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COMBINATION THERAPY AND ITS IMPLICATION ON CLINICAL EFFICACY OF ARTEMISININS- REVIEW

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ABSTRACT

Artemisinin and its derivatives have successfully been used in treatment of falciparum malaria infections in various parts of the world. More importantly, they have proved effective against strains resistant to conventional antimalarials such as chloroquine and mefloquine in those parts of the world where malaria is endemic. Only one clinically relevant artemisinin-resistant human malaria has been reported recently in South East Asia, although there are reports published on development of the rodent malaria parasite strains resistant to the drug earlier. This article reviews the implications of combination therapy on the pharmacokinetics and hence clinical efficacy of Artemisinins using relevant and published papers. It gives detailed account on the general chemistry and mechanism of action of the parent compound Artemisinin before considering its pharmacokinetics. Artemisinin-based combination treatments (ACTs) are now generally accepted as the best treatment options for uncomplicated falciparum malaria. They are rapidly and reliably effective. The article would focus on combination therapy & its implication on the pharmacokinetics & clinical efficacy of artemisinin & its derivatives and also presents the scientific rationale for the need of combining Artemisinins to enhance their clinical efficacy and also minimize the likelihood of the emergence of resistant strains of the malarial parasites.

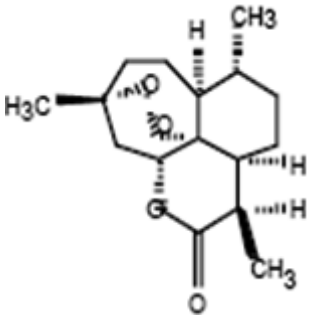
INTRODUCTION: The Chinese medicinal plant qinghao (Artemisia annua L.) has been used in traditional Chinese medicine for more than 2000 years [1]. The earliest reference to the plant goes back to "52 Prescriptions", found in the Mawangudi Tomb in an era dating back to 206 BC-AD 23. The first description of qinghao for treatment of malaria-related symptoms is found in "The Handbook of Prescriptions for Emergencies" by Ge Hong, who lived during AD 281-340 [2]. Isolation of the active moiety qinghaosu, however, took place at a considerably later time. Qinghaosu, meaning the extract of qinghao, was isolated in early 1970s from the leaves and flowering tops of the plant by Chinese scientists in their search for new antimalarial compounds. The compound showed good in vitro antimalarial activity and subsequent studies in animal models proved encouraging. By the end of the 1970s, several clinical studies conducted in China found qinghaosu to be an exceptional antimalarial agent with negligible toxicity and high efficacy against human malaria parasites, including those resistant to conventional malaria treatment [2, 3]. Since then, qinghaosu, now known as artemisinin in other parts of the world, has been used in treatment of predominantly falciparum malaria cases around the world [2, 4].

Artemisinin and its derivatives have successfully been used in treatment of falciparum malaria infections in various parts of the world [1, 2, 5, 6, 12-22]. More importantly, they have proved effective against strains resistant to conventional antimalarials such as chloroquine and mefloquine [5, 6]. No clinically relevant artemisinin-resistant human malaria has yet been reported, although there are some rumors recently about the emergence of resistant strains of the parasite in Cambodia, the world's epicenter for the emergence of drug resistant strains of microbials. Earlier while artemisinins were actively studied to be marketed, reports were published on development of the rodent malaria parasite strains resistant to the drug [2].

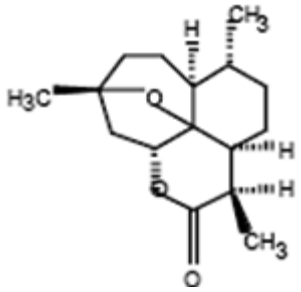
Artemisinin-based combination treatments (ACTs) are now generally accepted as the best treatments for uncomplicated falciparum malaria. They are rapidly and reliably effective. Efficacy is determined by the drug partnering the artemisinin derivative and, for artesunate- mefloquine, artemether- lumefantrine, and dihydroartemisinin- piperaquine, this usually exceeds 95% [7].

This review focuses on Combination therapy and its implication on clinical efficacy of artemisinin and the derivatives; but priori, it tries to present their chemistry and pharmacology, as well as their pharmacokinetics in the human body.

