Spotlights on new publications

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New drug targets VIII

Malaria: To discover a new potential anti-plasmodial drug target, a group of scientists from Tulane University, New Orleans, USA (**Chen *et al.,***) with corresponding author **Juan C. Pizarro**, investigated several chaperones of stress response pathway. Glucose-regulated protein (MW 78 kDa, GRP78) is a luminal molecule in the endoplasmic reticulum (ER) to maintain its hemostasis. It is worth mentioning that GRP78, also known as BiP, belongs to the heat shock protein, HSP70 family. To maintain ER hemostasis, GRP78 has essential roles in synthesis, folding and modification of membrane and secreted proteins. In stress conditions, there is accumulation of misfolded proteins triggering the unfolded protein response (UPR) to restore normal ER hemostasis. One of UPR pathways is to increase ER-folding capacity through GRP78 up-regulation. Meanwhile, it was shown that *Plasmodium* GRP78 is an essential chaperon (factor) for schizont and gametocyte stages. On the other hand, GRP78 has two binding domains, a nucleotide (NBD) and a substrate (SBD), and requires ATP hydrolysis to be activated. The investigators hypothesized that if an inhibitor is used to bind with NBD, the SBD fails to increase ATPase activity of GRP78. In their study, two factors were evaluated to select the best GRP78 inhibitor as potential anti-plasmodial drug target, binding affinity and sensitivity against Plasmodium strain. The binding affinity factor includes compound ability to bind with GRP78-NBD as well as preference selectivity to human GRP78 or *Pf*GRP78; the latter is associated with increased host cytotoxicity.

Screening in the previous studies for GRP78 inhibitors revealed four commercially available compounds; Apoptozole, MKT077, VER-155008 and Gilvocarcin V. Additional two GRP78 inhibitors, not previously used, were included (ARL-67156 and Elesclomol) as both were predicted to inhibit GRP78 in vitro. To achieve investigating the ability of screened compounds to bind with NBD of human and *Pf*GRP78, the investigators constructed synthesized compounds. Results revealed successful binding of the previously reported compounds with recombinant Plasmodium and human GRP78-NBD, but Gilvocarcin A showed the weakest binding affinity. While Apoptozole and MKT-077 showed similar binding affinities with both Plasmodium and human GRP78-NBD, VER-155008 showed higher affinity to bind with PfGRP78-NBD, but with higher selectivity to human GRP78. Also, their results revealed that binding of MKT-077 with *Pf*GRP78 was associated with significant thermal destabilization of GRP78, while that of apoptozole increased PfGRP78 thermal stability, suggesting the formation of a protein-compound complex. In the same time, to investigate the efficacy of GRP78 inhibitors as potential drug target against drug-sensitive and -resistant *P. falciparum* strains, *in vitro* growth inhibition assays were performed in different dilutions, and 50% inhibitory concentrations (IC_{50}) for each compound were calculated and compared with chloroquine as control. Three compounds showed strong anti-plasmodial activity; MKT-077 was the strongest, followed by Apoptozole and VER-155008 against both *Plasmodium* strains. However, only Apoptozole showed a statistically significant activity between sensitive and resistant *P. falciparum* strains. On the other hand, Gilvocarcin V and Elesclomol showed EC_{50} values in the same range of chloroquine.

Based on attained results, the investigators hypothesized that best inhibitor should have high parasite sensitivity (Apoptozole and MKT-077), high affinity (e.g. VER-155008), and overall higher protein complex stability as well as significant activity against resistant *Plasmodium* strains (Apoptozole). They attributed the limited binding affinity of Apoptozole to its limited affinity to phosphonucleotides (required for ATP hydrolysis). They also suggested the rigidity of the nucleotide-binding domain as a factor in the binding affinity of a compound to PfGRP78-NBD. To confirm their hypothesis, the investigators observed 33- and 23-fold decrease in PfGRP78-NBD' affinity towards ADP and ATP, respectively compared to human GRP78. However, mutant *Pf*GRP78-NBD (codon Y39) showed double increase in ADP binding affinity but failed to bind with Apoptozole suggesting the significant effect of Y39 mutation on the compound-binding site. The crystal structures of PfGRP78-NBD and the human counterpart were identified and used to investigate the differences in their binding affinities to ATP. They observed a major difference in their chemical structure which is responsible for the thermal stability of

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*Pf*GRP78-NBD. Surprisingly, codon Y39 was responsible for its thermal stability, as rigidity was pronounced in wild type compared to the mutant one.

