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List of abbreviations and symbols

BCNU	1,3-bis(2-chloroethyl)-1-nitrosourea (a GSH-R inhibitor)
BHA	butylated hydroxyanisole (a radical scavenger)
CM	cerebral malaria
CD36	cluster of difference 36 (integral membrane glycoprotein)
DFO	desferrioxamine, deferoxamine, desferal (a chelator)
ELAM-1	endothelial leukocyte adhesion molecule-1
G6PD	glucose-6-phosphate dehydrogenase
GSH	glutathione (reduced)
GSH-P	glutathione peroxidase
GSH-R	glutathione reductase
GSSG	glutathione (oxidised)
HeCNU	1-(2-chloroethyl)-3-(2-hydroxyethyl)-1-nitrosourea (a GSH-R inhibitor)
HMS	hexose monophosphate shunt
HRP	histidine-rich proteins
ICAM-1	intercellular adhesion molecule-1
IFN-g	interferon-gamma (a cytokine)
Il-1	interleukine-1 (a cytokine)
IRBC	infected red blood cells
NADPH	nicotinamide-adenine-dinucleotide phosphate (reduced)
O ₂ ^{•-}	superoxide radical
•OH	hydroxyl radical
P.	<i>Plasmodium</i>
PE	parasitised erythrocytes
PEG	polyethylene glycol
PfEMP1	<i>Plasmodium falciparum</i> erythrocyte major protein 1
PMN's	polymorphonuclear cells
R [•]	secondary radical
ROS	reactive oxygen species
SOD	superoxide dismutase
TNF	tumour necrosis factor (a cytokine)
VCAM-1	vascular adhesion molecule-1

Introduction

Malaria is an infectious disease, which world-wide affects more than 200 million people of which 1-2 million die each year (mainly children) [1]. The increasing and widespread resistance of parasites to the current antimalarials (a.o. chloroquine) is a major problem in the prevention and treatment of this disease.

Malaria is caused by protozoan parasites belonging to the genus *Plasmodium*. Four *Plasmodium* species are able to infect humans. *P. falciparum* causes the most dangerous form of malaria and is life threatening in an unprotected, non-immune population [2-5]. The disease is transmitted by the bite of an infectious female mosquito. The parasites enter the blood stream, migrate to the liver and from there infect red blood cells. The parasite proliferates in red blood cells, and the released progeny invades new red cells. The

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Keywords

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Cerebral malaria
Cytokines, Pro-oxidants
Anti-oxidants, Artemisinin
Desferrioxamine

Abstract

Malaria affects world-wide more than 200 million people, of which 1-2 million die every year. New drugs and treatment strategies are needed to face the rapidly increasing problems of drug resistance.

During a malaria infection, both host and parasite are under oxidative stress. Increased production levels of reactive oxygen species (ROS, e.g. superoxide anion and the hydroxyl radical) are produced by activated neutrophils in the host and during degradation of haemoglobin in the parasite.

The effects of ROS in malaria can be both beneficial and pathological, depending on the amount and place of production. Enhanced ROS production after the administration of pro-oxidants, which is directed against the intra-erythrocytic parasite, inhibits the infection both *in vitro* and *in vivo*. However, ROS are also involved in pathological changes in host tissue like damage of the vascular endothelial lining during a malaria infection (cerebral malaria).

Pro-oxidants support the host defense against the parasite when working in or near the infected cell but potentially cause vascular damage when working on or near the vascular lining. Examples of pro-oxidants are found among xenobiotics and food components. Important new drugs belonging to the class of pro-oxidants are artemisinin and its derivatives. Anti-oxidants potentially counteract these agents.

Treatment with anti-oxidants or chelators of metals to prevent their catalytic function in the generation of ROS may prevent vascular pathology. In addition, the iron chelator desferrioxamine, exhibits an antiparasitic activity, because iron is also essential for the proliferation of the parasite.

Cytokines play an important role in ROS-related pathology of malaria, though their mechanism of action is not completely elucidated. This field might bring up new treatment concepts and drugs.

Drugs which prevent host pathology, such as the cerebral complications might be life saving.

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erythrocytic cycle of the parasite causes the disease symptoms. For more details of the life cycle of the human malaria parasite, its differentiation and specific forms in the female mosquito and in the host the reader should refer to the literature [6].

A *P. falciparum* infection is characterised by an incubation period of about 12 days. Clinical manifestations are a.o. intermittent fever (every 48 hours), severe anaemia, shock, cerebral involvement, pulmonary oedema, hypoglycaemia and renal failure. Death occurs in 5-20% of patients that develop cerebral malaria. People that recover from cerebral malaria usually do not exhibit neurological sequelae (85% of children and 95% of adults) [4].

