Spotlights on new publications

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New drug targets VII

• **Schistosomiasis:** Motor control or disruption of neuromuscular control, is considered a successful strategy in treatment of parasitic helminths. For example, both Praziquantel (PZQ) and ivermectin lead to calcium influx and tetanic paralysis, while metrifonate inhibits acetylcholinesterase resulting in flaccid paralysis. In an attempt to resolve the situation of the sole licensed drug (PZQ) against some resistant strains of *Schistosoma* spp., a group of scientists from Canada and USA (**Nelly El-Sakkary *et al***.) conducted the present study. Their strategy is mapping of schistosome' nervous system followed by phenotyping studies aiming to disturb schistosome' neuromuscular functions. They used synapsin, as a neuronal marker, to investigate both central and peripheral nervous systems in *S. mansoni* adults and schistosomules; and use the obtained data as a guide to the discovery of new specific neurotransmitters, which would lead to the development of new drug targets. In trematodes, as with other organisms, functions of the central nervous system (cerebral ganglia) control all major body structures, i.e. somatic musculature, tegument, suckers as well as the reproductive organs are achieved by nerve fibers through chemical transmitters. Among them are acetylcholine, glutamate and the biogenic amines (BAs). Several studies were conducted for the first two transmitters, while little is known about BAs although they were previously reported as the major classical transmitter in the animal kingdom with a major role in schistosome motility. Invertebrate-specific BAs are derived from tyrosine and tryptophan or histidine, and they include several compounds, among which are octopamine (OA) and its precursor tyramine (TA). Both BAs were immunolocalized in snails for motor control, while synapsin was immunolocalized in arthropods for the same function. Interestingly BAs are present in mammals only in trace concentrations suggesting that in invertebrates they have major chemical control. Accordingly, the investigators' objective was OA immunolocalization in *S. mansoni* adults and schistosomules. This was to be followed by quantitative phenotyping studies to evaluate the effectiveness of some compounds that are structurally related to tyrosine-derived phenolamine and catecholamine neurotransmitters on schistosomules motility and length. The investigators hypothesized that this strategy may facilitate development of novel drug targets in schistosomiasis.

S. mansoni life cycle was maintained using *B. glabrata* snails, Dulbecco's modified Eagle medium and mice infection. OA immunolocalization in *S. mansoni* adults and schistosomules was achieved using primary specific OA antibody and monoclonal anti-*Drosophila'* synapsin antibody against negative controls without the primary antibody. Immunolocalization was visualized using confocal microscope and photographed using argon and helium-neon lasers to remove dyes and fluorophores. OA was found widely dispersed in the adult schistosome including the bi-lobed brain, longitudinal and transverse nerve chords along the length of the adult schistosomes, extending to the tegument. It is worth mentioning that several publications reported the presence of a single pair of cerebral ganglia in schistosomes and other trematodes. Interestingly, OA was strongly localized in two pairs of ganglia in schistosome' head, one just posterior to the oral sucker and the other anterior to ventral one. In males, OA was localized in nerve chords along the gynecophoric canal flaps and surface tubercles, while in females, it was localized in the ovary as well as the embryo within the *Schistosoma* egg. On the other hand, OA was only restricted to the developing cerebral ganglia and fine nerve fibers in the schistosomules.

The phenotypic studies were conducted on cultured schistosomules to investigate the effects of three phenolamine compounds; OA, its precursor (TA) and its methylated product (synephrine; SE) on schistosomules motility and length. The investigators also tested other structurally related catecholamines such as dopamine (DA), norepinephrine (NE) and metanephrine (ME), as well as three other BAs; histamine (HA), phenylethylamine (PE) and 5-hydroxytryptophan (5HT). Schistosomules motility and length were expressed as a fold change over the mean of each compound at baseline. Results revealed that phenolamines (OA, TA and SE) significantly increased schistosomules motility (5-22 folds) and length (20-25%), but SE was the strongest phenolamine, followed by TA, and the least was OA. As regards the catecholamines, ME was the strongest (14 fold motility and 25% length), followed by DA, while NE showed insignificant increased motility, but significantly increased length (25%). On the other hand, among the other three BAs, 5HT and PE insignificantly increased motility, and showed significant 10% increase in length, while HA showed no changes in schistosomules motility with significant 10% increase in length.

