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Review article

Antimalarial chemotherapy, mechanisms of action and resistance to major antimalarial drugs in clinical use: A review

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ABSTRACT

Malaria has remained the leading cause of death in children under five years of age and pregnant women in sub-Saharan Africa and other endemic countries. The discoveries of antimalarial drug especially the quinolones has led to the hope that malaria might be completely eradicated from the world. However, lack of proper understanding of the mechanisms of antimalarial drug action and resistance to major antimalarials currently in clinical use has doused our hope for malaria eradication in a near future. Here, the major antimalarials in clinical use, their modes of action and resistance profiles were reviewed. While drugs such as chloroquine were banned for reasons associated with resistance and safety in some countries like Nigeria, a proper understanding of their modes of actions in the malarial parasite could pave ways for discoveries and development of novel antimalarials with similar properties and targets. Other drugs such as the antifolates are still in use as Intermittent Preventive Treatments in Pregnancies (IPTPs) and Infants (IPTIs) respectively. Resistance to these drugs is driven by mutations of the drug target (DHFR and DHPS). Although Artemisinin combination therapies (ACTs) are widely in use in many malaria endemic areas, resistance to these combination regimens defined as delayed parasite clearance were since reported. Four credible single nucleotide polymorphisms (SNPs); N86Y, N1042D, S1034C, and D1246Y were detected in the *Plasmodium falciparum* Multidrug Resistance Transporter gene-1 (*Pf*MDR-1 gene) and implicated for artemisinin resistance while K76T mutation in the transmembrane domain of malarial parasites is associated with resistance to quinolone antimalarials.

Introduction

Malaria is often the most common cause of fever in endemic areas [1]. For the time being, people living in the poorest countries of the world are the most vulnerable to the dangers of malaria [2]. An estimated 3.4 billion individuals, almost half of the world's population are at risk of getting infected with the disease [2]. Additionally, immuno compromised individuals such as HIV/AIDS patients and travelers to endemic areas of the world as well as pregnant women and children under five years of age are primarily the most susceptible to the dangers of infection [3]. In 2012, an estimated 207 million cases and roughly 627,000 deaths were recorded due to malaria [4]. Six years later, an increase (228 million) in malaria cases was recorded in 2018 [5]. However, there is a significant decrease (405,000) in malaria deaths when compared to the year 2012. Similarly, an estimated 219 million cases and 435,000 global malaria deaths were recorded from 91 countries and areas where malaria transmission is ongoing,

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with 70% of the estimated cases and deaths recorded from 10 sub-Saharan African countries and India. Of these deaths, 403, 000 deaths accounting for 93% was recorded in Africa in 2017 [5], Moreover, almost 3.1 billion United States Dollars (USD) was invested in malaria control and elimination programs in 2017 [5]. Although, there are indications that malaria mortality had reduced by 42% due to an increase in prevention and control measures globally, and by 49% in endemic African region since 2000, it still remained a disease of public health concern [2].

Antimalarial drug discovery and chemotherapy

A typical drug discovery process was estimated to last for 8 – 15 years [6]. These include target identification to introduction of new drug into the market. Until late 1930s, quinine remained the gold standard antimalarial drug [7]. Following the discovery and extraction of quinine from Chinchona tree, a synthetic compound of quinine (chloroquine), with similar structure and mode of action was developed and proved effective against malaria in 1940s [8]. The success of which led to the hope that malaria might be completely eradicated from the world. Since then, chloroquine and other synthetic quinoline antimalarials such as mefloquine and amodiaquine remained the mainstay of malaria chemotherapy for more than half a century [7,8].

Artemisinins a different class of antimalarials were first discovered and extracted from sweet wormwood (*Artemisia annua*) [9]. Artemisinins have the ability to block the transmission cycle by inhibiting the development of gametocytes, inhibiting haemozoin formation and a number of other functions [9-11]. This class of antimalarials were proved to have broad stage specificity, ranging from rings through to trophozoites and schizonts in the malarial parasites.

Another class of antimalarials, the antifolates are used as a second-line therapy for malaria since 1971 [12]. These class of antimalarials are divided into the inhibitors of dihydropteroite synthase (DHPS) otherwise termed class I antifolates and inhibitors of dihydrofolate reductase (DHFR) also called class II antifolates. Both classes interfere with folate metabolism and *de novo* folate synthesis [12]. Malaria parasites had been proved to be unable to utilize exogenous folates, hence the folate pathway a good target for antimalarial drug discovery [13].

