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Characterisation of Equilibrative Nucleoside Transporter genes from *Trypanosoma cruzi* and the development of a nucleoside-based chemotherapy for Chagas' disease

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1. Introduction

- *Trypanosoma cruzi* is the causative agent of Chagas' disease.
- Like all parasitic protozoa, it lack the ability to synthesize purines de novo and rely exclusively on the salvage of purine from their hosts. Four ENT family genes were cloned from this parasite and expressed in *T. brucei* procyclic forms.
- Three of these were identified with a specific role in nucleoside salvage; TcrNB1(hypoxanthine/ guanine), TcrNT1 (inosine/guanosine) and TcrNT2 (thymidine transporter).
- Still under characterization TcrNB2.

2. Objectives

- Detailed characterization of TcrNB1 for purine nucleobase and nucleoside transporters in the presence of different concentration of test compound.
- Expression of TcrNB2 in a nucleobase-null background of *Leishmania mexicana* (NT3) to Identify the substrate for this transporter.

3. Methods

- TcrNB1 was expressed in in *T. brucei* procyclic forms using plasmid Phd1336,contains Blasticidin as selective marker.
- TcrNB2 in was expressed in *Leishmania mexicana* NT3 knockout using pNUS-HcN plasmid that contains a G418 resistance gene.
- 1 × 10^8 of the parasite were incubated with 0.05 or 0.1 µM of radiolabelled substrate, in the presence or absence of competitive inhibitor.

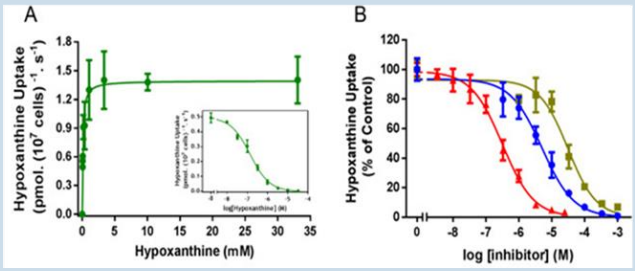

Fig.1. Hypoxanthine uptake in *T. brucei* Procyclic form TbNB-KO + TcrNB1 cells with 0.05µM of [3H]-hypoxanthine

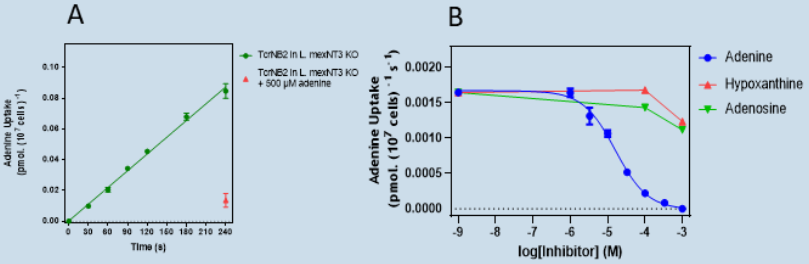

Fig2.(A): Time course uptake of 0.1µM [^{3}H]-Adenine uptake in TcrNB2 + *L. mexicana* NT3 KO. (B): Dose dependent in presence of unlabeled adenine

4. Results

- TcrNB1 displays high affinity for hypoxanthine and guanine, with Ki values of 93.8 ± 4.7 nM and 120 ± 20 nM, respectively through interactions with the ring nitrogen residues.
- It has a fairly strong selectivity for oxopurines over aminopurines.
- TcrNB2 was taken up 0.1 µM [^{3}H]-Adenine in the L.mex NT3 KO over 240 s.
- With dose dependent using 0.1µM [^{3}H]-adenine, TcrNB2 was inhibited by unlabeled adenine with EC_{50} 11.97 µM and displayed no inhibition with hypoxanthine and adenosine.

5. Conclusion

- TcrNB1 is a selective oxopurine nucleobase transporter.
- TcrNB2 shows it to be an adenine transporter with little or no affinity for other natural nucleosides or nucleobases.

6. References

1. Al-Salabi, M. and de Koning, H. (2005). doi: 10.1128/AAC.49.9.3682-3689.2005
2. Carter, N., Rager, N. and Ullman, B., 2003. doi.org/10.1016/B978-012473346-6/50012-0