Artemisinin resistance in *Plasmodium falciparum:* what is it really?

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Until very recently, artemisinin and its derivatives were the only commercially available antimalarial drugs for which there was no reported parasite resistance. Artemisinin combination therapies (ACTs) are currently relied upon for effective malaria treatment in most regions of the world in which the disease is endemic, and their continuing efficacy is crucial if control and elimination programmes are to succeed. The loss of effectiveness of artemisinin and its derivatives to drug resistance would constitute a major disaster in the fight against malaria. To properly assess the danger posed by artemisinin resistance, and therefore enable appropriate and proportionate responses, definitions of 'artemisinin resistance' and 'ACT resistance', at both the clinical and parasitological levels, are needed.

Artemisinin resistance as defined by delayed parasite clearance times

Artemisinin and its derivatives are highly potent antimalarial drugs, which are fast acting and possess short half-lives [1]. These characteristics, combined with their reported gametocidal activity [2], were often used as arguments for why resistance against artemisinin was unlikely to occur and as justification for their implementation in malaria control programmes worldwide [3]. Recent reports from Southeast Asia have described what appears to be an early sign of the development of parasite resistance to the drug [4,5]. In numerous studies in Southeast Asia, a subset of parasites are cleared from the blood more slowly than previously following ACT treatment, and this phenotype has been currently used to categorise 'artemisinin resistant' parasites. This has prompted the development of emergency containment initiatives aimed at curbing the spread of 'artemisinin resistant' parasites to other areas of the world (http://www.who.int/malaria/diagnosis_treatment/arcp/containment_project/en/index.html) [6].

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For every other antimicrobial, resistance is defined by clinical failures and/or decreased susceptibility *in vitro*. However, for the current definition of 'artemisinin resistance', based on delayed clearance of parasites, neither criterion is fulfilled. Nevertheless, parasite populations are clearly emerging that display delayed clearance times following treatment [7,8]. Here, we attempt to interpret the current definition of 'artemisinin resistance' in the context of ACTs and its implications for 'ACT resistance'.

Typically, drug resistance manifests as a gradual change in the slope of the clearance curve. However, this has not been observed for 'artemisinin resistant' parasites with delayed clearance after artemisinin treatment [5]. During the first 24 h following drug treatment, 'artemisinin sensitive' parasites show a ten times greater reduction in biomass than the 'artemisinin resistant' parasites (Figure 1). Following the second dose of the drug, there are no significant differences in the rate of parasite clearance between the two types of parasites (equal slope), suggesting that the efficacy of artemisinin is comparable in both 'resistant' and 'sensitive' parasites from the second dose onwards. Whereas the *in vitro* susceptibility of rapid and delayed clearance parasites are similar, late trophozoites and schizonts are more sensitive to drug than early trophozoites (rings) [9,10]. A plausible mechanism for this delay is increased tolerance to the drug in either early (0–24 h) or late (24–48 h) stage parasites. A previously published mathematical model suggested that increased tolerance occurred at the ring stage [11]. An alternative explanation is escape from artemisinin-induced death by late stage parasites during the first 24 h of treatment, leading to a surge in the number of circulating rings after 24 h. In either case, the 24-h delay could be due to epigenetic changes, as observed in tumour cells [12]. Further evidence for an epigenetic mechanism comes from observations of increased gene expression of histones and transcription factors at late stages in 'artemisinin resistant' parasites compared with 'sensitive' ones [13,14].

Another factor associated with altered artemisinin parasite clearance is the initial parasite density, a factor that could lead to longer parasite clearance times [15]. In many

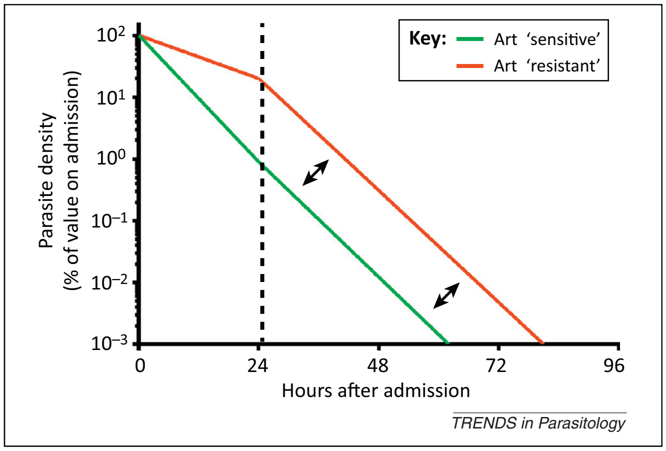

Figure 1. Parasite clearance in artemisinin sensitive and resistant parasite populations. Parasite clearance profile of artemisinin resistant and sensitive parasites populations as described in [5]. A broken line at 24 h shows a shift in clearance between populations, and arrows represent an identical slope thereafter. Abbreviations: Artartemisinin.

