**Spotlights on new publications**

Sherif M Abaza

Medical Parasitology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

**Corresponding Author:** Sherif M Abaza, **Tel.:** +20 1005 243 428, **E-mail:** smabaza@hotmail.com

**Received:** 3 August, 2020, **Accepted:** 15 August, 2020.

**Print ISSN:** 1687-7942, **Online ISSN:** 2090-2646, **Vol. 13, No. 2, August, 2020**

**New drug targets - XIII**

**Gene therapy:** Two important obstacles face control of neglected tropical diseases (NTDs); drug resistance and poverty of the affected population. Drug resistance includes other related factors such as side effects associated with prolonged regimens, mass treatment programs, low or inefficient drug potency against all parasitic stages, and parasitic gene mutations.

Epigenetics is the study of heritable phenotype changes without alterations of the DNA sequence, *i.e.*, changing gene activity without change in DNA sequence. It also means changing of gene code that affect its activity and expression, and subsequently affects the way cells read gene activity. An important utilized mechanism in the era of epigenetics is histone modification that affects gene activity and expression all through cell divisions; *i.e.*, change in cellular differentiation. Histone modification is defined as the structural adaptation of the chromosomal regions to preserve change in its activity state. As chromatin is the complex of DNA wrapping around histone proteins, therefore histone modifying enzymes will change gene expression as well.

During recent years (2017-2020), several studies were conducted to investigate the efficacy of drugs modulating histone acetylation/deacetylation process in the treatment of four important tropical diseases: schistosomiasis, malignant malaria, *Leishmaniasis*, and Chagas’ disease. This encouraged Italian reviewers (Rossella Fioravanti *et al.*) to summarize the results obtained due to using histone acetyltransferases (HATs) and histone deacetylases (HADCs) inhibitors. Given these data, the reviewers recommended further epigenetic studies for alternative gene therapy for NTDs.

Genomic studies of *P. falciparum* revealed ten histone acetyltransferases (HATs), seven histone deacetylases (HADCs), and eight bromodomain (BRD)-containing proteins. The latter is a conserved structural module in chromatin-associated proteins and HATs, *i.e.*, a protein domain known to recognize acetylsine residues on the histone. All *P. falciparum* HATs and HADCs showed high potentiality as drug targets. Among them, *PfGCN5* showed upregulation of virulence gene expression in stress conditions with crucial role in emergence of artemisinin resistance in *falciparum* malaria. Garcinol 1, a natural GCN5 inhibitor, showed significant lethal effects against erythrocytic asexual stages of both chloroquine-sensitive and -resistant *P. falciparum* strains. Presence of three non-identical residues in the crystal modelling structure of *PfGCN5* and its human counterpart encouraged the scientists to design novel selective specific *PfGCN5* inhibitors utilizing either virtual or high-throughput screening. Also, *in silico* docking studies conducted on the crystal structures of the BRD of *PfGCN5* using series of BRD inhibitors showed growth inhibition of *P. falciparum* erythrocytic stages. On the other hand, novel series of *PfHADACs* inhibitors were investigated *in vitro* against erythrocytic stages of *P. falciparum* and *P. berghei*. Two inhibitors showed sub-micromolar efficacy against chloroquine-resistant and –sensitive strains. One of the most efficient HDAC inhibitors (MC1742 12) displayed nano-molar activity against *P. falciparum* isolates, with extremely low toxicity against murine and human cell lines *in vitro*. In addition, the reviewers discussed the results of two studies that utilized combined therapy in treatment of *falciparum* malaria; primaquine with a pan HDAC inhibitor (vorinostat; SAHA), and the later with BIX-01294 (histone methyl-transferase inhibitor).

Genomic studies of *S. mansoni* identified twelve HDACs; three of class I, four of class II and five of class III, and all were cloned and characterized. It is worth mentioning that only the three HDACs class I showed expression at all life cycle stages, with *SmHDACB* transcripts being the most abundant. Besides, a gene encoding sirtuin homolog was also identified (*SmSIRT2*). As approved from the Food and Drug Administration (FDA), several HDAC inhibitors were investigated in treatment of schistosomiasis mansoni such as vorinostat (SAHA), panobinostat, belinostat, and romidepsin. They were tested against *S. mansoni* schistosomula, and adult worms for both viability and egg production. It was observed that SAHA displayed low inhibitory effects on all investigated assays, panobinostat was potent only against adult worm’ viability and egg productivity, belinostat was inactive against adult worm’ viability with low reduction in schistosomula viability and egg production. Notably,
romidepsin showed potency similar to praziquantel (100% against adult worm viability and egg production with neglected efficacy against juvenile schistosomula). Another study performed a high-throughput screening on 1500 HDAC inhibitors and identified three compounds with lethal potency against viability of both schistosomula and adults. Using virtual screening against the crystal structure of SmHDAC8, the most abundant HDAC in all developmental stages, enabled the investigators to identify several inhibitors with variable degrees of selectivity against human HDAC8. For S. mansoni sirtuin, two inhibitors against human SIRT1/2 (sirtinol and salermide) were investigated and induced schistosomula death and reduced adult worm stability and egg production as well due to DNA fragmentation. Another sirtuin selective inhibitor was identified (TCMDC- 143295) and a series of analogs was developed. All analogs showed improved potency and selectivity with no toxicity.