Quinolone Antimalarials

Quinoline is a heterocyclic aromatic organic compound mainly used as a building block for other molecules. This backbone is the basic structure for many antimalarial drugs commercially available and in clinical use [8,14]. Despite problems like drug resistance and associated side effects, quinolones are widely used for the treatment of severe malaria and malaria prophylaxis, in artemisinin combination therapy regimens, and for the development of novel quinoline drug candidates [15].

4-aminoquinolines

Quinine

An overdose of quinine is associated with a pathological condition otherwise termed as cinchonism or quinism. The bark of Cinchona tree was used to relate this condition. Mild cinchonism is associated by sweaty skin, tinnitus, hazy sight, abnormal hearing, headache, abdominal pain, rashes, lichenoid photosensitivity, dizziness, nausea, vomiting and diarrhoea [16]. In relation to other quinoline antimalarials, the mode of action of quinine is not well understood. The mode of quinine action has been hypothesized to be well related to chloroquine action inside the digestive vacuole of malaria parasite to inhibit the formation of haemozoin [7,16].

Chloroquine

Chloroquine has been the most widely used and the gold standard for malaria chemotherapy for decades [17]. The drug is cheap and fairly tolerated when related to other quinolones such as quinine [18,20]. During World War II, the development, testing and widespread use of chloroquine grew special significance. Before the exact mechanism of chloroquine action was identified, many analogs of the parent compound were synthesized in an attempt to improve its efficacy [21]. Several theories concerning the mode of action of chloroquine were proposed. This includes; the DNA-binding theory, how it affect protein synthesis, how it inhibit the metabolism of polyamine, haemoglobin degradation and formation of a heme-chloroquine complex, Of these, how chloroquine inhibit the conversion of toxic heme to a non-toxic haemozoin is the most widely accepted mechanism [8,15,22]. Other side effects associated with chloroquine uptake include dizziness, nausea, itching, temporary hair loss and diarrhea [16].

- **Mechanism of chloroquine action**

Despite the wide use of chloroquine and the amount of time invested on research, the exact mechanism of action of this gold standard antimalarial is still unclear. Although, many theories aimed at describing the exact mechanism of action of this quinoline antimalarial have been put forward, the most widely accepted was the one describing its role as an erythrocytic schizonticidal antimalarial that targets the haemoglobin degradation pathway of the parasite. Chloroquine in particular, was reported to be active against the blood stage development of malaria parasite's only, and not stages like hypnozoites, pre-erythrocytic or mature gametocytes [7]

Being a diprotic weak base, chloroquine in its unprotonated form penetrates membranes of infected RBCs, accumulate the acidic digestive vacuole and become deprotonated [7,22]. Deprotonation of chloroquine in the digestive vacuole prevents it from permeating out through the vacuole's membrane, hence its concentration **(Figure 1)**. Concentration of this drug in the digestive vacuole was shown to dimerize with ferriprotoporhyrin IX, leading to inhibition of heme polymerization **(Figure 2)**, thus resulting in the accumulation of heme-chloroquine complex in the parasite's digestive vacuole [15]. Accumulation of heme-chloroquine complex results in lysis of parasite membrane and inhibits the function of vital digestive enzymes like vacuole protease thus, leading to parasite death [8]. It is also expected that parasite feeding is affected by the accumulation of free heme [8]. Studies also disclose that chloroquine inhibit haemoglobin transport vesicle trafficking, with a resultant accumulation of haemoglobin and vesicles [15].

Figure 1. Accumulation and deprotonation of chloroquine in chloroquine sensitive Plasmodium parasites [23]

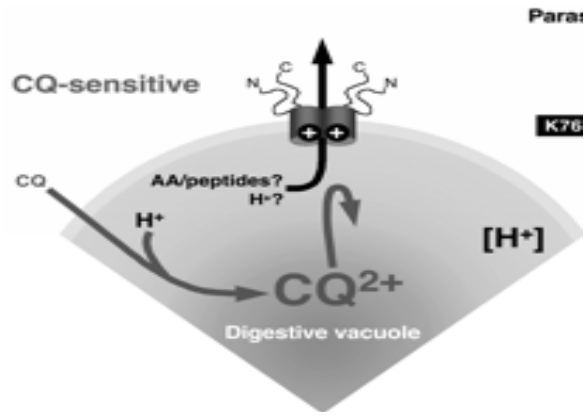
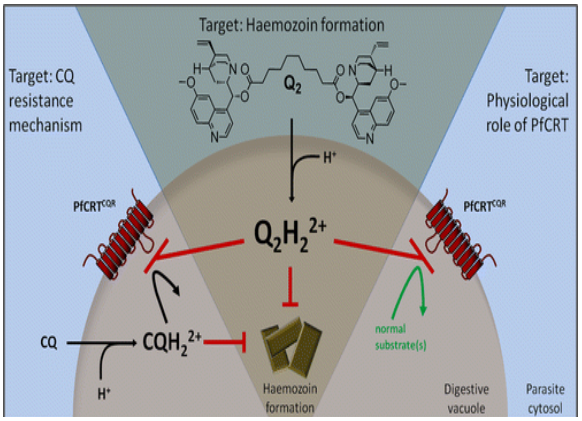


