Interaction of nucleoside inhibitors of HIV–1 reverse transcriptase with the concentrative nucleoside transporter–1 (SLC28A1)

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Human concentrative nucleoside transporter–1 (hCNT1) (SLC28A1) is a widely expressed, high–affinity, pyrimidine–preferring, nucleoside transporter implicated in the uptake of naturally occurring pyrimidine nucleosides as well as a variety of derivatives used in anticancer treatment. Its putative role in the uptake of other pyrimidine nucleoside analogues with antiviral properties has not been studied in detail to date. Here, using a hCNT1 stably transfected cell line and the two–electrode voltage–clamp technique, we have assessed the interaction of selected pyrimidine–based antiviral drugs, inhibitors of HIV-1 reverse transcriptase such as zidovudine (AZT), stavudine (d4T), lamivudine (3TC) and zalcitabine (ddC), with hCNT1. hCNT1 transports AZT and d4T with low affinity, whereas 3TC and ddC are not translocated, the latter being able to bind the transporter protein. Selectivity appears to rely mostly upon the presence of a hydroxyl group in the 3′-position of the ribose ring. Thus, hCNT1 cannot be considered a broad–selectivity pyrimidine nucleoside carrier; in fact, very slight changes in substrate structure provoke a dramatic shift in selectivity.

Introduction

Among antiviral nucleoside-derived drugs, some of the oldest in anti-HIV-1 therapy are pyrimidine nucleoside derivatives for which the major routes responsible for their transport into cells are not well understood. Three isoforms of concentrative nucleoside transporters (CNTs) (SLC28) have been identified. CNT1 (SLC28A1), which is a pyrimidine nucleoside-preferring transporter, CNT2 (SLC28A2), a purine nucleoside-preferring carrier protein and CNT3 (SLC28A3), a broad-specificity nucleoside transporter protein [1–4]. CNT1, as well as being responsible for the uptake of naturally occurring pyrimidine nucleosides into cells, is also a high-affinity transporter for a variety of pyrimidine nucleoside-derived drugs used in anti-cancer treatment, such as gemcitabine and the capecitabine-derived metabolite 5′-DFUR [5–7]. Heterologous expression of human CNT1 (hCNT1) in CHO-K1 cells results in increased sensitivity to 5′-DFUR, even when the endogenous nucleoside transport activity of this cell line, which is exclusively accounted for by equilibrative nucleoside transporters (ENTs), is pharmacologically inhibited [7]. Indeed,

expression of hCNT1 in cell lines derived from pancreatic adenocarcinomas similarly increased sensitivity to gemcitabine [8]. These observations highlight the important role that hCNT1 may play in chemosensitivity to anticancer drugs and raises the question of whether hCNT1 is similarly implicated in antiviral drug uptake.

CNT1 is expressed in the apical domain of the plasma membrane in intestinal and renal epithelial cells [9–13]. Thus, CNT1 plays a role in absorption and tubular re-uptake of pyrimidine nucleosides in the intestine and kidney, respectively. In hepatocytes, CNT1 is similarly located at the apical canalicular membrane and is likely to contribute to nucleoside salvage from bile [14]. CNT1 is also expressed in bone marrow macrophages [15–17]. Macrophages rely upon salvage pathways for nucleoside supply and indeed CNT1 activity and expression is highly regulated in this cell type following cytokine-triggered activation [17]. Overall, CNT1 tissue distribution suggests a putative role of this transporter protein in determining nucleoside-derived drug pharmacokinetics, particularly for

antiviral nucleosides, which are administered orally. Moreover, macrophages are also significant latent reservoirs for HIV-1 during antiviral treatment [18] and, consequently, changes in transporter expression following macrophage activation may also contribute to the modification of intracellular nucleoside-derived drug bioavailability.

In this study, we have addressed the question of whether hCNT1, in addition to its high-affinity transport of pyrimidine nucleoside derivatives used in anticancer therapy, is also a candidate for mediating the uptake of pyrimidine-based antiviral drugs. We have used an hCNT1 stably transfected cell line and the two-electrode voltage-clamp technique on *Xenopus laevis* oocytes expressing the hCNT1 transporter, to determine whether selected pyrimidine-based antiviral drugs, such as zidovudine (AZT), stavudine (d4T), lamivudine (3TC) and zalcitabine (ddC) do indeed interact with hCNT1.

Materials and methods

Nucleosides, nucleoside-derived drugs and other chemicals

Uridine ([5,6-^{3}H], 35–50 Ci/mmol) was purchased from Amersham Biosciences (Amersham, UK). Cytidine ([5-^{3}H(N)], 21.5 Ci/mmol) was from Moravek Biochemicals (Calif., USA). Uridine, thymidine, cytidine, 2′-deoxycytidine, AZT, d4T, ddC and 5′-DFUR were obtained from Sigma-Aldrich (Saint Louis, Mo., USA). 3′-Deoxythymidine was purchased from Fluka Chemie GmbH (Buchs, Switzerland). 3TC was kindly donated by the Hospital Universitari Germans Trias i Pujol (Badalona, Spain).

