Metabolism and digestion of p falciparum critical thinking examples

Technology, Development

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Abstract

Plasmodium falciparum is the parasite responsible for the deadly disease, malaria. The parasite is transmitted through the injection of sporozoites into the human blood stream. These sporozoites are transported in the blood stream to the liver. The sporozoites invade the hepatocytes and differentiate into merozoites. The merozoites invade the RBCs of the host; starting the blood stage of infection. The trophozoite stage ingests and degrades approximately 80% of the hemoglobin in the host cell. The hemoglobin is broken down into peptides due to enzymatic action by a metalloprotease and cysteine and aspartic proteases. Haematin is first released as a byproduct of heme digestion before being sequestered into crystals called haemozoin. These proteases perform the highly essential digestion of Haemoglobin, and

their inhibition results in death of the parasite. This is because free heme compromises cell and membrane integrity of P. falciparum. They are therefore very attractive targets for drug action. An antimalarial drug like Chloroquine, CQ, has been widely used as a synthetic quinoline. CQ is thought to bind to Ferriprotoporphyrin, (FP- remaining undigested heme), and is taken up highly selectively and concentrates in the infected erythrocytes. The development of resistance to P. falciparum infection has greatly hindered treatment by anti-malarial drugs. Studies have identified that the P. falciparum chloroquine resistance transporter (PfCRT) and PfMDR1 (multidrug resistance) proteins as the targets for potential mutations that result into resistance. Research is now focused on finding new chemotherapeutic agents to combat the parasite. Understanding the mode of Chloroquine resistance gives better insight into developing older classic 4-aminoquinolines. New derivatives of these drugs are believed to be able to combat resistant parasites.

Introduction

Plasmodium falciparum is the parasite responsible for the deadly disease, malaria. Tilley et al. (2007), reports that it causes approximately one million deaths annually. The parasite is injected into humans through the female Anopheles mosquito as it partakes of a blood meal. It is at the blood stage of parasitic infection that malaria pathology becomes evident (Tilley et al., 2011; Brayl et al., 2005). The infection is made worse by the adhesion of infected red blood cells, (RBCs), to walls of blood vessels as they avoid splenic clearance. This assignment therefore seeks to explore the way in

which metabolism and physiology of the parasite has evolved to allow for the digestion and the atypical processes which have been employed in the development of 4-aminoquinoline drugs.

Life cycle of P. falciparum

Figure 1: Life Cycle of P. Falciparum

The female anopheles mosquito injects into human skin, sporozoites of P. falciparum during a blood meal. These sporozoites are transported in the blood stream to the liver. The sporozoites invade the hepatocytes (Tilley et al., 2011). In the hepatocytes, the sporozoites differentiate into merozoites. The merozoites invade the RBCs of the host; starting the blood stage of infection. Merozoites change into trophozoites and then into schizonts. Schizonts are responsible for the release of new merozoites as they burst (Brayl et al., 2005). These merozoites infect new RBCs. The parasite stops the transportation of infected RBCs into the spleen, by coating the RBC with adhesive proteins. These adhesive-coated RBCs stick to the walls of small blood vessels, which may block the circulation system. This process is known as cytoadherance.

Merozoites may develop into gametocytes, which are capable of infecting mosquitoes. Microgametes are male gametocytes, while macrogametes are female gametocytes. These infect mosquitoes when they are ingested during a blood meal. In the mid-gut of the mosquito, both female and male gametocytes combine to form zygotes, which would then grow into ookinetes. The ookinetes are motile and pierce through the mid-gut walls and grow into oocysts. Oocysts release sporozoites after sometime, which

move to the mosquito’s salivary glands. When the mosquito takes a blood meal, the sporozoites are injected into the human bloodstream through the skin. In a mosquito, development takes approximately two weeks, during which it is not infective. After that, the mosquito can transmit sporozoites. Interestingly, the life cycle of the parasite can only occur at temperatures above 20 degrees Celsius (Brayl et al., 2005).

The Hemoglobin Digestive Process

P. falciparum, is the parasite responsible for the malaria, a disease that causes approximately one million deaths annually. It undergoes the process of erythrocytic schizogony which is responsible for the clinical manifestations of malaria. The trophozoite ingests and degrades approximately 80% of the hemoglobin in the host cell (Brayl 2005). As the trophozoite continues to develop, its endolysosomal system takes up the cytoplasm of the host cell using cytosomes which contains host hemoglobin. Cytosomes are formed by an invagination formed from the PVM (parasitophorous vacuolar membrane) and PPM (parasite plasma membrane) from which transport vesicles are pinched off. These vesicles fuse with the digestive vacuole (DV) and releases into contents into the DV.

