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Review Article

Burden of Drug Resistance in Malaria: Important Role of Antimalarial Combination Therapy

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Abstract

All antimalarial drugs available for the treatment and prevention of malaria are limited primarily by resistance and cross-resistance. The concept of combination therapy is based on the presence of two or more drugs with different mechanisms of action which enhances treatment efficacy and aids in deterring the emergence and spread of drug resistance. WHO has endorsed Artemisinin based Combination Therapies (ACTs) as a "policy standard", and these drug combinations are first-line treatment for all malaria infections in areas where *P. falciparum* is the predominant infecting species. The frequent failures of 7 day artemisinin monotherapy has been overcome through the administration of 3 day oral ACT drug combination treatment where artemisinin compounds are co-administered with long-acting antimalarial drugs which facilitate the elimination of the residual malarial parasites not killed by the artemisinin component. ACTs can improve the efficacy of failing individual component drugs, lower the incidence of malaria, and provide some protection for individual component drugs against the development of higher levels of resistance. Artemisinins are the most important class of antimalarial agents in the world today. These drugs are widely used, particularly for treatment of multidrug-resistant *P. falciparum* malaria. The first-generation artemisinins have their limitations, which include poor oral bioavailability, short half-lives, and recent development of drug resistance in Southeast Asia characterized by delayed parasite clearance. Second- and third-generation artemisinins will likely be cheaper, less prone to drug resistance, and have better pharmacokinetic properties. ACTs are more effective for treatment of *falciparum* malaria than other antimalarials, they are prescribed for limited periods of treatment, which enhances drug compliance, and they have a well-demonstrated ability to reduce transmission by decreasing gametocyte carriage. In the absence of an effective malaria vaccine, new combinations are also needed to protect patient populations in years to come. In this review, we will define the ideal and minimally acceptable characteristics of clinical partner drugs and combination treatments needed in the future. Continued investment over the next decade in discovery and development of new drugs and drug combinations are essential to combat malaria and avoid emergence of antimalarial drug resistance.

Keywords: Antimalarial Drugs; Malaria; Resistance; Combination Therapy (CT); Artemisinin based Combination Therapies (ACT); Artemisinin

Introduction

Drug Combination Therapies (CTs) are standard treatments for patients infected with Human Immunodeficiency Virus (HIV), Tuberculosis (TB) and Malaria [1]. In the case of malaria, the use of CT was driven by clinical necessity as patients routinely failed treatment with monotherapies due to drug resistance. The existing antimalarial drugs available for prophylaxis and treatment of malaria are limited due to drug resistance and cross-resistance among related drugs. Currently, the use of CTs to treat malaria, in the form of Artemisinin-based Combination Therapy (ACT), has emerged as the "policy standard" by WHO recommended since 2005 for first-line treatment for all malaria infections in areas where *P. falciparum* is the dominant infecting species [1]. The majority of drugs on market, however, have an ongoing role as antimalarials and their lifetimes could be extended if they were deployed in a rational planned manner by administering them in combination based on their Pharmacodynamic (PD) and Pharmacokinetic (PK) properties.

WHO has defined combination therapy as the synergistic or additive potential of multiple drugs to enhance efficacy and deter emergence of drug resistant parasites to individual combination partner drugs. Drug synergism has a number of outcomes that are favorable which include enhanced efficacy, decreasing drug doses to maintain efficacy and avoid toxicity, deterring drug resistance and lastly providing selective target or efficacy synergism against the malaria parasite. ACTs have been demonstrated to enhance efficacy for treatment of malaria, and these combination drugs have been shown to contain drug resistance [2].

Parasite resistance to antimalarial drugs is a major threat to global public health. Artemisinin and artemisinin analogues have been shown to be quick acting, highly efficacious antimalarials. These

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compounds are very potent, extremely fast-acting antimalarials with a broad therapeutic index. Artemisinin therapies are the only currently available mainstream drugs to treat drug resistant *falciparum* malaria. A molecular marker of artemisinin resistance consisting of mutations in the propeller domain of a kelch protein, K13 was discovered in 2014 to be associated with delayed parasite clearance *in vitro* and *in vivo*. These mutations in the K-13 protein have proven useful to track emergence and spread of artemisinin resistance [2,3]. The marker has been confirmed across 15 locations in Southeast Asia [4], in Haiti [5], Senegal [6] and Sub-Saharan Africa [7]. In addition, other molecular mutations near the K13 propeller region have been found in Kenya [8], Assam and Arunachal Pradesh [9], and a number of other locations [10]. For traditional drugs (chloroquine, amodiaquine, mefloquine, sulfadoxine-pyrimethamine, and quinine), the degree of resistance varies from drug to drug and from region to region.

