Chapter 1

The Impact of Pharmacokinetic Mismatched Antimalarial Drug Combinations on the Emergence and Spread of Drug Resistant Parasites

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Additional information is available at the end of the chapter

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1. Introduction

The concept of drug combination therapy is defined on the premise of combining drugs with similar half-lives in order to keep a constant dual pressure on current and reinvading parasites, which provides improved therapeutic efficacy and delays the development of resistance to the individual partner drug [1]. As the science of pharmaceutical technology and chemical synthetic capability advances is developed, the antimalarial drug combination has become more sophisticated and data driven, rather than empirically derived. The most significant problem with antimalarials is the growing incidence of drug resistance to *P. falciparum*. Drug resistance implies that there is a right shift in the relationship between drug concentration and efficacy. Although treatment failure in malaria usually results from poor patient drug compliance, inadequate dosing, pharmacokinetic (PK), and pharmacodynamic (PD) factors, some malaria infections will recrudesce and none of these factors will adequately explain why a recrudescence occurred. How parasites persist despite apparently adequate antimalarial treatment is an unanswered question [2, 3].

Combination therapy (CT) with antimalarial drugs is the simultaneous use of two or more blood schizonticidal drugs with independent modes of action and different biochemical targets in the parasite. Artemisinin based combination therapy (ACT) has been adopted in Asia since 1992 and in Africa since 2000 and ACTs have shown improved treatment efficacy with reductions in drug toxicity. ACTs are now the treatment of choice for *P. falciparum* malaria globally. They are more effective than non-artemisinin combinations or monotherapies, and their use reduces the probability of drug resistance emerging in *P. falciparum* parasites. Most of the antimalarial drugs and their combination formulations now in use were introduced before the modern era of dosage design based on PK principles to minimize

drug resistance [4]. Antimalarial dose regimens have tended to be empirically derived based on clinical studies with drug half-life, PK inter- individual variation, and drug exposure variables derived from experimental data or referred to by data from public trials. PK studies involve investigating the relationship between absorption, distribution, metabolism, and excretion of a drug. Many drugs share the same metabolic pathways or target the same receptors when considering matching their metabolism for selection of antimalarial combination drug partners. The ideal PK properties for an antimalarial drug have long been debated. From a resistance prevention perspective, the combination partners should have similar PK properties (PK half-life matching) [5].

Current recommended ACTs have repeatedly shown in a variety of clinical studies that the ACT partner drugs are well matched, efficacious well tolerated, and provide protection against new infections. ACTs are founded on the very novel mechanism of action of the artemisinin partner, which provides rapid parasite killing of the parasite biomass, quick reduction in clinical symptoms, broad spectrum killing against drug resistant parasites, and downstream decreases in formation of gametocytes, which in turn diminishes the spread of drug resistance genes [6]. An ideal drug combination would consist of drugs possessing complementary half-lives to provide growth inhibition from two drugs on existing and new infections. Drug possessing a short half-life are preferred to limit drug exposure at lower, non-growth inhibitory concentrations to new parasitic infections. By administering drugs with short half-lives, the exposure of drug to parasites is limited and the pressure to select for drug tolerance and resistance is minimized. However, as recently discussed, administration of PK mismatched antimalarial drug combinations does not significantly impact the spread of resistance, and administration of PK mismatched ACTs have a known risk of jeopardizing the efficacy of partner drugs where resistance has not yet been widely observed [7, 8]. Although PK matching has been required by the FDA for new anti-infective drug combinations, simply matching half-lives has not been considered sufficient [9].

PK mismatched drug combinations such as the ACTs have not been shown to have a significant risk of increasing drug resistance. Thus, the hypothesis that treatment with PK mismatched ACTs leads to drug resistance would seem to be rejected. In addition, clinical data shows that drug regimen that have significant PK mismatches have more mismatched regimens have consistently been associated with better and faster parasite killing than perfectly matched regimens. With that being said, there are examples of antimalarial drug combination that precede the ACTs with mismatched drug half-lives that have rapidly failed due to drug resistance. One of the best examples is the combination of mefloquine and sulfadoxine-pyrimethamine (Fansi-Mef). Mefloquine has a longer half-life than either sulfadoxine or pyrimethamine and drug resistance problems were predicted when this combination was introduced; this prediction was shown to be accurate.

Two aspects are important when choosing drug combination partners designed with the aim of containing the development and spread of drug resistance: mechanisms of action that are synergistic but different and a good PK match. Antimalarial drug combination treatment based on artemisinin drugs is recommended for first-line treatment worldwide due to high efficacy and a demonstrated ability to deter drug resistance. By considering factors beyond matching

half lives one could reasonably consider other influences such as drug mechanisms, other PK parameters beyond half-life, PD, and malaria epidemiology, and consideration of all of these factors should result in creation of more effective combination regimens that retain therapeutic and prophylactic efficacy in the face of drug resistance. No doubt, PK and PD matched drug combinations could enhance treatment efficacy and delay drug resistance, however, the pharmacological mismatch hypothesis as applied to antimalarial drugs is countered by clinical data.

The use of drug half-lives is an overly simplistic approach to explain why drug resistance has developed given the requirement to have active inhibitory concentrations of both partner drugs present at the same time to deter resistance to either drug partner. It is entirely possible to have partner drug concentrations trailing off below the minimum inhibitory concentration at a time when the calculated drug half-life suggests antiparasitic protection. Consequently, it appears more important that drug antimalarial activity profiles post-treatment are the critical factors to be matched, rather than simply their half-lives. The PD profile after dosing is dependent on dosage administered, the half-lives of the drugs administered, the PD parameters of the drugs administered, and any drug-drug interactions that may occur [1]. Many factors that might influence efficacy and resistance of antimalarial combinations for both drug treatment and chemoprophylaxis indications include convenience of the prescribed antimalarial regimen in PD matching. The PD matching parameter of drug killing activity can be quantified by assessing the drug time above the minimal inhibitory concentration (MIC) in plasma. Time above MIC for any antimalarial drug is a function of the maximum concentration (C_{max}) and drug clearance, which are again dependent on the dose administered and the formulation. The interaction between factors such as half-life, dosage, partner drug, and parasite drug sensitivity will yield hopefully a well-designed CT. In addition, those factors allow for mismatch in any of these variables by altering the relative dosages to achieve matched activity profiles post-treatment [1]. There are a variety of considerations to achieve a partner drug PD match to include different modes of action, different drug interactions of synergism and antagonism, avoidance of toxicity for any one partner drug, and CT or ACT-driven parasite killing.

While the PK mismatch hypothesis has been refuted for emergence of long-lived drug resistance, this hypothesis may be relevant for some geographic areas with endemic malaria [9]. In areas of high malaria transmission, the use of artemisinin derivatives might have little or no effect on malaria incidence. The use of drug combination treatment, however, may have an effect on the emergence of drug resistance in these endemic locations. While artemisinin compounds do not provide protection for longer-lived partner drugs given their rapid clearance, artemisinin drugs can diminish the probability of selection of drug resistance parasites initially and through enhanced antimalarial efficacy, artemisinin drugs can reduce transmission of drug resistant parasites and decrease gametocyte carriage. One of the most likely reasons why ACTs have deterred the emergence and spread of drug resistance may be related to the pharmacodynamic effects of artemisinin, particularly the reduction in parasite burden of 10,000 fold during each asexual replication cycle. Most importantly, the combination antimalarial drug partner will provide reciprocal protection to the artemisinin derivative from

drug resistance and toxicity. Rational consideration of PK/PD matching of drug partners should result in more effective combination regimens that retain therapeutic and prophylactic efficacy in the face of efficacy and resistance. Our view is that adopting a rational and objective method to simulate ACT or other CT drug effectiveness using PK/PD match and mismatch principles can play a valuable role in this process.

2. Current ACT partner drugs selected by comparative clinical trials

In the absence of an effective malaria vaccine, early and successful chemotherapy for malaria performs an essential role in reducing morbidity andmortality. Multidrug resistance has been reported from most parts of the world, and drug monotherapy or the use of some of the available combination chemotherapies for malaria is either ineffective or less effective. New antimalarial regimens are urgently needed and ACTs are widely advocated as the best antimalarial regimens possible today. ACTs have been shown to increase efficacy, shorten duration of treatment (and hence increase compliance), and they have been shown to decrease the risk of resistant parasites arising through mutation during therapy. There are five ACTs used broadly for first-line treatment of *P. falciparum* malaria globally, and these five artemisinin combination treatments have been shown to be much more effective than non-artemisinin-based combinations or monotherapies. The ACT options now recommended by WHO [2] for treatment of uncomplicated falciparum malaria include:

- artemether plus lumefantrine (AL),
- artesunate plus amodiaquine (AS-AQ),
- artesunate plus mefloquine (AS-MQ),
- artesunate plus sulfadoxine-pyrimethamine (AS-SP), and
- dihydroartemisinin plus piperaquine (DP).

