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THE ROLE OF ENZYME INHIBITION IN THE DEVELOPMENT AND DISCOVERY OF NEW ANTI-MALARIA DRUGS

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Abstract

Orally bioavailable drugs are significantly made up of drugs that functions basically as inhibitors of enzymes and are the most used in this present day as clinical therapeutic agents. The identification and optimization of drug candidates are the main focus of the discovery and development of drugs which acts by inhibiting specific enzymatic targets. The basis for the use of enzymes therapeutic targets originated from the high levels of association of the disease (target validation) and druggability (target tractability) which is a typical characteristic of this class of proteins. This article describes the existing practices and future directions in enzymological discovery of drugs, and emphasizing on the methods. With the rise in the spread of drug resistant strains of *Plasmodium falciparum*, malaria has remained a basic challenge to the world health as a whole. Malaria parasites that are resistant to drugs are seen to have evolved through an active site mutation in the drug targets or from biochemically induced alterations in the receptor for drugs. This challenge has since been made worse by a decrease in chloroquine sensitivity exhibited by *P. falciparum and vivax*. The host and the parasite inter-relationship can be made more complex by the complex parasitic life cycle which involves vertebrate and invertebrate hosts as well as varying locations within each of the hosts. Having a clearer comprehension of the metabolism of the parasite may give valid ideas to necessitate the development of new procedures which will be specifically targeted at the unique mode of metabolism of the parasite. The level of drug resistance elicited by specific stains of *P. falciparum* has led to the increasing interest in the search for new and better drugs that could act against the malaria parasite and also induce a negative effect on the drug targets. Inhibition of enzymatic catalysis has been utilized in drug development and design in order to a fast eradication of the endemic malaria health challenges in the world and specifically the most vulnerable sub-Saharan Africa regions. Many drugs designed on the basic knowledge of enzyme inhibition are currently undergoing clinical trials; such attempts include the use of bioinformatics tools in the study of the biochemical properties of some key metabolic enzymes to discover potential drug targets.

Keywords: *Plasmodium falciparum; Druggability; Parasite; Chloroquine: Metabolism*

Introduction

A molecule that binds to an enzyme and reduces its activity is known as an enzyme inhibitor. Since blocking an enzyme's activity can inactivate a pathogen or correct a metabolic imbalance, many drugs acts as inhibitors of enzymes [1]. Not all molecules that bind to enzymes can be regarded as inhibitors; activators of enzymes bind to any enzyme and increase their enzymatic activity, while substrates of enzymes bind and are converted to products in the normal catalytic cycle of the enzyme. Enzyme inhibition over the years has been applied in the design of drugs, chemotherapy, design of antibiotics and are also used the production of pesticides [2].

There are numerous drug molecules that act as inhibitors of enzymes, so discovering and improving these drugs is an active research aspect in the biochemical field as well as pharmacology. Specificity and potency are important factors in judging medicinal enzyme inhibitors. These two factors connote the ability of a drug to bind to other proteins and the dissociation constant, which is an indication of the needed concentration to inhibit the enzyme. Specificity and potency at its peak will ensure that drugs will elicit few side effects and thus low toxicity [2].

Antimalarial drug effect is initially characterized by the inhibition of parasite growth in culture media that has been exposed to drugs and compared to a drug-free control culture [1].

The unavailability of an effective prophylactic vaccine and the generation of drug-resistant strains has shown vividly the need for the development of new therapeutic approaches and identification of novel drug targets.

The Malaria parasite

Malaria is an infectious disease caused by bites of mosquitoes and it affects both humans and other animals. Malaria is caused by the transfer of parasitic protozoans, belonging to the *Plasmodium* genera. Symptoms of malaria include vomiting, tiredness, fever, and headaches (WHO 2014.). If not properly managed, people may have recurrences of the disease later. In those who are recent survivors of the infection, milder symptoms are exhibited at the point of reinfection.

