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Potential Role of Chloroquine in Macrophageal Iron Mobilization

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Abstract

Malaria anaemia is still a major killer of children in malaria endemic countries. We have shown from the published literature that malaria anaemia, especially mild to moderate anaemia is largely due to inflammation-induced hypoferreamia and immune-mediated destruction of parasitized and non-parasitized red blood cells. In this review, we speculate that the immunomodulatory and anti-inflammatory properties of chloroquine, a cheap, readily available antimalarial, may make it a very good candidate for investigation as a possible adjunct treatment for malaria-associated anaemia.

Keywords: Malaria; Anaemia; Chloroquine; Immune-modulation; Anti-inflammatory

Introduction

Anaemia remains a major global public health problem, affecting about 2 billion people, 89% of who live in developing countries [1,2]. It reduces the quality of life of children, and is a significant cause of morbidity and mortality in children and pregnant women [2,3]. While globally, the commonest cause of anaemia is iron deficiency [3-5], in malaria endemic areas, malaria contributes significantly to the pathogenesis of anaemia [3,5]. In such settings, poor response to treatment due to either inappropriate treatment or resistance to the antimalarial drugs by *Plasmodium* increase the risk of anaemia [6]. The complex pathogenesis of malaria-associated anaemia and the controversies generated by the perceived effect of iron administration on morbidity during malaria calls for alternative interventions to combat malaria-associated anaemia.

Chloroquine, a drug that has traditionally been used as a first line antimalarial has been noted to possess a number of actions separate from its antimalarial properties, which has seen it used in many settings. Its good safety profile, wide availability, and relatively low cost make it an attractive candidate for investigation on possible roles in combating malaria-associated anaemia. This review examines the pharmacology of chloroquine and relates this to the pathophysiology of malaria-associated anaemia to assess the possible use of chloroquine in the management of malaria anaemia.

Malaria-associated anaemia

Malaria-associated anaemia has been defined as a reduction in the haemoglobin or haematocrit below normal for age, sex and state of pregnancy, in the presence of malarial parasitaemia of any density in endemic areas [7].

The pathophysiology of malaria anaemia involves iron delocalisation mediated by cytokines and hepcidin, similar to the pathophysiology of anaemia of inflammation [8]. Erythrocytes are lost during malaria through hemolysis of parasitized red blood cells, erythrophagocytosis of parasitized and unparasitized RBC, hypersplenism, autoimmune hemolysis from the deposition of immunoglobulins on the surfaces of red blood cells, and hapten-induced intravascular haemolysis resulting in haemoglobineamia and haemoglobinuria [6,7,9-10].

Reduced erythropoiesis in malaria results from hypoactivity of the bone marrow with poor response to erythropoietin (EPO), probably mediated by a serum factor that suppresses the growth of the burst-forming unit-erythron (BFU-E) and the colony forming unit-erythron (CFU-E), and inadequate production of erythropoietin by inflammatory mediators such as tumour necrosis factor-α (TNF-α) [11,12]. Other mechanisms for reduced RBC production include malaria-induced dyserythropoiesis, especially in persons with recurrent malaria [13,14], and disturbances in serum cytokine levels involving raised serum TNF-α, IL-10 and IFN-γ [11,13,15,16].

Co-morbidities especially with bacteria and viruses which are consequences of the immunosuppressive effects of *P. falciparum* malaria also contribute to reduced RBC production leading to anaemia. Parvovirus B19 has been particularly suggested as an important contributor to malaria anaemia because of its high prevalence among children in developing countries, and the tropism of the virus for erythroid progenitor cells. Recent studies have found a significant association between co-infection with pavovirus B19 with severe anaemia in Ghanaian and Gabonese children with malaria [17,18].

Folate deficiency is thought to play a minor role in the development of malaria anaemia [19-21]. In contrast, iron deficiency might develop during malaria through reduced intake from anorexia, reduced absorption, and increased loss in haemoglobinuria. Furthermore, poor dietary intake of iron could result in background iron deficiency which becomes more obvious during malaria.

