A review on the effects of antimalarial agents on the hemoglobin and red cell indices

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ABSTRACT

Antimalarial agents have been succesfully used in the treatment and prevention of malaria infection. Malarial anaemia can be caused centrally through lowered red blood cell production or peripherally through destruction of erythrocytes via haemolysis or phagocytosis. Some antimalaria drugs have been implicated in reinforcing malaria-induced anaemia. Malaria infection can increase membrane porosity of the red blood cells. Malaria infection and treatment with antimalarial can affect hemoglobin and red cell indices to varying degrees.

Keywords: Malarial agents, hemoglobin, red blood cell indices and treatments

INTRODUCTION

Antimalarial drugs are used for the treatment and prevention of malaria infection [1]. Most antimalarial drugs target the erythrocytic stage of malaria infection, which is the phase of infection that causes symptomatic illness [2]. The extent of pre-erythrocytic (hepatic stage) activity for most antimalarial drugs is not well characterized. Treatment of the acute blood stage infection is necessary for malaria caused by all malaria species [3]. The efficiency of early diagnosis and prompt treatment as the principal technical components of the global strategy to control malaria is highly dependent on the efficacy, safety, availability, affordability and acceptability of antimalarial drugs [4]. The effective antimalarial therapy not only reduces the mortality and morbidity of malaria, but also reduces the risk of resistance to antimalarial drugs [5]. Anti malarial drugs can be classified according to anti malarial activity and according to structure.

According to anti malarial activity

- Tissue schizonticides in favour of causal prophylaxis: These drugs act on the primary tissue forms of the plasmodia which after growth within the liver, initiate the erythrocytic stage. By blocking this stage, further development of the infection can be theoretically prevented. Pyrimethamine and Primaquine have this activity [6].

- Tissue schizonticides for preventing relapse: These drugs act on the hypnozoites of P. *vivax* and P. *ovale* in the liver that cause relapse of symptoms on

reactivation. Primaquine is the prototype drug; pyrimethamine also has such activity [7].

- Blood schizonticides: These drugs act on the blood forms of the parasite and thereby terminate clinical attacks of malaria. These are the most important drugs in anti malarial chemotherapy. These include chloroquine,quinine, mefloquine, halofantrine,pyrimethamine,sulfa doxine, sulfones, tetracyclines etc [8].

- Gametocytocides: These drugs destroy the sexual forms of the parasite in the blood and thereby prevent transmission of the infection to the mosquito. Chloroquine and quinine have

gametocytocidal activity against P. *vivax* and P. *malariae*, but not against P. *falciparum*. Primaquine has gametocytocidal activity against all plasmodia, including P. *Falciparum* [9].

- Sporontocides: These drugs prevent the development of oocysts in the mosquito and thus ablate the transmission. Primaquine and chloroguanide have this action [9].Thus in effect, treatment of malaria would include a blood schizonticide, a gametocytocide and a tissue schizonticide (in case of P. *vivax* and P. *ovale*). A combination of chloroquine and primaquine is thus needed in all cases of malaria [10].

According to the structure

- Aryl amino alcohols: Quinine, quinidine (cinchona alkaloids), mefloquine, halofantrine.

- 4-aminoquinolines: Chloroquine, amodiaquine.

- Folate synthesis inhibitors: Type 1 – competitive inhibitors of dihydropteroate synthase – sulphones, sulphonamides; Type 2 – inhibit dihydrofolate reductase – biguanides like proguanil and chloroproguanil; diaminopyrimidine like pyrimethamine.

- 8-aminoquinolines: Primaquine, WR238, 605

- Antimicrobials: Tetracycline, doxycycline, clindamycin, azithromycin, fluoroquinolones

- Peroxides: Artemisinin (Qinghaosu) derivatives and analogues – artemether, arteether, artesunate, artelinic acid

- Naphthoquinones: Atovaquone

- Iron chelating agents: Desferrioxamine [11].

Red blood cell indices are blood tests that provide information about the hemoglobin content and size of red blood cells. Abnormal values

indicate the presence of anemia [12]. Red cells contain a special protein called hemoglobin, which helps carry oxygen from the lungs to the rest of the

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body and then returns carbon dioxide from the body to the lungs so it can be exhaled. Blood appears red because of the large number of red blood cells, which get their color from the hemoglobin [12].

