Research & Reviews: Journal of Pharmaceutical Analysis e- ISSN:2320-0812

Managing Malaria by Tackling Resistance to Artemisinin-Based Combination Therapy (ACT): Opportunities and Challenges

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Mini Review

Received: 14-Mar-2022, Manuscript No. JPA-22-56878; **Editor assigned**: 16-Mar-2022, PreQC No. JPA-22-56878(PQ); **Reviewed**: 28-Mar-2022, QC No. JPA-22-56878; **Revised**: 30-Mar-2022, Manuscript No. JPA-22-56878(R); **Published**: 06-Apr-2022, DOI:10.4172/2320-0812.11.02.001

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Keywords: Parasites; Kelch13; Artemisinin -Based Combination Therapies (ACTs); Multidrug-resistant malaria; Global malaria control

ABSTRACT

The emergence of artemisinin resistant *Plasmodium falciparum* parasites has the potential to cause a global public health crisis. Currently there are no drugs to replace the artemisinins. The most advanced drugs in the antimalarial drug development pipeline will still need about 5-6 years for approval by regulatory authorities. Resistance to artemisinin does not result in ACT treatment failures as long as the partner drugs are effective. Thus, public health workers still have one option to keep ACTs effective: improve surveillance for resistance to artemisinin and its partner drugs. This is crucial for faster selection of an effective partner drug in ACTs so as to prevent the emergence and spread of multi-drug resistant malaria.

INTRODUCTION

The resistance acquired by malaria parasites to Artemisinin (ART)–the backbone of ACTs (Artemisinin-Based Combination Therapies) which are the best available antimalarial medicines is a major threat to malaria eradication. ART resistance is strongly associated with nonsynonymous Single Nucleotide Polymorphisms (SNPs) (after amino acid residue 440) in the propeller region of *Plasmodium falciparum* Kelch13 (*PfK13*) gene (*Pf3D7*_1343700). Clinically, ART resistance is marked by delayed parasite clearance after a 3-day ACT course. *In vitro*, it is characterized by decreased sensitivity of tightly synchronized 0-3 hr post-invasion rings to the pharmacologically relevant dose (700 nM) of Dihydroartemisinin (DHA) in what is referred to as the Ring-stage Survival Assay (RSA). ART resistance first emerged in Cambodia about 2 decades ago and is now present throughout Southeast Asia (SEA). ACTs pair the short-lived, fast-acting ART derivatives with slower-acting but longer-lasting partner drugs (lumefantrine, amodiaquine, mefloquine, piperaquine and pyronaridine). Thus, ART resistance does not result in treatment failures with ACTs as long as the partner drug remains effective. However, resistance to the fast-acting ART exposes the partner drug to a parasite load much higher than it can handle. This creates a fertile ground for emergence of resistance to partner drug as well. Indeed, in recent years, multi-drug resistant *Plasmodium falciparum* parasites which are resistant to both artemisinin and partner drugs (Piperaquine (PPQ) and mefloquine) have increased in prevalence throughout SEA with resistance to the ACT of DHA+PPQ causing up to 50% treatment failures in some areas of Cambodia, Thailand, and Vietnam.

There are currently no drugs to replace the ACTs. So, for treatment of contemporary Malaria, the practice has been to replace a failed ACT by another ACT. This practice is helpful due to the observed curious opposing drug pressure (in the process of becoming resistant to drug X, the parasite becoming sensitive to drug Y) selection by ART partner drugs. However, in Laos and Thailand, there is already high failure rate to at least two of the five ACTs available, further reducing the already limited options. Thus, efforts need to be doubled to better characterize ART resistant parasites. Understanding the evolution of ART resistant parasites may catalyse innovative strategies to optimise the effectiveness of ACTs until new drugs are ready to replace the artemisinins.

LITERATURE REVIEW

Opportunities and challenges to maintain the effectiveness of ACTs

Various strategies have been suggested to win over artemisinin resistance. In this section, some of these options are discussed.

Increasing the duration of the treatment course

Inspired by the knowledge that only a small sub-set of ring stage *Plasmodium falciparum* parasites are resistant to ART, one of the strategies to cope with ACT resistance has been to increase the duration of the treatment course from 3 to 5 days. ACTs were formulated with ARTs, the fastest acting and exceedingly

potent antimalarials known, as the core antimalarial. However, their short-life is their biggest curse. In ACTs, they quickly kill the bulk of the parasites leaving their longer-lasting partners to deal with the few remaining parasites. However, resistance to ART exposes partner drugs to a much higher parasite load than they can handle. This is the reason for the spectre of parasites circulating in the blood after a 3-day treatment regimen. From this observation has emerged the idea to increase the treatment duration from 3 to 5 days. However, experts have argued that a longer therapy could hamper compliance and increase the chances of people not taking their treatment completely thus setting the stage for high-grade resistance.

