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Mini Review

Critical roles of thiol-mediated antioxidant detoxification systems in the pathophysiology of *Plasmodium falciparum*-infected erythrocytes

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ABSTRACT

Five species of intracellular protozoa of the genus *Plasmodium* cause malaria in human. The present review briefly highlighted the critical roles of thiol-mediated antioxidant detoxification systems in the pathophysiology of *Plasmodium falciparum*-infected erythrocytes that are required for the survival of the malarial parasite in hyperoxidative intracellular environment. Scientific search engines such as PubMed, Pubget, Medline, EMBASE, Google Scholar, ScienceDirect and SpringerLink were used to retrieve online publications from 1976 to 2015. Haemoglobin molecules that are taken up by the parasites' acid food vacuoles lead to the spontaneous oxidation of haem iron from Fe^{2+} to Fe^{3+}, formation of superoxide radicals ($O_2^{\bullet-}$), and subsequently, hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet-}OH$), which are highly reactive and cytotoxic oxygen intermediates. Additionally, toxic haem (ferri/ferroprotoporhyrin IX (FPIX) that is released upon haemoglobin digestion is biomineralized to form inert haemozoin. *P. falciparum* reduced glutathione (*Pf*GSH) is a cofactor for glutathione enzyme systems and mediates in direct reductive detoxification of the toxic byproduct of haemoglobin digestion-FPIX. The postulated role of *P. falciparum* glutathione *S*-transferase (*Pf*GST) in the development of drug resistance in malarial parasites is still being controversially discussed. However, selective inhibition of *Pf*GST and *P. falciparum* thioredoxin reductase (*Pf*TrxR) identifies novel drug targets and potential chemotherapeutic strategy to combating malaria.

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INTRODUCTION

Five species of intracellular protozoa of the genus *Plasmodium* cause malaria in human. The commonly encountered parasites include: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* [1,2], and recently, *P. knowlesi* [3-5]. Among these malarial parasites, *P. falciparum* remains the most virulent and common devastating human parasitic infection [6,7]. Reports showed that parasitic protozoan, especially *P. falciparum* infection, is affecting more than 500 million people and causing between 1.7 million and 2.5 million deaths each year of which 25% of children less than 5 years of age, pregnant women and non-immune individuals are preferentially affected [2,6,8-10]. Furthermore, *P. falciparum* malaria is the commonest type febrile illness in Africa; where an estimation of 1.8 billion US dollars account for direct costs for prevention and care as well as indirect costs such as loss in productivity in hyper-endemic areas [11].

Human beings usually are infected with malaria through bite of sporozoites-infected female mosquitoes (genius *Anopheles*); although malaria can be transmitted by transfusion of infected blood [12] and by sharing needles [13]. The parasites have a complicated life cycle that requires a vertebrate host for the asexual cycle and female *Anopheles* mosquitoes for completion of the sexual cycle [14]. The present review briefly highlighted the critical roles of thiol-mediated antioxidant detoxification systems in the pathophysiology of *P. falciparum*-infected erythrocytes that are required for survival of the malarial parasite in hyperoxidative intracellular environment it encounters during its development in mammalian and insect hosts.

Evidence Acquisition

Scientific search engines such as PubMed, Pubget, Medline, EMBASE, Google Scholar, ScienceDirect and SpringerLink were used to retrieve online publications from 1976 to 2015. Keywords such as '*Plasmodium falciparum*', 'malaria', 'glutathione detoxification', 'ligandins' 'ferri/ferroprotoporhyrin IX' 'thioredoxin' etc. were used to collate relevant articles. The results were then cross-referenced to generate a total number of 98 references cited in this review.

GENERAL CONCEPTS AND REVIEW

Malarial Infection and *Plasmodium falciparum* life cycle

During a mosquito blood meal, infectious sporozoites in the mosquito's saliva enter the host blood stream and invade the hepatocytes. In the hepatocytes, asexual multiplication

(exo-erythrocytic schizogony) leads to the production of several thousand merozoites. In one to two weeks, a single sporozoite can give rise to 30, 000 merozoites. The pre-erythrocytic stages induce no illness.

Unlike the *P. vivax* infection, which is characterized by relapses as a result of the presence of a dormant stage called the hypnozoite that remains in the liver, *P. falciparum* infection does not elicit relapses [2,13]. Therefore, the sporozoites of *P. falciparum* develop uniformly producing pre-erythrocytic schizonts, and simultaneously discharge all the merozoites that do not remain dormant as in the case of *P. vivax* infection [13].

The asexual erythrocyte cycle begins when a single merozoite invades a host erythrocyte and enclosed within a parasitophorous vacuole, which is separate and distinct from the host erythrocyte cytoplasm. The merozoites eventually released into the blood stream invade more erythrocytes. There are three observable morphologically distinct phases in parasitized erythrocytes. Firstly, the ring stage, which last for approximately 24 hours in *P. falciparum* infection, accounts for half of the metabolically non-descript intra-erythrocytic stage. It is followed by the trophozoite stage; a very active period during which most of the erythrocytes cytoplasm is consumed. Finally, the parasite undergo 4-5 rounds of binary divisions during the schizont stage, producing 8-36 merozoites that burst from the host cell to invade new erythrocytes, and thereby begins another round of infection. This phase of the infection (erythrocytic schizogony) is responsible for malaria pathogenesis.

The rupture of *Plasmodium*-infected erythrocytes is responsible for much of the morbidity and mortality associated with malaria during the asexual reproductive stage of the parasite. Intense fever occurs in 24-72 hour intervals, accompanied by nausea, headaches, and muscular pain among other symptoms. Furthermore, a variety of potentially fatal symptoms, including liver failure, renal failure and cerebral pathology are associated with untreated *P. falciparum*. These symptoms are consequences of the unique ability of the parasites to bind to endothelial surface; this adherence inhibits circulation and causes localized oxygen deprivation and sometimes hemorrhage [15,16].

Instead of producing new schizonts, some merozoites, after invasion of the erythrocyte, arrest their cell cycle and develop into male (micro) and female (macro) gametocytes, the forms that are required for transmission of the mosquito's parasite (asexual parasites do not survive following ingestion by the insect). Inside the mid-gut of the mosquito, fertilization occurs, producing zygotes, which develop into ookinetes. The ookinetes forms oocytes, which then grow, divide, and rupture to give rise to sporozoites, and subsequently migrate to the salivary glands. Then the infectious cycle of malaria can repeat itself.

