Biochemistry of malaria II: metabolism of nucleic acids

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Malaria is a major health problem in tropical parts of the world. With the emergence of drug-resistant strains of *Plasmodium falciparum* a rational biochemical approach to anti-malarial chemotherapy has become more desirable. As essential components of development and replication, nucleic acid precursors are one logical target for non-empirical antimalarial agents. In this paper, we discuss the metabolism of nucleic acids, including both purine and pyrimidine synthesis with special reference to our own work, in malarial parasites and their relation to host mammalian cells. Some key differences of these pathways between the host and malarial parasites are frequently described as a basis for possible chemotherapeutic targets and the development of antimalarial drugs. Based on the catalytic mechanism of enzyme dihydroorotate dehydrogenase action, we have designed and synthesized a quinone analog as a mechanism-based inhibitor. Quinone had moderate antimalarial activity against *P. falciparum in vitro* and *P. berghei in vivo.*

**Key words**: Malaria, Biochemistry, Nucleic acids.

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โรคตาเรียเป็นปัญหาทางสุขภาพที่สำคัญในประเทศไทย บุคคลที่มีการระบาดอย่างรวดเร็ว ของสายพันธุ์ที่ตื่นตัวเป็นสาเหตุให้เกิดอาการน้ำตาออกมากในหน้าตาโดยไม่ทราบสาเหตุ ซึ่งได้นำวิธีการทางชีวโมงมาใช้ในการพัฒนาการรักษาตาเรียที่มีการฟักผักถิ่นต้นที่มีความจำเป็นในการเจริญเติบโตและการเพิ่มข้อจินตนาการของน้ำตาเรียซึ่งเป็นสาเหตุหลักที่จะพัฒนาการรักษาตาเรียได้ สำหรับบทความนี้จะกล่าวถึงมาตรการรักษาตาเรียและวิธีการรักษาตาเรียในชีวิตประจำวัน อาจมีเหตุผลที่คนไข้ต้องรับการรักษาตาเรีย ไม่สามารถทำได้ด้วยการรักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียในชีวิตประจำวัน อาจมีการรักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประจำวัน การรักษารักษาตาเรียโดยใช้ยาในชีวิตประ...
Based on an estimation of the World Health Organization, perhaps 110 million clinical cases of malaria occur annually and approximately 1 million infected people die a year. Since the severity of the illness is markedly influenced by the species of *Plasmodium* causing human malaria: *Plasmodium vivax*, *P. ovale* and *P. malariae* rarely produce life-threatening diseases, whereas *P. falciparum* frequently causes severe disease. The presence of drug-resistant *P. falciparum* has made some antimalarial drugs unsuitable (e.g. chloroquine) in South East Asia and South America. Many new antimalarial drugs are being developed and some are being studied at the pre-clinical and clinical stages. Since these new drugs have been designed on an empirical basis, the mechanism of action of these new drugs remains to be identified. It is now possible to develop new antimalarial drugs rationally on the basis of accumulated knowledge of the biochemical processes of the parasite which can be used to define target metabolites and enzymes.

The complexity of the malaria life cycle has enabled most studies to illustrate the interesting biochemical and physiological processes of malarial parasites only in the intra-erythrocytic stages of *P. falciparum*. There were first successfully cultivated in vitro in 1976 by Professor Bill Trager of Rockefeller University. In the red cell, the intra-cellular parasite is dependent on host cell for certain basic nutrients (e.g. glucose, ribose), metabolites (e.g. hypoxanthine, folate) and enzymes (e.g. superoxide dismutase). The infected red cell is usually more permeable to nutrients, cations as Ca²⁺, and other substances, including toxic substances (e.g. antimalarial drugs). The knowledge of host cell-parasite relationship is now progressing well, e.g., it has been found that exogenous macromolecules can be directly accessed through a parasitophorous duct of malarial parasites.

The basic features of the biology of the malarial infection and the major principles and objectives of its treatment was recently reviewed in the journal. At present although there is little understanding of the biochemistry of malaria, recent knowledge concerning the advantages of the past experience and new technology has prompted us to discuss the metabolism nucleic acids, including both purine and pyrimidine synthesis, for a review of the biochemistry of malaria; however, we emphasize only the erythrocytic stage of the malaria parasite.

