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Review

Antimalarial drugs: Mode of action and status of resistance

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Malaria is a major global health problem, with an estimated 300 to 500 million clinical cases occurring annually. Malaria remains one of the leading causes of disease and death in the tropics, mainly of children under 5 years of age. The most prevalent and dangerous type of malaria is caused by *Plasmodium falciparum. P. vivax* is a common cause of malaria in Latin America, Asia, and Oceania, but not Africa. *P. malariae* and *P. ovale* are much less common. Antimalarials are used in three different ways: prophylaxis, treatment of falciparum malaria, and treatment of non-falciparum malaria. Prophylactic antimalarials are used almost exclusively by travelers from developed countries who are visiting malaria-endemic countries. The antimalarials in common use come from the following classes of compounds: the quinolines (chloroquine, quinine, mefloquine, amodiaquine, primaquine), the antifolates (pyrimethamine, proguanil and sulfadoxine), the artemisinin derivatives (artemisinin, artesunate, artemether, arteether) and hydroxynaphthaquinones (atovaquine).

Key words: Malaria, resistance, antimalarial drugs, plasmodium.

INTRODUCTION

Malaria is caused by infection with a single-cell parasite, *Plasmodium*. Four *Plasmodium* spp. cause malaria in human beings: *Plasmodium falciparum*, *P. vivax, P. ovale,* and *P. malariae. P. falciparum* is the most important because it accounts for the majority of infections and causes the most severe symptoms. Malaria remains one of the leading causes of morbidity and mortality in the tropics. According to the World Malaria Report (2011), there were 106 malaria endemic countries in 2010.

There were 216 million cases of malaria in 2010; 81% of these were in the World Health Organization (WHO) African region. An estimated 3.3 billion people are at risk of malaria. An estimated 655, 000 persons died of malaria in 2010. 86% of the victims were children under 5 years of age, and 91% of malaria deaths occurred in the African region. Antimalarials are used in three different ways: prophylaxis, treatment of falciparum malaria, and treatment of non-falciparum malaria. Prophylactic antimalarials are used almost exclusively by travelers from developed countries who are visiting malaria-endemic countries. Treatment protocols for falciparum malaria vary, depending on the severity of the disease; fast-acting, parenteral drugs are best for severe, life-threatening disease. In addition, treatment protocols for falciparum malaria vary geographically and depend on the resistance profiles for strains in particular regions. Non-falciparum malarias, in contrast, rarely are drug-resistant. In addition, *P. vivax* and *P. ovale* have dormant liver stages that can cause relapses months to years after an infection is cleared, so they need to be treated with an additional agent that can clear this stage. The antimalarials in common use come from following classes of compounds: the quinolines (chloroquine, quinine, mefloquine, amodiaquine, primaquine), the antifolates (pyrimethamine, proguanil and sulfadoxine),the artmisinin

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derivatives (artemisinin, artesunate, artemether, arteether) and hydroxynaphthaquinones (atovaquine). This review looks at the drugs in common use and their treatment regimens, pharmacokinetic properties, mechanism of action and resistance, as well as status of resistance.

CHLOROQUINE

Chloroquine was first synthesized in Germany, but it was not recognized as a potent antimalarial drug until the 1940s during the US World War II military effort. By 1946, it was found to be far superior to other contemporary synthetic antimalarials (Coggeshall and Craige, 1949). Chloroquine became the cornerstone of antimalarial chemotherapy for the next 40 years. It quickly became the drug of choice globally to treat uncomplicated *P. falciparum* infections, and it was used as part of the Global Malaria Eradication campaign launched by the WHO in 1955. Chloroquine is one of the least expensive antimalarials available and is still in widespread use. This drug can be taken both as a prophylactic and as a treatment.

Despite much research during the last 40 years, the exact mechanism by which chloroquine kills the malaria parasite remains controversial (Foley and Tilly, 1997; Foote and Cowman, 1994; Peters, 1997). This drug inhibits DNA and RNA biosynthesis and induces the rapid degradation of ribosomes and the dissimilation of ribosomal RNA. The inhibition of protein synthesis is also observed evidently as a secondary effect. It has been proposed that the inhibition of DNA replication is the general antimicrobial mechanism of action of chloroquine. Chloroquine accumulates in very high concentrations in the parasite food vacuole (Geary et al., 1986). Once in the food vacuole, chloroquine is thought to inhibit the detoxification of heme. Chloroquine becomes protonated (to CQ^{2+}) because the digestive vacuole is acidic (pH 4.7) and subsequently cannot leave the vacuole by diffusion. Chloroquine caps hemozoin molecules and prevents the further biocrystallization of heme, thus leading to heme buildup. Chloroquine binds to heme (or FP) to form what is known as the FP-chloroquine complex; this complex is highly toxic to the cell and disrupts membrane function. The actions of the toxic FP-chloroquine complex and FP result in cell lysis and ultimately the auto-digestion of the parasite cell. In essence, the parasite cell drowns in its own metabolic products.

Mechanism of resistance

Resistance to chloroquine was slow to develop, taking almost 20 years, despite extensive use of the drug, suggesting that mutations in several genes were required to produce the resistance phenotype. The mechanism of chloroquine resistance also is uncertain. Chloroquine-resistant parasites accumulate less chloroquine in the food vacuole than do sensitive parasites (Fitch, 1970) and one assumption is that chloroquine resistance is not based on the mode of action of the drug but on the access of the drug to the parasite food vacuole. Early studies indicated that chloroquine resistance was associated with an elevated level of drug efflux. Drug-resistant parasites were reported to release pre-accumulated chloroquine almost 50 times faster than chloroquine-sensitive isolates (Verdier et al., 1985; Krogstad et al., 1987). Furthermore, verapamil was shown to reduce the apparent rate of drug efflux from chloroquine-resistant parasites (Krogstad et al., 1987). Since verapamil is known to reverse the P-glycoprotein-mediated efflux of drugs in multidrug-resistant tumor cells (Martin et al., 1987), this led to the proposal that efflux of chloroquine by a plasmodial P-glycoprotein is responsible for chloroquine resistance.