P. vivax and *P. ovale* infections show respectively an incubation period of 13 and 17 or more days and again fever occurs every 48 hours. Other clinical manifestations are chronic anaemia and persistent splenomegaly.

The fourth parasite, *P. malariae*, causes chronic, often low grade, infection. The malaria infection is common in localised areas in the tropics. This infection has an incubation period of 28 days or longer and fever occurs every 72 hours. The infection may persist for 50 years or more, but is often without serious morbidity.

Much effort has been put into research for new antimalarials and the development of vaccines against the most threatening parasite *P. falciparum*. The search for new antimalarials is impeded by the lack of knowledge of the pathogenesis of the disease. In the early 1950s, Gilbert suggested that reactive oxygen species (ROS) play an important role in many human pathologies and malaria seems to be one of them [7].

Reactive oxygen species (ROS) is a collective noun for oxidising compounds such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), lipid peroxides, and other related species [8]. They are produced as a physiological response to a specific noxa. During a malaria infection massively recruited and activated monocytes and neutrophils produce increasing levels of ROS (oxidative stress), although other mechanisms are involved as well [9-11]. However oxidative stress during malaria is beneficial to the patient in the combat against its intra-erythrocytic parasite. Several studies have been described in which induction of oxidative stress by treatment with pro-oxidants proved to be effective against the infection [reviewed in 12]. On the other hand ROS play a role in the pathology of malaria. Excessive oxidative stress and particularly at unwanted places (e.g. vascular lining, blood brain barrier) will damage the defense system. Intra- and extracellular anti-oxidant systems are present to prevent damage, but they may fail during disease. Treatment with anti-oxidants reinforces these systems and protect the patient, especially during the life threatening phase of the disease [13-14].

An understanding of the role and mechanisms of action of oxidative stress may eventually lead to the development of new drugs and new concepts of treatment. This review will discuss oxidative stress and its role in malaria and also new strategies in the treatment and drug development that are based on the knowledge of oxidative stress in malaria, in order to prevent and to treat patients with this life threatening infection.

Principles of biosynthesis and biotransformation of ROS

The formation and biotransformation (detoxification) of ROS in biological systems is depicted in Figure 1. In this scheme the production of ROS is started when phagocytic cells are activated by immune modulators. One of the consequences of activation of macrophages is the generation of a respiratory burst. During activation of the respiratory burst, oxygen is taken up by the membrane-bound NADPH-oxidase complex and reduced to the superoxide radical [13-16].

Another process in which the $O_2^{\bullet-}$ is formed is the reduction of molecular oxygen in cells [15], not shown in Figure 1. The $O_2^{\bullet-}$ is reduced by superoxide dismutase (SOD) to H_2O_2 and this in turn is reduced to water by glutathione peroxidase (GSH-P) or by catalase. The oxidised glutathione (GSSG) is rapidly reduced by glutathione reductase (GSH-R), utilising NADPH generated by various intracellular reactions, including the hexose monophosphate shunt (HMS) [13-15].

SOD, catalase and GSH-P/GSH-R are important intracellular anti-oxidant enzymes; they are, however, much less prominently present in extracellular fluids. Catalase is present in liver cells and erythrocytes at high concentrations. Its reactivity is important when H_2O_2 concentrations are raised.