While all five species of *Plasmodium* have a haemolytic component usually of little consequence, *falciparum* malaria parasite multiply very rapidly and may occupy 30% or more of the erythrocytes causing a very significant level of haemolysis. One reason for this is that *P. falciparum* invades erythrocytes of all ages, of which *P. vivax* and *P. ovale* prefer younger erythrocytes, whereas *P. malariae* seeks matured erythrocytes [13]. There are evidence to suggest that geographical distribution of erythrocyte genetic traits such as the thalassaemias, sickle cell anaemia and glucose-6-phosphate dehydrogenase (G6PDH) deficiency correlate with reduced severity and incidence of malaria infection [17-19].

Haemoglobin Metabolism

During intra-erythrocytic development, *P. falciparum* ingests large amount of haemoglobin to meet its nutrient requirement [20] and to maintain osmotic stability within the host cell [21]. Specifically, the malaria parasite ingests 25 to 80% of total haemoglobin content [22,23]. Haemoglobin molecules, taken up by endocytosis undergo hydrolysis in the parasite's digestive acidic vacuole called the food vacuole. Cysteine and aspartic proteases are involved in haemoglobin proteolysis and have been reported to exhibit pH optimums within the range of 4.5-5.0 [24]. A pH homeostasis plays an important role in the pathophysiology of *falciparum* malaria, such as host cell exploitation and responses to antimalarial drugs [25]. Accordingly, baseline pH values and the mechanisms underpinning pH homeostasis in different parasite compartments have been of interest for several decades [25].

Endogenous production of reactive oxygen species (ROS) in parasitized erythrocytes is triggered following the digestion of haemoglobin and subsequent biochemical reactions in the parasites [26]. Haemoglobin molecules that are taken up by the parasites' acid food vacuoles lead to the spontaneous oxidation of haem iron from Fe^{2+} to Fe^{3+} (haemin) and the formation of superoxide radicals ($O_2^{\bullet-}$). The combination of $O_2^{\bullet-}$ and haemin inevitably leads to the generation of hydrogen peroxide (H_2O_2) and subsequently, hydroxyl radicals ($^{\bullet-}OH$), which are highly reactive and cytotoxic oxygen intermediates (Figure 1) [27]. Furthermore, toxic (ferroprotoporphyrin IX; containing Fe^{2+}) and hemin/hematin (ferriprotoporphyrin IX; containing Fe^{3+}) (FPIX) that are released upon haemoglobin digestion must be detoxified within the acid food vacuole to prevent downstream toxicity [19,28]. Most of the released FPIX is biomineralized (up to 90%; [29]) to form inert haemozoin. However, there are reports that substantial amount of FPIX (even as much as 50%; [30,31]) escapes biomineralization and has to be degraded or sequestered by other means to prevent membrane damage and parasite death [32-34].

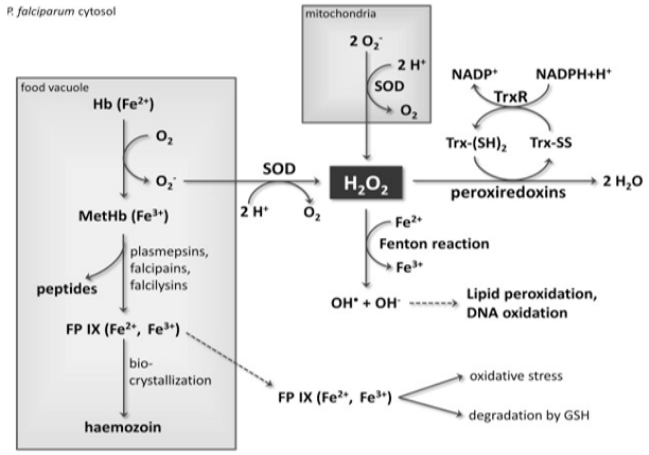

Figure 1. Sources of oxidative stress in *Plasmodium falciparum* [35]

However, some free FPIX (up to 50% [30,31]) from the food vacuole pass into the parasite compartment. The $O_2^{\bullet-}$ resulting from the oxidation of haem-iron of haemoglobin are either detoxified by superoxide dismutase (SOD) to yield H_2O_2 or in a spontaneous reaction with H_2O_2, lead to the formation of $^{\bullet-}OH$ [26,35] (Figure 1). In addition, ferric iron (Fe^{3+}) react with molecular oxygen via the Fenton reaction pathways to generate $^{\bullet-}OH$ (Equations 1 and 2) [36].

$$O^{\bullet-}_2 + Fe^{3+} \rightarrow O_2 + Fe^{2+} \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH \quad (2)$$

These radicals are highly reactive and cause, for instance, lipid peroxidation and DNA oxidative damage [35]. Additionally, H_2O_2 generated by the SOD activity has to be detoxified by reduction reaction to produce water. In *P. falciparum*, thioredoxin (Trx)-dependent peroxidase pathway plays this critical role of neutralizing ROS because the parasites lack catalase and glutathione peroxidase [35,37,38], which initially raised doubts about the relevance of reduced glutathione (GSH) in detoxification of ROS in *Plasmodium*.

Hyperoxidative Stress of Parasitized Erythrocytes

Malaria parasites are particularly vulnerable to oxidative stress during the erythrocytic life stages [34,37,39,40] as a result of acute phase response of the infected host immune system and intra-erythrocytic parasite's metabolic processes [26,41-43]. This is not surprising since the parasites live in a pro-oxidant intracellular environment that contains oxygen and iron; the key prerequisite for the formation of ROS via the Fenton reaction [36]. Additionally, in effort to eliminate or impede parasite fecundity, cytotoxic reactive oxygen and nitrogen species (RONS; e.g. peroxynitrite) arising from reaction of nitric oxide ($NO^{\bullet}$) with $O_2^{\bullet-}$ are deployed by the macrophages against invading *P. falciparum* [26,41], which exacerbate the oxidative stress of the already hyperoxidative parasitized erythrocytes. Expectedly, studies have shown that compromised $NO^{\bullet}$ metabolism may precipitate malaria complications by exacerbated cyto-adherence of infected erythrocytes through enhanced oxidation of redox-sensitive CD36 [44,45].