In the first part of this communication, we describe the characteristics of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules of the parasite which are different from those of the host cell; in the second part, we present purine and pyrimidine metabolism in the normal red cell as the basis for our discussion of the changes which occur following malaria parasite infection; and in the third part we consider purine metabolism in the malaria parasite and its relationship to the host red cell and also mention about possible chemotherapeutic targets and newly developed antimalarial agents.

**Characteristics of nucleic acids in malarial parasites**

Malarial parasites contain DNA, RNA and DNA-bound histones. An intra-erythrocytic ring-stage trophozoite contains about 10⁻¹⁵ g of DNA. The genome of the parasite contains four major bases: of adenine (A), cytosine (C), guanine (G), and thymine (T), shows methylation of cytosine only. It comprises ~3X10⁷ base-pairs (bp) of exceptionally A+T-rich (82%) sequence and extremely low G+C content (18%), which is the lowest reported for a living organism. These contents of the bases are also varied by species of *Plasmodium*, as shown in Table 1. Early studies have shown that primate malarial parasites contained 37% G+C; later studies have claimed that the content of 37% G+C was contaminated by host white cell. Analyses of the base composition of malarial parasite DNA, indicates *P. falciparum* is more closely related to rodent (P. berghei, P. chabaudi, P. vincke, P. yoelii) and avian (P. lophurae) malarial parasites than to primate (P. vivax, P. malariae, P. knowlesi, P. cynomolgi, P. fragile) malarial parasites. It is generally accepted that the G+C content of host mammalian cells is ~37%, which differs greatly from that of the malarial parasites.
Table 1. The G+C content of various malarial DNA. These values are estimated by the density at which they banded in Hoechst dye cesium chloride gradients. Symbols: +, present; -, absent.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>G+C content</th>
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<tr>
<td></td>
<td></td>
<td>18%</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>Man</td>
<td>+</td>
</tr>
<tr>
<td><em>P. berghei</em></td>
<td>Rodent</td>
<td>+</td>
</tr>
<tr>
<td><em>P. lophurae</em></td>
<td>Bird</td>
<td>+</td>
</tr>
<tr>
<td><em>P. knowlesi</em></td>
<td>Monkey</td>
<td>-</td>
</tr>
<tr>
<td><em>P. fragile</em></td>
<td>Monkey</td>
<td>-</td>
</tr>
<tr>
<td><em>P. cynomolgi</em></td>
<td>Monkey</td>
<td>+</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>Man</td>
<td>+</td>
</tr>
</tbody>
</table>

The RNA of the malarial parasite has been little studied; however, the RNA content of malarial parasite is estimated to be five times greater than the DNA content. Most of the malarial RNA is localized in abundant cytoplasmic ribosomes which are present in the intra-erythrocytic parasites. The ribosomal RNA (rRNA) of parasites is typically protozoan in its sedimentation values of 25S and 15S for 60S and 40S ribosomal subunit, respectively, while rRNAs of the mammalian cells are 28S and 18S. The base composition of the rRNAs from the parasites are found to have a 35% G+C content, whereas that of rRNAs from the host cells have a 64% G+C content. The pattern of rRNA synthesis and the existence of 45S, 37.2S, and 31.7S RNA species suggest that, in malarial parasites, the processing of rRNA may be typically eucaryotic. The characterization of messenger RNA (mRNA) appears to be like that of other eucaryotes in its size range and average chain length of about 1.2 kilobases (kb). The 3'-poly(A) content and average length resemble more closely those of mRNA from higher eucaryotes rather than the shorter chains of lower eucaryotes. Very little is known about transfer RNA (tRNA) in malaria parasites; however, the proportions of rRNA, tRNA and mRNA from *P. falciparum* (82:13:5) are very similar to those found in other organisms.