Bray et al. (1999) on the other hand have suggested that chloroquine resistance is caused by reduced affinity of chloroquine for heme, thereby reducing chloroquine uptake. Another proposal is that chloroquine is transported actively through the parasite by the Na^+/H^+ exchanger (NHE) and that resistance to chloroquine is mediated by mutations in the NHE (Wunsch et al., 1998) but this suggestion has been disputed. Wellems et al. (1990, 1991) analyzed a cross between a chloroquine-resistant and a chloroquine-sensitive strain of *P. falciparum*, and identified a chloroquine-resistance locus within a 400 kb segment of chromosome 7. Su et al. (1997) mapped the putative chloroquine-resistance locus to a 36 kb region and identified the open reading frames of 8 potential genes within this region. Initially, chloroquine resistance was thought to be caused by *cg2,* a gene coding for a polymorphic protein located at the parasite periphery. However, recent transformation studies have ruled out *cg2* and suggest another gene, *pftcr* within this region (Fidock et al., 1999).

Status of resistance

Chloroquine, soon after introduction in the 1950s, quickly became the main drug of choice globally to treat uncomplicated *P. falciparum* infections, for instance, as part of the Global Malaria Eradication Campaign launched by the (WHO) in 1955. However, *P. falciparum* did eventually develop resistance to chloroquine, and has spread to almost all the endemic countries today (Wellems and Plowe, 2001). Chloroquine resistant parasites in Africa were thought by some to share the same origin as the Indo-China strains, but by others to have developed locally as a result of mass drug administration plus intrinsic entomological, epidemiological, and parasitological factors that promoted local resistance selection (Diribe and Warhurst, 1985).

Current molecular studies suggest the Asian origin of African isolates, but at least four different foci of chloroquine resistance have so far been identified (Warhurst, 1995). Resistance to chloroquine has spread to almost all the countries thus limiting the effective use of this low cost antimalarial.

QUININE

Quinine is derived from the bark of the cinchona tree and was used for treating fevers as early as the 17th century, although not until 1820, it was the active ingredient of the bark, isolated and used in its purified form. Quinine is used as a treatment for uncomplicated and severe malaria in many different therapeutic regimens. Quinimax, which is a combination of quinine, quinidine, and cinchonine (all derived from cinchona bark), is also used (Deloron et al., 1985). Quinine must be administered for at least 7 days to non-immune populations (Bunnag and Harinasuta, 1986; Krishna and White, 1996; Bjorkman et al., 1991) but it is effective in immune populations (such as in sub-Saharan Africa) when given for 3 to 5 days because it appears to be potentiated by the host immune system (Miller et al., 1989). In the United States where quinine is not available commercially, quinidine (its D-isomer) is used. Quinine is also used in combination with antibiotics (tetracycline or doxycycline).

Mode of action

Quinine acts in a manner similar to that of chloroquine but with some differences; chloroquine causes clumping of the malaria pigment, whereas quinine antagonizes this process (Peters, 1987). In addition, quinine is a weaker base than chloroquine and has less affinity for heme, implying that mechanisms other than ion transport into the food vacuole and heme-drug interactions are required for the action of these drugs (Foley and Tilly, 1998). Quinine also interacts rather weakly with heme (Kd = 2.63×10^{-6} M) (Chou et al., 1980), but has been shown to inhibit heme polymerization (Slater 1992; Chou and Fitch, 1993) and heme catalase activity (Ribeiro et al., 1997). In the absence of a specific transporter, quinine is likely to be accumulated less efficiently in the food vacuole than chloroquine. Further work is required to determine whether the mechanism of action of quinine is more closely aligned to that of chloroquine.

Status of resistance

Although quinine treatment failure has been reported, many of these instances can be attributed to inadequate treatment. Reports of clinical quinine resistance have been sporadic, with the highest incidence occurring in Southeast Asia, where it was first reported in 1967 (WHO, 1967). In Thailand, treatment failure rates increased from 6% in 1978 to 14% in 1979 to 1980, and they were up to 38.5% in 1981 (Chongsuphajaisiddhi et al., 1983). A more recent report shows 23% recrudescence in pregnant women after quinine treatment (McGready et al., 1998), which may be an indication that quinine resistance in Thailand is stabilizing, perhaps because of the widespread use of quinine combinations and alternative drugs. In Africa, quinine resistance remains at very low levels, and even in Southeast Asia, cure rates with quinine combinations (for example, quinine-tetracycline) remain high (Watt et al., 1992; Looareesuwan et al., 1994; Bunnag et al., 1996).

MEFLOQUINE AND HALOFANTRINE

Mefloquine was developed in the 1970s by the United States Army in response to the increasingly poor cure rates of chloroquine, with clinical trials beginning in 1972 (Davidson et al., 1975; Trenholme et al., 1975; Rieckmann et al., 1974). Mefloquine has a very long half-life both in patients with malaria (10.3 to 20.5 days) (Karbwang and White, 1990; Na-Bangchang et al., 1995) and in healthy volunteers (13.8 to 27.5 days). Mefloquine is recommended for prophylaxis and therapy in chloroquine-resistant areas. Despite considerable publicity about possible neuropsychiatric side-effects of mefloquine, the same evidence is not conclusive (Choo, 1996).

Halofantrine is a tricyclic compound that was developed at approximately the same time as was mefloquine and has been used as a second-line agent; its use may be limited by its cardiotoxic side-effects and variable pharmacokinetics.

Mode of action

Mefloquine interacts relatively weakly with free heme, with reported Kd values ranging from 3.3×10^{-7} to 1.63×10^{-5} M (Chou et al., 1980; Chevli and Fitch, 1982). Mefloquine has been shown to inhibit heme polymerization *in vitro* with a similar (Slater and Cerami, 1992; Slater, 1993) or lower (Chou and Fitch, 1993; Raynes et al., 1996) efficiency than chloroquine (that is, close to millimolar levels). Given the lower basicity of mefloquine, it seems unlikely that it would reach the intravacuolar concentration required to inhibit heme polymerization. Furthermore, while chloroquine treatment of *P. berghei* infected mice was found to cause a decrease in hemozoin production, mefloquine and quinine had no effect (Chou and Fitch, 1993). Mefloquine

is also a much less potent enhancer of the peroxidase activity of heme than chloroquine (Sugioka and Suzuki, 1991) and has been shown to interfere with the ability of chloroquine to enhance heme-induced cell lysis (Dutta and Fitch, 1983).

