Combination Therapy in Light of Emerging Artemisinin Resistance

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Abstract Within less than a decade virtually all malaria-endemic countries have adopted one of the WHO-recommended artemisinin-based combination therapies (ACTs) for the treatment of falciparum malaria. In 2006, the first cases of clinical artemisinin resistance were reported from the Thai–Cambodian border. A number of factors are likely to have contributed to the development of artemisinin resistance in Southeast Asia. However, current evidence suggests that artemisinin resistance is simply a natural consequence of the massive deployment of ACTs in the region. The potentially devastating implications of resistance to a drug class to which there is currently no real alternative call for cost-effective strategies to extend the useful life spans of currently available antimalarial drugs. At the same time, major efforts to develop novel combination therapies not based on artemisinins are required.

1 Introduction

"The history of malaria contains a great lesson for humanity – that we should be more scientific in our habit of thought, and more practical in our habits of government. The neglect of this lesson has already cost many countries an immense loss in life and prosperity" [1].

With almost 800,000 deaths and hundreds of millions of clinical cases every year, much of what Sir Ronald Ross expressed almost exactly 100 years ago still holds true today [2]. In spite of major advances in the development of new artemisinin-based combination therapies (ACTs), the fact that malaria control is almost entirely reliant on a single class of antimalarials makes malaria control more

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vulnerable than ever before. Sir Ronald Ross was a British–Indian physician and entomologist, primarily noted for identifying the link between mosquitoes and malaria in the late nineteenth century for which he was awarded the Nobel Prize in Medicine in 1902. By that time, quinine was already firmly established in western medicine as the treatment of choice for malaria and the detection of the first cases of antimalarial drug resistance to quinine in South America was only a few years away. The discovery of the antimalarial properties of the bark of *Arbor febrifuga* (*Cinchona* spp.), a tree native to tropical South America, in the early seventeenth century had revolutionised malaria therapy. With the extraction of the main *Cinchona* alkaloids by Pelletier and Caventou in the early nineteenth century, the era of the "Peruvian bark" came to an end and the medicinal use of the bark was largely abandoned for the use of one of its main alkaloids, quinine [3]. Quinine was also the first antimalarial drug to which resistance was reported. In fact, the first reports of resistance (a series of treatment failures) emerged as early as in 1910 from South America [4, 5]. Surprisingly, throughout the twentieth century quinine resistance proved to have relatively little impact on the therapeutic use of the drug in most parts of the world and up till now it has never reached a level comparable to that seen with some of the synthetic antimalarials. Quinine is still widely used in malaria therapy and remains one of the most important partner drugs in antimalarial combination therapy. However, in recent years, the class of drugs that has drawn most of the attention and which is the basis for the majority of currently available combination therapies is the artemisinins.

Artemisinin is a sesquiterpene lactone extracted from sweet wormwood (*Artemisia annua* or Chinese: *qinghao*), a common plant native to temperate Asia, but naturalised and recently cultivated throughout the world. The first recorded use of the plant *qinghao* for the treatment of febrile illnesses dates back to the fourth century AD in China. Artemisinin was finally extracted and its antimalarial properties characterised in the early 1970s by Chinese scientists. Since then the use of the parent compound has largely been replaced by the use of its semisynthetic derivatives. Artesunate and artemether, the most commonly used artemisinin derivatives, are hydrolysed to dihydroartemisinin, which has a very short plasma half-life. This also means that virtually all artemisinin derivatives are likely to share an identical mode of action. Artemisinins are active against all asexual stages of malaria parasites and seem to exert some activity also against gametocytes [6]. Although the endoperoxide bridge seems to be vital for their antimalarial activity, the mechanism of action of the artemisinin compounds is still not fully understood [7].

More recently, fully synthetic peroxides have been developed as a promising alternative to currently used artemisinin derivatives. They contain the same peroxide bond that confers the antimalarial activity of artemisinins. One such peroxide, the ozonide OZ277 or arterolane, has recently entered Phase III clinical trials in the form of an arterolane maleate–piperaquine phosphate combination [8]. Originally, these compounds were developed as an alternative to circumvent the dependency on agricultural production of artemisinin. In the light of emerging artemisinin

resistance, their performance against artemisinin-resistant parasites may now decide their future more than anything else.

