

Critical Review

A Chemical Approach Towards Understanding the Mechanism and Reversal of Drug Resistance in *Plasmodium falciparum*: Is it Viable?

Kelly Chibale

Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

Summary

Genetic and biochemical approaches to studies of drug resistance mechanisms in *Plasmodium falciparum* have raised controversies and contradictions over the past several years. A different and novel chemical approach to this important problem is desirable at this point in time. Recently, the molecular basis of drug resistance in *P. falciparum* has been associated with mutations in the resistance genes, Chloroquine Resistance Transporter (PfCRT) and the P-glycoprotein homologue (Pgh1). Although not the determinant of chloroquine resistance in *P. falciparum*, mutations in Pgh1 have important implications for resistance to other antimalarial drugs. Because it is mutations in the aforementioned resistance genes rather than overexpression that has been associated with drug resistance in malaria, studies on mechanisms of drug resistance and its reversal by chemosensitisers should benefit from a chemical approach. Target-oriented organic synthesis of chemosensitisers against proteins implicated in drug resistance in malaria should shed light on mechanism of drug resistance and its reversal in this area. The effect of structurally diverse chemosensitisers should be examined on several putative resistance genes in *P. falciparum* to deal with antimalarial drug resistance in the broadest sense. Therefore, generating random mutations of these resistance proteins and subsequent screening in search of a specific phenotype followed by a search for mutations and/or chemosensitisers that affect a specific drug resistance pathway might be a viable strategy. This diversity-oriented organic synthesis approach should offer the means to simultaneously identify resistance proteins that can serve as targets for therapeutic intervention (therapeutic target validation) and chemosensitisers that modulate the functions of these proteins (chemical target validation).

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Address correspondence to Kelly Chibale, Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa. Fax: +27 21 689 7499. E-mail: chibale@science.uct.ac.za

INTRODUCTION

As in systems ranging from bacteria to cancer cells, *Plasmodium falciparum* (*P. falciparum*) malaria has not escaped multidrug resistance (MDR). Established quinoline antimalarial drugs exemplified by chloroquine (CQ) are no longer effective in most endemic areas. Mechanisms of CQ resistance have been the subject of intense debate over the last several years. One of the most controversial mechanisms is the efflux pump mechanism in *P. falciparum*, which has been compared to that of the multidrug resistance (MDR) phenotype observed in mammalian cancer cells (1). The MDR phenotype is determined by the presence of P-glycoprotein (P-gp), a member of the ATP-binding cassette (ABC) superfamily of active transporters encoded by two classes of *mdr* genes I and II, in the membrane that is responsible for pumping antitumour agents out of cells. It is an energy-dependent process that can be inhibited by drugs such as verapamil.

P-Glycoprotein Analogues and the Chloroquine Resistance Transporter (PfCRT) in Plasmodium falciparum

The potential role of ABC-transporters in quinoline drug resistance was first proposed when CQ resistance was reversed in vitro upon co-administration of verapamil and CQ (2). Several ABC-transporters have been identified in *P. falciparum* (3). Some data had earlier suggested that the *P. falciparum* MDR (*pfmdr1*) gene and related ABC-transporters are critical in mediating drug resistance in *P. falciparum*. However, contradictory evidence exists regarding the link between *pfmdr1* and the resistance phenotype. Although some research had earlier shown that overexpression of the *pfmdr1* gene was associated with some CQ-resistant parasites and mutations of this gene had been linked to the resistance phenotype, a genetic cross between the resistant and sensitive strains showed no linkage between the *pfmdr* genes and CQ resistance (4). More recent work has however shown that, although not the determinant of CQ resistance in *P. falciparum*, Pgh1 modulates sensitivity and resistance to mefloquine,

quinine and halofantrine through mutations or changes of expression levels (5). Then same mutations were shown to influence *P. falciparum* resistance towards CQ in a strain-specific manner.

Recently, the mechanistic basis for CQ resistance in *P. falciparum* has been linked to the polymorphic gene PfCRT that also controls the verapamil-sensitive component of resistance (6, 7). Based on available evidence, PfCRT is now being hailed as the main molecular determinant of CQ resistance. This transmembrane protein localizes to the parasite digestive vacuole, the site of CQ action. This finding is consistent with the earlier disclosed role for the *P. falciparum* digestive vacuole in CQ resistance (8). It has been suggested that CQ binding to hemozoin may be reduced due to changes in the pH of the digestive vacuole as a result of mutations in PfCRT (6). Indeed, access to hemozoin has been suggested as a basis of CQ resistance and CQ accumulation in the digestive vacuole is dependent on binding to soluble heme (9).