The injurious impact of free Hb has been ascribed to heme-driven oxidative processes and vascular dysfunction. A functionally intact clearance pathway is thus essential for rapid and efficient elimination and detoxification of free Hb and prevention of its deleterious effects [13].Chloroquine is a lysosomotropic weak base and accumulates within acidic cellular compartments. The pharmacologic action of chloroquine includes an increase in intralysosomal pH, preventing fusion of endosomes and lysosomes, and, consequently, disruption of intracellular trafficking [14]. This agent was widely used for the treatment of malaria. The efficacy of chloroquine as an antimalarial drug is owed to inhibition of heme catabolism in plasmodium parasites. By blocking polymerization of Hb-derived ferriprotoporphyrin IX, highly toxic heme-chloroquine complexes accumulate, thus limiting parasite survival. In contrast, preserving the Hb clearance pathway in malaria infected patients is critical. Oxidative heme toxicity to the blood-brain barrier has been intimately linked to some of the most severe cerebral complications of this disease [15]., and efficient Hb-iron recycling is critical to support erythropoiesis during severe anemia, which is one of the major worldwide causes of malaria death [16]. Chloroquine or its derivatives may interfere with an established pathway for Hb clearance, thereby inhibiting heme detoxification and, potentially, heme-iron recycling. Specifically, we found that chloroquine blocks Hb:Hp degradation by paralyzing lysosomal function and limiting heme access to HO-1, the primary enzyme of Hb-heme catabolism.

The effects of quinoline antimalarial drugs in red blood cells infected by *Plasmodium falciparum*

The most widely used antimalarial drugs belong to the quinoline family. The Plasmodium parasite digests hemoglobin, liberating the heme as a byproduct, which is toxic to the parasite [17]. It is detoxified by crystallization into inert hemozoin within the parasitic digestive vacuole. The measured crystal surface coverage is sufficient to inhibit further hemozoin crystal growth, thereby sabotaging hemedetoxification [18]. Bromoquine accumulates in the digestive vacuole, reaching submillimolar concentration, 1,000-fold. Such a dramatic increase in bromoquine concentration enhances the drug'sefficiency in depriving heme from docking onto the hemozoin crystal surface. Excess bromoquine forms a complex with the remaining heme deprived from crystallization. This complex is driven toward the digestive

vacuole membrane, increasing the chances of membrane puncture and spillage of heme into the interior of the parasites [19].

Denaturation of Hemoglobin and the Antimalarial Action of Chloroquine

Chloroquine kills malaria parasites by interfering with ferriprotoporphyrin IX (FP) detoxification and causing it to accumulate to lethal levels [20]. FP is produced when the parasites denature or degrade hemoglobin. It is detoxified by dimerization to β-hematin, which is a process that is promoted by unsaturated lipids [15]. Chloroquine treatment interferes with detoxification by causing unsaturated lipids in parasitized erythrocytes to be unavailable for the promotion of FP dimerization [17]. We have previously labeled this phenomenon "masking" and attributed it to an interaction of an unidentified substance with membrane lipids [10]. It is probable that the masking substance is an intrinsic membrane protein and that the parasite hydrolyzes it to unmask the lipids needed to promote FP dimerization. Normal erythrocyte membranes provide an example of lipid masking. They contain lipids capable of promoting FP dimerization; but they do not dimerize FP because the lipids are unavailable to perform this function, although they may be available to serve other functions. When the lipids are separated from the membrane proteins, however, they promote FP dimerization [17]. Malaria parasites feed on the erythrocyte cytoplasm from within their vacuoles. In the process, they also ingest vacuolar membrane. Initially, this membrane lines the inside of young endosomes and creates erythrocytoid bodies, which we define as inclusions of erythrocyte cytoplasm bounded by vestiges of the erythrocyte membrane [20]. As the endosomes mature, they become acidic and the extra membrane disappears. It is well known that chloroquine affects this part of the feeding process by impairing endosomal maturation and causing hemoglobin-laden vesicles with double membranes to accumulate [18].

The limitations of erythrocytes as drug carriers

A significant limiting factor for the use of RBCs as antimalarial carriers is that when present at therapeutically active concentration, the drug has to be innocuous for the cell physiology, which might not be an unsurmountable obstacle given the reduced metabolic activity of eryrthrocytes [21]. However, loading of some antimalarial drugs like clotrimazole had been observed to predispose RBCs to oxidative damage, an undesirable scenario because oxidized RBCs are rapidly taken up by hepatic reticuloendothelial system macrophages [22]. Another obstacle for the incorporation of antimalarial drugs into RBCs is drug loading itself, since most currently available protocols use a

harsh *ex vivo* isolation of erythrocytes followed by drug loading through diffusion [20]. Moreover, the physicochemical properties of each particular antimalarial drug will constrain the nanovector composition and the corresponding drug delivery mechanism [23]. As an example, the optimal approach for delivery of membrane-impermeable hydrophilic drugs such as fosmidomycin would be immunoliposomal fusion with the RBC membrane, which requires the incorporation of special fusogenic agents into highly fluid vesicles [24].