Unfortunately, the poor pharmacokinetic properties of artemisinins, particularly their short half-lives and unpredictable drug levels in individual patients, translate into substantial treatment failure rates when used as monotherapy, thereby suggesting their combination with longer half-life partner drugs. In the past decade, artemisinin and its semisynthetic derivatives have therefore become the most important basis for antimalarial combination therapies.

2 Combination Therapy

Combination therapy has a long history of use in the treatment of chronic and infectious diseases such as tuberculosis, leprosy, and HIV infections. More recently, it has also been applied to malaria treatment [9–11]. The theory underlying antimalarial combination therapy is that if two drugs are used with different modes of action, and ideally, also different resistance mechanisms, then the per-parasite probability of developing resistance to both drugs is the product of their individual per-parasite probabilities [12]. This is based on the assumption that throughout its history (e.g., chloroquine resistance has independently arisen only on a very limited number of occasions) this would make selection for resistance to a treatment combining two drugs with different modes of action extremely unlikely [13].

The WHO has recently defined antimalarial combination therapy as "the simultaneous use of two or more blood schizontocidal drugs with independent modes of action and thus unrelated biochemical targets in the parasite. The concept is based on the potential of two or more simultaneously administered schizontocidal drugs with independent modes of action to improve therapeutic efficacy and also to delay the development of resistance to the individual components of the combination" [6]. This definition specifically excludes a number of combinations commonly used in malaria therapy, such as atovaquone–proguanil or sulphadoxine–pyrimethamine, based on the assumption that the respective partners share similar modes of action and further reduces the number of currently available non-ACT combinations [14]. The WHO currently recommends five different ACTs (Table 1).

Compared to chloroquine, the cost of modern combination therapies is almost prohibitive. During the first years of deployment, the high cost of the new combination treatments therefore remained a major limiting factor. However, the past years have seen a major increase in donor funding. The Global Fund to Fight AIDS, Tuberculosis and Malaria alone has committed almost US $20 billion to support large-scale prevention, treatment and care programmes, including the massive deployment of combination therapies.

Table 1 List of ACTs recommended for the treatment of uncomplicated falciparum malaria by the World Health Organization [15]

Artemisinin derivative	Partner drug(s)	Formulation[a]	Resistance
Artemether	Lumefantrine	Coformulated	MDR
Artesunate	Amodiaquine	Coformulated	–
Artesunate	Mefloquine	Coblistered or codispensed	MDR
Artesunate	Sulfadoxine–pyrimethamine	Coblistered or codispensed	–
Dihydroartemisinin	Piperaquine	Coformulated	MDR

[a]The WHO recommends fixed-dose combinations over coblistered or codispensed formulations
MDR: recommended in areas of multidrug resistance (East Asia), artesunate plus mefloquine, or artemether plus lumefantrine or dihydroartemisinin plus piperaquine

3 Pharmacokinetic Mismatch and Compliance

In essence, the main concept behind combination therapy in malaria is to delay the development of resistance, to improve therapeutic efficacy, and to reduce malaria transmission. However, the optimal pharmacokinetic properties for an antimalarial drug (whether used in combination or as a single agent) have been a matter of debate. Ideally, antimalarial drugs should be present in the blood stream just long enough to cover the approximately three parasite life cycles (i.e., 6 days for *P. falciparum*) needed to eliminate all asexual parasites. In reality, this is difficult to achieve and a key to many limitations associated with ACTs seems to be the pharmacokinetic mismatch of the partner drugs [16]. A pharmacokinetic mismatch can also be a major factor contributing to resistance of the long-acting partner drug, which in the later stages of its presence in the blood stream is not protected by the short-acting artemisinins. This is not a problem as long as both drugs are fully efficacious on their own and as long as the drug levels of both drugs remain above the minimum inhibitory concentrations until all asexual parasites have been cleared. However, with a reasonable duration of drug administration, short half-life drugs will not be able to cover the minimum duration of drug exposure. At the same time, long half-life drugs will result inevitably in a long tail, during which the drug levels of the partner drugs will be below the minimum inhibitory concentrations and without protection from the artemisinin compound. This particularly applies to the use of ACTs in high transmission areas [17].

