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Nitric oxide bioavailability in malaria

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Abstract

Rational development of adjunct or anti-disease therapy for severe *Plasmodium falciparum* malaria requires cellular and molecular definition of malarial pathogenesis. Nitric oxide (NO) is a potential target for such therapy but its role during malaria is controversial. It has been proposed that NO is produced at high levels to kill *Plasmodium* parasites, although the unfortunate consequence of elevated NO levels might be impaired neuronal signaling, oxidant damage and red blood cell damage that leads to anemia. In this case, inhibitors of NO production or NO scavengers might be an effective adjunct therapy. However, increasing amounts of evidence support the alternate hypothesis that NO production is limited during malaria. Furthermore, the well-documented NO scavenging by cell-free plasma hemoglobin and superoxide, the levels of which are elevated during malaria, has not been considered. Low NO bioavailability in the vasculature during malaria might contribute to pathologic activation of the immune system, the endothelium and the coagulation system: factors required for malarial pathogenesis. Therefore, restoring NO bioavailability might represent an effective anti-disease therapy.

Pathogenesis of malaria

Malaria is one of the leading causes of morbidity and mortality, annually killing more than two million people worldwide [1]. Furthermore, patients with severe *Plasmodium falciparum* malaria still exhibit a 20–30% risk of mortality when given effective antiparasite therapy, providing the rationale for developing adjunct or anti-disease therapy that rescues ill patients from pathogenesis [2]. To develop adjunct therapy rationally, cellular and molecular definitions of the pathogenesis of *P. falciparum* malaria are needed. Nitric oxide (NO) is a molecule that has been proposed to have a crucial role in malaria pathogenesis [3] but its mechanism of action during the disease is controversial. On the one hand, it has been proposed that NO is produced in excess and kills the *Plasmodium* parasite [4], with the unfortunate side effects of mediating pathogenesis through oxidative damage or aberrant signaling in the brain [3], and contributing to anemia [5]. In this case, nitric oxide synthase (NOS) inhibitors or extracellular scavengers of NO might function as an adjunct therapy for severe *P. falciparum* malaria.

On the other hand, the findings that hypoargininemia [6] and low levels of plasma nitrates and nitrites (NOx) [7]—the stable degradation products of NO—correlate with the development of severe *P. falciparum* malaria pathogenesis suggest that levels of NO production might be low during malaria. In addition, the increased levels of free hemoglobin (Hb) in the blood of patients with severe *P. falciparum* malaria [8,9], an unavoidable consequence of parasite replication in red blood cells (RBCs), and the elevated levels of superoxide elicited by the infection, together with the well-documented scavenging of NO by Hb and superoxide, indicate that NO produced during malaria in the vasculature is probably removed rapidly. Studies of other diseases [10] report that low NO bioavailability contributes to pathologic activation of the immune system, the endothelium and the coagulation system; indeed, these factors are observed during severe *P. falciparum* malaria [11]. Therefore, restoring NO bioavailability or reversing the consequences of low NO bioavailability might represent an effective adjunct therapy for severe malaria.

Physiological role of NO

NO is an uncharged free radical that is produced during the enzymatic conversion of L-arginine to L-citrulline by members of the NOS family of proteins [12]. Three members of the family have been identified: endothelial NOS (eNOS or NOS3), neuronal NOS (nNOS or NOS1) and inducible NOS (iNOS or NOS2), with eNOS and nNOS both functioning in a calcium-dependent and -independent fashion, and iNOS functioning in a calcium independent fashion [12].

NO was identified originally as the endothelium derived relaxation factor that mediates vasodilation in a soluble guanylate cyclase (sGC)-dependent manner [13]. Because NO readily diffuses through cell membranes and can bind with high affinity to heme-containing proteins (e.g. sGC) or nitrosylate proteins, it has been reported to exhibit complex pleiotropic effects and is central to many aspects of physiology and pathology [14]. Adding to the complexity, proteins nitrosylated by NO can release NO, thus serving as a NO-transport mechanism [15], and Gladwin has proposed that the nitrite – the stable end product of NO degradation – can be converted by deoxyhemoglobin (deoxyHb) to NO and that this NO has an important role in ischemia–reperfusion injury in the heart and liver [10]. Besides NO concentration, the state of the NO species, its cellular location and the presence of superoxide, which reacts with NO to produce peroxynitrite, all determine which of the many pleiotropic roles NO has.

