A TENTATIVE FLOW CHART FOR THE BREAKDOWN OF HAEMOGLOBIN BY *PLASMODIUM*, HAEMOZOIN FORMATION, POTENTIAL THERAPEUTIC TARGETS AND EFFECT OF CHEMOTHERAPY {A REVIEW}.

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Abstract
Blood haemoglobin combines with oxygen available in the body to form oxyhaemoglobin, help transport and offload oxygen to various body tissues, support cellular respiratory events needed to drive metabolic activities in the body and support state of health. During Plasmodium malaria infection, the merozoite stage of malaria parasite in human or animal blood tissues, actively metabolizes and breakdown haemoglobin of the blood erythrocytes, forming free haem, while consuming the globin moiety. The resulting haem is consequently detoxified by polymerization to form haemozoin (malaria pigment), an adaptive process to evade toxicity by the intra-erythrocytic Plasmodium and enhance its survival - one of the potential chemotherapeutic targets along the pathway.
However, the produced haemozoin which is a stable pigment is toxic to the human or animal host. The host immune system reacts through phagocytic action of its polymorphonuclear leucocytes, which degrades the haemozoin into non-toxic forms and helps in reducing pathological damages to the tissues and accompanying symptoms. A designed flow chart for the process of haemoglobin metabolism and breakdown by Plasmodium parasite is tentatively presented.
Also, the effect of some antimalarials in the foregoing process and role in patients' recovery, through its inhibition of polymerization of haem, is reviewed and presented.

Key words: metabolism, haemoglobin, polymerization, haem, merozoites, toxicity.

INTRODUCTION

Malaria kills approximately 1.2 million people a year, most of them African and most of them children (TDRnews, 2007).Technically, malaria is currently endemic in some regions of Africa, while other areas are subject to repeated epidemics. More still, life expectancy at birth in the year 2006 was 50 years in sub Saharan Africa. In the second least healthy region, South Asia, it was 64 years, while in high income countries it was 79 (Weil, 2008).

The life cycle of *Plasmodium* malaria parasite commences with the inoculation of sporozoites into the blood stream, which later migrates to the liver to begin the non-erythrocytic or hepatic stage. During the erythrocytic stage of the malaria parasite life cycle, there is a huge amplification of the size of the parasites. Each of them at this stage termed sporozoite or the signet ring

develops into the schizont which contains deposits of malaria pigment granules (haemozoin) alongside the numerous merozoites formed from repeated divisions of the nucleus of each schizont. This results in the eventual release of the merozoites alongside haemozoin, as the encasing red blood cells ruptures. This haemozoin is toxic to its human or animal host (Turrini et al, 1993). The release of haemozoin is accompanied by the release of other metabolites and oxidants such as nitrogen oxides which cause damages to the host's tissues that manifest in observed pathologies (Green et al, 1990; Ozurumba and Ogundiniyi, 2011).

Chemotherapeutic agents that are capable of reducing pathologies associated with the invasion of red blood cells by the parasites has been one of the considerations for reducing morbidity and mortality associated with malaria (Murphy et al, 2006; Haldar and Mohandas, 2007).

Drugs derived from the ancient Chinese herb *Artemisia annua* may today be the most powerful weapon in the global war against malaria, but scientists are searching urgently for new drugs, given the possibility that, sooner or later, resistance to artemisinin may develop (TDRnews, 2007). Thus, affordable new antimalarials are needed in sub-saharan Africa, where first-line treatments such as cholroquine, sulphadoxine/ pyrimethamine are failing due to increasing parasite resistance. Drugs such as Lapdap for treating uncomplicated falciparum malaria and (developed by TDR and partners) co-artemether, a fixed combination of two drugs, artemether and lumefantrine (developed in the 1990s by Novartis) for treating acute uncomplicated malaria with a registered name of Coartem are engaged in disease endemic counties (TDRnews, 2003).

This paper attempt to review and examine the process of haemoglobin metabolism and breakdown by malaria parasite, with respect to malaria drug development, alongside the effects of chemotherapy in the process.

Thus, the objectives of this article are to:

.Examine the process involved in formation of malaria pigment during infection with *Plasmodium* malaria parasite.

