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European Journal of Pharmacology 479 (2003) 269–281
www.elsevier.com/locate/ejphar

Nucleoside transporters in the disposition and targeting of nucleoside analogs in the kidney

Lara M. Mangravite, Ilaria Badagnani, Kathleen M. Giacomini*

Department of Biopharmaceutical Sciences, University of California, San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143-0446, USA

Accepted 21 August 2003

Abstract

Systemic disposition of nucleosides and nucleoside analogs is dependent on renal handling of these compounds. There are five known, functionally characterized nucleoside transporters with varying substrate specificities for nucleosides: concentrative nucleoside transporters (CNT1–CNT3; Solute Carrier (SLC) 28A1–28A3), which mediate the intracellular flux of nucleosides, and equilibrative nucleoside transporters (ENT1–ENT2; SLC29A1–SLC29A2), which mediate bi-directional facilitated diffusion of nucleosides. All five of these transporters are expressed in the kidney. Concentrative nucleoside transporters primarily localize to the apical membrane of renal epithelial cells while equilibrative nucleoside transporters primarily localize to the basolateral membrane. These transporters work in concert to mediate reabsorptive flux of naturally occurring nucleosides and nucleoside analogs. In addition, equilibrative transporters also participate in secretory flux of some nucleoside analogs. Nucleoside transporters also serve in the targeting of nucleoside analog therapies to renal tumors. This review examines the role that these transporters play in renal disposition of nucleosides and nucleoside analogs in both systemic and kidney-specific therapies.

Keywords: Nucleoside transporter; Nucleoside analog; Renal cancer; Kidney; Drug disposition; Localization

1. Introduction

Nucleosides and nucleoside analogs are administered therapeutically in the treatment of cardiac arrhythmias, neoplasms, and viral infections. These compounds are hydrophilic and require facilitated transport to traverse cellular membranes. This movement is carried out primarily through interactions with nucleoside-specific transporter proteins of which there are two known families: sodium-dependent concentrative nucleoside transporters (CNTs; Solute Carrier (SLC) 28) and equilibrative nucleoside transporters (ENTs; SLC29). The presence of these transporters in various tissues throughout the body governs tissue specific disposition and pharmacokinetics of naturally occurring nucleosides as well as some synthetic nucleoside analogs. This review will focus on the five characterized nucleoside-specific transporters with regard to how they work in concert to mediate renal disposition of nucleosides and nucleoside analogs, as well as their role in targeting of nucleoside analogs in renal cancers.

Naturally occurring nucleosides are produced in the liver and peripheral tissues, circulate through the body for use by both energy-depleted and dividing cells, and are then actively reabsorbed within the kidney. Renal disposition of nucleoside analogs is more variable: some are reabsorbed but others are actively secreted (Table 1). The extent of reabsorption or secretion does not appear to be related to similarities in chemical structure between an analog and endogenous nucleosides. Deoxyadenosine, which differs from adenosine by one hydroxyl group, is actively secreted whereas ribavirin, which differs drastically in structure from endogenous nucleosides, is actively reabsorbed (Fig. 1) (Kuttesch and Nelson, 1982; Paroni et al., 1989). Unlike filtration, the processes of renal secretion and reabsorption require active transport.

Renal epithelial cells serve as a barrier between the tubular filtrate and the interstitium. As such, they are polarized with two distinct membranes: an apical or brush-border membrane that is in contact with the filtrate and a basolateral membrane that is in contact with the interstitium. Transepithelial flux of nucleosides in either

* Corresponding author. Tel.: +1-415-476-1936; fax: +1-415-502-4322.
E-mail address: kmg@itsa.ucsf.edu (K.M. Giacomini).

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doi:10.1016/j.ejphar.2003.08.076

Table 1
Renal handling of selected clinically administered nucleosides and nucleoside analogs

Therapeutic compound	Common indications	Active renal processes	Renal toxicities	References
Nucleosides				
Adenosine	Cardiac arrhythmia	Reabsorbed	–	(Kuttesch and Nelson, 1982)
Deoxyadenosine	–	Secreted	–	(Kuttesch and Nelson, 1982)
Synthetic analogs				
Gemcitabine	Pancreatic cancer, breast cancer, bladder cancer, non-small cell lung cancer	Metabolite poorly secreted	Mild proteinuria, common mild hematuria, common renal failure, rare	(Aapro et al., 1998; Abbruzzese et al., 1991)
Didanosine	HIV, HTLV	Secreted	Nephrotoxicity, rare	(Crowther et al., 1993; Hartman et al., 1990; Knupp et al., 1991)
Cytarabine	ALL, ANL, CML, Meningial leukemia	N/D	Renal failure, rare	(Slavin et al., 1978)
Ribavirin	Hepatitis C	Reabsorbed	Nephrolithiasis, rare	(Fontana, 2001; Paroni et al., 1989)
Zidovudine	HIV, HTLV	Secreted	–	(de Miranda et al., 1989)

HIV, human immunodeficiency virus; HTLV, human T-cell lymphotrophic virus; ALL, acute lymphocytic leukemia; ANL, acute non-lymphocytic leukemia; CML, chronic myelocytic leukemia. N/D, not determined. –, no toxicities reported.

the reabsorptive or secretory direction necessitates a minimum of two steps to traverse the epithelium: concentrative transport of nucleosides into the cells and then equilibrative transport out the other side. Thus, the subcellular localization of concentrative nucleoside transporters, which mediate influx, to the apical membrane would implicate them in the reabsorption of naturally occurring nucleosides and localization to the basolateral membrane would implicate them in the secretion observed for many nucleoside analogs.

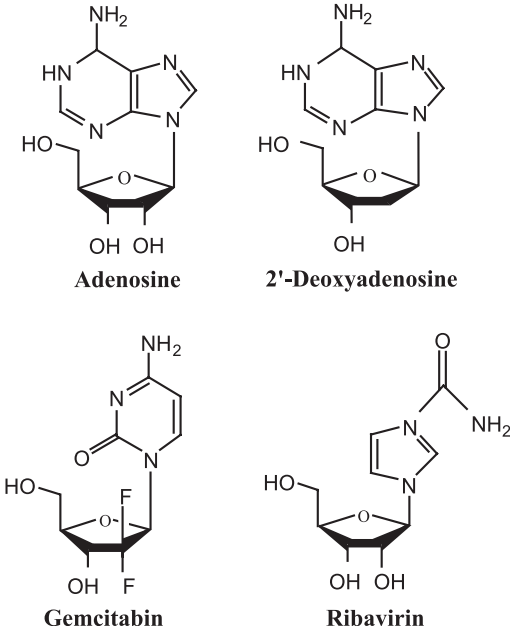

Fig. 1. Chemical structures of selected nucleosides and nucleoside analogs. Compounds have a purine (adenosine, deoxyadenosine, ribavirin) or pyrimidine (gemcitabine) base linked to a ribose or deoxyribose ring. Ribavirin is a guanosine analog. Gemcitabine is a cytidine analog.

Nucleoside transporters are expressed within the kidney and are expected to play an important role in the active processes that govern the renal disposition of nucleosides and nucleoside analogs. Historically, functional studies in animal tissue preparations suggested the presence of multiple nucleoside transport systems within renal epithelia (Le Hir and Dubach, 1984; Lee et al., 1990). Both sodium-dependent (concentrative) and sodium-independent (equilibrative) transport systems with varying substrate specificities have been described (Williams and Jarvis, 1990, 1991; Williams et al., 1989). Nucleosides can be categorized as either purine or pyrimidine nucleosides based on chemical structure (Fig. 1). Some nucleoside transport systems characterized in the kidney have a preference for purines, some prefer pyrimidines, and others appear to be indiscriminate. The first study of nucleoside transport systems in human renal brush-border membrane vesicles was carried out in this laboratory and identified the presence of both an equilibrative and a concentrative nucleoside transport system (Gutierrez et al., 1992). Further examination of the concentrative system indicated that the system preferred pyrimidine nucleosides although it also transported adenosine and guanosine (Giacomini et al., 1994; Gutierrez and Giacomini, 1994). The equilibrative transport system was not characterized.

Five nucleoside-specific transporters have been cloned and functionally characterized. These proteins are thought to be responsible for governing the systemic disposition of nucleosides and nucleoside analogs. All five of these transporters appear to be transcribed within the kidney and are

thus likely to play a role in renal disposition of endogenous and therapeutic nucleosides (Pennycooke et al., 2001; Ritzel et al., 2001). The relative importance of each of these transporters in the renal disposition of naturally occurring nucleosides and synthetic analogs is dependent on its substrate specificity and subcellular localization and level of expression within renal epithelia.

2. Concentrative nucleoside transporters

Three members of the mammalian CNT family have been cloned and functionally characterized to date: CNT1, CNT2 or SPNT (*S*odium-dependent *P*urine-selective *N*ucleoside *T*ransporter), and CNT3 (Ritzel et al., 1997, 1998; Wang et al., 1997b). These transporters differ in their substrate specificity and work in concert to mediate the renal transport of nucleosides and nucleoside analogs. CNT1 is pyrimidine preferring, CNT2 is purine preferring, and CNT3 is broadly selective. Concentrative nucleoside transporters couple inward flux of nucleosides to inward flux of Na^+. All three of the cloned nucleoside transporters appear to be expressed in the kidney to some degree (Table 2).

