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Current Opinion

In vitro evaluations of antimalarial drugs and their relevance to clinical outcomes

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Abstract

Plasmodium falciparum resistance to the former first-line antimalarials chloroquine and sulfadoxine/pyrimethamine has reached critically high levels in many malaria-endemic regions. This has spurred the introduction of several new artemisinin-based combination therapies (ACTs) that display excellent potency in treating drug-resistant malaria. Monitoring for the emergence of drug resistant *P. falciparum* is important for maximising the clinically effective lifespan of ACTs. Here, we provide a commentary on the article by Kaddouri et al., published in this issue of the *International Journal of Parasitology*, which documents the levels of susceptibility to ACT drugs and chloroquine in *P. falciparum* isolates from Mali. These authors report that some isolates approached a proposed in vitro threshold of resistance to monodesethyl-amodiaquine (the principal effective metabolite of amodiaquine, an important ACT partner drug), and establish baseline levels of susceptibility to the ACT drugs dihydroartemisinin and lumefantrine. The majority of clinical isolates manifested in vitro resistance to chloroquine. The authors also show good concordance between field-based assays employing a non-radioactive lactate dehydrogenase-based method of determining in vitro drug IC_{50} values and the well-established [^{3}H]hypoxanthine-based radioactive method. This work illustrates a good example of drug resistance surveillance, whose global coordination is being championed by the World Antimalarial Resistance Network. Our current opinion also more generally discusses the complexities inherent to conducting in vitro investigations with *P. falciparum* patient isolates and correlating these findings with treatment outcome data.

Keywords: *Plasmodium falciparum* malaria; In vitro assays; Drug resistance; Chloroquine; Artemisinin-based combination therapies

Malaria inflicts a heavy toll on the health and quality of life of the residents of intertropical countries, affecting one third of the world's human population (Sachs and Malaney, 2002; Guerra et al., 2008). Communities in Africa bear the brunt of this parasitic, blood-borne disease, with an estimated 90% of all malarial deaths occurring in African children below the age of 5 years (Hay et al., 2005). Efforts to control malaria have repeatedly faltered, in part as a result of the selection and spread of drug resistant *Plasmodium falciparum* parasites. These parasites have developed at least partial resistance to nearly every antimalarial regimen introduced to date, including the former first-line drugs chloroquine and sulfadoxine–pyrimethamine (Fig. 1) (Wongsrichanalai et al., 2002). In 2005, the World Health Organization (WHO) began recommending the use of artemisinin-based combination therapies (ACTs) as the preferred first-line antimalarials. These pair an extremely potent but short-lived artemisinin derivative (typically artesunate or artemether) with a longer-acting antimalarial such as lumefantrine, amodiaquine or mefloquine. Substantial financial support is being put into place to facilitate their distribution throughout endemic areas (Ashley and White, 2005; Bathurst and Hentschel, 2006; Grabowsky, 2008). While ACTs currently demonstrate excellent clinical efficacy, the history of antimalarial chemotherapy predicts that it is only a matter of time before parasite resistance emerges (Chretien et al., 2007).

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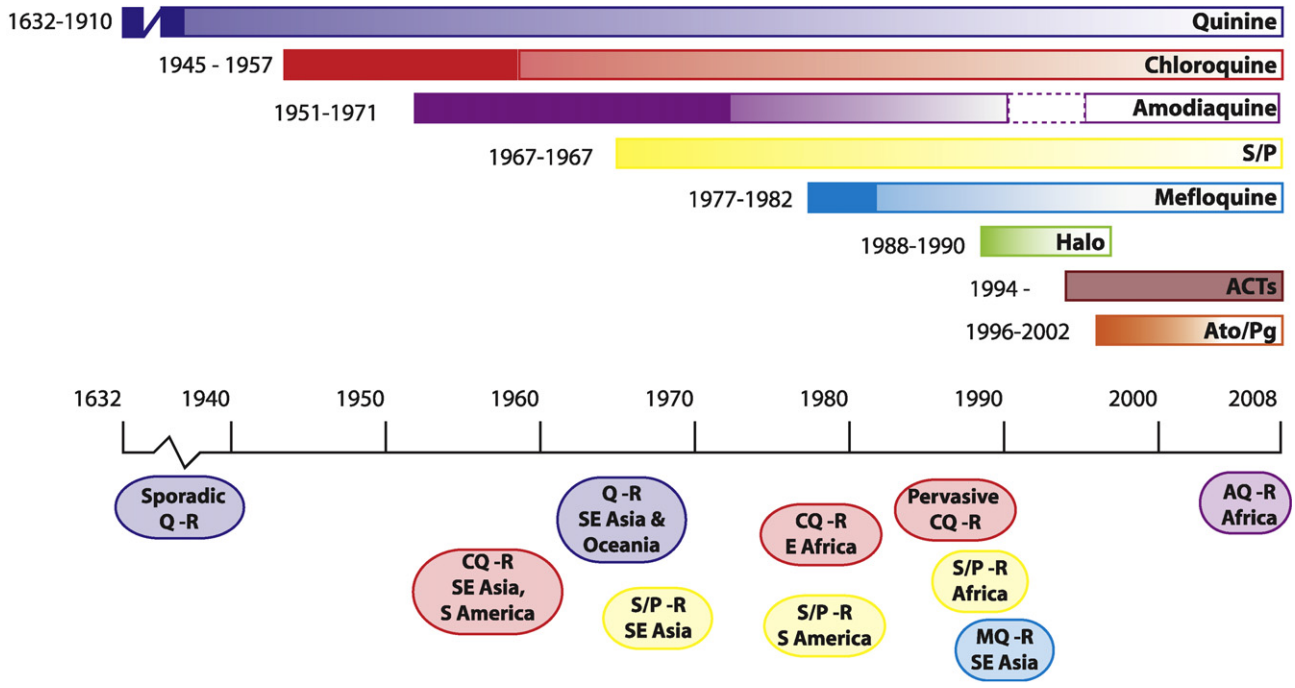