ACTs have been quite successful antimalarial drug combinations due to a variety of factors including: speed of action, resistance limited geographically to Southeast Asia, easy oral administration over a 3 day period which enhances patient drug compliance, and a proven ability to deter and, in some cases, reverse drug resistance to existing antimalarials such as mefloquine. ACTs have wide therapeutic indices, low drug toxicities, and remain efficacious despite significant PK profile mismatches. To enhance artemisinin drug efficacy and protect these compounds from emerging drug resistance, WHO has called for combination treatment with other partner drugs having different modes of action and extended half-lives [1,11]. In accordance with WHO's design, artemisinin analogues have been shown to rapidly decrease the parasite biomass, which leaves a small population of residual parasites alive to be killed by a long-acting partner drug. In addition, the likelihood of a simultaneous series of mutations against an artemisinin drug and a partner drug with a different parasite target is very small [12]. Therefore, for treatment of *falciparum* malaria, combining multiple drugs with different targets together is the best method to insure malaria infections are cured and resistance is deterred [11,12].

Existing Drug Resistance of Artemisinins and Other Antimalarial Drugs

Resistance of artemisinins

The artemisinin drugs are the only currently available mainstream drugs that do not have widespread problems with *P. falciparum* drug resistance. Initial reports of resistance to artemisinin on the border between Cambodia and Thailand have been published and follow-on studies have confirmed these findings [2, 3]. Other reports of artemisinin resistance have been noted in the Greater Mekong sub region formed by six countries to include Cambodia, Laos, Myanmar, Thailand, Vietnam, and China [11]. Research has characterized artemisinin drug resistance as a clinically observed delay in parasite clearance, and new developments in artemisinin drug resistance are as follows:

- Artemisinin resistance defined by delays in parasite clearance has been definitively demonstrated at Pailin, Cambodia and Mae Sot, Thailand [2].
- Studies to define the molecular mechanism and a genetic marker for artemisinin resistance are underway world-wide, and candidate marker genes that have been examined include single mutations and copy number mutations in *pfmdr1*, alteration in the sarco-endoplasmic reticulum calcium ATPase6 gene, *pfserca*, mutations in mitochondrial genes, and mutations in a de-ubiquitination enzyme, *pfubp1* [11].
- K13-propeller polymorphism has been confirmed as a molecular marker of artemisinin drug resistance [3]. The K13 propeller gene (PF3D7_1343700 or PF13_0238) mutations have been linked to both *in vitro* artemisinin resistance and *in vivo* slow parasite clearance rates, and these mutations have been observed in many areas of Southeast Asia [4-7].
- Polymorphisms in the K-13 gene have been observed in 15 locations in Southeast Asia [4], in Haiti [5], Senegal [6] and Sub-Saharan Africa [7].The presence of all of these markers in artemisinin resistant strains has been shown to correlate significantly with clinical manifestation of artemisinin resistance in Southeast Asia. There is no good correlation, however, for *in vitro* IC_{50} determinations of artemisinin antimalarial potency to delays in parasite clearance. The measurements of artemisinin drug levels in the blood or inside the erythrocyte may be a better, more effective assessment of drug resistance than drug concentrations measured in the plasma of treated patients.
- Many other molecular markers in and near the K13 propeller region have also been found to be associated with artemisinin resistance (Y493H, R539T, and I543T) [13].
- The rate of parasite clearance is a marker of infection *in vivo* that is a better assessment tool than the fraction of parasitemia positive cases by day 3. Parasite clearance is calculated by measuring the slope of parasite clearance on a log-linear curve, and this metric is not dependent on the initial parasitemia noted.
- There are ongoing efforts to monitor ACT efficacy in many countries around the world, and thus far, there are no confirmed reports of delays in parasite clearance in African patients treated with artemisinins.