Compliance also remains a key factor in the rational use of antimalarial drugs. In many settings, directly observed therapy is not an option. While rapid elimination reduces the selective pressure by avoiding a long tail of subtherapeutic concentrations, antimalarial drugs with a half-life of less than 24 h (such as artemisinins or quinine) need to be administered for at least 7 days to be fully efficacious. Although compliance with malaria treatment is difficult to assess in study settings and shows significant variations across different studies, there is a general consensus that antimalarial treatment regimens lasting up to 3 days are likely to give good compliance [18].

4 Coformulation

Another potential problem of treating patients with more than one antimalarial drug is the fact that many currently available combination therapies are not coformulated, greatly increasing complexity of treatment and the chances of misuse. Currently, the only widely used coformulated combination is artemether–lumefantrine. More recently, coformulated combinations of artesunate–amodiaquine and dihydroartemisinin–piperaquine have become available but coformulated combinations of many other ACTs are still unavailable. Even though coformulated, artemether–lumefantrine remains a relatively complex regimen (with an adult dose of four tablets twice daily for 3 days) and compliance, and therefore programmatic effectiveness, is not optimal [19].

5 ACT and Antimalarial Drug Resistance

Throughout the past 100 years, drug resistance has emerged as one of the biggest challenges for malaria control. The extensive deployment of antimalarial drugs since the introduction of chloroquine in the 1940s has provided a remarkable selection pressure on malaria parasites to evolve resistance mechanisms to virtually all available antimalarial drugs. In essence, it is continuous drug pressure that results in the selection of parasite populations with genetically reduced drug sensitivity. The widespread and indiscriminate use of antimalarial drugs places a strong selective pressure on malaria parasites to develop resistance. Malaria parasites can acquire high levels of resistance, both in the individual parasite as well as on a population basis. The high degree of resistance expressed by malaria parasites is at least in part attributable to their high diversity and genetic complexity, resulting in a variety of potential mechanisms to evade drug activity. This way *P. falciparum* has developed resistance to virtually all antimalarials in current use, drugs that once were considered the front line against the disease. However, the geographical distribution and extent of resistance to any single antimalarial drug show major variations. Originally believed to be limited to *P. falciparum*, antimalarial drug resistance is now also known to affect other species [20]. *P. vivax* has rapidly developed resistance to sulfadoxine–pyrimethamine in many parts of the world, whereas high-level resistance to chloroquine remains confined largely to Indonesia, East Timor, Papua New Guinea and other parts of Oceania [6].

In the late 1990s, combination therapy was introduced to overcome the quickening pace of drug resistance development in Southeast Asia. However, by that time drug resistance had reached many of the potential partner drugs or at least drugs structurally related to those used in combination with artemisinins. This particularly applies to mefloquine, an arylaminoalcohol, which had previously been used extensively as monotherapy in Southeast Asia. Based on large-scale field trials starting in 1983 mefloquine was introduced as standard therapy by the

Thai Ministry of Public Health as early as 1985 to overcome increasing chloroquine and sulfadoxine–pyrimethamine resistance [21]. Interestingly, mefloquine was used in combination with sulfadoxine–pyrimethamine initially before being deployed as monotherapy. Rising numbers of failures with the standard-dose mefloquine (15 mg/kg) resulted in an increase of the dose to 25 mg/kg and in 1995 mefloquine monotherapy had to be replaced by the combination of mefloquine with artesunate, making this combination the first ACT to be deployed on a large scale in Southeast Asia.

In an attempt to limit the impact of increasing levels of resistance to traditional antimalarial drugs, in 2001 the World Health Organization recommended that all countries experiencing resistance to conventional monotherapies, such as chloroquine, sulfadoxine–pyrimethamine, or mefloquine should use combination therapies, preferably based on artemisinin derivatives for the treatment of uncomplicated falciparum malaria [21]. However, although ACTs have shown high efficacy in the treatment of malaria in Southeast Asia, where transmission is typically low, concerns remain about their long-term implementation as first-line therapy in high-transmission areas in Africa [19, 22].

