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Drug resistant falciparum malaria and the use of artesunate-based combinations : focus on clinical trials sponsored by TDR

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Antimalarial drug resistance has now become a serious global challenge and is the principal reason for the decline in antimalarial drug efficacy. Malaria endemic countries need inexpensive and efficacious drugs. Preserving the life spans of antimalarial drugs is a key part of the strategy for rolling back malaria. Artemisinin-based combinations offer a new and potentially highly effective way to counter drug resistance. Clinical trials conducted in African children have attested to the good tolerability of oral artesunate when combined with standard antimalarial drugs. The cure rates of the different combinations were generally dependent on the degree of resistance to the companion drug. They were high for amodiaquine-artesunate, variable for sulfadoxine/pyrimethamine-artesunate, and poor for chloroquine-artesunate.

Key words Antimalarial drug resistance – artemisinin – fever clearance – parasite clearance

The global malaria burden is estimated at 300 to 500 million per year. There are between 1.5 and 2.7 million deaths per year, mostly in African children, that are attributable to *Plasmodium falciparum*[1,2]. Drug resistant *P. falciparum* is a serious problem, especially in sub-Saharan Africa and contributes to the increasing malaria-related morbidity and mortality[3,4]. Chloroquine resistance is wide-spread, sulfadoxine-pyrimethamine (SP) resistance is well established in southeast Asia, focal areas of Latin America, and is now emerging in east and central Africa[5]. Multidrug resistant *P. falciparum* has also emerged in certain areas, notably southeast Asia where resistance to chloroquine, SP, mefloquine and quinine is well documented[6]. Chloroquine (CQ) and SP are the two most commonly used antimalarial drugs because of their low cost. Currently available alternative, efficacious drugs (e.g. artemether-lumefantrine, atovaquone-proguanil) are considerably more expensive, and are unaffordable by many resourced strapped malaria endemic countries. The situation in many countries is critical and concerted, global action is required if we are to avert a malaria disaster[7].

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Drug resistance — consequences and challenges

Drug resistance has several deleterious consequences. From the clinical point of view, resistance leads to treatment failure and increased morbidity and mortality[8]. Anaemia is a particular problem in African children and is *per se* a significant cause of increased morbidity, the need for expensive and potentially dangerous blood transfusions, and mortality[9–11]. The grading of parasitological resistance to antimalarial drugs is well-known (RI, RII, and RIII). As resistance

increases there is little or no response to drugs and patients are at increased risk of severe malaria. Drug resistant parasites also produce more gametocytes compared to sensitive infections, a factor in aiding the transmission of resistant genes[12].

In malaria endemic countries, patients who fail treatment are often retreated with the same drug which also fails to clear parasites. This then leads to a downward spiral of chronic morbidity. There is clear evidence that the introduction of a new and efficacious antimalarial drug results in high treatment efficacy[13,14]. However, the efficacy of this new drug is also likely to decline over time. Following the introduction of SP, resistance and declining efficacy rose in parallel with increasing use. In Thailand, SP had a useful life span of only five years[6]. SP resistance is also emerging rapidly in Africa and is clearly not the answer to chloroquine resistant *P. falciparum*[15,16].

One factor that has contributed to the development of drug resistance is the limited number and types of antimalarial drugs that are available and the way they have been deployed. Drug policy in most countries is to use different drugs in sequence. In practice this often means using a drug beyond its useful life with the result that the second line drug is used so that by the time it is declared the first line drug, resistance has already developed. There are two ways to try to alleviate this problem. Firstly, develop new drugs that have different or novel modes of action (a long process), and secondly, use combinations of currently available drugs that have independent modes of action, in particular, artemisinin-based combinations.

Combating drug resistance through artemisinin-based combinations

There is evidence demonstrating that drug combinations with independent mechanisms of action and resistance enhance treatment efficacy and delay the development of resistance. This principle has been established for the treatment of tuberculosis and HIV-AIDS[17,18]. The scientific basis for using artemisinin-based combinations for treating malaria is sound for several reasons[19]. Combining different drugs with independent modes of action theoretically prevents the emergence of resistance because the probability that an infected patient will have parasites resistant to both drugs is greatly reduced (the product of the probability of resistance to either drug). Artesunate (AS) causes a rapid and substantial reduction in the parasite biomass, irrespective of their resistance to other antimalarials[20,21]. The remaining parasites are then killed off by high concentrations of the companion drug. In this way, exposure of parasites to sub therapeutic concentrations of the companion drug is minimised.

Extensive use of artesunate combined with mefloquine on the Thai-Myanmar border, an area of low transmission of multidrug resistant *P. falciparum* and chloroquine sensitive *P. vivax*, has produced three main effects over time. Efficacy against falciparum malaria has consistently exceeded 95%, the transmission of *P. falciparum* has been reduced, and the *in vitro* sensitivity of mefloquine has increased[22].