Many of the physiological effects of NO (Figure 1), such as vasodilation, inhibition of platelet aggregation and endothelial-cell activation, are mediated through the activation of sGC by NO, leading to increased production of guanosine 3',5'-phosphate (cGMP) [16]. In the vasculature, NO is produced by endothelial cell eNOS and targets smooth-muscle cells, causing vasodilation through the sGC–cGMP pathway. In addition, eNOS-derived NO has an important role in maintaining homeostasis by inhibiting the activation of endothelial cells and by modulating their expression of cell-adhesion molecules (CAMs) [17]. NO in the vasculature also modulates platelet function; platelets express eNOS, and NO inhibits platelet adhesion, activation and aggregation by both cGMP dependent and cGMP-independent pathways [18].

In the immune system, NO is a component of the innate immune response, and iNOS-derived NO is required for the control of several viral, bacterial and parasitic infections [19]. Despite all three NOS isoforms having similar NO-production rates of ~1 μM min^{-1} mg^{-1} protein [20], iNOS is responsible for the high-level production of NO by phagocytes because it is highly expressed after activation, comprising up to 1% of total protein [21]. Although the initial controversy regarding differing iNOS functions in human versus murine phagocytes has been settled [22] and the initial comparison of human and murine iNOS genes and promoter regions has shown them to be extremely similar [23], further detailed analysis of the iNOS genes of both species [24] is necessary to determine differences in their transcriptional regulation.

In addition to its antimicrobial role, NO can regulate immune-system function through cGMP-dependent and -independent mechanisms. In particular, several anti-inflammatory effects of NO have been established, including inhibition of T-cell and B-cell proliferation, and leukocyte rolling and adhesion on microvascular endothelial cells [19,25]. Furthermore, NO also modulates the production of cytokines, generally decreasing the amounts of pro-inflammatory cytokines [interleukin (IL)-1, IL-2, tumor necrosis factor (TNF) and interferon (IFN)-ɣ] and increasing the amounts of anti-inflammatory cytokines (IL-4, IL-13 and transforming growth factor-b) [19].

Finally, NO functions in both the peripheral nervous system (PNS) and the central nervous system (CNS). In the PNS, neurons use nNOS to produce NO that functions as a neurotransmitter, targeting smooth muscle and causing relaxation through the sGC–cGMP pathway. In the CNS, however, the role of NO is not fully understood, although it has been implicated in neuronal excitability (through both sGC–cGMP-dependent and -independent pathways), synaptic plasticity (through the sGC–cGMP pathway) and neurotransmitter release (through the sGC–cGMP pathway) [26]. Although nNOS is the primary isoform responsible for CNS NO production, eNOS and iNOS might also be involved, particularly in glial cells. Considering the involvement of NO in processes that are profoundly affected by malaria pathogenesis (e.g. endothelial activation, expression of CAMs, vascular leak, severe inflammatory response and coagulopathy) (Figure 1), it is easy to see why elucidating the mechanisms with which NO functions in malaria pathogenesis is an important line of research.

