AN ANALYTICAL EVALUATION OF *IN VITRO* DRUG-DRUG INTERACTION STUDIES FOR FIXED ARTESUNATE COMBINATION THERAPY

by

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List of Symbols and Abbreviations

ACT- artemisinin combination therapy

AE- arteether

APCI- atmospheric pressure chemical ionization

AS – artesunate

AQ- amodiaquine

AVQ – atovaquone

C- concentration

CC- calibration curve

cDNA – complimentary DNA

Cmax – peak plasma concentration

CMQ- carboxymefloquine

CQ- chloroquine

CR*PF* – chloroquine-resistant *Plasmodium faciparum*

CYP- cytochrome

deAQ- desetylamodiaquine

DDT – dichlorodiphenyltrichloroethane

DFN- diclofenac

dHA – dihydroartemisinin

DHER- dihydrofolate reductase

DMSO- dimethyl sulfoxide

DND*i* – Drugs for Neglected Diseases *initiave*

DNA- deoxyribonucleic acid

E- enzyme

EAC- enzyme activity change

EM- enzyme mediated

EC- electrochemical

ES- enzyme-substrate complex

ESI- electrospray ionization

FMO- flavin monooxygenase

FDA- food and drug administration

G6P- glucose-6-phosphate

G6PDH- G6P dehydrogenase

HLM- human liver microsome

HPLC- high pressure liquid chromatography

IC_{50} - half maximal inhibitory concentration

IRS – indoor residual spraying

ITN- insecticide-treated net

K_{dep} – sunstrate depletion rate constant

K_i - dissociation constant for inhibitor binding

K_M – Michealis Menten constant

LCMS – liquid chromatography-mass spectrometry

LOD- limit of detection

LOQ- limit of quantification

$MgCl_2$- magnesium chloride

MM- Michealis Menten

MOP- methoxypsoralen

MQ- mefloquine

NaCl- sodium chloride

NaOH- sodium hydroxide

$NADP^+$- nicotinamide adenine dinucleotide phosphate

NADPH- reduced nicotinamide adenine dinucleotide phosphate

NCE- new chemical entity

NEM- non-enzyme mediated

NME- new molecular entity

NTR – narrow therapeutic range

P- product

PG- proguanil

p*I*- isoelectric point

PLC- pilocarpine

PQ- piperaquine

PR- primaquine

PYR- pyrimethamine

QHS – artemisinin

RSP- risperidone

RSP-OH - dihydroxyrisperidone

S- substrate

SA – sulphonamide

TCP- tranylcypromine

TNF- tumour necrosis factor

TRP- tryptamine

UMMC- Universiti Malaya Medical Centre

UV- ultraviolet

V_{MAX} – maximum reaction velocity

WHO- World Health Organisation

PENILAIAN ANALITIKAL INTERAKSI DRUG-DRUG SECARA *IN VITRO* BAGI TERAPI KOMBINASI ARTESUNATE TETAP: ABSTRAK

Plasmodium falciparum yang rintang ubat kini merupakan suatu ancaman serius, terutamanya di kawasan-kawasan tertentu dunia di mana malaria adalah endemik. Oleh itu, pihak WHO mengesyorkan regimen kombinasi seperti artesunat-meflokuina dan artesunate-amodiakuina sebagai rawatan utama bagi penyakit malaria terutamanya di kawasan-kawasan di mana parasit falciparum adalah paling rintang. Walau bagaimanapun, kajian interaksi drug *in vitro* bagi kombinasi artesunat-meflokuina dan artesunate-amodiakuina belum pernah dijalankan.

Teknik experimen *in vitro* mikrosom hepar manusia digunakan untuk mengkaji metabolisme artesunat secara bersendirian dan juga dengan kehadiran mefloquina dan amodiakuina. Penentuan serentak artesunat, dihidroartemisinin dan artemisinin dalam sampel mikrosom dijalankan menggunakan kaedah kromatografi cecair tekanan tinggi yang dilengkapi dengan pengesan elektrokimia. Kajian ini menunjukkan bahawa apabila diinkubasi secara *in vitro* dengan mikrosom, artesunat dimetabolisme kepada dihidroartemisinin, dimangkin oleh enzim CYP450 .

Kajian *in vitro* ini juga menunjukkan bahawa tindak balas metabolisme artesunat tidak dipengaruhi oleh tindak balas metabolisme meflokuina ataupun amodiakuina pada kepekatan terapeutik. Ketiadaan interaksi di antara drug-drug ini memberi kesimpulan bahawa kombinasi artesunat-meflokuina dan artesunate-amodiakuina adalah rawatan anti-malaria yang berkesan dan selamat apabila digunakan pada kepekatan terapeutik. Walau bagaimanpun, kajian klinikal bagi kedua-dua kombinasi ini perlu dijalankan bagi menyokong penemuaan kajian ini.

AN ANALYTICAL EVALUATION OF *IN VITRO* DRUG-DRUG INTERACTION STUDIES FOR FIXED ARTESUNATE COMBINATION THERAPY: ABSTRACT

Drug-resistant *Plasmodium falciparum* has become a serious threat, especially in malaria-endemic regions of the world. In light of this, the WHO has recommended artemisinin combination therapy such as artesunate-mefloquine and artesunate-amodiaquine as first line treatment for uncomplicated falciparum malaria especially in regions harbouring the most resistant isolates. However, the *in vitro* drug interaction studies have not been conducted for both these combinations.

An *in vitro* experimental technique, utilizing human liver microsomes was used to study the metabolism of AS in the presence and absence of mefloquine and amodiaquine. Simultaneous determination of artesunate, dihydroartemisinin and artemisinin in microsomal sample was performed using high performance liquid chromatography- electrochemical (HPLC-EC) method. The study shows that artesunate is metabolised to dihydroartemisinin in the *in vitro* microsomal assay. The reaction is mediated by CYP450 enzymes present in the microsomal assay.

The metabolism of artesunate was not affected by mefloquine or amodiaquine at therapeutic concentrations using the *in vitro* microsomal technique. The absence of interactions between these drugs led to the conclusion that the artesunate-mefloquine and artesunate-amodiaquine combinations are ideal and safe treatments when used in therapeutic concentrations. However, further clinical studies must be done to confirm these findings.