Cell culture and nucleoside uptake measurements

A CHO-K1 cell line (hCNT1-CHO) expressing the human concentrative pyrimidine nucleoside transporter, hCNT1, has been used. This clone was generated as previously described [7] by stable transfection of CHO-K1 cells with a pCDNA3 vector incorporating a hCNT1 cDNA, cloned from human foetal liver (GenBank accession number U62966). hCNT1-CHO cells showed a typical hCNT1 activity pattern, characterized by high-affinity uptake of pyrimidine nucleosides and a lack of transport of purine nucleosides [7]. Cells were routinely cultured in Minimum Essential Medium (MEM)-Eagle supplemented with 4% (vol/vol) foetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine and a mixture of non-essential amino acids and antibiotics, as previously described [7].

hCNT1 activity was measured by incubating semi-confluent cell monolayers for 90 sec (initial velocity conditions) in an uptake buffer, supplemented with either sodium or choline chloride, in which the labelled pyrimidine nucleoside was added at a final concentration of 1 μM (specific activity 1 μCi/nmol). This method allows the calculation of Na$^+$-dependent uptake rates that are exclusively accounted for by the hCNT1 transporter. Incubations were stopped by rapid aspiration of the uptake buffer followed by immediate washing with a cold stop solution, as previously described [19,20]. To assess whether a selected nucleoside interacts with the hCNT1 transporter protein, Na$^+$-dependent uridine uptake was monitored in the presence of high concentrations of the putative inhibitor. Once inhibition by a particular nucleoside-derivative was observed, the substrate dependence of this effect was further monitored by using increasing concentrations of the inhibitor to determine IC$_{50}$ values. The Cheng–Prusoff equation was then used, when applicable, to calculate K_i values, as recently described by De Koning and co-workers, when monitoring the substrate recognition motifs of nucleobase transporters [21].

Expression of CNT1 in *X. laevis* oocytes and electro-physiological recordings

The hCNT1 cDNA was subcloned into a Bluescript vector, which was then linearized for cRNA synthesis, as previously described [6]. *X. laevis* oocytes were injected with 50 ng of cRNA. Oocytes were maintained at 18°C in Barth's medium for 3–5 days. The transportability of the selected pyrimidine nucleosides and nucleoside-derived drugs was then assessed, taking advantage of the fact that hCNT1 function is associated with substrate-induced Na$^+$ inward currents, using the two-electrode voltage-clamp technique [22]. The oocyte membrane potential (V$_m$) was held at –50 mV for continuous current recordings using Axoscope V1.1.1.14 software (Axon Instruments, Foster City, Calif., USA). To determine the current/voltage relationship, 11 pulses of potential between +50 and –150 mV (–20 mV decrement) were applied for 100 ms using pClamp 6 software (Axon Instruments). The apparent affinity constant ($K_{0.5}$) and the maximal current for saturating substrate concentrations (I$_{max}$) were then obtained by fitting the steady-state currents at each membrane potential to the equation: I = I$_{max}$ · [S]/($K_{0.5}$+[S]), where [S] is the substrate concentration, using the non-linear fitting method in SigmaPlot 8 (SPSS, Inc., Chicago, Ill., USA).

Statistical analysis

The unpaired Student's *t*-test has been used for the statistical comparison of experimental data. This analysis has been carried out using GraphPad Prism 4.0 software (GraphPad Software, Inc., San Diego, Calif., USA).

Results

The high-affinity interaction between the pyrimidine nucleoside cytidine and hCNT1 was assessed in our mammalian cell model by firstly monitoring cytidine uptake kinetics and secondly, the concentration dependence of cytidine-induced inhibition of Na^+-dependent uridine uptake (Figure 1A,B). Cytidine inhibited Na^+-dependent uridine uptake with an IC_{50} of 4.5 µM (Figure 1B). With the substrate/inhibitor concentration ratio used (1 to 100 µM), this yielded an almost identical K_i value (4.38 µM). Moreover, K_i was very close to the apparent K_m (3.1 µM) derived from the uptake kinetics shown in Figure 1A. This demonstrated that the hCNT1-CHO clone was a suitable cell model for the analysis of hCNT1 substrate selectivity.

The interaction between hCNT1 and nucleosides of pharmacological relevance was then assessed in hCNT1-CHO cells by monitoring the inhibition of 1 µM Na^+-dependent uridine uptake triggered by a panel of nucleosides used at high concentrations (Figure 2). Two naturally occurring pyrimidine nucleosides, cytidine and thymidine, significantly inhibited uridine transport when used at a concentration of 100 µM ($P<0.001$), far above their known apparent K_m for the transporter. Gemcitabine and 5′-DFUR, two pyrimidine nucleoside derivatives used in anticancer therapy, when assayed at the same concentration (100 µM) also inhibited transport, although inhibition by 5′-DFUR was markedly lower than that exerted by the other nucleosides. The antiviral drugs AZT, d4T, ddC and 3TC were used at a concentration of 5 mM to determine whether any interaction with the transporter occurs, even if of low affinity. AZT, d4T and ddC, but not 3TC, significantly inhibited hCNT1-mediated uridine uptake (AZT, $P<0.001$; d4T and ddC, $P<0.05$), AZT being the most effective. The concentration-dependence of this inhibitory action on hCNT1 function was then analysed for AZT, d4T and ddC (Figure 3). Whereas AZT almost completely abolished hCNT-1-mediated transport at the highest concentration used (85% inhibition at 10 mM) (Figure 3A), d4T and ddC, when used at the maximal concentrations allowed by their solubility limit (20 mM) exhibited approximately 80% inhibition of the Na^+-dependent uridine uptake (Figure 3B,C).