2.1. CNT1

CNT1 was the first member of the CNT family to be isolated and functionally characterized (Ritzel et al., 1997). CNT1 encodes a protein of 650 amino acids (~ 72 kDa) and is expressed in epithelial tissues (predominantly jejunum and kidney). CNT1 preferentially transports pyrimidine nucleosides (thymidine, cytidine, and uridine) and some pyrimidine nucleoside analogs but not the purine nucleosides, guanosine or inosine, or their analogs (Table 3). Interestingly, CNT1 does transport adenosine albeit at low capacity (Ritzel et al., 1997). It does not interact with either nucleobases (uracil, hypoxanthine) or nucleotides (UMP, UDP, UTP) (Lostao et al., 2000; Ritzel et al., 1997; Yao et al., 2002). Sequence analysis and topology studies suggest that CNT1 has 13 transmembrane domains with a cytoplasmic N-terminal tail and an extracellular C-terminal tail (Fig. 2) (Hamilton et al., 2001; Huang et al., 1994). CNT1 has two putative glycosylation sites in the C-terminal extracellular tail, at positions N606 and N654. Treatment with endoglycosidase F, a deglycosylation agent, indicates that at least one of these sites is glycoslyated (Hamilton et al., 2001). Identification of the molecular structure of CNT1 has allowed for extensive analysis of both the mechanism of transport and the substrate specificity of this protein. Transport is sodium-dependent, with a likely coupling ratio of 1:1 (Na^+/nucleoside). Transport also appears to be sensitive to membrane potential (Dresser et al., 2000; Lostao et al., 2000).

A wide variety of nucleoside analogs pass through the kidney and may be affected by the presence of CNT1 within renal epithelia. For this reason, an examination of the tolerated substrate substitutions that are compatible with transport is essential. To this aim, CNT1 has been expressed in multiple heterologous expression systems (oocytes, mammalian cells, and yeast) and examined for interaction with a variety of substrates. Standard assays in these systems involved determination of the uptake of radiolabeled nucleosides or nucleoside analogs and, often, the inhibition of uptake by unlabeled compounds. While this methodology provides a rapid way to test for interactions of compounds with transporters, it requires the use of radiolabeled substrate in order to determine definitively whether a substance is acting as a substrate or an inhibitor, a difficult and costly task. The advent of electrophysiological techniques has allowed for a much broader understanding of the substrate specificity of CNT1. Examination in this laboratory of the structural basis for substrate interaction of the rat ortholog, rCNT1, using electrophysiologic techniques has shown that pyrimidine analogs modified at the 3-, 4-, or 5-positions on the base continued to be transported by rCNT1 but modification at the 6-position negated substrate/transporter interactions (Dresser et al., 2000). 5′-Hydroxylation of the ribose was not essential for transport. Examination of human CNT1 via electrophysiology showed similar trends (Lostao et al., 2000; Mata et al., 2001). A summary of the substrates and inhibitors known to interact with rat and human CNT1, and the other cloned transporters discussed below, is provided in Table 3.

Table 2
Expression and localization of nucleoside transporters in the kidney

Transporter	Kidney expression		Localization in renal epithelia	
	mRNA	Protein	Apical	Basolateral
Concentrative nucleoside transporters (CNT)				
CNT1	++++	yes	***	–
CNT2	++	yes	**	*
CNT3	+	N/D	***	–
Equilibrative nucleoside transporters (ENT)				
ENT1	+++	N/D	*	**
ENT2	++	N/D	–	***

N/D: not determined. ++++, high expression; +++ to ++, intermediate expression; +, low expression; –, not present; ***, entirely localized; ** to *, partially localized.

2.2. CNT2 (SPNT)

The human ortholog of CNT2, termed SPNT1 or CNT2, was originally cloned in this laboratory from the kidney (Wang et al., 1997b). CNT2 is 72% identical to CNT1, and mediates sodium-dependent purine-selective nucleoside transport (Ritzel et al., 1998). The protein is predicted to have a similar secondary structure to the other cloned CNTs, and contains six putative N-linked glycosylation sites (only the four in the C-terminal tail are predicted to be extracellular) and six putative protein kinase C (PKC) phosphorylation sites. CNT2 has a wider tissue distribution than CNT1 and is most abundant in the GI tract where it appears to be responsible for the

Table 3
Substrate specificity of the rat (R) and human (H) orthologs of cloned nucleoside transporters

Substrate	CNT						ENT				References
	1		2		3		1		2		
	R	H	R	H	M	H	R	H	R	H	
Naturally occurring compounds											
Adenosine	26		6	8		15		50		140	(Che et al., 1995; Huang et al., 1994; Ritzel et al., 2001; Ritzel et al., 1997; Wang et al., 1997b; Ward et al., 2000; Yao et al., 1997)
Uridine	37	45	21	80		22	150	480	300	270	(Huang et al., 1994; Ritzel et al., 2001; Ritzel et al., 1997; Wang and Giacomini, 1997; Wang et al., 1997b; Ward et al., 2000; Yao et al., 1997)
Cytidine		34				15		680		5210	(Gerstin et al., 2002; Lostao et al., 2000; Ritzel et al., 2001; Wang et al., 1997a; Wang et al., 1997b; Ward et al., 2000; Yao et al., 1997)
Thymidine	14	16				21		240		620	(Fang et al., 1996; Ritzel et al., 2001; Ritzel et al., 1997; Wang and Giacomini, 1997; Wang et al., 1997b; Ward et al., 2000; Yao et al., 1997)
Inosine			28	5		53		200		50	(Loewen et al., 1999; Ritzel et al., 2001; Wang and Giacomini, 1997; Wang et al., 1997b; Ward et al., 2000; Yao et al., 1997)
Guanosine						43		140		2700	(Fang et al., 1996; Lostao et al., 2000; Ritzel et al., 2001; Ritzel et al., 1997; Ritzel et al., 1998; Ward et al., 2000; Yao et al., 1997)
Formycin B											(Che et al., 1995; Crawford et al., 1998a; Lostao et al., 2000; Ritzel et al., 2001; Wang et al., 1997b)
dA		*46*		*110*							(Gerstin et al., 2002; Graham et al., 2000; Lai et al., 2002)
Hypoxanthine									1000	700	(Ritzel et al., 2001; Ward et al., 2000; Yao et al., 2002)
Uracil									1800	2600	(Ritzel et al., 2001; Ritzel et al., 1998; Ward et al., 2000; Yao et al., 1997; Yao et al., 2002)
Adenine									2500		(Gerstin et al., 2002; Lum et al., 2000; Yao et al., 2002)
Thymine									1300	1700	(Yao et al., 2002)
UMP											(Lum et al., 2000)
AMP											(Gerstin et al., 2002)
ADP											(Gerstin et al., 2002)
ATP											(Gerstin et al., 2002)
Traditional inhibitors											
NBMPR							*0.004*	*0.005*		*2.8*	(Che et al., 1995; Crawford et al., 1998b; Fang et al., 1996; Griffiths et al., 1997a; Ritzel et al., 2001; Ritzel et al., 1997; Yao et al., 1997)
Dilazep								*0.06*			(Fang et al., 1996; Griffiths et al., 1997a; Griffiths et al., 1997b; Ritzel et al., 2001; Ritzel et al., 1997; Yao et al., 1997)
Dipyridamole								*0.005*		*.36*	(Che et al., 1995; Ritzel et al., 2001; Vickers et al., 2001; Ward et al., 2000; Yao et al., 1997)
Draflazine								.016		2.4	(Hammond, 2000)

absorption of orally administered therapeutic purine analogs such as ribavirin (Patil et al., 1998; Pennycooke et al., 2001). Significant amounts of CNT2 mRNA transcript are detected in kidney, liver, skeletal muscle, lymph nodes and mammary gland (Pennycooke et al., 2001). This protein preferentially transports purine nucleosides—adenosine, guanosine, inosine—and purine nucleoside analogs over pyrimidine nucleosides although it also transports uridine. CNT2-dependent nucleoside transport appears to be mechanistically very similar to CNT1-mediated transport. The ratio of Na^+ to nucleoside transport via CNT2 is 1:1 (Li et al., 2001). In addition, there is some residual nucleoside transport in the absence of Na^+ suggesting that the protein may transport other ions (Gerstin et al., 2002).

There are some significant species differences in substrate specificity for nucleosides and nucleoside analogs between rat and human CNT2, which is important to note since many functional studies have been done with the rat clone (Gerstin et al., 2002). Both orthologs selectively transport purine nucleosides but rat CNT2 prefers adenosine to inosine whereas human CNT2 prefers inosine to adenosine. The human ortholog is also less tolerant of ribose modifications, whereas the rat ortholog transports 5′-AMP and several ribose modified analogs including didanosine (2′,3′-dideoxyinosine) and fludarabine (2-F-Ara-AMP). Analogs with base modifications such as cladribine (2-chloro-2′-deoxyadenosine) are also less tolerated by human SPNT. Dehydroxylation at the 3′ position is not tolerated.