Figure 2. Effect of chloroquine on heme polymerization; the molecular mechanism of chloroquine action**.**

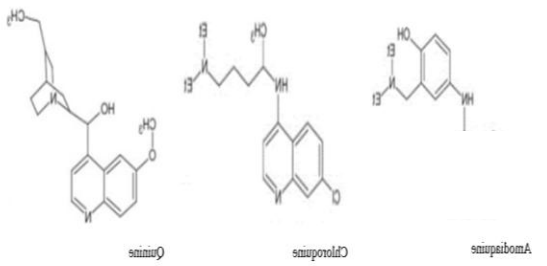


Source: http://phys.org/news/2014-01-strategy-emerges-drug-resistant-malaria.html).

Amodiaquine

Amodiaquine is structurally related to chloroquine **(Figure 3)**. At present, Amodiaquine is not in clinical use for malaria prophylaxis due to serious side effects of agranulocytosis and hepatitis reported [24, 25]. The toxicity of amodiaquine was first determined in experimental animal models and was explained by its 4-hydroxyanilino moiety. This was shown to undergo catalyzed oxidation to a reactive amodiaquine quinonimine, followed by the nucleophilic addition of glutathione [25]. The formation of amodiaquine quinonimine conjugate in experimental animals, and its ensuing binding to macromolecules of cells could affect cellular function either directly or by immunological responses that initiate hypersensitivity reactions and cause myelotoxicity [25].

Figure 3. Chemical structure of some 4-aminoquinolones



8-aminoquinolines

These are vital classes of antimalarial drugs with encouraging efficacy in the treatment of malaria and other emerging infectious diseases [26]. Primaquine, the only 8-aminoquinoline currently in use, is the most preferred drug of

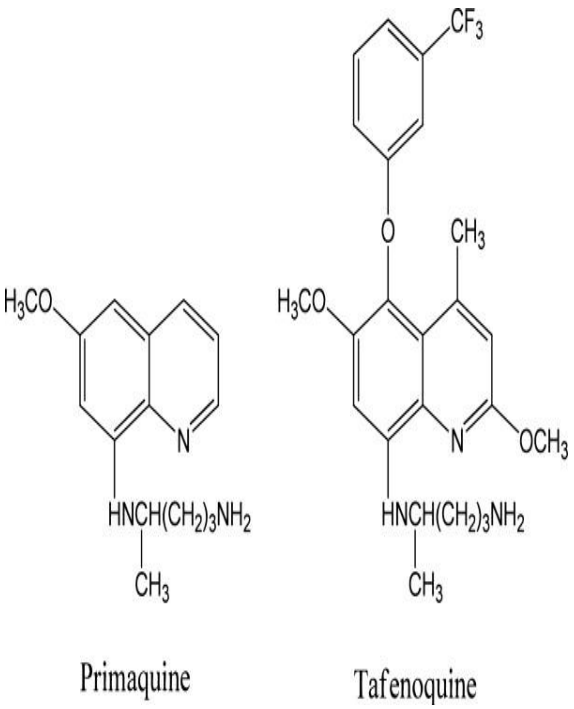
choice for the cure of hypnozoites due to *P. vivax*, *P. ovale* and early *P. falciparum* infections. Primaquine, is the only known drug capable of eliminating dormant liver stages of these parasites. The radical cure activity of primaquine was identified in the 1950s. Hundreds of *P. cynolmolgi*, a simian strain producing hypnozoites in infected monkeys were dosed with various aminoquinoline derivatives and the relapsing effects were determined [27].

Primaquine

To be active, primaquine, being a prodrug must first be metabolized. Due to uncertainties regarding primaquine's metabolic products that work against dormant liver stages, the exact mode of action of this drug is still unclear [28]. The drug was revealed to be widely used to cure relapsing forms of malaria due to hypnozoites produced by *P. vivax* and *P. ovale* [29].