Inhibition of hCNT1-mediated substrate translocation is not necessarily associated with transportability. To determine whether these three antiviral nucleoside derivatives are indeed translocated by hCNT1, nucleoside driven Na^+-inward currents were monitored in different *X. laevis* oocytes expressing hCNT1, using the two-electrode voltage-clamp technique. Water-injected oocytes did not show any nucleoside-driven Na^+-inward current (data not shown). Figure 4 shows representative electrophysiological records of currents generated by the naturally occurring nucleoside uridine and selected nucleoside derivatives at a clamped potential of −50 mV. Oocytes were continuously perfused with Na^+ buffer and a baseline current, which corresponds to the Na^+-leak current in the absence of substrate, was recorded. Addition of 0.5 mM uridine

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Figure 1. Interaction of cytidine with recombinant hCNT1 expressed in CHO-K1 cells
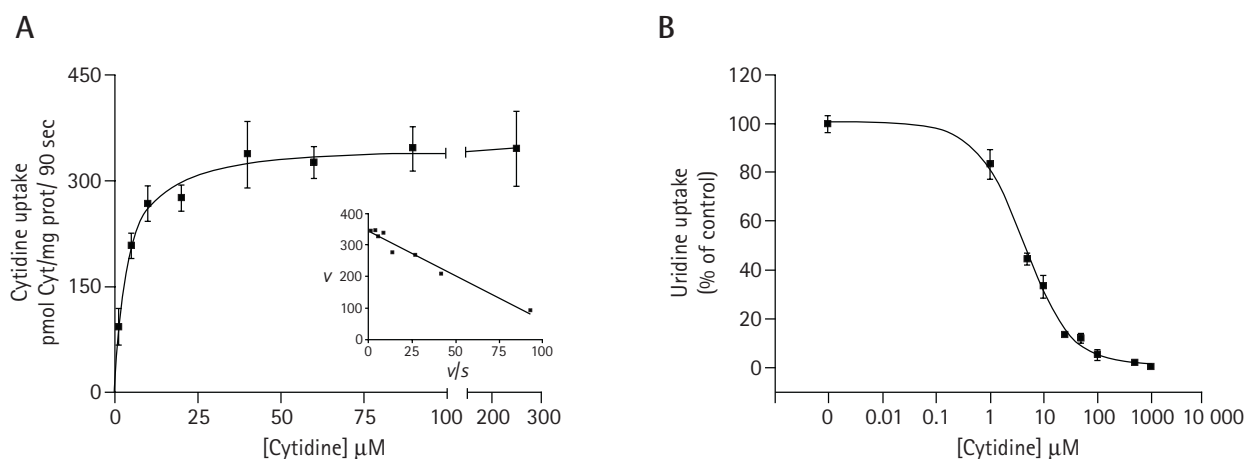
(A) Concentration-dependence of Na^+-dependent cytidine uptake by recombinant hCNT1. The inset shows the Eadie–Hoftsee plot (*v* versus *v/s*) that was used for the estimation of the apparent K_m value. Data are given as absolute uptake rates (pmol cytidine/mg protein/90 sec). Results are the mean ±SEM of four independent experiments. **(B)** Cytidine inhibition of Na^+-dependent uridine uptake by recombinant hCNT1. The uptake of 1 µM [³H] uridine was measured over 90 sec in the presence of increasing concentrations of cytidine. Results are shown as percentage of control (absence of inhibitor) values and are expressed as the mean ±SEM of three independent experiments.