The transformation of $O_2^{\bullet-}$ and H_2O_2 into the very

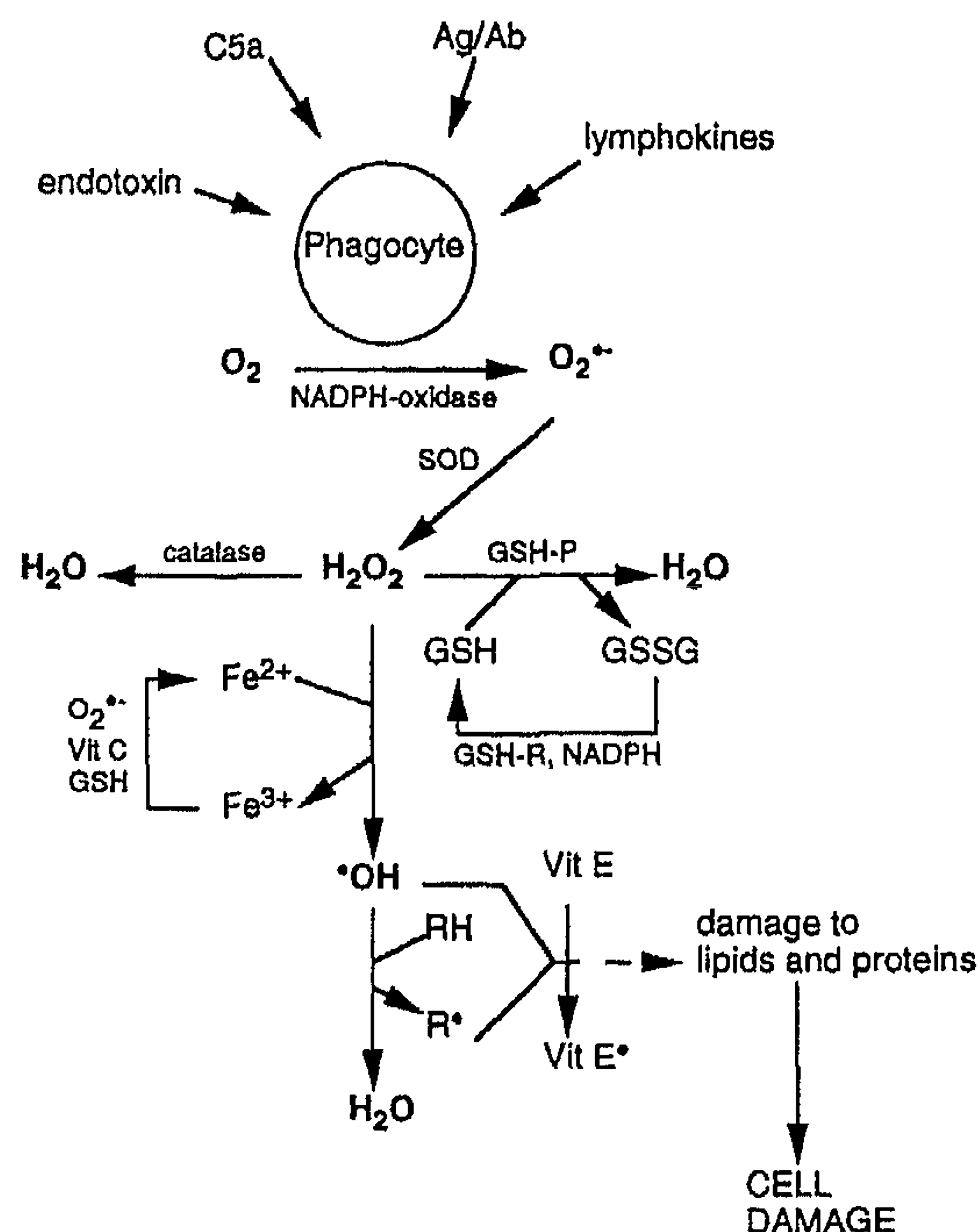


Figure 1

Formation and biotransformation of ROS in biological systems.

$O_2^{\bullet-}$, superoxide; SOD, superoxide dismutase; GSH, reduced glutathione; GSSG, oxidised glutathione; GSH-P, glutathione peroxidase; GSH-R, glutathione reductase; $\bullet OH$, hydroxyl radical; R^{\bullet} , secondary radical; Vit E, vitamin E; Vit E \bullet , vitamin E radical

reactive hydroxyl radical is catalysed by free transition metal ions (iron or copper) via the Haber-Weiss reaction [8 13]. This hydroxyl radical rapidly reacts at first encounter with e.g. lipids, proteins or DNA, often causing molecular and cellular damage [17 18].

An important intra- as well as extracellular anti-oxidant or radical scavenger is vitamin E (α -tocopherol) [19]. It reacts with peroxy and alkoxy radicals, thereby preventing reaction with other molecules. The ensuing α -tocopherol radical is only poorly reactive and ends the radical chain reaction.

Other important extracellular free radical scavengers which are not shown in this figure are β -carotene, ascorbate and urate [13 20]. In addition, several proteins (transferrin, caeruloplasmin, metallothioneins) inhibit formation of reactive species by strongly binding iron or copper [13 21 22].

Oxidative stress is the result of a disturbance in the balance of the naturally generated oxidants and anti-oxidants. This can be caused by an increase in the production of ROS and/or a decrease in the activity of anti-oxidant systems.

Malaria and oxidative stress

Production of ROS in malaria

During a malaria infection oxidative stress is increased by the increased production of ROS, both intra-erythrocytic and outside the parasitised erythrocyte.