The role of NO during malaria

Several hypotheses have been proposed regarding the role of NO during malaria pathogenesis. First, virtually all review articles about the killing of *Plasmodium* by the immune system, including a recent review by Riley and Stevenson [4], state that NO derived from iNOS-expressing monocytes or macrophages kills *Plasmodium* as part of innate immunity. Whereas the case for NO killing in leishmaniasis is well documented [27], there is little evidence to support its role in the killing of *Plasmodium* [28]. By contrast, most evidence (Table 1), including the lack of killing after incubation of *P. falciparum* with a saturated NO solu-

tion [29], suggests that the parasite possesses innate resistance to killing by NO and other reactive oxygen species (ROS). Rockett et al. observed that nitrosylated cysteine or glutathione kills *P. falciparum*—with 50% killing occurring at 40 mM and killing with NOx occurring in the range 10-30 mM—suggesting that NO-derived products rather than NO itself might be toxic to the parasite [29]. However, the amount of nitrosylated cysteine or glutathione required is two orders of magnitude greater than the total free-nitrosothiol levels observed in human plasma [30], and the NOx levels required are three orders of magnitude greater than the levels observed in *P. falciparum* malaria patients [7].

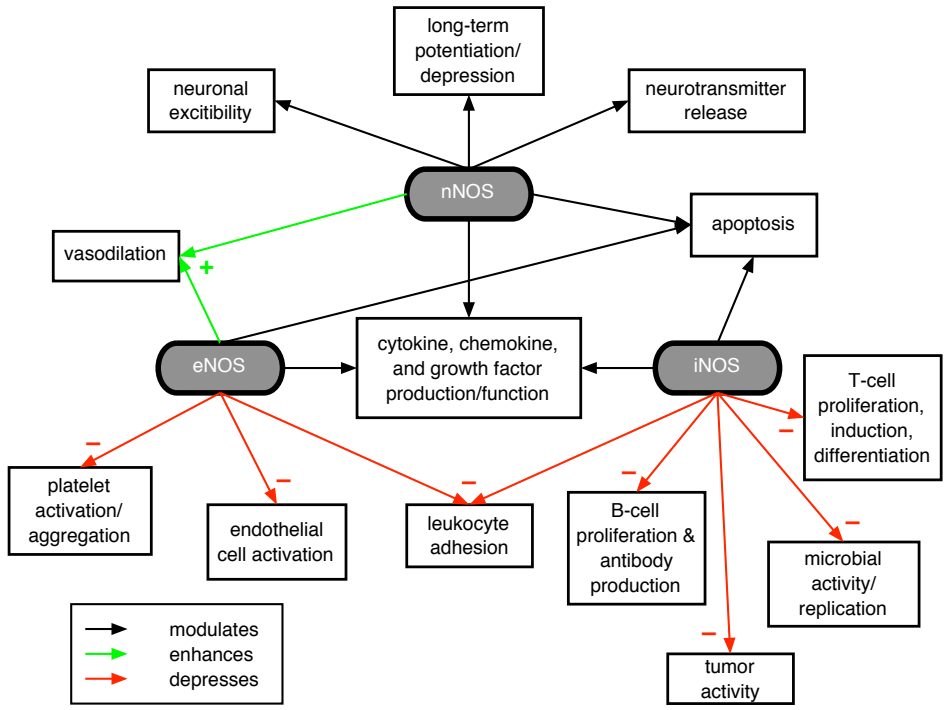

FIGURE 1. PHYSIOLOGICAL ROLES OF NO THAT ARE RELEVANT TO MALARIA PATHOGENESIS. OF PARTICULAR IMPORTANCE ARE THE HOMEOSTATIC AND ANTI-INFLAMMATORY PATHWAYS (RED ARROWS) THAT CAN BE DISRUPTED BY NO QUENCHING DURING MALARIA. GREEN LINES INDICATE ENHANCEMENT, RED LINES INDICATE DEPRESSION AND BLACK LINES INDICATE MODULATION.

The second hypothesis proposes that NO is overproduced during *Plasmodium* infection and that NO has a role in pathogenesis. For example, Clark et al. proposed that iNOS-derived overproduction of NO in the brain might disrupt the regulatory role of NO in the CNS, leading to the impaired consciousness of cerebral malaria (CM) [3]. Indeed, elevated levels of iNOS protein are detected in the cerebral-blood-vessel walls of patients who have succumbed to CM [3]. In addition, markedly increased levels of NOx have been observed in the cerebrospinal fluid (CSF) of children who died from malaria [31]. NO has also been implicated in the pathology of malaria anemia; plasma [32] and urine [5] NOx levels and ex vivo peripheral blood mononuclear cell (PBMC) iNOS activity [33] have been reported to correlate inversely with Hb levels.