Similar data do not exist from any other part of the world. India has malaria endemic zones similar to that of Thailand and the deployment of artemisinin-based combinations may also result in a similar beneficial effect. The question of whether deploying an artemisinin-based combination will also be useful in sub-Saharan Africa where the malaria transmission is more intense remains open.

Clinical trials of artesunate-based combinations

In 1998, WHO/TDR, USAID, and the Wellcome Trust agreed to commence a series of clinical trials to assess the efficacy and tolerability of artesunate combined with three standard antimalarial drugs in Africa and Latin America (Table 1). WHO-TDR was the coordinating and managerial body.

The three standard antimalarial drugs used were chloroquine, amodiaquine and SP. The criterion for using them in a particular country was that their efficacy was

Table 1. Countries and centres participating in the randomised controlled trials of artesunate combinations in Africa

Country	Executing institution	Drug	Enrolled
Gabon	Tuebingen University/A. Schweizer Hospital, Lambaréné, Gabon	AQ	220
Senegal	Hôpital Charles Nicolle, Rouen University, France & University of Dakar	AQ	321
Kenya	African Medical Research Foundation (AMREF)	AQ	400
Subtotal			941
The Gambia	Medical Research Council (MRC)	SP	600
Kenya	Kenyan Medical Research Institute (KEMRI)	SP	600
Uganda	Epicentre, France & Mbarara University	SP	425
Malawi	Queen Elizabeth Hospital, Blantyre	SP	450
Kenya	Wellcome Trust, Kilifi	SP	600
Subtotal			2675
Sao Tome	Lisbon University, Prince Leopold Institute, Antwerp & Sao Tome Ministry of Health	CQ	400
Burkina Faso	Centre National de Lutte contre le Paludisme (CNLP)	CQ	300
Côte d'Ivoire	Institute P. Richet, Bouaké	CQ	300
Subtotal			1000
Grand total			4616

at least 75%. Chloroquine was used in west Africa, and the other two drugs in several countries across Africa. Two trials were conducted in Latin America, using amodiaquine (AQ) in Colombia (trial ongoing) and SP in Peru. This report will focus on the African studies. These were randomised, double blind, placebo controlled trials that were conducted under Good Clinical Practices (GCP). Common clinical protocols and one analytical plan were used; the latter was designed so that an individual patient data (IPD) meta-analysis could be done. Collectively, they represent the largest series of antimalarial drug trials ever conducted. There were 11 sites in eight countries (Table 1).

The primary efficacy end points were the parasitological cure rates on Day 14 and 28. Secondary efficacy parameters were the rate of parasite and fever clearance, and the gametocyte carriage rates. Molecular genotyping was used to distinguish between recrudescent and fresh infections. Samples were also taken for: (i) polymerase chain reaction (PCR) to detect the genetic mutations associated with drug resistance; and (ii) population pharmacokinetic (PK) assessments. The PCR and PK data will be reported elsewhere. Artesunate(AS)/placebo was provided by Sanofi/Guilin, amodiaquine by Warner-Lambert/Parke-Davis (now Pfizer), and SP by the International Dispensary Association.

All the SP studies had three arms: SP alone, and two dosing regimens of artesunate. The CQ and AQ studies used three days of artesunate (Table 2). The dose of artesunate was 4 mg/kg daily for three days, and one day (SP studies only). To date, four studies have

Table 2. Study design of the randomised, double blind, placebo controlled efficacy and safety trials conducted in Africa and Latin America

Drug combination	Time (hours)		
	0	24	48
Two arm regimens : chloroquine or amodiaquine			
Arm 1	CQ/AQAS Placebo	CQ/AQAS Placebo	CQ/AQAS Placebo
Arm 2	CQ/AQAS	CQ/AQAS	CQ/AQAS
Three arm regimens : sulfadoxine/pyrimethamine			
Arm 1	SPAS Placebo	AS Placebo	AS Placebo
Arm 2	SPAS	AS Placebo	AS Placebo
Arm 3	SPAS	AS	AS

Drug dosages : CQ — 10/10/5 mg/kg once daily; AQ — 10/10/10 mg/kg once daily; SP — 25 mg/kg based on sulfadoxine component stat; AS — 4 mg/kg once daily x 3d.

