Antimalarial metabolism: what have we learnt so far?

Naazneen Khan¹* and Abdullah Chand²

¹ Evolutionary Genomics and Bioinformatics Laboratory, Division of Genomics and Bioinformatics, National Institute of Malaria Research, Sector-8, Dwarka, New Delhi-110077, India.
² CMJ University, Shillong, Meghalaya, 793 003, India.

Considering malaria is a highly devastating disease of mankind, total eradication of malaria seems to be an uphill task, the only relief from this disease is achieved by usage of antimalarial drugs. Since malaria is associated with humans from time immemorial, usage of traditional substances to most presently effective antimalarial have been recorded to cure this disease. With the advent of modern biological techniques and the understanding of the biochemical pathways of antimalarial metabolism thereby helping in designing successful usage of many antimalarials. Nevertheless, improper usages of certain drugs have led to the origin and spread of drug resistant malaria parasites (chloroquine resistant Plasmodium falciparum). Also, the genetic basis of antimalarial metabolism in humans is now well understood and frequent mutations in genes of malarial parasites are well associated with drug resistance. The entire scenario of antimalarial usage in the field have become complicated, partly due to poor understanding between antimalarial metabolism in humans and drug fighting mechanism in parasites, by which resistance to even combined therapy (e.g. Artemisinin Combination Therapy) have started emerging. Vital basic understanding from human and parasite population genetics (involving antimalarial both metabolizing genes in human and resistant genes in parasite) could be an ideal starting point to malaria control.

Key words: Malaria, antimalarial drugs, metabolization, population genetics, pharmacogenomics

INTRODUCTION

Malaria is considered as one of the three most deadly infectious diseases including Tuberculosis (T.B.) and Human Immunodeficiency Virus (HIV) (Riley and Stewart, 2013) which has resulted in high mortality worldwide with an estimated 3.3 million people at high risk malaria (World Health Organization, 2013). It affects, mainly, population residing in tropical and subtropical areas due to the presence of suitable temperature and rainfall which is required for the development of Plasmodium parasites in female Anopheles mosquitoes (Gallup and Sachs, 2001). Over 90% of malaria sufferers are borne by populations in Africa (Winstanley et al., 2004) and are mainly children (<5 years) and pregnant women. Outside Africa, malaria affects densely populated countries such as Indian subcontinent and Southeast Asia where the prevalence of P. falciparum, P. vivax and recently increased P. knowlesi are endemic.

Corresponding Author: Dr. Naazneen Khan, National Institute of Malaria Research, Sector-8, Dwarka, New Delhi-110077, India. E-mail addresses: naaz.27khan@gmail.com
Malaria is a devastating disease caused by different Plasmodium species, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae. Among the four species, Plasmodium falciparum is the most virulent and is a major cause of high morbidity and mortality in endemic regions (Boland, 2001). These parasites have complex life cycles in both human host and vector (Hyde et al., 2007) that starts with the inoculation of sporozoites into the human dermis which further infects the liver within 1-2 hr and invade hepatocytes, releasing merozoites in the blood stream. These merozoites undergo rapid multiplication and forms sexual stages that await ingestion by mosquitoes for further development of zygote that develops into oocyst forming thousands of sporozoites. Further, these sporozoites migrate to the salivary glands of mosquitoes and can be injected into new host upon next blood meal (Hyde et al., 2007). In order to hinder the pathogenesis and further transmission of malaria, each developmental stage of the parasite represents potential target for the development of antimalarial.

The most significant period in the history of malaria control was started when a conference was organized by World Health Organization, held in Kampala, Uganda in 1950 (Winstanley et al., 2004) which was named “Global Malaria Eradication Programme”. Initially, the campaign was started with the use of pesticide DDT and chloroquine, which was found to be successful in regions of low malaria transmission such as Sri Lanka, China, Latin America, Southern Europe, southern part of the United States, Southern region of Soviet Union (Sachs, 2002). Despite these successes, there was re-emergence of chloroquine-resistant Plasmodium parasites and DDT-resistant Anopheles mosquitoes in areas where it had been eliminated (Sachs, 2002). As a consequence, the concept of malaria eradication was dropped in favour of malaria control and the focus was totally shifted on the development of cost effective drug strategies. This programme was successful in countries with developed economy like Thailand having better health infrastructure (Chareonviriyaphap et al., 2000), but it failed in poor tropical countries that resulted in the withdrawal of Global eradication programme in 1972. Furthermore, the dramatic increase in cases of malaria led to the adoption of Roll Back Malaria (RBM) that was launched by a consortium of World Health Organization, World Bank, United Nation Development Programme and United Nation Children’s Fund (UNICEF) in 1998 (Brito, 2001).