CHAPTER 1: INTRODUCTION

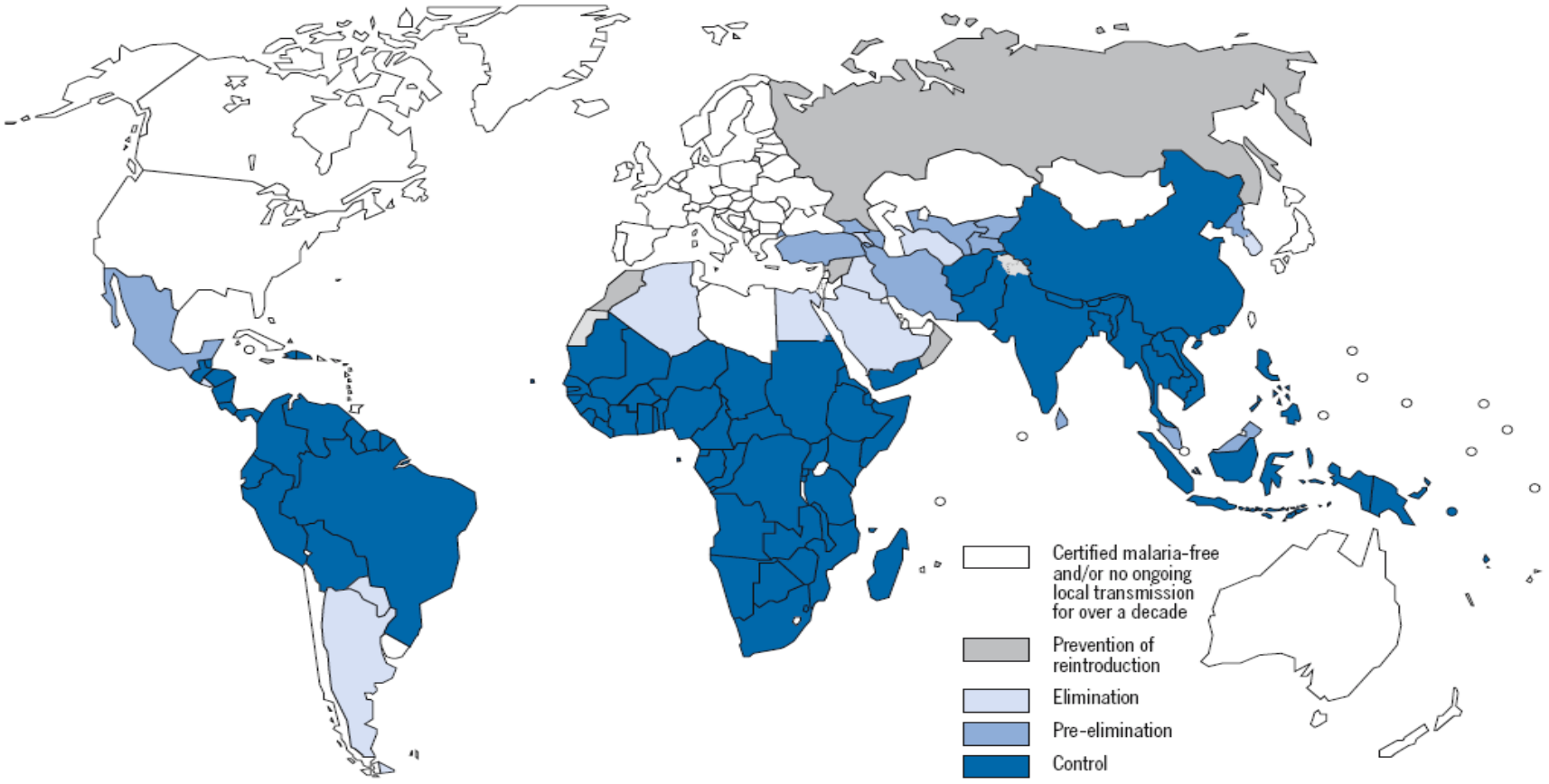
1.1. Global Malaria Scene

Malaria is a vector-borne infectious disease caused by protozoan parasites. It is widespread in tropical and subtropical regions of the world, including parts of the Americas, Asia, and Africa (Figure 1.1). Still, malaria continues to receive insufficient attention since industrial and financial powers are concentrated in temperate countries where malaria is no longer a threat. For this reason, malaria is considered a neglected disease (Carmargo, de Oliveira, Basano, & Garcia, 2009).

It has been documented that malaria pre-dates the evolution of man and the malarial parasite is known to have been a human pathogen for the entire history of its species (Joy, et al., 2003); (Hayakawa, Culleton, Otani, Horii, & Tanabe, 2008). The first evidence of malaria dates back to as far as 30 million years ago (Poinar, 2005) with historical records of the disease dating back to more than 3000 years ago (Sherman, 1998a); (Harrison, 1979); (Bruce-Chwat, 1988). However, the discovery of the causative agent of malaria only took place in 1880 by a French army doctor working in the military hospital of Constantine in Algeria , Charles Louis Alphonse Laveran (1845-1922). Laveran observed parasites for the first time, inside the erythrocytes of patients suffering from malaria and was awarded the Nobel Prize for his discovery.

Malaria causes about 250 million cases of fever and approximately 1 million deaths annually (World Health Organisation (WHO), 2008). Most of these cases occur in children under 5 years of age (Greenwood, Bojang, Whitty, Targett, & GA., Malaria, 2005). Approximately one infant dies of malaria every 30 seconds (Carmargo, de Oliveira, Basano, & Garcia, 2009). Pregnant women are also especially vulnerable. Pregnancy reduces a woman's immunity to malaria, making her more susceptible to

malaria parasite infections and other illnesses including severe anaemia and in extreme cases, even death.



[a] China, Indonesia, Phlippines, Solomon Islands, Sudan, Vanuatu and Yemen have subnational elimination programmes.

Figure 1. 1. Malaria-free and malaria-endemic countries in phases of control, pre-elimination, elimination, and prevention of re-introduction

Source: World Malaria Report 2008, World Health Organisation, 2008

For the foetus, maternal malaria is one of the leading causes of child mortality, with higher risks of spontaneous abortion, stillbirth, premature delivery and low birth weight (http://www.who.int/features/2003/04b/en/, 2003).

Uncomplicated malaria is the mild form of the disease which occurs as a febrile illness with headache, tiredness, muscle pains, abdominal pains, rigors (severe shivering), and nausea and vomiting. If left untreated, *P. falciparum* malaria can rapidly develop into severe malaria with anaemia (low haemoglobin in the blood), hypoglycaemia (low blood sugar), renal failure (kidney failure), pulmonary oedema (fluid in the lungs), convulsions, coma, and eventually death (World Health Organization (WHO), 2006).

Despite efforts to reduce transmission and increase treatments, there has been little change in malaria high-risk areas since 1992 (Hay, Guerra, Tatem, Noor, & Snow, 2004). If the prevalence of malaria continues, the death rate could double in the next twenty years (Bremen, 2001). Precise statistics are unknown because many cases occur in rural areas where people do not have access to hospitals or the means to afford health care. As a consequence, the majority of cases are undocumented (Bremen, 2001).

Malaria is presently endemic around the equator, in areas of the Americas, many parts of Asia, and much of Africa (Figure 1.1). In fact, in sub-Saharan Africa, malaria fatalities are about 85– 90% (Layne, 2005). Malaria is more common in rural areas than in cities; this is in contrast to dengue fever where urban areas present the greater risk (Van Benthem, et al., 2005). For example, the cities of Vietnam, Laos and Cambodia are essentially malaria-free, but the disease is present in many rural

regions (Trung H, 2004). By contrast, malaria in Africa is present in both rural and urban areas, though the risk is lower in the larger cities (Keiser J, 2004).

1.2. Malaria in Malaysia

Malaria is still the most important endemic disease in Malaysia, especially in remote areas. The first documented case of malaria fever (known then as 'Pinang fever') in Malaysia was in 1830 in Penang Island (Ward & Grant, 1830). Today, in spite of more than 30 years of active malaria control and eradication activities, the disease remains prevalent.