To eliminate the early stage of infection with *P. falciparum*, primaquine was now considered for prophylaxis against malaria, when parasite develops in the liver, thus preventing the clinical malaria [29]. Another antimalrial, a long-acting analogue of primaquine targeting the liver stage of malaria parasites; Tafenoquine **(Figure 4)** was recently approved by the United States Food and Drug Administration (US FDA) [30].

Figure 4. The chemical structure of primaquine and tafenoquine



Folate pathway as a drug target in malaria parasites

In the most virulent of all malaria parasites, *P. falciparum* [27], folate is synthesized *de novo* from the condensation of pteridines (Guanosine triphosphate GTP), *para*-amino benzoic acid (*pABA*) and Glutamate [31]. However, due to the absence of key enzymes in the folate biosynthesis pathway, humans are unable to synthesize folate *de novo*, hence rely on dietary intakes of preformed folates [32,33].

Although natural folate had been proved to differ from synthetic folic acid due to the reduction of di or tetrahydro forms of pteridine rings at positions 5,6,7 and 8, presence of additional glutamate residue that results in the formation of derivatives of polyglutamate and the existence of additional one carbon unit that is bound to the N5 or N5 nitrogen atom: methyl (CH3), formyl (CHO), methylene (=CH2) and methenyl (=CH+) [34]. The fully oxidized form of this compound is now termed folic acid, and all naturally occurring folic acids are called "folates" [32].

Mechanism of folate biosynthesis in *P. falciparum*

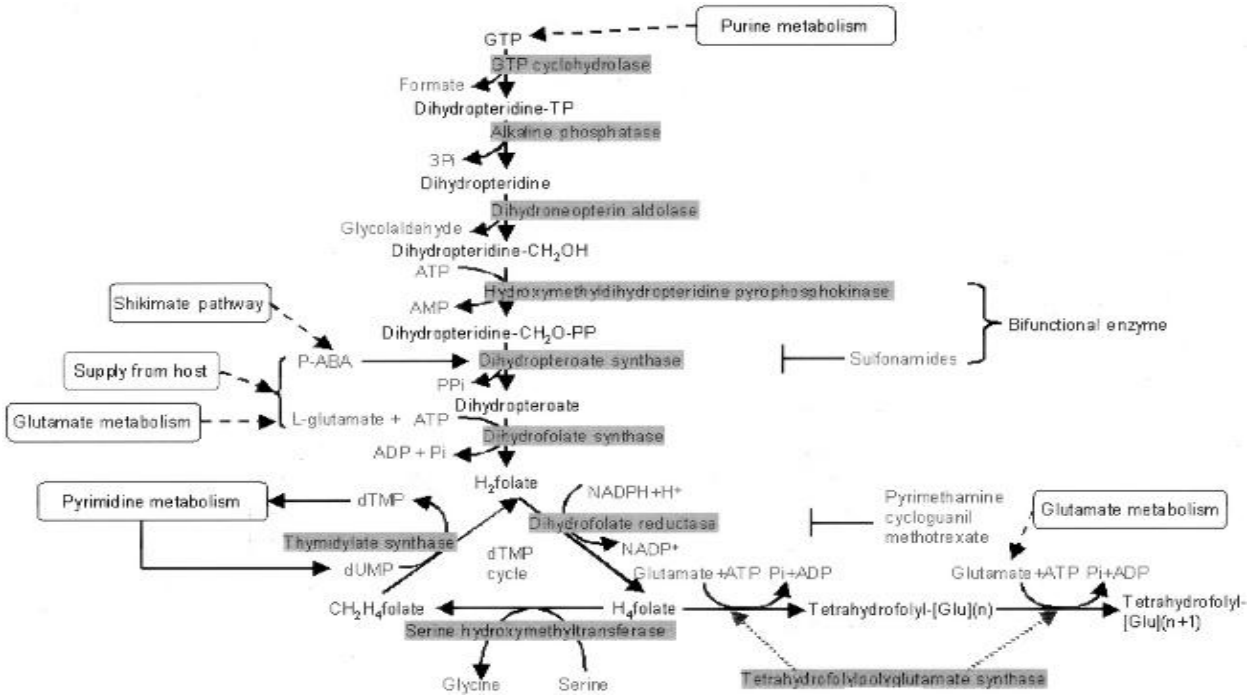
During erythrocytic and exo-erythrocytic schizogony, malaria parasites were revealed to extremely synthesize folate [35], a vitamin B that is soluble in water. Series of enzymatic activities in *P. falciparum* influence the synthesis of folate and its derivatives **(Figure 5)**. These are essential for the synthesis of nucleotides required for DNA replication, synthesis of the amino acids; glycine and methionine and the metabolism of histidine, glutamic acid and serine. Moreover, folate initiates protein synthesis in the parasite's mitochondria through formylation of methionine, making it crucial for parasite survival [13,32] Additionally, folate initiate the translation of mRNA [36].

