Spotlights on new publications

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New drug targets - XVIII

Apicomplexans: Pantothenate (Pan, vitamin B5) is the precursor of coenzyme A (CoA) synthesis, an essential cofactor required for oxidation and acetylation of the fatty acid (pyruvate) in the citric acid cycle. A potent class of pantothenamides (PanAms) was recently investigated as a novel promising anti-malarial drug. Through its acetylation process, CoA provides various metabolic functions essentially required for gene regulation, and proteins' post-translational modification. The necessary presence of CoA in several biological processes encouraged scientists to develop inhibitory compounds targeting its biosynthesis pathway as a broad anti-parasitic drug. In the present compilation, a group of scientists from Netherland and USA, **Laura E. de Vries** and her colleagues reviewed the biological and pharmacological aspects related to Pan synthesis, and salvage, as well as its metabolization to CoA in apicomplexans focusing on *Plasmodium* spp., and *T. gondii*. Since both are intracellular protozoa, they possess several strategies to utilize host nutrients by mediating new permeation pathways through permeable parasitophorous vacuole membrane, and/or membrane transporter proteins. The reviewers demonstrated in two figures that CoA is synthesized in all living creatures through a five-step reaction that requires Pan, four ATP molecules, and cysteine. Several enzymes are required for this five-step process including pantothenate kinase (PANK), dephospho-CoA kinase (DPCK), ketopantoate hydroxymethyl transferase (KPHMT), ketopantoate reductase (KPR), pantoate-β-alanine ligase (PBAL), phosphopantetheine adenylyl-transferase (PPAT), phosphopantothenoyl-cysteine (PPC) decarboxylase (PPCDC), and PPC synthetase (PPCS).

Genomic analyses of three *Plasmodium* spp. (*P. falciparum*, *P. yoelii*, and *P. berghei*), and *T. gondii* revealed genes encoding several of these enzymes, which prompted the reviewers to discuss previous studies that indicated potential dispensability of some of them. For example, both intracellular protozoa possess two genes encoding PANK enzyme, and simultaneous knockout of both genes showed that they were dispensable in *P. falciparum* and *P. yoelii*, essential only for the viability of erythrocytic stages in *P. berghei*, and lethal in *T. gondii*. Additionally, other *in vivo* studies in rodent models confirmed the dispensability of the genes encoding PPCS and PPCDC, indicating the possible salvage of Pan-CoA synthesis pathway in *P. berghei*. On the other hand, *T. gondii* possesses two other genes that encode three enzymes required for Pan synthesis. The first is a bifunctional gene encoding the mitochondrial KPHMT-KPR, and the second encodes the nuclear PBAL. The latter gene attracted much attention when inhibitory compounds designed against *M. tuberculosis*-PBAL blocked its pathway in *T. gondii*. However, two issues provided evidence supporting the potential use of PBAL inhibitors only in chronic toxoplasmosis. First, individual deletion of either gene resulted in a significant reduction of brain cysts *in vivo*. Second, isotope labeling experimental study in acute toxoplasmosis demonstrated absence of PBAL expression in cultured tachyzoites *in vitro*. This indicates that *T. gondii* bradyzoites are able to synthesize Pan. However, the main obstacle is to design a specific PBAL inhibitor with high permeability to cross through the blood brain barrier and *T. gondii* cyst wall.

Meanwhile, the mode of a possible PAN transport for CO-A synthesis remains elusive. In *Plasmodium* spp., higher expression of Pan transporters was observed in infected erythrocytes suggesting its potentiality as a promising drug target. Unfortunately, the identified palmitoyl acyltransferase (PAT) of *P. falciparum* and *P. berghei* was found to have essential functions in mosquito transmission in the former, and secretion of osmiophilic bodies in the latter. Hence, the role of PAT as a Pan transporter was denied. Accordingly, and based on another review published two years ago [Martin RE. The transportome of the malaria parasite. Biol Rev Camb Philos Soc 2020; 95(2):305-332.], the present reviewers stated that Pan of *Plasmodium* spp. requires highly divergent transporters.

Another obstacle in designing inhibitors targeting enzymes utilized in Pan-CoA biosynthesis pathway is cytotoxicity to the host, especially if the enzyme activity is not essentially specific for the parasite metabolism. This highlighted the importance of investigating inhibitors in animal models, to prove that they specifically target parasite enzymes only. Therefore,

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the reviewers excluded all dispensable enzymes and DPCK as promising *Plasmodium* drug targets. The latter was excluded due to its higher homology to the human equivalents. They proposed PfPPAT that exhibits weak homology to the human bifunctional PPAT/DPCK enzyme.

In this publication, previous studies conducted in the last two decades to investigate Pan analogs (PanAms) as inhibitory compounds targeting *Pf*PANK activity, were also reviewed. The reviewers discussed the possible mechanism of action reported for the investigated PnAms, Amb180780, KuWei173, and MMV689258, and concluded that PanAms were converted to CoA-PanAms inhibiting CoA-utilizing processes with generation of acetyl-CoA as a metabolite. Compiled from "**Pantothenate and CoA biosynthesis in Apicomplexa and their promise as anti-parasitic drug targets. PLoS Pathog 2021 Dec 30; 17(12):e1010124.**"

Pathogenic trypanosomatids: Several phenotypic studies demonstrated mitochondrial ultrastructure damage as a positive sign for successful efficacy of newly investigated drugs specifying the mitochondrion as a promising drug target. However, the molecular level of mitochondrial target remains unknown. In addition to its direct contributing role in organizing oxidative stress, the essential role played by the mitochondrial electron transport chains (METC) in eukaryotes' respiration encouraged scientists to propose it as promising drug target. Due to high homology to mammalian orthologues, the cytochrome complex was not preferred for drug intervention by studies that investigated new drugs in certain pathogens. However, *T. brucei* mitochondrion possesses a cytochrome-independent alternative oxidase (AOX) that was predominantly expressed in trypomastigotes and is absent in the mammalian host. It was reported that AOX replaced cytochrome oxidase (complex IV in METC). Neither AOX expression in *T. cruzi* and *Leishmania* spp., nor development of selective inhibitors were reported to date.

