Biomedical & Pharmacology Journal Vol. **4**(1), 21-28 (2011)

Lipid Metabolism in Plasmodium: Implication as Possible Target for Chemotherapy

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(Received: June 10, 2011; Accepted: July 09, 2011)

ABSTRACT

Lipid metabolism of the parasite is associated with alterations in fatty acids and cholesterol in the erythrocyte plasma membrane, which in turn are responsible for changes in permeability and fragility. The augmentation of all the membrane systems of the infected erythrocyte causes the lipid content to rise rapidly, but the parasite lipid composition differs from that of the erythrocyte in many respects. Phospholipid metabolism has been identified as an ideal target for novel anti-malarial chemotherapy due to its vital importance to the parasite. This paper attempts to review the underlying lipid metabolic pathways in the malaria parasite and their potential benefit as likely targets for novel anti-malarial chemotherapy.

Key words: Lipid metabolism, plasmodium, possible target, chemotherapy.

INTRODUCTION

The invasion of human erythrocytes by malaria parasite initiates Plasmodium development in a vacuole bound by erythrocyte-derived membrane, whose asymmetrical distribution of lipids is reversed in its orientation with respect to the parasite plasma membrane. The malaria parasite is incapable of synthesizing fatty acids *de novo*, but utilizes preformed fatty acids and lipids from the host. An enzyme capable of activating fatty acids, which is necessary for incorporation, into lipids has been localized to membranous structures found within the cytoplasm of the infected erythrocyte[1]. Lipid metabolism of the parasite may be associated with alterations in fatty acids and cholesterol in the erythrocyte plasma membrane, which in turn are responsible for changes in permeability and fragility[2]. A study evaluated the constitution of phospholipid classes and the content of cholesterol of various strains of *Plasmodium falciparum*-infected human erythrocytes grown in *in vitro* cultures in conjunction with drug susceptibility[3]. Study reveals that uninfected erythrocytes in the culture serve as a major source for the increased lipid content of malaria-infected cells. The alterations of the phospholipid composition of infected cells that results from parasite lipid metabolism are also reflected in the constitution of uninfected red cells, implying lipid exchange between infected and uninfected cells.

Glycerolipid and fatty acid biosynthetic pathways

The augmentation of all the membrane systems of the infected erythrocyte causes the lipid content to rise rapidly, but the parasite lipid composition differs from that of the erythrocyte in many respects: it is higher in diacyl phosphatidylethanolamine, phosphatidylinositol, diacylglycerols, unesterified fatty acids, triacylglycerols, hexadecanoic and octadecanoic fatty acids; but lower in sphingomyelin, phosphatidylserine, phosphatidylethanolamine,

cholesterol and polyunsaturated fatty acids. Active lipid metabolism accompany the membrane proliferation associated with feeding, growth and reproduction of malaria parasite.

Lipid metabolism is absent from normal mature human erythrocytes[4] but the phospholipid content increases by as much as 500% in the infected erythrocytes[5,6]. It has been previously shown that impairment of phospholipid biosynthesis with polar head analogs, which interfere with natural polar head incorporation either by substitution or competition[7-9] with fatty acids[10] is lethal to the intra-erythrocytic stage of *Plasmodium falciparum in vitro*.

In a study aimed at thoroughly studying the mechanism of action of compounds showing marked anti-malarial activity, the effect of the most active compounds on PC (phosphatidylcholine) and PE (phosphatidylethanolamine) biosynthesis was evaluated by measuring the incorporation of radioactive choline into PC as well as the incorporation of radioactive ethanolamine into PE using a cell harvester for rapid serial determination[11]. A study reported close correlation between impairment of phospholipid biosynthesis and inhibition of *in vitro* malaria parasite growth[12]. Compounds showing marked anti-malarial activity in the study, are assayed for their effects on phospholipid metabolism. It further revealed that the most active compounds are inhibitors of *de novo* phosphatidylcholine biosynthesis from choline. The report highlighted that specific anti-malarial effects of choline or ethanolamine analogs, are thus likely mediated by their alteration of phospholipid metabolism; and contended that *de novo* phosphatidylcholine biosynthesis from choline is a very realistic target for novel anti-malarial chemotherapy against pharmacoresistant strains.

Phospholipids are absolutely necessary for parasite membrane biogenesis and it has been shown that impairment of phospholipid metabolism is lethal to *Plasmodium falciparum in vitro*[7-10]. When considering the correlation between phospholipid metabolism impairment and parasite growth inhibition, molecules appear to segregate into two groups. The most whose IC_{50} is higher than 50μmol/L appear to be well distributed along the bisecting line (PL_{50}=IC_{50}). This contrasts with the second group that has a long alkyl chain and IC_{50} of less than 50μmol/L.