The controversy regarding the benefit of giving iron to children with malaria, following reports of increased morbidity and mortality among children living in malaria endemic areas who were given iron supplements, necessitates the search for a more acceptable therapeutic option for combating malaria anaemia. Besides, there have been conflicting reports on the benefits of iron supplementation during acute malaria attack. Results of a case control study from the Gambia

suggest that increase Hb following an episode of malaria infection was unlikely to be due to iron supplementation [22]. The study compared the absorption of isotope-labelled iron in children with either presumed iron-deficiency alone or anaemia post-malaria. While haemoglobin increase was significantly higher at both days 15 and 30 post malaria in the malaria group compared to the group with presumed iron deficiency alone, incorporation of isotope labelled iron was significantly reduced in the malaria group. This finding collaborates those from a randomised control trial in Tanzania [23] in which 100 children with Hb <5 g/dL and a positive smear for malaria parasite, were randomised to receive either iron supplementation in addition to malarial treatment (quininine plus fansidar), or only malarial treatment. The Hb rise at 2 weeks and 12 weeks, respectively, was 3.7 g/dL and 9.2 g/dL in the group that received iron supplementation plus malarial treatment, and 3.5 g/dL and 9.0 g/dL in the group that received only malarial treatment, suggesting that iron supplementation did not affect the recovery of haemoglobin level in children with malaria-associated anaemia in this study. However, other studies have reported beneficial effects among iron supplemented children with malaria [24-26].

Iron metabolism in malaria

Body's iron is regulated primarily by iron absorption from the small intestine. There are no known specialised excretory pathways for iron in the body, and variable amounts are lost from desquamation of skin and mucosal cells. The factors that determine that amount of iron absorbed include a physiological need for iron, dietary iron intake, bioavailability of dietary iron and the ability of the mucosal cell to adapt to body's dietary needs [27]. Under normal physiological conditions, about 1-2 mg of iron is absorbed from the intestine daily, but this can be upregulated in iron deficiency states. However, the bulk of the body's iron requirements are met by the recycling of iron by the reticulo-endothelial system (monocyte-macrophage system), which is responsible for the phagocytosis and catabolism of senescent erythrocytes.

A ferrireductase at the intestinal brush border reduces ingested iron from the ferric to ferrous form, which is then actively transported across the apical enterocyte membrane by divalent metal transporter 1 (DMT1), also called natural resistance-associated macrophage protein 2 (NRAMP-2). The iron in the enterocyte can either be transported out of the cell by the action of ferroportin 1 (an active iron exporter) and hephaestin (a ferrooxidase), or lost when the enterocyte sloughs off. Iron in the body is stored as ferritin (labile, readily accessible), haemoglobin, and hemosiderin (insoluble iron found mostly in macrophages). Macrophages acquire iron from various iron-containing proteins in the body such as transferrin, lactoferrin, haptoglobin-Hb complexes, senescent red blood cells and possibly direct uptake of ferric and ferrous iron [28,29]. The iron acquired by macrophages and hepatocytes are re-exported for use by the bone marrow for erythropoiesis.

Inflammation induces hypoferraemia through sequestration of iron by the reticuloendothelial system and decreased intestinal iron absorption [30,31], mediated by hepcidin, a disulfide–bonded 25 amino acid peptide produced in the liver [32,33]. Similarly, malaria-induced inflammation impedes iron flux within the macrophage-monocyte system making iron unavailable to the marrow cells for erythropoiesis [34]. The effect of *Plasmodium* infection on erythropoietin production is unclear. While animal studies suggest a vigorous host erythropoietin response to *Plasmodium* infection [13], humans have shown conflicting results (reviewed by Menendez et al. [7]). And as already discussed, disordered cytokine response, many of which remain elevated for weeks after successful treatment for malaria, contributes to the pathogenesis and persistence of malaria anaemia [35]. The resolution of malaria anaemia therefore could be the result of iron re-mobilization in the macrophage-monocyte system following resolution of the malaria-induced inflammatory process; in which case administration of exogenous iron in malaria anaemia might be of little benefit to the recipients, thus accounting for the conflicting reports from iron supplementation studies.

In the search for alternative therapeutic approaches to malaria anaemia, one of the first areas of interest would be the antimalarials because the discovery of an effective antimalarial with an additional property of enhancing haematologic recovery will be of immense economic and clinical advantage.

