Revisiting quinapyramine: Mechanism of uptake, action and resistance

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Introduction

Quinapyramine has been used in the treatment of veterinary trypanosomiases since 1950's. The use of quinapyramine to treat African Animal Trypanosomiasis was stopped in sub-Saharan Africa due to cross-resistance to diminazene, homidium and isometamidium [1]. However, the drug is still used in the treatment of *T. evansi*, *T. vivax* and *T. equiperdum* infections outside the tsetse belt of Africa [2]. Nevertheless, the mechanism of action and resistance to the drug is largely unclear.

Materials & Methods

We induced quinapyramine resistance in *T. equiperdum* and *T. evansi* bloodstream forms through adaptation to increasing concentration of the drug added to growth medium as described [3, 4]. Using Alamar blue assay, we investigated quinapyramine cross-resistance profile [3]. In addition, [3H]-adenosine uptake assay was used to study the involvement of P2 aminopurine transporter in quinapyramine uptake [5]. The localisation of quinapyramine and its effect on cellular morphology and cell cycle were studied using microscopy [4,6].

Results

Development of quinapyramine resistance

Development of resistance to quinapyramine was slow in both *T. equiperdum* and *T. evansi*, with parasites resistant to 50× EC_{50} generated in approximately 280 days. Adaptation quinapyramine resulted in growth defect and loss of kinetoplasts.

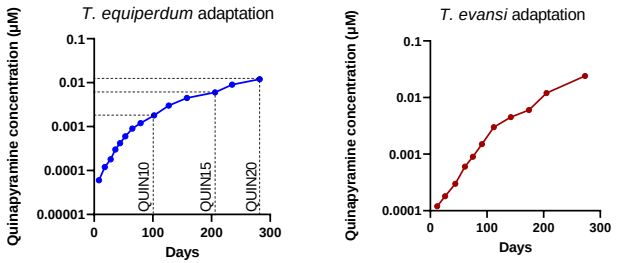


Fig 1. Adaptation of trypanosomes to increasing concentration of quinapyramine

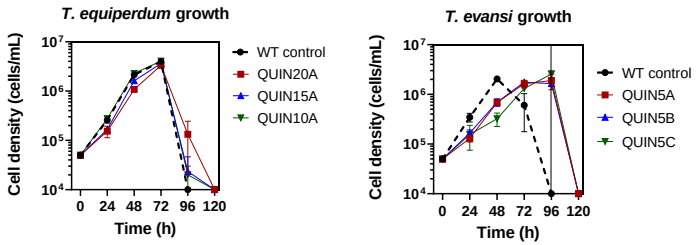


Fig 2. Growth defect in quinapyramine-resistant cell lines

Cross-resistance in quinapyramine resistant lines

Quinapyramine-resistant clones showed cross-resistance to diminazene, pentamidine, ethidium and isometamidium. However, there are variations among the different cell lines.

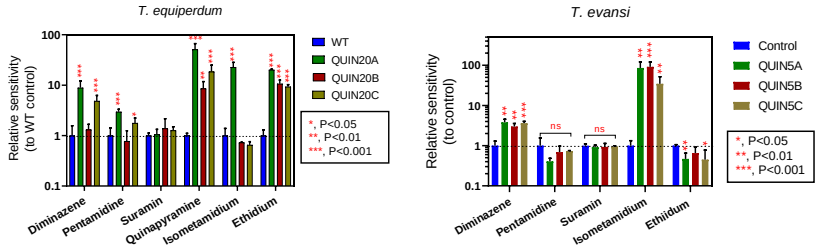


Fig 3. Cross-resistance profile of quinapyramine-resistant *T. equiperdum* and *T. evansi*

Sensitivity of other resistant lines to quinapyramine

We observed higher EC_{50} for quinapyramine in isometamidium-resistant *T. brucei* and diminazene-resistant *T. congolense* strains with low mitochondrial membrane potential [4, 5]. Using confocal microscopy, we have observed colocalization of quinapyramine with mitochondrial stain. In addition, the EC_{50} of quinapyramine was 2-fold higher in the P2 adenosine transporter knock-out (TbAT1-KO) compared to wild type. Further investigation revealed that quinapyramine inhibits P2-type [^{3}H]-adenosine uptake through the P2 transporter in a dose-dependent manner with *Ki* = 12.57 ± 0.68 (n=3; Fig 5).

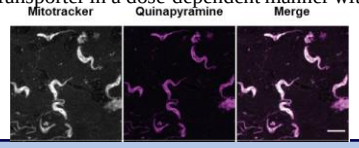


Fig 4. Accumulation of quinapyramine in the mitochondria of *T. brucei*.

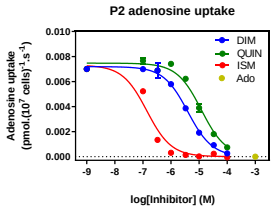
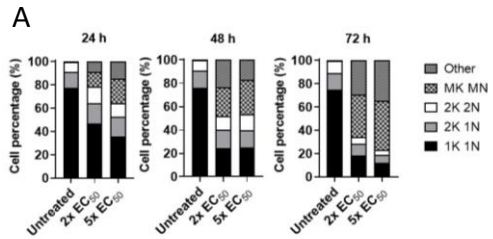
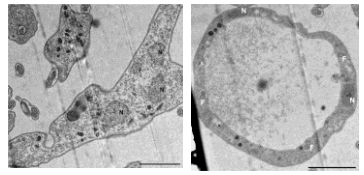


Fig 5. Inhibition of P2 [3H]-adenosine uptake by quinapyramine. This indicates that quinapyramine is yet another substrate of P2/AT1 transporter. DIM, diminazene; QUIN, quinapyramine; ISM, isometamidium; ADO, adenosine

Effect of quinapyramine on cell cycle and morphology





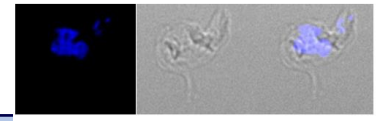


Fig 5. Effect of quinapyramine on cell cycle and cellular morphology in T. brucei. Majority of cells in culture treated with 2× and 5× EC_{50} quinapyramine containing multiple nuclei and/or kinetoplasts (MKMN) from 48 h (A). Treated cells imaged by fluorescence microscopy (B) and electron microscopy (C) appeared disfigured, lost their slender shape, and contained multiple flagellae. K, kinetoplast; N, nucleus; Other, other cellular aberrations e.g. no kinetoplast or nucleus etc.

Conclusion

We conclude that the P2 adenosine transporter contributes to quinapyramine uptake, and the drug targets mitochondria and kinetoplast, which possibly mediate its cross-resistance to other trypanocides. Trypanosomes treated with quinapyramine were disfigured, lost their slender shape, and contained multiple nuclei and flagellae. We propose that quinapyramine is trypanostatic over a wide range of concentrations, has minimal effects on G, S and M phase of cell cycle but inhibits the late-stage cytokinesis phase. Further investigations through flow cytometry, genome analysis, RNA-interference and metabolomics are on-going.

References

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