Lipid synthesis by malaria parasite is investigated by quantitatively measuring the parasite's ability to incorporate ^{14}C-labeled glucose carbon into various lipids *in vitro*[12]. Glucose is chosen as the substrate for lipid synthesis because glucose carbon serves as a primary source of the acetate units required for the *de novo* synthesis of fatty acids and sterols, as a source of the α-glycerol phosphate required for the synthesis of glycerides and phospholipids. Thin layer chromatographic procedures are used to separate the extracted lipid into neutral and phospholipids and to fractionate the neutral and phospholipids into various classes[13,14]. The individual neutral and phospholipid fractions are recovered from the plates and assayed for ^{14}C content with a lipid scintillation counter[15]. Results reveal that lipid synthesis observed for all three cell synthesis represent primarily a synthesis of phospholipids; in all instances between 90 to 99% of the total ^{14}C lipid activity is recovered from the phospholipids.

It has been shown that the malaria parasite is incapable of synthesizing lipids *de novo* and restricted to obtaining preformed fatty acids from the host. Several parasite enzymes involved in lipid biosynthesis from glycerides and free fatty acids as well as enzymes involved in the remodeling of lipid polar head groups have been identified[16]. A study to determine the *in vitro* incorporation of sodium acetate into the lipid classes of blood cells infected with malaria parasite reported that free fatty acids of both normal and infected plasma contain most of the activity found in their total lipids. The parasitized blood cells demonstrated greater incorporation of ^{14}C into their lipids than did plasma or normal blood cells. The phospholipid fractions of normal and parasitized blood cells possess most of the ^{14}C activity. A study reported a four-to-fivefold increase in the fatty acid content and a two-to-fourfold increase in phospholipid content of Plasmodium infected erythrocytes.

The high concentration of ^{14}C tagged free fatty acids found in the infected plasma is possibly due to the increased metabolism within the infected cells. It has been shown from experimental evidence

that sterols are produced to a greater extent in parasitized than in normal cells, since there is a significant amount of incorporation by sterols and sterol esters of the infected cells.

Several enzymes which are associated with the type II fatty acid synthetic pathway have been identified in Plasmodium and appear to be located in the apicoplast. Plasmodium homologues of enzymes involved in type II fatty acid synthesis has apicoplast targeting sequence and are sensitive to known inhibitors of type II fatty acid synthetase. This enzymatic biosynthetic pathway is a particularly attractive drug target for anti-malarial chemotherapy, since the human host synthesized fatty acids via different pathways utilizing different enzymes. A study revealed the discovery of two genes encoding type II fatty acid biosynthesis proteins: ACP (acyl carrier protein) and KAS III (beta-acetoacyl-ACP synthase III)[17]. The initiating steps of a type II system require a third protein: malonyl-co-enzyme A:ACP transcyclase (MCAT). The study described the identification of a single gene from *Plasmodium falciparum* encoding PfMCAT and the functional characterization of this enzyme. Another study presents evidence that two members of the *Plasmodium falciparum* acyl-CoA synthetase (PfACS) family are responsible for the activation of long-chain fatty acids by thio-esterification with CoA[18]. It described co-immunoprecipitation of ankyrin and of ACS1/3 indicating that at least a fraction of these proteins are physically associated with the infected erythrocytes and provide evidence for a novel specific interaction which suggests that such a binding brought these enzymes closer to the host erythrocyte membrane where exogenous fatty acids are available.

A study evaluated the constitution of phospholipid classes and the content of cholesterol of various strains of *Plasmodium falciparum*-infected human erythrocytes grown in *in vitro* cultures in conjuction with drug susceptibility[3]. Study reveals that uninfected erythrocyes in the culture serve as a major source for the increased lipid content of malaria-infected cells. The alterations of the phospholipid composition of infected cells that results from parasite lipid metabolism are also reflected in the constitution of uninfected red cells, implying lipid exchange between infected and uninfected cells.

The metabolism and dynamics of lipids in malaria infected erythrocytes have been extensively reviewed[19]. The phospholipid composition of infected is substantially different from that of normal red blood cells. This altered composition is achieved through various processes, of which *de novo* synthesis could supply all the needs of the parasite lipid anabolism provided adequate concentration of substrates is supplied. The *in vitro* culture system is obviously different from *in vivo* conditions where the large systemic increase in plasma fatty acids and triacylglycerols upon infection can serve the parasite's lipid metabolism.

