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

Mustafa M. Aldfer, Gustavo D Campagnaro, Manal J Natto, Abdulbaset Kabli, Richard J Burchmore and Harry P. de Koning

Institute of Infection, Immunity and Inflammation, College of Veterinary, Medical and Life Sciences, University of Glasgow, Glasgow G12 8TA, United Kingdom

Abstract:

Trypanosoma cruzi is the causative agent of Chagas' disease. Like all parasitic protozoa, *T. cruzi* lack the ability to synthesise purines de novo and rely exclusively on the salvage of purine from their hosts, using high affinity transporters in the plasma membrane. These transporters have remained largely uncharacterised but are expected to be encoded by genes of the family of the Equilibrative Nucleoside Transporters (ENTs), as they are in other protozoa. 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: a hypoxanthine/ guanine transporter TcrNB1; an inosine/guanosine transporter TcrNT1; a thymidine transporter, TcrNT2. However, the fourth gene encodes a putative transport protein with a so far unknown substrate. In this report we focused on characterization of TcrNB1 for purine nucleobase and nucleoside transporters with 0.05 µM of [^{3}H]-hypoxanthine in the presence of different concentration of test compound. TcrNB1 showed very high affinity for hypoxanthine and guanine with K_i values of 93.8 ± 4.7 nM and 120 ± 20 nM, respectively, and moderate affinity for adenine with K_i 3.74 ± 0.5 µM. TcrNB1 displayed much lower affinity for purine nucleosides than for nucleobases. We conclude that the TcrNB1 is a selective oxopurine nucleobase transporter. Our characterisation shows interactions between all four nitrogen positions of the purine with residues in the transporter binding site. In addition, expression of TcrNB2 in a nucleobase-null background of Leishmania mexicana, shows it to be an adenine transporter with little or no affinity for other natural nucleosides or nucleobases.