The first enzyme in *de novo* folate synthesis pathway in most organisms including *P. falciparum* is Guanosine triphosphate cyclohydrolase 1 (GCH1) and is responsible for the ring expansion reaction, converting Guanosine triphosphate (GTP) to dihydroneopterin triphosphate, a pteridine biosynthetic precursor [32]. High performance liquid chromatography (HPLC) using radiolabelled substrate revealed that dihydroneopterin triphosphate was synthesised from GTP in *P. falciparum* [37]. In the Shikimate pathway, *pABA* is synthesised from chorismic acid in two steps. In

the first instance, aminodeoxychorismate (ADC) synthase transfers the amide nitrogen of glutamine to chorismic acid to form 4-amino-4-deoxychorismate. Subsequently, the enzyme ADC lyase removes pyruvate from ADC and aromatises the ring to generate *pABA* [38-40]. Similarly, 6-pyruvoyltetrahydropterin synthase (PTPS) is the next enzyme that drive the generation of 6-hydroxymethyl-7,8 dihydroneopterin (HMDHP) in the P. falciparum folate biosynthesis pathway. This is activated when two phosphate groups from hydroxymethyl dihydropteridine pyrophosphokinase (HPPK) are added [38]. The third enzyme, Dihydropteroate synthase (DHPS), incorporates *pABA* to form dihydropteroate [41] by combining pteridine with *para*-amino benzoic acid (*pABA*) to form dihydropteroate [35]. However, during the synthesis of thymidylate, Dihydrofolate (DHF) is produced, through the reduction of the methylene group to form methyl. This process is followed by concurrent oxidation of tetrahydrofolate (THF) to DHF. For THF to be regenerated, dihydrofolate reductase (DHFR) is involved [41,32]. Folate in the form of 5,10-methylene tetrahydrofolate (MTHF) provides the methyl group that converts deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). Thymidylate synthetase is responsible for the methylation of deoxyuridine [31,43]. Additionally, the thymidylate synthetase dihydrofolate reductase (TS-DHFR) catalyzes two essential reactions, dTMP synthesis and the conversion of DHF to methylenetetrahydrofolate (CH_2H_4folate) [42].

When the *de novo* folate-synthesis is affected, malaria parasites upregulate their folate salvage pathway to obtain folate necessary for growth and development. In the human host, folate is available in the plasma principally as 5-methyltetrahydrofolate (MeTHF) [31]. Folate uptake by *P. falciparum* is a specific, energy-dependent and saturable process that can be inhibited by classical anion transport inhibitors such as probenecid and furosemide. These discoveries have highlighted the potential of blocking folate salvage transporters as a therapeutic strategy demonstrating the importance of folate salvage and transport in antifolate drug susceptibility and parasite survival [33].

Figure 5. Folate biosynthesis in the malaria parasite.



Antifolate antimalarials

Antifolates are ordinarily termed the nucleic acid biosynthetic inhibitors, a class of antimalarial drugs acting on enzymes of folic acid cycle [44]. Antifolates used in the treatment of malaria are divided into two classes; Class I antifolates which function as inhibitors of DHPS and class II antifolates which serve as inhibitors of DHFR [12].

Class I antifolates (inhibitors of DHPS)

Inhibitors of DHPS are sulpha based structural analogues of *p*ABA. They include Sulphonamides (Sulfadoxine) and Sulfones (Dapsone) [32]. Sulfadoxine and other sulpha drugs function by inhibiting the activities of DHPS [45]. Several trials confirmed that a single dose of sulfadoxine was an effective, although slow, schizonticide [46].

Class II antifolates (inhibitors of DHFR)

Inhibitors of DHFR compete with dihydrofolate in the active site of the enzyme to interfere with the synthesis of tetrahydrofolate. Drugs inhibiting DHFR include; Pyrimethamine, Proguanil and cycloguanil [37,46,47].

