Targeting the MEP Pathway for Antimalarial Drug Development

Children's Hospital of Philadelphia®
Division of Infectious Diseases

Andrew Borbi, Dana Hodge, Audrey R. Odom, MD, PhD

Penn
University of Pennsylvania

What is Malaria and Why Should You Care?

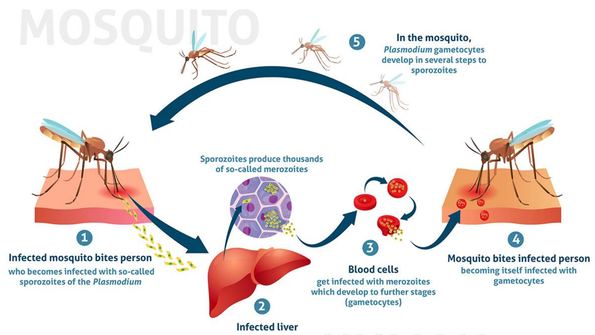


Figure 1

Malaria is a mosquito-borne disease caused by a parasite. People with malaria often experience fever, chills, and flu-like illness. Left untreated, they may develop severe complications and die. Malaria occurs mostly in poor tropical and subtropical areas of the world. In many of the countries affected by malaria, it is a leading cause of illness and death. In areas with high transmission, the most vulnerable groups are young children, who have not developed immunity to malaria yet, and pregnant women, whose immunity has been decreased by pregnancy. The costs of malaria – to individuals, families, communities, nations – are enormous. In 2019, an estimated 229 million cases of malaria occurred worldwide and 409,000 people died, mostly children in the African Region.

New antimalarials are urgently needed to treat malaria, which is caused by infection with the parasite Plasmodium falciparum. The methylerythritol phosphate (MEP) pathway for isoprenoid biosynthesis is a promising drug target for P. falciparum since this pathway is well validated and essential in the parasite but absent in humans.

What is the MEP Pathway?

The MEP pathway is used by the malaria parasite to synthesize isoprenoid precursors, namely IPP and DMAPP. Isoprenoids are a class of compounds that are vital to all organismal classes as they support many core cellular functions, such as aerobic respiration and membrane stability. Humans, however, use a different pathway, the mevalonate pathway, to synthesize isoprenoid precursors. This pathway for isoprenoid biosynthesis is thus a promising antimicrobial target, since this pathway is well validated and essential in several human pathogens but absent in humans.

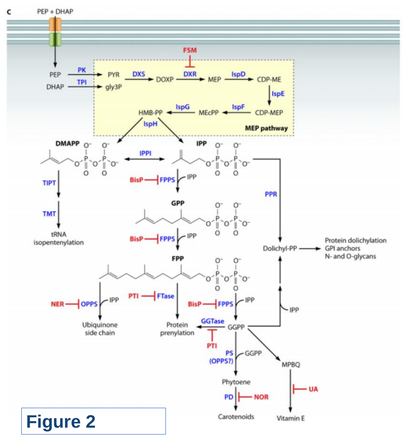


Figure 2

DXR as an antimicrobial target

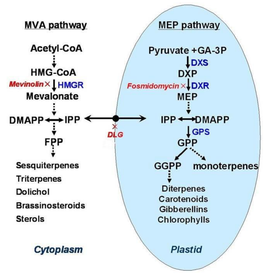


Figure 3

Development efforts targeting the MEP pathway aim to generate nontoxic compounds through the inhibition of a target that is not present in humans. DXR is a vital enzyme that is present in the MEP pathway but absent in humans. Fosmidomycin (FSM) is an antimicrobial drug that acts by inhibiting the DXR enzyme. It inhibits the synthesis of isoprenoids by Plasmodium falciparum and suppresses the growth of multidrug-resistant strains in vitro.