Reduced parasite susceptibility to artemisinins, in turn, renders the ACT partner drugs more vulnerable to the development of resistance. This ominous development, along with the previous emergence of parasite resistance to all currently used partner drugs, suggests that current ACT regimens will begin to fail. Replacements for ACTs and combination partners are urgently required. Ideally, at least one component needs to be as fast acting as the artemisinin derivatives to provide rapid relief of symptoms. The rise in *P. falciparum* resistance to artemisinin drugs is a significant global health issue [3-7], and there is a limited opportunity to contain this problem before it spreads to other locations outside of Southeast Asia assuming this phenomenon spreads geographically as opposed to arising *de novo* in a new location. ACTs are recommended as the first choice for treatment of uncomplicated malaria and the implementation of ACTs worldwide

Table 1: Dates of drug introduction and first reports of antimalarial drug resistance.

Antimalarial Drug	Introduced	First Reported Resistance	Difference (Years)	References
Quinine	1632	1910	278	[15]
Artemisinins	1971	2006	35	[13,14]
Chloroquine	1945	1957	12	[15]
Mefloquine	1977	1982	5	[15]
Proguanil	1948	1949	1	[15]
Sulfadoxine-pyrimethamine	1967	1967	0	[15]
Atovaquone	1996	1996	0	[15]

*Data were from Wongsrichanalai et al., 2002 [15] and WHO 2010 [13,14].

has been an essential component for treatment of malaria given the rise of resistance to other drugs. There are no other alternatives at present to ACTs that are similarly efficacious and well tolerated.

Resistance of other antimalarial medications

For other antimalarial medications (amodiaquine, chloroquine, mefloquine, quinine and sulfadoxine-pyrimethamine), the level of resistance, spread of resistance, and distribution of resistance geographically has historically varied. Chloroquine resistance of *P. falciparum* parasites has been noted in virtually every country endemic for malaria except Central America and the island of Hispaniola. Sulfadoxine-pyrimethamine resistance is widely prevalent in Africa, South America, and in Southeast Asia. Mefloquine resistance in *P. falciparum* has been reported in Southeast Asia and in areas of Africa and South America where mefloquine has been used as a single agent. As previously mentioned, the time required for malaria drug resistance to appear upon introduction of a new antimalarial drug is quite variable from 278 years for quinine to less than 1 year for atovaquone (Table 1) [1-3, 13-15].

Malaria drug resistance occurs due to mutations in genes coding for the actual drug target or in genes such as transporters associated with access to the drug target. Drug resistance parasites are selected if drug concentrations are high enough to inhibit sensitive parasites but insufficient to inhibit the growth of resistant parasites. Drug selection is increased by exposure of parasites to sub-therapeutic doses of drugs and may be influenced by factors that include 1) long drug half-life, 2) mutations arising from single mutations, 3) poor patient compliance, 4) immune response, and 5) how widely a drug is prescribed in a particular region. In general, it is likely that parasite, vector, and human variables play relevant roles [16].

Antimalarial drug resistance leads to a burden on public health due to extended illness particularly associated with recrudescence of disease and progression of disease from uncomplicated to severe malaria. Low levels of resistance have been shown to result in anemia, increases in gametocyte transmission, extended illness, and a higher probability of treatment failure for recurrent infections. In low transmission areas, the impact of drug resistance is particularly acute due to the lack of infrastructure and resources for diagnosis and treatment. The influences of drug resistance on public health include [16,17]:

1. Prolonged or recurrent illness,

2. Increased number of outpatient cases,

3. Increased progression to severe malaria,

4. Increased cerebral neurological sequelae associated with malaria infection,

5. Prolonged or worsening anemia and chronic effects of anemia,

6. Increased hospital admissions and death,

7. Increased gametocyte carriage and increased spread of drug resistant parasites,

8. Increased demand on diagnosis, and

9. Higher cost and lower cost-effectiveness of combination treatment.

As drug resistant parasites become more common in a geographic area, the time between the primary infection and a recrudescent infection will shorten, and eventually, symptoms of disease will not disappear after therapy. At this point, the incidence of malaria may rise in a low transmission setting, and rates of mortality will increase.

Prevention or Delay of Resistance by Antimalarial Combination Therapy

Drug combinations can accelerate response to therapy, increase cure rates and insure component drugs are protected against resistance. Stable parasite resistance to artemisinin compounds has been confirmed in the laboratory [3], which may support the hypothesis that such resistance is rare. In order to preserve the artemisinin compounds from development of drug resistance; they should only be used as a drug combination with another antimalarial. Artemisinin derivatives demonstrate desirable key performance parameters as combination partners given their very high rates of parasite killing, their lack of significant drug resistance outside of the Cambodia border region and they are generally well-tolerated clinically [18].