In 1901, the first organized anti-malarial campaign in Malaysia (then known as Malaya until 1957) was launched, spearheaded by Sir (Dr.) Malcolm Watson and it went on to be described as the first successful anti-malarial effort in the British Empire (Watson M. S., 1935). The campaign brought about a major reduction in malaria-caused deaths in Klang and Port Swettenham, from 368 deaths in 1901 to 59 deaths in 1902. A key activity that contributed significantly to the success of the campaign was the introduction of the drainage system, devised to control the *Anopheles* mosquitoes. This eventually led to other, better devised methods such as agitation ponds, automatic siphons and flush gates, some of which are still used today.

Prior to 1904, the management of malaria in Malaya was based on vector-control. However, with the growing global use of the anti-malarial drug quinine to treat malaria after its formal discovery by the Jesuit priests, drug therapy using quinine was used on a large scale. The discovery of synthetic anti-malarial drugs for treatment and prophylaxis from 1930 onwards propelled the malaria control efforts

further ahead. With the discovery of the effective insecticide dichlorodiphenyltrichloroethane (DDT) by Muller and Weisman in 1936 and further advances in chemotherapy, malaria eradication programmes were introduced worldwide. In Malaysia, the malaria eradication pilot project was launched in 1967 with the help of the WHO. Although the programme did not completely eradicate malaria in Malaysia, it successfully reduced annual death numbers of 250,000 in 1961 to 40,000 by the 1970es.

By 1980es however, difficulties in obtaining the large financial resources required for successful malaria eradication, development of successful insecticides and emergence of drug-resistant malarial parasites all led to malaria control programmes being established in place of malaria eradication programmes. Subsequently these programmes were integrated with other control programmes for diseases such as dengue, filariasis, Japanese encephalitis, typhus, plague and yellow fever. Among the strategies adopted in the malaria control programme were:

- Improving early diagnosis of malaria disease and its treatment
- Creating greater awareness of the seriousness of malaria
- Promoting the use of pyrethroid-treated mosquito nets
- Improving epidemiological data collection
- Improving and strengthening management and supervision
- Improving national and international training capabilities
- Operational research
- Development of malaria control staff
- Integration with other disease control programmes

In 1990, the number of reported malaria cases was 50,500 but this value decreased by 75% by year 2000 (Figure 1.2). Despite high numbers of reported cases, the number of malaria-caused deaths in 1990 was only 43 and only 35 in 2000 (World Health Organisation (WHO), 2008). With the development of anti-malarial drugs and the application of various methods of vector control, the number of reported malaria-related cases and incidence rates continues to undergo a steady decline (Figure 1.3).

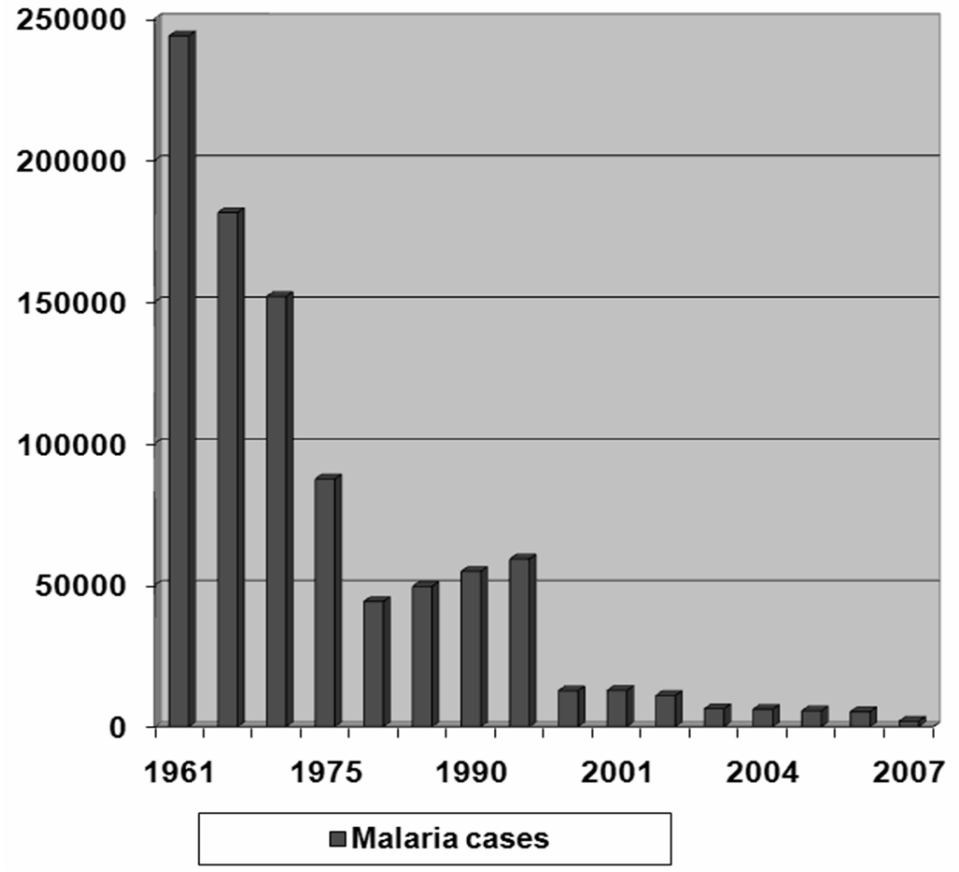


Figure 1. 2. Malaria Incidence from 1961 until May 2007

Source: Department of Public Health, Ministry of Health Malaysia, 2008

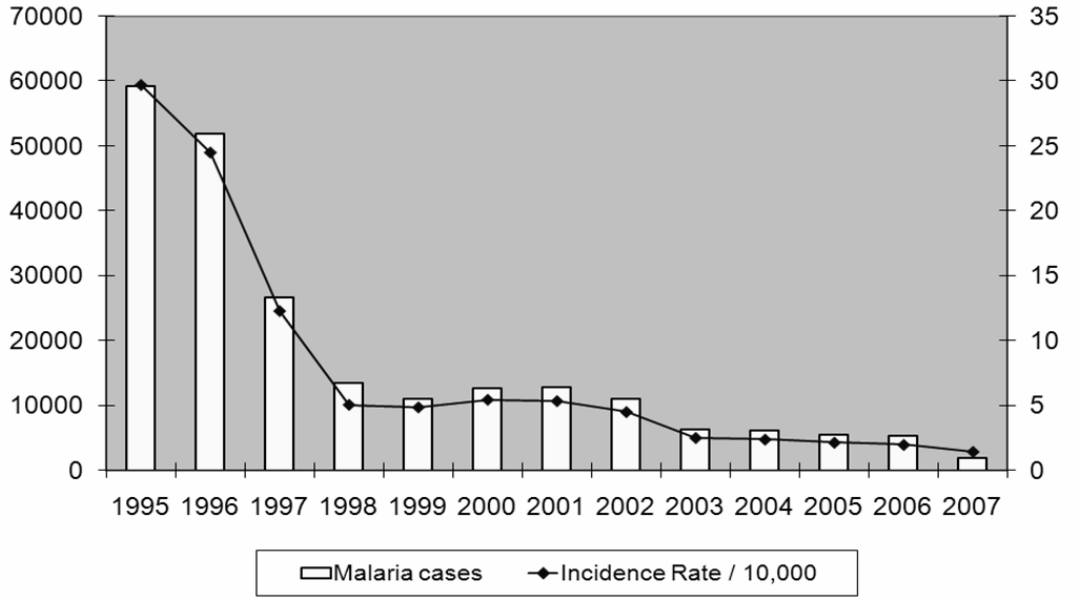


Figure 1. 3. Malaria Cases and Incidence Rate in from 1995- May 2007

Source: Department of Public Health, Ministry of Health Malaysia, 2008

Today, malaria is still a significant public health problem in Malaysia (Lim, 1998); (Ministry of Health Malaysia, 2002); (Ministry of Health Malaysia, 2004); (Jamaiah, Anuar, Najib, & Zurainee, 1998). The location of Malaysia at the equator, combined with optimal temperatures and humidity facilitates rapid growth and transmission of malaria parasites and vector (Rahman, Che' Rus, & Ahmad, 1997).

