Published in final edited form as:
Crit Care Med. 2009 February ; 37(2): 758–760. doi:10.1097/CCM.0b013e318194d5de.

Antioxidant therapy: Reducing malaria severity?*

Hans C. Ackerman, MD, DPhil, MSc,
Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Rockville, MD, Critical Care Medicine Department, National Institutes of Health Clinical Center, Bethesda, MD

Steven D. Beaudry, BS, and
Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Rockville, MD

Rick M. Fairhurst, MD, PhD
Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Rockville, MD

Keywords

oxidative stress; cytoadherence; *Plasmodium falciparum*; endothelium; artesunate; *N*-acetylcysteine; lactate; glucose-6-phosphate de-hydrogenase deficiency

Each year, infection with *Plasmodium falciparum* causes 300 – 600 million illnesses worldwide (1). A significant number of patients will progress to severe malaria with organ dysfunction, which is more common in the immunologically naïve: young children and travelers or other adults with only sporadic exposure to infected mosquitoes. Mortality from severe malaria ranges from 5% to 40%, and death often occurs shortly after hospital admission. Despite improved survival with artesunate treatment (2), mortality remains high in the first 24 hours of hospitalization, and therefore, adjunctive therapies are still urgently needed.

Neither hypovolemia, cardiogenic shock, nor vasodilatory shock is necessary for organ dysfunction in severe malaria (3). The impairment of tissue perfusion occurs within microvessels, where parasitized red blood cells (RBCs) adhere to endothelial cells and circulating immune cells via specific interactions between the parasite-encoded adherence ligand *P. falciparum* erythrocyte membrane protein 1 on the RBC surface and the host-encoded adhesion receptors cluster of differentiation 36 and intercellular adhesion molecule-1 (4). Adherence is associated with inflammatory cytokine secretion, adhesion molecule expression, tissue factor display, and platelet aggregation (5)—findings that are evident on postmortem examination of patients with severe malaria (6). Microcirculatory dysfunction may be worsened as nitric oxide is scavenged by cell-free hemoglobin released during hemolysis and by reactive oxygen species (7).

In severe malaria, reactive oxygen species are generated by parasite hemoglobin metabolism in RBCs, nicotinamide adenine dinucleotide phosphate oxidase in phagocytes, and nitric oxide synthase when the substrate arginine or cofactor tetrahydrobiopterin is lacking. Plasma hemoglobin released from lysed RBCs may also catalyze the generation of reactive oxygen species. Patients with severe malaria have increased reactive oxygen species products in urine

*See also p. 516.

The authors have not disclosed any potential conflicts of interest.

(8), decreased alpha-tocopherol in RBC membranes (9), and decreased deformability of RBCs under shear stress. Decreased deformability of RBCs is associated with mortality (10); this was the impetus for the study by Charunwatthana et al (8), who hypothesized that decreased deformability impedes the transit of RBCs through capillaries, impairing oxygen delivery. Because *N*-acetyl-cysteine (NAC) restores normal deformability of RBCs *in vitro* by repleting glutathione reserves (11), treatment with NAC was expected to improve RBC deformability and oxygen delivery *in vivo*.

However, oxidant stress in RBCs could offer some compensatory benefits to a patient with severe malaria. First, oxidative stress is a fundamental mechanism for killing phagocytosed pathogens, including *P. falciparum*. Treatment with a potent antioxidant might conceivably protect parasites from the oxidative burst of phagocytes and could antagonize the oxidation-mediated antimalarial effects of artesunate. *In vitro*, the parasiticidal effect of artesunate is reduced when given simultaneously with NAC, but is unaffected when NAC is given 2 hours after artesunate (12). In the present study, Charunwatthana et al administered NAC 2 hours after the first dose of artesunate and measured parasitemia every 6 hours to see whether NAC would impair parasite killing by artesunate.

Second, oxidative stress in RBCs may play a role in weakening adherence interactions between the parasitized RBCs and the microvascular endothelium. This is best illustrated by the classic protective effect of sickle cell trait against severe malaria. Infected RBCs from patients with sickle cell trait show aberrant display of the parasite-encoded adhesion ligand *P. falciparum* erythrocyte membrane protein 1 and reduced strength of adhesion to micro-vascular endothelial cells and blood monocytes *in vitro* (13). This phenotype may be reproduced in RBCs with glucose-6-phosphate dehydrogenase deficiency or other states of oxidative stress.

Third, although oxidative stress in infected RBCs might be beneficial, oxidative stress in endothelial cells causes adhesion molecule expression, apoptosis, and capillary leak. Endothelial cell injury by adherent parasitized RBCs is ameliorated by superoxide dismutase, ascorbic acid, or tocopherol *in vitro*, highlighting the potential therapeutic benefit of antioxidants as endothelial cell protectors (14).

So what will be the effect of a systemic antioxidant in severe malaria? In the study by Charunwatthana et al (8), adults with severe malaria were treated with artesunate and randomized to receive NAC (dosed as for acetaminophen overdose) or placebo. NAC had no effect on the primary or secondary end points of the study: lactate clearance time, coma recovery time, parasite clearance time, fever clearance time, or mortality. End products of oxidative stress (F2-isoprostanes) were found to be elevated in the urine of patients with severe malaria compared with uncomplicated malaria, but were unchanged by NAC treatment. Although red cell deformability was lower in fatal cases than in survivors of severe malaria, it was not changed by NAC treatment.