Figure 2. Inhibition of hCNT1-mediated uridine uptake by nucleosides and nucleoside analogues in CHO-K1 cells
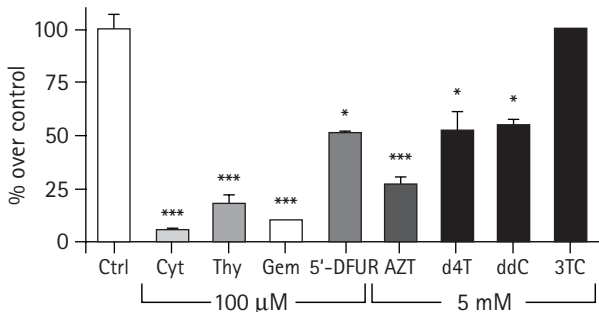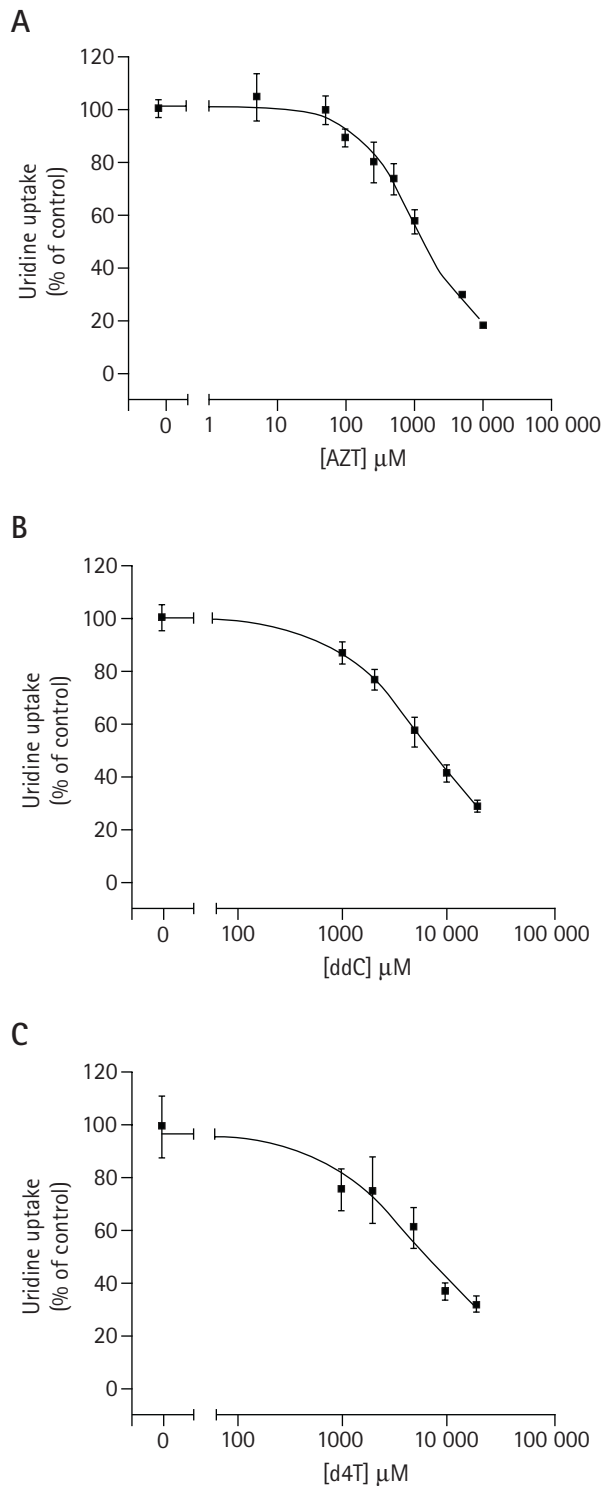

The Na⁺-dependent uptake of 1 μM [³H] uridine was measured over a 90 sec incubation time, either in the absence (Ctrl) or in the presence of high concentrations of competing non-radiactive nucleosides. Cytidine (Cyt), thymidine (Thy), gemcitabine (Gem) and 5′-DFUR were used at 100 μM, whereas AZT, d4T, ddC and 3TC were all used at 5 mM. Data are expressed as percentage of uptake versus control (absence of inhibitor) value. Results are expressed as the mean ±SEM of three independent experiments. Statistical significance was assessed using Student's *t*-test: ctrl versus inhibitor (*$P<0.05$; ***$P<0.001$).

Figure 3. Concentration-dependent inhibition of recombinant hCNT1-mediated uridine uptake by antiviral drugs

The Na⁺-dependent uptake of 1 μM [³H] uridine by recombinant hCNT1 expressed in CHO-K1 cells was measured over a 90 sec incubation time in the presence of increasing concentrations of **(A)** AZT, **(B)** ddC and **(C)** d4T. Results are shown as the percentage of the hCNT1-mediated uptake rates measured in the absence of inhibitor and are expressed as the mean ±SEM of three independent experiments.

evoked an inward current of ~150 nA (Figure 4A). The intensity of these Na⁺-inward currents varied among oocytes (Figure 4A–C) reflecting, as for other transporters, different hCNT1 expression levels. Washing out with Na⁺ buffer after perfusion of the nucleoside returned the current to the original baseline (not shown), as previously reported [22], but routinely, especially when different substrates were going to be perfused in a single oocyte, Na⁺-free solution was used for a fast removal of the Na⁺ and substrate. Inward current and the baseline current therefore disappeared when the oocyte was washed out with Na⁺-free buffer. Addition of Na⁺ buffer returned the current to baseline levels. AZT (Figure 4A) and d4T (Figure 4B), but not ddC (Figure 4C), elicited substrate-induced Na⁺-inward currents, thus demonstrating that AZT and d4T-mediated inhibition of Na⁺-dependent uridine uptake is indeed associated with transportability. Nervertheless, ddC inhibited the Na⁺-leak and uridine-induced currents (Figure 4C), indicating that although this nucleoside derivative is able to bind the transporter, it is not translocated. The apparent inhibition constant for ddC obtained by inhibition of the Na⁺-leak current was 5.5 mM.

The voltage-dependence of the hCNT1-mediated uptake of AZT and d4T was then determined by measuring substrate-driven Na⁺-inward currents at different membrane potentials. The derived I_{max} and $K_{0.5}$ values for AZT are shown in Figure 5. I_{max} was similarly voltage-dependent for both uridine and AZT (Figure 5A), although maximal current for the drug