Cycling ACTs and deploying Triple Artemisinin-based Combination Therapies (TACTs)

Since ART resistance alone is unable to cause treatment failures with ACTs, the selection of an effective partner drug for ART is crucial for maintaining the efficacy of ACTs. Hence to manage resistance in regions where failure to one ACT is established, ACTs are cycled. It is noteworthy that ART partner drugs (the pair of (amodiaquine and lumefantrine) and (piperaquine and mefloquine)) cause opposing selective pressures on *Plasmodium falciparum* Multi-Drug Resistance gene 1 (*PfMDR1*) and *Plasmodium falciparum* Chloroquine Resistance Transporter (P*f*CRT) haplotypes. This inspired the cycling of ACTs having different partner drugs to maintain effectiveness. However, high rate of failure of more than 2 different ACTs have already been reported in Laos, Thailand and Cambodia. In what appears to be a worrying situation, triple mutants (mutation in P*f*K13 (C580Y) as well as amplification of *PfMDR1* and plasmepsin 2) emerged following the replacement of DHA-PPQ with artesunate-mefloquine in Thailand, Laos and Vietnam. This suggests a dire need for better innovative strategies to curb the menace of multi-drug resistance.

Recently, in a screen of antimalarial drugs that are already in use, Kümpornsin, et al. [1] identified lumefantrine to be able to increase the killing activity of artemisinin against an artemisinin-resistant clinical isolate harbouring the P*f*kelch13C580Y mutation. Lumefantrine could thus be explored as a partner drug in ACTs to control ART resistance in SEA. While the mechanism of antimalarial action of lumefantrine is unknown, it might interfere with heme detoxification and facilitate the activation of ARTs by increasing the concentration of free heme [2]. Interestingly, lumefantrine is a part of the newly developed TACT regimen (artemether-lumefantrine plus amodiaquine). The opposing selective pressure between ACT partner drugs has been instrumental in the design of TACT. Notwithstanding this scientific knowledge, the swapping of DHA-PPQ for Artesunate-Mefloquine (AS-MQ) has been difficult to implement in Cambodia. Indeed, TACT combinations such as DHA-PPQ-MQ or Artemether-Lumefantrine (AL) plus Amodiaquine (AM) could serve as potential alternatives to tackle multi-drug resistance in this region. It is noteworthy that DHA-PPQ-MQ combination is already in use in Vietnam.

In addition to amplified copy numbers of plasmepsin II/plasmepsin III genes associated with piperaquine resistance, specific pfcrt mutations which evolved from the CQ resistant P*f*CRT Dd2 haplotype have now also been linked to PPQ resistance in Cambodia. Intriguingly, PPQ-resistant mutations have been found to re-sensitize parasites to Chloroquine (CQ), despite the presence of the K76T mutation. Similarly, a PPQ-

resistant *pfcrt* allele from the Chinese province Yunnan, with a genetic lineage different from that of Cambodia has also been reported to re-sensitize parasites to CQ. In light of this, using modelling simulations, Small-Saunders, et al. showed that a TACT of DHA-PPQ-CQ could be an effective regimen in SEA. This combination has the potential to force pfcrt into an evolutionary trap rendering it unable to generate new P*f*CRT mutations that could drive high-grade resistance to both PPQ and CQ [3].

Inclusion of gametocytocidal agent in ACTs

Apart from clearing asexual stages of the parasite, ARTs are also gametocytocidal. In a recent study, Witmer, et al. reported that the artemisinin-resistant phenotype of APL5G, P*f*K13C580Y is not confined to asexual blood stages but, additionally, expands to male sexual stages and directly influences transmission in the presence of DHA. They observed that in comparison to the wild-type NF54 strain, sexual commitment and exflagellation (activation of male gametocytes) is not compromised in ART resistant isolates. Further, sexual stage of P*f*C580Y was more likely to infect mosquitoes under DHA drug pressure than wild-type parasites [4]. This suggests that the continued use of artemisinin in the face of resistance could very likely result in an increase in the spread of ART resistance and underscores the need to include a gametocytocidal drug in ACTs. Primaquine; an 8-aminoquinoline gametocytocidal agent could be an option. However, Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency- the most prevalent enzyme deficiency affecting humans could be an issue. Although high doses of Primaquine are known to cause haemolysis in individuals with G6PD deficiency, as suggested by the WHO [5], a single low dose (0.25 mg/kg) of primaquine may be non-toxic while retaining its gametocytocidal potency. Another alternative is the incorporation of the Paul Ehrlich invented Methylene Blue (MB) (a multi-stage gametocytocidal agent) in ACTs.