Researchers postulate that the initiation of the hemoglobin digestion begins in the transport vesicles and is concluded in the DV (Brayl 2005). During digestion, the protein component of the hemoglobin is broken down into peptides due to enzymatic action by a metalloprotease and cysteine and aspartic proteases. Once the hemoglobin is broken into small peptides, they are removed from the endolysosomal system into the cytoplasm of the

parasite. Tilley et al., (2010) posit that once in the cytoplasm, cytosolic aminopepetidase hydrolyses the small peptides into liberated amino acids. This digestion process consumes a large amount of the parasite's energy, due to the recycling of the PVM and PPM and the creation of a suitable proton gradient for the optimum functioning of the protease enzymes (Tilley et al. 2010). A byproduct produced by this process is known as heamatin. Haematin is detoxified by its crystallization into haemozoin and then preserved in the digestive vacuole (Tilley et al. 2010).

The Digestive Vacuole and Proteases

Tilley et al., (2011) explains that the heme is digested by proteases. The free amino acids are used in the production of proteins while the remaining ones are exported out of the cell (Brayl et al., 2005). Tilley et al., clarifies that haematin is first released as a byproduct of digestion before being sequestered into crystals called haemozoin. The haemozoin is referred to as the malaria pigment and remains in the DV. Bonilla et al., (2007), highlights the proteases involved in haemoglobin digestion in the DV: four aspartic proteases; and plasmepsins (PfPM1, PfMP2, PfM2, PfHAP, PfPM4). Other proteases include proteases include: three cysteine proteases nown s Falcipains- PfFP1, PfFP2, PfFP3; and Falcilysin, a metalloprotease (Bonilla et al., 2007).

Plasmepsin I (PfPMP1), also known as aspartic haemoglobinase I initiates the breakdown of haemoglobin while PfPMP2 (plasmepsin II), splits the acid-denatured haemoglobin (Brayl et al., 2005). Falcipains cleave the globins which have been denatured into amino acids and small peptides (Brayl et al.,

2005). Falcipains do not act on FP or Hb. Since these proteases perform highly essential digestion of Hb, inhibiting them results in death of the parasite. They are therefore very attractive targets for drug action (Bonilla et al., 2007).

Figure 2: Proteases in the Digestive Vacuole, (Tulane, 2011).

Heme Detoxification

Heme detoxification utilizes a large amount of the parasite's energy. This is because it involves recycling of its PM and PVM, coupled with the creation of a proton gradient which is essential for the optimal function of proteases (Brayl et al., 2005). The heme which remains undigested is called (Ferriprotopoyphyrin IX, FP). FP causes the lysis of the parasite's membranes and suppression of various metabolic enzymes (Brayl et al., 2005). According to Tulane, (2011), three mechanisms have been identified through which heme detoxification occurs. The first mechanism is the sequestration of FP into haemozoin, HZ, a crystalline substance. Tulane, (2011), highlights that HZ is a chemically inert substance.

The other mechanisms are: degradation in the food vacuole by hydrogen peroxide; and breakdown facilitated by a glutathione-dependent process in the parasite's cytoplasm (Tulane, 2011). Hydrogen peroxide initiates oxidation of the poryphyrin ring which causes the opening and degradation of heme. The glutathione mediated process may occur when some heme is exported out of the DV into cell cytoplasm, where it is oxidized by reduced glutathione (Tulane, 2011). It is hypothesized that the pathway for HZ formation and the degradation pathways may operate simultaneously.

Tulane, (2011), suggests that in both pathways, free heme is turned into haemozoin and the rest is degraded. The process of HZ formation is not clear, but a few suggestions have been proposed (Tulane, 2011; Brayl et al., 2005). Certain lipids and membranes may assist in the solubilization of FP to prepare it for conversion into HZ (Tulane, 2011; Brayl et al., 2005).