6 Cross-resistance

The problem of antimalarial drug resistance is even further aggravated by the existence of cross-resistance among drugs with related chemical structures. This particularly applies to the 4-aminoquinolines (e.g. chloroquine–amodiaquine) and the arylaminoalcohols (e.g. mefloquine–lumefantrine) but also to artemisinin and its semisynthetic derivatives (e.g. artesunate–artemether). Malaria control has largely been relying on a small number of structurally related drugs essentially belonging to just a very few different classes. Once malaria parasites develop resistance to a single member of any of these classes, their sensitivity to most other antimalarials sharing a similar mode of action (i.e., typically belonging to the same class) is also compromised. This means that (e.g., in an area like Southeast Asia where the combination of artesunate and mefloquine is loosing its clinical efficacy) the introduction of ACTs using chemically related compounds, such as artemether–lumefantrine, may not be an option.

Interestingly, activity correlations derived from in vitro studies indicate that in spite of their different chemical structure there may be a certain level of cross sensitivity between artemisinin derivatives and certain arylaminoalcohols, currently the most commonly used partner drugs in ACTs [23]. The existence of this link is also supported by the potential role that the *P. falciparum* multidrug resistance 1 (*pfmdr1*) gene may be playing in simultaneously mediating the sensitivity to arylaminoalcohols and artemisinins [24].

7 Resistance to Partner Drugs

Preventing resistance to the partner drugs is obviously crucial. If the partner drug is not 100% successful in eliminating the parasites surviving the initial impact from the (subcurative) 3-day artemisinin treatment, ACTs are likely to select for artemisinin resistance. However, resistance has been reported against most of the commonly used partner drugs or at least to structurally closely related drugs resulting in activities considerably below 100%. Currently the most important partner drugs used in ACTs are lumefantrine, mefloquine, amodiaquine, and more recently piperaquine [22]. Mefloquine and lumefantrine are structurally related and belong to the class of the arylaminoalcohol antimalarials, whereas amodiaquine and piperaquine are both closely related to chloroquine.

Mefloquine is a synthetic antimalarial widely used throughout Southeast Asia as a combination partner for artesunate. It was introduced in Thailand in the mid 1980s. By the mid 1990s, resistance had reached a level that necessitated its combination with artesunate to reach adequate cure rates [21]. In spite of high levels of mefloquine resistance, particularly in Thailand, Cambodia, and Myanmar, mefloquine remains the most important ACT partner drug in the region.

Lumefantrine is commonly used coformulated with artemether and has never been used in monotherapy on any significant scale. Although clinical resistance to lumefantrine has not explicitly been reported, there is a strong indication of cross-resistance with mefloquine [25]. The use of lumefantrine is therefore not advisable in areas where high levels of mefloquine resistance have been reported.

Amodiaquine is a 4-aminoquinoline antimalarial originally developed in the 1940s. It is structurally closely related to chloroquine but due to its higher potency shows considerable activity also against chloroquine-resistant parasites. Resistance to both drugs also seems to be mediated by the same genetic mechanism [26]. Resistance to amodiaquine was reported soon after the advent of chloroquine resistance but has never reached its magnitude [27].

Although piperaquine, a bisquinolone antimalarial, is still considered to be a relatively new antimalarial drug throughout most of the malaria-endemic world, it has a long history of use in malaria treatment and prophylaxis in China [28]. Consequently, high levels of resistance have been reported from parts of southern China and resistance can relatively easily be induced in a *P. berghei* model [29].

8 Artemisinin Resistance

The statement "Resistance has arisen to all classes of antimalarials except, as yet, to the artemisinin derivatives" [6] in the WHO treatment guidelines from 2006 unfortunately does not hold true any longer. Clinical artemisinin resistance was first identified in 2006 in Ta Sanh, a small town in close proximity to the Thai–Cambodian border, a known hotspot of antimalarial drug resistance [30].

