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Malaria Parasite Pyrimidine Nucleotide Metabolism: A Promising Drug Target

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Abstract

Malaria is a major cause of morbidity and mortality in the tropical and subtropical endemic countries worldwide. This is largely due to the emergence and spread of resistance to most antimalarial drugs currently available, including the first-line treatment artemisinins. Thus, to fight this disease, there is an essential requirement to develop new antimalarial drugs for malaria chemotherapy. Plasmodium falciparum, the causative agent of the most lethal form of malaria in humans, cannot salvage preformed bases or nucleosides for pyrimidine synthesis and relies solely on pyrimidine nucleotides synthesized through the *de novo* biosynthetic pathway. In contrast, the human host cells have functionally operated both the salvage and *de novo* pathways. This mini review summarizes significant progress on understanding the pyrimidine nucleotide metabolism and the functional enzymes in the human parasite *P. falciparum*, which are different from the human host metabolic processes. Most recent information of the three-dimensional crystal structures and the catalytic mechanisms of the *de novo* pyrimidine enzymes: dihydroorotate dehydrogenase, orotate phosphoribosyltransferase, and orotidine 5'-monophosphate decarboxylase, as well as their inhibitors affecting these enzymatic activities are briefly reviewed in the context of their therapeutic potential against malaria.

Keywords: Malaria; *Plasmodium falciparum*; Pyrimidine nucleotide metabolism; Drug target; Drug development

Mini Review

Malaria, a major parasitic disease of humans, is caused by protozoa of the genus Plasmodium, classified in the phylum Apicomplexan. The disease afflicts 515 million clinical cases annually in 96 subtropical and tropical endemic countries. The death toll is reported at 1.3 million people each year, mostly young children in sub-Saharan Africa (90%) [1] of the five Plasmodium species that infect humans, including *P. vivax, P. malariae, P. ovale, and P. knowlesi, P. falciparum* is the most dangerous parasite and responsible for the majority of deaths. Based on the World Health Organization recommendation in 2001, artemisinin-based combination therapies are now used as the first-line drugs for treatment of *P. falciparum* malaria [2]. However by 2009, resistance to the drug treatment has been reported [3]. In addition to the lack of highly effective vector control and vaccines, the spread of drug-resistant malaria accompanied by a worldwide resurgence of the disease, it is necessary to develop quickly more effective novel antimalarial drugs, possessing a different mechanism of action, for malaria chemotherapy. This review highlights a better understanding of biochemical differences between the parasite *P. falciparum* and human metabolic processes, i.e., the pyrimidine nucleotide metabolic pathway, which may provide new candidate drug targets for intervention in the fight against this disease [4].

Nucleotide metabolism is one of the largest metabolic pathways, providing the building blocks for DNA and RNA synthesis in human cells. The nucleotides serve as key players in a wide range of cellular functions, including energy transduction, signal transduction, synthesis of biomolecules for carbohydrate and lipid metabolisms [5]. Purine and pyrimidine nucleotides are synthesized from *de novo* biosynthetic pathways or supplied *via* salvage pathways, where nucleobases, nucleosides and deoxynucleosides are recycled from nutrients or from degraded DNA and RNA. In human cells, both the *de novo* and salvage pathways are functioning at significant levels for the purine and pyrimidine nucleotide requirements, although the salvage pathways are more active than the *de novo* pathways [6]. This is true also for bacteria, plant and the free-living nematode Caenorhabditis elegans [7].

The *de novo* pyrimidine biosynthetic pathway is the most conserved metabolic pathway, and the six sequential enzymatic steps starting from bicarbonate ion (HCO_3-), glutamine (Gln), and adenosine 5'-triphosphate (ATP), providing uridine 5'-monophosphate (UMP) (Figure 1, in solid line box), have remained intact throughout evolution, although the primary structures of the enzymes responsible for the *de novo* synthesis deviate significantly among prokaryotes, parasitic protozoa, fungi, animals, and mammals including humans [8,9]. Unlike human host cells, *P. falciparum* parasites have very limited ability to salvage preformed pyrimidine bases and nucleosides (e.g., uracil, uridine, thymidine, cytidine) from the host cell and extracellular environment, but rely mostly on nucleotide synthesized through the *de novo* pathway. All the enzymes required for *de novo* synthesis of UMP, the first pyrimidine nucleotide metabolite acting as the precursor for all pyrimidine nucleotides synthesis , including dCTP, dTTP, CTP, TTP, and UTP, were detected in cell extracts from all Plasmodium species so far examined [10]. The genes encoding each enzyme in all steps of the *de novo* pathway were identified in the parasite genome [11].