Leishmania spp. possess four genes encoding class I/II HDAC and three genes encoding sirtuin homologs. The four inhibitors, previously investigated in schistosomiasis with FDA approval, showed poor efficacy against amastigotes and promastigotes of both L. donovani and L. amazonensis. Besides, they showed severe toxic effects towards cultured macrophages. Other inhibitors were tested as anti-Leishmania agents, however the results also showed low to moderate efficacy. It is worth mentioning that one of SAHA derivatives (MDG) showed significant potent efficacy against L. donovani and L. infantum intracellular amastigotes, with poor efficacy against promastigotes. On the other hand, bisnaphthalimidopropyl (BNIP) compounds showed potential inhibitory efficacy against Leishmania sirtuin, but with selectivity toward human SIRT1. After the recent identification and characterization of Leishmania sirtuin homologs in L. donovani, a selective inhibitor was developed (KH-TFMDI). It showed high potent inhibitory effects on the growth of L. amazonensis promastigote and amastigaste.

Because Leishmania and Trypanosoma species belong to the same kinetoplastid group, Chagas’ disease is treated by the same HDAC and sirtuin inhibitors as in leishmaniasis. Similarly, BNIPs exerted potent lethal activity against T. cruzi epimastigotes, and intracellular amastigotes, with low toxicity in vitro. However, it became ineffective when tested in experimentally infected mice. Also, KH-TFMDI showed significant potency against T. cruzi amastigote, trypomastigotes, and epimastigote as well. The present compilation was summarized from a review article “Targeting histone acetylation/deacetylation in parasites: an update (2017-2020). Curr Opin Chem Biol 2020 Jun 29; 57:65-74.”

Malignant malaria: Species of Plasmodium utilize proteases’ arsenal mainly for two essential events; hemoglobin degradation to be employed in protein synthesis and egress cascade to invade more erythrocytes. No doubt that falcipains and plasmepsins (PLMs), the major cysteine and aspartyl proteases in P. falciparum, respectively are important potential drug targets. Hundreds of studies were conducted to develop or synthesize falcipain inhibitors utilizing identification of falcipain crystal structure with high-throughput or virtual screening approaches. Structure activity relationship (SAR) studies were also conducted to identify selective specific inhibitors to falcipains. However, few studies were reported for PLMs, in spite of the fact that they not only contribute with falcipains in hemoglobin degradation, but they also act as maturation factors for synthesis of the rhoptry proteins. The latter are essential for maturation of three subtilisin-like proteases (SUBs) that play important roles, as processing proteins, in several events of egress and de novo RBCs invasion cascade. Among the ten aspartyl proteases (clan AA, family A1) detected in P. falciparum genome, only four PLMs; II, V, IX and X are promising drug targets. The first two (II and V) contribute in hemoglobin degradation, while the latter (IX and X) are involved in egress and de novo RBC’s invasion cascade. Except for PLM-V, Plasmodium PLMs share varying sequence homology with human aspartyl proteases, which means that searching for PLM inhibitors remains a difficult challenge. Also, this may explain the few studies conducted for development and/or synthesis of a selective PLMs inhibitor.

The present compilation discussed the study conducted by The Walter and Eliza Hall Institute of Medical Research, University of Melbourne, Australia (Paola Favuzza et al.). A collaborative team, thirty-two investigators, from Australia, USA and Switzerland discovered an oral bioavailable lead compound with selective in vitro and in vivo inhibitory effects on both PLMsIX and X. The investigators conducted a phenotype high throughput screening approach of a library including inhibitory compounds of aspartyl proteases against P. falciparum erythrocyte blood stages. The lead compound, termed WM382, showed significant lethal effects against P. falciparum erythrocyte stages. A single oral dose of WM382 after infection of mice liver with P. falciparum sporozoites caused protection of mice from developing erythrocytic stages. The investigators attributed this result to the essential roles played by PMLs IX and X, in egress of liver merozoites. Besides, complete cure was obtained in in vivo studies of experimentally P. berghei-infected mice and orally treated with WM382.