2.3. CNT3

CNT3-type transport characteristics were originally described in rabbit choroid plexus in this laboratory (Wu et al., 1992). hCNT3 and mCNT3 were identified in the EST database and simultaneously cloned from human mammary gland, human myeloid HL-60 cells, and mouse liver (Ritzel et al., 2001). CNT3 encodes a 691 amino acid protein (77 kDa) containing four N-linked glycosylation sites, all within the C-terminal tail. The amino acid sequence of CNT3 is 48% identical to CNT1 and 47% identical to CNT2. These three proteins are predicted to have very similar secondary structures. Mechanistically, CNT3 differs from CNT1 and CNT2

Table 3 (*continued*)

Substrate	CNT						ENT				References
	1		2		3		1		2		
	R	H	R	H	M	H	R	H	R	H	
Antineoplastic agents and metabolites											
Cytarabine	*1880*							1500		1200	(Che et al., 1995; Crawford et al., 1998a; Graham et al., 2000; Hyde et al., 2001; Vickers et al., 2002)
Gemcitabine		24						160		740	(Lostao et al., 2000; Mackey et al., 1999; Osato et al., 2003; Ritzel et al., 2001)
Cladribine			*13*	*371*				71			(Crawford et al., 1998a; Gerstin et al., 2002; Hyde et al., 2001; Ritzel et al., 2001)
Fluorouridine								50		220	(Crawford et al., 1998a; Hyde et al., 2001; Ritzel et al., 2001; Vickers et al., 2002)
5-Flurouracil											(Mackey et al., 2002; Mata et al., 2001)
Capecitabine											(Mata et al., 2001)
5dFU								18		340	(Dresser et al., 2000; Mackey et al., 1999; Mata et al., 2001; Vickers et al., 2002; Ward et al., 2000)
Fludarabine											(Hyde et al., 2001; Lostao et al., 2000; Ritzel et al., 2001)
Vidarabine											(Gerstin et al., 2002; Lum et al., 2000)
Trox											(Gourdeau et al., 2001)
Zebularine											(Ritzel et al., 2001)
Antiviral agents											
Zidovudine	500										(Huang et al., 1994; Mackey et al., 1998; Mata et al., 2001; Ward et al., 2000; Yao et al., 2001)
Zalcitabine	500							23000			(Huang et al., 1994; Ritzel et al., 2001; Ritzel et al., 1997; Ritzel et al., 1998; Schaner et al., 1997; Yao et al., 2001)
Didanosine			*46*	*19*				7400		2300	(Huang et al., 1994; Li et al., 2001; Ritzel et al., 2001; Ritzel et al., 1998; Yao et al., 2001)
Floxidine	*50*							50		320	(Crawford et al., 1998a; Gerstin et al., 2002; Ritzel et al., 2001; Vickers et al., 2002)
Lamivudine											(Graham et al., 2000; Huang et al., 1994; Lum et al., 2000)
Ribavirin								*1150*			(Dresser et al., 2000; Osato et al., 2003; Patil et al., 1998)
Acyclovir											(Lum et al., 2000; Schaner et al., 1997)
Gancyclovir											(Ritzel et al., 2001)
Stavudine											(Lum et al., 2000)

Yellow, known substrate; Red, known inhibitor; Blue, neither; White, unknown. Substrate specificity of mCNT3 (M) is given because no functional rat CNT3 ortholog has been reported to date. Values (in μM) are K_m if written in black, IC_{50} values if written in black and italicized, and K_i if written in blue, and EC_{50} if written in green. dA, deoxyadenosine; NBMPR, nitrobenxylthioinosine; 5dFU, 5′ -deoxy 5′ -fluorouridine; Trox, Troxacitabine (Che et al., 1995; Crawford et al., 1998a; Fang et al., 1996; Gourdeau et al., 2001; Graham et al., 2000; Hammond, 2000; Mackey et al., 1998; Schaner et al., 1997; Vickers et al., 2001; Wang et al., 1997a; Yao et al., 2001).

in two major ways. First, the Na^+/nucleoside coupling ratio for CNT3 is 2:1, as opposed to 1:1 for the other CNTs. In a physiological situation, this means that CNT3 concentrates nucleosides intracellularly 10 times more CNT1 or CNT2. Thus, CNT3-mediated transport of nucleosides or nucleoside analogs may be important even in tissues such as the kidney where lower levels of this transporter are expressed. Secondly, CNT3 is broadly selective for nucleoside substrates, transporting both purine and pyrimidine nucleosides. Therefore, it interacts with a much broader range of therapeutic nucleoside analogs and is expected to play an important role in absorption, distribution, and elimination of these drugs.

2.4. Amino acid determinants of specificity of concentrative nucleoside transporters

The molecular determinants of substrate specificity and translocation for all three cloned CNTs appear to be related. Removal of the N-terminus of CNT3, containing the intracellular tail and first three transmembrane domains, results in a truncated protein that retains function, indicating that translocation is independent of this portion of the protein (Hamilton et al., 2001). Examination of the conserved amino acid differences between these proteins in the remainder of the protein sequence has provided insights into regions responsible for substrate selectivity. This was originally examined in this laboratory with a series of chimeric proteins of the rat orthologs of CNT1 and CNT2 (Wang and Giacomini, 1997, 1999a,b). Using CNT1 as the template, the amino acids in transmembrane domain 7 were replaced with those from this region of CNT2, resulting in a broadly selective nucleoside transporter. Further studies determined that substitution of a single pair of conserved residues in CNT1 (S318G/Q319M) created a protein that tolerated both purine and pyrimidine nucleosides, implicating these residues in substrate binding and possibly in the translocation pathway of these proteins. Similar studies using the human orthologs showed that alteration of these two residues in

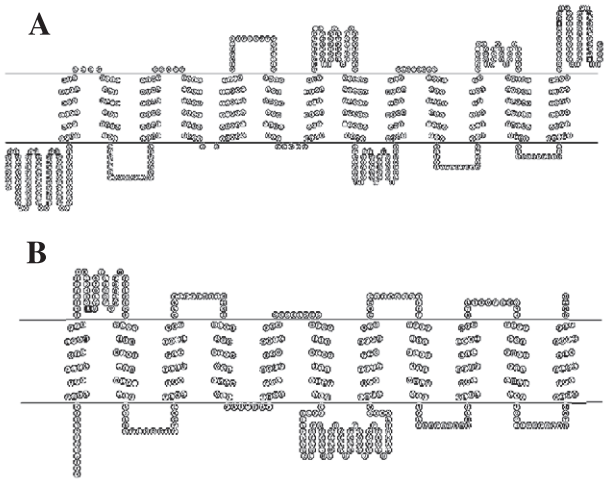

Fig. 2. Secondary structure of (A) CNT1 and (B) ENT1. All members of the CNT family are predicted to have 13 transmembrane spanning domains with a cytosolic N-terminal tail and an extracellular C-terminal tail. All N-linked glycosylation sites in each CNT are located within this C-terminal tail. All members of the ENT family are predicted to contain 11 transmembrane domains with a very short cytoplasmic N-terminal tail and extracellular C-terminal tail. ENTs are predicted to have two large loops—an extracellular loop between transmembrane domains 1–2 that is glycosylated and a cytosolic loop between transmembrane domains 6–7.

CNT1 confers the ability to transport purines to an otherwise pyrimidine-selective transporter (Loewen et al., 1999). In addition, mutations of conserved differences in a nearby region (S353T and L354V) selectively reduce pyrimidine transport, indicating that purine-selection and pyrimidine-selection may occur at different positions within these proteins. Examination of conserved residues at these positions in the broadly selective human CNT3 and its orthologs supports the importance of these four residues and the surrounding region in determination of substrate selectivity (Ritzel et al., 2001). Both pairs of residues in CNT3 contains one residue conserved with CNT1 and one residue conserved with CNT2 (G318/Q319 and S353/V354).

3. Equilibrative nucleoside transporters

Three members of the mammalian equilibrative nucleoside transporter (ENT, SLC29) family have been cloned to date: ENT1, ENT2, and ENT3 (Griffiths et al., 1997a,b; Hyde et al., 2001). ENT1 exhibits equilibrative nucleoside transport that is sensitive to inhibition by nanomolar concentrations of nitrobenzylthioinosine (NBMPR, termed *es*-type activity). Nucleoside transport by ENT2 is insensitive to inhibition by similar concentrations of NBMPR (termed *ei*-type activity). Both ENT1 and ENT2 are transcribed in the kidney but their respective protein levels have not been examined (Pennycooke et al., 2001). ENT3 sequence (accession number AF326987) has recently been identified within the GenBank database by its homology to members of the ENT family. While examination of the EST database indicates that ENT3 is expressed in kidney, this protein does not localize to the plasma membrane in renal epithelial cells (unpublished observation). Functional characterization of ENT3 has not been possible to date (Hyde et al., 2001). Similar database searches have identified a fourth family member, ENT4, in mouse and human (accession numbers NM146257 and NM153247). ENT4 has not yet been cloned, but does not appear to be transcribed in the kidney. For this reason, discussion of ENTs in this review will focus on ENT1 and ENT2.