The malaria parasite is haploid for most of its lifecycle, with zygote formation and meiosis occurring during the mosquito phase of development, as revealed by pulsed-field gradient electrophoresis, *P. falciparum* contains 14 chromosomes which are strikingly polymorphic in size ranging from 600 kb to approximately 3000 kb. These polymorphisms are very useful markers for typing isolates of *P. falciparum* obtained from endemic areas. The mechanisms of these variations have been found to be polymorphisms both in length and in sequence of the subtelomeric regions which are located at the ends of most parasite chromosomes. These are the result of aberrant DNA rearrangements during mitosis. The rearrangements seem to have no specific function and also occur in the DNA of many other eucaryotes. A number of genes encoding malarial antigens currently viewed as candidate vaccine molecules have been assigned to different chromosomes, e.g., chromosome 1 (ring-infected erythrocytic surface antigen, RESA), chromosome 3 (circumsporozoite antigen, CSP), chromosome 4 (dihydrofolate reductase), chromosome 7 (S antigen), and chromosome 14 (thrombospodin-related anonymous protein).

The intra-erythrocytic stage of *P. falciparum* (namely ring-stage and growing trophozoites, immature and mature schizonts) replicates itself 10-20 times during the last 4-6 hours (mature schizont stage) of its 48-hour life-cycle. If an average malarial parasite produces about 16 merozoite nuclei every 48 hours, the parasite doubling time is approximately six hours. An important consideration would be: what are the syntheses for the nucleic acids and the enzymes for such syntheses? Typically, DNA synthesis in the malarial parasite starts at 29.5-31 hours after invasion of the red cell by the parasite. The enzyme
responsible for chromosomal DNA replication in most eucaryotic cells is DNA polymerase-α in its association with primases. The enzyme has recently been found and partially characterized in malaria parasites. The malarial enzyme is strongly inhibited by aphidicolin and hydroxyurea as well as mammalian DNA polymerase-α, but a monoclonal antibody recognizing an epitope on the catalytic subunit of the enzyme show on cross-reactivity with the human enzyme. These may be potentially exploitable in terms of developing novel antimalarial chemotherapy based on the structural differences between the host and parasite enzymes. The sources of nucleic acid precursors, both purine and pyrimidine bases, will be discussed later.

Purine and pyrimidine metabolism of the host red cell

The mature red cell and reticulocyte contain little or no DNA. Although reticulocytes contains RNA they cannot synthesize it from exogenous uridine or adenosine. The normal red cell has a relatively limited network of purine salvage enzymes and no capacity for pyrimidine biosynthesis. The white cell contains both RNA and DNA and these can be synthesized from exogenous purine and pyrimidine bases and nucleosides. In this discussion, the metabolism of both purines and pyrimidines in the normal red cell is mentioned only as the basis for the knowing more about perturbation that occurs following malarial infection.

The mature red cell has no capacity for de novo of purines and can utilize preformed purines by salvage pathways for ATP and GTP synthesis as its major requirement. The purine bases, namely adenine, hypoxanthine and guanine, can be obtained directly from serum by the normal red cells using a transport system the details of which remain to be resolved. The purine nucleosides, namely adenosine and guanosine, can be salvaged from outside the red cells by the nucleoside transport which is associated with the glucose transporter in band 4.5 of red cell membrane protein with a molecular weight of 45-60 kDa. All purines, except inosine and hypoxanthine, that enter the red cell are then metabolized to ATP and GTP by a series of enzymatic reactions which still operate in the cells. The conversion of IMP to XMP and GMP is absent in the mature red cells, although such conversion can be found in the reticulocytes by the residual activities of IMP dehydrogenase and GMP synthetase. Furthermore, there is no interconversion between ATP and GTP in the mature red cells (Fig. 1). Interestingly, the purine analogs, such as erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), cofornycin and deoxycoformycin, all potent inhibitors of red-cell adenosine deaminase, have antimalarial effect both in vitro against P. falciparum and in vivo against P. knowlesi in monkeys.