Open and Important Questions Yet to be Answered

One open and important question that still remains to be answered relates to the fact that in 7-substituted 4-aminoquinolines, the length of the methylene spacer between two nitrogens in the side chain of CQ analogues is a major determinant of activity against CQ resistant *P. falciparum* (10–12). For example, in moving from CQ **1** to analogues **2–6**, the mechanisms of CQ resistance is somehow evaded (Fig. 1).

On this question, it was initially suggested that the resistance protein has 2 negative charges spaced 4 to 6 carbon atoms apart that bind the side-chain amino groups (11). However, it was

observed by others that these data do not account for the fact that the CQ resistance mechanism is much less effective for other aminoquinolines with side-chain amino groups separated by 4 carbon atoms as in amodiaquine and/or more recently ferroquine **6**. These observations were based on a study of 13 closely related 4-aminoquinolines and 9-aminoacridines, all with side-chain amino groups separated by 4 carbon atoms (13). It was found that the degree of resistance correlated with decreasing lipid solubility of the drug at physiological pH. In this regard, because mefloquine and halofantrine are more lipophilic than 4-aminoquinolines, it has even been suggested that the mutation(s) responsible for the mefloquine resistance may cause the resistance mechanism to favour handling lipophilic substrates yet mutation(s) causing 4-aminoquinolines may favor less lipophilic substrates.

In an attempt to answer the previous open and important question within the context of PfCRT, a model has been suggested in which PfCRT mutations may alter structurally specific drug interaction, directly or indirectly, affecting CQ flux across the digestive vacuole membrane (7). Of much interest is ferroquine **6** in which two methylene groups in the side chain of CQ are replaced by the hydrophobic ferrocenyl moiety but the two nitrogens in the side chain are still separated by 4 carbon atoms as in CQ. This compound is significantly more potent than CQ in vitro against a CQ-resistant strain of *P. falciparum* (14). A demonstration that CQ and analogues exemplified by **2–6** as well as other quinoline antimalarials interact differently with Pgh1 and/or PfCRT and corresponding mutants could go some way in answering these questions. Moreover, studies on the interaction of CQ and related 4-aminoquinolines with human P-glycoprotein could lead to 4-aminoquinolines drugs