Moreover, the investigators demonstrated PLM-X as a principal molecule required for the direct maturation of proteins involved in parasite development, and egress as well as de novo invasion of the egressed merozoites. The obtained results showed that PLM-X has an essential role in the processing, maturation and activation of SUBs 1 and 2, and subsequently
The development of novel antischistosomal drugs is of importance in view of resistance strains, its poor efficacy against juvenile schistosomes, and inability to prevent re-infection. Adekiya et al. (2020) reviews the molecular targets of the schistosomal tegument aiming to identify the most potential agents targeting these molecules. This will facilitate designing and developing a novel anti-schistosomal drug by the schistosomal tegument. It was reported that five TSPs were identified on Schistosoma genome. Two essential targets (PLM IX and X), will create a high threshold against resistance development. They concluded that WM382 is a safe effective antimalarial drug and recommended further studies to validate its use in clinical trials. Compiled from "Dual plasmepsin-targeting antimalarial agents disrupt multiple stages of the malaria parasite life cycle. Cell Host Microbe 2020 Apr 8; 27(4): 642–658.e12."

**Schistosomiasis:** The present compilation (Tayo Alex Adekiya et al.) reviews the molecular targets of the schistosomal tegument to identify the most potential proteins and/or receptors, and to highlight the potential targets among these molecules. This will facilitate designing and developing a novel anti-schistosomal drug to overcome three important obstacles in praziquantel (PZQ) treatment: emergence of resistant strains, its poor efficacy against juvenile schistosomes, and inability to prevent re-infection. Also, the present compilation discussed possibilities of utilizing surface functionalization of nanoparticles with antibodies or small peptides to specifically bind with the schistosomal tegument receptor(s).

On the other hand, researches in nanotechnology focus on nano-enabled drug delivery systems that increase drug bioavailability and therapeutic efficacy and reduce its side effect as well. Lipid-based nanoparticles (LBPNPs) such as liposomes, and nanodiscs, offer secure application of the encapsulated drug for the targeted delivery. Having a high affinity for the phospholipid bilayer of schistosomal tegument, LBPNPs gained much attention in treatment of schistosomiasis because of its well-absorbance. The reviewers summarized literature studies that utilized nanoparticles to deliver PZQ in an attempt to improve its therapeutic efficacy. The most reported nano-delivery system was using liposomes with significant reduction in worm burden; egg production and hepatic granuloma. However, none of these studies reported increase efficacy against juvenile schistosomes or prevention of re-infection.

*Schistosoma* adults possess a hepta-laminate tegumental surface that is continuously repaired and replaced every five days to survive against host immune response(s). The tegumental surface is provided by potential proteins, receptors and enzymes that are considered potential targets for nano-enabled drug delivery systems. Proteins include heat shock proteins (HSPs), microtubule related proteins (MRPs), schistosome glucose transporters (SGTPs), and tetraspanins (TSPs). It is well known that HSPs (mainly 16, 60 and 70) are molecular chaperones for protein folding to help schistosomes to survive in stress conditions. Also, HSPs are highly expressed upon increased temperature associated with the parasite passage from snails (22-28°C) to the mammalian hosts (33-37°C). It is worth mentioning that MRPs are assigned for mobility and schistosomes attachment and detachment. They include actin, tubulin, paramyosin, and dyneins. The latter was suggested to play an essential role in transporting of tegumental vesicles from sub-tegumental cells to the tegumental surface. While SGTPs facilitate glucose uptake directly from the host bloodstream for energy production, the main function of TSPs is to maintain the plasma membrane structure of the schistosomal tegument. They are members of integral membrane proteins expressed by the schistosomal tegument. It was reported that five TSPs were identified on Schistosoma genome. Receptors include aquaporins, acetylcholinesterase (AchE), and nicotinic type of acetylcholine receptors (nAChR). Aquaporins are responsible for influx and efflux control of water molecules within tegument compartments *i.e.* controlling schistosomiasis osmotic regulation. On the other hand, AchE and nAChR maintain the schistosomes ion channels and they are essential neurotransmitters. Finally, enzymes include glutathione S-transferase, thoredoxin peroxidase, protein disulfide isomerase, and glyceraldehyde-3-phosphate dehydrogenase. Each has its own function and collectively contribute to schistosome survival, growth, and development.