Parasitised erythrocyte

Several studies report on the increased oxidative stress in isolated infected red blood cells (IRBC). H_2O_2 production is reported in *P. berghei*-IRBC, and $O_2^{\bullet-}$ production in *P. falciparum*-IRBC [12 23]. In *P. berghei*- and *P. chabaudi*-IRBC increased levels of lipid peroxidation have been demonstrated [24]. It is suggested that ROS are produced as a result of the oxidation and degradation of ingested haemoglobin in the acid environment of the parasite's food vacuole. Acidification of the lysate in *P. falciparum*-infected erythrocytes (which also occurs during the digestion of host cell cytosol in the acid food vacuole of the parasite) results in the generation of H_2O_2 [25]. This is probably produced during the auto-oxidation of oxyHb to metHb [26]. Intact trophozoite-IRBC produce H_2O_2 and OH radicals about twice as much as normal erythrocytes, while no increase is detected at the ring stage when Hb-breakdown is limited. In the presence of reducing agents like ascorbate, extracts from parasitised erythrocytes caused a dose and time dependent DNA degradation [27]. These results suggest a progressive increase in intracellular iron levels during the further development of *P. falciparum*-IRBC. This leads to an increased production of H_2O_2 and OH radicals can be formed that may cause molecular and cellular damage [28].

Extra-erythrocytic production

Besides an enhanced intra-erythrocytic oxidative stress, increased production of ROS has also been observed outside the parasitised erythrocyte. Kharazmi et al. [10] demonstrated the capability of *P. falciparum* merozoites and soluble parasite antigens to generate ROS from blood monocytes and neutrophils of the host. Several *in vivo* studies showed

that during a *P. falciparum* infection ROS production by phagocytic cells was strongly increased and a more enhanced production was seen in severe and complicated infection than in milder cases [29 30]. During acute *P. knowlesi* infection in rhesus monkeys the \bullet OH production by monocytes was considerably elevated [11].

Malaria parasites and effects of ROS

ROS and the defense against malaria parasites

During the work on the establishment of continuous cultures of asexual blood forms of malarial parasites, it became clear that malarial parasites only tolerate very little oxygen *in vitro* and that they were sensitive to oxidant stress [31]. Other indications that parasites are sensitive to oxidative stress came from observations that certain genetic disorders, like sickle cell disease, thalassaemia and glucose-6-phosphate dehydrogenase deficiency (G6PD-deficiency), commonly found in some tropical areas, relatively protect against malaria. Alterations in haemoglobin-structure or the deficiency of the enzyme G6PD make red blood cells more susceptible to oxidative stress. This creates at the same time an unfavourable environment for the intra-erythrocytic parasite [32-34].

These findings lead to the research of analysis of the oxidative stress and the redox status of malaria-infected erythrocytes. This subject was summarised recently [12 35].

ROS, produced by activated monocytes, probably play an important role in the control of the parasitaemia. It has been demonstrated that phagocytic cells, like monocytes and polymorphonuclear cells (PMNs) damage the intra-erythrocytic parasites by their production of ROS [36 37]. Activated neutrophils and blood monocytes *in vitro*, are able to partially inhibit maturation of *P. falciparum* [38] and damage the erythrocyte membrane [39]. An increase in lipid peroxidation and sensitivity to hemolysis normally occurs during parasite maturation.

Defense of the malaria parasite against ROS

An important question is the way the parasite deals with oxidative stress. There are two possibilities; one, the parasite must develop its own defense, and two, stimulate that of the host cell to its own advantage. The essence of the defense is found in naturally occurring anti-oxidant systems.

As described in Figure 1, GSH and NADPH support the scavenging of ROS. Parasites have GSH-reductase activity, but little is known about GSH synthesis in the parasite [40 41]. In parasites, growing in glucose-6-phosphate dehydrogenase deficient -erythrocytes that were infected with *P. falciparum*, no GSH could be detected [42]. This implies that in infected erythrocytes the parasite may be totally dependent on host cell GSH synthesis. In patients infected with *P. vivax*, erythrocytic GSH and GSH-reductase activity decreased in proportion to the severity of the parasitaemia [43]. In a murine *P. berghei* infection, GSH can only be maintained in the reduced form with NADPH of the host cell [44]. The hexose monophosphate shunt activity, which provides NADPH, is increased in infected erythrocytes [35]. Whether parasites have their own hexose monophosphate shunt remains unclear. In addition, the parasite possesses both gluta-

mate dehydrogenase and isocitrate dehydrogenase which also produce NADPH [45].

Another important anti-oxidant enzyme is SOD. Endogenous SOD activity has been observed in three different rodent malaria parasites, *P. berghei*, *P. yoelii* and *P. vinckei* [46]. *P. falciparum* is also capable of producing SOD, however there is evidence that SOD produced by the host is "adopted" by the parasite [25-47]. The concentration of catalase increases in both the host and parasite compartment during *P. falciparum* infection [25]. H₂O₂ produced by the parasite during digestion of host cell cytosol, appeared to be partially handled by host catalase.