![Diagram of Purine and Pyrimidine Metabolism](image)

**Figure 1.** Purine salvage pathway in malaria parasites. The abbreviations used are as follows: AMP, adenosine monophosphate; AMPS, adenylosuccinate; ATP, adenosine triphosphate; dATP, deoxyadenosine triphosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; dGTP, deoxyguanosine triphosphate; IMP,inosine monophosphate; XMP, xanthosine monophosphate. The numbers used indicate the enzymes as follows: 1, adenosine deaminase; 2, purine nucleoside phosphorylase; 3, hypoxanthine-xanthine-guanine phosphoribosyl transferase; 4, adenylosuccinate synthase; 5, adenylsuccinate lyase; 6, nucleoside monophosphate kinase; 7, nucleoside diphosphate kinase; 8, adenosine phosphoribosyl transferase; 9, IMP dehydrogenase; 10, GMP synthase.
The red cell has no ability to de novo synthesis of pyrimidine because of the absence or very low levels of a number of enzymes responsible for its synthesis. In addition, there is no salvage pathway for utilization of the pyrimidine nucleosides, namely uridine and thymidine, even though both nucleosides can be taken up by the cell.

**Purine metabolism in the malaria parasite**

We have focused on the metabolism of purines for two purposes. One is to redirect attention to the remarkable deficiency of malarial parasites with respect to the biosynthesis of purines de novo, and the other is to discuss the salvage pathways they employ, in particular those that have unusual properties. These novel characteristics have been realized for most parasitic protozoans.

Based largely on two major technological requirements, the in vitro continuous culture of *P. falciparum* to permit sufficient quantities for biochemical analyses and the development of high-performance liquid chromatography (HPLC) to measure comprehensively and accurately all purine pathway intermediates, purine metabolism as well as pyrimidine and folate metabolism. Direct evidence of a lack of de novo purine synthesis in the intra-erythrocytic stage of the malarial parasite was provided by the lack of incorporation of radiolabelled precursor formate or glycine into purine nucleotides and by the absence of various enzymes of de novo synthesis.

Malaria infection results in a much greater ability of the red cell to salvage purines, owing to the existence of enzymes responsible for the synthesis of purine nucleotides. It has been suggested that the major purine incorporated into the parasite is hypoxanthine, the most abundant purine base contained in blood. Using hypoxanthine as the radiolabelled precursor, it was found that purine nucleotide metabolism in malaria-infected red cells is different from that of host red cells in two important metabolic aspects: active synthesis of GMP, GDP and GTP from hypoxanthine via inosine 5'-monophosphate (IMP); and the presence of an adenylosuccinate pathway to synthesize AMP, ADP and ATP from IMP. In addition, the parasite contains very high activity of adenosine deaminase for conversion of adenosine to inosine and hypoxanthine. It has been proposed that this enzyme may be the in vivo source of hypoxanthine for the malarial parasite. The purine salvage pathway in the malaria parasite is summarized in Fig.1. One important parasite enzyme involved in the purine salvage pathway is hypoxanthine-xanthine-guanine phosphoribosyltransferase (HGPRT) which has the ability to utilize hypoxanthine, guanine and xanthine as substrate, whereas the human host HGPRT lacks activity towards xanthine. This difference in substrate specificity leads to the development of chemotherapeutic agents specific for the parasite HGPRT (Davidson, Jr., personal communication).

**Pyrimidine metabolism in the malaria parasite**

As essential components of parasite development and replication, the metabolism of nucleic acid precursors is one logical component for the development of non-empirical (rational) antimalarials agents. Malaria parasites require purines and pyrimidines for DNA and RNA synthesis during exponential growth. The parasites use preformed purines, which are salvaged from the host, but they have to synthesize pyrimidine de novo (Fig.2). The requirement of low oxygen tension during intra-erythrocytic growth is proposed as being linked to dihydroorotate (DHO) oxidation of pyrimidine biosynthesis and a simplified electron transport system (ETS) of mitochondria containing dihydroorotate dehydrogenase (DHOase), ubiquinones, cytochromes band c, and finally, cytochrome oxidase (Fig.3). The intra-erythrocytic malarial parasites derive ATP from glycolysis and do not require oxygen for energy production. We have focused our attention on pyrimidine biosynthetic enzymes and mitochondrial metabolism because several lines of evidence suggested that there are some key differences between the malaria parasites and the mammalian host system.
Figure 2. Pyrimidine biosynthetic pathway in malaria parasites. The abbreviations and numbers used are as follows: 1, CPS II (carbamyl phosphate synthase II); 2, ATCase (aspartate transcarbamylase); 3, DHOase (dihydroorotase); 4, DHODase (dihydroorotate dehydrogenase); 5, OPRTase (orotate phosphoribosyl transferase); 6, ODCase (orotidine 5'-phosphate decarboxylase).