Although malaria incidence rates are higher in rural areas (86.5%) such as Sabah and Sarawak (Figure 1.4) and among the Orang Asli (33.1%) (Ministry of Health Malaysia, 2004), a recent study reported that migrant workers, namely from malaria-endemic countries such as Indonesia, India, Vietnam, Myanmar, and Pakistan (Lim, 1998); (Vijayakumari, 2006) are mainly responsible (up to 60% contribution) for the spread of malaria in Malaysian urban areas (Masitah, Nor aini, & Mas Ayu, 2008).

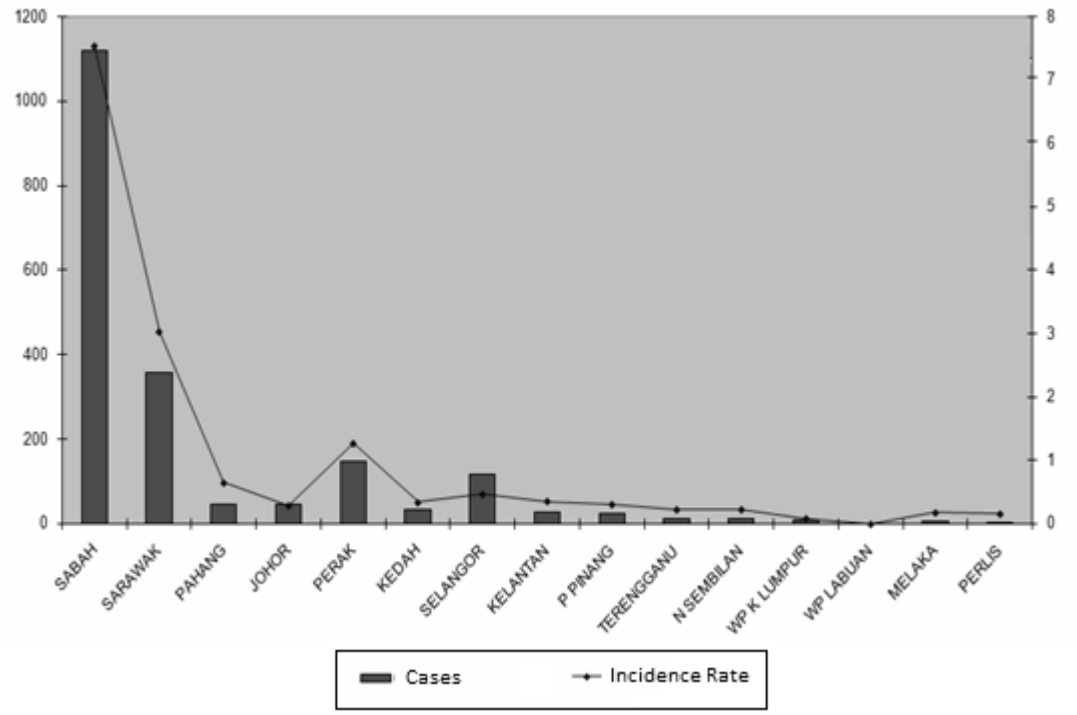


Figure 1. 4. Malaria Cases and Incidence Rate in Malaysia by State until May 2007

Source: Department of Public Health, Ministry of Health Malaysia, 2008

Another study reported that malaria recurrently occurred more often among foreign patients (57%) in University Malaya Medical Centre (UMMC) than Malaysian patients (43%) (Jamaiah I, 1998). Between the years 1983 to 1992, the number of malaria cases among foreigners in Malaysia had increased by 33%. With the annual increase of foreign workers influx into Malaysia, this percentage increase is not surprising.

In many malaria-endemic countries outside the African continent such as Malaysia, *P. vivax* coexists with *P. Falciparum* (Korsinczky, Fischer, Chen, Baker, Rieckmann, & Cheng, 2004). *Plasmodium* vivax is the most common strain of malaria parasite in Malaysia, followed by *P. Falciparum* (Figure 1.5). A study reported that *P. vivax*

was the most common infection in northern peninsular Malaysia, bordering Thailand, but *P. falciparum* and mixed infections also occurred (Rahman, Abu Hassan, Adanan, & Rashid, 1993).

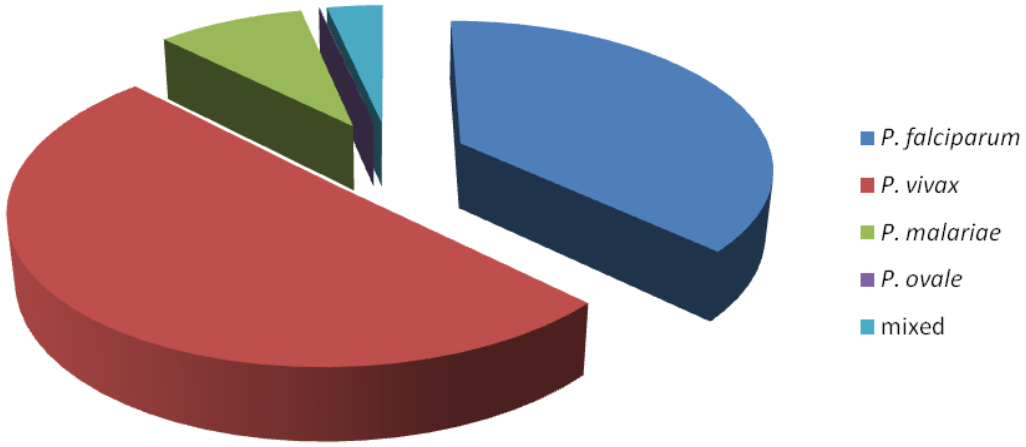


Figure 1. 5. Malaria Parasite Distribution in Malaysia until May 2007

Source: Department of Public Health, Ministry of Health Malaysia, 2008

1.3. The Mechanism of Malaria

The causative agent of malaria is a protozoan parasite of the genus *Plasmodium* (phylum Apicomplexa). In humans, the etiologic agents of malaria are the multistage *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi* (Mueller, Zimmerman, & Reeder, 2007); (Singh, et al., 2004). *P. falciparum* is the most common cause of infection and is responsible for about 80% of all malaria cases and 90% of malaria-caused deaths (Mendis, Sina, Marchesini, & Carter, 2001). The *Plasmodium* species, with the exception of *P.malariae* (which may affect the higher primates) are exclusively parasites of man.