Other proteins that might play a role in the oxidant defense of malaria parasites are histidine-rich proteins (HRP) [48-49, 50]. HRP are found in the knobs on the surface of *P. falciparum*-parasitised erythrocytes which are associated with the adherence to endothelial cells, but other functions of HRP have been proposed [51]. Histidine is an effective •OH scavenger and an efficient chelator of copper and iron [52]. In addition, histidine forms a tight complex with H₂O₂ [53]. All together these pathways reduce the exposure of the parasite and its host cell to the damaging effects of free radicals.

The host and the effects of ROS (cerebral malaria)

About one to two million patients each year, mainly children, die because of the complications of an infection with *P. falciparum*. The most prominent syndrome is cerebral malaria (CM). The clinical definition of CM in humans is the presence of *P. falciparum* parasites in the circulation and irrecoverable coma not explained by any other cause [4]. Post mortem, CM is characterised by the sequestration of parasitised erythrocytes (PE) in the microvasculature of the brain and the presence of petechiae and ring-haemorrhages, particularly in the white matter. The pathogenesis, however, is not yet completely understood. Most observations came from animal models, especially *P. berghei* infections in mice. As in human CM, cerebrovascular pathological changes (endothelial cell damage and petechiae) and sequestration of cells in post-capillary venules have been observed in rodent models of CM [54-56]. A striking difference between human and murine CM is the type of cell that sequesters; PE in human CM as composed to leukocytes in murine CM, although some reports mention also the sequestration of leukocytes in human CM [57]. In general, two hypotheses are put forward to explain the pathogenesis of cerebral malaria.

Erythrocyte adhesion and sequestration

The oldest of the two hypotheses is the mechanical hypothesis which focuses on the sequestration of infected red blood cells and the blockade of the cerebral microvasculature [5, 58]. PE express a receptor on their surface, *Plasmodium falciparum* erythrocyte major protein 1 (PfEMP1), which mediates the adherence of PE to the endothelial lining of the postcapillary venules of several organs [59-61]. Parasitised erythrocytes are able to bind to a number of adhesion molecules of endothelial cells, particularly of post-capillary venules (e.g. CD36, thrombospondin, intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1)) [62]. This is

supposed to cause vascular blockade, resulting in anoxia, acidosis and ischaemia. However, in addition to the mechanical obstruction of the venules, other factors seem to be required to induce the severe damage of the endothelial lining seen in CM [5].

Immunopathology and cytokines

A second concept is the inflammatory hypothesis, which is based on parasite-induced immunopathology [63]. In rodents, T-cells and macrophages play an important role in the immunopathology of CM. No CM occurs in congenital T-cell deficient mice or in normal mice treated with anti-T-cell antibodies [64-66]. A timely depletion of macrophages also prevents the development of the cerebral syndrome [67-69]. Malaria parasites are able to activate T-cells and mononuclear phagocytes to release cytokines. Of these cytokines, TNF (tumour necrosis factor) seems to be the most important in the pathogenesis of CM [70]. Both in human and murine CM, high TNF levels are associated with CM and/or severity of the disease [69, 71-73]. Grau et al. [74] were able to prevent death from CM in their mouse model (*P. berghei*-Anka) by treatment with anti-TNF antibodies.

TNF can induce several changes in endothelial cells, like the enhanced expression of endothelial cell receptors such as ICAM-1, VCAM-1 and ELAM-1 [62, 75]. Parasitised erythrocytes bind to endothelium *in vitro* and the severity of a *P. falciparum* infection is related to the degree of adherence of PE to the host's vascular endothelium [76].

Furthermore, TNF as well as lymphokines like interferon gamma (IFN-γ) can prime leukocytes to release ROS [77, 78]. At the same time, iron chelators and phenolic radical scavengers can inhibit TNF-release from macrophages [79]. This suggests that oxidant stress can amplify TNF-induced pathology, part of which can be mediated by ROS. During a *P. falciparum* infection an increased generation of ROS is observed. This explains both the role of TNF and of free radicals as important aspects of malaria pathology.

ROS produced by activated monocytes inhibit the growth of intra-erythrocytic parasites. However, the damaging effects of the ROS will not be limited to the PE since they also affect other host tissues. As described above, PE and leukocytes sequester in the brain. Local activation of T-cells and mononuclear phagocytes by parasite antigens and soluble products (malaria toxins) can result in an excessive production of cytokines, like TNF and ROS, which may damage the cerebral microvascular endothelium [5]. An increase in cerebrospinal fluid protein and lipid peroxidation products in patients with cerebral malaria compared to controls and in fatal cases of cerebral malaria compared to non-fatal cases has been observed [80].