Despite enormous efforts taken to eradicate and control malaria, it is still uncontrollable mainly because of poverty, low economic status and environmental conditions (temperature and rainfall) that lead to emergence of drug resistant parasites. In this review article, progress in the study of different drug metabolizing gene involved in antimalarial metabolism is presented with special emphasis on their mode of action and genetic overview.

**History of Antimalarial usage**

The effort for the eradication of malaria was started long ago before the use of Chloroquine. Several remedies were tried to treat malaria since time-immemorial which were totally based on the superstitious thought such as, opium laced beer, skull operations etc. In the nineteenth century, the first antimalarial “Quinine” was extracted from Cinchona bark and was used for more than 350 years to effectively treat malaria. It remained the mainstay of antimalarials till 1920’s, when more synthetic antimalarials became available. Although, Quinine was effective against malaria, the extraction cost was quite high and it also causes side effects (Brito, 2001). During this period, Chloroquine was introduced due to its effectiveness against all forms of malaria and cheap manufacturing cost. With the heavy usage of chloroquine, the cases of resistant Plasmodium falciparum emerged and become widespread in almost all areas of the world (Wellens and Plowe, 2001). Moreover, in the twentieth century, the discovery of Chinese herb “Qinghao” was marked as one of the greatest discoveries in the scientific world as its extracts form the basic ingredient for artemisinin and its derivatives that were found to be more effective than quinine. While quinine is effective against mature parasite stages, artemisinin acts against all asexual and sexual stages of parasites. Before artemisinin could be declared as an ideal antimalarial candidate, there was an emergence of artemisinin resistant parasites outbreaks in regions of Southeast Asia (Phyo et al., 2012). The emergence of resistivty to known drugs ignited the idea of combination therapies. World Health Organization has also recommended drug combinations to delay the emergence and spread of drug resistance. The first combination drug was “Fansidar” formed by amalgamating Sulphadoxine and Pyremethamine antimalarial compounds (Alessandro and Buttens, 2001). In recent years, WHO declared artemisinin combination therapies (ACT) as a first line treatment for uncomplicated Plasmodium falciparum (Li and Zhou, 2010).

**Antimalarial drug metabolizing pathways**

Since malaria is associated with humans from time immemorial, usage of various traditional substances to most presently effective drugs as antimalarial have been documented. These drugs help in curing the disease but at the same time persist in the body where they accumulate to toxic level which led to adverse drug reactions, therapeutic drug failure and susceptibility to other diseases. These drugs need to be metabolized and detoxified from the body as quickly as possible. Thus, the
Effective metabolism and detoxification of drugs is key to therapeutic drug efficacy and is carried out via two main reactions; Phase I and Phase II catalyzed by drug metabolizing enzyme. While Phase I reaction is primarily catalyzed by Cytochrome P450 enzymes (CYP), Phase II reactions are catalyzed by many enzymes viz. Acetyltransferase, Gucuronosyltransferase, Glutathionyltransferase and Sulphotransferases (Goldstein and Faleto, 1993). Furthermore, based on the effective metabolism by drug metabolizing enzyme, some drugs are metabolized by single enzyme (metoprolol by CYP2D6), whereas, others involve two or more enzyme for their metabolization (warfarin by CYP1A2, CYP2D6 and CYP3A4) (Lynch and Price, 2007). Hence, it was observed that the effective action of drug to its target mainly depends on its effective metabolism and detoxification. In this review article, we have discussed about the ten different antimalarial metabolized by one or more drug metabolizing enzyme and their mode of action:

**Primaquine**: The antimalarial Primaquine is one of the important drugs that is effective against the exo-erythrocytic stages of *Plasmodium vivax* and *Plasmodium ovale* and gametocytic stages of *Plasmodium falciparum* (Edwards et al., 1993).

Mode of action: Primaquine disrupts the metabolic processes of *Plasmodium* mitochondria by interfering with the function of ubiquinone as an electron carrier in the respiratory chain (Hill et al., 2006).

**Drug metabolizing enzyme**: Primaquine is primarily converted to metabolite carboxyprimaquine by CYP1A2 (Figure 1a). (Hill et al., 2006), and is also metabolized by CYP3A4 (Kerb et al., 2009).

**Artemisinin**: Artemisinin is an antimalarial extracted from *Qinghao* (blue-green herb) (Hsu, 2006) and is used in severe malaria and is effective against all the blood stages of *Plasmodium falciparum* including the youngest (ring form) stage of the parasite (WHO, 2006).