Mode of Action of Quinoline

Drugs containing quinoline have been used in the treatment of malaria since the separation of quinine from the Cinchona tree bark in the early nineteenth century. Since the 1920s, synthetic quinolines have been used in therapy. Chloroquine, CQ, has been widely used as a synthetic quinoline. CQ is thought to bind to Ferriprotoporphyrin, (FP- remaining undigested heme), and is taken up highly selectively and concentrates in the infected erythrocytes (Brayl 2005). This can be attributed to the proton-trapping system because CQ is a weak base, thus, when it is not charged diffuses easily into the acidic sections. In these sections, the CQ it attaches itself to the protons and is trapped. CQ is absorbed by FP 20 times faster than mammalian cells which contain acidic lysosomes (Brayl 2005). The complex formed by CQ and FP and its subsequent accumulation in the parasitic cells is responsible for its death. The CQ-FP complex causes the parasitic membranes to lyse through a mechanism of lipid peroxidation (Brayl 2005).

P. falciparum Drug Resistance and the Role of PfCRT

The development of resistance to P. falciparum infection has greatly hindered treatment by anti-malarial drugs. Studies have identified that the P. falciparum chloroquine resistance transporter (PfCRT) and PfMDR1

(multidrug resistance) proteins as the targets for potential mutations (Valderramos 2006). PfCRT is an important membrane protein located on the parasite's digestive vacuole (Rowena, 2009). Valderramos, (2006), associates mutations in PfCRT with reduced efficacy of CQ treatment and PfMDR1 with mefloquine treatment. A resistant parasite is unresponsive to treatment by these agents or less responsive than those parasites which are not resistant. It seems fitting in deed that the parasite would develop an adaptation in the transporter protein that is in charge of transporting the therapeutic agent that would kill it. Research studies by various scientists agree that PfCRT is the transporter protein responsible for CQ uptake into the digestive vacuole (Valderramos 2006; Brayl 2005; Rowena 2009)

There are three models which have been proposed to describe CQ resistance. These are: efflux of chloroquine; CQ leak out of DV membrane; and changes in pH at DV membrane (Valderramos 2006). Cecilia, (2010), describes the models above as the partitioning model, the channel model, and the carrier model respectively. The partitioning model suggests that the fast efflux of CQ by CQ resistant (CQR) parasites possess a pathway for CQ that is enhanced across the DV. The channel model postulates that mutated PfCRT contains a leak which allows protonated CQ to passively diffuse out of the DV, thus no accumulation of the drug. The carrier model presents PfCRT as a carrier molecule, subject to the demands of kinetics. Should there be a mutation in PfCRT, the molecule would not be able to carry CQ efficiently. Valderramos (2006), suggests that both the partitioning model and the channel model could work together with the carrier models to result in CQ resistance of P. falciparum.

The P. falciparum parasite mutates in the transporter protein in the DV of the parasite so as to be able to resist the action of quinoline drugs on the parasite. This mutation has been vital to its survival because the quinoline drugs form complexes with the undigested heme (FB), which then lyses the parasitic membranes, destroying it. Much research has been dedicated to find new ways to combat the mutating parasite in its bid for survival. This includes: finding new ways to use older drugs; redesigning currently existing drugs; and finding new drugs due to better understanding of the biological structure of the parasite (Biagini, et al. 2003). The use of 4-aminoquinoline drugs, for example, quinine, mefloquine, and amodiaquine has long been employed in malaria therapy. Their efficacy has been hindered by CQ resistance which has evolved in the parasites over time as an adaptation. New studies have demonstrated that the parasite experiences difficulty in developing a CQ resistance to drugs which bind to FP (Biargini, et al. 2003). Research therefore focuses on the creation of novel derivatives of 4-aminoquinolines which will then be able to combat malaria effectively.
For example, Amodiaquine (AQ) is effective against CQR-falciparum, but its use is not recommended due to resulting agranulocytosis and hepatoxicity on human subjects when used. However, Brayl, (2005) points out that research studies have resulted in fluoromodiaquine, an alternative to AQ which is safer (Brayl 2005; Biargini et al. 2003).

Conclusion

The digestion of haemoglobin in the trophozoite of P. falciparum takes place in its digestive vacuole. Earlier therapy with antimalarials specifically

targeted the PfCRT in the DV, inhbit this process, and thus, effectively destroy the parasite. As a result, the parasite has evolved to preserve this function, mainly believed to be a function of protein tansporter protein in the DV's mainly PfCRT and PfMDR1. Research is now focused on finding new chemotherapeutic agents to combat the parasite. Understanding the mode of CQ resistance gives better insight into developing older classic 4-aminoquinolines. New derivatives of these drugs are believed to be able to combat CQR parasites.

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