Notably, not only is the parasite itself under oxidative stress, but the host cell also shows oxidative alterations when infected with *Plasmodium* as demonstrated by changes in erythrocyte membrane fluidity, most probably, because of alterations in the composition of erythrocyte membrane lipid and protein cross-linking [46-49]. Haemochrome accumulation on the inner surface of parasitized erythrocytes, aggregation of erythrocyte band III and increased occurrence of auto-anti-band III antibodies suggest oxidative damage of the host erythrocytes by the malarial parasites [26,50], which is most apparent in erythrocytes infected with *Plasmodium* in late parasitic stages [47]. These modifications are reminiscent of those found in erythrocytes of humans with erythrocyte disorders such as sickle cell anaemia, α- and β-thalassaemias and G6PDH deficiency. Studies have shown that the underlying mechanism leading to these modifications are engendered by enhanced oxidative stress in the defective erythrocytes [51-53]. Interestingly, these erythrocyte disorders and G6PDH deficiency confer certain degree of resistance to *Plasmodium* infection and often limit the severity of the disease [19,54,55]. One hypothesis to explain this is that the increased oxidative stress within the defective erythrocyte causes an impaired infection and growth rate of the parasites [56,57]. Another hypothesis [47,48,58] suggest that defective erythrocytes infected with early stages of *P. falciparum* are more efficiently phagocytized by the host's immune system because of earlier occurrence of band III cross-linking which results in the early appearance of band III auto-antibodies. Thus, the recognition of parasitized erythrocytes by the host's immune system at an early stage of the infection ensures that parasitaemia of the infected individuals is kept low. Similar changes also occur in normal *Plasmodium*-infected erythrocytes but at a much later stage of the infection when most parasitized erythrocytes are already sequestered in the host's capillary system. However, since these modified erythrocytes are often no longer in circulation, they are not efficiently recognized by the host's immune system [13].

Diagnostic pathology reports have revealed substantial elevation of oxidative stress indicators in parasitized erythrocytes [41,43,59,60]. Notably, the polyunsaturated fatty acids (PUFAs) of erythrocyte biomembrane are particularly vulnerable to oxidative damage, exemplified by raised levels of end products of lipid peroxidation in serum of malarious individuals [40,61].

Plasmodium falciparum Thiol-Mediated Antioxidant Detoxification Systems

According to reports, the malarial parasites have evolved a complex network of NADPH dependent redox enzymes, with overlapping but also distinct functions, for neutralizing RONS and maintain comparatively reduced intracellular environment during blood stage infection, which are broadly classified in two groups [19,35,62]:

1. A complete glutathione system.
2. Specialized Trx system.

The first system is comprised of the GSH, flavo-enzyme glutathione reductase (GR), glutaredoxin (Grx) and Grx-like proteins, glutathione *S*-transferases (GSTs), γ-glutamylcysteine synthetase (γ-GCS), and a glutathione-dependent glyoxalase [35,62-64]. Although Vega-Rodríguez *et al.*, [65] suggested that *P. falciparum* glutathione (*Pf*GSH) biosynthetic pathway was essential for mosquito stage development of the parasite, the pathway may not be an appropriate target for antimalarials against blood stages of the parasite. However, the correlation between intracellular concentration of *Pf*GSH and the capacity of the parasite to withstand pro-oxidant challenges have been described [66,67]. The second system includes the Trx reductase (TrxR), several Trxs and Trx-like proteins, Trx-dependent peroxidases and imported human protein peroxiredoxin 2 (hPrx-2) [38,68-70]. Study showed that *P. falciparum* imports hPrx-2 from the human erythrocyte host to its cytosol for the primary purpose to scavenging cytotoxic peroxides and the abundance of hPrx-2 in the parasite increases significantly following chloroquine treatment [70]. It is worthwhile to note that there are other findings over the years, which have contradicted the reports of Vega-Rodríguez *et al.*,[65], in which these reports revealed that glutathione and Trx redox systems are potential targets for the development of new chemotherapeutics for eradication of malaria [71,72].

The tripeptide glutathione {(gamma-glutamyl-cysteinyl-glycine: GSH (reduced form); GSSG (oxidized form)} is the major low-molecular weight thiol buffer in most aerobic cells [19,26,73]. Generally, GSH is directly involved in antioxidant reactions, for instance, the termination of radical-based chain reactions involving single electrons transferred from thiyl radicals or disulphide radicals [74]. The ratio of GSH to GSSG is usually between 10:1 and 100:1 and maintained far on the side of reduced form of glutathione [75,76]. Specifically, GSH/GSSG redox ratio in intra-erythrocytic parasites displays highly reducing redox potential $E^{\circ}_{PfGSH} = -314$ mV when compared with the GSH/GSSG redox potential in other organisms [19,67]. Apart from the action of GR, which regenerates GSH from GSSG, there also exist GSSG-efflux pumps that export excess GSSG in order to maintain an adequate intracellular redox balance in the parasite (Figure 1). In cases of drug resistance, export of *Pf*GSH adducts are excreted from cells via pumps such as *P. falciparum* multidrug resistance (*Pf*MRP) proteins, which is within the drug/metabolite transporter superfamily of ATP transporters [19,38,76-78]. In addition, *de novo* biosynthesis of the tripeptide also contributes to sustaining sufficiently high intracellular *Pf*GSH levels [79]. Studies showed that *Plasmodium* also possesses the two enzymes that are responsible for the *de novo* biosynthesis of *Pf*GSH, notably, γ-GCS and glutathione synthetase, respectively [19]. Apart from its role as a general thiol redox buffer, *Pf*GSH acts as a cofactor for a variety of proteins including glutathione-dependent peroxidases, *Pf*GSTs, Grxs and glyoxalases [73,76]. The glyoxalase system is another vital cellular component crucial for survival of the parasite in that it catalyzes the conjugation of 2-oxoaldehydes such as methylglyoxal, a toxic metabolic by-product of glycolysis, with *Pf*GSH leading to the generation of non-toxic hydroxycarboxylic acids such as D-lactate, which are subsequently excreted from the parasite [19,80].