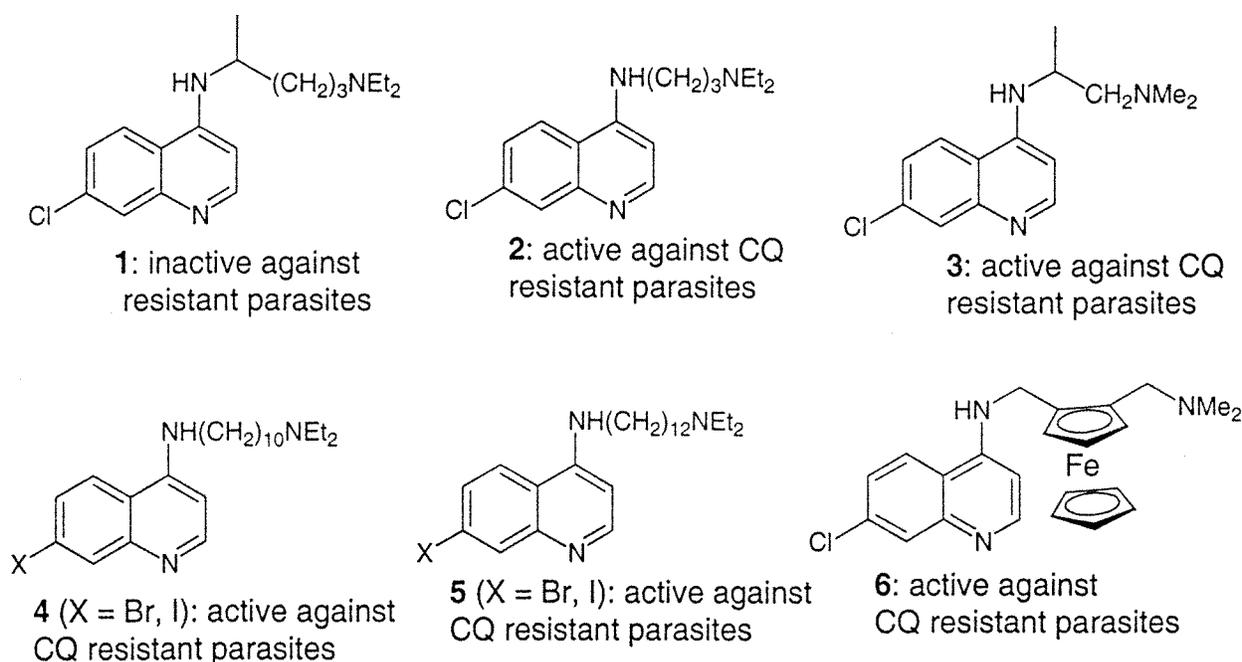


Figure 1. Chemical structures of CQ and derivatives active against CQ-resistant malarial parasites.

that selectively inhibit either the parasite ABC-transporter or the human enzyme.

The Need for a Chemical Approach: Target-Oriented and Diversity-Oriented Organic Synthesis

For over 10 years now, clearly work on mechanisms of resistance of *P. falciparum* to quinoline-containing drugs has been based on genetic and biochemical approaches. This work has been controversial and contradictory to say the least. On the other hand, there has not been a chemistry and/or chemical biology approach to this important question. Such an approach is less likely to be controversial and has the potential to resolve previous controversies. Available evidence suggests PfCRT (and to some extent Pgh1) and/or mutants to be viable targets for directing chemical libraries of potential chemosensitisers. However, for a chemical approach to be worthwhile, the effect of structurally diverse chemosensitisers should be examined on several putative resistance genes in *P. falciparum* in order to deal with antimalarial drug resistance in the broadest sense. So far, available evidence suggests that it is mutations in the already identified genes rather than overexpression that has been associated with drug resistance. Therefore, generating random mutations of these resistance proteins and subsequent screening in search of a specific phenotype followed by a search for mutations and/or chemosensitisers that affect a specific drug resistance pathway might be a viable strategy.

Identifying the mutant target for the chemosensitiser and/or the molecular sequence of the mutant gene could shed light on the resistance pathway. This diversity-oriented organic synthesis approach (15) will offer the means to simultaneously identify resistance proteins that can serve as targets for therapeutic intervention ("therapeutic target validation") and chemosensitisers that modulate the functions of these proteins ("chemical target validation") (16, 17). It is worth noting that the significance of either Pgh1 or PfCRT may be strain-specific and the scientific community will be ill-advised to narrowly focus only on these currently identified proteins. It has quite rightly been observed that changes associated with CQ resistance may also involve mutations in proteins other than PfCRT (7). Efforts should rather reflect the reality of drug resistance in malaria in the broadest sense. Although key PfCRT (or even Pgh1) mutations may be necessary for the elaboration of resistance to CQ in vitro, host immunity has been cited as a possible contributing factor to clinical outcomes. This conclusion was reached from recent evaluations of known polymorphisms in parasites from patients with uncomplicated malaria in Kampala, Uganda. It was found that the presence of 8 PfCRT and 2 Pgh1 mutations did not correlate with clinical response to therapy with CQ (18). Coupled with the enantiomer-specific sensitivity of K76I PfCRT mutations to quinine and quinidine, the selective verapamil-potentiating and -antagonizing effect of the quinidine and quinine response of K76I parasites, respectively, may suggest the importance of chirality in these mechanisms (19). At this juncture it is worth noting that the chemosentization of CQ-resistant parasites by

verapamil is not stereospecific. It has been shown that both the D and L forms are equipotent (20). It would be interesting to study the effect of both enantiomers of verapamil on the quinidine and quinine response of K76I parasites. Another point worth noting is that, even in the case of PfCRT, the verapamil-sensitive component of CQ resistance controlled by this protein may not necessarily extend to other chemosensitisers. Before other resistance genes in malaria are discovered, immediate work on target-oriented organic synthesis should focus on generating focused chemical libraries of rationally designed potential chemosensitisers in drug resistant *P. falciparum* that should be screened for chemosensitization activity. This should then be followed by investigating whether or not these molecules act by inhibiting PfCRT or Pgh1 or their mutants.

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