eP590
ePoster Viewing
Malaria

CHARACTERIZATION OF THE COMMERCIALLY-AVAILABLE FLUORESCENT CHLOROQUINE-BODIPY CONJUGATE, LYNXTAG-CQGREEN, AS A MARKER FOR CHLOROQUINE RESISTANCE AND UPTAKE.

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Objectives. Chloroquine (CQ) was a cheap, extremely effective drug against *Plasmodium falciparum* until resistance arose. Since then, much effort has gone into reviving chloroquine as a malaria chemotherapy. Central to this approach is the inhibition of CQ efflux from its site of action, the parasite digestive vacuole. CQ accumulation studies have traditionally relied on radiolabelled CQ, which poses several challenges. There is a need for a safe and biologically relevant substitute. We present here a commercially-available chloroquine-BODIPY conjugate, LynxTag- CQ_{GREEN}®, as a fluorescent indicator of CQ uptake.

Methods. Localization of LynxTag-CQ_{GREEN} in *Plasmodium falciparum* was assessed by confocal microscopy. Reinvasion IC_{50}s of this compound and CQ were determined in *P. falciparum* strains 3D7, 7G8 and K1. Suppression of LynxTag-CQ_{GREEN} efflux was measured after pretreatment with known reversal agents. Purified wild-type or mutant *P. falciparum* CQ resistance transporter (PfCRT), the digestive vacuole-localized transmembrane protein mediating the efflux of CQ, was reconstituted to liposomal membranes, and PfCRT-mediated transport of LynxTag-CQ_{GREEN} into the liposomal lumen was assessed with this defined system. Eight laboratory strains and twelve clinical isolates were sequenced for PfCRT and Pgh1 haplotypes and *pfmdr1* copy number, and CQ IC_{50}s determined. These data were analyzed with LynxTag-CQ_{GREEN} uptake/fluorescence by multiple linear regression to identify genetic correlates of uptake.

Results. LynxTag-CQ_{GREEN} localized primarily to the digestive vacuole of the parasite. The trend of its IC_{50}s against 3D7, 7G8 and K1 was comparable to that of CQ. Its uptake, as measured by fluorescence, was increased in K1 pretreated with chemoreversal agents. In the liposomal system, mutant PfCRT known to confer resistance to CQ increased penetrance of LynxTag-CQ_{GREEN} across the liposomal membrane compared to wild-type PfCRT as assessed by fluorescence. This increased permeability was inhibited by the PfCRT inhibitor verapamil. Uptake of the compound also correlated with the logIC_{50} of CQ and a mutation in Pgh1, F1226Y. R^2 for Pgh1 1226F parasites was 0.72; R^2 for 1226Y parasites was 0.676.

Conclusions. LynxTag-CQ_{GREEN} is a useful tool for the study of CQ uptake and permits direct visualization of localization.