GSH metabolism of *Plasmodium*-infected erythrocytes has been the focus of number of studies and have revealed the roles of the tripeptide (GSH) in the pathophysiology of parasitized erythrocytes, which are numerous but not only limited to its redox and antioxidant functions [34,65]. Müller, [38], proposed a correlation between intracellular *Pf*GSH levels and susceptibility to oxidative damage and reduced osmotic resistance of *Plasmodium*-infected erythrocytes, which corroborates recent reports [64]. These reports further asserted that *Plasmodium* caused the oxidation large amount of *Pf*GSH, which engendered the need for increased GSSG efflux to maintain an adequate GSH/GSSG thiol redox state in the infected cells (Figure 2).

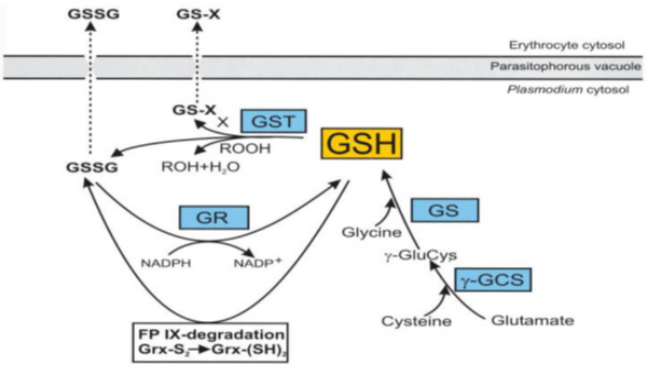

Figure 2: GSH metabolism in *P. falciparum*. Ferri/ferroprotoporhyrin IX (FPIX); xenobiotics (X) for export; GS-X adducts are excreted from cells via pumps such as multidrug resistance proteins [38].

Furthermore, Cappadoro *et al.*, [58] propounded that G6PDH-deficient erythrocytes also exhibit low GSH levels, which might be the explanation for low availability of NADPH for reduction of the tripeptide and consequential

rapid efflux of GSSG from the intracellular compartment of the invading parasite. These observations together suggested that one reason for the increased oxidative damage of *Plasmodium*-infected erythrocytes and G6PDH-deficient erythrocytes was the inadequate concentration of intracellular GSH.

Despite the efficient biomineralization of free haem into haemozoin following haemoglobin digestion by the parasite, a considerable amount of toxic FPIX remains free [30,31]. Free FPIX is toxic because its detergent-like properties interfere with membrane integrity and has the ability to undergo redox reactions causing the generation of ROS as a result of the presence of bound iron in FPIX. Therefore, FPIX needs to be sequestered and detoxified to prevent peroxidation of membrane structural components and ultimate parasite death. Several reports have shown that degradation and subsequent detoxification of free FPIX occurred through *Pf*GSH mediated non-enzymatic mechanism that was inhibited by chloroquine [31,81-84]. Consequently, failure to inactivate FPIX exterminates the *Plasmodium* parasite by oxidative damage to biomembrane structural components, digestive proteases and possibly other critical biomolecules [85]. Notably, it had been suggested that *Pf*GSH is involved in drug resistance [72]. Thus, the depletion of *Pf*GSH would result in a less efficient detoxification of free FPIX and consequently death of the parasite. The resistance of *P. falciparum* to chloroquine is one of the major drawbacks in the fight against severe malaria.

In addition to non-enzymatic detoxification of FPIX, other mechanisms of FPIX removal involves its sequestration by the parasite proteins. Proteins possibly involved in this process include histidine-rich protein 2 [86,87], glyceraldehydes-3-phosphate dehydrogenase, protein disulphide isomerase, *P. falciparum* GR (*Pf*GR) [88] and *P. falciparum* glutathione *S*-transferase (*Pf*GST) - referred to in this regard as 'ligandin' [19,83,89,90]. Apart from histidine-rich protein 2, which in complex with FPIX appears to develop peroxidase-like activity [91], most of the other FPIXs inhibit their binding proteins. Therefore, it is difficult to infer whether the affinity between FPIX and their binding proteins is actually a protective mechanism or whether the binding of FPIX has a deleterious effect on the parasites. In the case of glyceraldehyde-3-phosphate dehydrogenase, it is believed that the strong inhibition of the enzyme by FPIX is a mechanism by which the parasite is adapted to survive FPIX-induced oxidative stress. The inhibition of glyceraldehydes-3-phosphate dehydrogenase under elevated FPIX-induced oxidative stress results in glucose being primarily metabolized via the hexose monophosphate shunt rather than glycolysis and this provides increased levels of NADPH that are required for enzymatic antioxidant reactions in the parasite [16].

One of several intracellular antioxidant enzymes that are directly dependent on the availability of GSH is GST. GSTs catalyze the conjugation of GSH to the electrophilic centers of hydrophobic compounds, and thereby detoxify a wide range of mutagens, carcinogenic, pharmacologically active molecules and by-products of oxidative stress. All *Plasmodium* species studied exhibit GST activity as far as intra-erythrocytic stages [92] and *Pf*GST represent greater than one percent (1%) of the total cellular protein in the parasite [83,89]. The primary structure as well as the three – dimensional x-ray structure of *Pf*GST differ significantly from that of human GSTs, and by implication, *Pf*GST represents a novel GST isoform that cannot be assigned to any of the previously known GST classes [72,90]. Unlike higher eukaryotes, *Plasmodium* possesses only a single gene encoding GST. Consequently, the low number of GSTs expressed possible reflect that parasitic protozoa such as *Plasmodium* do not require a great number of xenobiotics detoxification enzymes and their GSTs have functions that are different from those found in eukaryotes. Nevertheless, inhibition of *Pf*GST is expected to disturb GSH-dependent conjugation processes, which promotes enhance levels of cytotoxic peroxides and increase in the concentration of toxic FPIX [92].