ROS and endothelial damage

The role of neutrophil-derived ROS in endothelial damage has been extensively studied. Endothelial damage in the rat, initiated by e.g. immune complexes, can be prevented by depleting animals of neutrophils, or by infusing SOD or catalase, radical scavengers or iron chelators (reviewed in [81]). These results suggest that •OH formation via iron and H₂O₂ is involved in endothelial damage. The protective

effect of iron chelation by desferrioxamine (DFO) on neutrophil-mediated killing of endothelial cells, is associated with the presence of DFO in the endothelial cells [82].

As mentioned before, an increase in redox-active iron may occur in malaria during the growth of *P. falciparum* because of the degradation of host cell haemoglobin. Also, an excess of free haemoglobin (normally bound to haptoglobin and cleared by the reticulo-endothelial system) might be able to catalyse the reduction of H_2O_2 to the $\cdot OH$.

Additional evidence for the role of oxygen-derived free radicals in brain injury, comes from ischaemic/reperfusion studies. In CM, the microvasculature is blocked with PE and periods of ischaemia and reperfusion might occur. Chan et al. [83], propose a role for hypoxanthine and xanthine oxidase in the reduction of reperfusion oxygen to $O_2^{\cdot -}$ and H_2O_2 . In the presence of transition metals like iron, hydroxyl radicals are formed which may stimulate peroxidation of membrane lipid in the brain. The iron chelator DFO appears to reduce brain injury in neonatal rats with hypoxic-ischaemic brain injury [84]. High DFO concentrations were achieved in the brain which might however protect the brain by other mechanisms than the chelation of iron [84-85].

Effects of experimental manipulation of the ROS status

Thus ROS are involved both in the control of the parasitaemia and in the pathogenesis of CM. This may have implications for the development of drug-treatment of malaria and its cerebral complication.

Manipulation with xenobiotics

Enhanced stress with pro-oxidants or anti-oxidant inhibitors

Several investigators used the susceptibility of malaria parasites to oxidative stress in administering pro-oxidants to try to inhibit infection. *In vitro* and *in vivo* studies show that agents known to generate ROS, like alloxan [86] and tert-butyl hydroperoxide [87], are able to inhibit *Plasmodium* development. Hunt and Stocker [12] reviewed the effects of pro-oxidants on different species of *Plasmodia*. The above mentioned compounds are however too toxic for human use. The artemisinin derivatives discussed below, are examples of drugs acting by this principle [88-90].

Another strategy by which oxidative stress on the parasite can be increased was explored by Zhang et al. [91]. They work on the development of inhibitors of anti-oxidant enzymes, thus decreasing anti-oxidant defenses and increasing oxidant stress. The two glutathione reductase inhibitors 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-(2-hydroxyethyl)-1-nitrosourea (HeCNU) were tested *in vitro*. Both compounds were able to inhibit the growth of *P. falciparum* in culture. Again, these types of compounds are useful in experimental pharmacology but are too toxic for human use.

Effects of anti-oxidants

Anti-oxidants may have a protective effect against the unwanted effects of ROS on the integrity of the vascular lining. Thumwood et al. [92] described that

administration of butylated hydroxyanisole (BHA), a well-known radical scavenger *in vitro* and *in vivo*, or the enzymes superoxide dismutase and catalase could prevent cerebral malaria in a murine model. After being fed a BHA containing diet the mice showed few or no cerebral symptoms and an important reduction in the development of haemorrhages, mononuclear infiltration and oedema in the CNS. Similarly, but more consistently protective effects were seen after repeated injections of BHA or by sustained release of BHA from osmotic pumps. The combination of intravenously administered polyethylene-glycol (PEG)-superoxide dismutase or PEG-catalase also protected mice against death from cerebral complications. Administration of two other anti-oxidants, vitamin E and trolox, partially protected against cerebral malaria.

Chelation of iron, a very important catalyst in oxidising processes, is another potential strategy to influence the development of CM. The iron chelator DFO might protect the central nervous system from ischaemic and haemorrhagic toxicity mediated by free radicals and especially hydroxyl radicals. DFO was added to a standard therapy with quinine and sulfadoxine-pyrimethamine to children with CM [93]. In the DFO group the rate of recovery to full consciousness was increased compared to the placebo group. The difference was more pronounced among patients with deep coma. The rate of parasite clearance from the blood was higher in the DFO treated group. However, DFO treatment did not prevent mortality. A larger clinical trial was recommended to determine the usefulness of DFO treatment in patients with CM.

ROS and food components

Several investigators investigated the control of malaria by dietary manipulation. Important food components in controlling dietary-induced oxidative stress are unsaturated fatty acids (pro-oxidants), vitamin C (ascorbic acid, pro- and anti-oxidant effects) and vitamin E (tocopherol, anti-oxidant).