The available data suggest therefore that, mefloquine interferes with a different step in the parasite-feeding process than chloroquine (Geary et al., 1986). Desneves et al. (1996) used the technique of photo affinity labeling to identify two high-affinity, mefloquine-binding proteins with apparent molecular masses of 22 to 23 kDa and 36 kDa in *P. falciparum* infected erythrocytes. The identities of these polypeptides have not been established yet, but they may be involved in mefloquine uptake or action. There is also increasing evidence to suggest a role for the plasmodial P-glycoprotein (P-glycoprotein homolog-1, Pgh-1) in mefloquine resistance. This raises the possibility that Pgh-1 may also be the target of action of mefloquine.

Mechanism of resistance

Mefloquine resistance in field isolates of *P. falciparum* is associated with amplification of the *pfmdr1* gene (Peel et al., 1993; Wilson et al., 1993; Cowman et al., 1994) and over-expression of its protein product Pgh-1 (Cowman et al., 1994). Moreover, selection for mefloquine resistance *in vitro* leads to amplification and over expression of the *pfmdr1* gene (Cowman et al., 1994; Wilson et al., 1989; Peel et al., 1994). This has led to the idea that Pgh-1 is responsible for at least some forms of mefloquine resistance. Resistance to halofantrine and quinine also increased during mefloquine selection, suggesting a similar underlying mechanism (Peel et al., 1993, 1994; Cowman et al., 1994). Resistance to mefloquine is not reversed by verapamil or chlorpromazine, but can be reversed by penfluridol (Peters and Robinson, 1991).

Status of resistance

Resistance to mefloquine has been rising inexorably ever since this drug was introduced in the 1970s (Boudreau et al., 1982; Espinal et al., 1985; Draper et al., 1985; Brasseur et al., 1990; Gay et al., 1990; Raccurt et al., 1991; Looareesuwan et al., 1992; White, 1994). In an area of Thailand where mefloquine was used intensively, substantial mefloquine resistance developed within 5 years of its introduction (White, 1994; Mockenhaupt, 1995). Cure rates with mefloquine have now dropped to 41% in some areas of Thailand (Nosten et al., 1991; Fontanet et al., 1993). Combination regimens with either artenusate or artemether have been introduced in an effort to stem the development of resistance to mefloquine (Price et al., 1995). There have been reports of intrinsic mefloquine resistance in regions of Africa where the drug had not been used (Oduola et al., 1992; White, 1994), although it now appears that this may have been due to pre-existing levels of quinine resistance. Resistance to mefloquine in the field was first noted in an area of Thailand (Boudreau et al., 1982) where quinine resistance was already widespread. The ease with which mefloquine resistance develops is exemplified by experiments, showing that mefloquine resistance readily can be induced by applying drug pressure during continuous passage of *P. berghei* through *Anopheles gambiae* (Fonseca et al., 1995).

Mefloquine has been used in higher doses as a solo treatment in much of Southeast Asia, although high levels of resistance to this therapy are now occurring there as well (ter Kuile et al., 1992). Mefloquine is still effective in most African countries and can be used in areas of chloroquine resistance. Mefloquine and halofantrine show a high degree of *in vitro* cross-resistance (Basco et al., 1995; Gay et al., 1990; Pradines et al., 1998; Rojas-Rivero et al., 1992; Basco and Le Bras, 1993), although evidence of *in vivo* cross resistance is limited. It indicates that increasing levels of resistance to mefloquine may limit the effective chemotherapy lifetime of both mefloquine and halofantrine.

ANTIFOLATES

Some of the most widely used antimalarial drugs belong to the folate antagonist class, albeit their role in malaria control is hampered by rapid emergence of resistance under drug pressure (Plowe et al., 1998). Inhibition of enzymes of the folate pathway results in decreased pyrimidine synthesis, hence, reduced DNA, serine, and methionine formation. Activity is exerted at all growing stages of the asexual erythrocytic cycle and on young gametocytes. Traditionally, antifolates are classified into two:

1. Type-1 antifolates (sulfonamides and sulfones) mimic p-aminobenzoic acid (PABA). They prevent the formation of dihydropteroate from hydroxymethyldihydropterin catalyzed by dihydropteroate synthase (DHPS) by competing for the active site of DHPS (a bifunctional enzyme in plasmodia coupled with 2-amino-4-hydroxy-6-hydroxymethyl-dihydropteridine pyrophosphokinase [PPPK]).
2. Type-2 antifolates (pyrimethamine, biguanides and triazine metabolites, quinazolines) inhibit dihydrofolate reductase (DHFR, also a bifunctional enzyme in plasmodia coupled with thymidylate synthase [TS]), thus preventing the NADPH-dependent reduction of H2folate (DHF) to H4folate (THF) by DHFR.

Mode of action

The antifolate drugs inhibit either dihydrofolate reductase

(DHFR) (pyrimethamine, cycloguanil) or dihydropteroate synthase (DHPS) (sulfadoxine). These are two key enzymes in *de novo* folate biosynthesis; inhibition of this metabolic pathway leads to the inhibition of the biosynthesis of pyrimidines, purines, and some amino acids. Antifolate antimalarial drugs interfere with folate metabolism, a pathway essential to malaria parasite survival.