Interestingly, molecular stability studies revealed that *Pf*GST exist as a tetramer (inactive)←→dimer (active) transition states, which is regulated by binding activity of physiologic GSH concentration ≈ 0.7 mM [72]. Recent studies have shown that the three dimensional structure of *Pf*GST displays some similarities to Mu-class GSTs [90,93]. However, *Pf*GST appears to have a larger hydrophobic binding pocket than the Mu-class GSTs that makes it more accessible by solvents [72]. Additionally, the C-terminus of *Pf*GST is truncated in comparison to other GST isoforms. Usually, the C-terminal part of Mu-class GSTs structurally restricts entry of substrates into the hydrophobic binding pocket, and thus, it is believed that the substrate specifically of *Pf*GST is less restricted, and thereby allows for the detoxification of wider range of molecules. This could explain why these parasites do not require as many GST isoforms as do other eukaryotes.

Thiol Antioxidant Systems: Attractive Targets for New Antimalarials

The postulated role of *Pf*GST in the development of drug resistance in malarial parasites is still being controversially discussed [89,92,94]. However, Ahmad and Srivastava, [95] noted that selective inhibition of *Pf*GST activity by protoporphyrin IX, cibacron blue and menadione, coupled with the unique nature of *Pf*GST, may open new vista of potential chemotherapeutic strategy, by serving as a novel drug target, to combating malaria [72]. In addition to cytosolic *Pf*GST, the presence of membrane bound GSTs, the so called membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) form, in *Plasmodium* was recently characterized using graph-

based information diffusion on compressed supergenomic networks as described [64]. Although evolutionally unrelated to the cytosolic GSTs, the MAPEG is a superfamily of detoxification enzymes that catalyze the conjugation of GSH to a broad spectrum of xenobiotics and hydrophobic electrophiles. Furthermore, the MAPEG exhibit no significant genome sequence homology with the *Plasmodium* cytosolic GSTs and other known parasite protein sequences. It is instructive to note that the parasitophorous vacuolar membrane antigen *Pf*EXP1 (*P. falciparum* exported protein 1), is the only known MAPEG with hematin substrate specificity to buffer oxidative stress produced through excess hematin [19,64]. Also, *Pf*EXP1 activity could predict the occurrence of resistant strains of *Plasmodium* since it is inhibited by artemisinin in a hematin concentration dependent manner [64].

Genetic and chemical tools have demonstrated that *P. falciparum* TrxR (*Pf*TrxR), in concert with the early stage of the thioredoxin redox cycle, and the enzyme involved in the rate-limiting step of glutathione synthesis-γ-GCS, are essential for the survival of malarial parasite [38,39,76]. Accordingly, some of the enzymes, particularly, *Pf*TrxR has also been proposed to be an attractive targets for the design of new antimalarials because of its structural and functional peculiarities that contribute to the antioxidant defense systems of the parasite [96,97]. Likewise, according to *in vitro* data from the reports of Gallo *et al*., [98], human GR (hGR) deficiency and drug-induced hGR inhibition confers protection on erythrocytes against malarial parasites by inducing enhanced ring stage phagocytosis rather than by impairing parasite growth directly.

CONCLUSIONS

Intra-erythrocytic *P. falciparum* ingests large amount of haemoglobin to meet its nutrient requirement, which results in endogenous production of cytotoxic RONS following the digestion of haemoglobin and subsequent biochemical reactions in the parasites. For the survival of *P. falciparum* in the hostile environment, the parasite is equipped with arrays of antioxidant processes and machineries that ensure the mitigation of intra-erythrocytic hyperoxidative stressors elicited by the generation of RONS. Notable among these antioxidant pathways are the thiol-mediated detoxification systems within the acid food vacuole of the parasite, which serve to prevent downstream toxicity from cytotoxic oxygen intermediates, and perhaps, involved in development of drug resistance in malarial parasites. Accordingly, the selective inhibition of thiol-mediated detoxification systems has been identified to be novel drug targets and potential chemotherapeutic strategy to combating malaria.

REFERENCES

1. Krotoski WA, Collins WE, Bray RS, Garnham PC, Cogswell FB, Gwadz RW, *et al*. Demonstration of hypozoites in sporozoite transmitted *Plasmodium vivax* infection. Am J Trop Med Hyg 1982; 31:1291-93.
2. Joseph V, Varma M, Vidhyasagar S, Mathew A. Comparison of the clinical profile and complications of mixed malarial infections of *Plasmodium falciparum* and *Plasmodium vivax* versus *Plasmodium falciparum* mono-infection. Sultan Qaboos Univ Med J 2011; 11:377–82.
3. Figtree M, Lee R, Bain L, Kennedy T, Mackertich S, Urban M, *et al. Plasmodium knowlesi* in Human, Indonesian Borneo. CDCP 2010; 16:6 pages.
4. Lee CE, Adeeba K, Freigang G. Human *Plasmodium Knowlesi* infections in Klang Valley, Peninsula Malaysia: A case series. Med J Malays 2010; 65:63-5.
5. Marchand RP, Culleton R, Maeno Y, Quang NT, Nakazawa S. Co-infections of *Plasmodium knowlesi*, *P. falciparum,* and *P. vivax* among humans and *Anopheles dirus* mosquitoes, Southern Vietnam. CDCP 2011; 17:12 Pages.
6. Bansal D, Bhatti HS, Sehgal R. Role of cholesterol in parasitic infections. Lipid Health Dis 2005; 4:10 doi: 10.1186/1476-511X-4-10.
7. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature 2005; 434:214-7.
8. Kochar DK, Singh P, Agarwal P, Kochar SK, Pokharna R, Sareen PK. Malarial hepatitis. J Assoc Physicians India 2003; 51:1069-72.
9. Adekunle AS, Adekunle OC, Egbewale BE. Serum status of selected biochemical parameters in malaria: An animal model. Biomed Res 2007; 18:109-13.
10. Onyesom I, Ekeanyanwu RC, Achuka N. Correlation between moderate *Plasmodium falciparum* malarial parasitaemia and antioxidant vitamins in serum of infected children in South Eastern Nigeria. Afr J Biochem Res 2010; 4:261-4.
11. Garba IH, Ubom GA. Total serum lactate dehydrogenase activity in acute *Plasmodium falciparum* malaria infection. Singapore Med. J 2005; 46:632-4.
12. Ali MSM, Kadaru AGM. *In vitro* processing of donor blood with sulphadoxine/pyrimethamine for eradication of transfusion induced malaria. Am J Trop Med Hyg 2005; 73:1119–23.
13. Tracy JW, Webster LT. Drugs used in the chemotherapy of protozoan infections. In: Goodman and Gilman's Pharmacological Basis of Therapeutics. Adam JG, Limbird LE, Gilman AG. (Eds). 10th Edition, McGraw-Hill, U.S.A. 2001.
14. www.parasitesinhumans.org/plasmodium-falciparum-malaria.html [retrieved on 1st August, 2015].
15. Smith JD, Craig AG. The surface of the *Plasmodium falciparum* infected erythrocyte. Curr Issues Mol Biol 1995; 7:81-94.
16. Cholera R, Brittain N, Gillrie M, Lopera–Mesa T, Diakita S, Arie T. Impaired cytoadherence of *Plasmodium falciparum*-infected erythrocytes containing sickle haemoglobin. Soc Trop Med Hyg 2007; 98:302-10.
17. Roberts DJ, Williams TN. Haemoglobinopathies and resistance to malaria. Redox Rep 2003; 8:304-10.
18. Taylor SM, Cerami C, Fairhurst RM. Hemoglobinopathies: Slicing the Gordian knot of *Plasmodium falciparum* malaria pathogenesis. PLoS Pathog. 2013; 9:e1003327.
19. Muller S. Role and regulation of glutathione metabolism in *Plasmodium falciparum.* Mol 2015; 20:10511-34.
20. Liu S, Zhang P, Ji X, Johnson WW, Gilliland GL, Armstrong RN. Contribution of tyrosine to the catalytic mechanism of isoenzyme of glutathione *S*-transferase. J Biol Chem 1992; 267:4296-9.
21. Lew VL, Tiffert T, Ginsburg H. Excess haemoglobin digestion and osmotic stability of *Plasmodium falciparum*-infected red blood cells. Blood 2003; 101:4189-94.
22. Goldberg DE. Haemoglobin degradation in *Plasmodium*-infected red blood cells. Semin Cell Biol 1993; 4:355–61.
23. Roth EF, Brotman DS, Vanderberg JP, Schulman S. Material pigment-dependent error in the estimation of haemoglobin content in *Plasmodium falciparum*-infected red cell: implications for metabolic and biochemical studies of the erythrocytic phases of malaria. Am J Trop Med Hyg 1986; 35:906–11.