Effects of artemether on hematological parameters

The characteristic peroxide lactone structure in the artemisinins is indispensable for their anti-malarial activity [25]. Splitting of this endoperoxide bridge by heme iron species results in the release of reactive oxygen species (ROS) that eventually cause parasite's death [26]. Although, this process takes place within plasmodium-infected erythrocytes, artemisinins distributed in other parts of the body could also be oxidized to generate ROS that will induce oxidative stress and cause toxicity [27]. In addition to attacking the causative organism of malaria, *Plasmodium falciparum,* the use of artemether may potentiate hematological abnormalities such as anemia [27]. Caution should therefore be observed in its use in people with anemic tendencies [28].

Parasite Clearance after Antimalarial Treatment

In the presence of heme Fe11, artesunate generate carbon centered free radicals that could damage the RBC membrane or cytoskeleton and thereby increase the rigidity of the infected RBC. The spleen has a lowered recognition threshold for the removal of rigid RBCs in malaria [29]. The reverse was found by acting on young ring-form parasites thereby preventing their development to mor rigid mature trophozoites and thereby attenuated the reduction in deformability associated with continued parasite growth [30]. Quinine, which acts predominantly on the mature trophozoites, had much less effect. The second possibility examined was that artesunate induces changes either in the parasite or in the RBC directly, which lead to increased antigenicity (either through increased expression of parasite antigens or the uncovering of cryptic host antigens) and thus increased opsonization [28]. The splenic threshold for removal of antibody-coated RBCs is also lowered in acute malaria.

The spleen plays a central role in circulating parasitized RBC clearance. After antimalarial treatment with artesunate in splenectomized patients, parasite clearance is delayed considerably although the parasites appear pyknotic and are usually not viable on culture. Parasite clearance after artesunate treatment is associated

with a significant rise in the number of "once-parasitized" RBCs, readily identified by their immunofluorescent staining with antibody against the P. falciparum-ring erythrocyte surface

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antigen [18]. The spleen presumably recognizes the dying or dead intraerythrocytic parasites and removes them as it does host nuclear remnants.

Effect of chloroquine and coartem on hemoglobin and red cells indices

Chloroquine and coartem when administered are carried in the blood stream where their actions are executed [26]. From results obtained from the literature reveal that these drugs do not have tremendous or serious adverse effects on blood parameter after 3 days of administration, the red blood cell count, Hb and PCV were not significantly altered following administration of the drugs [27]. Coartem appeared to have no deleterious effect on red blood cell, Hb and PCV when administered at the recommended doses [28]. But there is an indication that prolonged administration of coartem would possibly lead to anaemia [29]. This is in consonance with reports from preclinical data suggesting that repeated exposure to coartem may affect blood cell counts and predispose to anaemia [30]. However, chloroquine did not have any significant influence on the RBC, Hb and PCV but it rather lead to a reduction in MCV and MCH, a possible indication that administration of chloroquine for 3 days could lead to microcytic anaemia [32].

DISCUSSION

Malaria, a vector-borne disease transmitted by mosquitoes, causes flu-like symptoms in infected individuals [3]. If let uncheck, severe symptoms, including seizures, coma, and even death, may develop [5]. A particular health consequence, anaemia, is commonly found with malaria patients. Malarial anaemia can be caused centrally through lowered red blood cell production or peripherally through destruction of erythrocytes through haemolysis or phagocytosis [8]. Artemisinin-based combination therapy (ACT) is the typical treatment for uncomplicated Plasmodium falciparum malaria; however, it has been implicated in reinforcing malaria-induced anaemia [10]. Artesunate alone is commonly injected into severe malaria patients, but is also associated with acute delayed haemolytic anaemia. Due to the complex biological and ethical interactions, it is often difficult to attribute the cause of anaemia to malaria or malaria treatment specifically.

CONCLUSION

Malaria infection can increase membrane porosity of the red blood cells. Malaria infection and treatment with antimalarial can affect hemoglobin and red cell indices to varying degrees.

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