# Spotlights on new publications

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**Received:** 14 April 2018, **Accepted:** 21 April 2018.

## New drug targets VI

### • Schistosomiasis

Due to unavailability of asatisfactory vaccine against schistosomiasis, ineffectiveness of the sole licensed drug (Praziquantel; PZQ) against juvenile schistosomes, and presence of resistant strains in some endemic areas, a group of scientists from Australia and USA (**Madhu K. Sundaraneedi** *et al.*) conducted the present compilation. The study utilized the potent efficacy of acetylcholine esterase (AChE) in controlling neuronal functions by breakdown of acetylcholine, a neurotransmitter in both central and peripheral nervous systems of eukaryotic organisms. It is well known that schistosomes teguments contain large amounts of ACh to regulate host glucose uptake by the parasite using its tegumental nicotinic ACh receptors (nAChRs). The investigators observed that the inhibitory effects of AChE cause excessive ACh accumulation as well as over stimulation of its receptors, resulting inunorganized neuromuscular functions leading to death due to respiratory paralysis. Besides, there were several studies in the last seven years that utilized poly-pyridyl-ruthenium(II) complexes, as a potent group of AChEs, in treatment of several microbial pathogens. Meanwhile, ruthenium complexes are thought to be less toxic to human cells. Hence, the objective of their study was to evaluate the effectiveness of these compounds in treatment of schistosomiasis both *in vitro* and *in vivo*.

To achieve this, 13 ruthenium complexes were synthesized and screened for AChE inhibitory activity on soluble extracts from *S. mansoni* and *S. haematobium* adults and *S. mansoni* soluble egg antigen. However, a different pattern of inhibition was observed against *S. haematobium* extracts and not all the screened compounds showed correlated potency between the two species. Then the investigators evaluated *in vitro* the efficacy of the synthesized ruthenium complexes against the three intra-mammalian stages; schistosomula, adult and egg. In each stage, they selected the most effective compounds for a second experimental trial to find out the lethal dose. Accordingly, they infected *Biomphalaria glabrata* snails to obtain *S. mansoni* cercariae, and infected BALB/c mice. Adult and juvenile schistosomes were collected 7 weeks post-infection, and *S. mansoni* eggs were purified from infected livers. Inhibition assays against adult and juvenile schistosomes, cultured in Basch medium supplemented with antibiotics, were conducted in 24 and 96 well plates, respectively. Effect of complexes against *S. mansoni* egg hatching monitored by observing the motility of the released miracidia, while their effects on egg development were monitored by observing the released eggs from cultured pairs of *S. mansoni* adults, i.e. malformed or immature eggs. Based on their results, the investigators selected three compounds; Rubb7-tnl, Rubb12-tri and Rubb16-tnl. It was noticed that Rubb12-tri was also the most effective to inhibit AChE activity in *S. haematobium* extracts. Although Rubb16-tnl showed potent efficacy, it was not included in the next *in vitro* studies because it is characterized by longer chain lengths with more toxic effects on eukaryotic cells. Therefore, the investigators repeated their experiments using Rubb12-tri and Rubb7-tnl to assess their enzyme inhibitory effects. Versus negative controls (cultured schistosomes in the absence of both compounds), the investigators used the following parameters 1) measurement of surface AChE, 2) calculation of glucose uptake and glycogen storage, and 3) examination by scanning electron microscopy (SEM). To compare their cytotoxic effects, both compounds were incubated in human bile duct cell line H69, PZQ, and dichlorvos which is a metabolite released from dead schistosomes after using metrifonate as AChE inhibitor. A tolerability study was also conducted to determine the maximum tolerated dose of both compounds if administered to infected mice. Accordingly, the investigators conducted their *in vivo* assessment using five consecutive daily IV doses of 2 mg/kg (Rubb12-tri) and 10 mg/kg (Rubb7-tnl). Evaluation parameters used wereworm burden, hepatic egg burden, egg hatching index, egg development index, surface enzyme activity and glucose uptake ability.