Several lines of evidence argue against the hypothesis that NO is overproduced during malaria and that the excess NO functions systemically to mediate severe malaria pathogenesis. First, the presence of hypoargininemia in malaria patients suggests that NO production might be limited in malaria [6]. Human erythrocytes contain arginase and, consequently, the rupture of RBCs by the parasite will increase plasma arginase levels; this is a mechanism of hypoargininemia in sickle-cell patients [10]. Second, plasma and urine NOx levels correlate inversely with disease severity [7,34]. The results of some earlier studies seem to contradict this finding but these studies did not properly account for the effects of diet, dehydration and renal impairment [35]. It is important to note that the endpoint of all NO reactions is NOx, so it cannot be discerned what impact, if any, the NO had on physiological processes (e.g. binding to sGC or nitrosylation of a protein). Finally, PBMC iNOS levels [7,36] and iNOS mRNA levels [37] correlate inversely with disease severity. Collectively, these observations suggest that NO is not overproduced and that iNOS-derived NO might be beneficial rather than harmful.

Although controversial because studies in different regions report opposing results, several investigations report that polymorphisms in the promoter region of the iNOS gene (G-945C and/or C-1173T) of human malaria patients, which result in higher baseline NOS activity in PBMCs, are associated with protection from severe *P. falciparum* malaria [38]. If correct, these findings indicate that increased production of NO might be beneficial, either by killing the parasite or by preventing the development of disease. We propose that prevention of disease development is more likely because of the minimal evidence supporting parasite killing by NO (Table 1).

Table 1. Effect of NO on Plasmodium parasites and its involvement in malaria pathogenesis

Plasmodium species	Effect of NO on parasite	Effect of NO on pathogenesis
Plasmodium falciparum	No killing in vitro [29] or in vivo [69,70]	NO is beneficial iNOS protein levels and mRNA correlate inversely with disease [7,36,37]; plasma and urine NOx levels correlate inversely with disease [7,34]; hypoargininemia correlates with P. falciparum disease severity [6]
		NO is harmful CSF NOx levels correlate with death [31]; elevated iNOS levels in CM brains [3]; NOx levels and iNOS activity correlate inversely with total Hb levels [5,32,33]
		Controversial Role of NOS promoter mutations [38]
Plasmodium berghei	No killing [39,71][a]	iNOS or eNOS deficiency or inhibition has no effect [39,71][a]
Plasmodium chabaudi	Killing [72]	iNOS deficiency or inhibition increases mortality rate [72,73]
	No killing [73–76]	iNOS deficiency or inhibition has no effect [74–76]
Plasmodium yoelii	No killing [77]	NOS inhibition increases mortality rate [77]

[a]P. Sobolewski et al., unpublished.

Furthermore, studies in animal models do not support the hypothesis that NO overproduction mediates malaria pathogenesis or is antiparasitic (Table 1). Neither *Plasmodium berghei* ANKA (PbA)-infected iNOS-deficient mice nor mice treated with aminoguanidine (AG), a NOS inhibitor, are protected from

severe malaria, both exhibiting similar parasitemia, pathogenesis and mortality to controls [39]. These observations indicate that overproduction of NO does not have a pathogenic role during malaria pathogenesis.