Figure 4. Na$^+$ currents evoked in oocytes expressing hCNT1 by uridine and nucleoside-derivatives with antiviral properties
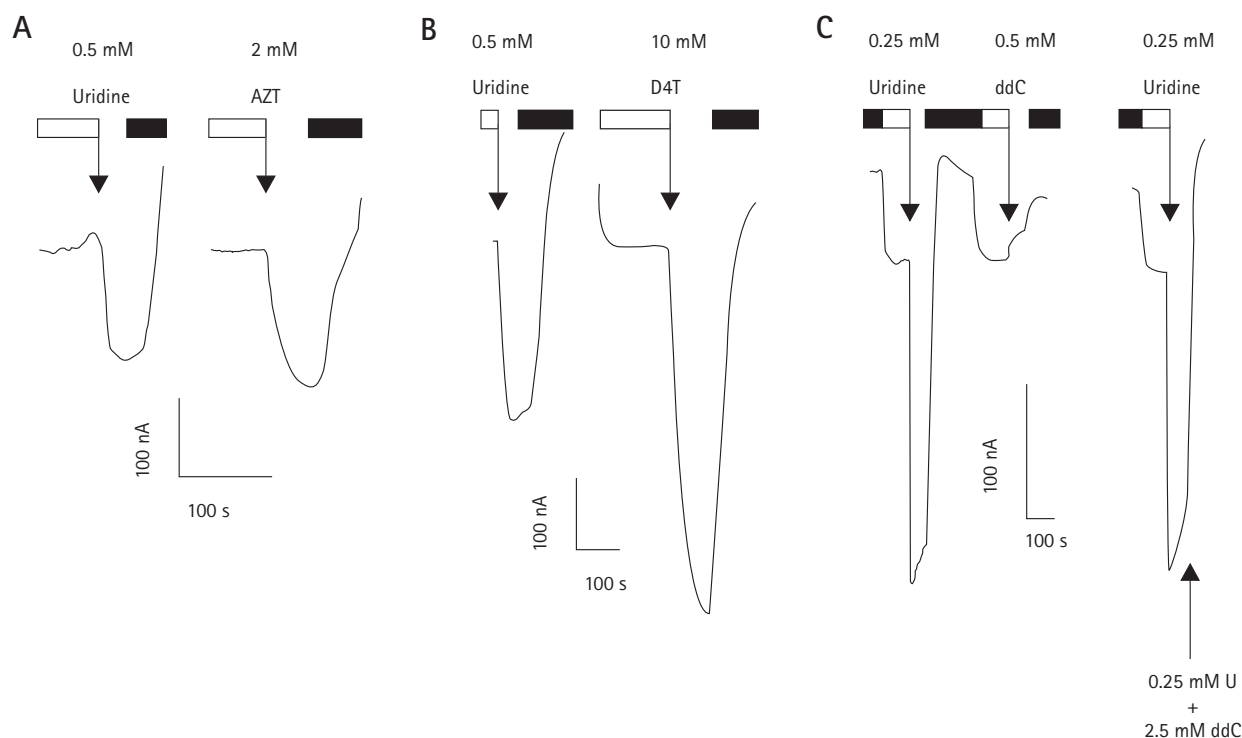
Each panel corresponds to a different oocyte. Oocytes were held at –50 mV and continuously perfused with Na$^+$ buffer in the absence of substrate (blank box). The current registered under that condition corresponds to the Na$^+$-leak current. Addition of uridine or antiviral drugs is indicated by an arrow. After perfusion with each nucleoside, oocytes were washed out in Na$^+$-free medium (black box). Both AZT and d4T induced Na$^+$-inward current indicating that they are transported **(A)** and **(B)**. ddC did not induce inward currents, even when added at higher concentrations (up to 5 mM) than those shown in this panel (not shown). Nevertheless, ddC reduced the Na$^+$-leak current and the simultaneous perfusion of uridine and ddC completely inhibited uridine-induced inward current at 2.5 mM **(C)**, which indicates that although it is not transported, it is able to bind the transporter.

was markedly higher (around twofold) than that of uridine. Similar behaviour was observed when d4T was used as substrate, although I_{max} values were three- to fivefold higher than those measured with the naturally occurring nucleoside uridine (not shown). $K_{0.5}$ for AZT was voltage-independent at physiological and hyperpolarizing membrane potentials (1.1 mM at –50 mV, Figure 5B), as it was for d4T (15.6 mM at –50 mV, data not shown).

The apparent K_m derived from these kinetic studies as well as the IC$_{50}$ values obtained from the cross-inhibition experiments (depicted in Figures 2 and 3) are shown in Table 1. For a better comparison between nucleosides, their chemical structures are also shown (Figure 6). Naturally occurring nucleosides and the two pyrimidine nucleoside derivatives used in anti-cancer therapy, gemcitabine and 5′-DFUR, showed IC$_{50}$ and apparent K_m values in the low-mid µM range. Constants for AZT reached 1 mM, whereas d4T kinetic values ranged from 4–15 mM. ddC, although not being transported, had similar IC$_{50}$ values to those of d4T.

All these nucleosides and derivatives differ in a number of structural features, but a significant difference between those that are efficiently transported and the antiviral nucleosides studied here is the lack of the hydroxyl group in the 3′ position of the sugar (Figure 6). To ascertain whether this is a key structural determinant for hCNT1 recognition, we compared the interaction of thymidine and 3′-deoxythymidine with hCNT1. Indeed, the lack of the hydroxyl group in the 3′ position of the deoxyribose is enough to dramatically increase the IC$_{50}$ value derived from the cross-inhibition experiments shown in Figure 7. IC$_{50}$ values were 12 µM and 737 µM for thymidine and 3′-deoxythymidine, respectively. Similar cross-inhibition experiments were then performed using cytidine and its corresponding ribose-modified nucleosides (data not shown). IC$_{50}$ values derived from these studies revealed that cytidine and 2′-deoxycytidine exerted a similar effect on uridine uptake (IC$_{50}$ values of 4.5 µM vs 8.6 µM, respectively), whereas, as shown earlier (Figures 3 and Table 1), 2′,3′-dideoxycytidine (ddC) inhibited uridine transport with an IC$_{50}$ value of nearly 6 mM.