Mechanism of resistance

This class of drugs includes effective causal prophylactic and therapeutic agents, some of which act synergistically when used in combination. Unfortunately, the antifolates have proven susceptible to resistance in the malaria parasites. Resistance is caused by point mutations in DHFR and DHPS, the two key enzymes in the folate biosynthetic pathway which are targeted by antifolates. Resistance to these drugs arises relatively rapidly in response to drug pressure and is now common worldwide. Resistance to DHFR and DHPS inhibitors is conferred by single mutations of the gene encoding for the respective enzyme, resulting in substitutions in the amino acid chain. There are areas of the DHFR and DHPS genes with identified mutations that are found in isolates that fail to respond to pyrimethamine/sulfa treatment. These occur principally at codons 108, 51, 59, 164, and also occasionally 50, 140, and the ``Bolivian repeat'' of the DHFR gene and codons 436, 437, 540, 581, and 613 of the DHPS gene (Plowe et al., 1998). There is a broad correlation between increased frequency of such mutations and resistance to pyrimethamine/sulfa drugs across the world (Wang et al., 1997).

Status of resistance

For all the antifolate drugs, a high number of mutations may be an indicator of clinical resistance, but as the number of mutations becomes smaller, the accuracy in predicting clinical resistance decreases. Resistance to the fansidar combination is widespread, especially in Southeast Asia.

Fansidar has been used as a second-line drug in areas of chloroquine resistance; the loss of fansidar effectiveness caused by increasing resistance has important implications for Africa, where the number of inexpensive alternatives is limited. The development of resistance to sulfadoxine-pyrimethamine by *Plasmodium* parasites is a major problem for the effective treatment of malaria, especially *P. falciparum.* Although the molecular basis for parasite resistance is known, the factors providing the development and transmission of these resistant parasites are less clear. In Tanzania, increasing rate of chloroquine resistance led to change in its first line treatment of uncomplicated malaria to sulfadoxine-pyrimethamine. This antifolate combination seemed to be an effective and reasonable alternative but resistance to sulfadoxine-pyrimethamine was rapidly gaining ground, facilitated by the slow elimination from the body. High level (45%) of sulfadoxine-pyrimethamine treatment failures were recorded in Muheza, North East Tanzania by Mutabingwa et al. (2001). Nazila et al. (2000) observed that when a combination of pyrimethamine and sulfadoxine was used in Kenya, drug resistant parasites were selected rapidly. A study of pyrimethamine-sulfadoxine effectiveness was carried out between 1997 and 1999 at Kilify on the Kenyan Coast, and it concluded that prevalence of triple mutant DHFR-double mutant DHPS combination may be an operationally useful marker for predicting the effectiveness of pyrimethamine-sulfadoxine as a new malaria treatment.

Gatton et al. (2004) worked on evolution of resistance to pyrimethamine-sulfadoxine in *P. falciparum*. Their findings indicate three stages in the development of drug resistance.

The first is the collection of existing parasites with genetic mutations in the DHFR and DHPS gene and it was driven by long half-life of pyrimethamine-sulfadoxine combinations. The second stage involves the selection of parasites with allelic types of higher resistance within the host during an infection. And in the third stage, clinical treatment failure becomes prevalent as the parasites develop sufficient resistance mutation to survive therapeutic doses of the drug combinations. They emphasized on importance of correct treatment of confirmed malaria cases to avoid development of parasite resistance to pyrimethamine-sulfadoxine.

ARTEMISININ DERIVATIVES

For nearly 2,000 years, a cold-water extract of sweet wormwood (*Artemisia annua,* ‘‘qinghao’’) has been used in China for the treatment of fevers. The active ingredient of this plant was isolated in 1970 by Chinese scientists. Artemisinin (or qinghaosu) and its derivatives (artesunate, artemether, and arteether) have been used extensively in China and Southeast Asia, where there are high levels of resistance to the majority of the quinoline-containing drugs and to all the antifolate drugs (Meshnick et al., 1996). The artemisinin-type compounds in current use are either the natural extract artemisinin itself or the semi-synthetic derivatives (dihydroartemisinin, artesunate, artemether). They achieve higher reduction rates of parasitaemia per cycle than any other drug known to date (White, 1997).

Mode of action

Artemisinin and its derivatives are sequiterpene lactones.

Once administered, the artemisinin derivatives are hydrolyzed rapidly to the biologically active metabolite dihydroartemisinin. The mode of action of the artemisinin drugs has not been completely elucidated. The present knowledge is reviewed by Meshnick et al. (1996) and Cumming et al. (1997). The structure of artemisinin is unusual, and its activity is thought to depend on the presence of the endoperoxide bond, as molecules without it have no antimalarial activity (Brossi et al., 1988). The endoperoxide bond may interact with iron or heme, decomposing into free radicals (Meshnick et al., 1993, 1996; Paitayatat et al., 1997). Unlike many redox reactions, this process is not reversible, so a single drug molecule will produce only one free radical. The effect of free radicals on the malaria parasite is still not fully understood. Because the concentration of free radicals is insufficient to cause general membrane damage, one theory is that a ‘‘specific free radical target’’ exists (Meshnick, 1994). The artemisinin free radical can form a covalent bond with either heme or other parasite proteins (Yang et al., 1993, 1994) and an initial hypothesis was that a heme-artemisinin compound might inhibit the production of hemozoin. No evidence, however, of reduced quantities of hemozoin in artemisinin-treated *P. falciparum* cultures has been found (Asawamahasakda et al., 1994). Artemisinin also has been shown to bind to 6 specific *P. falciparum* proteins, one of which is a member of the translationally controlled tumor protein family but the precise effect of this protein alkylation on the parasite is still to be determined.

Mechanism and status of resistance

Artemisinin-resistant strains have been developed both in *P. falciparum* cultures (Inselburg, 1985) and in *P. yoelii* mouse models (Chawira et al., 1986). There also have been some indications of increasing *in vitro* resistance in field isolates (Gay et al., 1994). *P. falciparum* resistance to artemisinins, which was confirmed on the Cambodia-Thailand border in 2009, is now suspected in parts of Myanmar and Viet Nam. However, Artemisinin-based combination therapies (ACTs) remain highly effective in almost all settings, so long as the partner drug in the combination is locally effective. Artemisinin derivatives have a gametocytocidal activity (Peters, 1993), a feature that, in combination with their pharmacokinetic and pharmacodynamic properties, may well delay the development of drug resistance in the field. Resistance is difficult to induce experimentally, and is labile (low levels of resistance are achieved after sustained drug pressure) but not retained once drug pressure is removed (Peters and Robinson, 1999). Available data suggest that resistance to this class of compounds would be multigenic and share similarities with the quinoline family, as demonstrated *in vitro* on a series of parasite isolates (Meshnick, 1999).