One interpretation of the data from animal models is that NO overproduction has a minimal role, if any, in the development of severe experimental malaria because neither inhibition of NOS nor use of NOS-deficient mice had a measurable effect on pathogenesis. However, it is rare for the rate of malaria mortality to be increased; to our knowledge, there is only one publication reporting more-rapid mortality in experimental (treated or knockout) mice compared with controls [40], and that article challenges a report of opposing results using the same polyclonal antiplatelet serum [41]. In addition, mice lacking other molecules with demonstrated efficacy at ameliorating experimental severe malaria (ESM) do not exhibit increased mortality rates. For example, we observed that IL-10 is present in the plasma of PbA infected mice and that IL-10$^{-/-}$-knockout mice become moribund at the same time as control mice [42,43]. However, treatment with this anti-inflammatory cytokine ameliorates the development of ESM [44]. Finally, an increased mortality rate is not expected in NOS-deficient mice if NO bioavailability during malaria is low owing to NO scavenging or low production; this is because decreasing NO production through knockout of NOS would not further diminish already minimal NO levels. To date, the preponderance of evidence indicates that NO production is diminished in subjects with severe *P. falciparum* malaria compared with in uncomplicated controls. It remains to be determined whether low NO bioavailability—caused by NO scavenging—during malaria contributes to malaria pathogenesis. There are two main candidates for NO scavenging: Hb and superoxide.

NO bioavailability is limited by free Hb

Based on the extensive literature about the role of free Hb both as a blood substitute [45] and during sickle-cell anemia [46], free Hb released during the asexual cycle of blood-stage *Plasmodium* might quench NO, thereby having an important role in limiting NO bioavailability during malaria. Low NO production as a consequence of hypoargininemia represents another mechanism [6]. During the course of malaria pathogenesis, there are two possible mechanisms of hemolysis. First, *Plasmodium* parasites rupture erythrocytes after completing their development within them, thus releasing progeny merozoites. The rupture will also release the remaining contents of the erythrocyte, which includes Hb. Second, there are more-complex, immune-system-mediated mechanisms of erythrocyte lysis leading to malaria anemia [47]. Complement-mediated erythrophagocytosis has long been postulated as a major mechanism of malaria anemia [48] but we speculate that other, non-phagocytic mechanisms such as T-cell-mediated and TNF-mediated cytotoxicity [49] might also be involved.

Free-Hb levels are markedly increased in patients with *P. falciparum* malaria [8], by up to an order of magnitude (15-μM heme) in acute cases [9], and we have observed elevated levels of cell-free Hb in the plasma in experimental *P. berghei* malaria. The in vivo effects due to free, natural molecular Hb in blood

are well defined within the research community interested in the development of molecular-Hb-based oxygen-carrying blood substitutes [45]. Free Hb is a powerful in vivo scavenger of NO, leading to vasoconstriction and impaired microvascular blood perfusion, which are major determinants of tissue and organism survival [50]. The efficiency of free Hb at scavenging NO is almost 1000-fold that of Hb packaged in RBCs [51]. NO reacts with the oxygen in oxyhemoglobin (oxyHb), generating methemoglobin [metHb or $Hb(Fe^{3+})$] and nitrate (Equation 1). NO also reacts with deoxyHb to form $Hb(Fe^{2+})NO$, which then reacts with oxygen to form metHb and nitrate (Equation 2).

$$Hb(Fe^{2+})O_2 + NO^{\cdot} \rightarrow Hb(Fe^{3+}) + NO_3^- \quad (1)$$

$$Hb(Fe^{2+}) + NO^{\cdot} \rightarrow Hb(Fe^{2+})NO + O_2 \rightarrow Hb(Fe^{3+}) + NO_3^- \quad (2)$$

Parasite-derived hemozoin (Hz) granules, which are vacuoles within the parasite where it stores the heme moiety of consumed Hb, are released with Hb during rupture of parasitized RBCs. Hz granules are unlikely to scavenge NO because the iron moiety of Hz is in the ferric Fe^{3+} state—as with metHb—which has a lower affinity (10 000 times) than the ferrous Fe^{2+} state for NO [20]. Recent evidence suggests that Hz increases the levels of lipopolysaccharide-induced and IFN-ɣ-induced iNOS transcription and NO production in PBMCs in vitro [33]; it remains to be determined whether this mechanism leads to increased NO production in vivo in *P.falciparum* patients.