Artemisinin drug resistance may be deterred by partnering artemisinin drugs with quickly cleared drug partners

Combining antimalarial drugs to minimize the potential for inducing parasite drug resistance has been shown to be effective in animal models to delay parasite resistance; however, delays in parasite resistance in human malaria by using combination therapy have not yet been demonstrated formally. A clear demonstration of formal proof is not easy to show experimentally. A growing body of circumstantial evidence exists, however, that supports the hypothesis that malaria drug resistance can be delayed. In theory, many factors might influence drug efficacy and emergence of drug resistance to antimalarial combinations (Table 2). Drug resistance can be deterred through rapid reduction in the parasite biomass provided by artemisinin compounds and drug partners in combination are shielded from development of drug resistance as multiple parasite mutations would be required for resistance to develop against two different drug targets. Development of novel antimalarial drug combinations with better matched PK profiles will provide

Table 2: Factors for and against the introduction of artemisinin-based combination therapy.

Factors for	Against
• The need to replace inadequate drug regimens that are leading to increased malaria-related morbidity and mortality. • Potential avoidance of the loss of available effective and affordable antimalarial drugs, especially in Africa. • Excellent efficacy (both clinical and parasitological clearance) of artemisinin derivatives despite extensive use with resistance reported only in discrete areas of Southeast Asia. • Potential reduction in transmission (especially of resistant mutants) due to the gametocytocidal effect of artemisinin derivatives.	• Highest cost • Problems of adherence to non-fixed combinations and their rational use, particularly in the home. • Lack of extensive clinical experience with most of the combinations currently under investigation. • Lack of evidence so far in Africa of its effectiveness in delaying the development of resistance. • Importance of not misusing artemisinin derivatives in view of their role in the treatment of severe malaria

complementary pressure on parasites to deter development of drug resistance [19].

Rapid drug clearance reduces the risk of drug resistance

Artemisinin analogues are very potent and rapidly decrease the parasite biomass, which leaves a small population of residual parasites alive to be killed by a partner drug. An efficacious partner drug typically has no problem eliminating the few parasites that remain. In addition, the likelihood of a simultaneous series of mutations against an artemisinin drug and a partner drug with a different parasite target occurring at the same time is very small [12]. Therefore, for *P. falciparum* treatment, combining multiple drugs with different targets together is the best method to insure malaria infections are cured and resistance is deterred [11,12]. Rapid drug clearance ensures residual drug does not act as a selective filter to insure survival of resistant parasites.

Artemisinin derivatives are particularly effective in combinations because of their high killing rates, minimal adverse effects, and absence of significant resistance outside of Southeast Asia. The data derived from human testing of artemisinin combination therapy regimens clearly shows that artemisinins have an amazing degree of activity as antimalarial drugs showing reductions in parasite number by a factor of approximately 10,000 fold per asexual cycle while other antimalarials are only capable of reducing parasite numbers by a factor of 100 to 1,000 fold per asexual cycle. Furthermore, artemisinins have activity against early and later stage parasites and thus they are capable of being used for treatment of uncomplicated and severe malaria [20].

The basic rationale for ACT deployment is easily understood, extremely simple and follows the following line of reasoning. If there are 10^{11} malaria parasites in a human infection and the mutation rate to provide resistance is 10^{-9}, there will be 100 parasites ($10^{11} \times 10^{-9} = 100$ parasites) carrying a newly arisen, spontaneous mutation encoding drug resistance. If that infection is drug-treated with monotherapy then some, many, or all of the 100 new resistant parasites will survive to recrudesce as a fully resistant infection. If drugs are combined as a two-drug combination, then simultaneous mutations in two genes are required to encode resistance (assuming the mechanism of resistance is independent for the two drugs) and thus the chance of being spontaneously resistant to both drugs is $10^{11} \times 10^{-9} \times 10^{-9} = 10^{-7}$, which is one infection in ten million. As a result, the use of combinations delays the input of mutations encoding resistance to either drug. The argument is robust to changes in the assumption about the number and mutation rate of parasites [21].