3.1. ENT1

ENT1 was first cloned from human placenta in 1997 (Crawford et al., 1998b; Griffiths et al., 1997a). Topology studies and sequence analysis indicate that this protein has 11 transmembrane domains, with a short internal N-terminal tail, a short external C-terminal tail, and two large loops: an extracellular, glycoslyated loop between the first two transmembrane domains and a cytoplasmic loop between transmembrane domains 6 and 7 (Fig. 2) (Sundaram et al., 2001a). ENT1 is broadly selective, transporting both pyrimidine and purine nucleosides and their analogs although with lower affinity than the CNTs. ENT1 is impermeable to nucleobases or nucleotide monophosphates (Lum et al., 2000; Yao et al., 2002). Pharmacological analysis indicates that ENT1 substrate binding is not affected by base modifications but is sensitive to dehydroxylation at the 2′, 3′ or 5′ positions of the ribose ring (Table 3) (Lum et al., 2000). Transport is inhibited by nanomolar concentrations of NBMPR (IC_{50} is 3.7 nM for uridine inhibition) though NBMPR is not itself a substrate (Griffiths et al., 1997a). In addition, transport is competitively inhibited by several coronary vasodilators: dilazep, draflazine, and dipyridamole. These compounds are structurally distinct from nucleosides but appear to bind to the same region of the transporter (Sundaram et al., 1998).

A number of studies have focused on mapping the substrate-binding region of ENT1. Mutagenesis studies indicate that transmembrane domains 3–6 of ENT1 are responsible for interacting with both the vasodilators and NBMPR, suggesting that this region of the transporter is also responsible for substrate binding (Sundaram et al., 1998, 2001b). In addition, deglycosylation at position 48 and mutagenesis of residue 33, both within the first extracellular loop, decrease affinity of ENT1 for NBMPR (Vickers et al., 1999, 2002). Further mutagenesis studies demonstrated that the conserved glycine at residue 179 in transmembrane domain 5 is important for both NBMPR binding and nucleoside transport, linking the two functions to the same region of the transporter (SenGupta et al., 2002).

Transcripts of ENT1 are expressed within the kidney and are expected to be responsible for the *es*-type transport

seen in membrane vesicle preparations from whole kidney tissue (Pennycooke et al., 2001). Recent studies have shown that ENT1 transport can be regulated by several signaling pathways present in renal epithelium including PKC and purinoceptor activity, indicating that renal nucleoside transport may be a dynamic process (Coe et al., 2002; Parodi et al., 2002).

3.2. ENT2

A transcript encoding a 456-residue protein (~ 50 kDa), termed ENT2, was simultaneously cloned in two separate laboratories from human placenta and the HeLa cell line (Crawford et al., 1998b; Griffiths et al., 1997b). ENT2 protein has a lower affinity than ENT1 for all nucleosides with the exception of inosine (Ward et al., 2000). ENT2 is a poor transporter of both guanosine and cytidine. ENT2 transport is only partially inhibited by NBMPR at micromolar concentrations (Yao et al., 1997). Recent studies indicate that ENT2 binds and translocates nucleobases via the same pathway as nucleosides (Yao et al., 2002). The region of the protein responsible for nucleobase transport appears to lie in transmembrane domains 5 and 6.

mRNA expression profiling indicates that ENT2 is less abundant than ENT1 in the kidney (Pennycooke et al., 2001). Thus, ENT2-mediated renal transport of nucleosides is likely to take a secondary role to ENT1-mediated transport, which has a higher expression level and a higher affinity for most nucleosides. However, ENT2 has a high affinity for inosine and hypoxanthine, two primary metabolites of adenosine. This may be important for termination of actions generated by adenosine-mediated pathways within the kidney.

4. Nucleoside transporter-mediated renal disposition of nucleoside analogs

Nucleoside transporters in the kidney have been identified primarily through reverse transcriptase-polymerase chain reaction (RT-PCR) and Northern blotting. mRNA transcripts of all five characterized transporters are present in human kidney. Transcripts of CNT2 and CNT3 appear to be present at significantly lower levels than those of CNT1 (Pennycooke et al., 2001; Ritzel et al., 2001). ENT2 transcripts are present at lower levels than ENT1. While CNT1 and CNT2 proteins have been identified in renal epithelium using protein-specific antibodies, protein levels have not been quantified to date (Felipe et al., 1998; Hamilton et al., 2001). CNT1 is likely the most abundantly expressed CNT in the kidney consistent with functional assays in human renal vesicles that identified a single pyrimidine-preferring concentrative transport system (Gutierrez and Giacomini, 1993; Gutierrez et al., 1992). The physiological relevance of high levels of pyrimidine-specific nucleoside transport in the kidney remains unclear.

To determine the role of nucleoside transporters in the renal disposition of nucleosides, we investigated the subcellular localization of these proteins. Transporters were tagged with green fluorescent protein (GFP) and stably expressed in two renal epithelial cell lines: MDCK and LLC-PK$_1$. Using this system, subcellular localization was determined directly via immunofluorescence and functionally via membrane-specific nucleoside transport (Fig. 3) (Mangravite et al., 2001, 2003). Studies examining subcellular localization of CNT1 and ENT1 have also been carried out by Lai et al. (2002) with similar results.

Our data indicate that the concentrative nucleoside transporters, CNT1, CNT2, and CNT3, localized predominantly to the apical membrane of MDCK where they control movement of nucleosides from tubular filtrate into epithelium (Fig. 4). While CNT1 and CNT3 were entirely confined to the apical membrane, CNT2 had significant presence on the basolateral membrane as well. In contrast, the equilibrative nucleoside transporters, ENT1 and ENT2, localized predominantly to the basolateral membrane where they likely control movement of nucleosides between the epithelial cytosol and the interstitium (Fig. 4). ENT2 was confined entirely to the basolateral membrane, whereas ENT1 was predominantly on the basolateral membrane with a small additional presence on the apical membrane.

Localization of CNTs to the apical membrane and ENTs to the basolateral membrane of renal epithelial cells implicates these transporters in active reabsorption of nucleosides and nucleoside analogs from the tubular filtrate. Nucleosides present in the tubular filtrate are actively removed in two steps. Nucleosides and nucleoside analogs within the filtrate come in contact with CNTs and are actively transported into the cytosol of the surrounding epithelium. As these nucleosides are concentrated within the cells, they are driven across the basolateral membrane via ENT1 and ENT2. This model of nucleoside transporters within the kidney explains the reabsorptive processes noted clinically for adenosine and other nucleosides but is in contrast to the secretory processes described for many clinically administered nucleoside analogs. Secretion may be explained by several factors. First, many of these analogs are not well tolerated by nucleoside transporters. Thus, they may not be effectively reabsorbed. Second, the renal epithelia is also home to a wide range of xenobiotic transporters and efflux pumps that mediate the efflux of compounds into tubular filtrate. Interactions with these pumps, which are broadly selective for substrates, would influence the overall renal disposition of nucleoside analogs. In fact, there is growing evidence that nucleoside analogs and their metabolites are substrates for some of the xenobiotic transporters found in the kidney. These active secretory processes are likely to play a major role in renal disposition of therapeutic

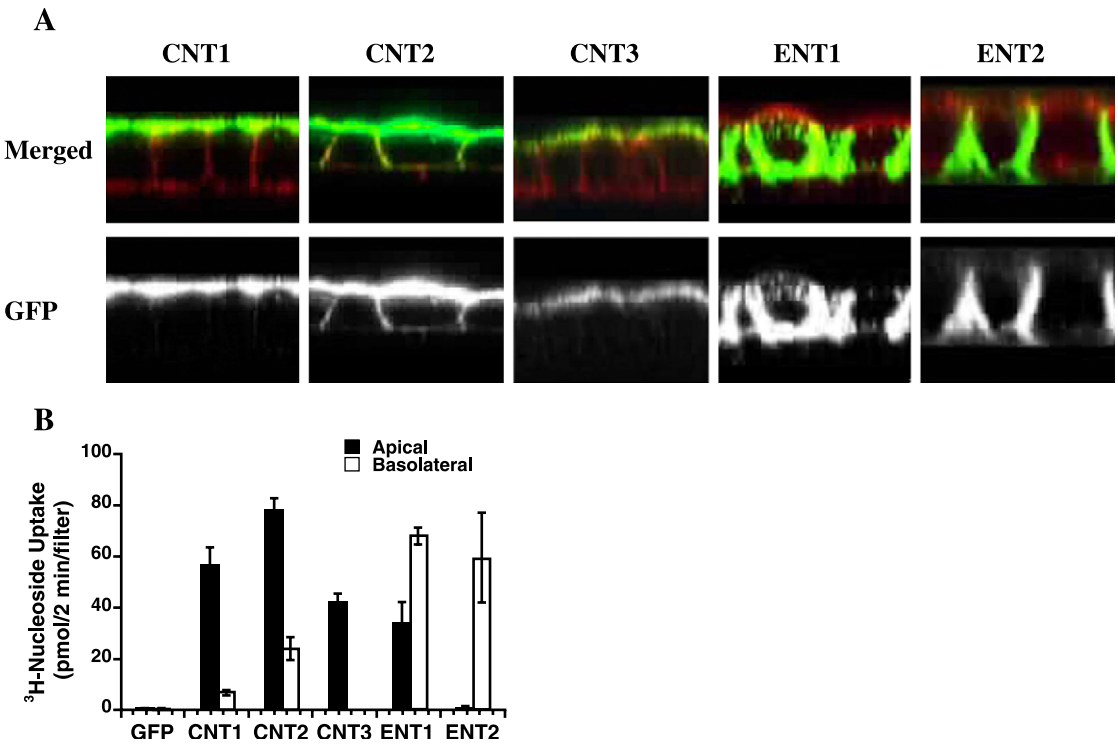

Fig. 3. Localization of nucleoside transporters in MDCK, a renal epithelial cell line (Mangravite et al., 2001, 2003). Cells were transfected with GFP-tagged CNT1, CNT2, CNT3, ENT1, or ENT2, and polarized by growth on permeable support. Localization was assay by (A) immunofluorescence or (B) functional transport. (A) Cells were fixed, stained for actin with Texas Red-conjugated phalloidin and visualized by confocal microscopy. Red, actin; Green, GFP. Top row shows both stains. Bottom row shows just GFP. Bar, 10 μm. (B) Cells were exposed to 10.1 μM nucleoside (0.1 μM ^{3}H-inosine or ^{3}H-thymidine and 10 μM nucleoside) in physiologic buffer for two min at either the apical or basolateral membrane. Thymidine was used to assess the function of CNT1 and inosine was used to assess the function of CNT2, CNT3, ENT1 and ENT2.