Confirming the role of free Hb in scavenging NO during ESM, we have observed marked in vitro quenching of NO by the plasma of mice six days after infection with *P. berghei*, and this quenching correlated well with the increase in plasma Hb levels. Moreover, the quenching was abrogated by cyanide–ferricyanide inactivation of the Hb, indicating that free Hb in plasma during malaria quenches NO. These results clarify previous observations that NOS inhibition and NOS deficiency did not affect ESM pathogenesis; NO bioavailability is already limited by NO quenching in the plasma and, thus, treatment with NOS inhibitors or the use of NOS-deficient mice does not decrease NO bioavailability further.

An argument against the role of free Hb in malaria pathogenesis is that free Hb is released during other diseases such as congenital hemolytic anemias without eliciting the impaired mental status and respiratory distress observed during severe *P. falciparum* malaria. However, less inflammation is elicited during these congenital anemia syndromes than during malaria, and there is no sequestration of parasites to cerebral and lung endothelium, as observed during *P. falciparum* infection. Gladwin's group proposes that many of the vascular complications common in hemolytic disorders are attributable to NO quenching by free Hb [10]. Indeed, the amelioration of sickle-cell anemia by administration of the NO donor sodium nitroprusside or by inhalation of NO gas indicates that NO quenching by Hb has a crucial role in the pathogenesis of sickle-cell anemia, which is another inflammatory disease [46].

If the low-NO-bioavailability hypothesis is correct, the malaria inflammatory response, in addition to increased endothelial activation (Figure 2), lacks feedback regulation by NO. This inflammatory re-

sponse also results in increased production of superoxide, which rapidly reacts with NO to form the labile radical peroxynitrite, thus decreasing NO bioavailability [52]. Furthermore, peroxynitrite is an extremely damaging oxidant [53], particularly in the brain [54] where its formation might reconcile Clark's hypothesis that NO is involved in pathogenesis, with low NO bioavailability functioning in the vasculature. Another potential toxic consequence of free Hb in plasma is the potent ability of Hb to generate radicals, which have been shown to cause vascular leak [45]. Similarly, metHb, produced as a result of NO scavenging by Hb, is labile and readily dissociates into free heme, which is a highly pro-inflammatory molecule [55]. A buildup of heme at sites of hemorrhage is a potent recruitment signal for inflammation at that site [55]. Thus, the low NO bioavailability, in concert with the malaria-specific inflammatory response, probably mediates the impaired consciousness that is observed in malaria but not in the other hemolytic disorders.