ATOVAQUONE

The antimalarial activities of hydroxynaphthoquinones were discovered during World War II. Atovaquone is the first effective compound in this class. Currently, it is being marketed as Malarone, which contains a fixed combination of atovaquone and proguanil.

Mode of action

Atovaquone {2-[trans-4-(40-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone}, hydroxynaphthoquinone is used for both the treatment and prevention of malaria in a fixed combination with proguanil. Whilst known to act primarily on mitochondrial functions, its mode of action and synergy with proguanil are not completely understood. This matter is further complicated by the diverse functions of mitochondria in various organisms and by technical difficulties with experiments. It is generally agreed that atovaquone acts on the mitochondrial electron transfer chain, although more recently, its activity and synergy with proguanil has been ascribed to its interference with mitochondrial membrane potential. Atovaquone inhibits cytochrome *c* reductase activity in *P. falciparum* (Fry and Pudney, 1992). Atovaquone is a ubiquinone analogue that binds to the cytochrome *bc1* complex of the parasite mitochondrial electron transport chain. The malaria mitochondria electron transport chain disposes of electrons generated by dihydroororate dehydrogenase during the synthesis of pyrimidines (Gutteridge et al., 1979) and the inhibition of this process by atovaquone may kill the parasite (Hammond et al., 1985). More recently, it has been shown in *P. yoelli* that atovaquone also dissipates the mitochondrial membrane potential of the parasite which may kill the parasite by initiating a process similar to apoptosis.

Mechanism and status of resistance

When atovaquone was first used in clinical trials in Thailand, the treatment failure rate was 33%, regardless of duration of therapy (Looareesuwan et al., 1999). This high level of treatment failure suggests that either a natural background of resistant mutants exists or resistance arises rapidly by the acquisition of point mutations in the cytochrome *b* gene. Mutations in cytochrome *b* have been found in atovaquone-resistant *Pneumocystis carini* and *P. yoelii* strains, indicating that they may be the cause of atovaquone resistance (Walker et al., 1998; Srivastava et al., 1999). Because of the high rate of treatment failure, atovaquone has been combined with other drugs, including proguanil, doxycycline, and tetracycline. All of these combinations yielded high cure rates (Looareesuwan et al., 1999; Redloff et al., 1996), and the atovaquone-proguanil combination (Malarone)

also is effective as a prophylactic.

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REFERENCES

Asawamahasakda W, Ittarat I, Chang CC, McElroy P, Meshnick SR (1994). Effects of antimalarials and protease inhibitors on plasmodial hemozoin production. Mol. Biochem. Parasitol. 67:183–191.

Basco LK, Le Bras J (1991). Reversal of chloroquine resistance with cyproheptadine in “wild” strains of *Plasmodium falciparum.* Trans. R. Soc. Trop. Med. Hyg. 85:204–205.

Basco LK, Le Bras J, Rhoades Z, Wilson CM (1995). Analysis of *pfmdr1* and drug susceptibility in fresh isolates of *Plasmodium falciparum* from sub-Saharan Africa. Mol. Biochem. Parasitol. 74:157-166.

Björkman A, Willcox M, Marbiah N, Payne D (1991). Susceptibility of *Plasmodium falciparum* to different doses of quinine *in vivo* and to quinine and quinidine *in vitro* in relation to chloroquine in Liberia. Bull. World Health Organ. 69:459-465.

Boudreau EF, Webster HK, Pavanand K, Thosingha L (1982). Type II mefloquine resistance in Thailand. Lancet ii:1335.

Brasseur P, Kouamouo J, Moyou RS, Druilhe P (1990). Emergence of mefloquine-resistant malaria in Africa without drug pressure. Lancet. 336:59.

Bray PG, Janneh O, Ward SA (1999). Chloroquine uptake and activity is determined by binding to ferriprotoporphyrin IX in *Plasmodium falciparum*. Novartis Fundam. Symp. 226: 252-264.

Bunnag D, Karbwang J, Na-Bangchang K, Thanavibul A, Chittamas S, Harinasuta T (1996). Quinine-tetracycline for multidrug resistant falciparum malaria. Southeast Asian J. Trop. Med. Public Health 27:15-18.

Chawira AN, Warhurst DC, Peters W (1986) Qinghaosu resistance in rodent malaria. Trans. Soc. Trop. Med. Hyg. 80: 477-480.

Chevli R, Fitch CD (1982). The antimalarial drug mefloquine binds to membrane phospholipids. Antimicrob. Agents Chemother. 21(4):581-586.

Chongsuphajaisiddhi T, Sabcharoen A, Attanath P (1983). Treatment of quinine resistant falciparum malaria in Thai children. Southeast Asian J. Trop. Med. Public Health 14:357-362.

Choo V (1996). Uncertainty about mefloquine will take time to resolve [news]. Lancet 347:891

Chou AC, Fitch CD (1993). Control of heme polymerase by chloroquine and other quinoline derivatives. Biochem. Biophys. Res. Commun. 195(1):42. August.

Chou AC, Chevli R, Fitch CD (1980). Ferriprotoporphyrin IX fulfills the criteria for identification as the chloroquine receptor of malaria parasites. Biochemistry 19:1543–1549.

Coggeshall LT, Craige B (1949). Old and new plasmodicide. In: Boyd ME, ed. A comprehensive survey of all aspects of this group of diseases from a global standpoint. Philadelphia: WB Saunders Publisher.

Cowman AF, Galatis D, Thompson JK (1994). Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the *pfmdr1* gene and cross-resistance to halofantrine and quinine. Proc. Natl. Acad. Sci. USA 91:1143–1147.