Combintion regimens with inhibitors of DHFR and DHPS

These class of antimalarials are used in combination for the treatment of malaria as they have different targets with a long half-life (3 to 4 weeks) and an advantage of single-dose therapy [41]. Sulfadoxine potentiated pyrimethamine in human *P. falciparum* infections, demonstrating that combined pyrimethamine and sulfadoxine (Fansidar®) was more effective than either drug used as monotherapy [46]. Sulfadoxine is used synergistically with pyrimethamine to inhibit the synthesis of tetrahydrofolate in malaria parasites [48]. Inhibition of the folate pathway cuts down the amount of folate derivatives that act as one carbon carriers in nucleotide synthesis and amino acid metabolism [38].

Sesquiterpene lactones (Artemisinins)

Chemically, artemisinin is a sesquiterpene trioxane lactone containing an endoperoxide bridge that is essential for their antimalarial activity [49]. This endoperoxide group is believed to be critical for antimalarial activity, since deoxy artemisinin lacks the peroxide bond and was proved not to possess antimalarial activity [50]. The ancient chinese book titled "*Zhou Hou Bei Ji Fang*" revealed that a pressed juice of a handfull of the leaves of the Qinghao plant, *Artemisia annua* soaked in two litres of water treats malaria [18]. A systemic bioassay guided screening of the leaves of this plant was later found to have 60-80% efficacy against rodent malaria [19].

Active ingredients of *Artemisia annua* are thought to be thermo-instable and lipophilic, because boiling is not required for the local preparation of *Artemisia annua* [51]. For this simple reason, **Li and Zhou** [51] reported the production of white solids during the ethereal extraction, which was 100% effective against mice *Plasmodium.* These compounds were then isolated and later characterised to be artemisinin [19,51]. Youyou Tu shared the 2015 Nobel prize for Medicine or Physiology for her discovery of artemisinin from ethereal extraction which opens a new beginning in the treatment of malaria and saves millions of lives [19].

Mechanism of action of artemisinin antimalarials

Although, the antimalarial mechanism of action of artemisinins is uncertain, previous findings indicate the broad stage specificity of artemisinin antimalarials to include; the haemoglobin digestion in the parasite's food vacuole, the mitochondrion, the translationally controlled tumour protein and Ca^{2+} pump localized in the endoplasmic reticulum otherwise termed *Pf*ATP6. These are an important aspect in the survival of this lethal parasite and serve as potential targets for artemisinin antimalarials [9]. Moreover, artemisinin has the ability to block the transmission cycle by inhibiting gametocyte development [10]. Following reports of decreased clinical efficacy manifested as delayed clearance of blood parasites made it imperative for the urgent understanding of the mechanism of action of artemisinins [52,53]

Free heme mediated activation of artemisinin antimalarials

Artemisinin activity is highly dependent on haemoglobin digestion [53]. During intra-erythrocytic development of the malaria parasite, haemoglobin is ingested and degraded in the acidic digestive vacuole (pH estimated at 5.0-5.4) [54, 55]. This process results in the formation of heme/hemin/haematin, an essentially toxic protein in the form of ferriprotoporhyrin IX and denatured globin fragments [54]. Heme irons were shown to be responsible for the bioactivation of artemisinins. Following artemisinin's interaction with intra-parasitic heme, its endoperoxide group is activated by iron or free heme to form heme-artemisinin adducts which eventually generate toxic free radicals that damage cellular components [49, 56-59]. While **Li and Zhou** [51] proposed that the generated free radicals mediate the antimalarial action of artemisinin, **Haynes et al.** [60] reported that the binding of artemisinin to free heme is not necessary for its antimalarial activity. Reaction of artemisinins with iron (heme iron or non heme) received much attention for the reason that

artemisinin contains a peroxy group, in which H_2O_2 is catalysed by ferrous iron or cupric copper to produce the HO free radical [51].

Furthermore, heme-artemisinin adducts were revealed to interact with the histidine rich protein II of *Plasmodium falciparum* (*Pf*HRP II), a putative heme polymerase, to inhibit the formation of haemozoin via heme polymerization [59].

Artemisinin Combination Therapies (ACTs)

Artesunate monotherapy had since been reported to clear malaria parasitaemia in a short period of time. However, rapid resistance and the short half-life of artemisinin has necessitated the combination of artemisinins with other class of antimalarials. In order to overcome resistance and promote the effectiveness of antimalarial drugs, artemisinin combination therapies (ACTs) was developed and implemented globally to serve as the principal approach for the treatment of malaria in regions where the disease is endemic [2,61].