Despite these advances, better treatments are required, and this leads us to the associated requirement to insure comparative efficacy assessments are well designed and easily interpreted. Given the performance of artemisinin combination therapy, the minimum acceptable cure rate has been at least 90%. This expectation is far above the cure rates achieved with malaria monotherapy in past years where cure rates of 70% were considered state of the art [2, 3]. The other side of this expectation is the idea that if cure rates fall below 90% a change in drug is therefore required. The overall effect of ACT use results in reduction in the probability of parasite recrudescence, reductions in the within-patient selection pressure, and prevention of parasite transmission. If the cure rate of any ACT is below 90%, the recommendation for the use of that combination for treatment should change, and a better ACT should be selected based on data from a comparative efficacy study demonstrating clinical superiority. In earlier "superiority trials", it was reasonable to plan a randomized comparison to test if there was a difference between the regimens being tested.

There is a wealth of published data on malaria drug studies with ACTs for treatment. One problem readily observed when comparing these studies is trying to compare data from studies with differing study endpoints. The WHO has updated its recommended study endpoints incorporating measures of both parasitological and clinical outcomes to standardize study design [3]. The majority of malaria drug studies conducted utilize clinical outcomes at either 14 or 28 days as the principal endpoint. This set of endpoints is too short, however, to account for the lingering presence of parasites still present as the presence or absence of residual parasites is an important public health parameter. Residual parasites still present in the blood of infected patients after treatment can result in anemia, recrudescent infection, and development of severe malaria. Longer-term studies conducted with an extended follow-up period can provide a more meaningful comparison of drug treatment in areas of high malaria transmission.

All ACTs in current clinical use are well designed and the studies supporting their use have been well conducted in various randomized comparative trials before being placed on market. Based on clinical experience with each partner drug in monotherapies and cautious consideration with various PK and PD factors for the combinations, the main comparative trials are summarized below along with discussion of non-ACT combinations previously in use.

2.1. Non-ACT combinations are no longer recommended for the treatment of malaria

There are a number of combination treatments that have been developed over the years that are not based on artemisinin drugs. These combinations include sulfadoxine-pyrimethamine plus chloroquine (SP-CQ) and sulfadoxine-pyrimethamine plus amodiaquine (SP-AQ). Given the broad distribution of resistance to antifolate compounds and to chloroquine, SP drug combinations are no longer effective. The combination of amodiaquine plus SP is more effective than either drug by itself, however, the efficacy of the amodiaquine sulfadoxine-pyrimethamine combination is usually inferior to ACTs, and this combination is no longer recommended for the treatment of malaria. Only Malarone (atovaquone-proguanil) is still recommended for chemoprophylaxis use [2].

2.2. ACTs recommended by the World Health Organization (WHO)

2.2.1. The 6-dose regimen of artemether-lumefantrine (AL) is superior to a 4-dose regimen

The data derived from one clinical trial conducted in Thailand from 1996-1997 (238 adults and children) showed a significantly higher rate of cure at day 28 with the 6-dose regimen of AL given over 3 days compared to the 4-dose regimen of AL also given over 3 days. To provide definitive data on this question of 4 doses versus 6 dose regimens, two six dose regimens comprised of a total of 480 mg of artemether and 2,880 mg of lumefantrine were assessed against a 4 dose regimen comprised of 320 mg of artemether and 1,920 mg of lumefantrine. This study was a double-blind trial 359 patients infected with uncomplicated multidrug resistant falciparum malaria. The study participants showed no differences in the fever and parasite clearance times, and there were no adverse effects noted with one regimen not observed in another. The six-dose regimens provided day 28 cure rates of 96.9% and

99.12%while the four dose regimen showed a cure rate of 83.3% (P < 0.001). Both of the six-dose regimens of AL were shown to provide a highly effective and very well tolerated treatment when compared to four-dose regimens of AL [10, 11].

2.2.2. A six-dose regimen of AL is not superior to treatment with AS-MQ

In another set of clinical trials (537 participants), the efficacy of a six-dose regimen of AL was compared to treatment with AS-MQ. For the AL regimen, two studies compared the antimalarial efficacy of AL to treatment with AS-MQ with an endpoint of day 28 parasitemia, and no differences in parasite or fever clearance time were detected. There were 11 parasitological failures in the AL treatment arm and none with AS-MQ [12]. The data from this study suggests that AS-MQ therapy is more effective than AL treatment [13].

2.2.3. The six-dose regimen of AL is similar to the efficacy achieved with AS-AQ treatment.

To test the hypothesis that AS-AQ is as effective as AL for treatment of acute uncomplicated malaria in Nigerian children, an open label, randomized controlled clinical trial was conducted in children aged 6 months to 10 years. The 132 patients in this trial were split into two groups of 66 and received 4 mg/kg of AS plus 10 mg/kg of amodiaquine daily or a weight based administration of a fixed dose AL tablet administered twice daily. Treatment with AS-AQ and AL was conducted for three days and clinical follow up was conducted until day 28. The cure rate in the protocol population at Day 28 for AS-AQ was 93%, and the cure rate for AL was 95% (OR –0. 71, 95% CI 0.12-3.99, rho= 0.66). The median survival time for the AL treatment group was 21 days and the means survival time for the AL treatment group was 28 days (Kaplan Meier product limit estimates, p=0.294). The polymerase chain reaction (PCR) corrected day 28 cure rates per protocol of the two patient populations were 98.4% for AS-AQ and 100% for AL. Both drug combinations were well tolerated, the efficacy comparison demonstrated that AS-AQ was as effective as AL, and both combinations were shown to be both efficacious and safe [14].

Another clinical trial was conducted in Burundi to compare children treated with AS-AQ versus AL with a 14-day follow-up period. A total of 295 children under 5years were included; 153 children were treated with AS-AQ, and 142 children were treated with the AL drug combination. Among the 295 children, 290 were followed up to 14 days. In the group of 149 children treated with AS and amodiaquine, 142 or 95.3% (95% CI: 91.9-98.7%) presented with adequate clinical and parasitological response, five or 3.3% presented with late parasitological failure, one or 0.7% with a late clinical failure and one or 0.7% with an early treatment failure. Among the 141 children treated with AL, 140 or 99.3% (95% Cl: 97.9-100%) presented with adequate clinical and parasitological response and one or 0.7% presented with a late parasitological failure. Side effects were comparable in both groups except for vomiting. Vomiting was more frequent in the AS-AQ treatment arm on Day 1 and Day 2 while the AL arm did not show this side effect. Both treatments decreased gametocyte carriage but did not achieve full clearance in all patients. During a consensus workshop, the Ministry of Public Health agreed that the combination of AS-AQ would be the best choice for first line treatment of uncomplicated falciparum malaria in Burundi including epidemic outbreaks [15].

2.2.4. The efficacy of DP is superior to AS-AQ for treatment of uncomplicated malaria

A comparative efficacy study was conducted to compare treatment with dihydroartemisinin-piperaquine (DP) to treatment with AS-AQ. This trial compared the efficacy of DP versus AS AQ in 334 patients infected with *P. falciparum*, P. vivax or both species of Plasmodium (185 were infected with *P. falciparum*, 80 were infected with *P. vivax*, and 69 were infected with both species) with a 42-day follow-up period. The overall parasitological failure rate at day 42 was 45% for AS-AQ and 13% for DP. Rates of both recrudescence of *P. falciparum* infection and recurrence of P. vivax infection were significantly higher after receipt of AS-AQ than after receipt of DP. By the end of the study, AS-AQ recipients were 2.95-fold more likely to be anemic and had a probability of carrying P. vivax gametocytes that was 14.5-times higher. DP demonstrated better efficacy and tolerability compared to AS-AQ for treatment of drug-resistant falciparum and vivax malaria infections. The extended post-treatment prophylactic effect provided by piperaquine delayed falciparum re-infection, diminished the incidence of vivax infection, decreased clinical anemia, and decreased the potential for vivax gametocyte carriage [16, 17].

2.2.5. The efficacy of DP is superior to a six-dose regimen of AL

Based on clinical trial data, Uganda introduced AL as the treatment of choice for uncomplicated malaria treatment. While AL is well tolerated and efficacious, it does have some drawbacks as a drug combination to include a requirement for administration with fatty foods, a twice-daily dosing regimen, and an increased risk of malaria re-infection in endemic areas. One of the new alternatives to AL for treatment is the combination of dihydroartemisinin and piperaquine (DP). In contrast to AL, DP is dosed once daily, not twice, and due to the long elimination half-life of piperaquine, this drug combination has a long period of prophylaxis post treatment.