Slower parasite clearance has also been associated with increased gametocyte carriage thereby increasing the risk of transmission of drug-resistant parasites. Given that it would be highly desirable for candidate drugs to have activity against both hepatic and sexual forms of the malaria parasite, it is surprising that few clinical trials, to date, have examined whether gametocyte carriage can be reduced following drug treatment. The only drugs found to be effective at reducing gametocyte carriage include artemisinin, ACTs, methylene blue, and primaquine.

Combination drugs with different mechanisms of action can delay drug resistance

Drug combinations can accelerate response to therapy, increase cure rates and insure component drugs are protected against resistance. The theory underlying the combination of two drugs for therapy relies on the fact that treatment with two drugs with different mechanisms of action and therefore different mechanisms of resistance have a probability of developing resistance to both drugs based on the product of their individual per probabilities of parasite drug resistance. If the probability of a *de novo* mutation to drugs A and B is one in a million then the probability of developing resistance to both is one in one trillion [17].

The rationale for ACT or combination therapy in general is twofold: 1) The combination of drugs is frequently more effective than monotherapy with one drug. 2) Given the low probability that a parasite develops a *de novo* mutation to one of the combination drugs during the infection, the now drug resistant parasite can still be killed by a partner antimalarial drug. Accordingly, drug combinations provide mutual protection that is believed to delay or prevent emergence of drug resistance. In order to obtain these two advantages of a combination therapy, the partner drugs in a combination must be independently efficacious when used to treat malaria.

Shorter dose regimens reduce induction of drug resistance

Due to the short half-lives of artemisinin analogues, monotherapy treatment must be conducted for 7 days with treatment every day. By combining an artemisinin analogue with a partner antimalarial drug with a long half-life, the duration of malaria treatment can be dropped from 7 days for artemisinin monotherapy to a 3-day combination regimen that provides increased efficacy and reduces the possibility of induction of drug resistance. The traditional antimalarial drugs, the quinolines (chloroquine, quinine, piperaquine, mefloquine, and primaquine) and the antifolates (sulfadoxine, pyrimethamine), have been utilized as long-acting ACT partner drugs (Table 3). When

Table 3: Drug classes of current antimalarial drugs according to chemical structure [19].

	Generic drug classes	Drug example
1	Arylaminoalcohols:	Quinine, quinidine (cinchona alkaloids), mefloquine, halofantrine, lumefantrine,
2	4-aminoquinolines	Chloroquine, amodiaquine (bisquinoline piperaquine)
3	8-aminoquinolines	Primaquine, tafenoquine (WR238605), 4-methyl primaquine diphosphate. (WR181023)
4	Folate synthesis inhibitors (Antifolates)	Type 1 - competitive inhibitors of dihydropteroate synthase – sulphones (dapsone), sulphonamides (sulphadoxine) Type 2 - inhibit dihydrofolate reductase - biguanides like proguanil and chlorproguanil; diaminopyrimidine like pyrimethamine; cycloguanil, trimethoprim
5	Peroxides (Sesquiterpene lactones)	Artemisinin (Qinghaosu) derivatives - dihydroartemisinin artemether, arteether, artesunate, artelinic acid
6	Antimicrobials:	Tetracycline, doxycycline, clindamycin, azithromycin, fluoroquinolones, chloramphenicol
7	Naphthoquinones (Respiratory chain inhibitors)	Atovaquone
8	Iron chelating agents	Desferrioxamine
9	Drug combinations	• Sulphadoxine-pyrimethamine ('Fansidar') • pyrimethamine + sulphadoxine + mefloquine ('Fansimef') • atovaquone + proguanil ('Malarone')

these slowly eliminated antimalarials are given in combination with artemisinin drugs, the duration of artemisinin treatment can be reduced to 3 days.

With this shorter 3-day treatment period, complete killing of all parasites requires the antimalarial activity of the partner drug persisting at parasiticidal concentrations. Thus, the partner compounds must be eliminated slowly. A slowly eliminated partner drug also protects the artemisinin component from development of drug resistance, while the partner drug is also partly protected by the artemisinin drug during the first 3 days of treatment. Mutual protection from drug resistance aids efforts to control malaria and this is very helpful in areas that have low-to-moderate endemic infection rates. To kill at least 90% of the infecting parasites parasitemia, a 3-day course of an artemisinin is necessary to provide coverage through three post-treatment asexual parasite growth cycles. This ensures that only approximately 10% of the infecting parasites are still present to be killed by the partner drug, which in turn reduces the probability of development of drug resistance.