The final step in their study was to search for drugs that modulate or inhibit tyrosine-derivative signaling investigated in schistosomules motility and length. Drugs (No.=28) were selected from compounds that affect OA

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signaling in invertebrates and adrenergic signaling in vertebrates. Schistosomules (7 days) were incubated in the presence and absence of the drug and their motility and length were compared. The investigators tested each compound three times and recorded the results for at least 15 schistosomules in each trial. Among the selected drugs, only 17 compounds showed effects on schistosomules motility or length, while 9 out of the 11 drugs that didn't alter schistosomules motility or length were found to act on adrenergic receptors in vertebrates. The investigators observed four points: 1) decreased motility was more associated with drugs inhibiting tyrosine-derivative signaling; 2) increased motility, with or without change in length, was induced by either agonistic or antagonistic drugs; 3) most drugs acting on adrenergic signaling which alter neither motility nor length were reported to act specifically on β-adrenergic receptors; and 4) OA receptors are more likely α-adrenergic receptors. According to the pronounced results on motility and length, the investigators selected only three drugs; chlorpromazine (CPZ), carvedilol (CAR) and propranolol (PR) to conduct concentration-response assays. Results revealed that CPZ effect on motility and length was biphasic; i.e. motility dramatically increased (50-fold), followed by paralysis at concentration of 100 μM and 500 μM, with increased length (~30%) in all concentration levels. The investigators attributed paralysis to muscle spasticity from the continuous muscle contraction. CAR showed paralytic effects in most concentrations tested with 20-30% length decrease only at higher concentrations. On the other hand, PR increased motility (17-24 fold) but with 30% length increase.

This interesting publication paved the way to certain facts: 1) in invertebrates, use of synapsin antibodies allows mapping of the nervous system controlling vital functions including muscle contraction, tegument innervation and reproduction; 2) in contrast to all other trematodes, only adult stages of schistosome have two pairs of cerebral ganglia, adjacent to oral and ventral suckers postulating their independent and coordinated control of various functions; 3) OA localization, which showed its wide distribution in both pairs of cerebral ganglia, the tegument tubercles, nerve fibers and neurons lining the caecum, gynecophoric canal flaps as well as the female reproductive tract, suggests the vital role of tyrosine signaling in schistosome' motility; 4) the variable obtained results in phenotyping studies revealed the complexity of the neuromuscular control in schistosomules and adult schistosomes, and this could be attributed to the presence of three muscle layers, circular, longitudinal and diagonal; and 5) phenolamine and catecholamine neurotransmitters alter schistosomules motility and shape, and this will facilitate discovery of new drugs which affect juvenile stages to be combined with PAZ.

Finally, the investigators concluded that mapping and phenotypic studies are considered the first step in development of novel drug targets against various invertebrates including helminthes and arthropods. They also recommended further *in vivo* studies as well as widening the search for more compounds to be investigated against schistosomules and adult schistosomes. **Compiled from** "Octopamine signaling in the metazoan pathogen *Schistosoma mansoni*: localization, small-molecule screening and opportunities for drug development." Disease Models & Mechanisms 2018 11: DOI 10.1242/dmm.033563, Published online 30 July 2018