The life cycle of *Plasmodium* is shown in Figure 1.6. The parasite's primary (definitive) hosts and transmission vectors are adult female mosquitoes of the *Anopheles* genus (Ross, 1897); (Holt, et al., 2002). Only female *Anopheles* mosquitoes (Figure 1.7) feed on blood, thus males do not transmit the disease. Out of the 380 species of *Anopheles* mosquito, only 60 can transmit malaria. Malaria parasites can also be transmitted by blood transfusions, although this is rare (Marcucci, Madjdpour, & Spahn, 2004).

Uninfected mosquitoes first ingest the malaria parasite by feeding on an infected human (host) carrier. During ingestion of a blood meal from an infected host, the parasites enter the female *Anopheles'* digestive system in the male and female sexual forms called gametocytes. Both male and female gametocytes then undergo rapid cellular division to form male and female gametes called microgametes and macrogametes respectively. The flagellated microgametes then fertilize the macrogametes by fusing with it in the mid-gut of the female *Anopheles*. The fertilization process produces an ookinete that penetrates the gut lining and produces an oocyst in the gut wall. When the oocyst ruptures, it releases sporozoites that migrate through the mosquito's body to the salivary glands, where they are then ready to infect a new human host. When the mosquito takes a subsequent blood meal, the motile, infective sporozoites are injected into the victim's skin capillaries, with the *Anopheles*' saliva and pass into the human's bloodstream. This type of transmission is occasionally referred to as anterior station transfer (Talman, Domarle, McKenzie, Ariey, & Robert, 2004).