Mode of action: The endoperoxide bridge of Artemisinin interacts with haem to produce free radicals that alkylate protein and damage the micro-organelles and membrane of the parasite. It also interrupts with the haemoglobin catabolism system causing inhibition of haemoglobin degradation and polymerization of haem to haemozoin which is used by parasite as its nutritional requirements (Pandey et al., 1999).

**Proguanil**: Proguanil is an antimalarial drug used for the treatment of malaria caused by *Plasmodium falciparum* (Pang et al., 1989).

Mode of action: Proguanil inhibits dihydrofolate reductase (DHFR) enzyme essential for synthesis of parasite DNA (Boggild et al., 2007).
Drug metabolizing enzyme: - Proguanil is converted to its active metabolite Cycloguanil by CYP2C19 (Figure 1c). Additionally, CYP3A4 have a limited role in the metabolism of Proguanil to Cycloguanil (Brentano et al., 1997).

Dapsone: - Dapsone is a 4, 4'-diaminodiphenyl sulphone and widely used for the treatment of Leprosy, Chloroquine resistant malaria and Dermatitis Herpetiformis. It is effective against the treatment of schizontidal and gametocidal activity of *Plasmodium falciparum*, but does not act against asexual form of the *Plasmodium vivax*. (Saha et al., 2003).

Mode of action: - Dapsone inhibits folic acid synthesis of parasite by inhibiting DHFR enzyme (Tingle et al., 1997).

Drug metabolizing enzyme: - Dapsone is metabolized by CYP2C9 (Tingle et al., 1997), CYP3A4 (Adedayo et al., 1998) and NAT2 (Louet et al., 1999) (Figure 1d).

Amodiaquine: - Amodiaquine is derived from quinoline and is more effective as compared to chloroquine against *Plasmodium falciparum* (Li et al., 2002).

Mode of action: - The mode of action against the parasite is same as that of chloroquine, by inhibiting polymerization of haem and thus, inhibiting formation of toxic haemozoin. (Hombhanje et al., 2004).

Drug metabolizing enzyme: - Amodiaquine is converted to N-Desethylamodiaquine by CYP2C8 (Li et al., 2002; Figure 1e).

Chloroquine: - Chloroquine is a dibasic drug, which diffuses down the pH gradient and accumulates in the acidic vacuole of the parasite *Plasmodium*. The high intravacuolar concentration of chloroquine inhibits the polymerization of the haem. As a result the haem which is released during haemoglobin breakdown builds up to poisonous levels, killing the parasite (Foley and Tilley, 1997).

Drug metabolizing enzyme: - Chloroquine is metabolized by CYP3A4 to N-desethylchloroquine (Cooper and Magware, 2008, Figure 1f).

Clindamycin: - Clindamycin is semisynthetic derivative of Lincomycin and is effective against many species including *Plasmodium* sp. (Lell and Kremsner, 2002).

Mode of action: - Clindamycin slowly accumulates inside the apicoplast of *Plasmodium* sp. and killing it ultimately (Lell and Kremsner, 2002).

Drug metabolizing enzyme: - Clindamycin is metabolized by CYP3A4 to Clindamycin sulfoxide (Wynalda et al., 2003, Figure 1g).

Halofantrine: - Halofantrine is a phenanthrene methanol derivative used as antimalarial drugs against uncomplicated chloroquine and multidrug resistant *Plasmodium falciparum* (Halliday et al., 1995).

Mode of action: - Halofantrine is blood schizonticides and affects only trophozoites and schizonts in the red blood cells. It binds to ferrirrotoporphyrin IX in red blood cells affected by *Plasmodium* and form toxic complexes that damage the parasite membrane (Nothdruft, 1993).

Drug metabolizing enzyme: - Halofantrine is metabolized by CYP3A4 to N-debutylhalofantrine (Baune, 1999; Figure 1h).

Mefloquine: - Mefloquine antimalarial is effective against the chloroquine resistant strains of *Plasmodium falciparum* (Riviere et al., 1985).

Mode of action: - The quinoline binds to high density lipoproteins (HDL) in the serum and delivered to the erythrocytes, where they interact with an erythrocyte membrane protein stomatin and are then transferred to the intracellular malaria parasite.

Drug metabolizing enzyme: - Mefloquine is metabolized by CYP3A4 (Khaliq et al., 2001, Figure 1i).

Sulphadoxine: - Sulphadoxine is a sulphonamide and is effective against *Plasmodium falciparum*. It is used in combination with other drugs to treat malaria ((Seaton et al., 2000).