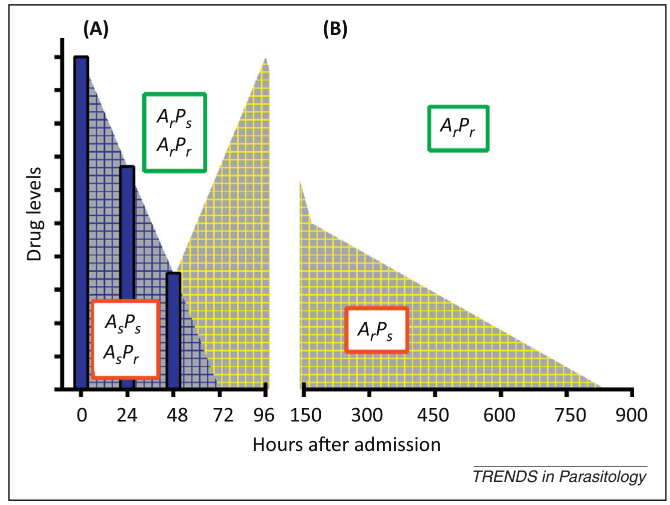

Figure 2. Selection of artemisinin combination therapy (ACT) resistant parasites. This scheme considers a malarious patient carrying parasites with all resistant phenotypic combinations. The selection of ACT resistant parasites is shown in a time-dependent manner during a given ACT course. The squared areas represent the effect of ACT components over time: in blue, **(A)**, three doses (blue bars) of artemisinin derivatives and in yellow, **(B)**, partner drugs. Four phenotype parasite populations are described as artemisinin resistant and sensitive (A_r and A_s, respectively) and partner drug resistant or sensitive (P_r and P_s, respectively). The red boxes show parasite populations killed given their phenotype, and green boxes show the parasite population that can escape antimalarial action at different times during ACT treatment.

of the studies of artemisinin resistance, parasite densities were higher for patients with slow clearance parasites compared with faster clearance [5,16]. Thus, the differences between the clearance rates of artemisinin 'resistant' and 'sensitive' parasites may be explained by factors other than direct susceptibility related genetic mutations.

Artemisinin resistance in the context of combination therapy

Artemisinin is administered in combination with other drugs (so-called 'partner drugs'), so the importance of resistance to the partner drug cannot be neglected. When used as monotherapy, a 7-day treatment regimen is required for complete cure [17]. Because the normal 3-day course of artemisinin given as part of ACT is not curative in itself [18], ACT failures could be due entirely to partner drug resistance.

We propose that the development of resistance to the partner drug is a necessary precursor to the development of artemisinin resistance. The rationale behind the partnering of artemisinin with a second, unrelated drug in an ACT, is that the probability of a parasite becoming resistant to both drugs is the product of the probabilities of becoming resistant to artemisinin [$P(A_r)$] and the partner drug [$P(P_r)$]: $P(ACT_r) = P(A_r) \times P(P_r)$ [19]. As each individual probability is $<<1$, the combined probability should be infinitesimally small [20]. However, if the pharmacokinetics of ACTs is taken into account, this argument fails. In conventional ACTs, the time windows in which each drug is active only overlap for short periods of time, with artemisinin reaching parasite killing concentrations in the blood far faster than the partner drug, so that parasite killing activities of the two drugs occur sequentially rather than concomitantly (Figure 2). This results in a long selective window for partner drug resistance selection in the post-treatment period, in which subtherapeutic levels of the partner drug are in circulation during its body elimination, creating optimal conditions for the development of partner drug resistance.

The process of selecting ACT-resistant parasites is described in Figure 2. There are four possible combinations of resistance and/or sensitivity to artemisinin (A) and its partner (P): sensitive to both drugs (A_s/P_s), resistant to one drug (A_s/P_r or A_r/P_s), and resistant to both drugs (A_r/P_r). If a population of parasites emerges that is resistant to artemisinin but not its partner (A_r/P_s), it would escape being killed by artemisinin initially, but would subsequently be killed by the partner drug. This possibility implies that resistance to artemisinin does not necessarily translate into resistance to ACTs.

In Southeast Asia, widespread mefloquine resistance preceded the introduction of ACT (artesunate plus mefloquine) [21]; thus, the emergence of dually resistant parasites (A_r/P_r) is much more likely to occur. This may soon also be the situation in Africa, where parasites may now be developing resistance to the new ACT partner drug lumefantrine through reinfections in patients with subtherapeutic partner drug levels following ACT treatment [22,23]. Thus, the most probable scenario for the development of ACT resistance is: $(A_s/P_s) \rightarrow (A_s/P_r) \rightarrow (A_r/P_r)$.

In summary, artemisinin resistance will arise within a parasite population in which partner drug resistance is already established rather than spontaneously and/or independently. This conclusion emphasises the importance of parasite population partner drug resistance in the development of artemisinin resistance under ACT treatment policies. Furthermore, the above-mentioned description shows that 'artemisinin resistance' and 'ACT resistance' do not necessarily define the same phenotype and need to be properly addressed in future research.

Concluding remarks and future perspectives

'Artemisinin resistance', as currently defined, is not yet of clinical importance. The causes for delayed parasite clearance times are likely to be multifactorial, and parasite densities at time of treatment initiation appear to be associated with this phenotype. Nevertheless, given the likelihood that true artemisinin resistance will develop [24], the process by which this may occur needs to be carefully considered. Understanding how resistance to ACTs may evolve and spread will aid efforts to increase the useful lifespan of this important antimalarial therapy. To postpone the development of clinically relevant artemisinin resistance, the only feasible and applicable strategy is to use efficient partner drugs in combination with suitable surveillance methods.

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