Gametocidal effect of artemisinins reduces drug resistance

One benefit from the artemisinin derivative component of ACT is its strong activity against gametocytes, which is the life stage of the parasite that is transmitted from humans to mosquitoes [4-6]. Combinations with artemisinin decrease gametocyte carriage and thus gametocyte transmission. In an area with multi-drug resistant parasites, ACTs have been shown to reduce gametocyte carriage nine-fold. As recrudescent infections are more likely to carry gametocytes, the selective transmission advantage of drug resistant parasites is reduced as cure rates are increased and the infections that do recrudesce are prevented from developing gametocytes after combination treatment has been initiated. Reduced transmission of malaria occurs if the treated patients are the main reservoir of gametocytes as they may be in low-transmission areas where effective antimalarial drugs are available. Reduced transmission results in reduced incidence of malaria which translates into reduced drug use. Thus, ACT use provides a deterrent to slow the rate of evolution of drug resistance [22].

All effective antimalarials prevent the development of gametocytes in *P. vivax, P. malariae* and *P. ovale* infections and the early-stage gametocytes (stages 1 to 3) of *P. falciparum*. The artemisinin derivatives inhibit development of more mature *P. falciparum* gametocytes. Gametocytemia is higherin recrudescent infections than in primary infections. In low transmission settings, this gametocytocidal effect and the high cure rates obtained with ACTs both contribute to reduce transmission and incidence of malaria.

Drug resistance is deterred by PK profile matching of combination drug partners

Antimalarial combinations should consist of drugs with similar PK profiles to provide complementary pressure on existing and new infecting parasites. From this perspective, drugs with shorter half-lives in general would be preferred to decrease the exposure of new parasites to sub-therapeutic drug levels, which may provide a selective filter to enhance survival of drug tolerant and drug resistant parasites. Once the artemisinin drug in an ACT has been eliminated, the partner drug is left unprotected and selective pressures on that partner drug, which in essence is acting as antimalarial monotherapy, will lead to emergence of resistance.

The implications of this "PK profile mismatch", particularly in areas of high transmission in Africa, requires more investigation and trade-offs between prevention of resistance and protection of patients from recrudescence and development of new infections may be considered. This does bring into question the need to preserve ACT drugs for long term use versus the need to provide a period of post-treatment prophylaxis. The paradox with some ACTs such as DP is the piperaquine component provides a long period of post-treatment prophylaxis but the extended half-life of piperaquine make this partner drug quite vulnerable to development of drug resistance, which will shorten the usable life of this drug combination. The safest approach from a perspective of preventing development of drug resistance is to use a drug partner that has a residual half-life as short as possible, while still enabling parasite clearance with a 3-day treatment. This ideal PK/PD matched combination may be difficult to develop given the limited range of antimalarial drugs available. When combinations are used, mismatched PK profiles can play a role in facilitating the development of resistance. Mismatched PK profiles allow parasites to evolve resistance sequentially as the longer half-life partner persists as a vulnerable monotherapeutic agent. The results

of mismatched PK can almost completely undermine the benefits of combination therapy.

Long Acting Partners Protect Artemisinins from Drug Resistance

Long-acting ACT partners deter development of drug resistance to artemisinin

Treatment of uncomplicated malaria can be achieved through a 3-day regimen of artemisinins combined with long-term acting drugs such as one or more of the quinoline or antifolate drugs. ACTs has been shown to be efficacious, the 3 day regimen enhances patient compliance, and the use of these drug combinations leads to decreased spread of parasite resistance [1]. The majority of artemisinin combination therapies in use today rely on a slowly eliminated partner drug such as piperaquine. The artemisinin analogue is quickly eliminated and is fully protected by the long acting partner drug during the period of drug therapy leading to no selective pressure on the artemisinins for existing and new infections. Artemisinin drugs are thus protected by those long-acting partners after the artemisinin analogue has been cleared, therefore, the risk of emergence of resistance to artemisinins has been reduced and the usable lifetime of the artemisinins can be extended for a longer period of time. This practice does place the long acting partner drug at risk which underscores the need for ongoing development of new partner drugs for ACT drug combinations. All of the current ACT partner drugs except lumefantrine were developed in the 1970s and 1980s. Even newly proposed partner drugs like pyronaridine were developed decades ago. Lumefantrine, the youngest partner drug, was developed in the mid-90s. No new partner drugs have come into use since then. Without the development of new partner drugs, it is likely existing ACT drug combinations will become useless with time. The combination of artesunate and sulfadoxine-pyrimethamine, for example, is no longer used in many areas due to the growing incidence of SP resistant parasites in many parts of the world.