Artemisinin-Chemistry and Pharmacology: Structurally, artemisinin is quite different from all previously known antimalarials. The compound is an unusually stable sesquiterpene lactone bearing a peroxy group (**Fig. 1**). The presence of the peroxide bridge is essential for artemisinin's antimalarial activity as a reduced form of the compound, deoxyartemisinin, lacks the antimalarial activity [2].



A



B

FIG. 1: CHEMICAL STRUCTURES OF ARTEMISININ (A) AND DEOXYARTEMISININ (B)

The white needle crystals of artemisinin are hardly soluble in water or oil and therefore formulations other than oral and rectal are not in clinical use [1, 2, 8]. However, since the peroxide bridge of the compound is stable under certain chemical reactions, several oil- and water-soluble derivatives of artemisinin have been synthesized. These include dihydroartemisinin, artemether, and artesunate, originally developed by the Chinese scientists, and arteether and artelinic acid [8].

Artemisinin is hydrophobic and passes biological membranes easily [2, 9, 10]. *In vitro* studies have suggested an uptake of artemisinin by both healthy and malaria infected red blood cells. It is known that artemisinin binds to hem, either in hemoglobin (inside red blood cells) or hemozoin (stored heme within the malaria parasites) [2, 8]. Once inside the cells, through an iron-mediated cleavage of the peroxide bridge, artemisinin free radicals are formed.

These free radicals are destructive to different parasite membranes, including mitochondria, rough endoplasmic reticulum, and plasma membranes, thereby killing them [2, 3]. It is believed that the hemin-rich internal environment of the parasites is one of the reasons for the selective toxicity of artemisinin toward the malaria parasites as hemin has been shown to interact with the compound [2].

Pharmacokinetics of Artemisinin: Artemisinin is primarily eliminated by enzymatic metabolism to presumably inactive metabolites, lacking the peroxide bridge [2, 6, 11]. Only trace amounts of the compound are detectable in urine in both healthy volunteers and malaria patients after oral administrations.

The ether and ester derivatives are metabolized to dihydroartemisinin, which accounts for most of the clinical effects of these derivatives after intake [6]. Despite a presumed high absorption, the oral preparations are believed to have a low bioavailability due to a significant first-pass extraction. Since intravenous administration of artemisinin is not possible, no information on its absolute bioavailability is available.

However, an oral formulation of artemisinin showed a 32% relative availability compared to an intramuscular suspension in oils [11]. The same extent of relative availability was found for suppositories compared to capsules [8]. Most pharmacokinetic parameters reported for artemisinin by various authors are consistently inconsistent in the pool of the literature [2, 12].

A study which is believed by the reviewers of this article to be more comprehensive reported an absorption lag-time of 0.5-2 hrs after oral intake, with peak plasma concentrations at 1-3 hours post-administration. It has a relatively short half-life of 1-3 hours [2]. Cytochrome P-450 enzyme 2B6 with some possible contribution of CYP3A4 and CYP2A6 have been suggested to metabolize the compound.

Rectal administration of artemisinin resulted in lower plasma concentrations of the drug compared to oral doses, although no significant difference was found in the elimination half-lives between the two administration routes [8]. Fraction bound artemisinin to plasma proteins averages around 80-85%. Artemisinin exhibits time- and dose-dependent kinetics in both healthy volunteers and malaria patients [2]. These include trends for a possible saturable first-pass metabolism and decreased plasma concentrations upon repeated administration of the substance.