Life cycle and Mechanism of action of plasmodium parasite

The hosts for the development of *P. falciparum* at different stages of its life cycle are humans and mosquitos. The life cycle includes the sexual reproduction and the asexual in mosquitoes and its asextual replication in humans. Mosquitoes get infected during their ingestion of human blood which contains mature forms of the gametocytes of *P. falciparum* [3]. The alteration of the stomach environment of the mosquito (e.g. sudden temperature reduction, change in pH and presence of compounds the triggers the formation of gametes) causes the transformation of gametocytes to gametes and their release from the erythrocytes (RBCs) [4]. A zygote can then be produced when there is a fusion between male and female gametes. The zygote develops into an ookinete that grows into an oocyst. An oocyst is a zygote protected by a hard spore [5]. Oocysts multiply into hundreds of infectious sporozoites (transmissible form the parasite) which are injected into a human during the next blood meals. The period for the development of the *P.falciparum* in the mosquito is approximately 7-14 days [6]. During the asexual life cycle in humans, parasites undergo two stages of development, the liver stage and the blood stage. The liver stage commences when an infected Anopheles mosquito bites a human, and sporozoites from the salivary glands of the mosquitoes are injected into the dermal tissue of humans and then finds its way to the bloodstream [7]. Sporozoites migrate to the liver and invade hepatocytes within 15 minutes to a few hours of the mosquito bite. Hepatic infection usually lasts 8 to 12 days and there are no occurrences of any clinical symptoms at this stage [8]. During asexual replication in the liver cells, sporozoites replicate and differentiate into many merozoites. Merozoites released from hepatocytes invade the host's erythrocytes (RBCs), and they start the blood stage of the malaria infection [9]. This takes approximately 48 hours for a parasite to grow to the point of maturation inside an RBC. Parasites develop from the ring stage to pigmented trophozoites and eventually undergo division to form an approximate average of 16 merozoites during the schizont stage [10]. The dark-brown pigment which is formed during trophozoiteand schizont stages is hemozoin, and this is produced by active uptake and digestion of the hemoglobin of hosts. When erythrocytess rupture, merozoites are

released into the blood stream for the continuation the asexual multiplication cycle by repeating the RBC invasion processes. After several asexual replication cycles, some of the ring stage parasites develop into male and female gametocytes. When gametocytes are taken up by a female Anopheles mosquito, sexual reproduction is instituted and the disease from that point on is transmitted [7, 8]

Mechanism of action of *Plasmodium*

P. falciparum in its erythrocyte stage invades the red blood cells where it forms a digestive vacuole (DV) which is alysosomal isolated acidic compartment. In the red blood cell, the parasite matures by ingesting haemoglobin from the cytoplasm of the host cell and makes deposition in the DV, where the degradation of proteins to its component peptides and heme take place and this is incorporated into the inert and harmless crystalline polymer hemozoin [11].

Types of enzyme inhibition

There are 2 types of enzyme inhibition;

Reversible and Irreversible Inhibition

Irreversible inhibitors usually interact with the enzyme and chemically alter it (e.g. via covalent bond formation). These inhibitors causes a modification in key amino acid residues needed for the activity of enzymes. In contrast, reversible inhibitors exhibit a non-covalent form of binding and different types of inhibition are produced and this depends on the chances of having the inhibitors bind to the enzyme, the enzyme-substrate complex, or both [12].

Reversible inhibitors bind to enzymes with a non-covalent form of interaction such as hydrophobic interactions, hydrogen bonds and ionic bonds. Series of weak bonds that occurs between the inhibitor and the active site combine to produce strong and binding with specificity. In contrast to the binding of substrates and irreversible inhibitors, reversible inhibitors generally do not undergo reactions that are chemically induced when bound to the enzyme and can be easily removed by diluting the complex or through dialysis [12].

