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Commentary

Ascorbic acid co-administration with artemisinin based combination therapies in falciparum malaria

Malaria infection poses a serious public health problem in endemic countries. As per World Malaria Report 2015, it is estimated that 3.2 billion people in 97 countries are at risk of being infected with malaria; 214 million cases of malaria with 4,38,000 malaria induced deaths are reported during the year[1]. Clinical consequences of malaria result primarily from parasitic invasion of red blood cells (RBCs). Inside the RBCs, the parasite metabolizes the host haemoglobin in the acidic environment of the parasite's food vacuole. This leads to production of free haem which contains Fe^{2+} atoms that can catalyze Fenton and Haber-Weiss reactions, generating the radicals which can cause extensive molecular damage[2]. Haemolysis of infected red cells releases free haem which may be responsible for an external oxidative stress on both infected and non-infected RBCs[2] and may be one of the factors contributing to destruction of normal red cells. Presence of pro-oxidants in plasma of patients suffering from acute falciparum malaria has been demonstrated by measurement of the erythrocyte thiobarbituric acid-reactive substance concentrations[3]. The parasite develops antioxidant mechanisms partly through reducing its own generation of reactive oxygen species and partly through increased synthesis of reduced glutathione and thioredoxin reductase system[2]. During intraerythrocytic development of *Plasmodium falciparum*, a large number of parasite proteins move beyond its own plasma membrane and associate with RBC membrane cytoskeletal proteins. Parasite and host red cell cytoskeletal protein interactions lead to formation of large molecular complexes which appear as electron dense "knobs" on the RBC surface. These changes lead to alterations in the rheological properties of infected red cells which become less deformable and more adhesive[4].

Artemisinin - based combination therapies (ACTs) are recommended by WHO as the first line treatment for uncomplicated *P. falciparum* malaria[5]. Artemether/lumefantrine (Coartem®) is one such ACT in which the artemisinin derivative is artemether and the second drug is lumefantrine - an aryl-aminoalcohol. Artemisinin derivatives have a quick onset of action and cause rapid reduction in parasitaemia. However, these have a short half-life and are not effective in complete elimination of the infection, and recrudescences of the infection have been observed after single drug therapy[6]. Hence, the addition of longer acting antimalarials was done in ACTs[6]. The exact mechanism of action of artemisinins in malaria infection is not completely understood. The active moiety is an 'endoperoxide bridge' within the molecule which is essential for the antimalarial action. Haem plays a predominant role in artemisinin activation, the active molecule binds to multiple parasite proteins and damages and disrupts parasite metabolic pathways and membrane transport channels through free radicals[7,8].

The source of haem required for artemisinin activation is the parasite's haem biosynthesis pathway at the early ring stage and from haemoglobin digestion at later stages. Thus high levels of haem in parasitized RBCs and preferential binding of drug to parasite proteins confer high specificity against malarial parasite within infected red cells as compared to normal red cells[8]. However, the high oxidant environment within and outside the red cells contributes to lysis of infected and to some extent the non-infected RBCs[2]. The artemisinin group of drugs are generally considered safe for patients, but in recent years an increasing number of reports of haemolytic anaemia following their administration have been reported in patients with severe falciparum malaria[9]. Artemisinins have

also been shown to alter the viscoelastic properties of red cells. Richards *et al*[10] tested viscoelasticity of RBCs of ten healthy female subjects using Coartem® in three different drug concentrations; low, normal (therapeutic dose) and high. There was a significant decrease in viscosity and elasticity at normal and high doses. The authors postulated a significant generation of free radicals at normal and high doses, which led to haemolysis and ultimately reduced viscoelasticity[10].

Ascorbic acid has antioxidant properties and is reported to mop up free radicals. Since malaria infection imposes tremendous oxidative stress on the host, the antimalarials are often prescribed with vitamin C or similar antioxidant supplements. The antioxidant effect in erythrocytes has been reported to depend upon the presence or absence of glutathione. In the presence of glutathione, ascorbic acid has synergistic anti-oxidant activity against haem-mediated cell toxicity[11]. In glutathione deficient red cells, as often happens in parasitized RBCs due to oxidative stress, ascorbic acid can react with iron or iron containing compounds to generate hydrogen peroxide or hydroxyl radical and accentuate the haemolytic mechanisms in malaria[11,12]. Vitamin C may have additional detrimental effects in malaria. Results from an experimental study have shown that concurrent administration of artemether and ascorbic acid compromised the rates of parasite clearance in *P. berghei* malaria infection in mice. This effect was more pronounced at higher doses of ascorbic acid. The high doses of vitamin C by itself could inhibit growth of malarial parasite to some extent[13].

In the article by McKoy *et al*[14] in this issue, the authors designed an *in vitro* study to observe the effects of co-incubation of artemether/lumefantrine combination (Coartem®) with vitamin C on the viscosity and elasticity of blood. The study was conducted on blood samples from 12 healthy female volunteers free from sickle cell disease/trait. Female volunteers were chosen to exclude intrinsic red cell confounding factor for haemolysis like glucose-6 phosphate dehydrogenase (G6PD) deficiency. The viscosity and elasticity of blood incubated with therapeutic concentration of Coartem® and low/high concentration of vitamin C were significantly reduced. The decrease was more pronounced with high dose of vitamin C. The decrease in viscosity and elasticity could probably result from decrease in haematocrit brought about by haemolysis. This study re-emphasises the need to further evaluate the role of ascorbic acid in malaria infection and its interaction with antimalarial drugs particularly artemisinins. This also raises concerns about concurrent administration of antioxidant supplements like ascorbic acid which can exert a dose-dependent pro-oxidant effect by increasing intracellular hydrogen peroxide generation, especially in an environment of free haem, a powerful generator of free radicals, as happens during the intraerythrocyte growth of malarial parasite[12]. The increased oxidative stress results in lipid peroxidation of red cell membranes causing structural and functional changes which lead to haemolysis. These observations acquire further relevance in view of the reported haemolytic complications.

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