Mode of action: - Sulphadoxine inhibits folic acid biosynthesis by blocking the formation of dihydrofolic acid and inhibiting dihydroprotoeate synthase (DHPS) enzyme (Ridley et al., 2002).

Drug metabolizing enzyme: - Sulphadoxine is metabolized by NAT2 (Fuchs, 2004; Melmon, 2000; Figure 1j).

Genetic overview of antimalarial metabolizing genes

Since the advent of new agricultural techniques, changes in environmental condition, subsistence strategies and dietary habits had sculpted the human genome that led to the emergence of different complex diseases. In order to cure or treat a disease, patients are administered drugs that are chemical in nature, hence their metabolism and detoxification in the body is essential. For effective metabolism, human body is armed with drug metabolizing enzyme for effective metabolism and detoxification of exogenous and endogenous compounds. We herewith, represented the detailed information about the drug metabolizing gene located on human chromosome with number of exons and introns involved in metabolism of anti-malarials.

CYP1A subfamily: - In humans, there are two members of CYP1A family: CYP1A1 and CYP1A2 that play a major role in the biotransformation of food and environmental pollutants. The human CYP1A2 is involved in the metabolism of antimalarial primaquine and spans a gene length of 7758 bp (Chromosome 15q24.1, position...
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75041184 – 75048941) with 7 exons that codes for protein of 516 amino acid (www.ncbi.nlm.nih.gov) and mainly expressed in the liver (Daly, 2003) (Figure 2).

**CYP2B subfamily**: The human CYP2B has only one member CYP2B6 that play a significant role in the metabolism of artemisinin. This gene spans a gene length of 27058 bp (Chromosome 19q13.2, position 41497204-41524301) with 9 exon (www.ncbi.nlm.nih.gov) that codes for protein of 491 amino acid (www.ncbi.nlm.nih.gov, Figure 3) which is highly expressed in liver, but also expressed in the brain, small intestine, kidney and lung (Nagata, 2002).

**CYP2C subfamily**: The human CYP2C has four members viz., CYP2C18, CYP2C19, CYP2C9, and CYP2C8 clustered at human chromosome 10q24. These four subfamily shares less than 82% amino acid identity and significantly contribute in different ways to metabolize drugs. Out of these four; CYP2C19, CYP2C9 and
CYP2C8 were found to be most divergent. The maximum homology is shared between CYP2C19 and CYP2C9 (90% homology), whereas, CYP2C8 share only 75% homology identity (Kudzi et al., 2009).

CYP2C8 is more divergent among all CYP2C family members as revealed from protein sequences. This gene involves in the metabolism of amodiaquine and is mainly expressed in human liver with expression level of less than 1 (Nagata and Yamazoe, 2002). The human CYP2C8 gene spans a gene length of 30,000 bp with 9 exons that code for 490 amino acid protein (www.ncbi.nlm.nih.gov, Figure 4A).

CYP2C9 is also one of the major CYP2C that comprised of one third of total hepatic P450 content. This gene plays a pivotal role in dapsone and spans a gene length of 50,734 bp (Chromosome 10q24, Position 96698415-96749148) and contains 9 exons that codes for protein of 490 amino acid and mainly expressed in liver (www.ncbi.nlm.nih.gov, Figure 4B).

CYP2C19 is the largest gene among human CYP450 involved in drug metabolism. The CYP2C19 gene plays a significant role in the metabolism of proguanil and spans a gene length of 90,209 bp (Chromosome 10q24.1-q24.3, position 96522463-96612671) and contains 9 exons that codes for protein of 490 amino acid (www.ncbi.nlm.nih.gov, Figure 4C), which is mainly expressed in the liver (Nagata and Yamazoe, 2002).

Cytochrome P450 3A (CYP3A):- The human CYP3A family composed of 25% - 30% of total hepatic cytochromes P450 (Shimada, 1994). The CYP3A gene family is the most important drug metabolizing gene which is involved in the metabolism of almost 60% of drugs. The CYP3A family comprised of 4 functional genes: CYP3A4, CYP3A5, CYP3A7 and CYP3A43 located within 218 Kb region of chromosome 7q22.1 (www.ncbi.nlm.nih). In addition to these four functional genes, two psuedogenes CYP3A5P1 and CYP3A5P2 are also present (Agarwal et al., 2008). CYP3A4 exhibit approximately 85% amino acid sequence similarity with CYP3A5 and CYP3A7 and approximately 75% amino acid sequence similarity with CYP3A43 (Domanski et al., 2000).