Table 3. Efficacy results of artesunate combination studies from selected studies that are either published or in press. For SP studies, only data for three days of artesunate are shown

Drugs/Countries	Day 14			Day 28		
	As 3 days	Standard drug	p-value	As 3 days	Standard drug	p-value
Amodiaquine (AQ)						
Kenya	175/192 (91)	140/188 (75)	< 0.0001	123/180 (68)	75/183 (41)	< 0.0001
Senegal	148/160 (93)	147/157 (94)	NS	130/159 (82)	123/156 (79)	NS
Gabon	92/94 (98)	86/96 (90)	0.016	80/94 (85)	70/98 (71)	0.019
Sulfadoxine Pyrimethamine (SP)						
Gambia	185/189 (97.9)	185/195 (94.9)	NS	181/187 (96.8)	173/193 (89.6)	0.005
Uganda	100/117 (85.5)	84/146 (57.5)	< 0.0001	68/116 (58.6)	55/144 (38.2)	0.001
Chloroquine (CQ)						
Burkina Faso	120/147 (81.6)	53/143 (37.1)	< 0.0001	71/145 (49)	27/142 (19)	< 0.0001

Figures in parentheses indicate per cent.

been published, and several are in press. The results of one day of AS are not reported here.

Results

The addition of artesunate to amodiaquine resulted in an increased cure rate in Gabon and Kenya but not in Senegal (Table 3). In the Gambia, cure rates were similar on Day 14 but were higher in the AS arm on Day 28. Cure rates of the AS arm for both days were significantly higher in Uganda where the SP alone cure rate was low (Fig. 1). Results from Kenya and Malawi were broadly similar (results to be published else-

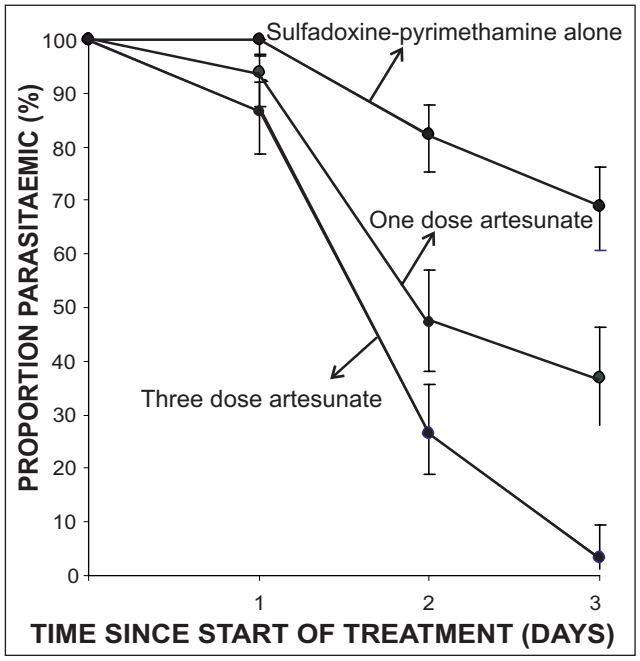

Fig. 1: Parasite clearance of artesunate with SP versus SP alone, although these data are from Uganda, they are representative of all the artesunate studies. The addition of artesunate increases the rate of parasite clearance

where). Chloroquine alone was poorly efficacious in Sao Tome (results to be published elsewhere) and Burkina Faso, and were improved by AS.

Parasite clearance was significantly faster in all trials with both three days and one day of artesunate compared to monotherapy (Fig. 2). Fever clearance was significantly faster in the SP trials but not always in the CQ and AQ studies. Gametocyte carriage was reduced in the SP and CQ studies by the AS regimens but the effect of AS in the AQ studies was inconsistent.

Tolerability was good in all studies. By group analysis, there was no increase in the proportion of patients reporting adverse events in the AS arms. Remarkable side effects in the AQ and AQ-AS arms were mild itching in nine patients (1%) and drug induced vomiting in 11 (1.2%). In the SP study in Kenya, 16 (2.7%) children developed mild, papular rashes. Serious adverse events (SAE) were few and mostly due to signs of severe malaria which developed in the first few days of treatment. One child with AQ induced vomiting was admitted to the hospital. Haematology results showed that the mean haemoglobin increased by Day 28 and was generally similar between the arms. In the AQ study, there was a decline in the mean neutrophil counts reaching a nadir on Day 21 and rising thereafter. Nine (6%) of 153 children developed asymptomatic neutropenia (<1,000) by Day 28. Biochemistry results were unremarkable. Raised liver enzymes present on Day 0 resolved over time.

Discussion

This series of clinical trials has shown that three days of artesunate combined with a standard antimalarial drug increased significantly the cure rates over the standard antimalarial drug when given alone. The exception was in Senegal. One day of artesunate with SP had no beneficial effect on cure rates. Toxicity was not increased by adding artesunate.