There are four classes of reversible inhibition

- **Competitive Inhibition**, in this form of inhibition, inhibitors compete with substrates for binding sites, because the substrate and the inhibitor both have an identical or overlapping sites to bind. As a result of the overlapping nature of the binding sites, a ternary complex—in which the substrate of the enzyme and the enzyme inhibitor would simultaneously bind to the enzyme is unable to be formed. Accordingly, in the enzyme-inhibitor complex, the enzyme is inactivated completely. A condition where there is a high concentrations of substrate can sufficiently overturn this type of inhibition (V_{max} remains constant), i.e., by out-competing the inhibitor. However, the apparent K_m will increase as it takes a higher concentration of the substrate to reach the K_m point, or half the V_{max}. The structure of competitive inhibitors are often similar to that of the structure to the real substrate[12].
- **Uncompetitive inhibition,** here the inhibitor can only binds to the substrate-enzyme complex. This type of inhibition leads to the decrease in Vmax (maximum velocity decreases as a result of removing activated complex) and apparent decrease in Km is also experienced (due to better binding efficiency as a result of Le Chatelier's principle and the effective elimination of the Enzyme-Substrate complex thereby causing a decrease in the Km which indicates a higher binding affinity) [12].
- **Non-competitive inhibition**, the binding of the inhibitor to the enzyme brings down its activity but has no effect on the substrate binding. As a result, the extent of inhibition depends greatly only on the inhibitor concentration and not the substrate. Vmax will be reduced due to the inability for the reaction to proceed further as efficiently as it should, but Km will remain the same as the actual substrate binding, by definition, will still be at optimal functionality [12].
- **Mixed Inhibition**, a mixed inhibitor binds to both the enzyme and at the same time the substrate of the enzyme. However, inhibitor's binding has an effect on the substrate binding, and vice versa. A reduction can be effected on this type of inhibition but cannot overcome by increment in substrate concentration. In as much as it is practically possible for mixed-

type inhibitors to bind in the active site, this type of inhibition generally results from an allosteric effects where the inhibitor binds to the enzyme but the binding is to an entirely different site. The binding of inhibitors to this allosteric sites changes the conformation (i.e., tertiary structure or three-dimensional shape) of the enzyme so that there will be a reduction in the affinity of the substrate for the active site [13].

Recent advances in malaria drug discovery

Efforts to the discovery of drugs that are geared towards the liver and transmission stages are in their infancy but attention is increasingly be directed to that field as targeting these stages could be instrumental in the complete eradication of malaria. Such approaches include;

- **Structural and mechanistic studies on ligand binding and enzyme inhibition using *Plasmodium falciparum* glutathione *S*-transferase**

  The Glutathione *S*-transferase enzyme of the *Plasmodium falciparum* (PfGST) is regarded a novel class of GST isoenzymes [14]. Since the amino acid constituents of the substrate binding site of PfGSTdiffers significantly from that of its human orthologs and there is only this one isoenzyme present in the parasite, PfGST is considered a highly important therapeutic target for the development of potent antimalarial drugs. GST activity has been noticed in all *Plasmodium* species so far studied as well as in all the intraerythrocytic stages of the parasite. The estimation of PfGST with regards to the total cellular protein has been seen to be >1%. The *P. falciparum* GST (PfGST) role in the development of drug resistance in malarial parasites has been postulated and is discussed controversially [15]. The primary structure as well as the three-dimensional X-ray structure of PfGST differ significantly from human GSTs, and indicate that PfGST cannot be attributed to any of the previously known GST classes on the basis of functionality, thus representing a novel GST isoform [16]. Furthermore, the parasite harbors only one GST enzyme and inhibition of PfGST is expected to disturb conjugation processes that are solely GSH-dependent in order to upregulate the levels of cytotoxic peroxides and to increase the concentration of toxic ferriprotoporphyrin IX (FP). PfGST is one of the most promising drug targets for the development of antimalarial drugs [15, 17].

- **Selection of potential inhibitors of plasmodium *falciparum* lactatedehydrogenase enzyme through docking studies**

  The inter-conversion of pyruvate to lactate in the final step of glycolysis is catalyzed by the LDH enzyme, and this is an important requirement for the production of energy in living cells. Ferriprotoporphyrin IX (hematin), one of the products of hemoglobin degradation by malarial parasites, intoxicates the parasite by instituting a competion with the NADH for the active site of *Pf*LDH; the survival of the parasite is dependent on the polymerization of hematin to hemozoin, which remains active in the food vacuole of the parasite and causes parasite death [11]. The derivatives quinolineare believed to form complexes with the dimerichematin thereby causing a prevention in the formation of hemozoin [18]. NADH analogs have been identified as potential inhibitors of *Pf*LDH in DrugBank [19]. A total of 50 compounds have been selected based on their interactions with an active site which share similarity with that of NADH; the three (*itraconazole*, *atorvastatin* and *posaconazole*) compounds that presented the best theoretical results were tested *in vitro* against *P. falciparum* blood parasites and against malaria in mice [1].