The bi-product of lipid metabolism are reactive oxygen intermediates such as superoxide, hydroxyl radical and hydrogen peroxide. These reactive oxygen intermediates (ROIs) damage Plasmodium lipids. Glutathione peroxidase is involved in the detoxification of these reactive oxygen intermediates. Oxidized glutathione is recycled and the reducing equivalents of NADPH generated probably by pentose phosphate cycle. However, it has been proposed that glutamate dehydrogenase provides the reduced NADPH needed for glutathione reductase[20]. Interestingly, the malaria parasite may supply the host erythrocyte with glutathione which could participate in protecting the host cell from oxidative damage[21]. It should be noted that the parasite lipid metabolism is intertwined with that of the host's because of the intimate relationship between the host and parasite.

The biosynthesis of sphingolipids *de novo* has been described in *Plasmodium falciparum*[22]. Studies of intra-erythrocytic development of *Plasmodium falciparum* have established that sphingomyelin is synthesized by a parasite-specific enzyme[23-25], and was important for parasite-mediated nutrient uptake[26]. However, in contrast to other eukaryotic cells, no discernible amounts of steryl esters are produced, and cholesterol is nearly absent in the malaria parasite[27].

The plasma membrane of infected erythrocyte contains more phosphatidylcholine and phosphatidylinositol and less sphingomyelin than the

plasma membrane of normal uninfected erythrocyte[28]. Large increases in the levels of palmitic (C16:0) and oleic (C18:1) acids and major decreases in the levels of polyunsaturated fatty acids such as arachidonic (C22:6) acids, occur as a result of malaria infection. This makes the phospholipid composition very similar to that of acids, such as arachidonic (C20:4) and docosahexanoic (C22:6) acids as a result of infection. Thus, the phospholipid composition is very similar to that of the parasite, indicating that there is intense dynamic phospholipid traffic between the erythrocyte membrane and the membrane of the intracellular parasite. These modifications must be as a result of parasite metabolism of erythrocyte lipids, since mature erythrocytes have negligible lipid synthesis and metabolism. Several studies have shown that the biosynthetic machinery of the parasite can provide all of the new phospholipid molecules.

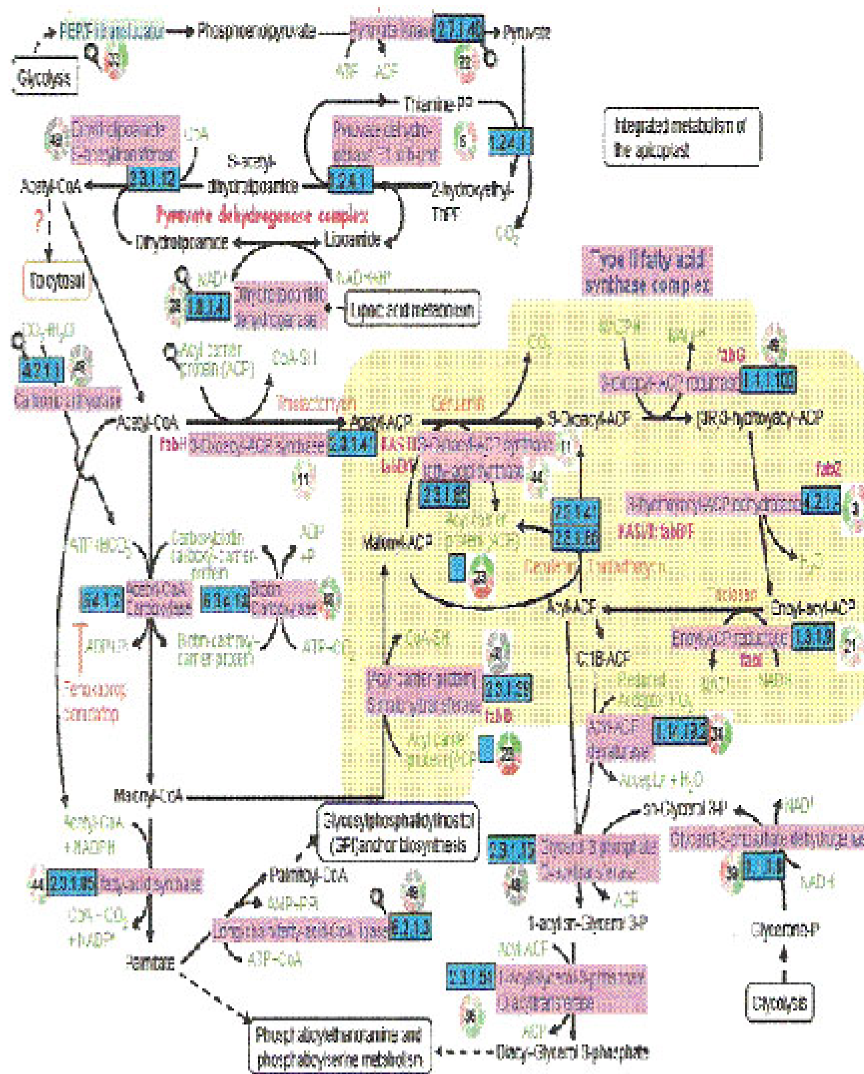

Fig. 1: Pathway of Lipid Biosynthesis in the Apicoplast of Malaria Parasite. (Retrieved on-line from PubMed-http://sites.buji.ac.il/malaria/maps/facidsynthesispath.html)