Developing HAD1 Mutants

Enhancer screen

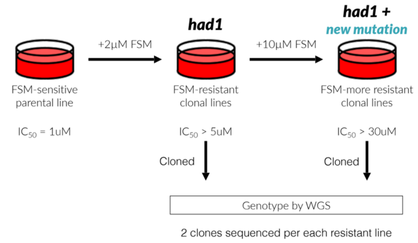


Figure 4

In an effort to learn about the mechanisms-of-action of FSM, the John lab grew wild-type parasites under selective pressure of the drug. The resistant parasites that developed as a result had a mutation in the HAD1 protein, which is believed to be a sugar phosphatase. The lab then placed these resistant parasites under greater selective pressure. This resulted in a mutation in the DXR enzyme itself, which is very rare.

How does the G103Y mutation affect enzyme function and FSM resistance?

Resistant line HN.10.52 (2 clones)

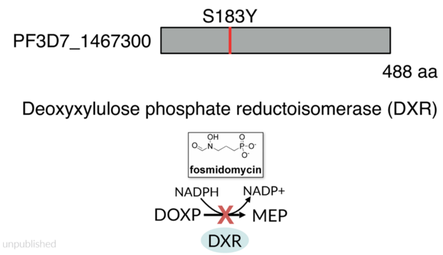


Figure 5

The new mutation was a change in the amino acid sequence: Serine183 to Tyrosine. Our goal was to determine the impact of this mutant DXR on enzyme function and sensitivity to fosmidomycin. To do this, we induced the same mutation in Escherichia coli DXR, which has close homology to the P. falciparum DXR. The mutation we plan to induce is to change a glycine residue to a tyrosine residue.

Sequence Alignment

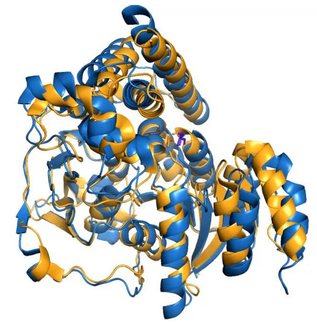
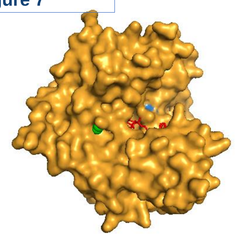


Figure 6

To ensure that the residue we were planning on mutating accurately corresponded to the mutated residue in the mutated P. falciparum DXR, we carried out a sequence alignment to see how conserved the sequences were between PfDXR and EcDXR.
We noticed that the sequences weren't highly conserved in that region of interest, so we also overlapped the crystal structures of FSM-bound PfDXR and EcDXR and noticed that the residues of interest lined up well.

Mutagenesis

Figure 7



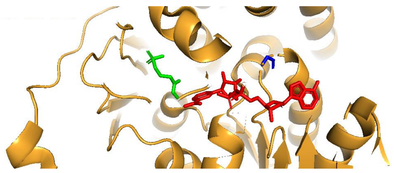


Figure 8

To induce the mutation, we designed primers and ran PCRs to develop a mutated version of the sequence that codes for DXR. We inserted this fragment into a plasmid and transfected wild-type E. coli with a plasmid containing the mutated protein. We se sequenced the DXR enzyme of our transfected cells to confirm that they had taken up our mutated plasmids. The next steps would be to grow the mutated protein using the transfected E. coli. Next, we would harvest cells, purify the protein, then evaluate its enzymatic activity and quantify its resistance to FSM.

What do we expect to learn

Enzyme assays will help determine what effects this mutation has on FSM binding;

Is the mutation preventing FSM binding?
In this case, the presence of FSM won't affect DXR activity.

Is the mutation allowing better binding of NADPH?
In this case, we would expect that a change in NADPH concentration will affect DXR activity.

Is the mutation affecting binding of DOXP?
In this case, we would expect that a change in DOXP concentration will affect DXR activity.

Selected Bibliography

1. Guggisbeg et al 2014. Euk. Cell 13(11): 1348-1359.
2. Guggisberg and Park et al. 2014. Nat. Commun. 5:4467.