A comparison of DP to AL was conducted in Kanungu, which is known to have moderate transmission of malaria. The 408 patients infected with uncomplicated falciparum malaria completed a 42-day follow-up, and the patients ranged in age from 6 months to 10 years. To differentiate recrudescent infections from new infections, the parasites were genotyped. DP-treated patients showed a much lower probability of parasitemia recurrence as only 12.2% developed recurrent parasitemia while 33.2% of AL-treated patients developed recurrent parasitemia (risk difference of 20.9%, 95% CI = 13.0-28.8%). There was no statistically significant difference between the two drug regimens due to recrudescent infections where AL treated patients showed recrudescence of 5.8% while DP treated patients showed recrudescence of 2.0% (risk difference of 3.8%, 95% CI= 0.2-7.8%). DP treated patients showed a diminished risk of gametocyte carriage after treatment as 4.2% showed gametocytemia after DP treatment while 10.6% showed gametocytemia after AL treatment ($p = 0.01$). Both AL and DP were shown to be safe and well tolerated [18, 19]. Similar studies were performed by other scientists in different regions and these studies indicated that daily treatment with DP is more efficacious than treatment with the six doses AL regimen. DP was also shown to be superior to AL for reducing the risk of recurrent parasitemia and gametocytemia, and it was shown to provide improved hemoglobin recovery [18-20].

2.2.6. The efficacy of DP is similar to AS-MQ

Multi-drug resistant falciparum malaria is a significant health problem in the Peruvian Amazon region. A randomized open label clinical trial was carried out comparing AS-MQ, the current first line treatment in this region of Peru, with DP. Total 522 patients with uncomplicated falciparum malaria were enrolled in this study and 260 were treated with AS-MQ and 260 with DP and followed for 63 days. Clinical and parasitological responses adjusted by PCR and estimated using per protocol analyses and Kaplan Meier survival tests were very good for both AS-MQ and DP. AS-MQ showed a 99.6% response while DP showed a 98.4% response (RR= 0.99, 95% CI =0.97-1.01). All of the incidents of recrudescence that were observed were attributed to parasitological failures that occurred late in the treatment period. All of the gametocytes observed were cleared in 28 days in the AS-MQ treatment group while gametocyte clearance in the DP treatment group required 35 days. New gametocytes were observed to appear more rapidly in patients treated with DP than in patients treated with AS-MQ. By day 7 eight patients treated with DP presented with gametocytemia while only two presented with gametocytemia in the AS-MQ group. Side effects of anxiety and insomnia were observed more frequently in patients treated with AS-MQ compared to DP. In summary DP was shown to be as efficacious as AS-MQ for treatment of falciparum malaria, and the DP combination is cheaper and better tolerated [21]. Other comparative efficacy studies have been conducted yielding similar data [22, 23].

2.2.7. Other clinical trials

A meta-analysis of data from 16 randomized trials (12 from Africa) compared the effect of adding 3 days of any artemisinin to one of the standard treatment regimens of CQ, SP, AQ, or MQ. The conclusion drawn in this study from all of the clinical trial data examined is the addition of 3 days of artemisinin to any of the standard antimalarial treatments significantly reduced parasitological failure on days 14 and 28. Gametocyte carriage was also reduced in the artemisinin treated patients.

In further studies, 3-day treatments with AL, AS-AQ, and DP was used for comparison of candidate regimens using 2-3 day courses of artemisinin-piperaquine (A-P). While initial parasite clearance was rapid and the A-P treatment was well tolerated, the 28-day cure rates for A-P were < 80% for 2-day treatments (2.4 mg/kg artemisinin, 14.4 mg/kg piperaquine) and 3 day treatment with artemisinin-piperaquine (3.2 mg/kg artemisinin and 16.0 mg/kg piperaquine) showed > 98% cure rates. These data supports further evaluation of 3-day treatment regimens with A-P for multidrug-resistant falciparum malaria. Similarly, data on the comparative efficacy of the chlorproguanil-dapsone -AS (CDA) combination are not yet available as clinical Phase III trials are still pending [17].

In conclusion, one non-ACT (atovaquone-proguanil) and five ACT combinations are recommended by the WHO to treat effectively drug resistant strains of Plasmodium [2]. In Southeast Asia, documented cases of resistance to artemisinins have been reported [24-26]. This resistance is characterized by delays in the parasite clearance time. Artemisinin resistance is related to widespread use particularly the indiscriminate use of artemisinin drugs as monotherapy. Due to the short half-lives of artemisinin compounds, cases of parasite reinfection have occurred during their use in monotherapy to treat malaria. The practice of prescribing

artemisinin drugs as monotherapy also enhances the probability of selecting for artemisinin resistant strains of Plasmodium. Artemisinins need, therefore, to be associated with effective antimalarials, which have a relatively long half-life. Not all combinations are good alternatives. Clinical trials comparing the efficacy of the AS-SP combination have shown problems with SP efficacy as monotherapy, and the AS-SP combination should not be administered in areas where cure rates for SP monotherapy are less than 80% [2]. AS-MQ and AS-AQ are both efficacious regimens, however, problems with tolerability with both drug combinations have been observed. The fixed-dose combination of AL remains very effective and well tolerated. It is recommended, however, that this combination be administered twice daily for three days with a fat-rich meal, which may limit adherence to treatment. In this context, the combination of DP shows equal or superior efficacy than other ACTs, and this combination appears to be a good alternative, particularly in malaria-endemic areas.

Antimalarials	**Half-life of artemisinin derivative**	**Half-life of long-term partner drug per full adult course (US$)**	**Regions currently in use purchase cost per course (US$)**
Artemether–lumefantrine (AL)	~ 3h	4–5 days	Africa, EM, SE Asia, WP and SA
Artesunate–mefloquine (AS-MQ)	< 1h	14–21 days	Africa, SE Asia, WP and SA
Artesunate–amodiaquine* (AS-AQ)	< 1h	9–18 days‡	Africa and EM
Chloroquine[1]	-	1–2 months	Africa, EM, SE Asia, WP and SA
Dihydroartemisinin–piperaquine (DP)	45 min	~5 weeks	SE Asia
Artesunate-SP (AS-SP)	< 1h	4 days (S) or	Africa, EM (IPT in Africa, EM and WP)
Atovaquone-proguanil (Malarone)	NA	~8 days (P) 73h (Ato) or 14h (Prog)	Africa, SE Asia, EM and WP

*This refers to the $t_{1/2}$ of the active metabolite monodesethyl-amodiaquine; the $t_{1/2}$ of amodiaquine is ~3hr.

[1]These former first-line antimalarials are included as a reference. EM, eastern Mediterranean; IPT, intermittent preventive treatment; NA, not applicable; P, pyrimethamine; S, sulphadoxine; SA, South America; SE Asia, Southeast Asia; $t_{1/2}$, half-life; WP, Western Pacific. SP = sulfadoxine-pyrimethamine

Table 1. Plasma half-lives of drugs used in artemisinin-based combination therapies [28]

None of the comparative efficacy trial designs for these combinations followed the PK match hypothesis that the drugs contained in a regimen should have elimination half times that match and that the partner drugs should not have any clinically significant negative PK

interactions [27]. The elimination half-lives of partner drugs should match because the matching elimination kinetics is especially important in areas of high malaria transmission due to exposure of parasites to declining concentrations of one drug in the combination leading to development of resistance. All six artemisinin drug combinations show a high degree of PK mismatch (Table 1).

3. Matching drug half-lives to deter drug resistance

Resistance to antimalarials by falciparum parasites has been an ongoing global public health concern since chloroquine and mefloquine resistance emerged [28]. The emergence of multi-drug resistant parasites has triggered the use of combination antimalarial regimens. The need for effective treatments has resulted in use of antimalarial combinations thrown together that have often worked better than monotherapies, though sometimes only temporarily. Many factors that might influence efficacy and resistance of antimalarial combinations for both chemotherapy and chemoprophylaxis indications include convenience of the prescribed antimalarial drugs rather than a focus on matching PK parameters. Therefore, the ideal drug combination would consist of drugs with complementary half-lives to insure each drug provides inhibitory activity to protect the other drug(s) in a combination from emerging drug resistance associated with exposure to sub-therapeutic monotherapy. To avoid such exposure to sub-therapeutic drug levels, drugs with shorter half-lives would be a better choice to avoid exposure of new parasites to sub-therapeutic drug levels. Once the artemisinin component has been eliminated, the partner drug is therefore left unprotected once the artemisinin has been eliminated from the body, and selective pressures on that partner drug will lead to development of resistance.

The implications of this "PK 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. 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 development of resistance. Mismatched PK profiles allow parasites to evolve resistance sequentially as the longer half-life partner persists as a vulnerable monotherapeutic agent (Figure 1). The results of mismatched PK profiles can almost completely undermine the benefits of combination therapy.

