Identification of inhibitors of PfCCT, a key enzyme of Plasmodium falciparum membrane biosynthesis

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Résumé

During its life cycle in the human erythrocyte, Plasmodium falciparum, the parasite responsible of malaria, relies on phospholipids to build the membranes necessary for daughter cell development. The parasite membranes are composed of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) which together represent approx. 80% of the total membrane lipids. In P. falciparum, PC and PE are synthesized by the parasite's machinery through the de novo CDP-choline and CDP-ethanolamine (Kennedy) pathways using choline or ethanolamine as precursors. Our studies focus on the two cytidylyltransferases : PfCCT and PfECT. These enzymes catalyze the rate-limiting step of their respective pathway and both contain two cytidylyltransferase domains. Here we focus on the biochemical characterization and the inhibition of PfCCT. Interestingly, both catalytic domains of PfCCT are active while site-directed mutagenesis revealed that only one domain of PfECT is active, suggesting substantial evolutionary differences within this protein family3. Recently, we obtained the 3D crystal structure at 2.4 Å resolution of the C-terminal catalytic domain of PfCCT in complex with its reaction product CDP-choline. By virtual screenings of commercial compounds using docking tools, we identified molecules that competitively inhibit PfCCT activity. We are also developing a second approach for the identification of PfCCT inhibitors by fragment-based drug design. Primary screening of fragment library (230 molecules) has been performed by fluorescence-based thermal shift assay followed by Nuclear Magnetic Resonance Saturation Transfer Difference (NMR STD) as secondary screen to eliminate false positive ligands. Co-crystallization of the protein-fragments complexes will then be used for the optimization process, allowing subsequent rational design of inhibitors of this key enzyme of P. falciparum membrane biosynthesis.

Mots-Clés: Malaria, Structure based drug design, Phospholipid metabolism

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