Potential role of chloroquine in macrophageal iron mobilization

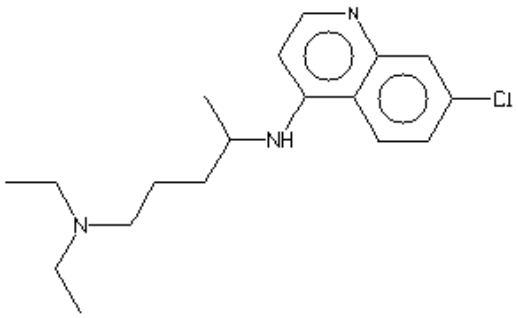
Almost globally abandoned as an antimalarial because of high rates of resistance, chloroquine could be an important adjunct treatment for malaria anaemia. It is widely acknowledged that even in areas where chloroquine resistance is high; the use of chloroquine could still confer some clinical and parasitological benefit. One of the properties that gives chloroquine this advantage is its antipyretic actions which provide relief to the patients. In addition to being cheap, safe and widely available, chloroquine is likely to return to use in much of the regions where it has previously been abandoned. For example, there have been reports of the return of chloroquine efficacy in Malawi 12 years after its withdrawal from use [36]. It is likely that similar experiences will be reported in other areas where chloroquine had been abandoned due to high resistance. Another reason chloroquine is attractive for investigation of a possible effect in macrophageal iron mobilization is because chloroquine has several pharmacological properties in addition to its antimalarial actions. As already mentioned, chloroquine has antipyretic properties; it also has anti-inflammatory activities for which it is used in many inflammatory conditions such as rheumatoid arthritis and connective tissue diseases.

Chloroquine pharmacodynamics

Chloroquine is a 7-chloro-4-[[4-(diethylamino)-1-methylbutyl]amino] quinoline- (molecular formula: C18H26ClN3), and has the following structural formula (Scheme 1).

It is a weak base and raises the endocytic and lysosomal pH of eukaryotic cells [37]. Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract, with only a small proportion appearing in the stool [38]. Peak plasma levels of 60-90 ng/mL are attained within 1-6 h following a single oral dose of 300 mg base of chloroquine, although there is considerable inter-individual variation, and depending on whether or not it is taken with food [38-40]. The absorption is also very good when given intramuscularly and via nasogastric tube. Chloroquine has a strong affinity for blood constituents particularly thrombocytes and granulocytes which reduces the plasma concentrations [41,42]. In addition, about 46-74% of chloroquine in the plasma is bound to plasma proteins mainly albumin and α-acid glycoprotein [43,44]. It is also avidly bound to several tissues in the body. In animals, from 200 to 700 times the plasma concentration may be found in the liver, spleen, kidney, and lung [45], and some melanin-containing tissues such as the retina, the

inner ear and hair follicles [46]. The brain and spinal cord, in contrast, contain only 10 to 30 times the amount present in plasma [45].



Scheme 1: Chloroquine.

Excretion of chloroquine is quite slow, but is increased by acidification of the urine. Chloroquine undergoes appreciable degradation in the body. The main metabolite is desethylchloroquine, which accounts for one fourth of the total material appearing in the urine; bisdesethylchloroquine, a carboxylic acid derivative, and other metabolic products as yet uncharacterized are found in smaller amounts. Slightly more than half of the urinary drug products can be accounted for as unchanged chloroquine. Chloroquine is eliminated slowly from the body and can be detected in the urine for more than a year after intake [47]. It has a multiexponential elimination pattern with an initial elimination phase with a half life of 3-6 days followed by a slower phase with a half life of 12-14 days. Terminal elimination of half-lives of chloroquine and its metabolite diethylchloroquine of up to 2 months have been reported, although it is thought that this half life is of minor importance in the elimination of the drug from the body. Between 45% and 56% of the total dose of chloroquine is eliminated from the urine within 3-13 weeks and about 8-10% are eliminated in the feaces [47].