Coartem (artemether-lumefantrine) is one of the first combination treatments with artemisinin drug to be formulated as a fixed dose to enhance patient compliance. Data on drug resistance profiles [29] suggests this combination may be vulnerable to certain mutations in the pfmdr1 multi-drug resistance transporter gene found in *P. falciparum*. Mutations in the pfmdr1 gene from Y to N (position 86) have been shown to lead to increased tolerance to Coartem facilitating parasite re-infection. While the 86N mutation appears to be acting to enhance drug tolerance

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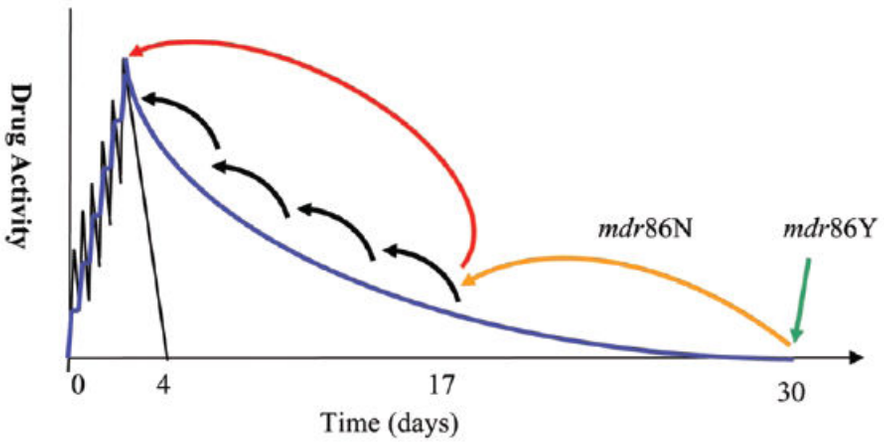

Figure 1. Likely evolution of resistance to artemether-lumefantrine (AL), the graph represents the drug levels in the serum of a patient starting the 3-day Coartem therapy regimen, an artemisinin-based combination therapy (ACT) comprising lumefantrine *(blue)* and artemether *(black).* The drugs have decayed to non-therapeutic levels when they cross the *X*-axis (defined as the concentration at which the drug, if present as a monotherapy, would be unable to prevent infection by drug-sensitive parasites). The *pfmdr* 86N mutation's *(orange arrow)* increased tolerance of lumefantrine allows it to infect a person 17 days post-treatment, compared with 30 days post-treatment for the *mdr*86Y mutation. Two scenarios are given for subsequent mutational steps. Scenario 1 is a single large increase in drug tolerance *(red arrow),* in which the presence of artemether in the ACT is powerless to stop the spread of this mutation. The increased tolerance of lumefantrine allows it to infect a person 4 days post-treatment and to spread through the parasite population, displacing the 86N form, which can infect no earlier than 17 days post-treatment. Scenario 2 is a subsequent increase in tolerance, which occurs as a series of small increments *(black arrows);* the presence of artemether in the ACT will slow this process when it reaches day 4, and Coartem may remain clinically effective for a longer period [29].

rather than act as a mechanism of drug resistance, parasites with this mutation will spread this drug resistance allele more readily than wild-type parasites with the 86Y *pfmdrl* gene. (Figure 1).These observations raise a new additional point: can ACT use accelerate the development of resistance to artemisinin derivatives? The presence of the *pfmdrl* 86N allele has also been shown to confer less sensitivity to artemisinin *in vitro* [29].

While artemisinin or an artemisinin derivative may not have selected for resistance at pfmdr1 86N due to the short half-life of artemisinin drugs, lumefantrine has a much longer half-live of 5 days could be driving selection at this allele resulting in increases in drug tolerance to both partners. This study does support the principle that understanding mechanisms of resistance at the molecular level to ACT partner drugs is essential to maintain drug efficacy of these combination treatments. ACTs, like other drugs, have a finite useful life span, which likely varies from short in Southeast Asia to longer in Africa. Development of new ACT drugs with better-matched PK rates of clearance is clearly a priority to avoid emergence of drug resistance to both drugs in the combination. To observe resistance emergence with clarity, early detection is clearly essential to facilitate longer term planning on first-line drug choices [30].

Mathematical modeling of associations between drug clearance and parasite drug resistance have been proposed based on a division of parasite resistance into three categories, *Res0*, *Res1*, and *Res2*. Parasites in the *Res0* group have wild-type susceptibility to antimalarial drugs, and they are readily killed. Parasites in the *Res1* group have increased tolerance to antimalarial drugs, but they can still be killed. Parasites in the *Res2* group are resistant to drug treatment

and cannot be killed. The rate of parasite evolution in the *Res2* group is commonly underestimated if the effects of drug clearance are not taken into account. There are also two phases of drug resistant development related to the speed of resistance development, which are intrinsic to this model of drug resistance. In the first phase, Phase A, *Res1* drug tolerant parasites are spreading and replacing Res0 drug sensitive parasites, however, *Res2* parasites are not present. In Phase B, parasites capable of surviving drug treatment are more quickly selected which leads to emergence of Res2 parasites, which cannot be killed, and clinically, results in treatment failure. Clinical treatment of malaria parasites in Phase A is commonly successful, and the transition from Phase A to Phase B is not readily observable without drug resistance testing of parasite populations. This underscores the need for parasite drug surveillance programs to sample parasite populations and assess any transition from wild type to drug resistant [31].

The addition of this aspect of PK to model of drug resistance has resulted in several novel findings, as detailed earlier. 1) A long elimination half-life at therapeutic concentrations results in long periods of chemoprophylaxis, so drug half-life is a potent selective force increasing the rate at which resistance evolves. 2) Parasite drug resistance arising from mutations at a particular gene locus may take place in two phases, Phase A and B, where drug tolerance evolves into overt drug resistance. 3) The duration of Phase A and Phase B may be quite variable depending on the transmission rates in a particular geographic area, which underscores the need for active drug surveillance programs. 4) Artemisinin drug combination therapy has been shown to slow down the rate of parasite drug resistance, however, successful introduction of an ACT is dependent on the absence of drug resistance to either of the partners in the ACT as existing resistance to either partner will result in rapid clinical failure [31-33].

All currently used combinations of artemisinins have mismatched drug elimination rates, and the pharmacokinetic half-life differences between the short-lived artemisinins and the long-lived quinolines such as piperaquine and mefloquine is a cause for concern regarding the long-term development and spread of drug resistance [8]. The elimination half-life of artemisinins ranges from 0.4-2.6 hours, but the elimination half-life of quinolines range from 4 days-2 months. The elimination half-life for sulfadoxine-pyrimethamine (SP) ranges from 4-8 days, and the elimination half-life of mefloquine (MQ) ranges from 14-21 days. Accordingly, there is an extended period when SP and MQ are "unprotected" given the short half-life of AS (Table 1) [32-34]. This sort of mismatched PK profile occurs with other combinations such as AL, AS-AQ, DP, and the combination of atovaquone-proguanil used in Asia and Africa [8, 35]. Artemisinin derivatives are protected from the rapid development of resistance as these drugs circulates in the presence of a much more long-lived partner, but the long-lived partner is not protected by the artemisinins [36].

Combining artemisinins with quinoline compounds has provided a mixed outcome. While the mechanisms of action of artemisinin and quinoline compounds provide a synergistic outcome, the elimination half-life differences between these compound classes is a risk for development of drug resistance. One example of this concern, which has proved to be on target, is the rise of AS-MQ clinical treatment failures in Southeast Asia in areas with high levels of MQ resistance. The combination of AS and MQ represents a drug combination with a significant mismatch in elimination half-lives (< 1 hour for AS and 14-21 days for MQ). From a PK

perspective, artemether and lumefantrine have a better match in terms of half-life as lumefantrine has a half-life of 4-5 days [37]. Despite this better match in half-lives, AL is slightly less efficacious than AS-MQ in areas with a high prevalence of multidrug resistant parasites [38, 39]. AS-AQ has been recently introduced in regions of Sub-Saharan Africa. AS-AQ also suffers from a PK mismatch due to the long half-life of amodiaquine, which ranges from 9-18 days [40]. A number of studies in Africa have shown increases in drug failures associated with resistance to amodiaquine [41]. Drug resistance is likely to increase and spread given AS-AQ has been adopted as the treatment of choice in a number of countries in Africa [8].

The introduction of mefloquine into Southeast Asia resulted in development of drug resistance in a period of 4 years. This lead to efforts to create a combination drug comprised of mefloquine and sulfadoxine pyrimethamine (MQ-SP or FansiMef). This drug combination ultimately failed, and this failure was predicted in advance due to the mismatch in drug half-lives between mefloquine and sulfadoxine pyrimethamine. Mefloquine has a half-life of 14-21 days while pyrimethamine, the longer-lived of the two antifolate drugs, has a half-life of 10 days. This mismatch left mefloquine unprotected from development of parasite drug resistance during a period of sub-therapeutic treatment in the bottom half of the elimination period [42]. In contrast, the introduction of artesunate combined with mefloquine lead to significant reductions in malaria deaths, reductions in drug resistant parasites, and documented reversals in mefloquine drug resistance [43]. If SP could not have been expected to protect MQ against tile emergence of resistance because of a PK mismatch, why did the AS-MQ drug combination with an even more profound half-life mismatch (AS <1 hour, MQ 14-21 days) succeed [5]?

