Enheten för Infektionssjukdomar, Institutionen för Medicin, Solna

Plasmodium falciparum drug transporter genes in emerging malaria multidrug resistance

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Hillarpsalen, Retzius väg 8, KI, Solna

Fredagen den 15e april 2011 kl 14.00

av

Maria Isabel Mendes Veiga

Huvudhandledare:
Dr José Pedro Gil
Karolinska Institutet
Institutionen för fysiologi och farmakologi

Bihandledare:
Professor Anders Björkman
Karolinska Institutet
Institutionen för Medicin Solna

Professor Koji Lum
Department of Anthropology,
Binghamton University, USA

Fakultetsopponent:
Associate Professor Roland A. Cooper
Old Dominion University
Department of Biological Sciences
Norfolk, USA

Betygsnämnd:
Docent Teresa Frisan
Karolinska Institutet
Institutionen för Cell- och Molekylärbiologi

Professor Arne Holmgren
Karolinska Institutet
Institutionen för Medicinsk Biokemi och Biofysik

Professor Dan I. Andersson
Uppsala biomedicinska centrum BMC
Institutionen för medicinsk biokemi och mikrobiologi

Stockholm 2011

ABSTRACT

Malaria is caused by an intracellular protozoan parasite of the genus *Plasmodium.*
The use of chemotherapy, the foremost tool available for the control of the disease, has been challenged in the last decades by the development and spread of drug resistance among malaria parasites. A clear understanding behind the mechanisms of parasite resistance is required for the improvement of treatment efficacy, policy assessment and in the development of new drugs.

A common strategy used by parasites in achieving resistance involves decreasing drug accumulation inside the cell. This is typically accomplished by increasing the availability of transporter proteins that mediate the efflux of the active compound.

The goal of this thesis was to better understand the involvement of drug transporter genes in the molecular mechanisms underlying drug susceptibility in *Plasmodium falciparum* malaria. The approaches involved clinical drug trials, clinical isolates and extensive studies of laboratory *P. falciparum* parasites.

The contribution of *pfmrp1* polymorphisms in *in vivo* parasite drug response was studied in *P. falciparum* infected patients from drug efficacy clinical trials. After SP treatment, recrudescent infections selected for parasites that had a lysine at amino acid position 1466 in *pfmrp1,* thus providing the first indication that this transporter gene may have a role in *P. falciparum* antifolate drug responses *in vivo*.

We examined the effect of the ACT partner drug, mefloquine, on the intra-erythrocytic cell cycle of *P. falciparum* laboratory parasites having different *in vitro* drug susceptibilities, while in parallel investigating the expression of four pivotal drug transporter genes: *pfcrt, pfmdr1, pfmrp1* and *pfmrp2*. This study revealed a delay in the cell cycle of the parasite after drug pressure, accompanied by gene induction of the transporter genes studied.

The genetic background of the drug transporter genes *pfcrt, pfmdr1, pfmrp1* and *pfmrp2* were further studied at length in field isolates collected at the Thai-Myanmar border, a historically known epicenter of resistance. The isolates were characterized *in vitro* for their sensitivity against a broad range of ACT relevant antimalarials. Correlation analyses revealed novel candidate markers for multidrug resistance against structurally unrelated antimalarial drugs used extensively in ACT regimens worldwide.

In conclusion, these studies reinforce the concept of malaria drug resistance as a multi-factorial and complex phenomenon that may involve not only the parasite's handling of the incoming drug, but also concomitant responses of its basic physiology.

ISBN 978-91-7457-277-3