Progress towards understanding structures, catalytic mechanisms and regulation of the mammalian and human enzymes for the *de novo* pyrimidine pathway has been significant in recent years [5,9]. Some key differences on the functioning organization of the enzymes, and their genomes including the six enzymes of the pathway from precursors HCO_3-, Gln, and ATP to UMP synthesis warrants a closer

look. The first three enzymes (carbamoyl phosphate synthetase, CPS II; aspartate transcarbamoylase, ATC; dihydroorotase, DHO) of the parasite were readily separated into three different molecular masses by analytical gel filtration chromatography [12], which is consistent with the presence of three discrete monofunctional enzymes. This is similar to that found in another species of protozoa, Crithidia fasciculata, and in many prokaryotic systems [12,13]. The characteristics are different from the humans, wherein the CPS II, ATC and DHO activities are carried on a 243-kDa multifunctional protein, namely CAD [14]. The malarial DHO enzyme has been purified from *P. falciparum* and its gene has been cloned, expressed and characterized in detail by our groups [15]. The DHO is a Zn^{2+} enzyme belonging to the amidohydrolase family, sharing characteristics of both mammalian type I and eubacterial type II DHO by overall amino acid sequence homology, structural characteristics, kinetic and inhibitor properties [15,16]. At present, the malarial CPS II and ATC enzymes are still poorly characterized and regulation of these pyrimidine enzymes is unknown, in contrast to the human CAD enzyme [9].

Recent studies have mainly attended on dihydroorotate dehydrogenase (DHOD), the fourth enzyme in the pathway, particularly as a target for antimalarial agents [16-18]. The *P. falciparum* DHOD has been characterized, and immunogold labelling localized DHOD in the inner membrane of mitochondrion [18]. The three-dimensional crystal structure of the parasite enzyme has been elucidated and compared to the human DHOD structure [19,20]. Crystal structures of human and parasite DHOD identifies completely different binding sites for the antiproliferating leflunomide inhibitor. The overall structure is α/β-barrel, similarly to that of other type II DHOD of eukaryotic origin. It contains flavin mononucleotide coenzyme, ubiquinone binding site and active site for dihydroorotate substrate. Furthermore, the *de novo* pathway is tightly coupled to the mitochondrial electron transport system (Mt-ETS) through the DHOD and its coenzyme Q (CoQ) (Figure 1). The MtETS are valuable targets in malaria chemotherapy [21].

In our laboratory, we have characterized in detail the functional, kinetic, and structural properties of orotate phosphoribosyltransferase (OPRT) and orotidine 5'-monophosphate decarboxylase (OMPDC), the fifth and sixth enzymes of the *de novo* pathway [22-24]. The OPRT and OMPDC enzymes were purified directly from *in vitro* culture of *P. falciparum*. The native enzymes obtained from the parasite are organized in an α2β2 heterotetrameric quaternary structure having two subunits each of OPRT and OMPDC [22]. We have also expressed both genes in *E. coli* [23,24]. Co-expression recombinant *P. falciparum* OPRT and OMPDC genes are also exhibited the *in vitro* α2β2 complex formation [25,26]. Most recently, the parasites' low complexity region is involved in the protein-protein interaction during the α2β2 heterotetrameric formation of the malarial OPRT and OMPDC enzymes, [(OPRT)2(OMPDC)2], as identified by means of a unique insertion of low complexity amino acid sequence characterized by single amino acid repeat, which was not seen in their homologous enzymes from other organisms [27]. Furthermore, three-dimensional crystal structures of the parasite OPRT and OMPDC have been elucidated [28-32], and compared to the recently characterized human OPRT and OMPDC structures [5], which are carried on a 52-kDa bifunctional UMP synthase protein [8,9]. Thus, the inhibitors of OMPDC have been designed and chemically synthesized for therapeutic potential against malaria by using the structure-based drug-design approach of the parasite enzyme [33].

Surprisingly, there is little information on the sequential enzymatic steps after UMP synthesis before yielding dCTP, dTTP, CTP, TTP, and UTP, which are the building nucleotide blocks for nucleic acid synthesis in the parasite (Figure 1, broken line box). Genes are present in the parasite genome but few enzymes have been studied to date. Ribonucleotide reductase (RNR) of *P. falciparum* catalyzes the production of deoxyribonucleotides from ribonucleotides, which is tightly associated with the thioredoxin reductase enzyme [34]. *P. falciparum* TMP kinase (TMPK), catalyzing the synthesis of dTTP and TTP, is classified as type I enzyme by amino acid sequence but has high efficiency in phosphorylation of azido-dTMP and dGMP as well as *E. coli* type II TMPK, sharing characteristics of both types [35]. Additionally, CTP synthetase (CTPS) catalyzes the production of CTP from UTP, is the only known enzyme for cytosine nucleotide *de novo* synthesis in *P. falciparum* [36]. Typically, CTP reacts with phosphocholine to form CDP-choline, which can combines with diacylglycerol to form phosphatidylcholine (PC) and other phospholipids by operating Kennedy pathway.