Mechanisms of action of chloroquine

Chloroquine is primarily used as an antimalarial drug. It is a potent schizontocidal drug which is highly effective against the asexual forms of all the four species of *Plasmodium* that cause malaria in man – *P. falciparum, ovale, vivax* and *malariae*. In addition, it is active against the gametocytes of *P. vivax, ovale* and *malariae* but not against the gametocytes of *P. falciparum* [47]. It is thought that the mechanism of action of chloroquine against *Plasmodium* relates to its inhibition of the enzyme that polymerises and detoxifies ferriprotoporphyrin IX in the parasite food vacuole [48,49]. Chloroquine accumulates in the acid food vacuole of the intra-erythrocytic stage malaria parasite. The food vacuole primarily serves to degrade ingested red cell haemoglobin to provide the growing parasite with needed amino acids. This process releases haem which in the soluble form is harmful to biological membranes and inhibits a variety of enzymes. To avert this potential danger, the parasite detoxifies the haem by incorporating it into an insoluble crystalline compound called haemozoin or malaria pigment. A component of the *Plasmodium trophozoites* promotes the polymerization of haem to form haemozoin. This heme polymerase activity is inhibited by chloroquine [49] by forming molecular complexes with plasmodial DNA, thereby inhibiting plasmodial DNA synthesis [50,51].

Other pharmacologic effects of chloroquine

In addition to the above antimalarial actions, there is abundant evidence that chloroquine has several other actions on a number of cell types. For example, AtT-20 cells (a mouse pituitary gland tumour cell line) secrete ACTH by cleaving the precursor to ACTH and b-endorphin. These hormones are stored in secretory granules and discharged only in the presence of a secretagogue. Chloroquine blocks the storage of newly synthesized ACTH in the secretory granules and instead diverts it to the outside of the cell [52]. Chloroquine also inhibits a number of thiol-containing enzymes including alcohol dehydrogenase, a mechanism thought to be responsible for chloroquine-induced retinopathy [53], and blocks the actions of endogenous as well as exogenous histamine in guinea-pigs [54], blocks histamine induced broncho-constriction in animal models [55] and decrease antigen-induced bronchoconstriction in guinea pig trachea [56]. It has also been shown to have a steroid-sparing effect [57]. In patients with asthma, administration of hydroxychloroquine (a derivative of chloroquine) at a dose of 300-400 mg/day (max 6.5 mg/kg) in adult asthmatics, including steroid dependent asthmatics, led to improvement of symptoms [58]. Chloroquine has also been noted to inhibit the replication of a number of viruses such as HSV-1 virus [59], HIV-1 and several AIDS related opportunistic microorganisms [60,61]. It is thought that the inhibitory action of chloroquine on HIV acts at several targets in the HIV life cycle, including inhibition of the HIV-1 integrase and Tat-mediated transactivation, and reduction of iron stores within cells affecting reverse transcription [62-64]. Other anti-HIV effects of chloroquine include inhibition of post-transcriptional maturation of gp120 [65]. It has been suggested that chloroquine is effective in inhibiting the effects of the lethal factor of anthrax [66,67].

Chloroquine has an anti-mutagenic effect [68]. It binds strongly to nucleic acids particularly to CG sequence of DNA, reinforcing its structural configuration and preventing mutagenesis, and improves the cell mechanism of DNA repair from the damage induced by alkylating therapy [69,70]. By inhibiting phospholipase A2 and tumour necrosis factor, chloroquine acts as an immunomodulator [71,72]; and also act as a lysosome-stabilizing agent.

Chloroquine and iron metabolism

The role of chloroquine in iron metabolism is still poorly understood. However, there is abundant evidence to suggest that many of the effects of chloroquine on organisms results from the interference with intracellular free iron which deprives the organisms from the iron needed for metabolism. Chloroquine, being a weak base, accumulates in acid intracellular compartments increasing the intracellular pH. Legssyer and co-workers have shown that chloroquine significantly reduces incorporation of iron into the liver, spleen and alveolar macrophages of animals loaded in vivo with iron dextran [73]. They assessed the haematological parameters and iron load in male Wister rats that had either been loaded with iron using iron dextran, or made iron deficient by being fed with iron depleted diet over a 5 week period. Chloroquine was administered to these rats beginning one week prior to iron-loading or depleting schedule till the end of the experiment. They found that the uptake of iron by the bronchoalveolar macrophages after iron dextran loading in rats treated with chloroquine was about 400% lower than in rats not treated with chloroquine. Furthermore, chloroquine interferes with the transferrin-transferrin receptor pathway and phagocytosis, and inhibits the uptake of radioactive iron in a dose dependent manner in both neuronal and

glial cells in vitro, and significantly reduced nitrite release in primary cultures of macrophages from iron loaded rats treated with chloroquine [73].

