Abstract of Dissertation submitted by Talaam Keith Kiplangat
Mitochondria as a Potential Target for the Development of Prophylactic and Therapeutic Drugs against *Schistosoma mansoni* Infection

マンソン住血吸虫のミトコンドリアは予防と治療効果が期待できる創薬標的である

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Introduction

Praziquantel (PZQ) is the only drug available for treatment of schistosomiasis. However, PZQ does not offer protection against infection and does not completely kill adult parasites. Parasites resistant to PZQ can be induced experimentally in infected mice, and reduced susceptibility have been reported to occur in various endemic areas. Given these shortfalls of PZQ, the development of new drugs for the treatment and prevention of schistosomiasis is needed. Given the complexity schistosomes' life cycle, they have evolved efficient mechanisms for the smooth transitions among environments of varying hosts and free-living stages, where their mitochondria are known to play key roles. Drug development targeting the mitochondrial respiratory chain has been explored but little information is available about the impact caused by disruption of mitochondria-related processes in prophylaxis and treatment of *S. mansoni* infection. In this study, we investigated the *in vitro, ex vivo*, and *in vivo* antischistosomal activities of several compounds reported to inhibit mitochondria-related processes and demonstrated the potential use of these compounds for prevention and treatment of *S. mansoni* infection.

Materials and Methods

In this study, we performed initial screening of 116 compounds that have been reported to inhibit mitochondrion-related processes (such as ETC, cellular respiration, and membrane potential), classical antiparasitic agents, and a small number of molecules used for treatment of human disease against cercariae of *S. mansoni*. We evaluated microscopically motility and viability by comparing parasites in the presence of 10 μM compounds to those in wells containing vehicle (DMSO) at three different time points (≤1 h, 18 h, and 41 h). Motility was scored using a 5-point scale (4, normal motility; 3, reduced motility; 2, uncoordinated minimal motility, 1, severe reduction in motility; 0, total absence of mobility). A subset of 8 compounds including atovaquone, nitazoxanide, flusulfamide, fenpyroximate, plumbagin, amiodarone, pyrvinium pamoate, and ascofuranone, were selected according to their efficacy against cercariae as well as their commercial availability and presence in amounts sufficient for *in vivo* experiments. Mefloquine and PZQ were used as positive controls.

These selected compounds were screened against schistosomula (*in vitro*), and adult *ex vivo* at 10 μM and determining changes in motility of the parasites as mentioned above. Effects of these selected compounds on adult oxygen consumption rate (OCR) were determined at 50 μM concentrations using a Seahorse XFe24 extracellular flux analyzer.

In vivo prophylactic and therapeutic nature of the selected compounds were determined using atovaquone, 100 mg/kg body weight; nitazoxanide, 50mg/kg; ascofuranone, 100 mg/kg; flusulfamide, 5 mg/kg; fenpyroximate, 2 mg/kg; plumbagin, 2 mg/kg; pyrvinium pamoate, 2 mg/kg; amiodarone, 50mg/kg; mefloquine, 100 mg/kg; PZQ, 100mg/kg; and vehicle (1× phosphate-buffered saline [PBS] containing 3% [vol/vol] ethanol and 7% [vol/vol] Tween 80). The worm burden was calculated using the following formula:

$$\text{Worm burden (\%)} = \frac{(NW_{\text{neg}} - NW_{\text{tre}})}{NW_{\text{neg}}} \times 100$$

Results

After 41 hours of exposure, 48 compounds out of 116 showed motility score 2.0 or less; of these, 37 compounds showed complete inhibition of cercariae motility (with scores of 0.0) and another 11 compounds showed mean scores of 0.1 – 2.0. Most of the compounds are known to be inhibitors of complex I, II and III of electron transport chain. Complete inhibition of schistosomula motility was observed after incubation for 8 hours with atovaquone; 24 hours with amiodarone, nitazoxanide, mefloquine, or plumbagin; and 48 hours with pyrvinium pamoate. Upon exposure to selected compounds, the pair of *S. mansoni* adults separated where male and female were examined individually. Amiodarone was less effective against both male and females. Atovaquone and fenpyroximate were not effective against females of *S. mansoni*. The remaining being effective to both male and females. In prophylactic assays, the worm burden was significantly suppressed ($p < 0.05$) following prophylaxis with each of the selected compounds, such that hosts exhibited worm burdens ranging between 1.9 and 15.0%. A reduction of worm burden below 50% was achieved in mice treated with ascofuranone, plumbagin, pyrvinium pamoate, amiodarone, nitazoxanide, and mefloquine. A significant reduction in OCR was observed after addition of nitazoxanide, atovaquone, ascofuranone, pyrvinium pamoate, mefloquine, amiodarone, or fenpyroximate.

Discussion

Depending on the life cycle stage, helminths can perform aerobic (oxygen) and anaerobic (fumarate) respiration. The majority of the anti-cercarial compounds identified in the present work are molecules that target complex III, and include ascofuranone, atovaquone, and their derivatives suggesting that *S. mansoni* at this stage depends on an active aerobic respiratory chain to survive. Schistosomula were insensitive to inhibitors of complex I, II and III but sensitive to a complex II+III inhibitors suggesting that simultaneous inhibition of oxygen and fumarate respirations is required to cause lethality in this stage. Pyrvinium pamoate and ascofuranone, inhibit fumarate respiration in parasitic helminths, inhibited parasite motility suggesting that *S. mansoni* employ active fumarate respiration. Schistosomula transformed *in vivo* (subcutaneously) were susceptible to all the selected compounds, suggesting differential dependency on mitochondrial respiration between *in vitro*- and *in vivo*-transformed schistosomula. Significant reduction in OCR observed with atovaquone, ascofuranone, pyrvinium pamoate, mefloquine, amiodarone, and fenpyroximate indicates that these compounds are, in fact, *S. mansoni* respiratory chain inhibitors. In this study, we demonstrated for the first time (to our knowledge) that inhibitors of mitochondria-related processes have potential for use in chemoprophylaxis. In conclusion, the mitochondrion of *S. mansoni* is a good drug target space; the results obtained in the present study provide starting points for the development of new drugs for the prevention and treatment of schistosomiasis.