Cytochrome P450 3A4 (CYP3A4):- CYP3A4 gene is involved in metabolism of wide variety of antimalarial. It is located at chromosome 7q21.3-q22.1 and spans a gene length of 27,592 bp with 13 exons (www.ncbi.nlm.nih, Figure 5) that codes for protein of 502 amino acids which is mainly expressed in the liver and intestine. In adults CYP3A4 is the dominant CYP3A enzyme that plays a dominant role in the metabolism of numerous drugs (Hirota et al., 2004).

N-acetyltransferase (NAT):- The human N-acetyltransferase is a broad spectrum drug metabolizing enzyme that catalyzes N-acetylation and O-acetylation of
arylamine carcinogens and heterocyclic amines present in the exogenous chemicals including therapeutic drugs, food and environmental pollutants. These enzymes are located in the cytosolic liver, gut and many other tissues of mammalian species (Dupret and Lima, 2005).

In humans, there are two functional N-acetyltransferase (NAT) genes, NAT1 and NAT2 and one pseudogene NATP. These functional NAT genes are located at chromosome 8 and share 87% nucleotide and 81% amino acid sequence identity (Windmill et al., 2000).

**N-Acetyltransferase 2 (NAT2)**: Human NAT2 gene spans a gene length of 9.9 kb (Chromosome 8, positions 18,248,755 – 18,258,723) (NCBI Build 37.1 assembly, Figure 6) and consists of a two exons, separated by a 9 kb intron. Only the second exon consists of single open reading frame (ORF) of 873 bp that codes for 290 amino acid and mainly expressed in liver (Fusseli et al., 2007).

**Figure 5.** Gene location, Exon, Intron of drug metabolizing gene CYP3A4 located on human chromosome 7.

**Figure 6.** Gene location, Exon, Intron of drug metabolizing gene NAT2 located on human chromosome 7.

The prospect of antimalarial usage lies in pharmacogenomics

With the availability of human genome sequence, interindividual variation with respect to drug response has become clear (Brockmoller and Tzevtkov, 2008) and helped in understanding the fact that not all individual can
equally respond to a particular drug which supports the genetic basis of drug metabolism. In order to know the genetic basis of gene involved in drug metabolism, the inter-individual variation is very important. This difference in response to drugs might be a consequence of variation involved in drug metabolism, drug transporter or drug receptor gene. Furthermore, these variations have considerable effects on phenotypes, either as extensive metabolizer (metabolism of drug is very fast and results in low or no drug effects) or as poor metabolizer (metabolism of drug is very slow and results in accumulation of drug inside the body). However, it is difficult to find, whether the whole gene family or only one gene is involved in the inter-individual variation. Recently, "Pharmacogenomics" emerged as a new field to study variation in genome related to drug response. This field helped not only in understanding the mechanism of drug metabolism but also in understanding the interaction of drugs with component of parasite (i.e. *Plasmodium*) and host. Additionally, Pharmacogenomics holds the promise of personalized drug treatment, based on underlying genetic variability of an individual (Wang and Weinshilboum, 2008). Thus, it is very interesting to understand that the success of any drug treatment depends on the individual pharmacogenomic vulnerability as well as the sensitivity of pathogen to drug. By improving our deeper understanding and awareness of genetic variability in drug metabolizing gene, parasite biology and vector behavior, it is possible to reduce the emergence and spread of drug resistance as well as being helpful for the development of safe, cheap and efficient drug.

**CONCLUSION AND RECOMMENDATIONS**

Malaria remains a major worldwide public health threat as a result of increased drug resistance. The effective action of drug depends on two main aspects of drug metabolism i.e. pharmacokinetics and pharmacodynamics. Individual susceptibility for drug response are modulated by genetic polymorphism in the drug metabolizing gene which can lead to drug failure, emergence of resistance, increased toxicities, decreased safety and adverse drug reactions. Interestingly, majority of antimalarial are metabolized by CYP3A4 drug metabolizing gene. Despite enormous number of studies focusing on pharmacokinetic interaction of antimalarials with drug metabolizing gene (Daniyan *et al.*, 2008; Khaliq *et al.*, 2001), little information is known regarding relationship between antimalarial and drug metabolizing enzyme polymorphism. Moreover, study related to genetic polymorphisms, drug toxicity and environmental exposure found to be a promising area of research in controlling the pathogenesis and eradication of the disease.

**ACKNOWLEDGEMENT**

The authors wish to thanks the Indian Council of Medical Research (ICMR) for Junior and Senior Research Fellowships.

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