Food components and parasitaemia

Vitamin E deficient mice are protected against *P. yoelii* infection with diets containing fish oil. Dietary vitamin E supplementation enhanced the infection and killed the mice [94]. Protection was also seen in chloroquine-resistant malaria in mice after feeding a vitamin E-deficient diet supplemented with menhaden-fish oil [95]. Addition of menhaden or fish oil to a normal chow diet had a strong, but incomplete, protective effect in normal mice against *P. yoelii* and *P. berghei* infection respectively [96-97].

Dietary supplementation with highly unsaturated fatty acids of fish oils results in the incorporation of these substances into the host erythrocyte and/or parasite membranes. In the absence of vitamin E, such membranes would be very vulnerable to oxidative stress. In an experimental set up it might be possible to decrease the vitamin E content of the host plasma sufficiently to block the erythrocytic stage of the parasite, without depleting the tissue tocopherol stores and running the risk of serious side effects [98]. A severe or absolute vitamin E deficiency might not be required to obtain full protection against the parasite. In many areas where malaria is endemic, the vita-

min E status of the population is low. Supplementation with highly unsaturated fatty acids could be of some benefit against malaria.

Addition of unsaturated tropical (plant) oils to a vitamin E deficient diet did not protect against a *P. berghei* infection in mice [98]. This can be explained by the fact that fish oil contains much more polyunsaturated fatty acids than tropical plant oil. Thus, not only is the vitamin E status important, but also the peroxidisability of the dietary fatty acids incorporated in membranes. However, the exact biochemical mechanism by which unsaturated fatty acids act remains unclear. Certain prostaglandin derivatives decrease the growth of malarial parasites in mice [99], so an effect of fish oil in modulating prostaglandin metabolism must be considered. At the same time, vitamin E-deficiency alone can also have an antimalarial effect in mice [100].

Vitamin C with its well-known anti-oxidant effects can also exert strong pro-oxidant effects in the presence of metals such as iron or copper [98]. Ascorbic acid treatment marginally enhanced the development of young parasites, but was highly deleterious to advanced forms of *P. falciparum* [101]. This deleterious effect on late stage parasites is probably due to its pro-oxidant action that synergises with increased levels of iron-containing structures, that are produced during growth and differentiation of the parasite. Until now, no effect of dietary supplementation of vitamin C on the course of malaria infected mice has been observed.

Food components and protection against CM

The effects of dietary supplementation or restriction were not only studied in the course of infection but also in development of CM. Anti-oxidants are expected to protect against the vascular complications of malaria.

Dietary-induced oxidative stress has recently been demonstrated to protect against CM in a murine model [102]. Mice were fed a diet, supplemented with menhaden-fish oil and deficient of vitamin E for four weeks before and during infection with *P. berghei* ANKA. Mice receiving this diet showed complete survival for 14 days post infection and 6-day parasitaemias were decreased. The antimalarial effect could be prevented by supplementing the diet with vitamin E or with either of two synthetic anti-oxidants, N,N'-diphenyl-p-phenylenediamine or probucol. These results contradict with the studies in which anti-oxidant treatment results in protection against CM [92]. Different cytokines (IL-1, TNF) may play a mediatory role in the prevention of CM following administration of diets supplemented with fish-oil and deficient of vitamin E [97 103 104]. However, the exact mechanism is not clear yet.

New drugs, acting on oxidative stress

Glutathione reductase is an important intracellular anti-oxidant enzyme. The structure of the enzyme is known, and appears to be an interesting target for the design of novel antimalarial drugs. Zhang et al. [91] disturbed the pro-oxidant-anti-oxidant balance in favour of the former by the administration of glutathione reductase inhibitors. What is important is the fact, that glutathione reductase is not essential for the

normal function of the erythrocytes. Inhibitors must act preferentially in the infected erythrocyte without affecting normal function of other host tissues.

Promising new antimalarial drugs are artemisinin (qinghaosu) extracted from the leaves of *Artemisia annua* L and its derivatives [88 89]. Artemisinin and its derivatives artemether (an oil-soluble methyl ether) and artesunate (a sodium succinyl salt) are produced for clinical use in several countries. The results of comparative studies in which a qinghaosu compound was compared with another antimalarial, show a reduction of the fever clearance time (17% ~ 7.7 h) and of the parasite clearance time (32% ~ 19.8 h) for the qinghaosu compound, but also a higher recrudescence rate (reviewed in [105]). These compounds play a role in the treatment of severe malaria. Maeno et al. [106] studied the effects of artesunate on rhesus monkeys experimentally infected with *Plasmodium coatneyi*. They found in addition to a reduced parasitaemia, a reduced rate of sequestration of PE in cerebral microvessels.