The most common antimalarials used as combination therapies with artemisinin derivatives are: Artemether plus Lumefantrine (A+L), Artesunate plus Amodiaquine (AS+AQ), Artesunate plus Mefloquine (AS+MQ), Artesunate plus sulfadoxine and pyrimethamine (AS+SP) and dihydroartemesinin plus Piperaquine (DHA+PQ). In many countries the use of AL or AS-AQ was implemented as the first-line treatment for uncomplicated malaria. To define the baseline characteristics of ACTs, an efficacy trial with good numbers of artemisinin-based combinations were carried out in many countries since the last two decades [62].

Safety and adverse side effects of some antimalarial medicines

Antimalarial drugs like all other medicines must be adjudged safe for use in humans, in additional to their efficacies, before acquiring approval from regulatory bodies (e.g. U.S. Food and Drug Administration (FDA) in the United States and National Agency for Food and Drug Adminstration and Control (NAFDAC) in Nigeria). Some antimalarials, especially those administered as prophylaxis to pregnant women during antenatal clinics must also be safe for both the mother and the fetus [63]. Due to some adverse side effects, studies have revealed that the malaria medicine, primaquine, although safe in other group of individuals, is not recommended for use by pregnant women, due to potential risk of hemolytic effects in the fetus. Similarly, an overdose of quinine by pregnant women may induce abortion, and an unsuccessful abortion is expected to result in deafness and hypoplasia of the optic nerve in the children [64]. So far, Artemisinin derivatives are classified as the most effective antimalarials in clinical use. In uncomplicated malaria, a 4 mg/kg/day of Artemisinin derivatives are served for 3 days. In severe malaria however, clinical studies have revealed that intravenous artesunate is the drug of choice in any trimester of pregnancy [65]. Due to their short half-lives, as well as the need to counterbalance the partial loss of efficacy due to drug resistance, Artemisinin derivatives are used in combination with other class of antimalarials [65]. These are safe and very well-tolerated in the general population without any deleterious side effect. However, the risk of miscarriage, still birth and congenital malformations associated with prolong use, similar to those presented by quinine therapy was reported [65]. In combination regimens, it is essential to have a clear understanding of safety, toxicity and tolerability of drugs among the population. A uniform standard in assessing the safety and tolerability of antimalarial drugs will be useful in the formulation and implementation of malaria treatment policies that are based on the drug effectiveness, safety and tolerability [66].

Antimalarial drug resistance

Antimalarial drug resistance is a "*shift to the right of the dose-response curve, thus requiring higher drug concentrations to achieve the same parasite clearance*" [67]. It was reported that *P. falciparum* has developed ample resistance to all classes of drugs used in malaria treatment, possibly with the exception of artemisinin combination therapies [67]. However, there is an emerging evidence of resistance to artemisinin derivatives [1,52].

Resistance to this classical antimalarial currently in clinical use (ACTs) is defined "as a delayed parasite clearance following treatment with an artesunate monotherapy, or after treatment with an artemisinin-based combination therapy (ACT)" [67].

The prerequisite for antimalarial drugs action is their abilities to their target and get distributed in the intracellular compartment. Similarly, their solubility, abilities to permeate cell membranes, and the likelihood to bind to transporter proteins that responsible to regulate

drugs trafficking through intra-cellular determines drug efficacy [68]. Normally, eukaryotic cells avoid the toxicity of xenobiotics through transferring them into the digestive vacuoles for further processing or remove them extracellularly. In *P. falciparum*, two types of transporters are responsible for mediating drugs into the digestive vacuoles; P-glycoprotein related transporters (which include *P. falciparum* multidrug resistance transporters (*Pf*MDR 1 and 2) and *P. falciparum* multidrug resistance associated protein (*Pf*MRP)) and drug metabolite transporter (DMT) system that represents the *P. falciparum* chloroquine resistance transporter (*Pf*CRT) [67-70]. Drugs are trafficked from the cytosol to the intravacuolar compartment by P-glycoprotein related transporters and in the opposite direction by drug metabolite transporter [68].

Point mutations in certain genes in these transporter systems as well as those of *P. falciparum* dihydrofolate reductase (*Pf*DHFR) and *P. falciparum* dihydropteroite synthase (*Pf*DHPS) have been implicated in antimalarial drug resistance [68-70]. However, *Pf*MDR-2 was believed to be involved in translocation of heavy metals and has nothing to do with multidrug resistance [68].