24. Goldberg DE. Haemoglobin degradation. Curr Top Microbiol Immunol 2005; 295:275-91.
25. Lanzer M, Rohrbach P. Subcellular pH and Ca^{2+} in *Plasmodium falciparum*: Implications for understanding drug resistance mechanisms. Curr Sci 2007; 92:1561-70.
26. Bozdech Z, Ginsburg H. Antioxidant defense in *Plasmodium falciparum*-data mining of the transcriptome. Malaria J 2004; 3:23.
27. Liochev SI, Fridovich I. Superoxide and iron: partners in crime. IUBMB Life 1999; 48:157-61.
28. Sigala PA, Goldberg DE. The peculiarities and paradoxes of *Plasmodium* heme metabolism. Annu Rev Microbiol 2014; 68:259–78.
29. Egan TJ, Combrinck JM, Egan J, Hearne GR, Marques HM, Ntenteni S. Fate of haem iron in the malaria parasite. *Plasmodium falciparum.* Biochem. J 2002; 365:343-7.
30. Loria P, Muller S, Foley M, Tilley L. Inhibition of the peroxidative degradation of haem as the basis of action of chloroquine and other quinoline antimalarials. Biochem J 1999; 339:363-70.
31. Zhang J, Krugliak M, Ginburg H. The fate of ferriprotoporphyrin IX in malaria infected erythrocytes in conjunction with the mode of action of antimalarial drugs. Mol Biochem Parasitol 1999; 99:129-41.
32. Atamna H, Ginburg H. Origin of reactive oxygen species in erythrocytes infected with *Plasmodium falciparum*. Mol Biochem Parasitol 1993; 61:231-41.
33. Tilley L, Loria P, Floey M. Chloroquine and other quinoline antimalarials. In: Antimalarial Chemotherapy. Rosenthal PJ. (Ed). Totowa, NJ. Humana Press. 2001; pp 87-122.
34. Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H. Oxidative stress in malaria parasite infected erythrocytes: host – parasite interactions. Int J Parasitol 2004; 34:163–89.
35. Jortzik E, Becker K. Thioredoxin and glutathione systems in *Plasmodium falciparum*. Int J Med Microbiol 2012; 302:187-94.
36. Puppo A, Halliwell B. Formation of hydroxyl radicals from hydrogen peroxide in the presence of iron: Is haemoglobin a biological Fenton reagent? Biochem J 1988; 249:185-90.
37. Muller S, Lieban E, Walter RD, Krauth–Siegel RL. Thiol-based radox mechanism of protozoan parasites. Trends Parasitol 2003; 19:320–8.
38. Muller S. Redox and antioxidant systems of the malaria parasite: *Plasmodium falciparum*. Mol Microb 2004; 53:1291-305.
39. Hunt NH, Stocker R. Oxidative stress and the redox status of malaria–infected erythrocytes. Blood Cells 1990; 16:499-526.
40. Simoes AP, Van den Berg JJ, Roelofsen B, Op den Kamp JA. Lipid peroxidation in *Plasmodium falciparum*-parasitized human erythrocytes. Arch Biochem Biophys 1992; 298:651-2.
41. Sibmooh N, Yamanont P, Krudsood S, Leowattana W, Brittenham G, Looareesuwan S, *et al.* Increased fluidity and oxidation of malarial lipoproteins: relation with severity and induction of endothelial expression of adhesion molecules. Lipid Health Dis 2004; 3:15 doi: 10.1186/1476-511X-3-15.
42. Pradines B, Ramiandrasoa F, Fusai T, Hammadi A, Henry M, *et al*. Generation of free radicals and enhancement of hemin-induced membrane damage by a catechol iron chelator in *Plasmodium falciparum*. J Biol Sci 2005; 5:463-71.
43. Idonije OB, Festus O, Okhiai O, Akpamu U. Comparative study of the status of a biomarker of lipid peroxidation (malondialdehyde) in patients with *Plasmodium falciparum* and *Plasmodium vivax* malaria infection. Asian J Biol Sci 2011; 4:506-13.
44. Gruarin P, Primo L, Ferrandi C, Bussolino F, Tandon NN, Arese P, *et al.* Cytoadherence of *Plasmodium falciparum*-infected erythrocytes is mediated by a redox-dependent conformational fraction of CD36. J Immunol 2001; 167:6510-7.
45. Fritsche G, Larcher C, Schennach H. Regulatory interactions between iron and nitric oxide metabolism for immune defense against *Plasmodium falciparum* infection. J Infect Dis 2001; 183:1388-94.
46. Destro Biso LG. Genetic resistance to malaria, oxidative stress and haemoglobin oxidation. Parassitol 1999; 41:203-4.
47. Giribaldi G, Ulliers D, Mannu F, Arese P, Turrini F. Growth of *Plasmodium falciparum* induces stage-dependent hemichrome formation oxidative aggregation of band 3, membrane disposition of complement and antibodies, and phagocytosis of parasitized erythrocytes. Br J Haematol 2001; 113:492-9.