Figure 3. Proposed relationship among O₂ utilization, electron transport system and pyrimidine biosynthesis.
Evidence of functional pyrimidine biosynthesis de novo in the intra-erythrocytic stage of the malaria parasite comes largely from these lines of investigation. Firstly, _P. falciparum_ growing in vitro culture can incorporate radioactive bicarbonate into pyrimidine bases of DNA and RNA. In the parasite, it has been demonstrated that there is very little incorporation of orotate, uridine and thymidine into DNA and RNA, whereas these pyrimidines can be easily taken up by the _P. falciparum_-infected red cell (Krungkrai, unpublished results). (13) Secondly, the enzymes responsible for salvaging preformed pyrimidine bases have never been demonstrated; those enzymes are thymidine kinase, (14) uridine kinase and cytosine kinase. (21) Thirdly, all the enzymes required for uridine 5'-monophosphate (UMP) synthesis de novo (see Fig. 2) have been detected in extracts from several species of _Plasmodium_. (20-23)

The first three enzymes of pyrimidine biosynthesis [carbamyl phosphate synthase II (CPS II), aspartate transcarbamylase (ATCase), dihydroorotase (DHOase)] in the malaria parasites _P. berghei_ and _P. falciparum_ are found to be separated on individual proteins by using a Superose 12 gel filtration column on a fast protein liquid chromatography (FPLC) system in the presence of stabilizing agents and mixtures of protease inhibitors. In a control experiment with host mammalian enzymes under the same conditions, the three enzymatic activities were eluted at the same position on the FPLC system of apparent _M_r > 670 kDa. (22) Our result is similar to what is found in most procaryotic systems but differs from the analogous mammalian systems in which the first three enzymes are associated with a single multifunctional protein. (26) We have extensively characterized the third enzyme DHOase catalyzing the reversible reaction of carbamyl aspartate (CA) and dihydroorotate (DHO), and the mechanism of catalytic action has been proposed as follows: the action of an active-site Zn^{2+} ion which can coordinate to a putative tetrahedral intermediate form following attack by the ureido amino function at the β-carboxyl group of CA (in the forward direction) or following attack by water at this carboxyl group (in the backward direction) (Fig. 4). (21)

![Diagram](image)

**Figure 4.** Proposed malarial DHOase enzymatic catalysis.
The fourth enzyme of the pathway, dihydroorotate dehydrogenase (DHODase), has been shown to be a particulate protein and is extensively characterized. By analogy to other systems the DHODase is expected to be associated with the outer surface of the inner membrane of the mitochondria in the malaria parasite. The last two enzymes, orotate phosphoribosyltransferase (OPRTase) and orotidine 5'-phosphate decarboxylase (ODCase), catalyze the final steps of de novo synthesis of uridine 5'-monophosphate (UMP) in mammalian cells and exist as bifunctional protein, but in malaria parasites these enzymes have been described as discrete entities. From the lines of evidence, it appears that de novo biosynthesis of pyrimidine in malaria parasites is catalyzed by six discrete enzyme activities, different from that in the analogous mammalian process. Furthermore, while the malaria parasites are totally dependent on the de novo synthesis because they lack relevant salvage enzymes (for instance, thymidine kinase), in contrast, the host mammalian cells can either use preformed pyrimidine bases and nucleosides or synthesize their own. These key differences suggest that the malaria parasites may be sensitive to novel antimalarial drugs that act on pyrimidine de novo synthesis. One such compound, hydroxynaphthoquinone A566C, possibly inhibiting DHODase activity, is being developed and tested in a human trial.