The endoperoxide bridge of these compounds seems to be essential for antimalarial activity, and strong evidence exists that the antimalarial activities of these drugs depend on the generation of free radical intermediates that alkylate and oxidise proteins and oxidise lipids [107 108]. In the presence of the free radical scavengers ascorbic acid and vit E, the antimalarial activity is decreased *in vitro* [109 110] and *in vivo* [111]. Haem and free iron (abundantly present in infected red blood cells) seem to be responsible for the activation of the drugs and the selective toxicity to malaria parasites. Artemisinin is also activated *in vitro* by haem and free iron [112-114], while chloroquine, which binds haem, antagonises the antimalarial activity [115].

Clinical perspectives in malaria prevention and therapy

Despite all the efforts made, malaria is still the world's most devastating human infection. Since 1960, chloroquine-resistant and multidrug-resistant strains of *P. falciparum* have spread and the degree of resistance has increased [116]. New strategies focus on vaccines, life style, food habits and on development of new drugs.

The first malaria vaccine, SPf66, claimed some success in several phase II en III trials in Latin America as well as in Africa [reviewed in 117]. In Tanzania, the estimated vaccine efficacy against clinical malaria was 31% while the incidence of mild side effects after three doses was less than 6%. There is however much skepticism and little reason for optimism with respect to the SPf66 vaccine. The second trial in the Gambia [118] revealed no protection by SPf66 and the results of a third trial in Thailand are about to become available.

The approach discussed in this review focuses on the oxidative stress that occurs in malaria and the generation by and susceptibility of malaria parasites to ROS. The production of ROS seems to be part of the natural host defense against malarial parasites and several investigations have tried to increase this natural defense by administration of pro-oxidants. In recent studies, oxidative stress was increased by the addition of polyunsaturated fatty acids to the diet and

indeed parasitaemia was inhibited. However, in most studies the diet was fed for several weeks which makes it a less realistic approach. In addition, ROS are also involved in host tissue pathology. An increase in oxidative stress could increase host tissue pathology. The role of dietary restriction and supplementation and its significance in the treatment of cerebral malaria is not yet elucidated.

ROS play a dual role in malaria. Besides their protective effect against malarial parasites, ROS produced by activated leukocytes sequestered in the postcapillary venules might contribute to the pathology observed in severe and cerebral malaria. In contrast to pro-oxidants, anti-oxidants could play a role in the treatment of malaria pathology. The protective results obtained with the radical scavenger BHA and the anti-oxidant enzymes SOD and catalase are promising.

Another promising approach could be the use of an iron chelation therapy. The iron chelator desferrioxamine has been available for clinical use for a period of time and is used in the treatment of iron and aluminum overload. The discovery of the central role of iron in $\cdot\text{OH}$ production and the role of free radicals in many human pathologies stimulated the interest of many investigators for DFO [119]. In malaria, DFO was first shown to inhibit parasitaemia, by depriving the parasite's enzymes (e.g. ribonucleotidase) of essential iron [120-122]. In addition, DFO might also protect against cerebral damage by chelating iron and thus inhibiting hydroxyl radical formation. A major disadvantage of DFO is its low gastrointestinal absorption and penetration into cells. The therapeutic efficacy of iron chelators might be increased by the development of new, oral iron chelators which are able to penetrate cells more easily.

As stated before, a major problem in the treatment of malaria is the development of resistance of the parasite to most drugs. Most current antimalarial drugs like chloroquine, were developed on the basis of their action against asexual erythrocytic forms of malaria parasites, which are responsible for clinical illness [123-124]. Primaquine is the only drug clinically used to eradicate tissue forms of plasmodia that cause relapses. Compounds that are being used against chloroquine-resistant and multidrug-resistant strains of *P. falciparum* include mefloquine and halofantrine. Both compounds are related to quinine.

New important drugs, acting by increasing the oxidative stress and clinically tested at present are the artemisinin-derivatives as discussed above. A promising class of drugs might be derived in the future from the glutathione reductase inhibitors, as discussed above.

It is reasonable to assume that new antimalarials, including pro-oxidants, that act upon the parasite will eventually induce resistance. This is not true for compounds not acting upon the parasite, but preventing host pathology in another way, like anti-oxidants could do on the vascular complications. A second advantage could be that by preventing pathology, the patients own immune-system might be able to overcome the infection, leading to development of immunity to the parasite. If we consider new strategies in the prevention and treatment of malaria based on the oxidative stress that occurs in malaria, substances that can efficiently bind or deplete intracellu-

lar Fe^{3+} should be highly considered in the treatment of severe disease.

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