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FUNCTIONAL COMPARISONS OF HUMAN AND RAT CONCENTRATIVE NUCLEOSIDE TRANSPORTERS CNT1, CNT2 AND CNT3

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ABSTRACT

Title: Functional comparisons of human and rat concentrative nucleoside transporters CNT1, CNT2 and CNT3
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Purpose: To compare the functional activities of human and rat CNT1, CNT2 and CNT3 transporters, and to profile the inhibitory effects of major nucleoside and nucleotide analog drugs on these transporters for identifying interspecies differences.
Methods: Human and rat CNTs were transiently transfected in polarized MDCK-II cell monolayers under the same conditions. Transport of [^{3}H]uridine was characterized in terms of linearity, K_m and V_{max}. Cis-inhibition of uridine transport by adenosine was evaluated to obtain IC_{50} values for all rat and human CNT transporters. Inhibition effects of over 10 nucleoside / nucleotide analog drugs on these transporters were evaluated. Further IC_{50} assessments were performed on drugs such as ribavirin, gemcitabine and trifluorothymidine which either showed distinct inhibitory profiles on the three isoforms or between human and rat orthologs. Amino acid sequence alignment analysis were conducted to identify segments of amino acids that could contribute to difference among the three human CNT isoforms and their rat orthologs.
Results: Figure 3 is showing the transporter-mediated cellular accumulation of 2 µM [^{3}H]uridine in polarized MDCK cells transfected with human and rat CNTs. There were no significant differences between human and rat CNT1 or CNT3, while rat CNT2 exhibited 43% more uridine uptake than human CNT2. IC_{50} values of adenosine on inhibiting uridine uptake are listed in Table 1, along with K_m and V_{max} of uridine transport mediated by each transporter. There were less than 2-fold differences in the K_m of uridine between human and rat for CNT1 and CNT3, but a greater than 2-fold difference between human and rat for CNT2. Nucleoside/nucleotide analogs exhibited distinct profiles in inhibiting uridine transport mediated by these transporters. For example, ribavirin did not inhibit human and rat CNT1 (IC_{50} values > 1000 µM), while it was a potent inhibitor of human CNT2 (IC_{50}=5.7 µM) and less potent in inhibiting rat CNT2 (IC_{50}= 207 µM) (Fig 4). In contrast, gemcitabine was a moderate inhibitor of human and rat CNT1 (IC_{50}=69.6 and 48.7 µM) but did not inhibit CNT2.
Conclusions: Our studies compared functional activities of human and rat CNT transporters in transporting uridine. Nucleoside drugs exhibited different profiles in inhibiting these transporters. Notably, there was a substantial difference between human and rat CNT transporters in terms of inhibition or trans-stimulation by certain nucleoside analogs.

BACKGROUND

Nucleoside analogs have been widely developed as anti-cancer and anti-viral agents. Bioavailability of these agents can be determined by a number of physiological processes, one of which involves cellular uptake by membrane transporters. Solute carrier family 28 (SLC28) also known as the Concentrative Nucleotide Transporter (CNT) family, is comprised of three isoforms, CNT1-3, which are Na^+-dependent symporters that can mediate the transport of naturally occurring nucleosides and nucleoside analogs. Functionally, CNT1 primarily transports pyrimidine nucleosides (uridine, thymidine, and cytidine) and adenosine; CNT2 primarily transports purine nucleosides (adenosine and guanosine) as well as uridine; and CNT3 transports both purine and pyrimidine nucleosides. Although naturally occurring nucleosides show similar specificity by species orthologs, there is evidence suggesting that synthetic nucleoside analogs may show differences in specificity. By comparing difference in the amino acid sequence between species, we can speculate on the importance of some of the residues involved in nucleoside analog specificity.

Fig 1. Localization of CNT (SLC28) and ENT (SLC29) family transporters.

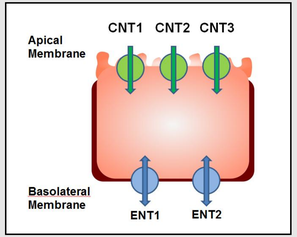
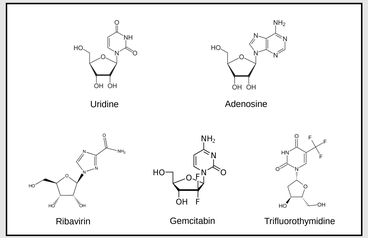


Fig 2. Chemical structures of some nucleoside and nucleoside analogs used in this study.



OBJECTIVE

Our objectives are to compare functional activities of human and rat CNT1, CNT2, and CNT3 transporters, and to profile the inhibitory effects of major nucleoside and nucleotide analog drugs on these transporters in order to identify interspecies differences.