There are also reports indicating an auto-induction effect caused by artemisinin & its derivatives artemether and, although less convincingly, artesunate [13]. The lower plasma concentrations toward the end of the treatment period are believed to be due to an increase in the first-pass extraction of the drug, affecting its bioavailability. Unchanged artemisinin elimination half-lives during pre- and post-induction states imply the compound to be a high extraction drug with little effect of the induction on its systematic clearance [6, 8]. A semi-physiological pharmacokinetic model for artemisinin incorporating auto-induction of metabolism and saturable first-pass hepatic extraction which consisted of a pharmacokinetic component and an enzyme component, with the former influencing the concentrations of the enzyme and the latter influencing the concentration of artemisinin has been developed as described below (**Fig. 2**) [13]:

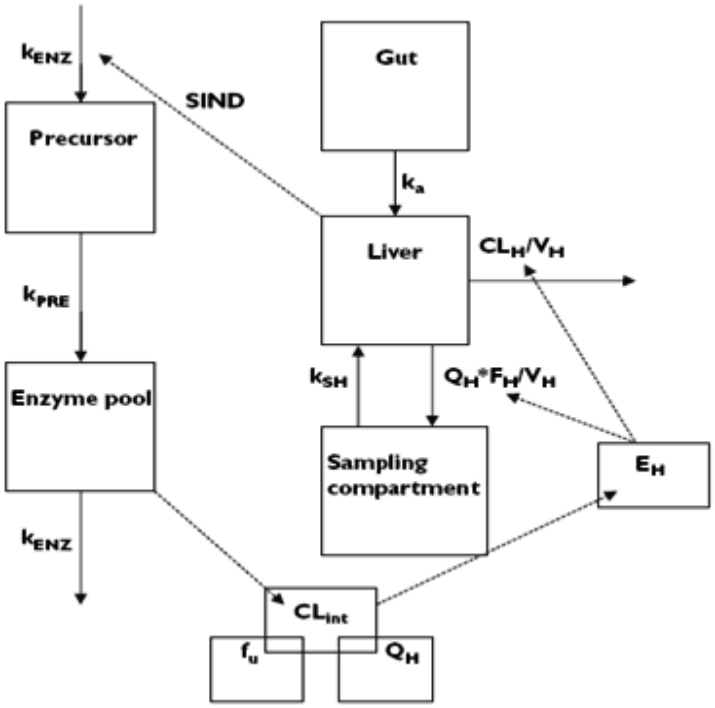


FIG. 2: SCHEMATIC DIAGRAM OF THE INDUCTION MODEL APPLIED TO SALIVA ARTEMISININ CONCENTRATION DATA

k_{ENZ}: Zero-order production rate constant for the enzyme precursor or first order elimination rate of the metabolizing enzymes, k_{PRE}: first-order production rate constant for the metabolizing enzymes, CL_{int}: intrinsic clearance, f_u: plasma unbound fraction, Q_H: hepatic plasma flow, E_H: extraction ratio, F_H: bioavailability from the liver compartment to the sampling compartment, k_a: absorption constant rate, k_{SH}: transfer rate constant of artemisinin from the sampling compartment to the hepatic compartment (set equal to Q_H/Vs, VS being the volume of distribution of the sampling compartment), CL_H: hepatic clearance, V_H: volume of the liver compartment (set equal to 1), S_{IND}: slope of the inducing effect of artemisinin hepatic concentration on the production rate.

The capillary and saliva sampling have been suggested as promising replacements for venous sampling in pharmacokinetic studies of artemisinin. Indications of a putative arterio-venous concentration difference were reported in the same study [2].

In most studies, it was shown that there was a time-dependent decrease of Dehydroartemisinin (DHA), a derivative of Artemisinin, plasma concentrations after the same repeated doses and a steady state plateau blood drug concentration did not appear after several of the same repeated doses (**Fig. 3**) [2, 3, 4, 7, 8, 13].

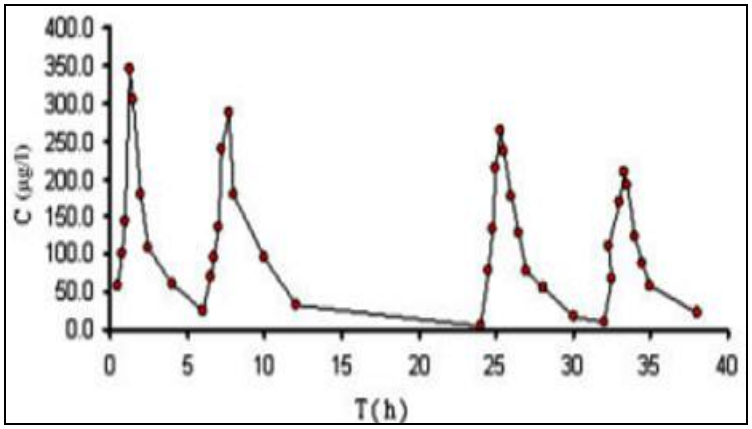


FIG. 3: MEAN DHA PLASMA CONCENTRATION- TIME CURVE OF 320MG DHA (EIGHT ARTEKIN TABLETS) GIVEN ORALLY FOUR TIMES IN HEALTHY VOLUNTEERS
Data represent mean±SD (n=6)