48. Williams TN, Weatherall DJ, Newbold CI. The membrane characteristics of *Plasmodium falciparum*–infected and –uninfected heterozygote alpha (o) thalassemic erythrocytes. Br J Haematol 2002; 118:663-70.
49. Omodeo-Sale F, Motti A, Basilico N, Parapini S, Olliaro P, Taramelli D. Accelerated senescence of human erythrocyte cultured with *Plasmodium falciparum*. Blood 2003; 102:705-11.
50. Parker PD, Tilley L, Klonis N. *Plasmodium falciparum* induces reorganization of host membrane proteins during intraerythrocytic growth. Blood 2004; 103:2404-6.
51. Shalev O, Repka T, Goldfarb A, Grinberg L, Abrahamov A, Olievieri NF. Deferiprone (L1) chelates pathologic iron deposits from membranes of intact thalasaemic and sickle red blood cells both *in vitro* and *in vivo*. Blood 1995; 86:2008-13.
52. Beppu M, Ando K, Kikugawa K. Poly-N-acetyllactosaminyl saccharide chains of band 3 as determinants for anti-band 3 auto antibody binding to senescent and oxidized erythrocytes. Cell Mol Biol 1996; 42:1007-24.
53. Asian M, Thornley-Brown D, Freeman BA. Reactive species in sickle cell disease. Ann NY Acad Sci 2000; 899:375-91.
54. Chan TK. Glucose-6-phosphate dehydrogenase (G6PD) deficiency: A review. HK J Paediatr (New Series) 1996; 1:23-30.
55. Fibach E, Rachmilewitz E. The role of oxidative stress in hemolytic anemia. Curr Mol Med 2008; 8:609-19.
56. Eaton JW, Eckman JR, Berger E, Jacob HS. Suppression of malaria infection by oxidant-sensitive host erythrocytes. Nature 1976; 264:758-60.
57. Friedman MJ. Erythrocytic mechanism of sickle cell resistance to malaria. Proc Matt Acad Sci USA 1978; 75:1994.
58. Cappadoro M, Giribaldi G, O' Brien E, Turini E, Mannu F, Ulliers D. Early phagocytosis of glucose-6-phosphate dehydrogenase dependent erythrocytes parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. Blood 1998; 92:2527-34.
59. Chikezie PC, Chikezie CM, Uwakwe AA, Monago CC. Investigation on methaemoglobin concentration of three human erythrocyte genotypes infected with *Plasmodium falciparum*. J Appl Sci Environ Manag 2009; 13:5-8.
60. Chikezie PC. Comparative methaemoglobin concentrations of three erythrocyte genotypes (HbAA, HbAS and HbSS) of male participants administered with five antimalarial drugs. Afr J Biochem Res 2009; 3:266-71.
61. Griffiths MJ, Ndungu F, Baird KL, Muller DPR, Marsh K, Newton CRJC, *et al.* Oxidative stress and erythrocyte damage in Kenyan children with severe *Plasmodium falciparum* malaria. Br J Haematol 2001; 113:486–91.
62. Boumis G, Giardina G, Angelucci F, Bellelli A, Brunori M, Dimastrogiovanni D, *et al.* Crystal structure of *Plasmodium falciparum* thioredoxin reductase, a validated drug target. Biochem Biophys Res Commun 2012; 425:806–11.
63. Kehr S, Sturm N, Rahlfs S, Przyborski JM, Becker K. Compartmentation of redox metabolism in malaria parasites. PLoS Pathog 2011; 6:e1001242.
64. Lisewski AM. *Plasmodium* spp. membrane glutathione *S*-transferases: Detoxification units and drug targets. Microb Cell 2014; 1:387-9.
65. Vega-Rodriguez J, Franke-Fayard B, Dinglasan RR, Janse CJ, Pastrana-Mena R, Waters AP, *et al.* The glutathione biosynthetic pathway of *Plasmodium* is essential for mosquito transmission. PLoS Pathog 2009; 5:e1000302.
66. Lüersen K, Walter RD, Muller S. *Plasmodium falciparum*-infected red blood cells depend on a functional glutathione *de novo* synthesis attributable to an enhanced loss of glutathione. Biochem J 2000; 346:545–52.
67. Kasozi D, Mohring F, Rahlfs S, Meyer AJ, Becker K. Real-time imaging of the intracellular glutathione redox potential in the malaria parasite *Plasmodium falciparum*. PLoS Pathog 2013; 9:e1003782.
68. Kanzok SM, Schirmer RH, Turbachova I, Iozef R, Becker K. The thioredoxin system of the malaria parasite *Plasmodium falciparum*-glutathione reduction revisited. J Biol Chem 2000; 275:40180-6.
69. Nickel C, Rahlfs S, Deponte M, Koncarevic S, Becker K. Thioredoxin networks in the malarial parasite *Plasmodium falciparum*. Antioxid Redox Signal 2006; 8:1227–39.