MATERIALS AND METHODS

- Human and rat CNTs were transiently transfected in polarized MDCK-II cell monolayers and allowed to properly localize for 48 hours prior to performing apical transporter assays.
- Radiolabelled [^{3}H]uridine was used to characterize human and rat CNT transporters in terms of linearity, K_m and V_{max}.
- IC_{50} values were determined using [^{3}H]uridine (2 µM) as a substrate and adenosine as a reference inhibitor.
- Additional nucleoside/nucleotide inhibitors such as ribavirin, gemcitabine and trifluorothymidine were evaluated against the reference substrate [^{3}H]uridine.
- Amino acid homology alignments were conducted to speculate on potential residues that may contribute to these differences.

RESULTS

Fig 3. Comparison of apical uptake of [^{3}H]uridine by human and rat CNTs.

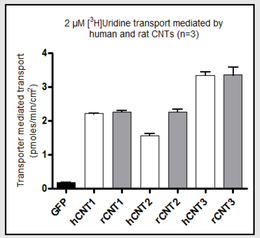


Fig 4. Comparison of inhibition / trans-stimulation of human and rat CNT by using nucleoside analogs in the presence of [^{3}H]uridine.

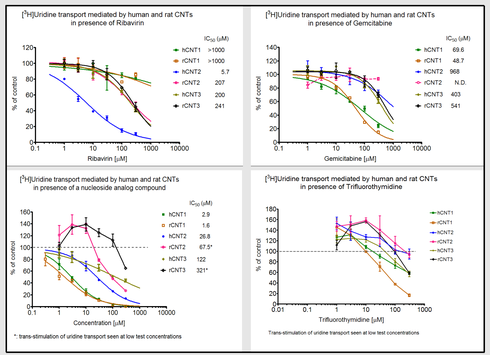


Fig 5. Inhibition of [^{3}H]uridine uptake by 100 µM nucleoside analogs

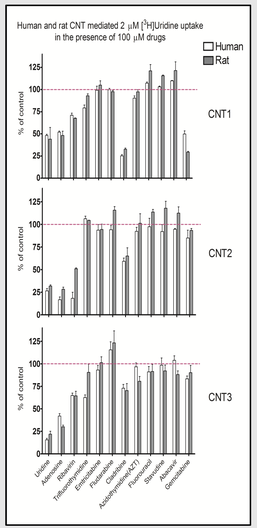
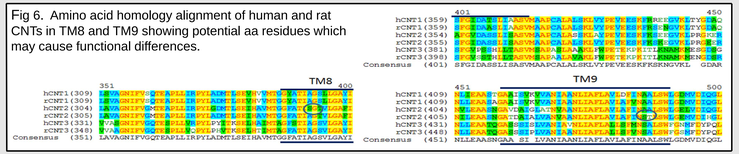


Table 1. K_m, V_{max} and IC_{50} comparison of human and rat CNT transporters using [^{3}H]uridine as a substrate and adenosine as an inhibitor.

Transporter	Uridine Transport		Inhibition by Adenosine
	K_m (µM)	V_{max} (pmole/min/cm^2)	IC_{50} (µM)
hCNT1/SLC28A1	27.1	29.8	116.6
rCNT1/Slc28a1	19.6	49.7	55.0
hCNT2/SLC28A2	28.0	35.9	17.4
rCNT2/Slc28a2	57.9	36.7	22.7
hCNT3/SLC28A3	11.9	27.8	58.2
rCNT3/Slc28a3	22.6	24.2	22.6

RESULTS (con't)

Fig 6. Amino acid homology alignment of human and rat CNTs in TM8 and TM9 showing potential aa residues which may cause functional differences.



- Cellular accumulation of [^{3}H]uridine showed no significant differences between human and rat for either CNT1 or CNT3. However, under the same experiment conditions, rat CNT2 exhibited 43% more uridine uptake than the human CNT2 ortholog (Fig. 3).
- K_m and V_{max} of [^{3}H]uridine apical uptake and IC_{50} values using the reference inhibitor adenosine were determined for human and rat CNT1-3 (Table 1).
- Ribavirin was a more potent inhibitor for human CNT2 (IC_{50}= 5.7 µM) than for rat CNT2 (IC_{50} = 207 µM) and did not inhibit human or rat CNT1 (IC_{50} > 1000 µM) (Fig. 4).
- Gemcitabine was a moderate inhibitor of human and rat CNT1 (IC_{50} = 69.6 µM and 48.7 µM, respectively), a weak inhibitor of human and rat CNT3, but did not appear to inhibit CNT2.
- Certain nucleoside analogs trans-stimulated uridine transport at low concentrations. This phenomenon was transporter and species-dependent.
- The trans-stimulation could be allosteric modulation of urdine transport through interacting with different binding sites.

CONCLUSIONS

- For uridine transport, there is no significant species difference between human and rat for CNT1 and CNT3. There is a noticeable difference for CNT2.
- Modulation of uridine transport by certain nucleoside analog drugs has shown species difference for all three transporters
- Trans-stimulation of uridine transport by certain nucleoside drugs were observed. The stimulation is dependent on the species, transporter and compound used.
- Our data suggest that CNTs may have multiple binding sites.

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