Whether the combination treatment failures are principally related to PK mismatch or not, the solution to the mismatch problem is conceptually straightforward: formulate and administer combination therapies in which pharmacokinetic profiles superimpose. PK match is not the only relevant factor and other factors, which influence treatment failure, such as poor patient compliance with therapy regimens, combination drug interaction, PD profiles, and drug related toxicity, should still be considered.

4. PK mismatch does not lead to emergence of ACT resistance

4.1. Simply matching PK half-lives is not sufficient to design the best drug combinations

Artemisinin derivatives are particularly effective when used as combination therapies because they are well tolerated, have excellent parasiticidal properties, but there is currently early evidence of clinical drug resistance [24-26]. PK matching has been required by regulatory agencies such as the FDA for anti-infective drug combinations; however, simply matching the half-lives of prospective ACT partner drugs will not be adequate to provide dual pressure on falciparum parasites [5]. One example of this is the chloroquine which has a very long half-life of 4-6 weeks, however, drug elimination is not constant and drug concentration above the minimal inhibitory concentration is likely much shorter. This leads to exposure of parasites to sub-therapeutic drug concentrations. The time when antimalarial drugs persist at sub-therapeutic concentrations is defined as the “selective window”. During this selective window

period, the concentration of antimalarial drugs is sufficient to inhibit the growth of drug sensitive parasites but insufficient to prevent the growth of drug resistant parasites. As chloroquine has a long period of sub-therapeutic drug concentration (and hence a long selective window) this explains why chloroquine is vulnerable to emergence of drug resistant parasites.

When exposed to two drugs, parasites would need to develop mutations at two different genes simultaneously, and the likelihood of this occurring (i.e., $10^{-6} \times 10^{-6} = 10^{-12}$) becomes extremely small [44]. The probability of selecting a mutant parasite with resistance to both drugs is very unlikely. Quick parasite killing by artemisinin compounds results in very few parasites left to become drug resistant, however, artemisinin monotherapy must be administered for 7 days which does not work well in practice given poor adherence to this regimen, and 7 day regimens of artemisinin compounds have been shown to fail in 10% of subjects [45]. ACT combinations must include a drug partner which has a half-life greater than 24 hours. By combining an artemisinin drug with a slowly eliminated compound such as MQ (half-life of 20 days) given for 3 dayscomplete protection is provided for the artemisinin derivatives (< 1 hour half-life) from drug resistance. MQ, however, is left unprotected, which demonstrates the mismatch in drug partner PK profiles. The number of residual parasites exposed to MQ alone, following two asexual cycles, is a tiny fraction of those present at the peak of the acute symptomatic infection. In such regimens, therefore, the PK profile mismatch is unavoidable.

The residual parasites left after artesunate killing are subsequently exposed to elevated concentrations of mefloquine. Despite any presence of drug tolerant parasites, the concentrations of mefloquine present are likely sufficient to kill the remaining residual parasites. However, the long elimination half-life of mefloquine will provide a filter to select for the survival of drug resistant parasites newly acquired which will enhance the spread of drug resistant parasites. In low transmission areas of northwestern Thailand where mefloquine resistant parasites are present, the use of artesunate-mefloquine was very efficacious in treatment of drug resistant parasites and inhibiting malaria incidence [43, 46]. AS-MQ will likely be useful in other regions such as in Africa where higher transmission rates of malaria are present.

There are major concerns that mismatched PK profiles will allow parasites to evolve resistance to the longer half-life partner, undermining the benefits of ACTs. Drug combinations with mismatched PK profiles do not decrease the spread of resistance and drug partners should have complementary PK profiles to insure drug pressure from both partners is sufficient to avoid selecting for drug resistance. Artemisinin 'resistance' or 'tolerance' has developed in Southeast Asia manifested by delayed parasite clearance and reduced sensitivity to ACT [3]. *In vivo* data derived from PK/PD studies should provide the basis for defining the time above the minimum inhibitory concentration required for treatment and further characterize the selective windows that partner drugs provide. Both critical pieces of PK/PD data will help drive partner selection of drug combination. Each new combination derived will present with advantages and disadvantages based on cost, tolerability, PK profile matching, and ease of administration.

Drug combination therapy has been experimentally assessed for treatment of multidrug resistant tuberculosis, which in a variety of ways, mimics some of the challenges faced today for antimalarial drug development. Just as in antimalarial drugs, the hypothesis that PK-profile mismatch could explain the emergence of drug resistance was tested using two common anti-tuberculosis drugs, rifampin, and isoniazid. The hypothesis being tested was the larger the PK profile mismatch, the greater the size of isoniazid and rifampin resistant bacterial subpopulations would become. To test this hypothesis, investigators examined the sterilizing and bactericidal effects of hollow-fiber-system studies in experiments lasting up to 42 days. These experiments were designed to mimic the PK of rifampin and isoniazid administered to patients. Rifampin was administered first, followed by isoniazid 0, 6, 12, and 24 hours later.

Statistical analysis of the different combination regimens showed that the 12 hour and 24 hour mismatched regimens consistently killed better than the PK matched regimens for both sterilizing effects and bactericidal effects ($p<0.05$). The results from this experiment suggest changing order of administration or scheduling of drug administration could result in enhanced microbial killing. A number of analysis of variance calculations were conducted in this study, and the authors concluded that rifampin-resistant and isoniazid-resistant subpopulations were not significantly increased due to PK mismatch. Thus, the PK mismatch hypothesis was rejected to explain development of drug resistant tuberculosis. Instead, the authors concluded that sequential administration of anti-tuberculosis drugs following a particular schedule is the best new paradigm for accelerating killing of M. tuberculosis. In addition, the authors concluded that current efforts aimed at better PK matching to decrease tuberculosis resistance emergence are likely futile and counterproductive [9].

4.2. PK mismatch does not lead to emergence of resistance to ACTs

Antimalarial drugs of the quinolines, antifolates, and artemisinin classes have been deployed in various combination therapies with each other to both increase the efficacy of treatment as well as delay the incidence of drug resistance. In most of the ACTs currently in use or being evaluated, e.g. AS-MQ, the partner drug is eliminated slowly. The partner drug is therefore unprotected once artemisinin has been eliminated from the body, and this lack of protection for the long-lived partner creates selective pressure for emergence of new drug-resistant infections. The consequences of using drugs with mismatched PK profiles are not understood particularly in regions of high malaria transmission found in Africa. The safest approach is to combine drugs with the shortest elimination half-life capable of clearing parasites in a 3-day drug treatment regimen. Given the array of drugs on hand, this is not easy to do.

All of the antimalarial partner drugs on market have half-lives ranging from 5 days to 30 days, which leaves these partner drugs open to the eventual development of drug resistance [7]. Conversely, the use of long-lived partner drugs does provide a very good post-treatment prophylactic effect, which reduces the probability of future new infections. This extended post treatment prophylactic effect may come at the expense of the longevity of the drug combination treatment as drug resistance arising from PK profile mismatch could limit the time a particular combination therapy is effective. Drug combinations designed to deter emergence of drug resistance should have two important features; different, synergistic mechanisms of action and

a compatible PK profile. As reviewed by Hastings and Watkins [7], drug combinations with PK profile mismatches do not deter the spread of drug resistant parasites and could cause problems with the efficacy of currently used still effective drugs. This underscores the need for compatible PK profiles among combination drug partners ideally with the shortest half-life possible [8].

In another study, the *in vitro* susceptibility of parasites to MQ was followed in patients treated with the AS-MQ drug combination. Although the combination of AS and MQ constitutes a PK mismatch, there has been no decline in the *in vitro* susceptibility of parasites to MQ during a 5-year study period; in fact, the sensitivity to MQ has increased significantly. This contrasts with a 40% decrease in efficacy *in vivo* to MQ in the 5 years before the study [47]. Cure rates with AS-MQ remain almost 100%. The protective effect of AS on MQ when both drugs are administered in a combination regimen is believed to be against resistant parasites, which ensures high cure rates. In addition, the considerable reduction of parasite biomass by artesunate, which reduces the chance of selecting MQ-resistant mutant-parasites, and artesunate driven reduction in parasite transmission through reduction of gametocyte carriage rates are additional benefits provided by this combination [48, 49]. These factors reduce the selection of MQ-resistant parasites [50]. This study has shown that through a 5-year study period that artesunate-mefloquine remained effective with no decrease in malaria drug sensitivity observed. This data may have been superseded by recent reports of artesunate drug resistance associated with a new molecular marker, mutations in the propeller region of a kelch protein, K-13 [24-26, 46].