Pyrimidine biosynthetic enzymes as drug targets

In order to exploit fully these pharmacological targets, it is necessary to pursue detailed mechanistic and structural characterization of these enzymes of pyrimidine biosynthesis. We have studied novel forms of DHODase and DHODase from malarial parasites. The malarial DHODase has a monomeric structure with a native molecular mass of 38 kDa. Not withstanding the marked physical differences, the characterization of kinetic parameters of the malarial DHODase suggest that the protozoan, mammalian and bacterial DHODase enzymes share a common catalytic mechanism.

The malarial DHODase has been purified to apparent homogeneity by detergent solubilization followed by anion-exchange, dye-agarose affinity and gel filtration chromatography. It is membrane-bound and active in monomeric form with a molecular weight of 55 kDa. The DHODase contains about 2 mol of iron and 2 mol of acid-labile sulfur per mole of enzyme, and does not contain a flavin cofactor. It is interesting to note that the optimum activity of DHODase requires ubiquinone coenzymes CoQ, CoQ, CoQ or CoQ. The cofactor and kinetic analyses of the enzyme-catalyzed reaction suggest that the enzyme catalyzes DHO oxidation through the inter-

![Diagram](image)

Figure 5. Proposed malarial DHODase enzymatic catalysis.
mediary of a single [2Fe-2S] iron-sulfur cluster with ubiquinone acting as an obligatory electron transfer (Fig.5). Our results support the hypothesis of the linkage of pyrimidine biosynthesis to the electron transport system and limited oxygen utilization in malaria parasites by DHODase via ubiquinones.

Recently, we have reported that there is a cytosolic form of DHODase which catalyzes the reduction of orotate to DHO. The physiological significance of this form remains to be elucidated. Interestingly, the purified malarial DHODase has ability to generate a superoxide radical during oxidation of dihydroorotate to orotate as is evidenced by the following. The rate of dichlorophenol-indophenol, cytochrome c and nitroblue tetrazolium reductions by DHODase were significantly inhibited by enzyme superoxide dismutase (SOD) or theonyltriﬂuoroacetone (specific iron chelator of the enzyme). This was also conﬁrmed by activity staining on non-denaturing polyacrylamide gel electrophoresis of the purified enzyme. Our results are consistent with the finding of the existence of manganese-SOD in the mitochondria of the parasite, suggesting that the production of the superoxide radical may occur in vivo.

There is a need for new antimalarials with modes of action different from those of drugs currently in use or even promising drugs since the spread of multi drug-resistant P. falciparum is very rapid. Recent work has modiﬁed our knowledge about the malarial enzymes of pyrimidine biosynthesis, these enzymes may be good targets for the development of antimalarial drugs. We have carried out a systematic study testing known compounds affecting the enzymes of the pathway and also having potent antimalarial activity against P. falciparum grown in vitro and P. berghei in mice. Orotate and a series of ﬁve substituted derivatives are found to inhibit competitively the purified DHOase and DHODase enzymes in the malarial parasite (Table 2). As mentioned previously, the malarial DHODase catalyzes the reversible reactions of CA and DHO. The following results on the kinetic parameters and inhibitory effect of orotate analogs on the malarial DHODase are from the reaction of DHO to CA (the backward reaction). The order of effectiveness as inhibitors for malarial DHODase is as follows: F-OA>HN₃-OA, CH₂-OA>OA>Br-OA>I-OA, whereas OA, F-OA and NH₃-OA are the most effective inhibitors for malarial DHODase.