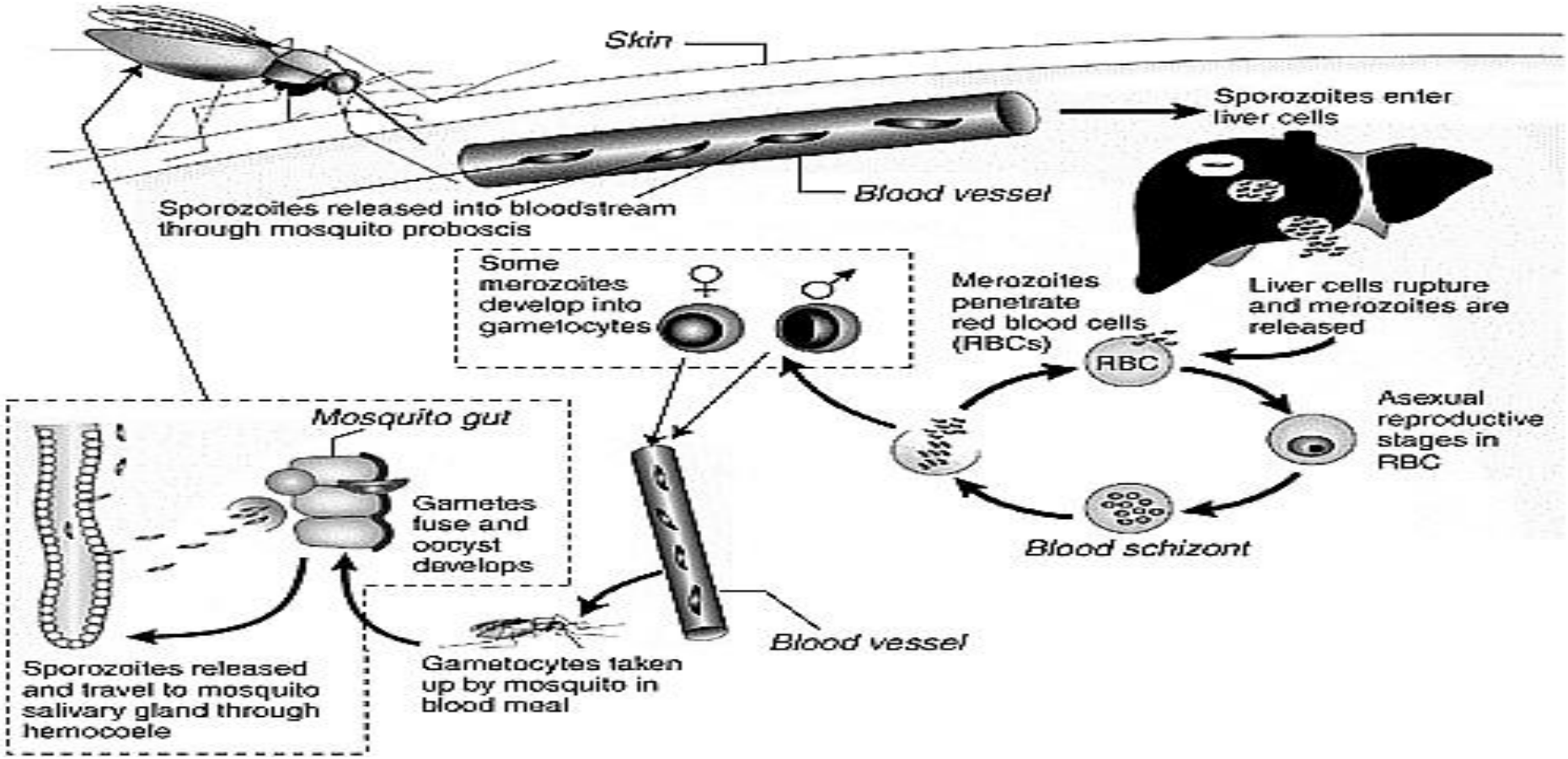


Figure 1. 6. Lifecycle of *Plasmodium falciparum*

Source: http://www.nap.edu/books/0309092183/xhtml/images/p2000af94g137001.jpg

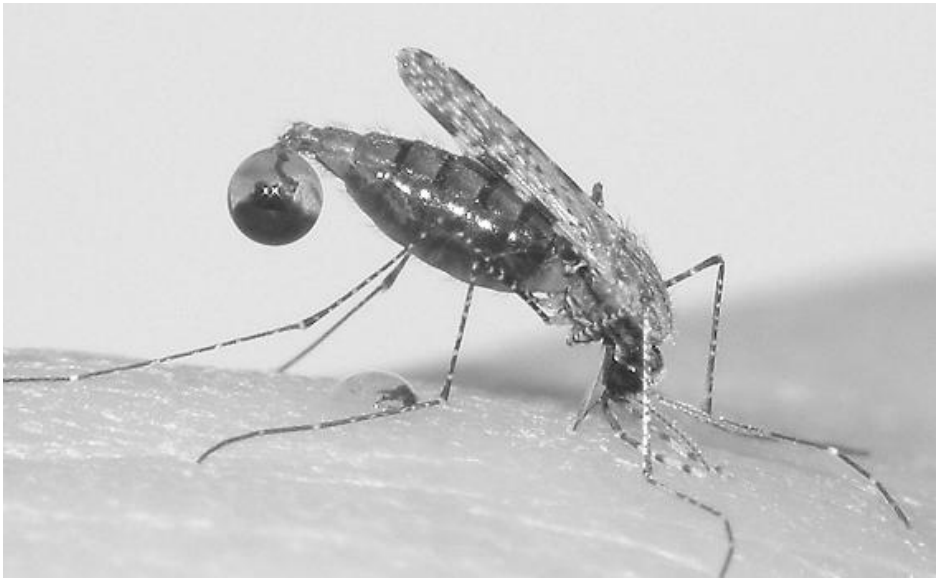


Figure 1. 7. A Feeding female *Anopheles* mosquito

Source: http://www.arbovirus.health.nsw.gov.au

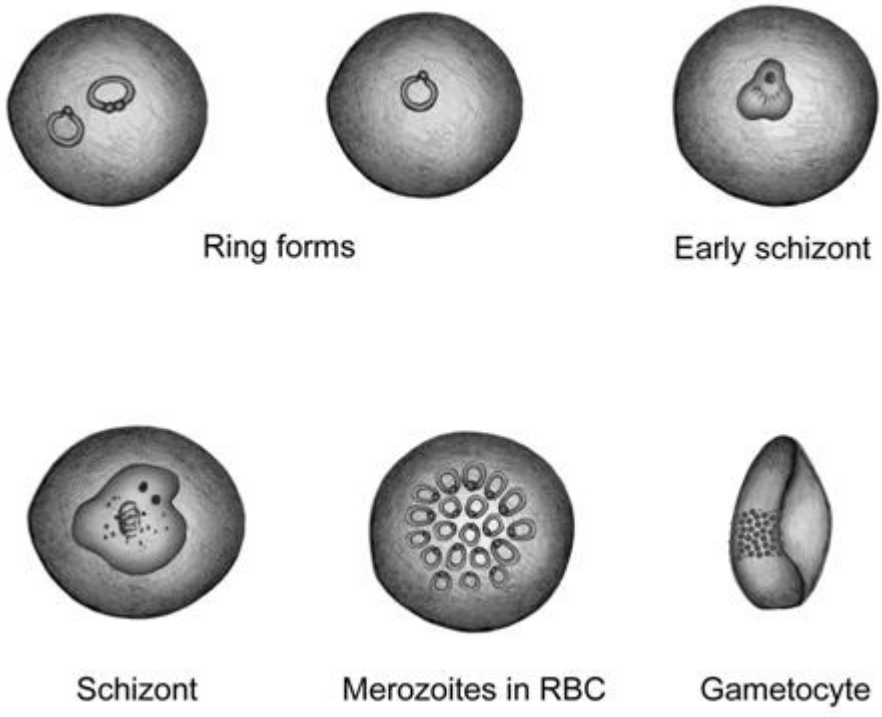


Figure 1. 8. Different Life Stages of *Plasmodium falciparum*

Source: http://img.medscape.com

Once in the human body, the malaria parasite develops via two phases: an exoerythrocytic and an erythrocytic phase. The exoerythrocytic phase involves infection of the hepatic system, or liver, whereas the erythrocytic phase involves infection of the erythrocytes, or red blood cells. When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver. Within 30 minutes of being inoculated into the human host, the sporozoites disappear from the blood. Many are destroyed by the human body's phagocytes but some enter the hepatocytes (parenchyma cells of the liver) directly or via the Kupffer cells. In the hepatocytes, the sporozoites multiply asexually and asymptomatically in a process called exoerythrocytic schizogony for a period of 6–15 days. During this process, multinucleated schizonts are formed. The cytoplasm of each schizont divides in a manner that results in the encapsulation of each nucleus, such that each encapsulated nucleus forms a merozoite. About 2000 to 40,000 merozoites can be formed in the liver depending on the species of *Plasmodium*. The merozoites then undergo maturation in the hepatocytes. The merozoite-containing hepatocytes eventually rupture, releasing the mature merozoites into the blood. Despite the numerous defence mechanisms present in the internal body systems, the merozoites escape from the liver undetected. This is done by wrapping themselves in the cell membrane of the infected host liver cell (Sturm, et al., 2006). The released merozoites then invade the erythrocytes, thus beginning the erythrocytic stage of the life cycle (Bledsoe, 2005). Figure 1.8 shows the different life stages of the *Plasmodium falciparum*.

In the erythrocytic stage, the released merozoites invade the erythrocytes present in the sinusoids of the liver while some are phagocytised. Within the erythrocytes, the merozoites undergo a trophic period, in which they enlarge and again multiply

asexually, periodically breaking out of their host erythrocyte to invade fresh erythrocytes. Several such amplification cycles occur, significantly destroying many erythrocytes in order to release mature merozoites. Following the lysis of erythrocytes to release mature merozoites, a glycolipid with many properties of bacterial endotoxins is released, causing a cascade reaction. The glycolipid activates cytokines such as tumour necrosis factor (TNF) and interleukins II-1, II-6 and II-8 (Stein, 1983) and other unidentified pyrogenic substances that leads to paroxysms of malarial fever and malaise Thus, classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells.

Some *P. vivax* and *P. ovale* sporozoites do not immediately develop into exoerythrocytic-phase merozoites, but instead produce hypnozoites that remain dormant for periods ranging from several months (6–12 months is typical) to as long as three years. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in these two species of malaria (Cogswell, 1992).

The *Plasmodium* parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and erythrocyes and is relatively invisible to immune surveillance. However, circulating infected erythrocytes are destroyed in the spleen. To avoid this fate, the *P. falciparum* parasite displays adhesive proteins on the surface of the infected erythrocytes, causing the erythrocytes to adhere to the walls of small blood vessels, thereby sequestering the parasite from passage through the general circulation and the spleen (Chen, Schlichtherle, & Wahlgren, 2000). This "adherence" is the main factor that gives rise to hemorrhagic complications of malaria. High endothelial venules (the smallest branches of the circulatory system) can be blocked by the

attachment of masses of these infected erythrocytes. The blockage of these vessels causes symptoms such as in placental and cerebral malaria. In cerebral malaria the sequestrated erythrocytes can breach the blood brain barrier possibly leading to coma (Adams, Brown, & Turner, 2002).

Some merozoites in the blood turn into male and female gametocytes. If a mosquito pierces the skin of an infected person, it potentially picks up gametocytes within the blood. Fertilization and sexual recombination of the parasite occurs in the mosquito's gut, thereby defining the mosquito as the definitive host of the disease. New sporozoites develop and travel to the mosquito's salivary gland, completing the cycle. Pregnant women are especially attractive to the mosquitoes due to hormonal, metabolic, or mechanical (increased expression of adherence factors in placenta enhancing the conditions for *P. falciparum* development) changes (Lindsay, Ansell, Selman, Cox, Hamilton, & Walraven, 2000). Malaria in pregnant women is a major cause of stillbirths, infant mortality and low birth weight (van Geertruyden, Thomas, Erhart, & D'Alessandro, 2004), particularly by *P. falciparum* infections, but also by other species infection, such as *P. vivax* (Rodriguez-Morales, et al., 2006).

1.4. Methods in Malaria Control

Methods used to prevent the spread of disease, or to protect individuals in areas where malaria is endemic, include prophylactic drugs, mosquito eradication, and the prevention of mosquito bites. The continued existence of malaria in an area requires a combination of high human population density, high mosquito population density, and high rates of transmission from humans to mosquitoes and from mosquitoes to humans. If any of these is lowered sufficiently, the parasite will eventually disappear

from that area, which occured in North America, Europe and much of Middle East. However, unless the parasite is eliminated globally, it could re-establish if conditions revert to a combination that favours the parasite's reproduction. Many countries are observing an increasing number of imported malaria cases due to extensive travel and migration.