.Design and present a tentative flow chart of the process of formation of malaria pigment.

.Highlight some potential chemotherapeutic targets along the process of malaria pigment formation.

RED CELL INVASION BY MEROZOITES

The fact that sporozoites exist in circulation, albeit only briefly, without entering the erythrocytes suggest that they do not posses the equipment for invasion, or that the red blood cells lack the required apparatus for reception or both (Miller et al, 1997). It is generally believed that initial merozoite attachment to red cells is a random process while initiation of invasion requires re-orientation of the parasite until the apical end is in contact with the red cells. However, this does not rule out the possibility of a common occurrence of multiple invasions rather than being an event that occurs by chance (Simpson et al, 1999). Several factors are known to enhance multiple invasions. One of such factors is the lack of free mixing in areas where the sequestered schizonts are located which makes the pool of susceptible erythrocytes much smaller than the total red cell number.

The works of Bannister et al (1977), Kilejian et al (1977) and Waters et al (1990) has shown that invasion of erythrocytes by malaria merozoites is a receptor – mediated event, involving attachment between receptors on merozoites and ligands on the erythrocytes in a specific and sequential manner. Following binding of the merozoite receptors to erythrocytes ligands, invagination occurs leading to the formation of an endocytotic vacuole. However, the parasite only attaches briefly and disengages if the erythrocyte is susceptible to invasion.

MALARIA PARASITE METABOLISM, TENTATIVE FLOW CHART FOR BREAKDOWN OF HAEMOGLOBIN BY PLASMODIUM MALARIA PARASITE AND CONSEQUENT FORMATION OF MALARIA PIGMENT (HAEMOZOIN).

Basilico et al (1998) showed in their study that during erythrocytic schizogony, merozoites actively metabolizes or degrades the haemoglobin of the red blood cell, forming a free haem in the process while consuming the globin moiety. They later detoxify the resulting haem by polymerization to form haemozoin. Monti et al (1999) described the polymerization of haemoglobin derived "ferric –protoporphyrin: IX [Fe(III) PPIX]" (Iron III haem), to inert haemozoin as being a crucial and unique process for intraerythocytic Plasmodia to prevent haem toxicity and thus good target for new antimalarials.

In a follow-up study by Loria et al (1999), it was reported that malaria parasites feed by degrading haemoglobin in an acidic food vacuole (the physiological site of haemozoin formation) at a pH of 5.2, producing free haem moites as the by- product. This free haem is later detoxified by converting it into a stable, crystalline black brown pigment known as "malaria pigment" or "haemozoin" (Pandey et al, 1996). Furthermore, the later showed from their findings that the possible route for haem degradation is through a "rapid peroxidative decomposition". A parasite – specific enzyme "haem polymerase" is involved in the formation of haemozoin. Meshnick (1996) reported form his study that most of the haem which is released during haemoglobin degradation is incorporated into the haemozoin. The polymerization of haem may not occur spontaneously under the reaction conditions corresponding to food vacuoles of the malaria parasite. Their analysis of the fate of the haem molecules in *P. falciparum* infected erythrocytes showed that only about one third of the haem is polymerized to form haemozoin, while the remaining two third appeared to be degraded by a non-enzymatic process which leads to an accumulation of iron in the parasite. Pandey and Tekwani (1996) in their work, reported that synthetic peptides containing a repetitive pentapeptide sequence (Ala-His-His-Ala-Asp) of a malarial histidine-rich protein: mhrp represents the haem binding site of the mhrp and possibly the site of nucleation for haem polymerization (Pandey et al, 1997).

Olliaro and Yuthavong (1999) reported that haemoglobin digestion, alongside redox processes, free radical formation and reactions accompanying haem release, followed by its polymerization into haemozoin are the major processes occurring in the digestive vacuole of Plasmodium parasite.