The reviewers discussed studies that dealt with all these potential targets and suggested them as novel anti-schistosomal drugs utilizing nano-delivery systems. Some of these targets were reviewed in details, such as SGTPs 1 and 4; aquaporins, AchE, nAChR, and TSPs. Therefore, nanoparticles functionalized with antibodies or antibody-like ligands or aptamers, with high specificity to the potential targets will enable scientists to design novel anti-schistosomal drugs. Aptamers have emerged as promising molecules developed from short single-stranded oligonucleotide (RNA or DNA ligands) or peptides that bind to their...
target molecules; whatever its size, with high specificity, affinity, and versatility. Compiled from "A review of nanotechnology for targeted anti-schistosomal therapy. Front Bioeng Biotechnol 2020; 8: 32."

**Visceral leishmaniasis (VL):** Leishmaniasis is one of the major neglected tropical diseases caused by more than twenty *Leishmania* species. Variable clinical presentations in VL are reported, as well as several cases with missed diagnosis. Even if cases are accurately diagnosed, failure of treatment due to drug resistance or several undesirable side effects from prolonged treatment are also repeatedly reported. There is an urgent need to discover novel anti-*Leishmania* compounds effective against several *Leishmania* spp., with low cost, and short time of treatment.

Drug delivery systems have been considered in the last two decades to decrease drug toxicity and reduce time of treatment as well. Among the recently developed delivery systems is the micelle, a thermo-reversible co-polymer with hydrophilic and hydrophobic segments. Several studies utilized micelles, as delivery systems for treatment of various diseases, reported their stability and efficient targeting ability. On the other hand, clioquinol (ICHQ), a quinoline-derived molecule, showed promising *in vitro* results against both stages of *L. amazonensis* and *L. infantum*, and anti-leishmanial activity in treating experimentally *L. amazonensis*-infected BALB/c mice. In murine cutaneous *Leishmaniasis*, four treatment regimens were investigated; ICHQ, ICHQ- Poloxamer 407-based-micelles (ICHQ-M), Amphotericin B, and AmpB-based liposomal formulation, versus Miltefosine as a control drug. Results showed that ICHQ and ICHQ-M regimens demonstrated the highest reductions in all tested parameters; lesion size and number, parasite burden in the infected organs, and development of anti-leishmanial Th1-type response (IFN-γ, IL-12, TNF-α, and GM-CSF), and with no toxicity.

Besides, being inexpensive and easily manufactured encouraged a group of investigators from Brazil and Peru (*Grasiele SV Tavares et al.*) to investigate the efficacy of micelles to deliver ICHQ for treatment of VL. Because *L. infantum* is the major species responsible for VL in South America, the investigators subcutaneously infected four groups of BALB/c mice with *L. infantum* stationary promastigotes. This was followed by subcutaneous injection of ICHQ, or ICHQ-M, while the other two groups were utilized as control. A group was treated with oral Miltefosine, whereas the other was administered a subcutaneous injection of empty micelles (untreated). The investigated parameters included parasitological (parasite burden) and immunological (cytokine levels). Significant higher levels of IFN-γ, IL-12, GM-CSF, nitrite and IgG2a isotype antibody levels, with low IL-4 and IL-10 production were observed in all the investigated regimens. Also, significant reductions in the parasite burden were obtained in comparison to untreated group. However, comparison between the three treated groups, showed that ICHQ-M was the most effective in induction of Th1 immune response, and reduction in the parasite burden. The investigators recommended further studies to validate ICHQ-based micelles in clinical trials. Compiled from “A clioquinol-containing Pluronic® F127 polymeric micelle system is effective in the treatment of visceral leishmaniasis in a murine model. Parasite 2020 Apr; 27: 29.”

**Chagas’ disease:** Calcium has several functions in eukaryotes and its role as a signaling messenger is well-documented in pathogenic trypanosomatids. Modulation of proliferation and stage differentiation in *Leishmania* and *Trypanosoma* spp. was demonstrated both *in vivo* and *in vitro* by Ca$^{2+}$ signaling through several surface glycoproteins receptors. In addition, Ca$^{2+}$ binding proteins have essential roles in hemoflagellates because they are critically important for flagellar activity, propagation as well as adhesion to host cells. Because Ca$^{2+}$ is required for the fusion of the host cell lysosome to the parasite plasma membrane, it is proposed that Ca$^{2+}$ increase the capacity of *Leishmania* amastigotes and *Trypanosoma* trypomastigotes to invade host-cells. Another reported function of Ca$^{2+}$ signaling is immunoevasion of the host immune response by contributing to release of variant surface glycoproteins. It is worth mentioning that Ca$^{2+}$ signaling in *Leishmania* spp. is necessary for the long-term adaptive responses against environmental stressors in the mammalian host.