The C_{max} of the first dose was 1.7 times that of the fourth dose in the repeated dosage regimen. Generally speaking, when the same repeated doses were given at the same time intervals t, and $t=t_{1/2}$, then after 4–5 repeated doses, the plasma drug concentration nearly reached a steady-state concentration in the human body [4]. This phenomenon suggested the auto induction of hepatic drug metabolizing enzymes for DHA, and the results were similar to other artemisinin drugs [2, 13].

The results of most studies have shown that food intake probably has no substantial influence on the pharmacokinetics of orally administered artemisinin [14] (see **Fig. 4** below). Inter-individual variation is large as is intra-individual variation. With poor bioavailability, small absolute changes of absorption have large relative effects [15].

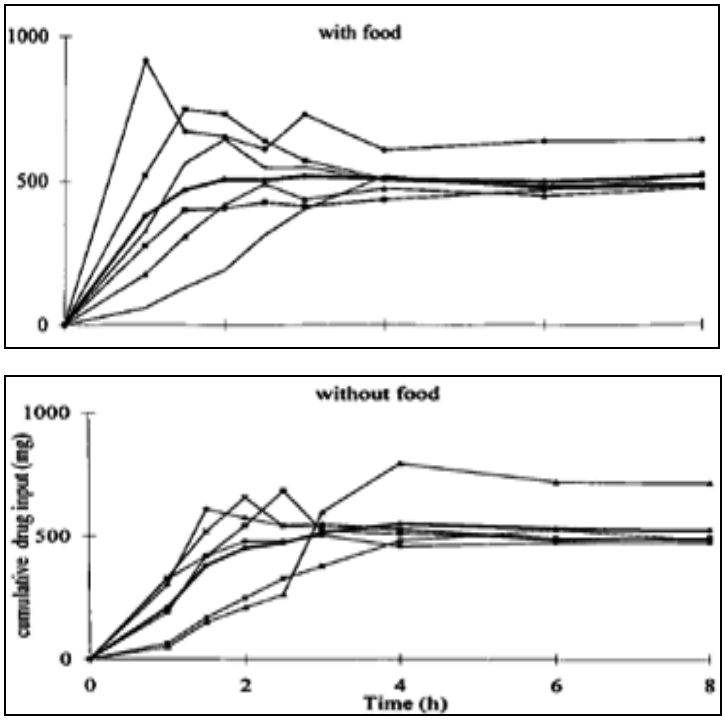


FIG. 4: CUMULATIVE AMOUNT OF ARTEMISININ REABSORBED AFTER ADMINISTRATION WITH FOOD AND WITHOUT FOOD ASSUMING 100% BIOAVAILABILITY
Each symbol represents results for an individual patient

There are reasons to suspect that food would have an influence on the pharmacokinetics of artemisinin. Artemisinin is poorly soluble in both water and oil. The milieu of the gastrointestinal tract is watery; this is changed by food intake, and thus a change in bioavailability might be anticipated. Moreover, food intake increases intestinal and liver blood flow [15].

However, it is hard to predict the direction of possible changes on the basis of purely theoretical considerations and in vitro data. No change was observed in area under the curve (AUC); the parameter most likely to reflect bioavailability. It is thus unlikely that bioavailability is changed very much by food; this conclusion is strengthened by the fact that none of the other measures of absorption (e.g., absorption rate) shows a change after food intake [2, 15].

Another important pharmacokinetic factor influenced by food is liver blood flow, and therefore bioavailability and/or systemic clearance. Because only trace amounts of unchanged artemisinin in urine were found, enzymatic, and thus most probably, hepatic, metabolism seems to be the main route of elimination of artemisinin [15].