DISCUSSION

ART resistance has substantially compromised the efficacy of ACTs across SEA. More worryingly, it has now emerged also in Africa. Thus, in Rwanda and Uganda, P*f*K13 mutations (R561H, A675V and C469Y) resulting in delayed clearance phenotype *in vivo* have been reported and proposed as candidate markers of ART resistance [6,7]. Further, the R561H mutation has recently been characterized to also mediate a comparable or even higher ART resistance than that seen for C580Y *in vitro* (4.7% vs. 4.6% RSA survival on the Asian Dd2 parasites and a 6.6% vs. 4.8% RSA survival on African 3D7 parasites) [8]. Four African countries account for over half of global malaria mortality: Nigeria (31.9%), the Democratic Republic of Congo (13.2%), United Republic of Tanzania (4.1%) and Mozambique (3.8%). The emergence of multi-drug parasites in SEA is a wakeup call for improved surveillance towards faster response in Africa. Despite Nigeria contributing the highest percentage of global malaria-related deaths, no data on the therapeutic efficacy study of ACTs is available. Further, molecular surveillance studies have found no validated K13 polymorphism associated with ART resistance. It is good to know that an efficacy study to monitor resistance to the ACT currently in use in Nigeria (artemether-lumefantrine (co-artemether)) is ongoing (https://nimr.gov.ng/elemenor-7171).

Aside from surveying for resistant mutations, there is need for their characterization so as to know the trajectory of resistance, fitness cost incurred as well as if resistance is transmissible. In the study by Stokes, et al. the genes of Asian and African parasite isolates were edited to introduce those ART resistance mutations which are found in SEA (C580Y and R539T) and in Africa (R561H). Apart from P*f*3D7 and a Ugandan isolate (UG659) whose genes were edited to carry the R561H and C580Y mutations respectively, ART resistance exerted a very high *in vitro* fitness cost on African malaria parasite isolates. This relative negative fitness cost may translate into a slower dissemination of ART resistance in the high transmission setting of Africa. However, the study also reported that resistance can be achieved in some African strains of the parasite (notably a P*f*3D7 parasite isolate carrying R561H mutation) and that such resistance can be achieved at levels comparable to or above those seen in SEA parasites. Although R539T had a better RSA survival than C580Y, when these mutations were introduced in SEA parasite isolates, the latter exhibited a lesser fitness cost than the former which may be the reason why C580Y is the most widely spread variant in SEA. RSA survival of R561H was comparable to that of C580Y on Asian Dd2 parasites (4.7% versus 4.6%) and higher on African 3D7 parasites (6.6% versus 4.8%). Further, it had no impact on fitness of 3D7 parasites. This suggests that the degree of resistance associated with specific mutations is influenced also by the genetic background of the parasite. The emergence of R561H mutation in Africa is therefore a cause for major concern as results by Stokes, et al. reveal a phenotype that may favour R561H spread [8].

CONCLUSION

Limited options are currently available to keep ACTs effective until new drugs are ready to replace them. Though KAF609; a drug at the end stage of development performed very well during early clinical trials, it may not become available before 2026. Therefore, improved surveillance for ART and partner drug resistance is crucially needed to help policymakers decide on making a right choice from the limited options currently available. Also, only a few of the K13 mutations reported so far have been validated by *in vitro* gene-editing experiments to confer ART resistance *in vitro*. More studies are therefore required to know whether or not these mutations can confer *in vitro* ART resistance. Further, there is need to characterize such novel mutations i.e. scoring their level of resistance using standard RSA, comparing their rate of transmission with wild-type parasite isolates as well as assessing their fitness. It must indeed be clear that the parasite in its attempts to resist drugs has the choice to mutate only those residues that do not jeopardize its very survival. For instance, the Kelch protein is crucial as it facilitates haemoglobin transport from the cytosol into the food vacuole. All mutations that the parasite can afford to have must in no way abolish haemoglobin transport function of this protein. Knowledge of these facets of the range of mutations that the parasite can afford to have could inspire both faster surveillance and appropriate response.

CONFLICT OF INTEREST

None declared

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