Severe malaria increases the list of heme oxygenase-1-protected diseases

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'...the anti-inflammatory molecule heme oxygenase-1 prevents the development of experimental cerebral malaria.'

Malaria is the most widespread and lethal parasitic disease in the world today, and claims the lives of at least 1 million people every year. The disease is caused by parasitic protozoa of the genus *Plasmodium*, whose life cycle involves a vertebrate host and a mosquito vector. The parasite undergoes two different stages of infection within the mammalian host. The first stage occurs inside hepatocytes and is clinically asymptomatic. The symptoms associated with the disease appear only during the subsequent stage of infection, which occurs inside erythrocytes.

In humans, clinical manifestations of malaria can range from mild to severe malaria, the latter comprising a series of syndromes that include, among others, cerebral malaria (CM) and acute respiratory distress syndrome. Interestingly, although four *Plasmodium* species infect humans (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*), only one of these parasites, *P. falciparum*, causes fatal disease. The reason(s) for this difference are yet to be fully elucidated. The sequestration of infected erythrocytes in vital organs, a feature unique to *P. falciparum* infection, has been identified as a major factor. However, whether this feature causes disease due to mechanical blockage of blood flow or to the release of inflammatory mediators in vulnerable regions caused by local infected-erythrocyte rupture, is still a matter of controversy [1], and increasing evidence is suggesting a role for inflammatory mediators in malaria-associated pathology [2]. Thus, it has been proposed that the pathogenesis of malaria might not differ much from other clinically overlapping systemic diseases caused by other pathogens or even nonpathogen-dependent inflammatory syndromes [3].

Recently, we have used mice infected with *Plasmodium berghei* ANKA, a rodent model of malaria pathology, to demonstrate that the anti-inflammatory molecule heme oxygenase (HO)-1,

which degrades heme to generate biliverdin, iron and carbon monoxide (CO), prevents the development of experimental CM (ECM) [4]. Moreover, we demonstrated that exposure to the end-product of HO-1 activity, CO, also protected mice against ECM [4].

The anti-inflammatory effects of HO-1 activation were first reported in experimental sepsis [5], and later extended to inflammation in general [6]. Again, in these cases, CO was shown to be the mediator molecule between HO-1 activity and protection against inflammation [6]. More recently, it was postulated that the inhibitory effects of CO observed in systemic inflammation, and the role of TNF-induced HO-1, might also play a role in a malarial context [3]. In fact, HO-1 was detected in histological sections from human sepsis and malaria cases [7].

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Similarly to nitric oxide (NO), CO inhibits the generation of various proinflammatory cytokines by inhibiting the nuclear factor- κ B pathway [8]. Interestingly, NO, which induces HO-1 [9,10], was also shown to protect mice from ECM [11]. The protective effects of CO inhalation, in the context of ECM in mice, were associated with the inhibition of blood-brain barrier disruption, brain microvascular congestion and hemorrhage, neuroinflammation, and the suppression of activated CD8⁺ T-cell sequestration in the brain, but without any effect in the parasitemia [4]. These findings not only provide new insights into the pathogenesis of CM but also contribute evidence to the idea that death caused by CM is more inflammatory in nature than simply the result of mechanical obstruction. These findings also raise the question concerning their protective effect in other malaria-associated pathologies. Our preliminary studies suggest that CO might also counteract the development of a respiratory distress-like syndrome in mice [Pamplona and Mota, Unpublished Data]. Again, this strongly

points to a common genesis in apparently distinct malaria-associated syndromes [3]. Moreover, these findings also suggest, as previously proposed [3], that severe malaria does not appear to be a unique disease, instead presenting many features common to other cytokine-induced pathologies or inflammatory syndromes. In fact, activation of HO-1 expression has been shown to rescue several inflammatory states, including skeletal muscle contractile failure [12], experimental autoimmune encephalomyelitis (EAE) [13], endotoxin-induced endothelial cell activation [14], uveitis [15], focal ischemia–reperfusion cardiac injury [16], and rejection of transplanted tissue [17]. Even some features that appear to be very specific to CM, such as blood–brain barrier compromise, also occur in other systemic inflammatory states, such as severe childhood influenza [18]. In view of such a broad protection conferred by HO-1, we speculate that a basic, common mechanism underlies this protective effect.

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In our recent findings concerning CM, the protective mechanism of CO seems to be mediated by the binding of CO to hemoglobin, preventing its oxidation and the generation of free heme, a molecule we showed to contribute to the development of ECM in mice [4]. Interestingly, the reported protective mechanism of NO in ECM also appears to involve the binding of NO to hemoglobin to prevent the generation of free heme [11]. In fact, using different combinations of mouse and *P. berghei* strains, we have correlated the level of free heme and the development of CM [4]. However, it seems unlikely that

protection against certain inflammatory states such as EAE [13] and uveitis [15] would be dependent on free hemoglobin. Thus, whether another more universal mechanism of protection is operating remains to be elucidated.

The fact that exogenous CO leads to a decrease in neuroinflammation and protects blood–brain barrier integrity, suppressing the onset of ECM in mice without affecting parasite load [4], raises the important issue of whether it should be considered as a novel potential adjunct therapy together with the classical anti-malarial treatments. At present, there is controversy in using adjunctive therapies in the treatment of CM, since a series of adjuncts have been tested in randomized controlled trials, and none successfully reduced mortality [19]. Nevertheless, since CO can fully protect mice from developing ECM when administered before the onset of ECM symptoms [4], it is conceivable that children below 5 years of age (at high risk of developing fatal disease) who are admitted to hospital with *P. falciparum* infection could receive CO by inhalation. Moreover, pharmacological administration of CO to tissues using CO-releasing molecules may, in the future, prove to represent a viable alternative to the inhalation of gaseous CO. Most of the data from experimental models of inflammatory diseases such as sepsis and now, CM, pave the way in considering CO as a potential therapeutical molecule, and research in this direction should be pursued.

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