- **Antigenic target of *Plasmodium* species for vaccine research**

  The various developmental stages involved in the parasitic (Plasmodium species) disease represent numerous opportunities for the direction of targets to the series of availableantigens that thus lead to a potential elicition of immuneresponses [20, 21]. Regarding the *P.falciparum* life cycle,

each stage of parasitic diseases relating to humans could have a drug and vaccine developed in order to specifically target the antigen [22].

Conclusion

The ingenuity and resilience of the malaria causing parasite in self preservation, irrespective of the threat, requires a consistent evaluation of control programs, development and intense search for new antimalarial. An ideal antimalarial drug combination to stop the continual spread of resistant parasites is urgently needed. A better understanding of the metabolism of the parasitemay lead to the development of novel therapeutic targets which takes advantage of the uniqueness of specific malarial enzymes that can be effective against the malaria parasite.

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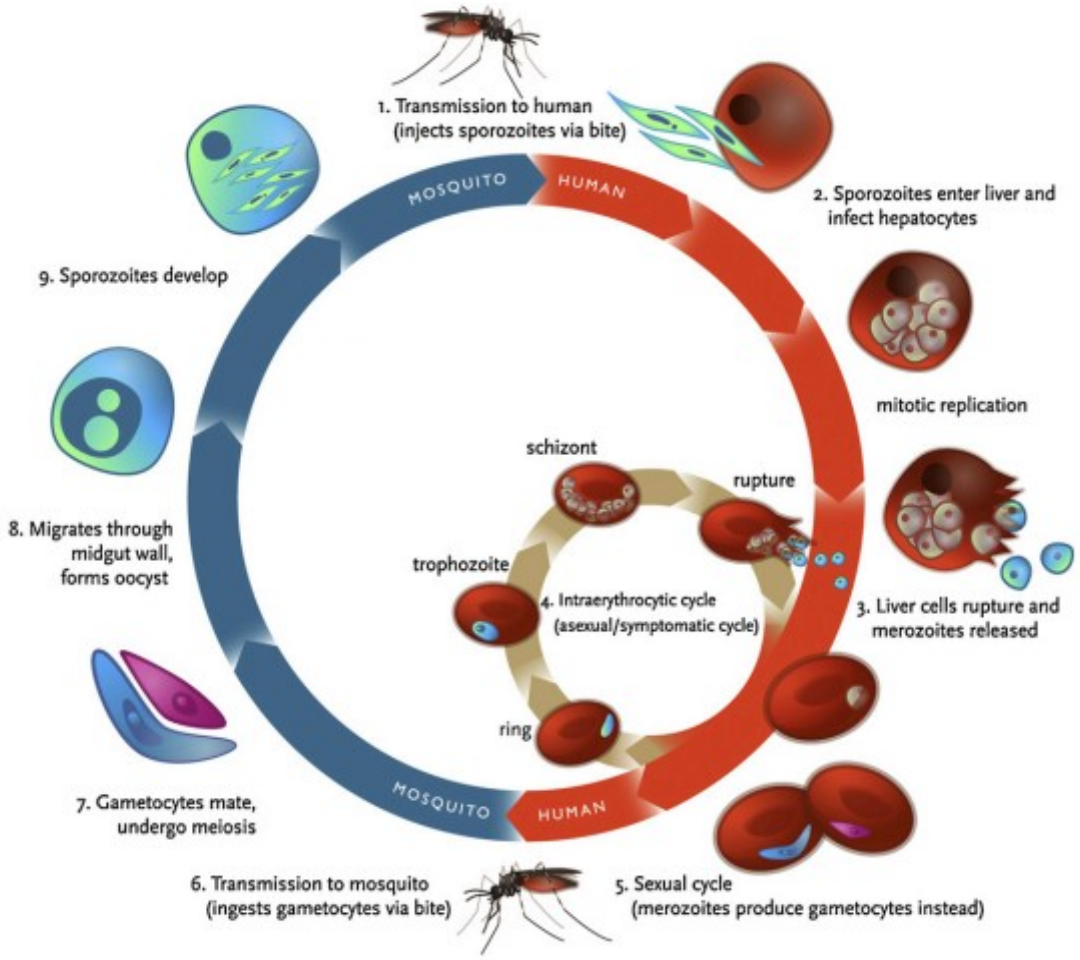

Figure 1. Life cycle of *plamodium falciparum*. Source: (Klein, 2013).