Assessing the potential of nucleoside transporters to deliver drugs against the various Trypanosoma species responsible for African animal trypanosomiasis

Marzuq A. Ungogo[1], Mustafa M. Aldfer[1], Manal J. Natto[1], Kayhan Ilbeigi[2], Jamal Ibrahim Asseri[1], Richard J. S. Burchmore[1], Guy Caljon[2], Serge Van Calenbergh[3] and Harry P. De Koning[1]

[1]School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK
[2]Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Antwerp, Belgium
[3]Laboratory for Medicinal Chemistry (Campus Heymans), Ghent University, Gent, Belgium

Animal African trypanosomiasis is a complex disease of many different domestic animals, multiple trypanosome species and multiple modes of transmission, as well as vast geographical areas from African and the Middle East to South America and Asia. To control this disease, there is only a handful of old drugs. However, not only has resistance been reported to these drugs, there is an innate difference in sensitivity to them in the various species, in part linked to differences in transporters such as TbAT1/P2 and TbAQP2, which are absent from the *T. congolense* and *T. vivax* genomes but responsible for the uptake of diamidine and melaminophenyl arsenical drugs in brucei-group trypanosomes. Yet, at point of care, it is usually not known which (single or multiple) species of trypanosome have infected the animal, often leading to sub-optimal treatment. A single treatment active against all relevant *Trypanosoma* species and tolerated by the various hosts, would be a genuine breakthrough. Our groups have been working towards a nucleoside drug treatment with that desirable profile. One potential bottleneck would differences in nucleoside transporters, which could cause some nucleosides to be excluded from specific species, making them insensitive. We have thus developed a bespoke Leishmania mexicana cell line ('SUPKO') from which the NT1.1/NT1.2 locus and the NT2 gene were deleted, creating a null background for the heterologous expression of Trypanosoma nucleoside transporters. We have used this system to express T. congolense and T. vivax transporters of the ENT family and found that TcoAT1 and TvxNT3 are broad specificity nucleoside transporters and that their expression sensitises this Leishmania cell line to specific nucleoside analogues. Detailed characterisation using [^{3}H]-adenosine showed these carriers are high affinity, with Km values of 0.42 and 1.41 µM for TcoAT1 and TvxNT3, respectively. The interactions these transporters make with adenosine showed close similarity to the those of the *T. brucei* P1 transporters and we identified 'allowed' modifications that did not impede uptake through any of these carriers.