nucleoside analogs. It is likely that ENT1 on the basolateral membrane may serve in concert with apical xenobiotic pumps to mediate secretion of therapeutic nucleoside analogs.

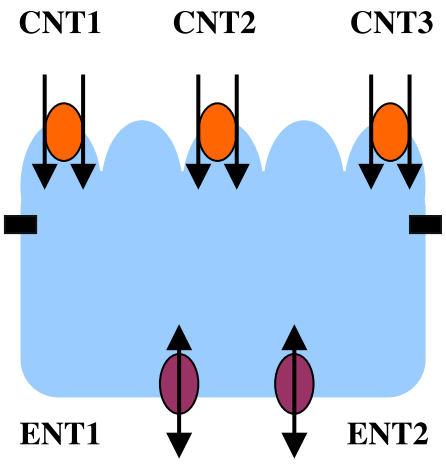

Fig. 4. Model of nucleoside transporters in renal epithelia. Primary localization of transporters are displayed visually in a representative renal epithelial cell. Rectangles represent tight junctions, separating the apical (top) from basolateral (bottom) membranes. Dual arrows on the concentrative transporters indicate cotransport of substrate and sodium.

5. Nucleoside transporters in renal cancer chemotherapy

In addition to their significance in the renal disposition of systemic therapies, nucleoside transporters could have a major impact in chemotherapeutic treatment of renal neoplasms. Ninety percent of renal cancers occur within the cortex and originate predominantly within the proximal tubule, which is the site of localization of reabsorptive transporters. Immunohistochemistry studies of CNT1 in rat kidney sections indicate that this is the precise location of CNT1 and, presumably other concentrative nucleoside transporters, within the kidney (Hamilton et al., 2001).

Systemic chemotherapy is not indicated for renal cortical tumors as they often present with multi-drug resistance phenotypes, possibly due to the presence of multiple efflux pumps within this tissue. First line therapy is surgical removal: partial, simple or radical nephrectomy (Godley and Taylor, 2001). However, survival rates are low, in part because diagnosis often does not occur early enough to prevent metastasis. For this reason, all patients are encouraged to enroll in clinical trials. There are approximately 80 clinical trials currently ongoing in the United States examining novel therapies for renal cancer (http://www.nci.nih.gov/clinicaltrials). The usefulness of several nucleoside analogs known to be effective in solid tumor therapy is being examined. Gemcitabine, capecitabine (a pro-drug of

5-fluorouracil), 5-fluorouracil, and fludarabine administration alone or in combination with other therapies are all currently in clinical trials. With all five known and functionally characterized nucleoside transporters expressed within the proximal tubule, it is reasonable to think that nucleoside analogs may provide an effective line of defense in renal cancer.

Based on the proposed renal model of nucleoside transporter-mediated nucleoside movement discussed above, an antineoplastic analog selectively transported by CNTs could easily gain and retain access to renal cortical tumors. For example, gemcitabine is preferentially transported by CNT1 (K_m is 24 μM) over ENT1 (K_m is 160 μM) or ENT2 (K_m is 740 μM) (Mackey et al., 1999). There are no known interactions between gemcitabine and any efflux pumps or xenobiotic transporters, although the active species, gemcitabine triphosphate (Gem-TP), may undergo active efflux. In addition, relative expression levels of nucleoside transporters in renal carcinomas may be a very important factor in the chemotherapeutic efficacy of these nucleoside analogs.

Expression of nucleoside transporters appears to be a dynamic event. Transcription and translation of nucleoside transporters are regulated by various cellular and systemic processes. Normal cellular demand for endogenous nucleosides varies with cell cycle. As such, expression of CNT1 has been shown to be cell cycle regulated (Valdes et al., 2002). ENT1 is regulated by PKC, an internal signal present in renal cells (Coe et al., 2002). Within macrophages, proliferation and activation alter both CNT and ENT expression (Soler et al., 2001). It is not surprising, then, to discover that nucleoside transporters are alternatively expressed in cancerous cells. Studies of mRNA transcript expression in individual biopsies from kidney cancer tumors indicate two important points (Pennycooke et al., 2001). First, tumor cells have lower levels of mRNA transcripts of concentrative nucleoside transporters than do normal tissue. CNT1 expression is very low or negligible in most biopsy samples. CNT2 expression levels decrease minimally. Secondly, there is enormous interpatient variability in transporter expression levels. ENT1 transcript levels varied widely in normal kidney tissue with a 64-fold difference in expression observed across 15 samples. In addition, the difference in ENT1 transcript levels between matched kidney and renal tumor samples varied widely among subjects.

Little work has been done to determine the relationship between nucleoside transporter expression and nucleoside analog cytotoxicity in renal carcinomas but there has been some significant work of late looking at resistant phenotypes in leukemias. Transport of nucleoside analogs represents the first step in tumor cytotoxicity (Fig. 5). The internalized analog is then phosphorylated by a series of nucleoside kinases to the active triphosphorylated metabolite. Several studies have attempted to correlate cytotoxicity of nucleoside analogs with mRNA expression of ENT1, the predominant NT expressed in blood cells, in neoplastic cells. Cytarabine and fludarabine chemotoxicity

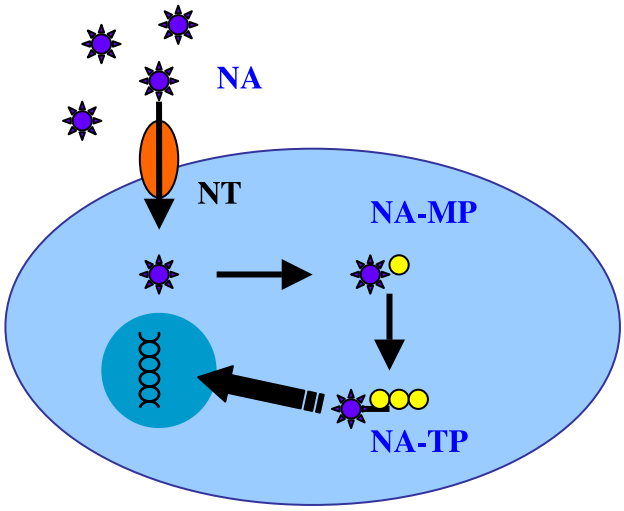

Fig. 5. Simplified diagram of mechanisms of activation of antineoplastic nucleoside analogs. Nucleoside analogs (NA) gain entry into cells via nucleoside transporters and are activated by a series of three consecutive phosphorylation steps to active triphosphorylated forms (NA-TP). NA-TPs can act by causing DNA chain termination, inhibiting ribonucleotide reductase, incorporating into RNA, and inducing apoptosis.

correlated to increased ENT1 expression in several studies (Gati et al., 1998; Molina-Arcas et al., 2003; Stam et al., 2003; Wright et al., 2002). As nucleoside analogs are used more and more for treatment of solid tumors such as renal carcinoma, which express multiple nucleoside transporters, these studies will need to be extended to the other nucleoside transporters.

If relative expression of nucleoside transporters does correlate to therapeutic efficacy of antineoplastic nucleoside analogs, then a diagnostic test for the presence of these transporters may prove helpful. An immunohistochemical technique for quantification of ENT1 protein levels in tumors is currently being developed for primary breast cancer and lymphomas (Mackey et al., 2002; Reiman et al., 2002). This strategy could prove especially useful in renal cancers, as first line therapy in all cases is nephrectomy. Removed tissue could be assayed for markers involved in resistant phenotypes and a more rational chemotherapeutic regime may ensue.

6. Future studies

The work outlined in this review has provided a basis for understanding the role of nucleoside transporters within the kidney. Nucleoside transporter distribution in renal epithelium clearly implicates these proteins in the reabsorptive flux of naturally occurring nucleosides and nucleoside analogs, a process that affects systemic exposure to these compounds. It also implicates these transporters in the targeting of nucleoside analog therapies within renal cancers. Efficacy of antineoplastic treatment with nucleoside analog therapies is highly variable. Much of the current

work in the field is aimed at understanding the mechanisms for this variability. Inherent nucleoside analog-specific resistance is often indicative of altered expression of nucleoside transporters. Understanding the mechanisms that govern transporter expression is essential to map the pathways by which tumor cells alter transporter expression.