Figure 5. $K_{0.5}$ and I_{max} for AZT as a function of membrane potential

A

B

C
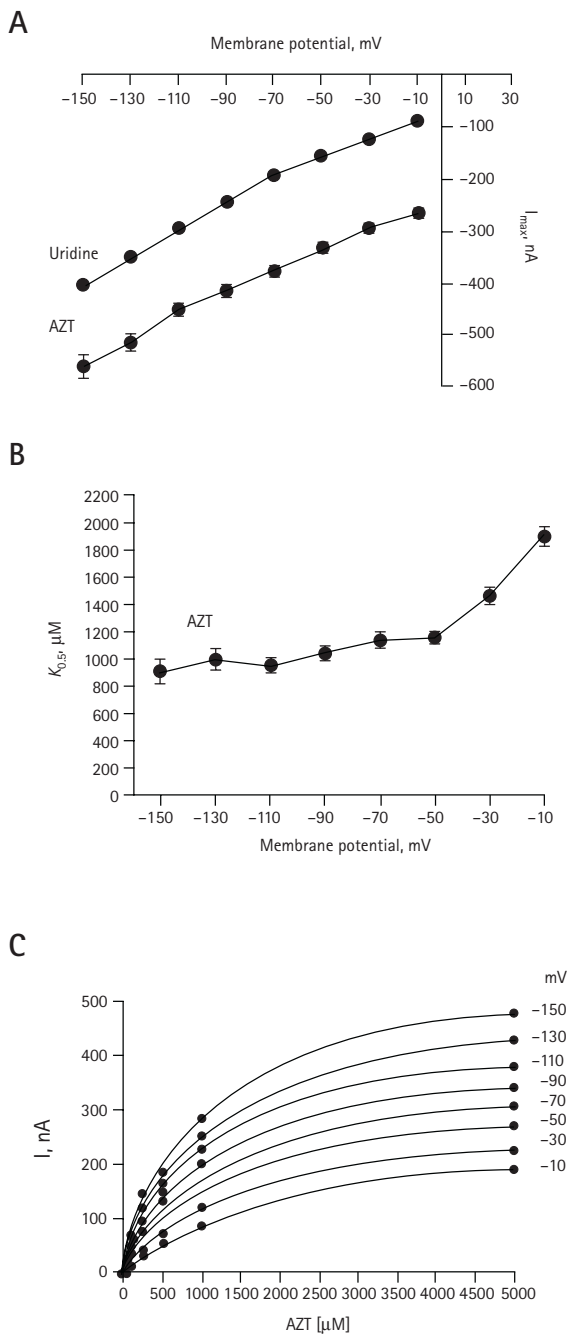
Experiments were routinely performed in hCNT1-expressing oocytes 3 days after cRNA injection. $K_{0.5}$ and I_{max} for AZT were obtained at every membrane potential by fitting the steady-state currents obtained at seven different concentrations (from 50–5000 µM) to the equation described in Materials and methods. A representative experiment using a single oocyte expressing hCNT1 is shown. The error bars correspond to fitting error. **(A)** I_{max}/V curve. Maximal current for uridine was obtained using a saturating uridine concentration (0.5 mM). At each membrane potential, I_{max} for AZT was 1.5- to twofold higher than that for uridine and increased with more negative potentials from –360 nA at –50 mV to –560 nA at –150 mV. **(B)** $K_{0.5}/V$ curve. $K_{0.5}$ values were comparable at each membrane potential between –50 (1160 ±29 µM) and –150 (906 ±94 µM) mV. Similar results were obtained with oocytes from five different frog donors. **(C)** Fit of the data used to obtain I_{max} and $K_{0.5}$ of AZT in Figure 5A and B.

Table 1. Kinetic constants of hCNT1 interaction for selected naturally occurring nucleosides and nucleoside analogues used in anticancer and antiviral therapies

|	IC_{50}, µM	K_m, µM
Cytidine	4.5 ±0.3	3.1 ±1.4
Thymidine	12.55 ±3.3	ND
AZT	1004 ±260	963 ±330
d4T	4525 ±521	15 600 ±4700
ddC	5730 ±384	NT
3TC	>>20 000	NT
Gemcitabine	ND	17 ±5
5′-DFUR	ND	209 ±135

Apparent K_m and IC_{50} values for nucleosides, antiviral and anticancer drugs. IC_{50} values were obtained from experiments depicted in Figures 1, 3 and 7. Apparent K_m values for AZT and d4T were calculated by fitting the data obtained using the two-electrode voltage-clamp technique in *X. laevis* oocytes expressing hCNT1. Apparent K_m values for gemcitabine and 5′-DFUR have been taken from Lostao *et al.* (2000) [6] and Mata *et al.* (2001) [7]. The apparent K_m value for cytidine was calculated from data shown in Figure 1A. For a better understanding of putative structural features conferring nucleoside interaction with hCNT1, the chemical structures of all compounds used in this study are also shown (Figure 6). Values are expressed as mean ±SD. ND, not determined; NT, not transported.