Malignant malaria: Dihydro-pyrimidinones (DHPMs) are heterocyclic synthetic products of the Biginelli reaction. DHPMs are now widely used in pharmaceutical industry as calcium channel blockers, antihypertensive and anticancer drugs, as well as for antiviral and antifungal activities. Besides, few recent publications reported the use of some DHPMs in treatment of *falciparum* malaria. The objective of the present compilation which was conducted by a team of investigators from Brazil (**Rogerio *et al***.) with correspondence to **Cedric S Graebin**, is to synthesize several DHPMs to be evaluated in treatment of *P. falciparum in vitro* and *in vivo*. The study was designed as follow: 1) synthesis of thirty DHPMs compounds; 2) cytotoxicity evaluation of these compounds using kidney African Green Monkey cells; 3) hemolysis assay to investigate if the synthesized compounds at various concentrations can induce RBCs hemolysis; 4) *in vitro* evaluation as anti-plasmodial agents against chloroquine-resistant strains of *P. falciparum* (W2 strain); 5) *in vivo* evaluation of the most *in vitro* effective compounds against *P. berghei*–infected mice; and finally 6) *in silico* target fishing approach to investigate the biological targets at the molecular level of only three compounds that showed both *in vitro* and *in vivo* efficacy against malaria parasites. Parameters used are: 1) determination of MDL_{50} (colorimetric estimation of viable cells and survival) using spectrophotometer for cytotoxicity evaluation; 2) determination of hemolysis rate against control (RBC plus saponin which causes 100% hemolysis), and absorbance of the RBCs supernatant was measured using spectrophotometer; 3) quantitative measurement of histidine-rich protein II by specific monoclonal antibodies in sandwich ELISA, followed by calculation of IC_{50} using dose-response curves for *in vitro* evaluation; and 4) parasitaemia reduction% in treated mice compared to two other groups, untreated controls and mice treated with chloroquine for *in vivo* evaluation.

The investigators succeeded to synthesize thirty compounds; nine of them are novel. Most compounds were 100% pure, as measured by high-performance liquid chromatography, whereas few had 83-99% purity range. Out of the 30 compounds investigated *in vitro*, only 17 showed anti-plasmodial activities, and nine of them at concentrations below 10 μM. Cytotoxicity results showed that non-effective DHPMs or those with little *in vitro* efficacy against *P. falciparum* showed lower toxicity and high MDL_{50} values, and vice versa. According

to these results, the investigators suggested that both mechanisms for anti-plasmodial activity and toxicity are similar. Hemolysis assay revealed that none of these compounds induced any degree of RBCs hemolysis at 62 uM concentration. However, only five compounds induced some degree of RBCs hemolysis ranging from 0.95-22.6% at 1000 μM. Accordingly, the investigators selected only three compounds with good *in vitro* anti-plasmodial activity with lower cytotoxicity in the *in vivo* studies. Similarly, they were active with 33-60% reduction of parasite burden on the 8th day post-infection. Although their anti-plasmodial activities were less than the comparable drug (chloroquine), the investigators recommended slight modification in the synthesized DHPMs to obtain more effective *in vivo* results. Finally, *in silico* target fishing studies utilized two *in silico* approaches from the available informatics; structure-based pharmacophore target fishing and molecular docking databases using PharmMapper server and ChEMBL database, respectively. Therefore, performing these studies allowed the investigator to identify the similarity in the chemical structure of the active three compounds with the chemical and target information from the available databases. PharmMapper predicted up to 300 potential protein targets for the three selected DHPMs, and only two proteins were identified in *P. falciparum* (L-lactate dehydrogenase and protein kinase 5). Furthermore, ChEMBL database showed that the selected three compounds are potent inhibitors of some human protein kinases. The investigators concluded that the synthesized DHPMs could be used as anti-plasmodial drugs because they have high affinity to bind with protein kinase 5 and glycogen synthase kinase 3β. The latter is another protein kinase essential for completion of the asexual erythrocytic cycle for *P. falciparum*. **Compiled from** "Synthesis and molecular modelling studies of pyrimidinones and pyrrolo [3,4-d]-pyrimidinodiones as new antiplasmodial compounds." Mem Inst Oswaldo Cruz. 2018; 113(8): e170452, Published online 18 Jun 2018.