Dihydroartemisinin -piperaquine (DP) is an ACT with a very large PK profile mismatch as piperaquine is characterized by the longest mean terminal elimination half-life when compared to other long-lived partners (Table 1). The cumulative risk of any parasitological failure, however, was greater for the AL, AS-MQ, and AS-SP combinations than for DP, which reflects the post-treatment prophylactic effect piperaquine given its long biological half-life of piperaquine. Various formulations of DP have been shown to be highly effective for treatment of uncomplicated *P. falciparum* and vivax malaria. A number of trials have shown that DP is similar or superior to AL, AS-MQ, AS-AQ, and AS-SP for treatment of malaria in children and adults in Asia and Africa. Fortunately, DP does not have the same selective pressure, and likely not the same mechanisms of resistance as chloroquine and amodiaquine. Piperaquine offers many advantages as a combination partner for treatment of uncomplicated malaria including superior efficacy, lower toxicity, and long-duration prophylaxis. Accordingly, the DP combination offers significant benefits over other available ACTs making it a prime option for the management of uncomplicated malaria [16].

Another important finding which does impact on the pharmacological mismatch hypothesis as applied to antimalarial drugs is clinical data that shows that more mismatched regimens have consistently been associated with better and faster microbial killing than perfectly matched regimens. Pharmacokinetically, the combination of artemether and lumefantrine is better matched compared to other ACTs (Table 1). It appears to be slightly less efficacious, however, than AS-MQ in areas with a high prevalence of multidrug resistant parasites [39]. In one clinical trial (537 participants), the efficacy of a six-dose regimen of AL was compared to AS-MQ. No differences in day 28 parasitemia were noted, and no differences in parasite or fever clearance times were detected. There were, however, 11 parasitological failures with AL

and none with ASMQ [12]. The results of this clinical trial suggest that AS-MQ therapy is more effective than AL therapy [13, 51]. This also suggests that solutions that rely on minimizing PK mismatch, such as fixed dose combinations, and the design of regimens that rely on better PK profile matching to close the monotherapy "window," will likely be ineffective solutions for combating drug resistance.

4.3. The Impact of PK Matching on Malaria Parasite Epidemiology

Antimalarial drug resistance usually starts in low transmission areas. Patients infected with falciparum malaria from regions with low or intermediate malaria transmission are generally symptomatic and require full treatment with ACT drugs to achieve a cure. Accordingly, the treatment of these patients is more likely to select for drug resistant parasites. By contrast, patients infected with falciparum malaria from regions with higher rates of transmission have a greater innate immunity developed through sequential infections, which may or may not be fully treated in low transmission areas, and it is in such areas that the benefits of antimalarial combinations would be greatest [49].

Optimized treatment plans must consider malaria transmission intensity as the probability of a new infection occurring during the post-treatment drug elimination phase equates to a trade-off in terms of the elimination half-life required for an ACT partner. Exposure of patients to new malaria infections in high transmission areas occurring every day or every week may translate to a need for longer post-treatment prophylaxis rather than complete parasite eradication. Longer half-life drugs have enhanced risk for driving parasite drug resistance. For patients who are exposed to intermittent infections in low transmission areas, complete parasite eradication to deter drug resistance may be the more important requirement and mismatched PK profiles between partner drugs may be of less consequence [17].

The rejection of the PK mismatch hypothesis for emergence of long-lived drug resistance may not be applicable, however, to all malaria areas. In areas of high malaria transmission, use of artemisinin derivatives might have little or no effect on malaria incidence. The elimination half lives of partner drugs should match because the matching elimination kinetics is especially important in areas of high malaria transmission due to exposure of parasites to declining concentrations of one drug in a drug combination, which selects for resistance. Semi-immune individuals commonly harbor sexual and asexual parasite forms, which, in turn, increase the number of gametocytes present, which may offset any gains in transmission reduction through ACT treatment in endemic areas [49].

5. PK profile matching is one of many strategies to prevent or slow the spread of drug resistance

All CTs and ACTs currently recommended by the WHO rely on partner drugs that are already compromised by resistance including the artemisinins [24-26]. As ACTs are rolled out, optimizing their effectiveness and slowing their resistance will require selection of partner drugs based on PK and PD parameters, and such trials will target the right drugs to the right

settings, which consider the broader impacts of different treatment regimens. New WHO policies are made based on good clinical research, and the mission to replace failed malaria drugs with more efficacious ACTs is being accomplished. However, maximizing the benefit and prolonging the life of ACTs will also require learning from the past. New strategies are needed to prevent ACTs from established drug resistance existing against partner drugs. Better understanding of the drug mechanisms of action, the PK profile match, PD profiles, drug toxicity, and malaria epidemiology will be required to design and deploy well-suited combination therapies that provide prolonged prophylactic efficacy while still deterring resistance. To delay the onset of resistance, the drugs used in combination should have compatible PK and PD profiles, no adverse pharmacological interactions, and no additional toxicity.

Artemisinin resistance has been confined to Southeast Asia [24-26], however, there is no reason why artemisinin resistance could not break out in other regions. Clinical research has shown the combination of artemisinin with an efficacious partner drug can improve cure rates to an adequate level even in areas where partner drug resistance has been observed [25]. Previous models demonstrating the benefits of ACT in delaying the onset of resistance largely assumed parasites are either completely sensitive to the drug, or else completely resistant [52]. This is an overly simplistic approach to modeling drug resistance, and knowledge of PK/PD mechanisms can guide development of more realistic models of drug resistance [53]. While PK profiles describe the disposition of drugs by the human body, PD parameters actually measure how drugs act on the parasite. PD parameters that are relevant include fever clearance (FCT) and parasite clearance times (PCT), time above the minimum inhibitory concentration (MIC), the half maximal inhibitory concentration (IC50) or accumulation of drug inside the parasite working at the drug target. This can be described by defining PK parameters after treatment and combining that data with PD parameters *in vivo*. Unlike models based strictly on differences in *in vitro* drug sensitivity or resistance, these combined PK/PD models do throw light on what properties define a good CT, and therefore, we believe that a combination of PK/PD considerations can usefully contribute to the rational design of drug combinations.

Antimalarial drugs are primarily deployed as combination therapy as a mechanism to prevent or slow the spread of resistance. Hastings and Hodel have defined six key PK/PD considerations for potential combination therapies [1]:

5.1. The half-lives and activity profiles of partner drugs

Mutual protection between partner drugs is dependent on having active concentrations of both drugs at the same time during treatment, and post-treatment. It, therefore, appears more important that drug antimalarial activity profiles post-treatment are the critical factors to be matched, rather than simply their half-lives. One way in which matching of drug killing can be quantified is by their time above the minimal inhibitory concentration (MIC) a concept originally used in bacteriology but now being increasingly used in malaria.

The time above the MIC is determined by the pharmacokinetic profile of a drug, and a number of parameters such as the maximum concentration attained and drug clearance affects this parameter. The MIC itself has been approximated by many using *in vitro* data derived from drug potency testing where the IC_{99} is used to approximate the MIC. Determining the time

above the MIC can be approximated graphically by plotting the MIC concentration against the drug concentration versus time curve. By considering the interaction between a variety of PK and PD factors, combination drugs can be designed to minimize the effect of an adverse parameter by altering dosing to achieve a better PK/PD match post-treatment. Maximal concentrations of both drugs optimize clinical effectiveness and help protect against resistance being driven through drug failures [31]. The natural variation in PK/PD may largely undermine matching done on average PK/PD values.

5.2. Natural variation in population PK/PD

The variation in drug sensitivity for a parasite is a key PD parameter assessed *in vitro* by drug potency testing with an IC50 endpoint (the half-maximal inhibitory concentration). By assessing, the average IC50 values against a battery of parasites from different regions, better PD matches can be made when considering the variation that naturally may occur particularly when drug potency is assessed against multi-drug resistant parasites. Understanding the variation in parasite drug sensitivity is an important component for creating a PD profile match [54]. Constructing a balance between drug half-lives, dosing, and sensitivity of parasites to antimalarial drugs must be conducted based on population averages. Use of average values does have its drawbacks, as there is considerable variability in both PK and PD parameters in a population. Mismatches in PK and PD parameters will no doubt occur which may affect individual treatment of patients and may affect the extent to which mutual drug protection can be provided by each partner drug. Most arguments for matching half-lives are overoptimistic in nature and fail to consider how factors such as natural variation in PK/PD can affect a match made solely on one PK parameter. In conclusion, it is only plausible to make approximate matches based on drug half-lives. Most drugs outside the artemisinin class have relatively long half-lives, and they can be approximately matched with the limitations listed above.

