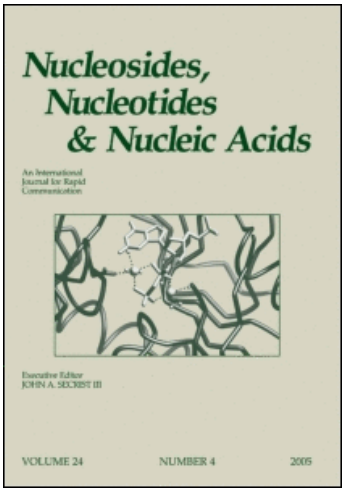

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Membrane Transport of Nucleoside Analogues in Mammalian Cells

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MEMBRANE TRANSPORT OF NUCLEOSIDE ANALOGUES IN MAMMALIAN CELLS

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ABSTRACT: This paper attempts to summarize what is known, from rapid kinetic studies of cell membrane transport, about the mechanism by which nucleoside analogues permeate the plasma membrane of mammalian cells.

GENERAL BACKGROUND: Nucleoside analogues are becoming increasingly important in the treatment of cancer[1-8] and of viral infections[1-5,9-11]. Essentially all of these antineoplastic and antiviral agents must initially permeate the plasma membrane and thereby gain access to the interior of cells before they can begin to exert their biological effects. The ability of these nucleoside-like drugs to traverse biological membranes can clearly have an important impact on their pharmacokinetics, disposition and *in vivo* biological activity. For example, the sensitivity of different types of human leukemic cells to cytosine arabinoside (araC) has been correlated both with the density of nucleoside transport sites present on these cells and with influx rates of araC among these cell types[12-15].

Mammalian cells possess specific carrier proteins for the membrane transport of physiological nucleosides[6-8,16-19] and nucleobases[6,16,17,20,21]. Several different types of mammalian nucleoside transport systems of broad permeant specificity have been identified, differing mainly in whether they are concentrative or nonconcentrative and in whether or not they are inhibited by nanomolar concentrations of 6-[(4-nitrobenzyl)-thio]-9-β-D-ribofuranosylpurine (NBMPR)[8,19]. The concentrative, sodium-dependent type of nucleoside transporter appears to be insensitive to inhibition by NBMPR.

As indicated above for araC, therapeutic nucleoside analogues often utilize a nucleoside transporter to enter cells[6-8,16-19]. This report summarizes past findings, as well as results from our laboratory, concerning the mechanisms by which a number of nucleoside-like drugs permeate the plasma membrane of mammalian cells.

PREVIOUS STUDIES ON NUCLEOSIDE ANALOGUE TRANSPORT IN MAMMALIAN CELLS: A considerable number of experimental studies have been published concerning the transport kinetics of nucleoside analogues in mammalian cells. However, the methodologies used in many of these earlier studies were unable to address the critical first few seconds of nucleoside influx into cells and, as a result, often monitored the subsequent intracellular metabolism of the nucleoside rather than its initial transport[6-8,16-18]. With the recent development of effective, rapid sampling techniques, true initial velocities of nucleoside transport have become accessible[8,16-19]. As a result, the transport kinetics of many physiological nucleosides and nucleoside analogues have been rigorously determined, and the results of these kinetic analyses have been used to elucidate the mechanism of cell permeation of these compounds. Among the seventeen nucleoside analogues* listed in TABLE 1, it was concluded that sixteen of them permeate the membrane of cells by a saturable process indicative of carrier-mediated transport. The transport K_m values determined for these latter permeants ranged between 8 and 15,500 μM. The cellular influx of ten of these nucleoside analogues was shown to be sensitive to inhibition by NBMPR, while five permeants were not examined for this trait. Of the thirteen nucleoside analogues so investigated, the cellular transport of all but N^6-L-phenyl-isopropyladenosine was found to be inhibited by other nucleosides. In spite of the variety of mammalian cell types used in these transport studies, it is notable that the majority of these nucleoside analogues appeared to permeate the cells under study by means of an NBMPR-sensitive, nonconcentrative nucleoside transporter. By contrast,

*5'-Methylthioadenosine is a naturally occurring co-product of polyamine biosynthesis and thus is not, strictly speaking, a nucleoside analogue. However, it is included in TABLE 1 because it is a uniquely 5'-modified nucleoside which has been demonstrated to be a permeant of the erythrocyte nucleoside transporter.

formycin B has been found to enter mouse intestinal epithelial cells via an NBMPR-insensitive, sodium-dependent nucleoside transporter, and lipophilic N^6-L-phenylisopropyladenosine has been concluded to permeate cells by nonfacilitated diffusion.