The investigators discussed advantages and disadvantages of *Pf*GRP78 rigidity. Being rigid, the molecule does not allow for discovery of a suitable inhibitor. This is better achieved by computational screening since active site flexibility is highly expensive. Computational screening (using *in silico* bioinformatics), allows quick screening for the suitable inhibitors. So with bioinformatics, it is easier to manipulate rigid stable molecules than flexible ones. In addition, the investigators suggested that *Pf*GRP78' low affinity for phosphonucleotides can be an advantage during computational screening. In conclusion, Apoptozole is established as a promising novel drug target against *falciparum* malaria, but its binding affinity is only increased with mutant Y39 strains. The investigators recommended further studies to investigate the structure interaction(s) in PfGRP78-NBD–Apoptozole complex. Compiled from **"Repurposing drugs to target the malaria parasite unfolding protein response." Sci Rep 2018; 8: 10333.**

Intestinal microsporidiosis: This clinical disease is reported worldwide specially in HIV patients and generally in immunocompromised ones caused by *Encephalitozoon* species. Drug resistance was repeatedly linked with two multidrug resistance genes. *E. intestinalis* is amitochondriate, lacking organelles for energy production, i.e. oxidative phosphorylation through glycolysis. Triosephosphate isomerase (TIM) is an important glycolytic enzyme responsible for reversible interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP). Previous studies proposed TIMs inactivation using established safe drugs, e.g. omeprazole, rabeprazole and sulbutiamine as a new strategy to develop novel drugs for treatment of Chagas' disease, African sleeping sickness, amoebiasis, and giardiasis. Sulfhydryl compounds were also suggested to inactivate TIM through derivatization of its cysteine residues (Cys). From Mexico, **Itzhel García-Torres *et al.*** succeeded for the first time to characterize *E. intestinalis* TIM both at functional and structural levels. To achieve this, gene encoding TIM was successfully synthesized according to DNA sequence obtained from NCBI database, while over-expression and purification of *Ei*TIM was performed using *E. coli* BL21 DE3pLysS culture cells. This was followed by measurement of melting point for EiTIM' thermal denaturation and quantitative determination of derivatized free Cys as well as its structural characteristics and folding properties with and without addition of three sulfhydryl reagents (MMTS, MTSES and DTNB), at increased concentrations. In addition, assays to investigate the efficacy of three commercial already approved drugs; omeprazole, rabeprazole and sulbutiamine to inactivate recombinant *Ei*TIM were conducted.

Results revealed that 1) *Ei*TIM showed high catalytic activity in interconversion of DHAP and GAP isomers; 2) *Ei*TIM is efficiently inactivated by all sulfhydryl compounds tested in a concentration-dependent manner; however, it was partially inactivated by addition of MMTS (60%) and totally inactivated by the NTSES and DTNB (100%); 3) All sulfhydryl compounds didn't significantly affect human TIM; 4) Derivatization of at least 3 Cys per subunit in EiTIM leads to loss of enzymatic activity; 5) In the structural studies, there was significant alteration in *Ei*TIM global stability, and 6) Sulbutiamine was the most effective safe approved drug, even at low concentrations and showed more efficacy than tested sulfhydryl compounds.

The mechanism of action of the safe drugs was documented from several reports to cause derivatization of Cys residues. Accordingly, the investigators suggested that the mechanism of TIM inactivation in sulfhydryl compounds is similar. One more important feature of sulbutiamine is its ability to crosses blood-brain barrier; therefore, is a promising drug in the treatment of microsporidial encephalitis. Future studies were recommended to asses sulfhydryl compounds and other safe drugs that cause derivatization of Cys residues against TIM enzyme from other Encephalitozoon species. Compiled from **"First characterization of a microsporidial triosephosphate isomerase and the biochemical mechanisms of its inactivation to propose a new druggable target." Sci Rep 2018; 8: 8591.**