Table 2. Effect of orotate analogs on the malarial DHOase and DHODase enzymes.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Symbols</th>
<th>DHOase Kₘ (µM)</th>
<th>DHOase Kᵢ (µM)</th>
<th>DHODase Kₘ (µM)</th>
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<td></td>
</tr>
<tr>
<td>orotate</td>
<td>OA</td>
<td>-</td>
<td>860</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td>5-fluoroorotate</td>
<td>F-OA</td>
<td>-</td>
<td>65</td>
<td>-</td>
<td>35</td>
</tr>
<tr>
<td>5-amino(orotate</td>
<td>NH₃-OA</td>
<td>-</td>
<td>142</td>
<td>-</td>
<td>44</td>
</tr>
<tr>
<td>5-methylorotate</td>
<td>CH₂-OA</td>
<td>-</td>
<td>166</td>
<td>-</td>
<td>158</td>
</tr>
<tr>
<td>5-bromo(orotate</td>
<td>Br-OA</td>
<td>-</td>
<td>2200</td>
<td>-</td>
<td>380</td>
</tr>
<tr>
<td>5-iodoorotate</td>
<td>I-OA</td>
<td>-</td>
<td>&gt;3500</td>
<td>-</td>
<td>464</td>
</tr>
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</table>
As may be observed from Table 3, F-OA has the most potent antimalarial activity against both *P. falciparum* in vitro and *P. berghei* in mice. CH₃-OA and NH₂-OA also have potent antimalarial activity. Our results on the inhibitory effect and antimalarial activity of orotate analogs suggest that the two enzymes, DHOase and DHODase, in the malaria parasite may be good targets for the design of new classes of antimalarial drugs.³⁰⁻³³

<table>
<thead>
<tr>
<th>Orotate analogs</th>
<th><em>P. falciparum</em> [IC₅₀ (µM)]</th>
<th><em>P. berghei</em> [ED₉₀ (mg/kg)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td>N.E.</td>
<td>N.E.</td>
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<tr>
<td>F-OA</td>
<td>0.06</td>
<td>2.5</td>
</tr>
<tr>
<td>CH₃-OA</td>
<td>6.0</td>
<td>&gt;25.0</td>
</tr>
<tr>
<td>NH₂-OA</td>
<td>50.0</td>
<td>&lt;10.0</td>
</tr>
<tr>
<td>Br-OA</td>
<td>N.E.</td>
<td>not test</td>
</tr>
<tr>
<td>I-OA</td>
<td>N.E.</td>
<td>not test</td>
</tr>
</tbody>
</table>

IC₅₀: concentration of compound producing 90% inhibition of *P. falciparum* grown in vitro. ED₉₀: dose of compound giving 90% inhibition of parasitemia for 4-day suppressive test in mice infected with *P. berghei*. N.E.: no effect on parasite morphology at 1.0 mM for *P. falciparum* or at dose of 25 mg/kg for *P. berghei*.

In collaboration with Drs. A. Cerami and G.B. Henderson (Rockefeller University, New York), we have synthesized a quinone analog 2-chloromethyl-3-methyl-1,4-naphthoquinone, a mechanism-based inhibitor for malarial DHODase. Some kinetic parameters of the inhibitory effect against the purified DHODase have been identified. The quinone has K₅₀ and K₉₀ values of 7µM and 0.86 min⁻¹, respectively. It shows moderate antimalarial activity against *P. falciparum* in vitro with 50% inhibitory concentration of 10µM. In vivo antimalarial activity of the quinone against *P. berghei* infected mice at a dose of 17.5 mg/kg body weight daily for four days cleared parasitemia in infected mice, in comparison with chloroquine therapy with a standard dose of 25 mg/kg.¹⁰⁻¹² The efficacy of quinone is somewhat comparable to that of chloroquine. Series of the quinone analogs as mechanism-based inhibitors are being studied.

**Conclusion**

Much effort is being spent to develop malaria vaccines, but despite the vast amount of knowledge gained about the parasite's life-cycle and many surface antigens, the prospects of success are as yet uncertain. The vaccine candidates have not been as immunogenic as was hoped. Our results, together with that of other investigators, suggest that the pyrimidine biosynthetic pathway and mitochondrial metabolism may be a promising target for the design of antimalarial drugs. The attractiveness of these targets will become clearer as the identities and specificities of all enzymes in the pathway become clearer.

**A perspective**

At present, the utility of most antimalarial drugs is threatened or impaired by the emergence of multidrug-resistant malaria parasites. Therefore, improvements in chemotherapy for treatment of malaria are urgently required. The new developments will require better understanding of the mechanisms of drug action and drug resistance and also the basic biochemistry of the malaria parasite.

**Acknowledgements**

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