As of February 2015, artemisinin resistance has been confirmed in five countries of the Greater Mekong Sub-region (GMS): Cambodia, The Lao People's Democratic Republic, Myanmar, Thailand, and Viet Nam. In majority of sites, patients with artemisinin-resistant parasites still recover after treatment, if they are treated with an ACT containing an effective partner drug. However, along the Cambodia-Thailand border, *P. falciparum* has become resistant to almost all available antimalarial drugs. There is a real risk that multidrug resistance will soon emerge in other parts of the Mekong sub-region as well.

The emergence of artemisinin resistance has raised new challenges for patient care, as resolution of disease will likely take a longer period of time. Prolonged parasite clearance may affect treatment outcomes for severe and complicated malaria, which is currently treated with injected artesunate. Oral artemisinin monotherapy and ACT treatment has been associated with increases in parasite clearance time which is also known to be associated with increases in gametocyte carriage. The implications for transmission of gametocytes are not yet clearly understood in areas with artemisinin resistant parasites. Data on transmission of artemisinin resistant parasites and data on how infective the resulting sporozoites may be are not yet available. The loss of artemisinin efficacy raises concerns as this phenomenon may lead to failure of the artemisinin component to fulfill its role in reduction of the parasite biomass and providing some protection to its partner drug.

Development of second and third generation artemisinin-derived drugs less vulnerable to drug resistance

With data showing early-stage resistance to artemisinin drugs, researchers have focused on designing more potent analogues of artemisinin less vulnerable to drug resistance with increased metabolic stability. Research has shown that the lactone carbonyl group in artemisinin can be removed completely without any detrimental effect on drug activity. In the latter half of the 1990s, a series of such C-10 carba-analogues were synthesized [23], based on deoxoartemisinin [24], which is eight-fold more active than artemisinin *in vitro*. The effect of including substituents at other positions in the artemisinin scaffold has also been extensively investigated. One analogue, incorporating a methyl group at the 3-position, proved particularly effective, perhaps because this substituent enhanced the stability of a (putative) primary radical which was generated by endoperoxide cleavage. Conversely, chemists have found that introducing substituents on the α-face of the artemisinin molecule should be avoided. This portion of the artemisinin molecule is believed to support the 'triggering' mechanism of the drug. The presence of an α-substituent would disrupt the tight binding between artemisinin and heme, which is proposed to be crucial to artemisinin activation. The most extensive manipulations of the artemisinin 'lead compound' have revealed that even when the B and D rings were removed completely, the resultant molecule retained antimalarial activity.

Major pharmaceutical companies are, for the first time in recent history, beginning to take an interest in developing a new generation of antimalarial drugs derived from artemisinin. The German pharmaceutical company Bayer has synthesized and screened several semi-synthetic nitrogen-containing derivatives, in collaboration with Richard Haynes of the Hong Kong University of Science and Technology. One of these compounds, artemisone, is currently undergoing phase II clinical trials [25]. Jonathan Vennerstrom of the University of Nebraska, with support from the Medicines for Malaria Venture (MMV) and Hoffman-La Roche, has been designing a new generation of synthetic drugs, which are inspired by artemisinin, but not derived from it. These drugs, for example OZ277 [24], retain the 1,2,4-trioxane ring found in the lead compound. The first clinical trials of OZ277 and combination with piperaquine took place in Thailand earlier this year [26, 27].

Potential risk of induction of drug resistance for long-acting partners in an ACT

As artemisinin derivatives are eliminated rapidly from the body, drug combinations including artemisinin derivatives given for 3 days must be co-administered with a slowly eliminated partner drug. These drugs are commonly older antimalarial drugs, which provide protection for the artemisinin component against *de novo* artemisinin resistance mutations as long as drug adherence is maintained. As recently discussed by Hastings and Watkins [28], PK mismatched drug combinations do not significantly affect the spread of resistance, and PK/PD mismatched ACTs have a known risk of jeopardizing the efficacy of partner drugs where resistance has not yet been shown such as piperaquine [29] or mefloquine [30].