Efforts to eradicate malaria by eliminating mosquitoes have been successful in some areas. In the United States of America, the draining of wetland breeding grounds, better sanitation, and the use of the pesticides eliminated mosquitoes and led to the reduction of malaria cases (http://www.cdc.gov/malaria/history/eradication_us.htm, 2004). Before pesticides were used, malaria was successfully eradicated or controlled also in several tropical areas by removing or poisoning the breeding grounds of the mosquitoes or the aquatic habitats of the larva stages.

Sterile insect technique is also emerging as a potential mosquito control method. Progress towards genetically modified insects suggests that wild mosquito populations could be made malaria-resistant. Researchers at Imperial College London in 2000, created the world's first transgenic malaria mosquito (Catteruccia, et al., 2000) and the first *Plasmodium*-resistant species was produced by a team at Case Western Reserve University in Ohio in 2002 (Ito, Ghosh, Moreira, Wimmer, & Jacobs-Lorena, 2002). Following that, a separate published study found that a chemical produced by sea cucumber *Cucumaria echinata*, impaired the development of the malaria parasites produced by transgenic mosquitoes (Yoshida, et al., 2007).

Indoor residual spraying (IRS) is the practice of spraying insecticides on the interior walls of homes in malaria-infected areas. After feeding, many mosquito species rest on nearby surfaces while digesting the blood meal. Therefore, insecticide-coated

walls of dwellings could eliminate mosquitoes before they inject another victim, transferring the malaria parasite. The first and historically most effective insecticide used for IRS was DDT. Initially it was used exclusively to combat malaria. However, its use quickly spread to agriculture. This large-scale use led to the evolution of resistant mosquitoes in many regions of the world. The DDT resistance shown by the *Anopheles* mosquitoes is comparable to antibiotic resistance exhibited by bacteria. Despite its effectiveness, awareness of the negative consequences of DDT overuse led it to be banned from agricultural applications in many countries. It is likely that DDT may now be more effective method of malaria control since its use has been limited or banned for some time.

Mosquito nets hinder direct contact between mosquitoes and people, greatly reducing the infection and transmission of malaria. Nevertheless, nets are not perfect barriers. As such, they are often treated with insecticides (insecticide-treated nets, ITN) designed to kill the mosquito before it has time to search for a way past the net. ITN are estimated to be twice as effective as untreated nets (Hull, 2006) and offer greater than 70% protection compared to absence of net (Bachou, Tylleskär, Kaddu-Mulindwa, & Tumwine, 2006).

1.5. Treatment of Malaria

For most infectious diseases for which there are effective vaccines, a single infection confers long-standing protective immunity. However, this type of protective immunity does not exist for malaria. There is currently no licensed vaccine that will prevent malaria (Plowe, Alonso, & Hoffman, 2009), but this is an active field of

research. Vaccines for malaria are under development, with no completely effective vaccine yet available. However, there are antimalarial medications.

Malaria is treated with antimalarial drugs. Antimalarials can be classified as prophylactic and therapy drugs. Prophylactic drugs are taken as prevention and require continuous administration to reduce the risk of infection. Therapy drugs on the other hand are taken when the person is already infected with *Plasmodium*. However, strategies for combating malaria change rapidly, and when drugs are administered in combination, it can be difficult to identify which agents are prophylactic and which are therapeutic. Another approach for classifying antimalarials is to group them by mechanism of action and by chemical structure. Based on this type of classification, there are the following three groups of antimalarials: the quinolines, antifolates, and the artemisinin derivatives.

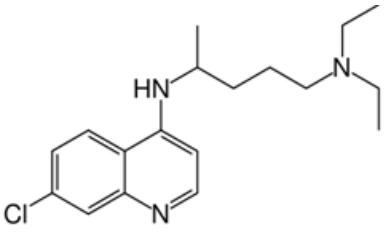
1.5.1. Quinolines

The first quinoline antimalarials were alkaloids extracted from the cinchona tree. There are 3 groups of quinolines developed as antimalarials. They are the 8-aminoquinolines, 4-aminoquinolines, and quinolinemethanols. The first synthetic antimalarial was an 8-aminoquinoline called plasmaquine (later called Pamaquine) that was found to be very effective but too toxic. A less toxic analogue called primaquine (PR) was synthesized and is still utilized until today to destroy liver reservoirs of *Plasmodium.* Figure 1.9 shows the structure of major quinoline drugs.

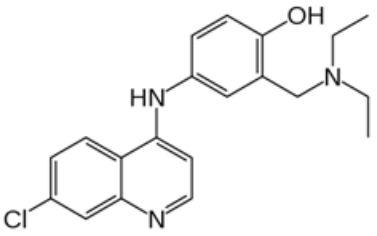
1.5.1(a) Quinine

Quinine is a natural white crystalline alkaloid with a bitter taste and has antipyretic (fever-reducing), antimalarial, analgesic (painkilling), and anti-inflammatory properties. It is a stereoisomer of quinidine. Quinine was the first effective treatment for malaria caused by *P. falciparum*. It remained the antimalarial drug of choice until the 1940s. Since then, many other effective antimalarials have been introduced, although quinine is still used to treat the disease in certain critical situations.

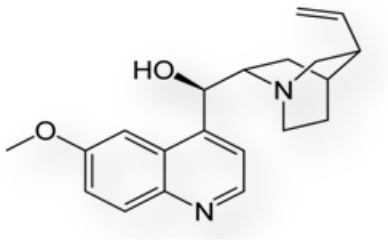
Quinine is an alkaloid that acts as a blood schizonticidal and weak gametocide against *P. vivax* and *P. malariae*. As an alkaloid, it is accumulated in the food vacuoles of the *Plasmodium* species, especially *P. falciparum*. It acts by facilitating an aggregation of cytotoxic heme. Quinine is less effective and more toxic as a blood schizonticidal agent than another aminoquinoline, chloroquine (CQ). However it is still very effective and widely used in the treatment of acute cases of severe *P. falciparum*. It is especially useful in areas where there is known to be a high level of resistance to CQ, mefloquine (MQ) and pyrimethamine (PYR). Quinine is also used in post-exposure treatment of individuals returning from an area where malaria is endemic. Quinidine is a direct derivative of quinine. It has similar anti-malarial properties to the parent compound. Quinidine is recommended only for the treatment of severe cases of malaria.



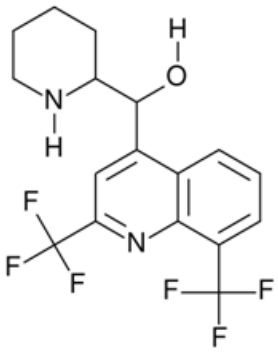
chloroquine



amodiaquine



quinine



mefloquine

Figure 1. 9. Structures of Major Quinoline Drugs

1.5.1(b) Chloroquine (CQ)

CQ is perhaps one of the earliest and effective 4-aminoquinolines to have been produced. It is metabolized by hepatic CYPs 2C8, 3A4 and 2D6 into N-desethylchloroquine (Projean, et al., 2003), which retains half of CQ's antimalarial properties (McChesney & Fitch, 1984). CQ until recently was a widely used antimalarial due to its efficacy and low risk of side effects in prescribed doses. It was often combined with PR tablets. Since CQ also has a significant anti-pyretic and anti-inflammatory effect when used to treat *P. vivax* infections, it may still remain useful even when resistance is more widespread. A slightly different drug called nivaquine or CQ phosphate has also been used.