In addition, there is inherent interpatient variability in systemic response to clinical nucleoside analog therapies. Single nucleotide polymorphisms within the genes that encode nucleoside transporters may affect transporter function and expression. In this laboratory, we have screened an ethnically diverse population for genetic variation in the exons and flanking intronic regions of CNT1, CNT2, ENT1, and ENT2 (Leabman et al., 2003). Both nonsynonymous variants, which alter the amino acid sequence of the transporters, and synonymous variants, which do not alter the amino acid sequence, were found in all four cases. We have identified 13 nonsynonymous variants in the coding region of CNT1, the primary CNT expressed in the kidney. Phenotypic characterization of these variants is underway. Only two nonsynonymous variants were identified in the coding region of ENT1, the primary ENT in the kidney (Leabman et al., 2003). Heterologous expression of these variants in yeast and kinetic analysis indicated that both variants were functionally identical to the reference ENT1 (Osato et al., 2003). These studies represent a starting point in the analysis of genetic influence on variation in transporter function. Variation in splice sites and promoter regions may also influence transporter expression or function. Understanding these mechanisms could lead to simple clinical diagnostic tests that would predict patient response to nucleoside analog therapies and improve therapeutic response.

In addition, there is a need to understand how nucleoside transporters may affect the basic physiological processes of the kidney. Renal processes are closely monitored and rapidly altered to maintain proper systemic levels of fluid and electrolytes via a series of signaling pathways, including adenosine signaling. Adenosine signaling is essential in modifying key kidney functions including glomerular filtration rate, solute excretion, and renin release (Spielman and Arend, 1991). Extracellular adenosine acts on adenosine receptors (A_1, A_{2A}, A_{2B} and A_3) present throughout the kidney including the epithelium. Nucleoside transporters may play a role in terminating adenosine-mediated signal by removing adenosine from the external site of action. Very little is known about the significance of coexpression of adenosine receptors and nucleoside transporters within renal epithelia. These proteins may be regulated in a synergistic fashion to control adenosine-signaling pathways.

Nucleoside transporters within the kidney govern systemic distribution of naturally occurring nucleosides and therapeutic nucleoside analogs. Knowledge of the substrate specificity of each of these transporters and their subcellular localization within renal epithelial cells has greatly enhanced our understanding of the role these proteins play within the kidney. Examination of protein expression levels and spatial localization throughout the nephron will aid in developing this model of systemic nucleoside distribution and in understanding the relevance of nucleoside transporters to renal cancer therapy and basic physiologic functions within the kidney.

Acknowledgements

We would like to thank Jennifer Gray and Ryan Owen for comments on this manuscript. This work was supported by National Institute of Health (NIH) grants GM-61390, GM-42230 and GM-36780. I.B. was supported in part by a National Science Foundation Graduate Research Fellowship.

References

Aapro, M.S., Martin, C., Hatty, S., 1998. Gemcitabine—a safety review. Anticancer Drugs 9, 191–201.

Abbruzzese, J.L., Grunewald, R., Weeks, E.A., Gravel, D., Adams, T., Nowak, B., Mineishi, S., Tarassoff, P., Satterlee, W., Raber, M.N., Plunkett, W., 1991. A phase I clinical, plasma, and cellular pharmacology study of gemcitabine. J. Clin. Oncol. 9, 491–498.

Che, M., Ortiz, D.F., Arias, I.M., 1995. Primary structure and functional expression of a cDNA encoding the bile canalicular, purine-specific Na^+-nucleoside cotransporter. J. Biol. Chem. 270, 13596–13599.

Coe, I., Zhang, Y., McKenzie, T., Naydenova, Z., 2002. PKC regulation of the human equilibrative nucleoside transporter, hENT1. FEBS Lett. 517, 201–205.

Crawford, C.R., Cass, C.E., Young, J.D., Belt, J.A., 1998a. Stable expression of a recombinant sodium-dependent, pyrimidine-selective nucleoside transporter (CNT1) in a transport-deficient mouse leukemia cell line. Biochem. Cell. Biol. 76, 843–851.

Crawford, C.R., Patel, D.H., Naeve, C., Belt, J.A., 1998b. Cloning of the human equilibrative, nitrobenzylmercaptopurine riboside (NBMPR)-insensitive nucleoside transporter ei by functional expression in a transport-deficient cell line. J. Biol. Chem. 273, 5288–5293.

Crowther, M.A., Callaghan, W., Hodsman, A.B., Mackie, I.C., 1993. Dideoxyinosine-associated nephrotoxicity. Aids 7, 131–132.

de Miranda, P., Good, S.S., Yarchoan, R., Thomas, R.V., Blum, M.R., Myers, C.E., Broder, S., 1989. Alteration of zidovudine pharmacokinetics by probenecid in patients with AIDS or AIDS-related complex. Clin. Pharmacol. Ther. 46, 494–500.

Dresser, M.J., Gerstin, K.M., Gray, A.T., Loo, D.D., Giacomini, K.M., 2000. Electrophysiological analysis of the substrate selectivity of a sodium-coupled nucleoside transporter (rCNT1) expressed in *Xenopus laevis* oocytes. Drug Metab. Dispos. 28, 1135–1140.

Fang, X., Parkinson, F.E., Mowles, D.A., Young, J.D., Cass, C.E., 1996. Functional characterization of a recombinant sodium-dependent nucleoside transporter with selectivity for pyrimidine nucleosides (cNT1rat) by transient expression in cultured mammalian cells. Biochem. J. 317 (Pt. 2), 457–465.

Felipe, A., Valdes, R., Santo, B., Lloberas, J., Casado, J., Pastor-Anglada, M., 1998. Na^+-dependent nucleoside transport in liver: two different isoforms from the same gene family are expressed in liver cells. Biochem. J. 330, 997–1001.

Fontana, R.J., 2001. Uric acid nephrolithiasis associated with interferon and ribavirin treatment of hepatitis C. Dig. Dis. Sci. 46, 920–924.

Gati, W.P., Paterson, A.R., Belch, A.R., Chlumecky, V., Larratt, L.M., Mant, M.J., Turner, A.R., 1998. Es nucleoside transporter content of acute leukemia cells: role in cell sensitivity to cytarabine (araC). Leuk. Lymphoma 32, 45–54.

Gerstin, K.M., Dresser, M.J., Giacomini, K.M., 2002. Specificity of human and rat orthologs of the concentrative nucleoside transporter, SPNT. Am. J. Physiol., Renal. Physiol. 283, F344–F349.

Giacomini, K.M., Markovich, D., Werner, A., Biber, J., Wu, X., Murer, H., 1994. Expression of a renal Na^+ nucleoside cotransport system (N2) in *Xenopus laevis* oocytes. Pflugers Arch. 427, 381–383.

Godley, P.A., Taylor, M., 2001. Renal cell carcinoma. Curr. Opin. Oncol. 13, 199–203.

Gourdeau, H., Clarke, M.L., Ouellet, F., Mowles, D., Selner, M., Richard, A., Lee, N., Mackey, J.R., Young, J.D., Jolivet, J., Lafreniere, R.G., Cass, C.E., 2001. Mechanisms of uptake and resistance to troxacitabine, a novel deoxycytidine nucleoside analogue, in human leukemic and solid tumor cell lines. Cancer Res. 61, 7217–7224.

Graham, K.A., Leithoff, J., Coe, I.R., Mowles, D., Mackey, J.R., Young, J.D., Cass, C.E., 2000. Differential transport of cytosine-containing nucleosides by recombinant human concentrative nucleoside transporter protein hCNT1. Nucleosides Nucleotides Nucleic Acids 19, 415–434.

Griffiths, M., Beaumont, N., Yao, S.Y., Sundaram, M., Boumah, C.E., Davies, A., Kwong, F.Y., Coe, I., Cass, C.E., Young, J.D., Baldwin, S.A., 1997a. Cloning of a human nucleoside transporter implicated in the cellular uptake of adenosine and chemotherapeutic drugs. Nat. Med. 3, 89–93.

Griffiths, M., Yao, S.Y., Abidi, F., Phillips, S.E., Cass, C.E., Young, J.D., Baldwin, S.A., 1997b. Molecular cloning and characterization of a nitrobenzylthioinosine-insensitive (ei) equilibrative nucleoside transporter from human placenta. Biochem. J. 328, 739–743.

Gutierrez, M.M., Giacomini, K.M., 1993. Substrate selectivity, potential sensitivity and stoichiometry of Na^+ nucleoside transport in brush border membrane vesicles from human kidney. Biochim. Biophys. Acta 1149, 202–208.

Gutierrez, M.M., Giacomini, K.M., 1994. Expression of a human renal sodium nucleoside cotransporter in *Xenopus laevis* oocytes. Biochem. Pharmacol. 48, 2251–2253.

Gutierrez, M.M., Brett, C.M., Ott, R.J., Hui, A.C., Giacomini, K.M., 1992. Nucleoside transport in brush border membrane vesicles from human kidney. Biochim. Biophys. Acta 1105, 1–9.