The intra-erythrocytic stages of *Plasmodium falciparum* accummulate triacylglycerol, produced using oleate and diacylglycerol as substrates[27,29]. In contrast to other eukaryotic cells, neither steryl esters nor cholesterol esters, the second neutral lipid species reported to be important for a related apicomplexan, *Toxoplasma gondii* are detected in *Plasmodium falciparum*.

The genome of *Plasmodium falciparum* contains the genes for fatty acid synthase II (FAS II) pathway[30]. Previous studies have identified and located the FAS II enzymes in the apicoplast[31,32]. In the FAS II pathway each reaction is catalysed by a discrete enzyme[33]. Malonyl-ACP is the starting point and is produced from malonyl CoA and ACP catalyzed by the enzyme Malonyl CoA: ACP transacetylase (fabD).

The enzymes of the fatty acid biosynthetic pathway, in the apicomplexan parasites including the Plasmodium species, are predicted to be localized in the apicoplast; based on the N-terminal extensions suggesting such a localization[34]. Malonyl-CoA itself formed from acetyl-CoA and a single acetyl-CoA carboxylase (ACCase) is predicted to be localized to the apicoplast in the Plasmodium genome. Among the enzymes of the FASII pathway in *Plasmodium falciparum*, fabI or enoyl-ACP reductase, which catalyses the final step in the chain elongation cycle, has been investigated in great detail from the viewpoint of identifying potent inhibitors. The enzyme catalyses the conversion of trans-2-acyl-ACP to acyl-ACP and requires NADH as the cofactor. The creation of transgenic *Plasmodium berghei* parasites with the Pfenoyl-ACP reductase replacing the endogenous counterpart, is another interesting development to serve as an *in vivo* mice model[35] for studying drug efficacy. Three condensing enzymes β-ketoacyl-ACP synthases (PfKAS I, II, III) essentially involved in chain initiation and fabB/F (I,II) involved in chain elongation are also being investigated as possible drug targets.

Isoprenoid biosynthetic pathway

The malaria parasite genome provides evidence for the presence of the non-mevalonate pathway for isopentenyl pyrophosphate (IPP) biosynthesis. The presence of 1-deoxy-D-xylose-5-phosphate (DOXP) synthase in the parasite genome has been reported[36]. Isoprenoids, consisting of isopentenyl pyrophosphate (IPP) repeat units form prosthetic groups of some enzymes and are involved in the synthesis of ubiquinone and dolichol. The DOXP pathway has been fully characterized[37]. The mechanism of export of IPP is not clear, but the close association of the apicoplast with the mitochondrion is visualized to facilitate its import and ubiquinone biosynthesis in the mitochondrion. Thus inhibitors of DOXP reducto-isomerase may have anti-malarial activity[36]. The formation of isopentenyl diphosphate and dimethylallyl diphosphate, both central intermediates in the biosynthesis of isoprenoids in *Plasmodium falciparum*, occurs via the methylerythritol phosphate (MEP) pathway.

Interestingly, metabolic profiles show that DOXP and CDP-ME (4-[cytidine-5-diphospho]-2C-methyl erythritol) are highly accumulated when compared to the other intermediates, mainly in the trophozoite and schizont stages. It is considered that both DOXP and CDP-CE could act as a metabolite reserve, which might be used during schizogony to sustain high demand of isoprenoids, and both intermediates might be key metabolites of the MEP pathway in *Plasmodium falciparum*. The metabolic results are correlated with the transcript profiles of genes involved in the MEP pathway. Results indicate that MEP pathway metabolite peak preceded maximum transcript abundance during the intra-erythrocytic cycle. The MEP pathway associated transcripts are mostly altered by the drug, indicating that parasite is not strongly responsive at the transcript level. A combined analysis of metabolic and transcription profiles may be a useful procedure for the identification of candidate enzymes as novel drug targets[38].

CONCLUSION

A better understanding of the parasite's lipid metabolism may lead to the development of novel therapeutic strategies which exploit the uniqueness of the malaria parasite.

This lends credence to the existence of novel mechanisms and pathways to malaria infection, thus describing a new intervention strategy in the fight against malaria.

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