Discussion

This study demonstrates that the so-called 'high-affinity' pyrimidine-preferring nucleoside transporter CNT1 is not in fact a broad-selectivity carrier for pyrimidine-derived drugs. hCNT1 is responsible for the translocation of selected anticancer drugs, thus conferring chemosensitivity to those cells expressing the transporter [7,8]. However, among the selected antiviral drugs used in this study none appears to interact with high affinity with hCNT1. This was evident even when the concentrations tested were above those expected *in vivo*.

Although AZT and d4T are recognized by hCNT1 with relatively low affinity, they appear to be translocated with higher V_{max} values than those found for naturally occurring nucleosides and selected fluoro-pyrimidines. We have recently demonstrated that I_{max} values for cytidine and its analogue gemcitabine are comparatively low and independent of membrane potential, thus suggesting decreased constant rates for the fully loaded nucleoside/Na⁺/transporter complexes [22]. This occurs despite their apparent high binding affinity for the carrier protein [6,22]. These two nucleosides carry an NH_2 group at the 4 position of the pyrimidine ring, whereas other nucleosides that show voltage-dependence of their I_{max} values, including the antiviral drugs assayed here, carry an O in that position. Little is known about the structural determinants of the pyrimidine ring necessary for substrate recognition and translocation. However, when measuring

Figure 6. Structures of naturally occurring nucleosides and nucleoside analogues used in anticancer and antiviral therapies
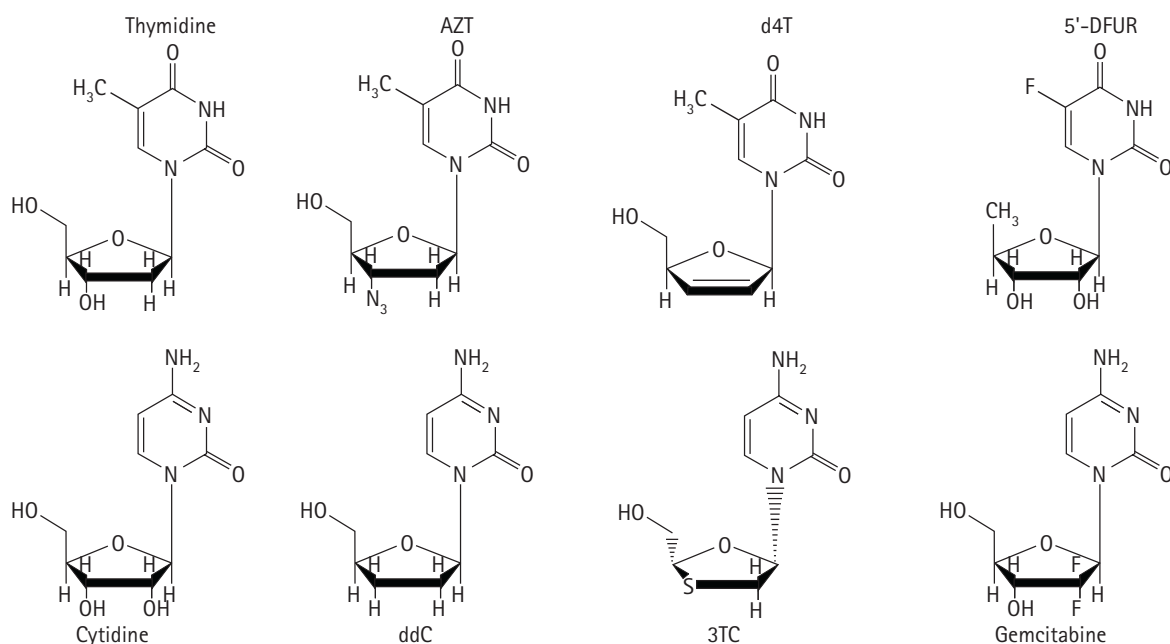

substrate selectivity of the rat orthologue of CNT1 by electrophysiological techniques at a single membrane potential, it has been shown that a change in the N from the 5 to 6 position of the azacytidine molecule results in a loss of transportability [23].

Moreover, in a recent contribution analysing the uridine-binding motifs of hCNT1, it has been shown that N at position 3 also contributes to substrate binding [24]. Our previous data [22] and those presented here for the antiviral drugs, suggest that an N or an O at position 4 would probably modify the voltage dependence of the I_{max} values, without significantly modifying substrate recognition itself. Nevertheless, since nucleobases do not interact with CNT transporters it is likely that substrate recognition relies mostly upon the ribose ring structure, as recently reported for the *Toxoplasma gondii* high-affinity nucleoside transporter TgAT2 [25]. Indeed, a primary role for the ribose in determining substrate transportability is supported by the fact that all antiviral drugs assayed here, which are either not transported or poorly transported by hCNT1, lack the 3′ hydroxyl group of the sugar. Thus, this position appears to be a key structural determinant for nucleoside recognition by the human orthologue of CNT1, irrespective of whether the pyrimidine nucleoside incorporates a ribose or a 2′-deoxyribose. Our comparative analysis of the interactions of thymidine, 3′-deoxythymidine, cytidine, 2′-deoxycytidine and ddC

with hCNT1 supports this hypothesis. This agrees with a recent contribution by Cass and colleagues in which the uridine-binding motifs recognized by the human orthologue of CNT1 were analysed by measuring cross-inhibition of uridine uptake by a set of uridine analogues [24].