Hamilton, S.R., Yao, S.Y., Ingram, J.C., Hadden, D.A., Ritzel, M.W., Gallagher, M.P., Henderson, P.J., Cass, C.E., Young, J.D., Baldwin, S.A., 2001. Subcellular distribution and membrane topology of the mammalian concentrative Na^+ nucleoside cotransporter rCNT1. J. Biol. Chem. 276, 27981–27988.

Hammond, J.R., 2000. Interaction of a series of draflazine analogues with equilibrative nucleoside transporters: species differences and transporter subtype selectivity. Naunyn-Schmiedeberg's Arch. Pharmacol. 361, 373–382.

Hartman, N.R., Yarchoan, R., Pluda, J.M., Thomas, R.V., Marczyk, K.S., Broder, S., Johns, D.G., 1990. Pharmacokinetics of 2′,3′-dideoxyadenosine and 2′,3′-dideoxyinosine in patients with severe human immunodeficiency virus infection. Clin. Pharmacol. Ther. 47, 647–654.

Huang, Q.Q., Yao, S.Y., Ritzel, M.W., Paterson, A.R., Cass, C.E., Young, J.D., 1994. Cloning and functional expression of a complementary DNA encoding a mammalian nucleoside transport protein. J. Biol. Chem. 269, 17757–17760.

Hyde, R.J., Cass, C.E., Young, J.D., Baldwin, S.A., 2001. The ENT family of eukaryote nucleoside and nucleobase transporters: recent advances in the investigation of structure/function relationships and the identification of novel isoforms. Mol. Membr. Biol. 18, 53–63.

Knupp, C.A., Shyu, W.C., Dolin, R., Valentine, F.T., McLaren, C., Martin, R.R., Pittman, K.A., Barbhaiya, R.H., 1991. Pharmacokinetics of didanosine in patients with acquired immunodeficiency syndrome or acquired immunodeficiency syndrome-related complex. Clin. Pharmacol. Ther. 49, 523–535.

Kuttesch Jr., J.F., Nelson, J.A., 1982. Renal handling of 2′-deoxyadenosine and adenosine in humans and mice. Cancer Chemother. Pharmacol. 8, 221–229.

Lai, Y., Bakken, A.D., Unadkat, J.D., 2002. Simultaneous expression of hCNT1-CFP and hENT1-YFP in Mardin-Darby canine kidney (MDCK) cells: localization and vectorial transport studies. J. Biol. Chem. 277 (40), 37711–37717.

Leabman, M.K., Huang, C.C., DeYoung, J., Carlson, E.J., Taylor, T.R., De La Cruz, M., Johns, S.J., Stryke, D., Kawamoto, M., Urban, T.J., Kroetz, D.L., Ferrin, T.E., Clark, A.G., Risch, N., Herskowitz, I., Giacomini, K.M., Investigators of Pharmacogenetics of Membrane Transporters, 2003. Natural variation in human membrane transporter genes reveals evolutionary and functional constraints. Proc. Natl. Acad. Sci. U. S. A. 100, 5896–5901.

Lee, C.W., Cheeseman, C.I., Jarvis, S.M., 1990. Transport characteristics of renal brush border Na^+ and K^+ dependent uridine carriers. Am. J. Physiol. 258, F1203–F1210.

Le Hir, M., Dubach, U.C., 1984. Sodium gradient-energized concentrative transport of adenosine in renal brush border vesicles. Pflugers Arch. 401, 58–63.

Li, J.Y., Boado, R.J., Pardridge, W.M., 2001. Differential kinetics of transport of 2′,3′-dideoxyinosine and adenosine via concentrative Na^+ nucleoside transporter CNT2 cloned from rat blood–brain barrier. J. Pharmacol. Exp. Ther. 299, 735–740.

Loewen, S.K., Ng, A.M., Yao, S.Y., Cass, C.E., Baldwin, S.A., Young, J.D., 1999. Identification of amino acid residues responsible for the pyrimidine and purine nucleoside specificities of human concentrative Na^+ nucleoside cotransporters hCNT1 and hCNT2. J. Biol. Chem. 274, 24475–24484.

Lostao, M.P., Mata, J.F., Larrayoz, I.M., Inzillo, S.M., Casado, F.J., Pastor-Anglada, M., 2000. Electrogenic uptake of nucleosides and nucleoside-derived drugs by the human nucleoside transporter 1 (hCNT1) expressed in *Xenopus laevis* oocytes. FEBS Lett. 481, 137–140.

Lum, P.Y., Ngo, L.Y., Bakken, A.H., Unadkat, J.D., 2000. Human intestinal es nucleoside transporter: molecular characterization and nucleoside inhibitory profiles. Cancer Chemother. Pharmacol. 45, 273–278.

Mackey, J.R., Mani, R.S., Selner, M., Mowles, D., Young, J.D., Belt, J.A., Crawford, C.R., Cass, C.E., 1998. Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. Cancer Res. 58, 4349–4357.

Mackey, J.R., Yao, S.Y., Smith, K.M., Karpinski, E., Baldwin, S.A., Cass, C.E., Young, J.D., 1999. Gemcitabine transport in xenopus oocytes expressing recombinant plasma membrane mammalian nucleoside transporters. J. Natl. Cancer Inst. 91, 1876–1881.

Mackey, J.R., Jennings, L.L., Clarke, M.L., Santos, C.L., Dabbagh, L., Vsianska, M., Koski, S.L., Coupland, R.W., Baldwin, S.A., Young, J.D., Cass, C.E., 2002. Immunohistochemical variation of human equilibrative nucleoside transporter 1 protein in primary breast cancers. Clin. Cancer Res. 8, 100–106.

Mangravite, L.M., Lipschutz, J.H., Mostov, K.E., Giacomini, K.M., 2001. Localization of GFP-tagged concentrative nucleoside transporters in a renal polarized epithelial cell line. Am. J. Physiol., Renal Physiol. 280, F879–F885.

Mangravite, L.M., Xiao, G., Giacomini, K.M., 2003. Localization of human equilibrative nucleoside transporters, hENT1 and hENT2, in renal epithelial cells. Am. J. Physiol., Renal Physiol. 284, F902–F910.

Mata, J.F., Garcia-Manteiga, J.M., Lostao, M.P., Fernandez-Veledo, S., Guillen-Gomez, E., Larrayoz, I.M., Lloberas, J., Casado, F.J., Pastor-Anglada, M., 2001. Role of the human concentrative nucleoside transporter (hCNT1) in the cytotoxic action of 5′-deoxy-5-fluorouridine, an active intermediate metabolite of capecitabine, a novel oral anticancer drug. Mol. Pharmacol. 59, 1542–1548.

Molina-Arcas, M., Bellosillo, B., Casado, F.J., Montserrat, E., Gil, J., Colomer, D., Pastor-Anglada, M., 2003. Fludarabine uptake mechanisms in B-cell chronic lymphocytic leukemia. Blood 101, 2328–2334.

Osato, D.H., Huang, C.C., Kawamoto, M., Johns, S.J., Stryke, D., Wang, J., Ferrin, T.E., Herskowitz, I., Giacomini, K.M., 2003. Functional characterization in yeast of genetic variants in the human equilibrative nucleoside transporter, ENT1. Pharmacogenetics 13, 297–301.

Parodi, J., Flores, C., Aguayo, C., Rudolph, M.I., Casanello, P., Sobrevia, L., 2002. Inhibition of nitrobenzylthioinosine-sensitive adenosine transport by elevated D-glucose involves activation of P2Y2 purinoceptors in human umbilical vein endothelial cells. Circ. Res. 90, 570–577.

Paroni, R., Del Puppo, M., Borghi, C., Sirtori, C.R., Galli Kienle, M., 1989. Pharmacokinetics of ribavirin and urinary excretion of the major metabolite 1,2,4-triazole-3-carboxamide in normal volunteers. Int. J. Clin. Pharmacol. Ther. Toxicol. 27, 302–307.

Patil, S.D., Ngo, L.Y., Glue, P., Unadkat, J.D., 1998. Intestinal absorption of ribavirin is preferentially mediated by the Na^+ nucleoside purine (N1) transporter. Pharm. Res. 15, 950–952.

Pennycooke, M., Chaudary, N., Shuralyova, I., Zhang, Y., Coe, I.R., 2001. Differential expression of human nucleoside transporters in normal and tumor tissue. Biochem. Biophys. Res. Commun. 280, 951–959.

Reiman, T., Clarke, M.L., Dabbagh, L., Vsianska, M., Coupland, R.W., Belch, A.R., Baldwin, S.A., Young, J.D., Cass, C.E., Mackey, J.R, 2002. Differential expression of human equilibrative nucleoside transporter 1 (hENT1) protein in the Reed–Sternberg cells of Hodgkin's disease. Leuk. Lymphoma 43, 1435–1440.

Ritzel, M.W., Yao, S.Y., Huang, M.Y., Elliott, J.F., Cass, C.E., Young, J.D., 1997. Molecular cloning and functional expression of cDNAs encoding a human Na^+ nucleoside cotransporter (hCNT1). Am. J. Physiol. 272, C707–C714.