*In vitro* results revealed that treated worms showed significant decreased levels of both surface and somatic AChE activity as well as glycogen content compared to controls, suggesting impaired glucose uptake in the presence of ruthenium compounds. Cytotoxicity assay showed satisfactory results in comparison with PZQ and dichlorvos. Besides, *in vivo* results revealed significant reduction in worm burden (42%) in mice treated with Rubb12-tri, whereas the other compound showed non-significant decreased worm burden. Similar results were obtained for AChE inhibitory activity, while glucose uptake activity was not significantly different in both compounds compared to control group. Regarding egg burden, there was no decrease in egg counts, but egg viability showed significant reduction in hatching capability and egg development (i.e. increased immature and misshapen eggs with increased eggshell malformation) with both compounds. SEM showed flattened dorsal tubercles of

 *DOI: 10.21608/puj.2018.3532.1015*

the male treated parasites, at the site of glycogen storage. The investigators discussed their results and attributed the effects of ruthenium compounds to the paralysis induced by AChE inhibition and cholinergic accumulation, as the effect was pronounced on adult worms. Concerning no significant decrease in egg burden despite the significant decrease in worm burden, the investigators attributed these results to the possibility of ruthenium drugs stimulating schistosome reproductive tract motility releasing all under-developed eggs with significant increase in misshapen and immature eggs. Besides, under-nourished worms due to impaired glucose uptake lost their ability to produce eggs. Finally, the investigators recommended further studies to modify ruthenium compounds for development of more effective and selective drugs against schistosomiasis to overcome the limitations of current monotherapy. Compiled from **"Polypyridylruthenium (II) complexes exert anti-schistosome activity and inhibit parasite acetylcholinesterases."PLoS Negl Trop Dis, 2017 Dec; 11(12): e0006134.**

## • Leishmaniasis

Control of leishmaniasis faces two major obstacles; the first is treatment failure because most of anti-leishmanial drugs are highly toxic with long term of administration, which obliges patients to stop chemotherapy. The second obstacle is HIV coinfection which was reported during the last two decades, with instability of host immune response. Therefore, search for a new drug target to control leishmaniasis becomes one of the main objectives in the majority of publications in Brazil, a very high endemic country for visceral and cutaneous leishmaniasis. In a previous compilation [Spotlights on new publications; New drug targets IV (PUJ; 2016, 9: 112-117], the reviewers discussed the probability of proteomic approach for identification of new proteins essential for *Leishmania* intracellular survival and virulence. In the present compilation, **Patrícia ST Veras**, as a corresponding author, published a new report with a team of Brazilian investigators (**Carlos ES Guedes** *et al.*). Three facts which were previously observed initiated the study idea; 1) translocator protein (TSPO), one of those proteins detected in their proteomic analysis, participates in apoptosis and has a major role in a range of functions of cellular processes such as proliferation, respiration, steroidogenesis as well as host immune response, 2) CBA mouse macrophages are able to control leishmaniasis *major*, but are still susceptible to *L. amazonensis*, with different immune-inflammatory profiles in both infections, indicating role of macrophages in controlling leishmaniasis, and 3) macrophages infected with *L. amazonensis* contain TSPO which is associated with a ligand of lower relative abundance of peptides in comparison with those infected with *L. major*, which may explain its role in susceptibility to leishmaniasis *amazonensis* These observations stimulated the investigators to search for one of these compounds with specific ligands to modulate TSPO actions to be used in killing *L. amazonensis* in infected macrophages. They selected PK11195 due to several reasons, the most important of which is its ability to increase free radical production in neuronal cells through activation of the mitochondrial permeability. Also, it was used as a marker of cerebral lesions and as an immunomodulator due to its pro-apoptotic properties, as a potential chemotherapeutic anticancer agent. As an anti-parasitic therapy, PK1195 was previously reported to reduce *P. falciparum* proliferation rate in infected cells, and *T. gondii in vitro*.