Why was NAC ineffective? In a pilot study (15), 30 patients were randomized to NAC or placebo, and at 24 hours a greater proportion of patients in the NAC-treated arm had normal lactate levels compared with the placebo arm (10 of 15 vs. 3 of 15, $p = 0.01$); however, the placebo group had higher baseline parasitemia and bilirubin and lower Glasgow coma scores than the treatment group. Baseline differences in disease severity could have overestimated the benefit, if any, of NAC treatment. As a consequence, the current study may have been under-powered to detect the true effect of NAC treatment on severe malaria. Antimalarial treatment was also different in the current study: NAC was given 2 hours after artesunate, a pro-oxidant drug, compared with NAC being given simultaneously with quinine in the pilot study. Furthermore, genetic polymorphisms that influence RBC oxidative stress (such as

glucose-6-phosphate dehydrogenase deficiency) were not accounted for, but could have modified the response to antioxidant therapy.

In hindsight, it is unclear what effect NAC should have had on severe malaria. Oxidative stress is considered beneficial for parasite killing and for weakening the adherence between infected RBCs and endothelial cells or monocytes. On the other hand, oxidative stress causes endothelial injury and impairs RBC deformability. The results of the current study are neutral. Does this close the book on antioxidants for severe malaria? No, but future strategies might specifically target antioxidants to the endothelium while enhancing oxidative stress in infected RBCs.

On second thought, combining a systemic antioxidant NAC with an RBC-specific pro-oxidant artesunate may have accomplished just that.

References

1. Snow RW, Guerra CA, Noor AM, et al. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature 2005;434:214–217. [PubMed: 15759000]
2. Dondorp A, Nosten F, Stepniewska K, et al. Artesunate versus quinine for treatment of severe falciparum malaria: A randomised trial. Lancet 2005;366:717–725. [PubMed: 16125588]
3. Planche T, Onanga M, Schwenk A, et al. Assessment of volume depletion in children with malaria. PLoS Med 2004;1:e18. [PubMed: 15526044]
4. Ockenhouse CF, Ho M, Tandon NN, et al. Molecular basis of sequestration in severe and uncomplicated *Plasmodium falciparum* malaria: Differential adhesion of infected erythrocytes to CD36 and ICAM-1. J Infect Dis 1991;164:163–169. [PubMed: 1711552]
5. Francischetti IM, Seydel KB, Monteiro RQ. Blood coagulation, inflammation, and malaria. Microcirculation 2008;15:81–107. [PubMed: 18260002]
6. Taylor TE, Fu WJ, Carr RA, et al. Differentiating the pathologies of cerebral malaria by postmortem parasite counts. Nat Med 2004;10:143–145. [PubMed: 14745442]
7. Gramaglia I, Sobolewski P, Meays D, et al. Low nitric oxide bioavailability contributes to the genesis of experimental cerebral malaria. Nat Med 2006;12:1417–1422. [PubMed: 17099710]
8. Charunwatthana P, Faiz MA, Ruangveerayut R, et al. *N*-acetylcysteine as adjunctive treatment in severe malaria: A randomized double blinded placebo controlled-clinical trial. Crit Care Med 2009;37:516 – 522. [PubMed: 19114891]
9. Griffiths MJ, Ndungu F, Baird KL, et al. Oxidative stress and erythrocyte damage in Kenyan children with severe *Plasmodium falciparum* malaria. Br J Haematol 2001;113:486–491. [PubMed: 11380421]
10. Dondorp A, Angus BJ, Hardeman MR, et al. Prognostic significance of reduced red blood cell deformability in severe falciparum malaria. Am J Trop Med Hyg 1997;57:507–511. [PubMed: 9392587]
11. Nuchsongsin F, Chotivanich K, Charunwatthana P, et al. Effects of malaria heme products on red blood cell deformability. Am J Trop Med Hyg 2007;77:617– 622. [PubMed: 17978059]
12. Arreesrisom P, Dondorp AM, Looareesuwan S, et al. Suppressive effects of the anti-oxidant *N*-acetylcysteine on the anti-malarial activity of artesunate. Parasitol Int 2007;56:221–226. [PubMed: 17544319]
13. Cholera R, Brittain NJ, Gillrie MR, et al. Impaired cytoadherence of *Plasmodium falciparum*-infected erythrocytes containing sickle hemoglobin. Proc Natl Acad Sci USA 2008;105:991–996. [PubMed: 18192399]
14. Taoufiq Z, Pino P, Dugas N, et al. Transient supplementation of superoxide dismutase protects endothelial cells against *Plasmodium falciparum*-induced oxidative stress. Mol Biochem Parasitol 2006;150:166 –173. [PubMed: 16930739]
15. Watt G, Jongsakul K, Ruangvirayuth R. A pilot study of *N*-acetylcysteine as adjunctive therapy for severe malaria. QJM 2002;95:285–290. [PubMed: 11978899]