Four credible single nucleotide polymorphisms (SNPs); N86Y, N1042D, S1034C, and D1246Y were detected in *Pf*MDR-1 gene, in which asparagine at codons 86 and 1042, serine at codon 1034, and aspartic acid at codon 1246 of *Pf*MDR-1 protein had been replaced by tyrosine, aspartic acid, cystine, and tyrosine, respectively [68]. Each amino acid has certain physiochemical property, namely, side chain volume, side chain charge, and hydrolipophilicity index. Therefore, their substitution in the structure of any transporter changes the physiochemical properties of that transporter and consequently affects its potential to bind to and transfer different drugs [68,71]. The non-mutant form of *P. falciparum* (chloroquine sensitive) mediates transfer of drugs, such as, quinoline, mefloquine, halofantrine and lumefantrine from the cytosol into the vacuole. However, a resistant strain sways these drugs away from their target site of action in the cytosol in what is termed the "charged drug leak" **(Figure 6)** [25].

*Pf*CRT, a drug metabolite transporter system is made up of 424 amino acids arranged in 10 α-helical transmembrane domains (TDMs) **(Figure 7)** oriented inside the digestive vacuole membrane and N-termini which are exposed to the cytosol [14]. *Pf*CRT function in the efflux of alkaloids, amine compounds, divalent cations and amino acids, and peptides that result from the vacuolar digestion of globin [68].

Figure 6. Charged drug leak model of chloroquine resistance mechanism [23].

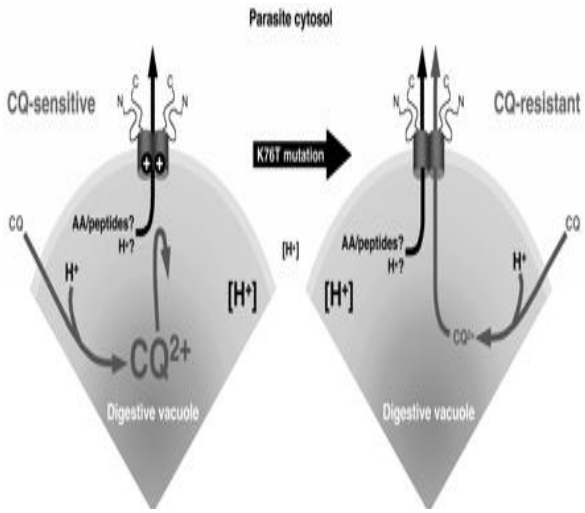
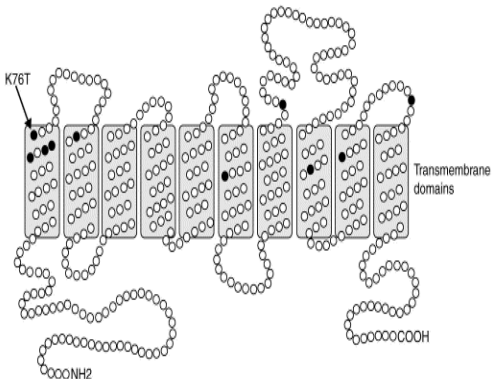


Figure 7. Transmembrane properties of PfCRT in the food vacuole.



So far, 32 plausible point mutations have been identified in *Pf*CRT gene, and their ubiquity alters the physiochemical properties of *Pf*CRT gene and the phenotype of certain strains of *P. falciparum*, thus conferring resistance to chloroquine [8]. Of these, single nucleotide polymorphisms in the K76T domain (substitution of lysine with threonine at codon 76 of *Pf*CRT gene) was implicated as a critical component of Chloroquine resistance and suggest that CQ access to ferriprotoporphyrin IX is determined by drug–protein interactions involving this mutant residue [14]. Sulfadoxine-pyrimethamine (SP) resistant parasites rapidly spread from Southeast Asia and South America in the 1970s–1980s and to Africa in

the last two decades [46]. Unlike Chloroquine where resistance was due to a mutated transporter protein, SP resistance is driven by mutations of the drug target (DHFR and DHPS) [46, 41].

Conclusion

This review has shown that understanding the safety, modes of action and adverse effects of antimalarial drugs is essential in developing novel antimalarials. Similarly, identifying the resistance profiles of malaria parasites to antimalarial drugs is imperative. In this review, the major antimalarial drugs in clinical use were compiled with a view to improve our understanding of their targets, safeties and efficacies as well as resistance profiles.

Conflicts of interest: None

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