Theoretically, biliary excretion is another possible route of elimination. The influence of changes in liver blood flow on pharmacokinetics depends on the relationship between liver blood flow and the intrinsic capacity of the liver to metabolize a drug (the so-called "intrinsic clearance"). When intrinsic clearance is high compared to liver blood flow, the rate-limiting factor in drug clearance is liver blood flow; changes in liver blood flow are thus expected to have an influence on pharmacokinetic parameters.

When intrinsic clearance is low compared to liver blood flow, changes in liver blood flow do not affect clearance. Because no differences were found in the pharmacokinetics of artemisinin after food versus those before food, liver blood flow has no influence on the elimination or the bioavailability of artemisinin. Artemisinin is therefore probably a so-called low-clearance drug [4, 15].

Artemisinin based Combination Treatments (ACTS): ACTs are combinations of an artemisinin derivative and another structurally unrelated and more slowly eliminated antimalarial agent. They are now generally accepted as the best treatment options for uncomplicated falciparum malaria, but not in all parts of the world. Artesunate- sulfadoxine- pyrimethamine and artesunate- amodiaquine are effective in some areas, but in other areas resistance to the partner precludes their use.

There is still uncertainty over the safety of artemisinin derivatives in the first trimester of pregnancy, when they should not be used unless there are no effective alternatives. Otherwise, except for occasional hypersensitivity reactions, the artemisinin derivatives are safe and remarkably well tolerated [1, 2, 7, 8].

The adverse effect profiles of the artemisinin-based combination treatments are determined by the partner drug. Most malaria endemic countries, including Ethiopia, have now adopted artemisinin-based combination treatments as first-line treatment of falciparum malaria, but in most of these countries, only minorities of the patients that need artemisinin-based combination treatments actually receive them largely due to the higher cost.

Effectiveness of the Fixed-dose combinations of artemisinin derivatives: Malaria is a (eukaryotic) protozoan parasite of red blood cells that may reach burdens as high as 10^{13} in the blood of the human host, although most symptomatic infections are caused by between 10^{7} and 10^{12} parasites [7]. The theoretical rationale underlying combination drug treatment of tuberculosis, leprosy, HIV infection and many cancers is now well known, and the same general principle is now widely accepted for malaria.

If two drugs are used with different modes of action, and therefore different resistance mechanisms, then the parasite probability of developing resistance to both drugs at the same cell division is the product of their individual per parasite probabilities [7]. This is of particular relevance to malaria because on any one day there are only about 10^{17} malaria parasites in the entire world. Most identified mechanisms of antimalarial drug resistance result from genetic mutation. Mutation rates for eukaryotes are of the order of 1 in 10^{6} divisions but viable resistant mutant parasites are selected at much lower frequencies.

The highest frequencies documented for the de novo emergence of mutations conferring drug resistance in acute malaria in humans are for atovaquone and pyrimethamine at around 1 in 10^{12} parasites. So if the per parasite probability of developing resistance to two drugs (A and B) are both high at 1 in 10^{12}, then a simultaneously resistant mutant (i.e., resistant to both A and B) will arise spontaneously every 1 in 10^{24} parasites. But because there is a cumulative total of less than 10^{20} malaria parasites in existence each year, such a simultaneously resistant parasite would arise spontaneously roughly once every 10,000 years, provided the drugs always confronted the parasites in combination [7].

Thus, provided the de novo per parasite probability of developing resistance is not much higher than 1 in 10^{12} cell divisions and both drugs are present together at inhibitory concentrations, then combinations markedly delay the emergence of resistance. But for ACTs, because the artemisinin derivatives are eliminated rapidly (due to their short half-lives), and the partner drugs are eliminated slowly, there is complete protection only for the artemisinin derivative.

The combination still provides good protection against the emergence of resistance to the partner drug, but once resistance has developed the residual concentrations of unprotected partner drug do provide a selective filter enhancing the spread of resistance to the partner compound [7]. **Figure 5** shows the pharmacokinetic- pharmacodynamic rationale for ACTs using artesunate- mefloquine as an example.