Fig. 1. Emergence of resistance to the principal antimalarials. Each coloured bar represents an antimalarial monotherapy or combination. Years to the left of each bar represent the date the drug was introduced and the first reported instance of resistance. Ovals below the time line denote the approximate periods when resistance spread though different geographical regions. Quinine, chloroquine and sulfadoxine/pyrimethamine remained effective for considerable periods after the first reported instances of resistance. Halofantrine has had limited usage since about 1998 due to cardiotoxicity concerns. Amodiaquine was removed from the list of approved antimalarials in 1990 due to concerns over serious side effects, but was reinstated in 1996 because the perceived benefits outweighed the risks (dashed border). Field trials of artemisinin (Qinghaosu) and its derivatives were first implemented in China in the early 1970s. Artemisinin has a low radical cure rate when used alone in a short course, presumably due to its very short half-life in vivo. Since 1994, artemisinin and its derivatives have been used in combination therapies (ACTs). No clinical resistance to ACTs has yet been demonstrated. Timelines were derived from (Wongsrichanalai et al., 2002; Hyde, 2005; Tinto et al., 2008) and references therein. ACTs, artemisinin-based combination therapies; AQ, amodiaquine; Ato/Pg, atovaquone/proguanil; CQ, chloroquine; Halo, halofantrine; MQ, mefloquine; Q, quinine; R, resistance; S/P, sulfadoxine/pyrimethamine.

The paper by Kaddouri et al. (2008) in this issue of the *International Journal for Parasitology* uses an in vitro approach to assay clinical isolates of *P. falciparum* for their degrees of susceptibility to chloroquine and several ACT component drugs (lumefantrine, dihydroartemisinin and the active monodesethyl metabolite of amodiaquine). This study provides baseline efficacy data that are necessary for subsequent detection of the emergence of resistance to ACT drugs. Reflecting back on chloroquine, it is striking that four decades elapsed between the early reports of emerging clinical resistance and the identification of the primary genetic determinant, *pfcrt* (Fidock et al., 2000; Wellems and Plowe, 2001). Only then could a simple PCR-based molecular assay be designed to determine the prevalence of chloroquine-resistant strains of *P. falciparum*, revealing their origins and widespread dissemination throughout nearly all malaria-endemic regions (Djimdé et al., 2001; Wootton et al., 2002). Advances in recent years have now equipped us with powerful genetic and genomic tools (that include allelic exchange and transgene expression, quantitative trait loci analysis, haplotype mapping, microarrays and whole genome sequencing; Ekland and Fidock, 2007), a better understanding of the molecular mechanisms of antimalarial drug resistance (Woodrow and Krishna, 2006), and the capacity to exchange and analyse data as never before. Translating this into more effective management and control of drug-resistant malaria presents an incredible scientific challenge. Meeting this challenge will be further complicated by issues such as the distribution of ineffective or counterfeit drugs, regional differences in implementation programs, and the limited funds available for basic malaria research. In this article, we will discuss some of the issues confronting researchers investigating antimalarial drug resistance in vitro with patient isolates and how the paper by Kaddouri et al. (2008) fits into this context.

A high rate of clinical failure defines the ultimate measure of resistance, however, there are many challenges associated with clinical studies of drug resistance, including costs and logistical difficulties. The ability to detect drug resistant parasites can be obscured by multiple factors including poor patient compliance, patient variability in drug absorption or metabolism, the general health and nutritional state of the patients, the rate at which re-infections are acquired, the genetic complexity of the resistance phenotype, and the degree of pre-existing immunity to plasmodial parasites. As an example of the apparent impact of immunity, a study conducted in Mali in 2001