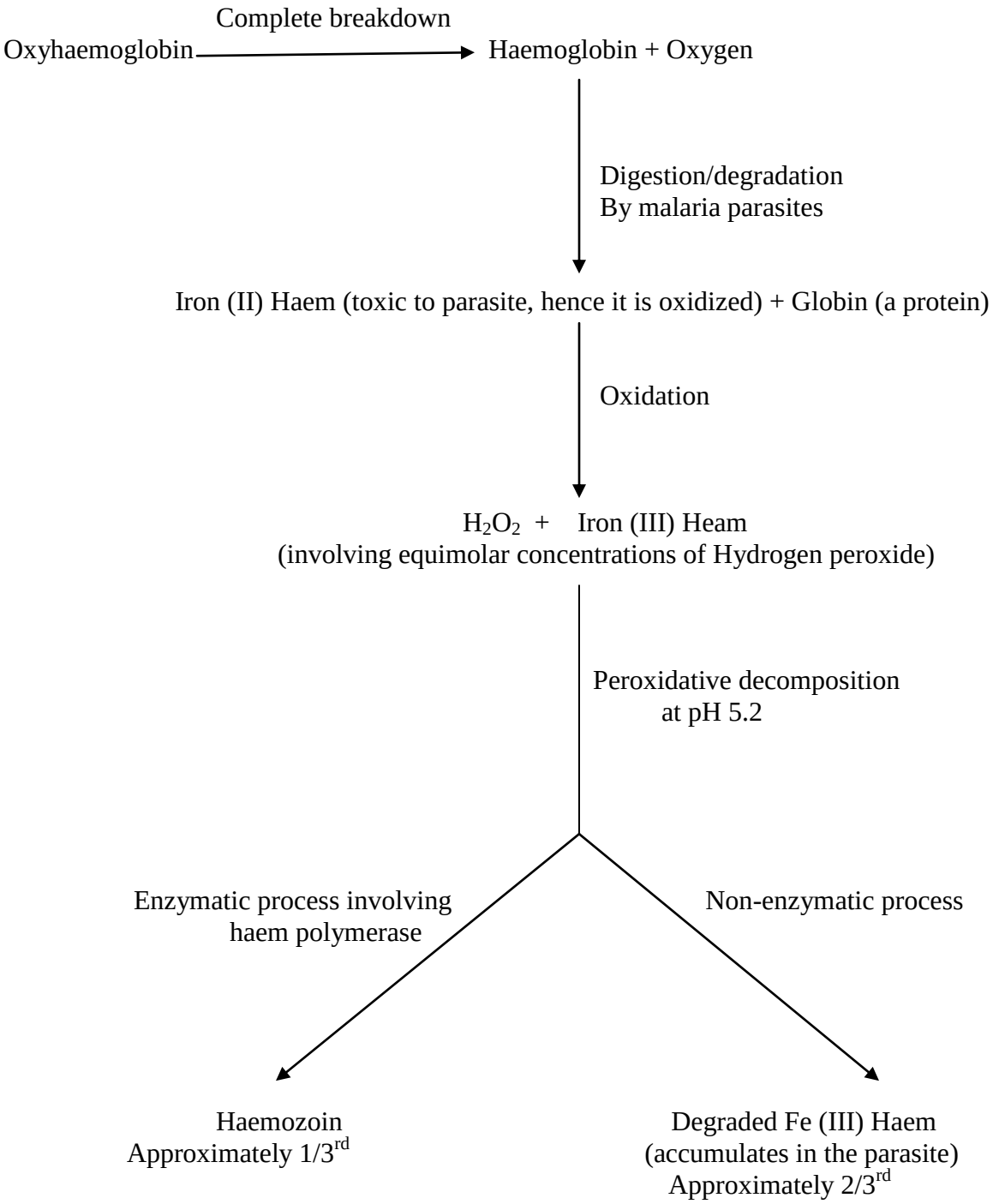

Figure 1: The tentative representation the flow chart for haemoglobin breakdown by *Plasmodium* malaria parasite.

POTENTIAL CHEMOTHERAPEUTIC TARGETS IN THE TENTATIVE FLOW CHART

The processes described above are potential chemotherapeutic targets in the malaria parasites that can be used as the basis for screening compounds in order to identify new leads, which in turn, will qualify for lead optimization work. Babior et al (2000) in his study reported that neutrophils and perhaps other phagocytes contain 3 neutral proteases – cathepsin G, elastase and proteinase 3, which all reside in the azurophil granules of the neutrophils. They are called neutral proteases because they best act under neutral pH, which is the pH of normal tissues, unlike lysosomal proteases which are most active in acidic environment. These neutrophilic proteases are employed chiefly to degrade substances such as haemozoin that the neutrophils have digested.