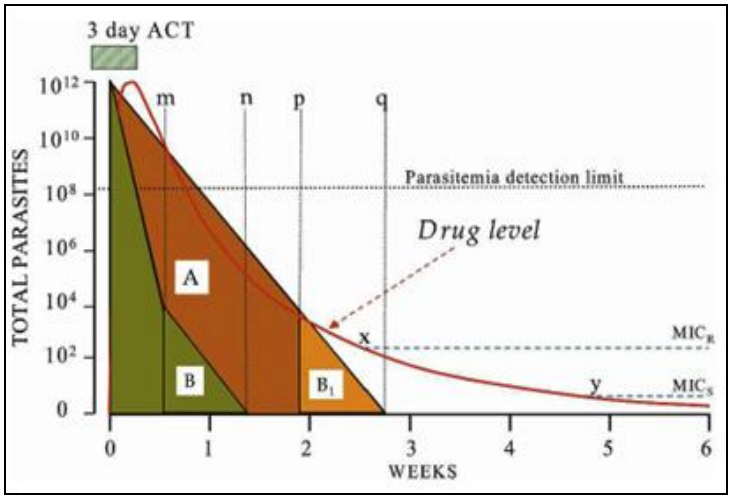


FIG. 5: THE PHARMACOKINETIC-PHARMACODYNAMIC PROFILE OF ARTEMISININ COMBINATION TREATMENT IN FALCIPARUM MALARIA

The individual patient parasite burden (approximating to 2% parasitemia in an adult) is shown on the vertical axis in a logarithmic scale, and the concomitant profile of drug concentrations is shown as a curved red line. The total numbers of parasites exposed to the drugs are shown as triangles, the area of which corresponds to total numbers in the blood. In this example the ACT partner drug is mefloquine. The treatment is given for 3 days, which covers two asexual cycles and the effect of the artesunate is a 100,000,000-fold reduction in parasite burden.

This leaves approximately 10,000 parasites (dark green triangle B) remaining for residual concentrations of mefloquine (from points *m* to *n*) to remove. If no artesunate had been given, the mefloquine would have reduced the parasite burden more slowly (light brown large triangle), and the number of parasites corresponding to B (i.e., B1) would have been exposed to lower mefloquine concentrations (from points *p* to *q*). In this example these concentrations would be insufficient to inhibit growth of the most resistant parasites prevalent (minimum inhibitory concentration; MIC_R) and so, whereas the ACT would cure all infections provided these blood concentrations were achieved, there would be treatment failures with mefloquine monotherapy. MICs refer to the most sensitive MICs for artesunate and mefloquine respectively. The time from points *x* to *y* on the mefloquine elimination curve represents the window of selection (about 16 days in this example) during which newly acquired infections with sensitive parasites cannot establish themselves whereas resistant parasites can.

Treatment failure rates are higher and parasite clearance times longer with ACTs in Western Cambodia than elsewhere, the epicenter of drug resistance in Southeast Asia [7]. However, elsewhere, Artemisinin derivatives are particularly effective in combinations because of their high killing rates (parasite reduction ratios, RR, of 10,000 fold per cycle), lack of adverse effects, and absence of significant resistance [2]. Artemisinin and its derivatives are the most rapidly eliminated of all antimalarials with half-lives of approximately 1 Hour [1, 2, 7, 12, 16-23].

The “ideal” pharmacokinetic properties for an antimalarial drug have been a subject of much discussion. Nevertheless, from a resistance prevention perspective, the combination partners should have similar pharmacokinetic properties to provide optimum mutual protection. Slow elimination of the partner drug allows 3-day regimens to be given, but at the price of providing days or weeks of sub-therapeutic blood levels that provide a selective filter for resistant parasites acquired from elsewhere, and thereby encouraging the spread of resistance, (Fig. 5).

On the other hand these residual “prophylactic” levels suppress new infections giving a period of post treatment prophylaxis (PTP) which, in high transmission settings, may improve clinical and hematological recovery. Rapid elimination ensures that the residual concentrations do not provide a selective filter for resistant parasites, but rapidly eliminated drugs (if used alone) do not provide any PTP, must be given for 7 days, and adherence to 7-day regimens is poor. Incomplete treatment encourages resistance.

