Enheten för Infektionssjukdomar, Institutionen för Medicin, Solna

Molecular basis for the mechanisms of action and resistance to artemisinin combination therapy in *Plasmodium falciparum*

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ABSTRACT

Plasmodium falciparum malaria remains a leading cause of death among children in Africa. To improve treatment efficacy and delay development and spread of antimalarial drug resistance artemisinin artemisinin-based combination therapy (ACT) is now globally recommended as first-line treatment of uncomplicated *P. falciparum* malaria as a cornerstone in modern malaria control.

The aim of this thesis is to improve the understanding of the molecular basis of potential evolution of *P. falciparum* resistance to ACT.

After the worldwide introduction of ACT several reports demonstrate that the multidrug resistance protein 1 (*pfmdr*1) and chloroquine resistance transporter (*pfcrt*) genes are under selective pressure. This thesis describes the *in vivo* selective process for *pfmdr*1 haplotype coding for aminoacids 86N, 184F 1246D in reinfections after artemether-lumefantrine treatment. The selective window is within 35 days after treatment during the elimination phase of the partner drug.

PFMDR1 homologue model structures unveiled the functional interference of 86N, 184F and 1246D in antimalarial drug transport. This was further supported by *in vitro* susceptibility of *P. falciparum pfmdr*1 transfectants clones to aminoquinolines indicating that PFMDR1 may act as a vacuolar importer.

Since the resistance mechanisms of *P. falciparum* to the major ACTs are largely unknown other candidate genes were analysed. Therefore the multidrug resistance-associated 1 (*pfmrp1*) gene diversity in *P. falciparum* and its potential contribution to decreased ACT sensitivity was studied. Some 21 nonsynonymous and 6 synonymous single nucleotide polymorphisms were identified. The polymorphism I876V appears to be significantly ($P<0.05$) selected in reinfections after artemether-lumefantrine. The structural role of I876V polymorphism and impact for PFMRP1 transport was then studied in bacterial ABC transporter homologue, MsbA, and shown to be related to the nucleotide binding region of ABC transporters.

To investigate mechanism of action of artemisinins in *P. falciparum*, parasite's calcium homeostasis was studied using techniques of live single cell imaging and flow cytometry. Our work suggests that artemisinin triggers Ca^{2+} signalling- dependent cell death in *P. falciparum*. Parasite cell death was partially rescued (31%) by the Ca^{2+} chelator Bapta.

In conclusion, *P. falciparum* is adapting to the new ACTs. Complex mechanisms of *pfmdr*1/*pfcrt* are being selected by partner drugs and may represent entry points towards alarming evolution of tolerance and resistance to ACT.

Key words: *Plasmodium falciparum*; ACT; antimalarial resistance; drug selection; evolution; drug transporters

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