In human parasite *P. falciparum*, the *de novo* pyrimidine pathway is known to be linked with the *de novo* folate biosynthesis *via* thymidylate synthetase (TS) enzyme (Figure 1) [37]. The parasite TS is a part of the bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS), a validated target for antifolate drugs used in malaria chemotherapy. The three-dimensional crystal structure of the parasite DHFR-TS enzyme and substrate channeling domains have been resolved [38]. It is well recognized that the *de novo* folate pathway is operating in the parasite, like in bacteria, whereas the human host is incapable of *de novo* synthesis [37,38].

Furthermore, functional and kinetic properties of carbonic anhydrase (CA) enzyme were studied in *P. falciparum* [39]. The parasite CA catalyzes the interconversion of HCO_3- and CO_2, having catalytic properties distinct from that of the human host CA isozyme I and II. However, low amino acid sequence identity in the primary structures and phylogenetic analyses of the *P. falciparum* CA is being tapped in preclinical phase for drug development [40-42]. Importantly, the CA supplies HCO_3- as substrate for the first enzyme CPS II of the *de novo* pyrimidine biosynthetic pathway, linking the parasite CA to the pyrimidine pathway as shown in Figure 1 [42].

In conclusion, artemisinin-resistant parasites have already emerged and spread in Southeast Asian region by 2014 [43,44]. This phenomenon entails novel measures for malaria treatment and control. Fortunately, one triazolopyrimidine inhibitor, namely DSM265, targeting the parasite fourth enzyme DHOD of the *de novo* pyrimidine pathway would prove promising as it progresses to clinical phase I trials for drug development [45]. Moreover, as structure-based design of antimalarial drug development continues to be tapped three-dimensional crystal structure of the parasite enzyme, especially for the sixth enzyme OMPDC [30], the possibility of modulating potential toxicity through the pyrimidine pathway might have therapeutic potential against malaria [46].

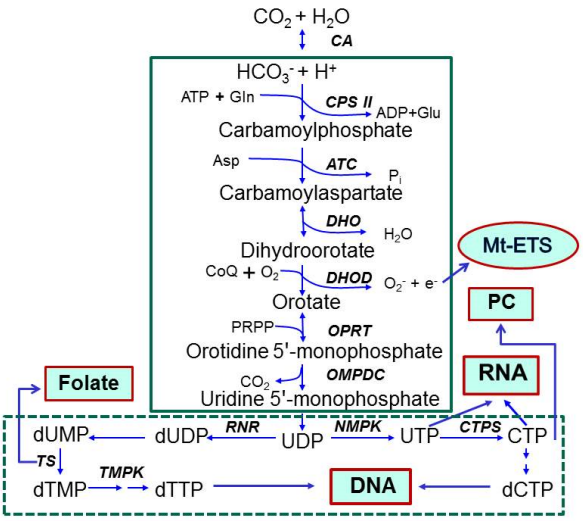

Figure 1: The *de novo* pyrimidine nucleotide biosynthetic pathway in *P. falciparum*. The pyrimidine nucleotides are important metabolites for DNA, RNA, folate, and phosphatidylcholine (PC) synthesis. The enzyme carbonic anhydrase (CA), providing HCO_3^- as substrate for CPS II, is associated with the pyrimidine pathway. The mitochondrial electron transport system (Mt-ETS) is linked to the enzyme DHOD of the pathway, functioning as electron disposal. The pyrimidine *de novo* pathway is closely linked to the folate biosynthesis by thymidylate synthetase (TS). CTP converts to PC by forming CDP-choline and then combining with diacylglycerol. Abbreviations are as follows: CA, carbonic anhydrase; CPS II, carbamoylphosphate synthetase II; ATC, aspartate transcarbamoylase; DHO, dihydroorotase; DHOD, dihydroorotate dehydrogenase; OPRT, orotate phosphoribosyltransferase; OMPDC, orotidine 5'-monophosphate decarboxylase; NMPK, nucleoside monophosphate kinase; RNR; ribonucleotide reductase; CTPS, CTP synthetase; TS, thymidylate synthetase; TMPK, thymidylate kinase; PC, phosphatidylcholine. The solid line box indicates the six sequential enzymatic steps from Gln, ATP and HCO_3^- for UMP synthesis. The broken line box shows the sequential steps after UMP synthesis to provide dCTP, dTTP, CTP, TTP and UTP.

Conflict of Interest

The author declares that there is no conflict of interest.

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