Even 7-days regimens of artemisinin derivatives (as monotherapy) are associated with approximately 10% failure rates. Thus, to be highly effective in a 3-day regimen, the terminal elimination half-life of at least one drug component must exceed 24 hours (longer for less active drugs) such that concentrations in the fourth drug-exposed asexual cycle (7 to 8 days after starting treatment) are still sufficient to suppress multiplication of the most resistant parasites prevalent [7].

Provided there is not high-level resistance to the partner drug, then ACTs provide complete protection for the artemisinin derivatives from selection of a de novo resistant mutant if adherence is good (i.e., no parasite is exposed to artemisinin during one asexual cycle without the partner being present), but this does leave the partner's slowly eliminated "tail" unprotected by the artemisinin derivative.

However, because artemisinin and its derivatives reduce parasite numbers by approximately 10,000-fold per 2- day asexual cycle, the residual number of parasites exposed to the slowly eliminated partner drug alone, after 2 asexual cycles of artemisinin exposure, is a tiny fraction (< 0.0001%) of those present at the peak of the acute symptomatic infection (Fig. 5). Furthermore, these residual parasites are exposed to relatively high levels of partner drug and, even if susceptibility was reduced, these levels are usually sufficient to eradicate the infection [12].

But the long elimination phase "tail" of the partner drug does provide a selective filter for resistant parasites acquired from elsewhere, and thereby contributes to the spread of resistance once it has developed. Although the greatest use of antimalarials is in high-transmission areas, historically resistance has emerged and spread most rapidly in low transmission settings. This illustrates the important role of host immunity in delaying the emergence and spread of resistance [7, 12, 21-23].

The main obstacles to the success of combination treatment in preventing the emergence of resistance will be inadequate treatment (e.g., substandard drugs, incorrect dosing, unusual pharmacokinetics, poor adherence) and, as for antituberculous drugs, use of one of the combination partners alone.

This is why blister packing has been encouraged and fixed dose combinations are now being developed and recommended. Cost is a major obstacle to ensuring adequate treatment because patients may not have enough money to purchase a full course of treatment or, once they feel better, will keep the remaining prescribed drugs for themselves or a family member when they next fall ill. Poor quality drugs are common in tropical areas of the world and counterfeit medicines are a major concern. Antimalarials are available widely in the market place, and often sold at incorrect doses or without correct advice.

Even when a correct course is obtained adherence to antimalarial treatment regimens is often incomplete. Irrespective of the epidemiologic setting, ensuring that patients with high parasitemias receive a full course of treatment with adequate doses of ACTs would be an effective method of slowing the de novo emergence of antimalarial drug resistance [23].

Ideally, to ensure the maximum useful therapeutic life, there should be no resistance to the partner drug in an ACT, yet on the Northwestern border of Thailand, an area of low transmission where mefloquine resistance had developed already, systematic deployment of artesunate- mefloquine combination therapy was dramatically effective both in stopping resistance, and also in reducing the incidence of malaria .

In fact, mefloquine resistance declined after widespread deployment of artesunate- mefloquine. In this setting before ACTs were introduced when mefloquine monotherapy was used, resistant parasites had a survival and transmission advantage, which was negated by the artesunate- mefloquine combination treatment. Mefloquine resistance develops rapidly because gene amplification is a relatively frequent mitotic event in *P. falciparum*, but it may also go rapidly as de-amplification is also frequent.

But for the other drugs and resistance mechanisms involving mutations, deploying an ACT containing a failing drug may not lead to a reversal of resistance, and could eventually leave the artemisinin component inadequately protected as resistance to the partner worsens [7]. Four ACTs are currently recommended by WHO and the selection by a specific national government will be based on the prevalence of

resistant parasitic strains and the cost among others. These ACTs include:

- Artesunate- mefloquine,
- Artesunate- sulfadoxine- pyrimethamine (sp),
- Artesunate- amodiaquine, and
- Artemether- lumefantrine.

Ethiopia has adopted Arthemeter-lumefantrin, with a fixed dose combination ratio of 80/480mg, commonly called Coartem, as the first line treatment for falciparum malaria since the year 2003.

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