Cumming JN, Ploypradith P, Posner G (1996). Antimalarial activity of artemisinin (Qinghaosu) and related trioxanes: Mechanism(s) of action. Adv. Pharmacol. 37:253- 297.

Davidson MW, Griggs Jr, BG, Boykin DW, Wilson WD (1975). Mefloquine, a clinically useful quinolinemethanol antimalarial which does not significantly bind to DNA. Nature 254:632-634.

Deloron P, Le Bras J, Ramanamirija JA, Coulanges P (1985). *Plasmodium falciparum* in Madagascar: *In vivo* and *in vitro* sensitivity to seven drugs. Ann. Trop. Med. Parasitol. 79(4):357-365.

Desneves J, Thorn G, Berman A, Galatis D, La Greca N, Sinding J, Foley M, Deady LW, Cowman AF, Tilley L (1996). Photoaffinity labeling of mefloquine-binding proteins in human serum, uninfected erythrocytes and *Plasmodium falciparum*-infected erythrocytes. Mol. Biochem. Parasitol. 82(2):181-194. November.

Diribe CO, Warhurst DC (1985). Chemotherapy and drug resistance in malaria. Biochem. Pharm. 34:3019-3027.

Draper CC, Brubaker G, Geser A, Kilimali VA, Wernsdorfer WH (1985). Serial studies on the evolution of chloroquine resistance in an area of East Africa receiving intermittent malaria chemo suppression. Bull. World Health Organ. 63:109–118.

Dutta P, Fitch CD (1983). Diverse membrane-active agents modify the hemolytic response to ferriprotoporphyrin IX. J. Pharmacol. Exp. Ther. 225(3):729-734.

Espinal CA, Cortes GT, Guerra P, Arias AE (1985). Sensitivity of *Plasmodium falciparum* to antimalarial drugs in Colombia. Am. J. Trop. Med. Hyg. 34:675–680.

Fidock DA, Nomura T, Talley KA, Cooper AR, Dzekunov MS, Ferdig TM (2000). Mutations in the *P. falciparum* digestive vacuole trans membrane protein PfCRT and evidence for their role in chloroquine resistance. Mol. Cell. 6(4):861-871.

Fitch CD (1970). *Plasmodium falciparum* in owl monkeys: Drug resistance and chloroquine binding capacity. Science 169:289-290.

Foley M, Tilley L (1997). Quinoline antimalarials: Mechanisms of action and resistance. Int. J. Parasitol. 27:231-240.

Fonseca L, Vigario AM, Seixas E, do Rosario VE (1995). *Plasmodium berghei*: selection of mefloquine-resistant parasites through drug pressure in mosquitoes. Exp. Parasitol. 81:55–62.

Fontanet AL, Johnston DB, Walker AM, Rooney W, Thimasarn K, Sturchler D, Macdonald M, Hours M, Wirth DF (1993). High prevalence of mefloquine-resistant falciparum malaria in eastern Thailand. Bull. World Health Organ. 71:377–383.

Foote SJ, Cowman AF (1994). The mode of action and the mechanism of resistance to antimalarial drugs. Acta Trop. 56:157-171.

Fry M, Pudney M (1992). Site of action of the antimalarial hydroxynaphthoquinone, 2-[trans-4-(49-chlorophenyl) cyclohexyl]- 3-hydroxy-1,4-naphthoquinone (566C80). Biochem. Pharmacol. 43:1545–1553.

Gay F, Ciceron L, Litaudon M, Bustos MDG, Astagneau P, Diquet B, Danis M, Gentilini M (1994). *In vitro* resistance of *Plasmodium falciparum* to qinghaosu derivatives in West Africa. Lancet 343:850-851.

Gay F, Bustos DG, Diquet B, Rivero LR, Litaudon M, Pichet C, Danis M, Gentilini M (1990). Cross-resistance between mefloquine and halofantrine. Lancet 336:1262.

Geary TG, Divo AD, Jensen JB, Zangwill M, Ginsburg H (1990). Kinetic modeling of the response of *Plasmodium falciparum* to chloroquine and its experimental testing *in vitro*: Implications for mechanism of action of and resistance to the drug. Biochem. Pharmacol. 40:685-691.

Geary TG, Bonanni LC, Jensen JB, Ginsburg H (1986). Effects of combinations of quinoline-containing antimalarials on *Plasmodium falciparum* in culture. Ann. Trop. Med. Parasitol. 80(3): 285-291.

Hammond DJ, Burchell JR, Pudney M (1985). Inhibition of pyrimidine biosynthesis *de novo* in *Plasmodium falciparum* by 2-(4-t-butylcyclohexyl)- 3-hydroxy-1,4-naphthoquinone *in vitro*. Mol. Biochem. Parasitol. 14:97-109.

Inselburg J (1985). Induction and isolation of artemisinine-resistant mutants of *Plasmodium falciparum.* Am. J. Trop. Med. Hyg. 34:417-418.

Karbwang J, White NJ (1990). Clinical pharmacokinetics of mefloquine. Clin. Pharmacokinet. 19(4):264-279.

Miller KD , Greenberg AE, Campbell CC, (1989). Treatment of severe malaria in the United States with a continuous infusion of quinidine gluconate and exchange transfusion. New Engl. J. Med. 321:65-70 .

Krishna S, White NJ (1996). Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. Clin. Pharmacokinet. 30(4):263-299.