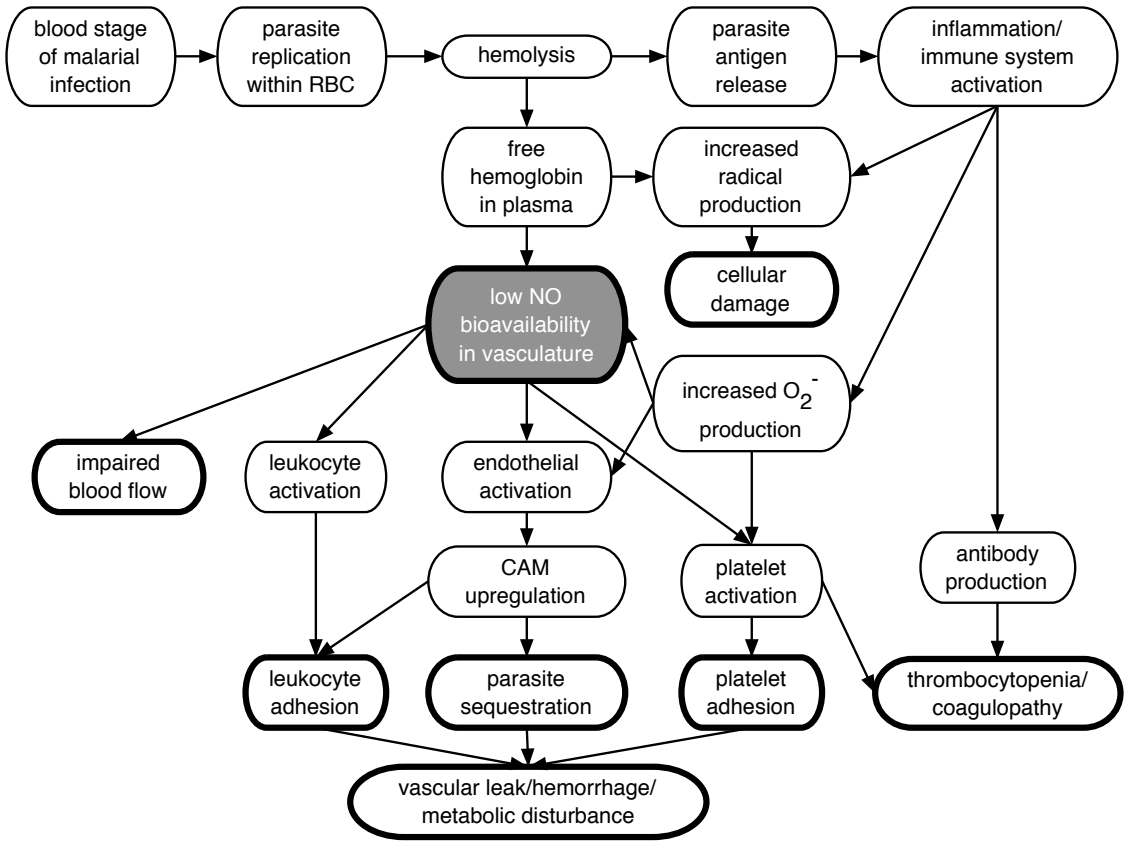

Figure 2. Hypothesis of low NO bioavailability. Image illustrates the key role that NO bioavailability might have in malaria. Restoring NO bioavailability by reducing quenching or providing exogenous NO might ameliorate the downstream consequences (e.g. vascular leak and thrombocytopenia) during malaria of impaired NO bioavailability due to Hb and superoxide scavenging. Thick ovals represent end-stage hallmark effects of malaria pathogenesis.

NO bioavailability is limited by superoxide

The rupture of infected erythrocytes caused by the release of parasite progeny also results in the release of malaria glycosylphosphatidylinositol (GPI)-anchored proteins and Hz. Both GPI and Hz elicit a strong pro-inflammatory response in monocytes and neutrophils [56,57] that leads to increased production of ROS, including superoxide, causing oxidative stress [58]. Studies of several fields, including ischemia–reperfusion injury, cardiovascular disease [59], diabetes [60] and sickle-cell anemia [61], have implicated oxidative stress in reducing the bioavailability of NO [62] and in pathogenesis. Of particular relevance to malaria is the fact that NO scavenging by superoxide has been implicated in endothelial dysfunction [59], platelet activation [63] and regulation of immune response [25].

Possible sources of vascular superoxide include phagocyte NAD(P)H oxidase (NOX2), endothelium NAD(P)H oxidase (NOX4), xanthine oxidase and uncoupled members of the NOS family. Increased phagocytic NAD(P)H oxidase activity is expected to occur as a result of the host inflammatory response. Increased endothelial production of superoxide has been observed in response to proinflammatory cytokines such as TNF [64] and IL-1 [65] that are upregulated during malaria pathogenesis [43]. Xanthine oxidase functions in the pathology of sickle-cell anemia [61] and might be an important component of innate immunity [66]. Finally, members of the NOS family can produce superoxide following depletion of arginine or tetrahydrobiopterin (a necessary cofactor) [12], which might exacerbate the effects of the hypoargininemia observed during *P. falciparum* malaria [6]. Superoxide might also mediate Hb scavenging of NO because Hb is not only a potent NO scavenger by itself but also a generator of superoxide by autooxidation of oxyHb. Within the erythrocyte, autooxidation of oxyHb is balanced by the presence of superoxide dismutase (SOD), catalase and metHb reductase [67] but, in plasma, this process is uncontrolled. Thus, it is likely that malaria inflammation results in increased NO quenching by superoxide.