However, due to improper use, parasite resistance developed rapidly, rendering the drug useless in many malaria endemic countries. Although the emergence of drug resistant parasitic strains is rapidly decreasing its effectiveness, it is still the first-line drug for malaria treatment in most sub-Saharan African countries. It is now suggested that it be used in combination with other antimalarial drugs to increase effectiveness.

1.5.1(c) Piperaquine (PQ)

Piperaquine (PQ), a bisquinoline, was used in China in the late 1970es and 1980es as malaria prophylaxis and treatment of CQ-resistant falciparum malaria due to its good tolerability and efficacy. It is structurally similar to CQ and highly lipophilic and hydrophobic (Sim, Davis, & Ilett, 2005); (Ahmed, et al., 2008). PQ exhibits elevated absorption and bioavailability in healthy volunteers when taken with moderately fatty meals (D'alessandro, 2009) maximising its therapeutic effects.

Due to its rather extended elimination half-life (20 -33 days) (Hung, et al., 2004); (Tarning, et al., 2005); (Nguyen, et al., 2009), it is often co-administered with dihydroartemisinin (dHA) in ACTs. It is found to be effective against both *falciparum* and *vivax* malaria, with a mechanism that acts through chemical inhibition of parasite heme detoxification (Davis, Hung, Sim, Karunajeewa, & Ilett, 2005).

1.5.1(d) Amodiaquine (AQ)

Amodiaquine (AQ) has been used as falciparum malaria prophylaxis for over 40 years (Foley & Tilley, 1998). It is a 4-aminoquinoline produced as an alternative to CQ. It is shown that AQ is highly effective in inhibiting growth of *P.falciparum in vitro* (Ekweozor, Aderounmu, & Sodeinde, 1987). It is intrinsically more active than CQ against *P. falciparum* parasites, which are moderately CQ resistant. The drug is therefore increasingly being considered as a replacement for chloroquine as a first line drug in Africa because of widespread CQ resistance.

Because of major side effects, mainly agranulocytosis, observed during prophylactic use of the drug, AQ is now only recommended for treatment of malaria for which no serious cases of toxicity have been reported (Laurent, et al., 1993). After oral administration, AQ is rapidly absorbed and metabolized into mainly *N*-desethylamodiaquine (DEAQ) with other minor metabolites, 2-hydroxyl-DEAQ and *N*-bisdesethylAQ (bis- DEAQ) (Churchill, Patchen, Campbell, Schwartz, Nguyen-Dinh, & Dickinson, 1985); (Churchill, Mount, Patchen, & Björkman, 1986); (Mount, Patchen, Nguyen-Dinh, Barber, Schwartz, & Churchill, 1986). Although the formation of DEAQ is rapid, its elimination is very slow with a terminal half-life of over 100 h (Winstanley, Edwards, Orme, & Breckenridge, 1987); (Laurent, et al., 1993). AQ and DEAQ both have antimalarial activity, but AQ is 3 times more active than its metabolite DEAQ (Churchill, Patchen, Campbell, Schwartz, Nguyen-Dinh, & Dickinson, 1985); (Li, Björkman, Andersson, Ridderström, & Masimirembwa, 2002), produced through metabolism catalysed by hepatic enzyme CYP2C8 (Li, Björkman, Andersson, Ridderström, & Masimirembwa, 2002). However, since AQ is rapidly cleared and the metabolite DEAQ attains high plasma concentrations for a

long time, AQ is considered a prodrug. AQ is currently used in combination with artemisinin derivatives.

1.5.1(e) Mefloquine (MQ)

Quinolinemethanols, structural analogs of quinine (Hofheinz & Merkli, 1984) are potent drugs against both *P.falciparum* and *P.vivax*. Although some of the earlier compounds exhibited appreciable photosensitivity (Pullman, Eichelberger, Alving, Jones, Craige, & Whorton, 1948), a derivative with elevated efficacy and negligible photosensitivity was successfully developed. This derivative called mefloquine (MQ) is still utilized to treat malaria.

MQ is an orally-administered antimalarial drug used as a prophylaxis against and for treatment of malaria. MQ is a very potent blood schizonticide and active against the erythrocytic stages of Plasmodium species. It is the drug of choice to treat malaria caused by chloroquine-resistant *Plasmodium vivax* (Maguire, Krisin, Marwoto, Richie, Fryauff, & Baird, 2006). MQ interferes with transportation of haemoglobin products and other substances from the host cell to the parasite's food vacuole. However, the drug has no effect against the exoerythrocytic (hepatic) stages of the parasite.

MQ is metabolized by the hepatic CYP3A4 into carboxymefloquine and hydroxymefloquine. The rather long half-life of MQ of about 20-30 days (Schwartz, et al., 1982); (Desjardins, Pamplin, von Bredow, Barry, & Canfield, 1979) and sub-therapeutic concentrations of the drug which can remain in the blood for months after treatment, may contribute to emergence of parasite resistance (Karbwang & White, 1990); (Nosten & Price, 1995). Parasite resistance towards MQ is evident especially

in Thailand and other parts of Southeast Asia. Therefore, MQ is now used in combination with artemisinin derivatives such as artesunate (AS) or artemether in order to stem further development of resistance (Price, et al., 1995). The sustained use of the AS-MQ combination has, in fact, reduced falciparum malaria transmission and progression of drug resistance in western Thailand (Nosten, et al., 2000); (Woodrow, Haynes, & Krishna, 2005).

1.5.2. Antifolates

Antifolates act by inhibiting the enzyme dihydrofolate reductase (DHFR) in the *Plasmodium* parasite (Yuthavong, 2002). DHFR is important in folate synthesis that forms the essential folate cofactor, fully-reduced tetrahydrofolate (Gregson & Plowe, 2005). Tetrahydrofolate is vital for the production of purine and pyrimidine bases, which are necessary monomers in DNA synthesis. Low levels of tetrahydrofolate are known to arrest DNA replication in *Plasmodium* (Triglia & Cowman, 1999). Sulphonamides (SA), sulfones, pyrimethamine (PRY) and proguanil (PG) are among the most widely used antifolates (Figure 1.10). However, rapid emergences of resistance among *Plasmodium* towards these drugs are reducing its effectiveness (Olliaro & Yuthavong, 1999).

PYR is used in the treatment of uncomplicated malaria. It is used in cases of CQ-resistant *P. falciparum* strains when combined with sulphadoxine (a type of SA). It acts primarily on the schizonts during the hepatic and erythrocytic phases. Sulphadoxine acts on the schizonts during the hepatic and erythrocytic phases. It is mainly used for treating *P. falciparum* infections and is less active against other *Plasmodium* strains. However usage is restricted due to the long half life of the