Ritzel, M.W., Yao, S.Y., Ng, A.M., Mackey, J.R., Cass, C.E., Young, J.D., 1998. Molecular cloning, functional expression and chromosomal localization of a cDNA encoding a human Na^+/nucleoside cotransporter (hCNT2) selective for purine nucleosides and uridine. Mol. Membr. Biol. 15, 203–211.

Ritzel, M.W., Ng, A.M., Yao, S.Y., Graham, K., Loewen, S.K., Smith, K.M., Ritzel, R.G., Mowles, D.A., Carpenter, P., Chen, X.Z., Karpinski, E., Hyde, R.J., Baldwin, S.A., Cass, C.E., Young, J.D., 2001. Molecular identification and characterization of novel human and mouse concentrative Na^+ nucleoside cotransporter proteins (hCNT3 and mCNT3) broadly selective for purine and pyrimidine nucleosides (system cib). J. Biol. Chem. 276, 2914–2927.

Schaner, M.E., Wang, J., Zevin, S., Gerstin, K.M., Giacomini, K.M., 1997. Transient expression of a purine-selective nucleoside transporter (SPNTint) in a human cell line (HeLa). Pharm. Res. 14, 1316–1321.

SenGupta, D.J., Lum, P.Y., Lai, Y., Shubochkina, E., Bakken, A.H., Schneider, G., Unadkat, J.D., 2002. A single glycine mutation in the equilibrative nucleoside transporter gene, hENT1, alters nucleoside transport activity and sensitivity to nitrobenzylthioinosine. Biochemistry 41, 1512–1519.

Slavin, R.E., Dias, M.A., Saral, R., 1978. Cytosine arabinoside induced gastrointestinal toxic alterations in sequential chemotherapeutic protocols: a clinical-pathologic study of 33 patients. Cancer 42, 1747–1759.

Soler, C., Garcia-Manteiga, J., Valdes, R., Xaus, J., Comalada, M., Casado, F.J., Pastor-Anglada, M., Celada, A., Felipe, A., 2001. Macrophages require different nucleoside transport systems for proliferation and activation. FASEB J. 15, 1979–1988.

Spielman, W.S., Arend, L.J., 1991. Adenosine receptors and signaling in the kidney. Hypertension 17, 117–130.

Stam, R.W., den Boer, M.L., Meijerink, J.P., Ebus, M.E., Peters, G.J., Noordhuis, P., Janka-Schaub, G.E., Armstrong, S.A., Korsmeyer, S.J., Pieters, R., 2003. Differential mRNA expression of Ara-C-metabolizing enzymes explains Ara-C sensitivity in MLL gene-rearranged infant acute lymphoblastic leukemia. Blood 101, 1270–1276.

Sundaram, M., Yao, S.Y., Ng, A.M., Griffiths, M., Cass, C.E., Baldwin, S.A., Young, J.D., 1998. Chimeric constructs between human and rat equilibrative nucleoside transporters (hENT1 and rENT1) reveal hENT1 structural domains interacting with coronary vasoactive drugs. J. Biol. Chem. 273, 21519–21525.

Sundaram, M., Yao, S.Y., Ingram, J.C., Berry, Z.A., Abidi, F., Cass, C.E., Baldwin, S.A., Young, J.D., 2001a. Topology of a human equilibrative, nitrobenzylthioinosine (NBMPR)-sensitive nucleoside transporter (hENT1) implicated in the cellular uptake of adenosine and anti-cancer drugs. J. Biol. Chem. 276, 45270–45275.

Sundaram, M., Yao, S.Y., Ng, A.M., Cass, C.E., Baldwin, S.A., Young, J.D., 2001b. Equilibrative nucleoside transporters: mapping regions of interaction for the substrate analogue nitrobenzylthioinosine (NBMPR) using rat chimeric proteins. Biochemistry 40, 8146–8151.

Valdes, R., Casado, F.J., Pastor-Anglada, M., 2002. Cell-cycle-dependent regulation of CNT1, a concentrative nucleoside transporter involved in the uptake of cell-cycle-dependent nucleoside-derived anticancer drugs. Biochem. Biophys. Res. Commun. 296, 575–579.

Vickers, M.F., Mani, R.S., Sundaram, M., Hogue, D.L., Young, J.D., Baldwin, S.A., Cass, C.E., 1999. Functional production and reconstitution of the human equilibrative nucleoside transporter (hENT1) in *Saccharomyces cerevisiae*. Interaction of inhibitors of nucleoside transport with recombinant hENT1 and a glycosylation-defective derivative (hENT1/N48Q). Biochem. J. 339, 21–32.

Vickers, M.F., Young, J.D., Baldwin, S.A., Ellison, M.J., Cass, C.E., 2001. Functional production of mammalian concentrative nucleoside transporters in *Saccharomyces cerevisiae*. Mol. Membr. Biol. 18, 73–79.

Vickers, M.F., Kumar, R., Visser, F., Zhang, J., Charania, J., Raborn, R.T., Baldwin, S.A., Young, J.D., Cass, C.E., 2002. Comparison of the interaction of uridine, cytidine, and other pyrimidine nucleoside analogues with recombinant human equilibrative nucleoside transporter 2 (hENT2) produced in *Saccharomyces cerevisiae*. Biochem. Cell Biol. 80, 639–644.

Wang, J., Giacomini, K.M., 1997. Molecular determinants of substrate selectivity in Na^+ dependent nucleoside transporters. J. Biol. Chem. 272, 28845–28848.

Wang, J., Giacomini, K.M., 1999a. Characterization of a bioengineered chimeric Na^+ nucleoside transporter. Mol. Pharmacol. 55, 234–240.

Wang, J., Giacomini, K.M., 1999b. Serine 318 is essential for the pyrimidine selectivity of the N2 Na^+ nucleoside transporter. J. Biol. Chem. 274, 2298–2302.

Wang, J., Schaner, M.E., Thomassen, S., Su, S.F., Piquette-Miller, M., Giacomini, K.M., 1997a. Functional and molecular characteristics of Na^+ dependent nucleoside transporters. Pharm. Res. 14, 1524–1532.

Wang, J., Su, S.F., Dresser, M.J., Schaner, M.E., Washington, C.B., Giacomini, K.M., 1997b. Na^+ dependent purine nucleoside transporter from human kidney: cloning and functional characterization. Am. J. Physiol. 273, F1058–F1065.

Ward, J.L., Sherali, A., Mo, Z.P., Tse, C.M., 2000. Kinetic and pharmacological properties of cloned human equilibrative nucleoside transporters, ENT1 and ENT2, stably expressed in nucleoside transporter-deficient PK15 cells. Ent2 exhibits a low affinity for guanosine and cytidine but a high affinity for inosine. J. Biol. Chem. 275, 8375–8381.

Williams, T.C., Jarvis, S.M., 1990. Na^+ dependent purine and pyrimidine nucleoside transporters in bovine outer renal cortex brush-border-membrane vesicles. Biochem. Soc. Trans. 18, 684–685.

Williams, T.C., Jarvis, S.M., 1991. Multiple sodium-dependent nucleoside transport systems in bovine renal brush-border membrane vesicles. Biochem. J. 274, 27–33.

Williams, T.C., Doherty, A.J., Griffith, D.A., Jarvis, S.M., 1989. Characterization of sodium-dependent and sodium-independent nucleoside transport systems in rabbit brush-border and basolateral plasma-membrane vesicles from the renal outer cortex. Biochem. J. 264, 223–231.

Wright, A.M., Paterson, A.R., Sowa, B., Akabutu, J.J., Grundy, P.E., Gati, W.P., 2002. Cytotoxicity of 2-chlorodeoxyadenosine and arabinosylcytosine in leukaemic lymphoblasts from paediatric patients: significance of cellular nucleoside transporter content. Br. J. Haematol. 116, 528–537.

Wu, X., Yuan, G., Brett, C.M., Hui, A.C., Giacomini, K.M., 1992. Sodium-dependent nucleoside transport in choroid plexus from rabbit. Evidence for a single transporter for purine and pyrimidine nucleosides. J. Biol. Chem. 267, 8813–8818.

Yao, S.Y., Ng, A.M., Muzyka, W.R., Griffiths, M., Cass, C.E., Baldwin, S.A., Young, J.D., 1997. Molecular cloning and functional characterization of nitrobenzylthioinosine (NBMPR)-sensitive (es) and NBMPR-

insensitive (ei) equilibrative nucleoside transporter proteins (rENT1 and rENT2) from rat tissues. J. Biol. Chem. 272, 28423–28430.

Yao, S.Y., Ng, A.M., Sundaram, M., Cass, C.E., Baldwin, S.A., Young, J.D., 2001. Transport of antiviral 3′-deoxy-nucleoside drugs by recombinant human and rat equilibrative, nitrobenzylthioinosine (NBMPR)-insensitive (ENT2) nucleoside transporter proteins produced in Xenopus oocytes. Mol. Membr. Biol. 18, 161–167.

Yao, S.Y., Ng, A.M., Vickers, M.F., Sundaram, M., Cass, C.E., Baldwin, S.A., Young, J.D., 2002. Functional and molecular characterization of nucleobase transport by recombinant human and rat equilibrative nucleoside transporters 1 and 2. Chimeric constructs reveal a role for the ENT2 helix 5–6 region in nucleobase translocation. J. Biol. Chem. 277, 24938–24948.