Krogstad DJ, Gluzman IY, Kyle DE, Oduola AMJ, Martin SK, Milhous WK, Schlesinger PH (1987). Efflux of chloroquine from *Plasmodium*

falciparum: mechanism of chloroquine resistance. Sci. 238:1283-1285.
Looareesuwan S, Chulay JD, Canfield CJ, Hutchinson DB (1999). Malarone (atovaquone and proguanil hydrochloride): A review of its clinical development for treatment of malaria. Malarone Clinic Trials Study Group. Am. J. Trop. Med. Hyg. 60:533-541.
Looareesuwan S, Viravan C, Vanijanonta S, Wilairatana P, Suntharasamai P, Charoenlarp P, Arnold K, Kyle D, Canfield C, Webster K (1992). Randomised trial of artesunate and mefloquine alone and in sequence for acute uncomplicated falciparum malaria. Lancet 339:821–824.
Martin SK, Oduola AM, Milhous WK (1987). Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. Science 235:899-901.
McGready R, Cho T, Cho JJ, Simpson JA, Luxemburger C, Dubowitz L, Looareesuwan S, White NJ, Nosten F (1998). Artemisinin derivatives in the treatment of falciparum malaria in pregnancy. Trans R. Soc. Trop. Med. Hyg. 92:430-433.
Meshnick SR (1994) Free radicals and antioxidants. Lancet 344:1441-1442.
Meshnick SR (1999). Artemisinin antimalarials: Mechanisms of action and resistance. Med. Trop.(Marseille. 58(Suppl. 3):13-17.
Meshnick SR, Yang YZ, Lima V, Kuypers F, Kamchonwongpaisan S, Yuthavong Y (1993). Iron-dependent free radical generation from the antimalarial agent artemisinin (qinghaosu). Antimicrob. Agents Chemother. 37:1108-1114.
Meshnick S, Taylor TE, Kamchonwongpaisan S (1996). Artemisinin and the antimalarial endoperoxides: From herbal remedy to targeted chemotherapy. Microbiol. Rev. 60:301-315.
Miller KD, Greenberg AE, Campbell CC (1989). Treatment of severe malaria in the United States with a continuous infusion of quinidine gluconate and exchange transfusion [see comments]. New Engl. J. Med. 321: 65-70.
Mockenhaupt FP (1995). Mefloquine resistance in *Plasmodium falciparum*. Parasitol. Today 11:248–253.
Mutabingwa T, Nzila A, Mberu E, Nduati E, Winstanley P, Hills E, Watkins W (2001). Chlorproguanil-dapsone for treatment of drug resistant falciparum malaria in Tanzania. Lancet 358:1218-1223.
Na-Bangchang K, Molunto P, Banmairuroi V, Thanavibul A, Karbwang J (1995). Pharmacokinetics of mefloquine when given as a single and two divided-dose regimens. Int. J. Clin. Pharmacol. Res. 15:215-220.
Nosten F, Ter Kuile F, Chongsuphajaisiddhi T, Luxemburger C, Webster HK, Edstein M, Phaipun L, Thew KL, White NJ (1991). Mefloquine-resistant *falciparum* malaria on the Thai-Burmese border. Lancet 337:1140–1143.
Nzila AM, Mberu EK, Sulo J, Dayo H, Winstanley PA, Sibley CH, Watkins WM (2000). Towards an understanding of the mechanism of pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: Genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites. Antimicrob. Agents Chemother. 44:991-996.
Oduola AMJ, Sowunmi A, Milhous WK, Kyle DE, Martin RK, Walker O, Salako LA (1992b). Innate resistance to new antimalarial drugs in *Plasmodium falciparum* from Nigeria. Trans. R. Soc. Trop. Med. Hyg. 86:123–126.
Paitayatat S, Tarnchompoo B, Thebtaranonth Y, Yuthavong Y (1997). Correlation of antimalarial activity of artemisinin derivatives with binding affinity with ferroprotoporphyrin IX. J. Med. Chem. 40:633-638.
Peel SA, Bright P, Yount B, Handy J, Baric RS (1994). A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (*pfmdr*) of *Plasmodium falciparum in vitro*. Am. J. Trop. Med. Hyg. 51: 648–658.
Peel SA, Merritt SC, Handy J, Baric RS (1993). Derivation of highly mefloquine-resistant lines from *Plasmodium falciparum in vitro*. Am. J. Trop. Med. Hyg. 48:385–397.
Peters W (1997). Drug resistance in malaria parasites of animals and man. Adv. Parasitol. 41: 1-62.
Peters W (1987). Chemotherapy and Drug Resistance in Malaria. Academic Press, London.
Peters W, Robinson BL (1991). The chemotherapy of rodent malaria. XLVI. Reversal of mefloquine resistance in rodent *Plasmodium*. Ann. Trop. Med. Parasitol. 85:5–10.
Peters W, Robinson BL (1999). The chemotherapy of rodent malaria. LVI. Studies on the development of resistance to natural and synthetic endoperoxides. Ann. Trop. Med. Parasitol. 93:325-329.
Peters W, Robinson BL, Milhous WK (1993). The chemotherapy of rodent malaria. LI. Studies on a new 8-aminoquinoline, WR 238,605. Ann. Trop. Med. Parasitol. 87:547–552.
Plowe C, Kublin JG, Doumbo OK (1998). *P. falciparum* dihydrofolate reductase and dihydropteroate synthase mutations: Epidemiology and role of clinical resistance to antifolates. Drug Resistance Updates 1:389-396.
Pradines B, Rogier C, Fusai T, Tall A, Trape JF, Doury JC (1998) *In vitro* activity of artemether against African isolates (Senegal) of *Plasmodium falciparum* in comparison with standard antimalarial drugs. Am. J. Trop. Med. Hyg. 58:354-357.
Price RN, Nosten F, Luxemburger C, Kham A, Brockman A, Chongsuphajaisiddhi T, White NJ (1995). Artesunate versus artemether in combination with mefloquine for the treatment of multidrug-resistant falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 89:523–527.
Raccurt CP, Dumestre-Toulet V, Abraham E, Le Bras M, Brachet-Liermain A, Ripert C (1991). Failure of falciparum malaria prophylaxis by mefloquine in travelers from West Africa. Am. J. Trop. Med. Hyg. 45:319–324.
Radloff PD, Phillips J, Nkeyi M, Hutchinson D, Kremsner PG (1996). Atovaquone and proguanil for *Plasmodium falciparum* malaria. Lancet 347:1511-1513.
Raynes K, Foley M, Tilley L, Deady L (1996). Novel bisquinoline antimalarials: Synthesis, antimalarial activity and inhibition of haem polymerisation. Biochem. Pharmacol. 52:551–559.
Ribeiro MCde A, Augusto O, Ferreira AMda C (1997). Influence of quinoline-containing antimalarials in the catalase activity of ferriprotoporphyrin IX. J. Inorg. Biochem. 65:15–23.
Rieckmann KH, Trenholme GM, Williams RL, Carson PE, Frischer H, Desjardins RE (1974). Prophylactic activity of mefloquine hydrochloride (WR 142490) in drug resistant malaria. Bull. WHO 51:375-377.
Rojas-Rivero L, Gay F, Bustos MDG, Ciceron L, Pichet C, Denis M, Gentilini M (1992). Mefloquine halofantrine cross-resistance in *Plasmodium falciparum* induced by intermittent mefloquine pressure. Am. J. Trop. Med. Hyg. 47:372-377.
Slater AF, Cerami A (1992). Inhibition by chloroquine of a novel haem polymerase enzyme activity in malaria trophozoites. Nature 355(6356):167-169.
Slater AFG (1993). Chloroquine: Mechanism of drug action and resistance in *Plasmodium falciparum*. Pharmacol. Ther. 57:203–235.
Srivastava IK, Vaidya AB (1999). A mechanism for the synergistic antimalarial action of atovaquone and proguanil. Antimicrob. Agents Chemother. 43:1334-1339.
Su XZ, Kirkman LA, Fujioka H, Wellems TE (1997). Complex polymorphisms in an 330 kDa protein are linked to chloroquine resistant *P. falciparum* in South-East Asia and Africa. Cell 91:593-603.
Sugioka Y, Suzuki M (1991). The chemical basis for the ferriprotoporphyrin IX-chloroquine complex induced lipid peroxidation. Biochim. Biophys. Acta. 24; 1074(1): 19-24.
Ter Kuile FO, Nosten F, Thieren M, Luxemburgerer C, Edestein MD, Chongsuphajaisiddhi T, Phaipun L, Webster HK, White NJ (1992). High-dose mefloquine in the treatment of multidrug-resistant falciparum malaria. J. Infect. Dis. 166: 1393–1400.
Trenholme CM, Williams RL, Desjardins RE, Frischer H, Carson PE, Rieckmann KH, Canfield CJ (1975). Mefloquine (WR 142,490) in the treatment of human malaria. Science 190:792–794.
Verdier F, Le Bras J, Clavier F, HatinI, Blayo M (1985). Chloroquine uptake by *P. falciparum* infected human erythrocytes during *in vitro* cultures and its relationship to chloroquine resistance. Antimicrob. Agents Chemother. 27:561-564.
Draper CC, Brubaker G, Geser A, Kilimali VA, Wernsdorfer WH (1985).