5.3. Dosages and toxicity

Combining two drugs always increases the risk of toxicity, but it is important to quantify this increased risk and to describe how it can be mitigated. There are two main types of toxicity associated with malaria drugs. One type is dose- or concentration -dependent adverse drug reactions (ADRs), which are mostly predictable and consistent between patients because they are explained by the known pharmacological action of the drug. The second type is dose or concentration independent, and this type of toxicity is largely unpredictable and dependent on the metabolism, immune system, or genetics of individual patients. Changes in dosing of a combination with decreased concentrations of each partner drug requires drug efficacy modeling using both PK and PD parameters to answer the questions associated with the outcomes of reducing the doses of partner drugs in a combination treatment which may have an impact on both efficacy and drug resistance [54].

5.4. Mechanisms of action

Independent mechanisms of action provide complementary pharmacodynamic effects, and, indeed, drug combinations may provide benefits of drug synergy against malaria parasites that go beyond simple additive effects (drug combinations may also have antagonistic effects which are not helpful for combinatorial efficacy). Combining drugs with additive or synergistic action should also increase parasite clearance post-treatment and hence may speed the resolution of symptoms. One good example of such a drug combination is Malarone™, the combination of atovaquone and proguanil. The addition of proguanil, a weak antifolate drug, provides significant synergy to the action of atovaquone against the malaria parasite. Atovaquone as a single agent is quite vulnerable to development of malaria drug resistance (one single base pair mutation in the malaria parasite's *cytb* gene equates to complete drug resistance to atovaquone). Another well-known example is the synergy observed between artesunate and mefloquine; the combination of the two drugs together provides greater efficacy than the action of each individual drug. Drug synergy can provide significant benefits to overall efficacy, but any observed synergy should not necessarily be used to decrease the doses of each partner drug in a combination except where necessary to diminish toxicity. The ideal approach is to dose each drug at a monotherapeutic dose in a combination to maintain efficacy of the combination even when drug resistance is present against one partner drug.

5.5. PK Profiles

Drugs in combination treatments may have different pharmacokinetic profiles when dosed together compared to when they are dosed individually. Process of drug elimination and clearance should include an examination of the effects on drug disposition of dosing two drugs at the same time. If drugs share the same conversion or elimination pathways post absorption, then their actions could become non-additive but in unpredictable ways. Conversely, if the same elimination pathway saturates for both drugs, and both parent forms are active, then drug half-lives may be extended and anti-malarial synergy may arise. In essence, the consequences of PK interactions are difficult to predict and may be dependent on exact metabolic pathways utilized for drug elimination, which determines whether active metabolites have antiparasitic activity. PK interactions can be readily assessed in both *in vitro* and animal models prior to administration to patients in clinical trials. Early drug safety and pharmacokinetic studies are generally conducted using healthy volunteers, and the pharmacokinetic effects of malaria infection cannot be assessed definitively in these early studies.

5.6. Common mechanisms of resistance

Combination therapy provides benefits to patient care based on the need for a parasite to develop resistance to both drugs to avoid being killed. Intuitively, cross-resistance between the constituent drugs in a CT will undermine this effect and modelling shows that even small amounts of cross-resistance may have an adverse effect on the usable lifespan of both drugs in a combination [55]. Combining drugs from the same class into a combination treatment is not the best practice [2, 4] given mutations may arise that provide resistance to both drugs in the combination. This has been shown to occur with antifolate drug combinations such as

sulfadoxine-pyrimethamine. A more significant drawback to using drugs from the same class is the likelihood that both drugs actually share the same drug target and potentially a similar mechanism of drug toxicity.

PK/PD matching of partner drugs is mainly achieved through deployment of appropriately matched partner drugs in combination to enhance efficacy and reduce the probability of selecting for drug resistance. In many models of drug resistance, this is modeled as purely a parasite specific component that will inform whether a patient will potentially be cured by a malaria drug. A PK/PD model based on drug mechanism of action is structured differently and the potency of a drug against the malaria parasites (as assessed by the IC50) is one of many possible variables that may influence treatment outcome. In order to model why individual patients fail treatment, it is essential to know how a particular drug combination actually clears an existing infection. Putting resistance into this matrix of variables will inform the PK/PD parameters associated with treatment success and failure. Accordingly, rational and objective models based on PK/PD parameters are the best tools available to project whether a particular combination treatment is a good choice for a particular area [1].

Treatment of malaria disease today is focused on using combination therapies, and defining PK/PD parameters accurately to achieve the best drug match which is essential to reduce the speed at which resistance develops. Therefore, three more PK/PD considerations for potential combination therapies are required which we will discuss in detail below.

5.7. Partner drug-drug interaction

Drug-drug interactions occur mainly during drug absorption and during the phase of drug distribution. These processes are dependent on active transport pathways, plasma protein binding of drugs, drug biotransformation, and excretion. Most drugs are given orally, and subsequently, drug absorption occurs through the intestinal mucosa, mainly in the duodenum. The bioavailability of drugs is dependent on a P-glycoprotein found in the enterocytes of the intestine.

The *Pglycoprotein* can actively pump back drugs back into the intestinal lumen, which in turn drops the bioavailability of any orally dosed compound. Metabolism of compounds is dependent on the activity of cytochrome P450 (CYP450) enzymes found in enterocytes, which can metabolize drugs before they reach systemic circulation. Induction or inhibition of the *Pglycoprotein* and enterocyte CYP450 enzymes can thus influence drug bioavailability. In patients infected with falciparum malaria, concomitant administration of oral artemisinin and mefloquine has been shown to significantly increase the AUC and decrease the oral clearance of artemisinin. In contrast, concomitant oral administration of dihydroartemisinin (DHA) and mefloquine to healthy volunteers has been shown to have no effect on either partner drug, except for a slight increase in the absorption rate of mefloquine. Similarly, no alteration in the pharmacokinetic s of either atovaquone or proguanil was observed following concomitant treatment with artesunate (AS) in healthy adult volunteers [56].

5.8. Drug-tolerant parasites

ACTs may also decrease transmission of drug-resistant parasites by reducing the number of gametocytes carried by patients. Optimal combination plans must consider the intensity of malaria transmission because the likelihood of a new infection after the drug elimination phase translates into a consideration between shorter-acting or longer acting combination drugs. Patients living in endemic areas who experience new infections on a regular basis may benefit more from a longer period of post-treatment prophylaxis than absolute eradication of all infecting parasites. This benefit may be worth using a longer duration partner drug that is more prone to develop drug resistance and hence have a shorter life span. Overall, the goal of deterring drug resistance must be achieved through elimination of parasite transmission. In 1996, Kyle et al [57] described the dormancy theory, in which some of the parasites, called "sleeping beauties" exposed at ring stage *in vitro* become metabolically inactive and resume growth after removal of the drug. Others have since confirmed this phenomenon [58]. Until recently, it was reasonable to assume that, with a correct prescription (dosage and administration), the artemisinin derivatives were universally effective against *P. falciparum*. Long-term eradication strategies must encompass drug treatment with either ACTs or low dose primaquine to achieve reductions in parasite transmission. Development of novel gametocidal drugs is clearly a priority to insure that drugs are available to achieve the "last mile" of malaria eradication [59].

6. PK/PD profile matching will deter resistance to ACTs

The principle problems in deterring the emergence of malaria drug resistance are associated with the use of malaria monotherapy or incomplete treatment. Not only are there well-known problems with drug compliance associated with patient treatment of malaria there are also issues of poor drug quality and access to malaria drugs in the marketplace without physician oversight. Broad resistance to all antimalarial drugs in various geographic regions, now including the artemisinins, has occurred historically [24-26], and therefore, the treatment of malaria disease by CT must employ an array of methods to find the most appropriate combination partner drugs for a particular geographic region. We have suggested the use of PK and PD parameters for selection of the most appropriate partner drugs, and there are some studies where this approach has been taken to find the best PK/PD match.

PK/PD matching between combination partners has been evaluated in a few studies. The artemisinin derivative, dihydroartemisinin (DHA), and MQ were investigated in zero healthy Thai males in a study designed as a three-way crossover study [60]. Patients in this study were distributed into three treatment groups dosed with three different combinations of DHA and mefloquine. In the first group, patients were administered a single oral dose of 300 mg of DHA, the patients in the second group were given a single oral dose of 750 mg of MQ, and the patients in the third group were given 300 mg of DHA and 750 mg of MQ. The patients in all three groups tolerated their treatments well. Oral DHA was shown to be quickly absorbed and metabolized within 8-10 hours while MQ showed a different pattern. The absorption and

clearance of MQ was slow compared to DHA, however, the absorption rate of MQ was faster when co-administered with DHA suggesting a synergistic process.