The majority of studies conducted in developing novel drugs hypothesized that potential drug target should selectively disturb a specific mechanism involved in the parasite with minimal effects on the host cells. A group of scientists from Venezuela and USA (*Gustavo Benaim and his colleagues*) observed differences in the mechanism(s) involved in intracellular Ca$^{2+}$ homeostasis between host mammalian cells and *T. cruzi*. In all pathogenic trypanosomatids, three intracellular organelles contribute together by diverse mechanisms at the plasma membrane level to regulate Ca$^{2+}$ homeostasis. They are unique mitochondrial, an endoplasmic reticulum with Ca$^{2+}$ pump, and acidocalcisomes. The latter are acidic vacuoles that are filled with Ca$^{2+}$, polyphosphates and other ions, identified only in trypanosomatids that infect specialized cells such as platelets and mast cells. The unique mitochondrion contains pyruvate dehydrogenase phosphatase (PDP) to sensitize environmental Ca$^{2+}$ concentration. In the low Ca$^{2+}$ concentration, PDP, utilizing the Krebs cycle, produces energy metabolism required to activate endoplasmic reticulum Ca$^{2+}$ pump. This will increase accumulation of Ca$^{2+}$ inside the acidocalcisomes, to be released to the cytoplasm by specific receptors.
Accordingly, the three organelles act in concert whenever a change in intracellular Ca\(^{2+}\) has occurred, to return the concentration to the cytoplasmic basal level. However, this is limited because of their capacity volume. Accordingly, parasite plasma membrane will be responsible for the long-term regulation of intracellular Ca\(^{2+}\) concentration by Ca\(^{2+}\) extrusion regulated by calmodulin (Calm). Although Calm of mammals and all trypanosomatids have 89% homology, the reviewers claimed that differences between both Calms deserve special attention, because there are 16 amino acid substitutions in the latter, and it is encoded by three genes tandemly arranged.

After discussing different mechanisms involved in the general homeostatic systems between host mammalian cells and T. cruzi, the reviewers discussed several potential anti-Trypanosoma drugs that act on the three intracellular organelles of T. cruzi without affecting host Ca\(^{2+}\) homeostasis. Five groups of drugs were discussed; the commonly used anti-arrhythmic drug (Amiodarone and its derivatives), inhibitors of ergosterol synthesis, the new anti-tuberculosis drug (SQ109), the well-known anti-parasitic drug (Miltefosine), and calcium channel blockers (CCBs).

Several studies were conducted to investigate the effects of Amiodarone and its derivatives in treatment of Chagas’ disease. From, the obtained results, it was concluded that Amiodarone has direct potent effects on T. cruzi mitochondrion and acidocalcisomes with rapid Ca\(^{2+}\) release to the cytoplasm. A recent study trial conducted in military working dogs in Texas (USA) showed 95.3% increased survival. Combined treatment was also used; Amiodarone with Itraconazole, an ergosterol synthesis inhibitor. Besides, Amiodarone derivatives such as Dronedarone and Amioder were investigated. While Amioder gave similar results to Amiodarone, more rapid effects were noticed using Dronedarone, beside its lower IC50. Another inhibitor of ergosterol synthesis, Posaconazole showed potent efficacy in treatment of Chagas’ disease, with significant increase in basal intracellular Ca\(^{2+}\) concentrations in cultured epimastigotes.

Regarding the recently tested anti-tuberculous drug (SQ109), it is already in phase II-III clinical trials and has given promising results in treatment of resistant tuberculosis. It was investigated in treatment of Chagas’ disease because it inhibits ergosterol synthesis with direct major parasite lethal effects through mitochondrial collapse and rapid alkalinization of acidocalcisomes. Also, Miltefosine showed similar results on T. cruzi mitochondrion and acidocalcisomes, with synchronous action on Ca\(^{2+}\) permeability in the plasma membrane. On the other hand, CCBs showed significant inhibitory effects on growth of L. infantum promastigotes and T. cruzi epimastigotes in vitro. The reviewers recommended further studies to validate their potentiality in treatment of Chagas’ disease. Compiled from “Disruption of intracellular calcium homeostasis as a therapeutic target against Trypanosoma cruzi. Front Cell Infect Microbiol 2020 Feb; 10: 46".