imidazoles such as ketoconazole,[187] antibiotics such as oxytetracycline, chlortetracycline, minocycline, thiamphenicol, the mitochondrial-specific dye Janus Green, the riboflavin antagonist 8-(methylamino)-7-methyl-10-ribitylisoalloxazine,[175] and oxines such a 8-hydroxy-5-methylquinoline[186] against *P. falciparum* in culture. This synergism suggests that the drugs themselves may exert oxidant stress on the infected erythrocyte. Indeed, minocycline, which contains a *p*-aminophenol function capable of "redox cycling" is the most potent of the tetracycline antibiotics against malaria.[188,189]

There is mounting evidence that many oxidant antimalarials work in partnership with reactive forms of iron. The strongest evidence stems from the dramatic antagonistic effects of simultaneous administration of the powerful iron chelator desferrioxamine (Figure 7). This chelator binds all six coordination positions of ferric iron,[190] thereby preventing any redox reactions on the part of the metal.[167,191] As indicated above, both *tert*-butyl hydroperoxide and hydrogen peroxide cause selective destruction of *P. vinckei* malaria-infected murine erythrocytes in vivo. When administered concurrently with desferrioxamine, however, the antimalarial and hemolytic effects of these agents are completely blocked.[106,107] Thus, the *tert*-butylalkoxy and *tert*-butylperoxy radicals[192-195] produced in reactions between *tert*-butyl hydroperoxide and iron or "hemozoin" might be important contributors to this an-

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timalarial efficacy. The net iron catalyzed reaction is shown below:

$$2t\text{-BuOOH} \xrightarrow{[Fe]} t\text{-BuO}\cdot + t\text{-BuOO}\cdot + H_2O$$

In uninfected red cells, desferrioxamine will block hydroperoxide-induced lipid peroxidation, but not membrane protein cross-linking.[196] Similarly, desferrioxamine completely abrogates the antimalarial and hemolytic effects of alloxan and divicine, two "redox cycling" antimalarials.[145,147] These observations suggest that free iron may ordinarily be present within infected cells or that it is generated in the oxidant-mediated degradation of hemoglobin.[109,111,197] Regardless of its origin, iron is a necessary participant in the damage of infected cells by some oxidant drugs. Chelation of this free iron likely prevents deleterious iron-dependent reactions such as the oft-invoked "Fenton reaction"[198,199] which yields the dreaded hydroxyl radical:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH\cdot + OH^-$$

Finally, several nutritional experiments indirectly support the antimalarial action of oxidants by enhancing or inhibiting their effects. For example, 2(3)-*tert*-butylated hydroxyanisole (BHA) was found to prevent the antimalarial effect of both alloxan and divicine against *P. vinckei* in vivo[113] while dietary (0.75%) BHA enhanced parasitemias in *P. chabaudi*.[200] By contrast, riboflavin deficiency,[201] either nutritionally[202-204] or chemically induced,[205,206] decreased parasitemia in *P. falciparum*, *P. lophurae*, and *P. berghei*. Several riboflavin antagonists[207-209] (Figure 8) also exhibit antimalarial effects against *P. vinckei* and *P. falciparum*. Riboflavin deficiency is associated with decreased red cell GSH, decreased activity of the FAD-dependent enzyme, GSH reductase,[54] and increased methemoglobin.[210] Dutta et al.[211] hypothesized that the malaria parasite may have a greater dependence on riboflavin for antioxidant metabolism than does the host erythrocyte.

V. Conclusion

The combination of malaria parasite and host erythrocyte is unusually oxidant sensitive. Not only does the parasite itself exert oxidant stress on the host cell but parasitism also increases the susceptibility of the red cell to exogenous oxidant damage. This oxidant sensitivity may be attributable, in part, to dependence of the parasite on host erythrocyte for generation of reducing equivalents and, in part, to the accumulation of iron-rich byproducts of hemoglobin catabolism in the infected cell. It appears that natural selection has exploited this situation; high frequencies of G-6-PD deficiency occur in many areas of endemic malaria. Because of their diminished ability to survive oxidant stress, enzyme-deficient red cells are more liable to premature lysis before parasite maturation, a factor which limits the rate of development of infection.

More recently, a number of oxidant drugs have been found to have impressive antimalarial potency. These drugs include direct oxidants (i.e. peroxides) and agents which undergo oxidation/reduction cycling (i.e. quinones). Most notably, the novel endoperoxide sesquiterpene lactone, artemisinin, has been found to be the active principle of an ancient Chinese folk remedy for malaria. This drug and its analogues, which are effective both in vitro and in vivo, represent the latest addition to the growing list of oxidant antimalarials.

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 antimalarials. In view of the growing problem of drug resistance and the present lack of an effective vaccine against any species of human malaria, the development of novel therapeutics (perhaps with oxidant effects directed against the infected erythrocyte) should be vigorously pursued.

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