Mobilization of iron from transferrin and ferritin depends on an acidic environment. At higher pH, iron remains bound to transferrin, and therefore unavailable. Chloroquine therefore interferes with intracellular free iron availability without affecting the level of iron complexed into organic molecules [74]. Intracellular ferritin iron is used for heme synthesis through a process requiring proteolytic ferritin degradation in a lysosomal-like compartment [75]. Chloroquine by raising the pH of the monocytes, limits iron availability. The growth inhibitory effects of chloroquine on legionella pneumophilia [37], *Histoplasma capsulatum* [76] and *Franscisella tularensis* [77] depend on its ability to limit iron availability in the phagolysosome. Other organisms that are inhibited by chloroquine through iron deprivation are HIV-1 and HSV [64,65].

Chloroquine and post malaria anaemia

Is there a role for chloroquine in improving the erythropoietic response post malaria? It is difficult to say with certainty what the role chloroquine would play in enhancing haemopoietic response during malaria. But it is obvious that whatever role it would play will depend on the relative importance of each of the multifarious effects of chloroquine and the pre-morbid status of the patient, and other features of the index illness. In a patient who was already iron-deficient prior to malaria, chloroquine administration could worsen the anaemia because of its tendency to create an intracellular iron deficient state. On the other hand, in pre-morbidly iron sufficient patients, the importance of chloroquine-induced iron deprivation will be lower than its immune modulatory effects. Thus the anti-inflammatory properties of chloroquine and its effect on oxidative stress could advantageously reduce malaria-induced hemolysis and modulate most of the inflammatory effects of malaria, thus allowing for a faster haematologic recovery (Figure 1).

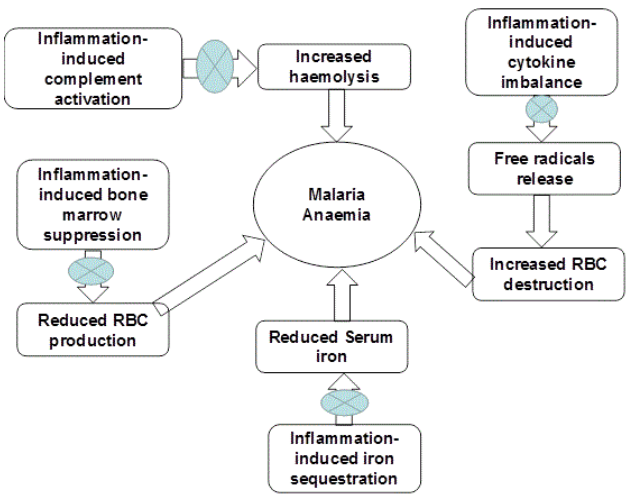


Figure 1: Potential points of action of chloroquine to enhance erythropoietic recovery post-malaria anaemia.

Increased serum levels of TNF-α, IFN-γ and nitric oxides depress erythropoiesis via bone marrow depression, dyserythropoiesis and erythrophagocytosis, and also by direct inhibition of erythropoiesis. Chloroquine on the other hand interferes with the pathways responsible for the production of these products. It is likely therefore that by inhibiting the production of these toxic chemicals, chloroquine minimises the erythropoietic insults and aids erythropoietic recovery post-malaria.

Co-morbidities during malaria contribute to malaria-associated anaemia. The inhibitory effects of chloroquine on many micro-organisms could contribute to the resolution of malaria anaemia by reducing the incidences of concurrent infections. The different actions of chloroquine might appear paradoxical; however, this could be Nature's back-up mechanism in which chloroquine while restricting access to life-giving iron for the pathogens, helps to alleviate some of the negative consequences of the prevailing infection. Since many of the actions of chloroquine are still poorly understood, this drug merits further investigation on any possible role in enhancing haematopoietic recovery post-malaria.

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