The malaria parasite's chloroquine resistance transporter: a multidrug resistance carrier?

R.E. Martin,[1,2] R.L. Summers,[1] M. Nash,[1] R.V. Marchetti,[1] T.J. Dolstra[1] and K. Kirk,[1] [1]Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia and [2]School of Botany, The University of Melbourne, Parkville, VIC 3010, Australia.

Malaria remains a major infectious disease in many parts of the world; an effective vaccine is not yet available and the parasite has developed resistance to most of the antimalarial drugs currently in use. When it was introduced in the 1940s, the affordability, low toxicity, and effectiveness of chloroquine (CQ) caused a revolution in the control of the disease. Around 15 years later, however, chloroquine-resistant (CQR) parasites had emerged and by the 1990s resistant strains were prevalent in most regions where malaria is endemic. CQR parasites accumulate much less CQ than do their CQ-sensitive (CQS) counterparts and it is this marked decrease in drug accumulation that underlies the phenomenon of CQ resistance. CQ resistance has been attributed primarily to mutations in the chloroquine resistance transporter (PfCRT), an integral membrane protein localised to the parasite's internal digestive vacuole (believed to be the site of CQ action) and a member of the Drug/Metabolite Transporter (DMT) Superfamily. Furthermore, mutations in this protein also modulate the parasite's susceptibility to a number of other clinically important drugs. However, the mechanism by which mutant PfCRT confers reduced drug accumulation within the digestive vacuole, and hence resistance, has been unclear.

We have expressed PfCRT in *Xenopus* oocytes, achieving a robust heterologous system for the functional characterization of this protein. Achieving expression was not straightforward; the coding sequence was codon-harmonised to facilitate correct folding of the protein and a number of putative trafficking motifs were removed to prevent its retention at internal membranes. Without these changes, PfCRT was not expressed at significant levels in the oocyte plasma membrane. Using this system, we undertook direct measurements of [^{3}H]CQ transport *via* PfCRT and provided a clear demonstration that the resistance-conferring form of PfCRT ($PfCRT^{CQR}$) has the ability to transport CQ out of the digestive vacuole whereas the sensitive form of the protein ($PfCRT^{CQS}$) does not (Martin *et al.*, 2009). We also showed that the transport of CQ *via* $PfCRT^{CQR}$ is inhibited by verapamil, a drug long-recognised for its ability to reverse CQ-resistance *in vitro*. Moreover, CQ uptake was inhibited by a number of quinoline antimalarials (including quinine and amodiaquine) as well as the antiviral agent amantadine (which exhibits some antimalarial activity *in vitro*, particularly against CQR parasites). By contrast, piperaquine and artemisinin (both clinically effective against CQS and CQR strains) were without effect. A focus of our recent work has been on determining whether verapamil, quinine, or amodiaquine are also transported *via* mutant forms of PfCRT.

There has been some conjecture as to whether PfCRT behaves as a channel or a carrier (*e.g.* Sanchez *et al.*, 2007). We found that the transport of CQ *via* $PfCRT^{CQR}$ is saturated by low (clinically relevant) concentrations of the drug (the apparent K_m(CQ) was 245 μM; Martin *et al.*, 2009). To place this in context, the addition of 100 nM CQ to the extracellular solution is estimated to result in a CQ concentration of around 2 mM in the digestive vacuole of CQS parasites and 200 to 500 μM in the digestive vacuole of CQR parasites - due to the accumulation of the drug *via* 'weak-base trapping' in this acidic compartment. A characteristic of saturable transport is the ability of unlabelled substrate to *cis*-inhibit the uptake of a radiolabelled substrate. We observed *cis*-inhibition of [^{3}H]CQ transport by unlabelled CQ in oocytes expressing $PfCRT^{CQR}$ and likewise found that the inhibition of $PfCRT^{CQR}$ by a number of different compounds, including quinine, verapamil, and amantadine, was concentration-dependent. Furthermore, we have recently shown that CQ uptake in oocytes expressing $PfCRT^{CQR}$ displays another hallmark of carrier-mediated transport - a marked dependence on temperature (Summers & Martin, 2010). Taken together, the saturability and strong temperature-dependence of CQ transport *via* $PfCRT^{CQR}$, along with the placement of the protein within the DMT superfamily of carrier proteins, support the view that PfCRT is a carrier. Moreover, the interaction of mutant forms of the protein with a number of different drugs suggests that PfCRT can function as a multidrug resistance carrier. The finding that $PfCRT^{CQR}$ behaves as a carrier has significant implications for the treatment of CQR parasites with CQ or CQ-like drugs. In particular we relate this to the example of Guinea-Bissau, where high doses of CQ are routinely used to cure CQR malaria (Ursing *et al.*, 2007).

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