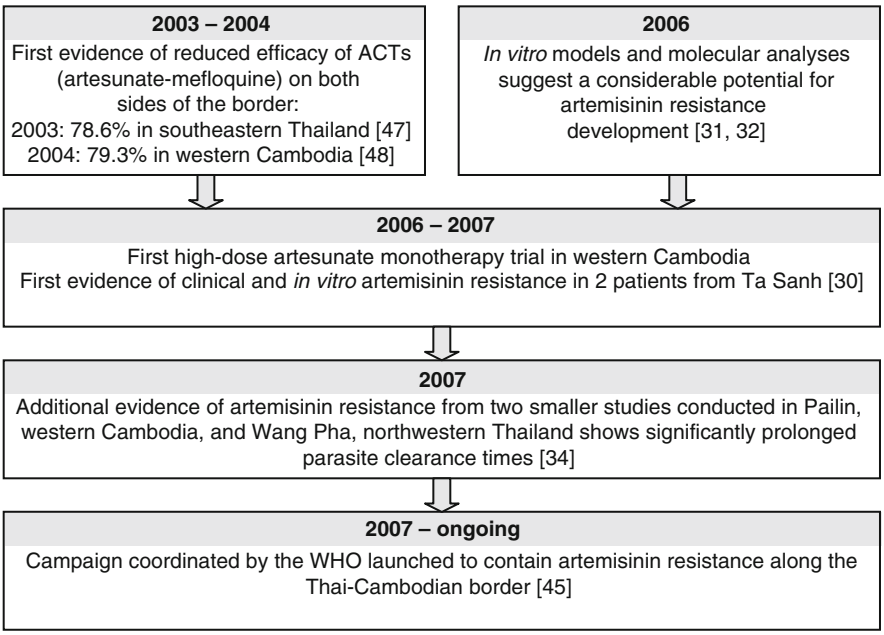

Fig. 1 Evidence of emerging artemisinin resistance along the Thai–Cambodian border

Even before the discovery of clinical resistance, in vitro models and molecular analysis had suggested a considerable potential for resistance development [7, 31, 32]. Moreover, ex vivo data and clinical treatment response seem to indicate that artemisinin sensitivity is also compromised in western Thailand [33, 34] (Fig. 1).

Not since the discovery of chloroquine resistance in the 1950s has malaria control been reliant upon the efficacy of a single class of drugs as much as it does currently. In the past 10 years, virtually all falciparum malaria-endemic countries have adopted some kind of ACT as first- or second-line therapy for uncomplicated falciparum malaria. In the absence of a defined artemisinin resistance mechanism and mechanism of action, as well as data from clinical trials using the new synthetic peroxides, it is hard to tell what impact the recent developments in Southeast Asia will have. However, in the current situation losing a single drug to resistance could potentially endanger virtually all malaria control efforts worldwide.

9 Defining Artemisinin Resistance

Defining artemisinin resistance remains a major challenge. The most commonly used definition of antimalarial drug resistance dates back to 1973 and defines resistance as “The ability of a parasite strain to survive and/or multiply despite

the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject" [38]. Although, – with minor modifications, – this definition remains valid as of today, it was developed for traditional monotherapies and in its original version has therefore only limited applicability to modern combination treatments. The most obvious problem in defining resistance to combination therapies is the fact that, with two or more combination partners, resistance might arise to a single component without ever becoming clinically evident. Since in ACTs artemisinins are generally responsible for the initial reduction in the parasite burden, the most obvious clinical parameter indicating reduced susceptibility to artemisinins is prolonged parasite clearance [39, 40]. Although data from ACT trials can provide important initial data on compromised drug sensitivity, a detailed assessment of treatment response to artemisinins requires extensive controlled monotherapy studies with artemisinin derivatives (including basic pharmacokinetics and a reliable way of excluding reinfections). These studies are currently being conducted in Asia and Africa. Ex vivo drug sensitivity data can be extremely helpful in interpreting geographical or temporal trends but need to be interpreted in a broader context with clinical data [33]. In spite of a number of interesting leads, as yet reliable molecular markers of artemisinin resistance have not been identified [31]. Artemisinin resistance can have major implications for malaria control programs in the affected countries and should therefore only be considered after careful analysis of treatment response parameters and treatment success in relation to ex vivo drug sensitivity.