Although the addition of artemisinin derivatives can improve the efficacy of certain conventional antimalarial agents in areas where parasites have developed high-level resistance to these drugs, reintroduction of these conventional drugs in ACTs is questionable or controversial [31]. In Thailand and Cambodia, high-level resistance to mefloquine is quite prevalent [32], but artesunate–mefloquine is widely deployed in these areas. In China, extensive use of piperaquine has resulted in parasites that are more 'resistant' to the drug [33], but DHA–piperaquine is still very effective in treating malaria parasites. Because the resistance to amodiaquine and chloroquine are highly correlated and the efficacy of Fansidar® (SP) in treating *falciparum* malaria is waning in many African countries, ACTs with these partner drugs are still being tested [34,35]. Even though deployment of such ACTs with a failing partner drug may seem to reverse the resistance to the partner drug, as in the case of artesunate–mefloquine in Thailand [36], the effectiveness of ACTs might be compromised with the use of an inappropriate partner drug [37].

There are a number of factors that affect the choices of antimalarial drug combination partners to inhibit parasite resistance; two or more drugs with antimalarial efficacy, different parasite targets and mechanisms of action, at least additive and hopefully synergistic properties in combination, and a good pharmacokinetic match. In an ideal world, antimalarial drugs possessing short half-lives would be best to decrease the probability of exposing a re-invading parasite to sub-therapeutic concentrations of drug that may induce selection for resistance.

Gametocytocidal effect of artemisinins to prevent resistance

In vitro, artemisinin has been found to kill not only asexual blood stages but also the early stages of gametocytes of *P. falciparum*. The effect of artemisinin depends on the concentration of the drug as well as the initial parasitemia level. The best gametocidal outcome is found where the *in vitro* IC_{50} ranges from 10-20nM and the initial parasitemia level are no higher than 1%. Resistance of *P. falciparum* to other antimalarial drugs, e.g., chloroquine and pyrimethamine, has not been shown to affect susceptibility of its asexual and sexual stages to artemisinin [38]. In addition, Adjuik et al. undertook a meta-analysis of individual data of patients from 16 randomized trials (n=5,948 participants) that studied the effects with artesunate combinations used to treat *P. falciparum* malaria infections. Parasite clearance was observed to be significantly faster with artesunate. In participants with no gametocytes at baseline, artesunate reduced the gametocyte count on day 7, with larger effects at days 14 and 28. Adding artesunate for 1 day (shown in six trials) was associated with fewer failures by day 14 and day 28. In these trials, gametocytes were reduced by day 7. The 3 days of artesunate combination treatment substantially reduced treatment failure, recrudescence, and gametocyte carriage [39].The treatment of patients with artemisinin drugs has been shown to reduce gametocyte production by 8 to 18-fold [40].

This, in turn, reduces the probability that gametocytes carrying drug resistance genes will be transmitted, and, theoretically, artemisinin drug use may diminish malaria transmission rates. Fast parasite clearance observed with first-generation artemisinin drugs was also associated with decreased gametocyte carriage thereby reducing the risk of transmission of drug-resistant parasites.

Conclusion

New drug combination therapy is derived from the premise that administering two or more drugs at the same time that have synergistic or additive effects with independent mechanisms of action and different targets will lead to enhanced efficacy and diminished drug resistance. There is an unprecedented interest in developing new antimalarial compounds to treat malaria. New funding, tools, and partners from all sectors, combined with new leadership have emerged. We must take advantage of this momentum as eradication of malaria will inevitably take many decades. Given the long lead times involved in product development, solid investments in R&D now are critical to ensuring that the world has the right healthcare technologies for eradication (including medicines, vaccines, and vector control tools). Operational and market research must continue so that an understanding of current dynamics in the antimalarial market can inform the effective implementation of future tools. Antimalarials play a critical role in ending suffering and saving lives - they are the tip of the spear in the pursuit of eradicating this ancient scourge. There are a number of non-governmental organizations such as Medicines for Malaria Venture and the Bill and Melinda Gates Foundation and governmental consortiums such as the Global Fund that are committed to playing a leading role developing the next generation of medicines and ensuring that these innovations make a significant public health impact. In the absence of an effective malaria vaccine, the development of new antimalarial drugs - most likely derived from, or inspired by, artemisinin - will continue to be the foundation of drug combinations in the fight against malaria.

Conflict of Interest

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author and are not to be construed as official, or as reflecting the views of the Department of the Army or the Department of Defense.

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