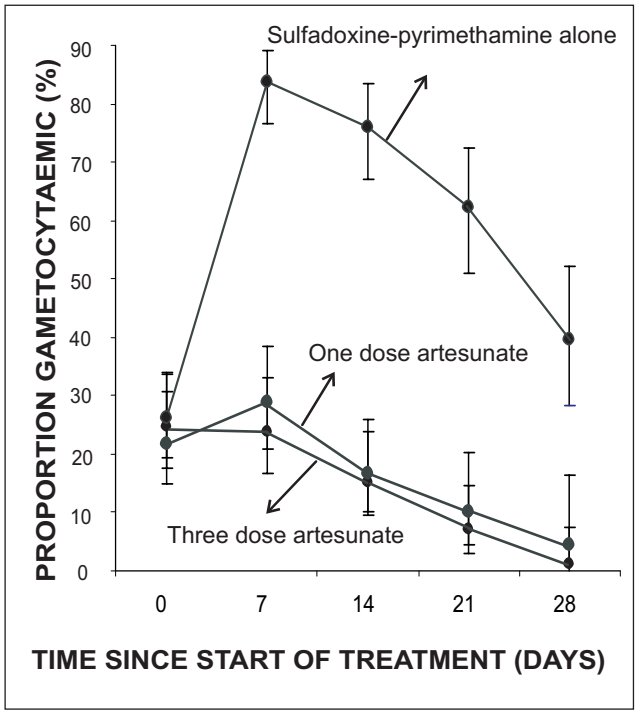

Fig. 2: Gametocyte carriage of SP alone and combined with one or three days of artesunate (Data from Uganda)

These efficacy results are encouraging for those countries where the background rates of resistance to standard antimalarial drugs are not high. Unfortunately, for Kenya and Burkina Faso, amodiaquine and chloroquine combinations, respectively, can not be recommended because the absolute cure rates were still modest despite the artesunate. We are unable to explain the lack of a significant increase in the cure rate in Senegal. The combination had a superior pharmacodynamic effect in terms of reducing the rate of parasite reduction but this did not translate into a lower failure rate. In the Gambia, there was no difference in the cure rate on Day 14 but by Day 28, a significant effect was seen for three days of artesunate. Whilst many *in vivo* studies follow patients up to Day 14 for convenience, it is better to extend this to at least 28 days to detect late resistant infections. PCR was performed in these studies to differentiate resistant infections from new infections. The effect of artesunate was unchanged but failure rates decreased because new infections always contributed to the recurrent parasitaemias during follow-up (these data will be published elsewhere).

All study drugs were well tolerated and there was no evidence of increased toxicity because of the addition of artesunate. Early, drug induced vomiting requiring retreatment is an important consideration for malaria control programmes. The proportion was very small in the AQ studies but no one in the SP studies had drug induced vomiting. Most of the early withdrawals from the study were due to patients developing danger signs of severe malaria.

For amodiaquine two side effects have been well described in the literature, hepatitis and neutropenia. Risk estimates, which are based on weekly AQ prophylaxis, are 1 in 15,650 and 1 in 2,000, respectively[23]. We did not detect any case of clinical or biochemical hepatitis, but the sample size lacked power to detect hepatitis at the reported rates. There was, however, a decline in the mean neutrophil counts that was independent of drug arm, a reassuring finding in that the artesunate did not add to the neutropenia. A small number of children remained neutropenic by Day 28, the clinical significance of this is unclear. They were afebrile and asymptomatic and one child was parasitaemic. None of the children were followed-up after Day 28, thus, we can not comment on the evolution of the neutropenia. The limited data available in the literature show that falls in the total white cell and neutrophil counts have occurred with chloroquine and sulfadoxine/pyrimethamine. In a systematic review of efficacy and safety data from published and unpublished studies showed that the tolerability profile of amodiaquine monotherapy was similar to those of CQ and SP[24]. Published data on AQ treatment induced neutropenia and hepatitis are few. Of pertinence are reports of a case of asymptomatic hepatitis in a normal volunteer following two doses of amodiaquine and artesunate, a decline in mean absolute neutrophil counts following AQ, SP, AQ-SP combined, and AQ induced neutropenia[24–26]. More research is required to define and characterise the risk of neutropenia. Longitudinal studies are also needed to assess the safety of repeated AQ/AQ-AS use because redosing will happen in practice once these drugs are deployed widely.

Notable SP related side effects were a small number of patients who developed mild cutaneous eruptions that resolved spontaneously or with antihistamine treatment. These reactions most probably represented mild SP allergy. Artesunate allergy is rare but well described, so this can not be excluded definitively[27]. Serious SP drug reactions such as G-6-PD related haemolysis, hepatitis, erythema mutliforme did not occur in these studies but the sample size was inadequate to detect these rare toxicities[28].

For malaria endemic countries that are considering a change of their current first line antimalarial drug, the artemisinin derivatives are a good option where the background rate of resistance to the companion drug is low. Long-term deployment studies are now warranted to assess the public health impact of these combinations and assess their safety through pharmacovigilant systems.

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Note : Since the article was written, the result of all the TDR coordinated trials as well as trials from Thailand have been analysed as an individual patient data meta analysis. The analysis shows that the addition of three days of oral artesunate improved the efficacy of single agent drugs for treating uncomplicated falciparum malaria.

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