Schneider's insect medium supplemented with antibiotics and heat-inactivated fetal bovine serum was used to culture promastigotes of *L. amazonensis*, *L. braziliensis* and *L. major* for several passages up to the stationary growth phase.To determine IC50 of PK1195, TSPO ligand PK11195 was prepared in 100% ethanol, and incubated in serial dilutions with the cultured promastigotes for 48 h. AlamarBlue® was used to determine cell viability, and its absorbance was measured using a spectrophotometer. Macrophages were obtained by peritoneal lavage of mouse cavities and cultured in supplemented Dulbecco's modified Eagle's medium (DMEM), and AlamarBlue® was also used to determine the viability of uninfected macrophages when tested against serial dilutions of PK1195. Then, macrophages were infected with *L. amazonensis* promastigotes, and treated with the lethal concentrations of PK1195 in two separate experiments (early and late treatment). Subsequent treatment was done to evaluate its efficacy to kill promastigotes; the second experiment treatment was conducted after further 96 h, enough time for promastigotes → amastigotes transformation, to test efficacy to kill amastigotes. After treatment, infected treated macrophages were stained with H & E, and counted, and viability was also assessed using AlamarBlue®.Viability of parasites in macrophages after early and late treatment was assessed by counting the number of promastigotes after incubation in Schneider's medium for five days. As a control drug, infected macrophages were treated with 2.1 μM amphotericin B, whereas control cells were incubated with ethanol. To determine if the effect of PK11195 on parasite viability was reversible, infected macrophages incubated with PK11195 for 6, 12, 24, or 48 h, were subsequently washed and incubated with PK11195-free DMEM for another 48 h, and the number of viable promastigotes was counted. To determine the mechanism of TSPO ligand PK11195, free oxygen radicals, nitric oxide and pro-inflammatory cytokines (IL-6, IL-10, TNF-α, IL-12, IFN-γ) were determined quantitatively. Furthermore, the study was completed using transmission electron microscopy (TEM) to demonstrate intracellular morphological changes caused by treatment with PK11195.

The obtained results revealed that the median IC50 value was 14.22 μM (concentration ranged from 0.20 to 400 μM) for treatment of *L. amazonensis* with PK11195, whereas lower values were reported for *L. braziliensis* and *L. major* (3.51 and 8.23 μM, respectively). The selective index of

the lethal dose of PK1115 which measures viability of the infected macrophages was 13.7 indicating its safety in use for experimental animals. In time- and dose-dependent manner, there was significant reduction in the percentage of infected macrophages (97.75% reduction versus 86.88% in the control group). Similar significant reductions were also observed in parasite number/infected macrophage (1.41 and 1.16 at concentrations of 75 and 100 µM, respectively), versus 6.37 for the control group. When infected macrophages were subsequently treated (early stage), there was significant reductions in number of promastigotes (91.08%, 99.09% and 100%) at concentration of 100 µM after 24 h, 75 µM and 100 µM after 48 h, respectively. Whereas, when treated after further incubation for 96 h, there was complete clearance of amastigotes (100% reduction) at concentrationof 75 µM for 48 h and either 50 or 75 µM for 72 h. Interestingly, 100% reduction was also obtained when the infected macrophages were treated with amphotericin B at concentration of 100 µM after 24 h and 75 µM after 48 h. Also, PK11195 showed pronounced irreversible reduction in parasite viability of 97.15% and 100% after treatment for 24 h and 48 h, respectively. Moreover, there was significant reduction in $O_2$ production (3.5-4.5-fold in comparison to untreated controls). In contrast, no change was detected in NO production and the evaluated inflammatory cytokines, suggestingthat killing of intracellular parasites is not through inflammatory mediators and confirming PK11195 mechanism of action. TEM results revealed that the intracellular parasites showed marked apoptotic morphological alterations such as cytosolic disorganization, appearance of double membrane vacuoles containing degraded material, and marked swelling of mitochondria and kinetoplasts. In addition, multi-vesicular bodies with remarkably high electro-density were observed after 24 h and 48 h of treatment suggestive of dead parasites. The investigators confirmed the high affinity of PK11195 to bind with TSPO and its potentiality as new chemotherapeutic agent for treatment of cutaneous leishmaniasis. As regards mechanism of PK11195 actions, the investigators hypothesized the interaction and incorporation of PK11195 within wall lipid bilayersalters membrane permeability. Finally, the investigators recommended further studies to evaluate the efficacy of TSPO-PK11195 ligand *in vivo*. Compiled from **"*In vitro* evaluation of the anti-leishmanial activity and toxicity of PK11195." Mem Inst Oswaldo Cruz, 2018; 113(4): e170345**.