In confirmation of the role of superoxide in limiting NO bioavailability during malaria pathogenesis, our group and others have observed marked protection of mice infected with *P. berghei* by treatment with pegylated SOD (PEG–SOD) and pegylated catalase (PEG–CAT), compared with PEG–CAT treatment alone or vehicle controls [68]. All three groups had similar parasitemia and, thus, the results cannot be attributed to differences in infection or parasite replication. Furthermore, we observed a marked reduction in the profound thrombocytopenia that is a hallmark of malaria, suggesting either that NO bioavailability is increased by the PEG–CAT+PEG–SOD treatment (because NO is a potent inhibitor of platelet activation) or that superoxide contributes to malaria thrombocytopenia.

NO bioavailability and resistance to severe malaria

Any hypothesis regarding malaria pathogenesis must account for the fact that only 1–2% of the individuals infected with *P. falciparum* develop severe malaria and succumb to the disease. The aforementioned

clinical studies correlating iNOS promoter polymorphisms with NO production and survival from severe malaria suggest that elevating NO production might be a mechanism to overcome NO scavenging and prevent the development of disease. However, the presence of hypoargininemia and low levels of plasma NOx in patients with severe P. falciparum malaria indicates that these patients might have insufficient amounts of NOS substrate to elevate NO production. A variety of other factors such as malarial inflammation probably functions in concert with free-Hb toxicity to mediate malaria pathogenesis. Differences in the inflammatory response (e.g. decreased production of pro-inflammatory cytokines) might inhibit the pathogenic process (Figure 2) independently of free-Hb levels. Furthermore, decreasing the immune response might reduce hemolysis, thus decreasing free-Hb levels and ameliorating free-Hb exacerbation of the inflammatory response. Differences in activation of the coagulation cascade might inhibit the pathogenic process independently of free-Hb levels but might also decrease vascular leak and petechial hemorrhage, thus decreasing both inflammation at the hemorrhage site and release of free Hb. Finally, more-efficient means of clearing plasma Hb or increased expression of antioxidants such as SOD and catalase might be beneficial by reducing NO scavenging. Thus, increasing the levels of bioavailable NO, either by increasing the release of NO in the vasculature or by mitigating NO scavenging, might serve as an adjunct or anti-disease treatment for malaria.

Restoration of NO bioavailability is being tested therapeutically for diseases such as acute respiratory distress, cardiovascular disease, diabetes and sickle-cell anemia. We have observed that treatment with a NO donor results in marked protection of mice infected with *P. berghei* compared with vehicle controls. Both groups had similar parasitemia; thus, the results cannot be attributed to differences in infection, parasite replication or parasite killing by NO. We conclude that treatment with the NO donor restored NO bioavailability by overcoming the quenching that occurs during the course of malaria. The NO can reach one of its targets (sGC) and carry out its homeostatic role. Overall, our interpretation of data from malaria and other diseases is that NO bioavailability is limited during malaria and exacerbates malaria inflammation, endothelial activation and coagulopathy (Figure 2).

Concluding remarks

To develop effective adjunct or anti-disease treatment for malaria, the mechanisms of malaria pathogenesis must be understood. We believe that the data indicate that NO bioavailability is low within the vasculature during malaria because levels of NO production are low owing to hypoargininemia and increased quenching of NO by free Hb and superoxide. NO has a potent role in vascular homeostasis that is disrupted during malaria pathogenesis. Restoring NO bioavailability by diminishing NO quenching, providing exogenous NO or restoring arginine levels might, therefore, be an effective anti-disease therapy. It remains to be determined whether restoring NO bioavailability within the vasculature late in the disease is beneficial. Finally, it is still unclear whether, as proposed by Clark et al. [3], NO produced within tissue such as the brain might function in pathogenesis.

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