70. Koncarevic S, Rohrbach P, Deponte M, Krohne G, Prieto JH, Yates J, *et al.* The malarial parasite *Plasmodium falciparum* imports the human protein peroxiredoxin 2 for peroxide detoxification. Proc Natl Acad Sci USA 2009; 106:13323–8.
71. Müller S, Gilberger TW, Krnajski Z, Lüersen K, Meierjohann S, Walter RD. Thioredoxin and glutathione system of malaria parasite *Plasmodium falciparum.* Protoplasma 2001; 217:43-9.
72. Tripathi T, Rahlfs S, Becker K, Bhakuni V. Glutathione mediated regulation of oligomeric structure and functional activity of *Plasmodium falciparum* glutathione *S*-transferase. BMC Struct Biol 2007; 7:67, 10 pages.
73. Sies H. Glutathione and its role in cellular functions. Free Radic Biol Med 1999; 27:916-21.
74. Frey PA. Radicals in enzymatic reactions. Curr Opin Chem Biol 1997; 1:347-56.
75. Zitka O, Skalickova S, Gumulec J, Masarik M, Adam V, Hubalek J. *et al.* Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumor patients. Oncol Lett 2012; 4:1247-53.
76. Patzewitz EM, Wong EH, Muller S. Dissecting the role of glutathione biosynthesis in *Plasmodium falciparum*. Mol Microbiol 2012; 83:304–18.
77. Oude ERP, Ottenhoff R, Liefting WG, Schoemaker B, Groen AK, Jansen PL ATP-dependent efflux of GSSG and GS-conjugate from isolated rat hepatocytes. Am J Physiol 1990; 258:G699-G706.
78. Homolya L, Varadi A, Sarkadi B. Multidrug resistance- associated proteins: export pumps for conjugated with glutathione, glucuronate or sulfate. Biofactors 2003; 16:499–526.
79. Griffith OW. Biologic and pharmacologic regulation of mammalian glutathione synthesis. Free Radic Biol Med 1999; 27:922–35.
80. Urscher M, Alisch R, Deponte M. The glyoxalase system of malaria parasites–Implications for cell biology and general glyoxalase research. Semin Cell Dev Biol 2011; 22:262–70.
81. Dubios VL, Platel DF, Pauly G, Tribouley-Duret J. *Plasmodium berghei*: Implication of intracellular glutathione and its related enzyme in chloroquine Resistance *in vivo.* Exp Parasitol 1995; 81:117-24.
82. Atamna H, Ginsburg H. Heme degradation in the presence of glutathione. A proposed mechanism to account for the high levels of non-heme iron found in the membranes of hemoglobinopathic red blood cells. J Biol Chem 1995; 270:24876–83.
83. Liebau E, Bergmann B, Campbell AM, Teesdale–Spittle P, Brophy PM, Luersen K, *et al.* The glutathione *S*-transferase from *Plasmodium falciparum*. Mol Biochem Parasitol 2002; 124:85-90.
84. Deharo E, Barkan D, Krugliak M, Gokenser J, Ginsburg H. Potentiation of the antimalarial action of chloroquine in rodent malaria by drugs known to reduce cellular glutathione levels. Biochem Pharmacol 2003; 66:809-17.
85. Ducharme J, Farinotti R. Clinical pharmacokinetics and metabolism of chloroquine: focus on recent developments. Clin Pharmacokinet 1996; 31:257-74.
86. Choi CY, Cerda JF, Chu HA, Babcock GT, Marletta MA. Spectroscopic characterization of the hem-binding sites in *Plasmodium falciparum* histidine–rich protein 2. Biochem 1999; 38:16916-24.
87. Masayuki H, Keichi Y, Hiroyuki O, Ryiochi K. Synthesis and properties of Aib-containing of histidine-rich proteins 2 from *Plasmodium falciparum*. Nippon Kagakki Koen Yokoshu 2006; 86:881.
88. Campanale N, Nickel C, Daubenberger CA, Wewan DA, Gorman JJ, Klonis N. Identification and characterization of heme- interacting proteins in the malaria parasite, *Plasmodium falciparum.* J Biol Chem 2003; 278:27354-61.
89. Harwaldt P, Rahlfs S, Becker K. Glutathione *S*-transferase of the malaria parasite *Plasmodium falciparum* characterization of a potential drug target. Biol Chem 2002; 383:821-30.
90. Fritz-Wolf K, Becker A, Rahlfs S, Harwaldt P, Schirmer RH, Kabsch W, et *al.* X-ray structure of glutathione *S*-transferase from the malaria parasite *Plasmodium falciparum*. Proc Natl Acad Sci USA 2003; 100:13821-6.
91. Machima R, Tilley L, Simos MA, Papalexis V, Raftery MJ, Stocker R. *Plasmodium falciparum* histidine- rich protein 2 (PfHRP2) modulates the redox activity of ferriprotoporphyrin IX (FePPIX): peroxidase like activity of the PfHRP2–FePPIX complex. J Biol Chem 2002; 277:14514-20.
92. Deponte M, Becker K. Glutathione S transferase from malarial parasites- structural and functional aspects. Methods Enzymol 2005; 401:240–52.
93. Perbandt M, Bumeister C, Walter RD, Betzel, C, Liebau E. Native and inhibited structure of a Mu class-related glutathione *S*-transferase from *Plasmodium falciparum*. J Biol Chem 2004; 279:1336-42.
94. Mangoyi R, Hayeshi R, Ngadjui B, Ngandeu F, Bezabih M, Berhanu A, *et al.* Glutathione *S*-transferase from *Plasmodium falciparum*–interaction with malagashanine and selected plant natural products. J Enzyme Inhib Med Chem 2010; 25:854-62.
95. Ahmad R, Srivastava A. Inhibition of glutathione *S*-transferase from *Plasmodium yoelii* by protoporphyrin IX, cibacron blue and menadione: Implications and therapeutic benefits. Parasitol Res 2008; 102:805-7.
96. Turrens JF. Oxidative stress and antioxidant defenses: a target for the treatment of diseases caused by parasitic protozoa. Mol Aspects Med 2004; 25:211–20.
97. Theobald AJ, Caballero I, Coma I, Colmenarejo G, Cid C, Gamo MJ, *et al.* Discovery and biochemical characterization of *Plasmodium* thioredoxin reductase inhibitors from an antimalarial set. Biochem 2012; 51:4764–71.
98. Gallo V, Schwarzer E, Rahlfs S, Schirmer RH, Van Zwieten R, Roos D, *et al.* Inherited glutathione reductase deficiency and Plasmodium falciparum malaria–a case study. PLoS ONE 2009; 4:e7303.

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