The evidence that slight changes in nucleoside structure may determine such a dramatic shift in substrate recognition and transportability may be physiologically and pharmacologically relevant. Nucleosides and most of their analogues are absorbed in the intestine and efficiently reabsorbed in the renal tubule, due to the polarized localization of nucleoside transporters CNT proteins are located at the apical side, whereas the equilibrative nucleoside carriers (SLC29) have been found at the basolateral side of epithelial cells [9,11,12,26]. The exclusion of certain pyrimidine nucleoside derivatives from recognition by the high-affinity, pyrimidine-preferring nucleoside transporter hCNT1 would compromise their disposition unless alternative carriers recognize these compounds. ENT transporters may be candidates for this physiological role. Although interaction of AZT with hENT1 has been described by cross-inhibition studies, this analogue seems to be a substrate of the hENT2 isoform [27]. ddC also appears to be recognized by ENT1 and ENT2, although the reported apparent K_m value for ENT1-mediated uptake was 22 mM [27]. Moreover,

Figure 7. Interaction of thymidine and 3′-deoxythymidine with recombinant hCNT1 in CHO-K1 cells
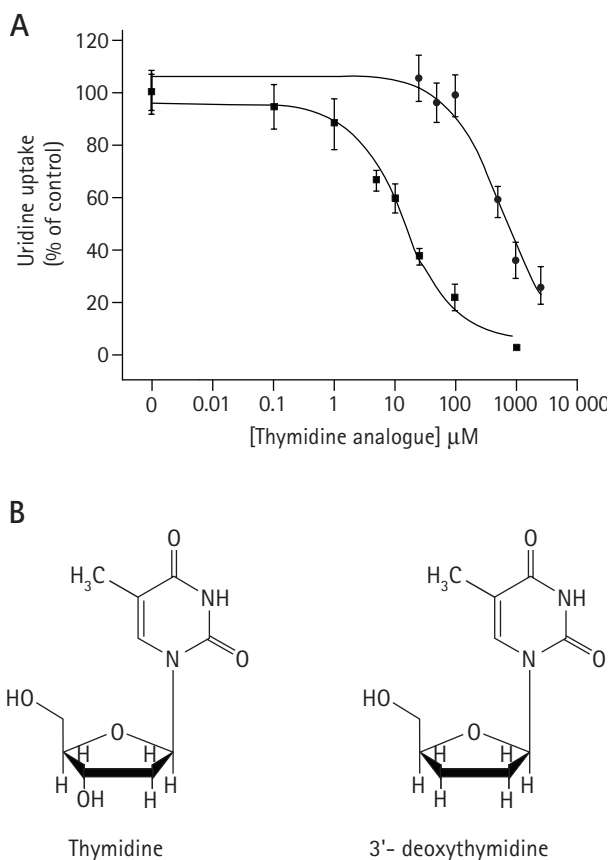
The uptake of 1 µM [³H] uridine was measured over 90 sec in the presence of increasing concentrations of thymidine (■) and 3′-deoxythymidine (●). Data are given as the percentage of uptake rates measured in the absence of inhibitors. The results are the mean ±SEM of three independent experiments. For a better understanding of the structural constraints for hCNT1 recognition, the structures of thymidine and 3′-deoxythymidine are also shown.

AZT and ddC are also substrates of the human concentrative nucleoside transporter hCNT3, but probably with low affinity, as reported here for hCNT1 [28]. Carriers belonging to the SLC22 family, mostly organic anion transporters (OATs) and, to a lesser extent, organic cation transporters (OCTs), have been implicated in the uptake of some nucleoside derivatives, such as AZT [29–31]. High affinity transport of AZT has been reported for OAT1, OAT2, OAT3 and OAT4, with apparent K_m values in the 20–150 µM range [31–33]. Whereas OAT1, OAT2 and OAT3 show mainly basolateral localization in renal epithelial cells; OAT4 is located at the apical side [34]. However, their exact role in AZT disposition is somewhat uncertain, because they are all anion exchangers with as yet undefined stoichiometry [34]. It has been suggested that exchange involves one organic anion/one dicarboxylate,

and, as for OAT3, it may be indirectly coupled to the Na⁺ gradient [35]. Moreover, although OAT4 is the only member of this family located at the apical side of renal epithelial cells, it is not yet known whether this isoform is expressed in enterocytes [36]. Thus, the exact driving force for AZT uptake remains to be fully elucidated and it also remains to be seen which would be suitable high-affinity carriers responsible for the apical uptake of this and other antiviral drugs. The apical location of hCNT1 in enterocytes would favour the view that this transporter might play a minor role in AZT absorption if luminal drug concentrations reach much higher levels than in plasma. Nevertheless, the apparent K_m value for AZT is definitely far above those concentrations encountered in humans.

In summary, this study demonstrates that the high-affinity nucleoside transporter hCNT1 is not in fact a broad-selectivity pyrimidine nucleoside carrier, particularly for those derivatives lacking a hydroxyl group at the 3′ position of the ribose ring, as in the case of selected inhibitors of HIV-1 reverse transcriptase. It is also apparent that very slight changes in substrate structure provoke a dramatic shift in selectivity. Thus, hCNT1 is probably not a suitable target to improve drug therapy with nucleoside HIV reverse transcriptase inhibitors. Hence, the role of other transporters, such as OATs, in antiviral drug targeting requires further research.

Acknowledgements

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