Therefore, **Yasmine Pedra-Rezende** and her Brazilian colleagues proposed the mitochondrial import system as a molecular mitochondrial drug target in pathogenic trypanosomatids. Their review included two parts. First, they discussed their previous studies investigating the efficacy of naphthoquinone (β-lapachone derivatives) as a potent trypanocidal targeting *T. cruzi* mitochondrial membrane potential. Results revealed that four β-lapachone derivatives (N 1-4) disturbed oxygen consumption as well as the activities of cytochrome C complexes (II-IV). Notably, ROS production was directly associated with ultrastructural mitochondrial damage in using derivatives (N 2-4). In compound N1, ROS production was not due to mitochondrial damage. Moreover, Quinones, Tafenoquine and clerodane diterpene showed similar mechanism of action when investigated targeting *L. donovani* mitochondrial membrane potential.

Second, the reviewers discussed how kinetoplastids accommodate their mitochondria' morphology and content in response to the host environmental stress conditions, i.e. unique dynamic mitochondria. The majority of membrane proteins were imported utilizing several molecules that contributed to protein transfer through biological membranes. These were termed archaic translocase of the outer membrane (ATOM), translocase of the inner membrane (TIM), and the sorting and assembly machinery (SAM). The reviewers tabulated all isoforms of ATOM, TIM, and SAM reported in the last decade with the results of their encoding genes deletion. They also drew a figure representing their localization (inner or outer membrane) for the mitochondrial import protein system in *T. brucei*.

In most eukaryotes, Tom40, the mitochondrial outer membrane protein, is the molecule responsible for importing processes. However, it is absent in kinetoplastids, and replaced by archaic translocase import channel complex. Several ATOM isoforms that were identified in pathogenic trypanosomatids including ATOM11, ATOM12, ATOM14, ATOM19, ATOM40, and ATOM69, were functionally involved in mitochondrial protein import system. Besides, a specific peripheral pATOM36 protein was identified mediating and organizing the functions of protein import complexes. Its localization at the tripartite attachment complex, a structure linking mitochondrial genome (kDNA) to kinetoplastids' basal body of the parasite, suggested its essential role in separating the replicated kDNA for both daughter cells after mitosis. This highlights kinetoplastids' pATOM36 as a promising drug target. On the other hand, TIMs are highly conserved molecules in all eukaryotes. Previous studies identified and characterized seven small TIMs with cysteine-rich motifs in *T. brucei* (*Tb*Tim9, *Tb*Tim10, *Tb*Tim11, *Tb*Tim12, *Tb*Tim13, *Tb*Tim8/13, and *Tb*Tim17). All were essential for parasite viability, survival, growth, and virulence.

In fact, deletion of ATOM, and TIM encoding genes resulted in inhibition of mitochondrial import protein complexes with subsequent inhibition of essential molecules required for parasite survival, viability, and development. The reviewers claimed that although kinetoplastids ATOM, and TIM showed some similarities with mammalian orthologues, scientists should search for isoforms with conserved sequences different from those of mammalian orthologues to develop selective inhibitors. Compiled from "**Is the mitochondrion a promising drug target in trypanosomatids? Mem Inst Oswaldo Cruz 2022 Feb 21; 117:e210379.**"

Malignant malaria: Utilizing flux balance analysis, a computational polymer-metal hybrids (PMH) technology, plus a metabolic network for estimation of pathogen essential genes, a group of investigators from Nigeria and Germany **(R. Afolabi *et al.*)** predicted 5 out of 21 potential drug targets in *P. falciparum*. The computational approach highlighted these targets since they have no homology to their human orthologues. They included RNA pseudouridylate synthase putative (RPuSP), pseudouridylate synthase, 6-pyruvoyltetrahydropterin synthase, DNA-lyase, and long-chain-fatty-acid-CoA ligase. The investigators selected the first drug targets for two reasons. First, *P. falciparum* RPuSP is a member of RsuA family of enzymes that proved its essentiality for protein synthesis and cell growth. It catalyzes the modification of uridine to pseudouridine in small ribosomal RNA subunit for protein synthesis. Second, no previous studies working on molecular docking of *Pf*RPuSP were recorded. Using homology modelling, the investigators succeeded to predict its 3D structure, and characterized its physicochemical properties. It was reported that *Pf*RPuSP is hydrophilic in nature and has an *in vivo* half-life of less than five hours after overexpression in *E. coli*. Docking-based virtual screening of the 3D crystal structure against a compound library of 5621 molecules constructed from PubChem and ChEMBL databases was conducted to select compounds with high binding affinity. According to their calculated binding energies, eleven compounds were selected followed by manual docking studies. Only seven compounds that exhibited remarkable interactions with *Pf*RPuSP active binding sites were proposed as potential novel anti-malarial drugs. Absorption, distribution, metabolism, excretion and toxicology (ADMET) properties of the seven selected compounds confirmed their drug-like properties. The investigators recommended further studies to validate the efficacy of these seven compounds *in vitro* and to investigate their affinity and inhibitory potency on *Pf*RPuSP in experimental animal models. Compiled from "**Computational identification of *Plasmodium falciparum* RNA pseudouridylate synthase as a viable drug target, its physicochemical properties, 3D structure prediction and prediction of potential inhibitors. Infect Genet Evol 2022 Jan; 97:105194.**"