Serial studies on the evolution of chloroquine resistance in an area of East Africa receiving intermittent malaria chemosuppression. Bull. World Health Organ. 63:109–118.
Walker DJ, Wakefield AE, Dohn MN, Miller RF, Baughman RP, Hossler PA, Bartlett MS, Smith JW, Kazanjian P, Meshnick SR (1998). Sequence polymorphisms in the Pneumocystis carinii cytochrome b gene and their association with atovaquone prophylaxis failure. J. Infect. Dis. 178:1767-1775.
Wang P, Read M, Sims PF, Hyde JE (1997b). Sulfadoxine resistance in the human malaria parasite *Plasmodium falciparum* is determined by mutations in dihydropteroate synthetase and an additional factor associated with folate utilization. Mol. Microbiol. 23:979-986.
Warhurst DC, Hall A, Tjokrosonto S (1995). RI-quinine-fansidar resistant falciparum malaria from Malawi. Lancet 2(8450):330.
Watt G, Loesuttivibool L, Shanks GD, Bordreau EF, Brown AE, Pavanand K, Webster HK, Wechgritaya S (1992). Quinine with tetracycline for the treatment of drug resistant falciparum malaria in Thailand. Am. J. Trop. Med. Hyg. 47:108–111.
Wellems TE, Panton LJ, Gluzman IY, Rosario VE, Gwadz RW, Walker-Jonah A, Krogstad DJ (1990). Chloroquine-resistance not linked to *mdr*-like genes in a *Plasmodium falciparum* cross. Nature 345:253-
Wellems TE, Plowe CV (2001). Chloroquine-resistant malaria. J. Infect. Dis. 184:770-776.
Wellems TE, Walker-Jonah A, Panton LJ (1991). Genetic mapping of the chloroquine-resistance locus on *Plasmodium falciparum* chromosome 7. Proc. Natl. Acad. Sci. USA. 88:3382-3386.
White NJ (1994). Mefloquine in the prophylaxis and treatment of falciparum malaria. Br. Med. J. 308:286–287.
White NJ (1997). Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. Antimicrob. Agents Chemother. 41:1413-1422.
Wilson CM, Serrano AE, Wasley A, Bogenschutz MP, Shankar AH, Wirth DF (1989). Amplification of a gene related to mammalian *mdr* genes in drug-resistant *Plasmodium falciparum*. Science 244:1184–1186.
Wilson CM, Volkman SK, Thaithong S, Martin RK, Kyle DE, Milhous WK, Wirth DF (1993). Amplification of *pfmdr1* associated with mefloquine and halofantrine resistance in *P. falciparum* from Thailand. Mol. Biochem. Parasitol. 57:151–160.
World Malaria Report (2011). WHO Press, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland pp. 1-137.
Wunsch S, Sanchez CP, Gekle CP, Wortmann LG, Wiesner J, Lanzer M (1998). Differential stimulation of the Na1/H1 exchanger determines chloroquine uptake in *Plasmodium falciparum*. J. Cell Biol. 140:335-345.
Yang YZ, Asawamahasakda W, Meshnick SR (1993). Alkylation of human albumin by the antimalarial artemisinin. Biochem. Pharmacol. 46: 336-339.
Yang YZ, Little B, Meshnick SR (1994). Alkylation of proteins by artemisinin. Effects of heme, pH, and drug structure. Biochem. Pharmacol. 48:569-573.