Apart from the processes occurring in the parasite digestive vacuole, other classes of potential chemotherapeutic targets are enzymes involved in macromolecular and metabolite synthesis including those responsible for membrane processes and signaling.

The reports of Davis and Icke (2002) indicated that malaria parasite may appear distorted if the patient has been treated, or has had inadequate or ineffective prophylaxis. Some antibiotics such as tetracycline negatively affect the parasites' physiology. This would consequently, affect the ability of merozoites to actively metabolize or degrade haemoglobin (its major source of nutrition) into haemozoin. Hence, parasite metabolism is affected by chemotherapy, which therefore influences outcome of diagnosis and status (vis-à-vis prognosis) of the infected individual.

Various points on this tentative flow-chart for haemoglobin breakdown by *Plasmodium* malaria parasite seem to be potential targets for the development of chemotherapeutic interventions against malaria; with some other salient associated pathology related antimalarial chemotherapeutic agents whose mechanisms of association in the breakdown process of haemoglobin by *Plasmodium* parasite was not part of the scope of this review- and which could be developed to support the fight back against the dreaded disease called malaria that has plagued mankind for long.

EFFECT OF ANTIMALARIAL CHEMOTHERAPY ON MALARIA PIGMENT AND ASSOCIATED HAEM MOLECULES

Iron chelation (a term applied to grouping of compounds, and in this case- in which iron is inclusive) therapy with "desferrioxamine", the only compound of this nature that is available for use in humans, has clinical activity in both uncomplicated and severe (complicated) malaria in humans. Mabeza et al (1999) stated that 'a considerable number of iron (III) chelators, designed for purposes other than treating malaria have been shown to exhibit antimalarial activity in-vitro'; apparently through a mechanism of with-holding iron from vital metabolic pathways of the intra-erythrocytic parasite while that of certain Iron (II) chelators with antimalarial activity appears not to be through withholding of iron, but through the formation of toxic complexes with iron.

Goldberg et al (1990) suggested that an influx of chloroquine is thought

to raise vacuolar pH in malaria parasites, thereby inhibiting the functions of proteases responsible for haem polymerization into haemozoin. This rise in vacuolar pH above the normal level leads to reduced activity of the parasite food vacuole as they become unable to effectively degrade haemoglobin and ultimately starved of amino acids (Homewood et al, 1972).

The relatively new antimalarial-artemisinnin, is activated by intraparasitic haem and iron into a free radical which then alkylates specific malarial proteins leading to the death and clearance of such parasites (Meshnick, 1996). Pandey et al (1997) complimented the earlier mentioned finding when they reported from an in-vitro study that synthetic peptides corresponding to a repetitive sequence of malaria-histidine rich protein, bind haem and inhibit haemozoin formation.

Basilico et al (1998) confirmed that Quinoline containing anti-malarials such as chloroquine, inhibit the formation of haemozoin and its polymer beta-haematin, forming electronic pi-pi interactions with haem monomers. This instructively, indicates that once the polymerization of haem can be prevented, a process that requires the breakdown of haemoglobin into haem, the ability of the malaria parasite to complete its life cycle could be inhibited. “Then, we could be talking about another essential and unique pharmacological target.”

Then, in a study carried out by Loria et al (1999), it was shown that chloroquine and quinacrine are efficient inhibitors of the peroxidative destruction of haem, while epiquine, a quinoline compound with very low antimalarial activity, has little inhibitory effect. Furthermore, it was reported that chloroquine enhances the association of Iron (II) haem with membranes, while epiquinine inhibits this association, and that treatment of parasitized erythrocytes with chloroquine leads to a build-up of membrane associated haem in the parasite.

CONCLUSION

In the light of the foregoing, the review and consequent design of process of haemoglobin breakdown, I am of the opinion that the designed and presented tentative flow chart, suggested pharmacological targets and effects of chemotherapy on the process could provide insights, for more understanding of the discussed process and possibly enhance capacity for further exploitation of certain points in the pathway for designing effective drugs.

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