10 The Causes of Artemisinin Resistance

The general view has been that several factors protect artemisinins from the development of resistance: the short plasma half-life of both the parent compound and its active metabolite, the rapidity with which the drugs clear asexual parasites, and the presence of an effective partner drug from a different class of antimalarials, which is expected to protect the artemisinin component [41]. It has been hypothesised that the de novo emergence of antimalarial drug resistance can be prevented by use of combination therapies [12, 42]. The assumption is that because of the logarithmic distribution of parasite numbers in human malaria infections, inadequately treated high biomass infections are a major source of de novo emergence of resistance. However, the recent events suggest that the hope that resistance can actually be prevented through combinations may have been overly optimistic.

Much of the blame for emerging artemisinin resistance in Southeast Asia has been assigned to the extensive use of artemisinin monotherapy. Compliance issues and counterfeited or substandard tablets that contain smaller amounts of or less active ingredients are considered to be additional sources of drug pressure [43]. Specific epidemiological, pharmacokinetic, and parasite factors in Southeast Asia have also been implicated in the development of artemisinin resistance [44]. Consequently, even before artemisinin resistance had been reported for the first

time the WHO banned artemisinin monotherapy in an attempt to protect the artemisinins and to slow down the development of resistance. Interestingly Vietnam, a country with one of the longest histories of artemisinin monotherapy use in Southeast Asia, was by far not he first to report artemisinin resistance. In Vietnam, artemisinins have been used for malaria control since 1989 and although the current national guidelines recommend the use of ACTs, artemisinin and artesunate are still widely available as monotherapy through the private sector [45]. Vietnam is also one of the few countries where artemisinin monotherapy can be linked directly to a highly successful malaria control program. Between 1991 and 2006, malaria cases in the country have diminished from 1,672,000 clinical cases with 4,650 deaths, originally, to 91,635 with 43 deaths [46].

Although the development of artemisinin resistance is likely to have been a complex multifactorial event, the actual explanation for artemisinin resistance is likely to be rather simple. ACTs have simply gone down the same road that all previous antimalarials have. Artemisinin resistance is probably a natural consequence of the extensive deployment of ACTs and was bound to happen sooner or later. "Preventing" artemisinin resistance was never a real option.

11 Measures to Limit the Spread of Resistance

When the first evidence of artemisinin resistance became available in 2006, the World Health Organization launched an ambitious campaign to contain artemisinin resistance along the Thai–Cambodian border. These efforts involve the early detection and rapid treatment of all malaria infections on both sides of the border, preferably with a non-ACT combination therapy. In addition, the deployment of insecticide-treated nets to decrease malaria transmission and the screening and treatment of migrants were intensified, together with a more thorough mapping of the geographic boundaries of artemisinin resistance [15, 35]. Recently, Maude et al. concluded that containment of artemisinin-resistant malaria could also be achieved by eliminating malaria using ACT [47]. However, as ACTs are more effective against infections with artemisinin-sensitive parasites resulting in a relative increase in the proportion of artemisinin-resistant parasite isolates, this approach would require malaria elimination down to the last parasite. Unfortunately, this is unlikely to ever happen in a landlocked environment surrounded by malaria-endemic countries.

Although the history of malaria control teaches us that the Thai–Cambodian border has always been a hotspot of antimalarial drug resistance development, it also teaches us that sooner or later resistance is likely to emerge independently in other parts of the world. With the unprecedented deployment of ACTs throughout the malaria-endemic world, artemisinin resistance is likely to eventually emerge in other parts of the world.

The potentially devastating implications of resistance to a drug to which there is currently no real alternative calls for cost-effective strategies to extend the useful

life spans of currently available antimalarial drugs while at the same time investing into major efforts to develop novel compounds as a replacement for the artemisinins.

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