Pharmacodynamically, the combination of DHA and MQ resulted in a synergistic schizonticidal activity when tested *ex vivo* using blood from treated subjects. The maximum activity (E_{max}) of DHA was increased by a factor of two, and the (E_{max}) of MQ was increased by 20 fold compared with each drug alone. The area under effect-time curve (AUEC) of DHA was increased by factor of 4, and the AUEC of MQ was increased by a factor of two compared with each individual drug alone. The AUEC of MQ during the first 24 hours ($AUEC_{0\text{-}24h}$) was increased approximately 50-fold in the presence of DHA [61]

The dynamic profiles of both DHA and MQ were shown to coincide generally with their kinetic profiles in this study. The co-administration model for AS and MQ provided a more accurate estimation of the relative contributions of the benefit to therapy without any enhancement of adverse effects of each individual drug. The model-independent PD measures showed significant increases in the AUEC and the maximum response (E_{max}) of both DHA and MQ when they were given in combination compared with each individual drug alone. MQ was not shown to have a significant effect on the maximal effect of DHA, while DHA co-administration dramatically increased the E_{max} of MQ [61].

The combination of artemether and lumefantrine is a new and very well tolerated oral antimalarial drug effective even against multidrug resistant falciparum malaria. The artemether component is absorbed rapidly and slowly biotransformed to dihydroartemisinin, and both the parent and metabolite were eliminated with half-lives of approximately 1 hour. Both artemether and DHA provide rapid reduction of the parasite biomass and quick resolution of symptoms. Lumefantrine has a half-life of 3-6 days with highly variable absorption dependent on co-administration with fat, which enhances absorption. The role of lumefantrine is to clear the few parasites that actually remain after artemether treatment, and lumefantrine plasma concentrations on day 7 (or the area under the curve) have been shown to correlate with treatment outcomes. [62, 63].

Traditionally, the selection of doses of artemisinin drugs in ACTs has been empirical in the absence of an established PK/PD relationship. PK/PD matching of artemisinins has been studied in detail particularly when artemisinins are administered in combination treatment. Based on PK/PD modeling, Hoshen et al. postulated that a small fraction of malaria parasites become cytostatic or dormant because of chemotherapeutic pressure. At this stage, the growth and development cycle of the parasite is halted, which results in the presence of parasites that are not drug susceptible to further dosing until the dormant state ends. This hypothesis suggests that the antimalarial activity of AS would be slower entailing either frequent doses or an extended period of treatment and surveillance. Based on modeling, the authors developed a strategy for selecting rational models for antimalarial chemotherapy [64].

Hoshen et al. [64] experimentally modeled pharmacokinetic and clinical data derived from a variety of regimens utilized to treat Thai patients treated with AS combined with mefloquine. The model assumed no PD interaction between AS and mefloquine, however, the parasites in many areas of Thailand have been shown in a variety of studies to already be resistant to

mefloquine. The model produced suggested that treatment with either AS or mefloquine administered as monotherapy would fail, however, treatment with a daily dose of an AS-MQ drug combination for 3 days would be successful. The predictions of this study were on target with clinical outcomes as shown in a variety of studies examining the efficacy of the AS-MQ combination. This study yielded some very useful data, and the results merit a wider application for other drug combinations [64].

Angus et al. studied the dose-response relationship of AS used to treat acute falciparum malaria in adult Thai patients. This is the only study to date to report the dose response relationships of an artemisinin antimalarial agent used for patient treatment. In this study, patients received single oral doses of AS followed by mefloquine. The proper PD model was applied with dose, the measured drug concentrations of AS and its active metabolite DHA in the plasma, and parasite clearance values (the half maximal parasite clearance time or PC_{50}, the time required for 90% of the parasites to be cleared or the PC_{90}, and the overall parasite clearance time or PCT). There was considerable variance in the PC50 and PC90 values observed in patients dosed with mefloquine alone or with low doses of AS.

The EC_{50} required to achieve the half-maximal parasite clearance (PC_{50}) was shown to be 0.7 mg/kg, and the dose required to achieve 90% parasite clearance was shown to be 1.2 mg/kg (the PC_{90}), and a dose of 1.4 mg/kg was required to achieve 100% parasite clearance (PCT). The E_{max} was achieved at 28.6 hours, and the EC50 was estimated to be 1.6 mg/kg. These results suggest that a dose of AS of 2 mg/kg is the lower limit of a maximum effective dose. Current practice includes a dose of 4 mg/kg of AS per day over a 3-day period in combination with a partner drug. This dose has been shown to be both safe and effective for treatment of uncomplicated falciparum malaria. For the artemisinin compounds, it is not clear which pharmacokinetic parameters is the most relevant related to a pharmacodynamic outcome. Additional studies will be needed to understand the relationship between concentration and effect for these compounds [65].

For the majority of antimalarials, parasite killing is not known to be strictly dependent on sustaining drug concentrations above the minimum inhibitory concentration or MIC. Therefore, getting a good match between pharmacokinetic and pharmacodynamic profiles to avoid the emergence of drug resistance is made more complex by the relationship between the nature of the antiparasitic action of a drug and the length of time required to achieve parasite killing. These factors interact with the selective window of a prospective antimalarial drug, which defines the period where sub-therapeutic levels of drug are present. This period of selectivity should be covered by a partner drug to avoid drug resistance.

There are two possibilities here; parasite killing of an antimalarial drug is dependent on the time that drug concentrations are maintained above the MIC (which might be as long as several 48-hour parasite life cycles), or the antiparasitic activity of an antimalarial drug might be dependent on total drug exposure as measured by AUC. The period of time during terminal drug elimination when drug levels fall below the MIC is called the "selective window". This window has been defined for pyrimethamine where a single dose of drug selects drug-resistant parasites after a period of 52 days. The selective window has also been defined for lumefantrine where a single dose of drug results in lumefantrine resistant parasites after a period of 30 days

[5]. The ACTs in current use and recommended by the World Health Organization include, AS-SP, AS-AQ, AS-MQ, AL and DP, which are currently the most popular ACTs on market. For all of these drug combinations, the partner drug lasts far longer than the artemisinin component, and drug resistance has already been observed for each partner drug in various regions of the world to different degrees. ACT partner drugs may protect against induction of resistance to artemisinin drugs, and artemisinin compounds may prevent selection of parasites initially that are drug resistant, however, once artemisinin compounds are cleared, the partner drug is left with no protection and parasites will be exposed to sub-therapeutic drug levels during this "selective window" period.

Models have been created to analyze the probabilities of selection of drug resistant parasites, and these models suggest selection of drug resistant parasites is dependent on the fraction of patients with residual drug present from recent infections and not on the probability of a new infection occurring during the drug clearance phase. Accordingly, transmission intensity may not have a direct effect on selecting for drug resistant parasites [7].

Understanding the pharmacokinetic and pharmacodynamic profiles associated with drugs in the post-treatment period is clearly important. While the lower transmission rates found in Asia result in decreased use of drugs, these patients do present with symptomatic infections that must be treated. This, in turn, results in exposure of malaria parasites in these regions to malaria drugs. In contrast, patients in Africa are semi-immune to malaria infections and they may harbor reservoirs of parasites that are not treated as frequently. This may protect the population from selection for drug resistant parasites. While the use of artemisinin combination therapy has provided benefits to reversing declines in efficacy of partner drugs such as mefloquine in Asia, there may be consequences to the use of mismatched antimalarial drugs together in combination not yet observed [66].

We cannot predict with any confidence the fate of artemisinin drugs in Africa or the fate of their partner drugs. It may be that artemisinin drugs can reverse resistance to partner drugs, similar to the outcome of AS-MQ in Asia, or it is possible that these partner drugs and artemisinin compounds may fail in areas of Africa with higher malaria transmission rates. Factors associated with drug resistance such as the interaction of pharmacodynamics, pharmacokinetics, malaria epidemiology, and immunology should be considered when designing the next generation of antimalarial combination treatments. Conducting drug trials over extended periods of time will be necessary to understand how these variables interact, and the use of molecular markers of drug resistance for combination partners will be essential to monitor the emergence of drug resistance early prior to the emergence of treatment failures. A recent development in Malawi regarding the use of chloroquine showed drug resistance to chloroquine had disappeared 10 years after the government withdrew this drug from use. This provides a novel opportunity to examine the question of how long ACTs can deter the re-emergence of chloroquine resistance in this country [66]. Long-term studies of combination treatment with chloroquine or treatment with chloroquine analogues like piperaquine may assist in determining the best method of extending the life of existing drugs. [16, 19].

7. Conclusion

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 clinical evidence with a confirmed molecular marker to *P. falciparum* poor oral bioavailability, and short half-lives. Second- and third-generation artemisinins will likely be less expensive with improved properties. ACTs are more effective in malarial patients than other antimalarials in their ability to overcome recrudescence and minimize noncompliance through short duration treatment periods. To date, no clinically significant drug interactions have been observed with artemisinin combinations, which still require further study. The pharmacokinetic profiles of the artemisinins have been evaluated extensively in animals but not as extensively in humans, which is an unmet need. These compounds exhibit variable PK profiles and lack established PK/PD relationships to support defining rational dosage regimens for malaria treatment. Future efforts should focus on understanding all of the parameters required to conduct a valid PK/PD match between an artemisinin compound and an appropriate partner drug [67].

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