SELECTION OF HUMAN ERYTHROCYTES FOR TRANSPORT STUDIES: For the studies conducted in our laboratory, several considerations seemed to recommend the use of human erythrocytes as a model system for investigating the cellular transport of nucleoside analogues. First, these cells are of human origin and thus offer the advantage of avoiding any species-related differences of the nucleoside transporter which might otherwise obscure our attempt to understand the human pharmacology of nucleoside-like drugs. Second, fresh human erythrocytes are readily available in pure form, with a minimum of preparative manipulations or trauma to the cell membrane. Third, these cells appear to contain only a single type of nucleoside transporter: the NBMPR-sensitive, nonconcentrative system[8,19]. Fourth, this nucleoside transporter present in human erythrocytes appears to be similar to that found in other human tissues, such as leukocytes[13] and umbilical cord endothelial cells (C. Jurgensen and G. Wolberg, personal communication). Moreover, the observed ability of NBMPR to protect mice against the cytotoxicity caused by certain nucleoside analogues[8,19] indicates that this same type of nucleoside carrier is importantly operative in intact animals of at least one species. Fifth, human erythrocytes also possess a purine nucleobase carrier which is functionally distinct from the nucleoside carrier[20,21]. This circumstance has proven particularly valuable in our transport studies with the antiviral "acyclic nucleosides" (see below).

TRANSPORT STUDIES OF NUCLEOSIDE ANALOGUES IN HUMAN ERYTHROCYTES: We have used rapid kinetic techniques to investigate the mechanism by which a number of nucleoside analogues of therapeutic interest permeate the membrane of human erythrocytes (TABLE 2). Among the compounds studied, 5-iodo-2'-deoxyuridine, 3-deazaadenosine and guanine arabinoside appeared to enter human erythrocytes exclusively by means of the NBMPR-sensitive, nonconcentrative nucleoside transporter, with K_m values ranging from 150 to 3400 μM at 37°C. By contrast, the more lipophilic nucleoside analogues 3'-azido-3'-deoxythymidine and 2',3'-dideoxythymidine appeared to traverse the erythrocyte membrane solely by nonfacilitated diffusion, i.e., independently of any carrier

TABLE 1

Membrane transport properties of nucleoside analogues characterized previously by rapid sampling techniques with mammalian cells

Nucleoside analogue	Influx inhibited by		K_m (μM)[a]	Cell type	Ref.
	NBMPR	Nucleosides			
2'-Deoxycoformycin	+	+	>>100 μM[b]	Human erythrocytes	22
2-Chloroadenosine	+	+	23	Human erythrocytes	23
Tubercidin	+	+	18	Mouse L5178Y cells	24
6-Azauridine	+	+	3,600 (pH 6.0) 15,500 (pH 7.4)	Human RPMI 6410 cells	25
3-Deazauridine	+	ND[c]	520	Human RPMI 6410 cells	26
Cytosine arabinoside	+	+	51	Human lymphocytes	13
			142	Human neutrophils	
			255	AML/AMML leukocytes	
			197	AUL leukocytes	
			214	ALL leukocytes	
5-Azacytidine	+[d]	+	290	Rat Novikoff hepatoma cells	27
Tricyclic nucleoside	+[d]	+	10	Rat Novikoff hepatoma cells	28

5'-Methylthio-adenosine	+	+	184	Human erythrocytes	29
5'-Deoxyadenosine	ND[e]	+	115	Mouse L1210 cells	30
8-Azidoadenosine	+	+	80	Human erythrocytes	31
2-Fluoroadenine arabinoside	ND	+	317	Mouse intestinal epithelial cells	32
Tiazofurin	ND	ND	170	Human erythrocytes	33
8-Bromoguanosine	ND	ND	8	Mouse splenocytes	34
6-Thiopurine arabinoside	ND	ND	1000	Mouse L1210 cells	35
Formycin B	-	+	80	Mouse intestinal epithelial cells	36
N^6-L-Phenylisopropyl-adenosine	-	-	∞[f]	Rat Novikoff hepatoma cells	37

[a] These kinetic parameters were determined at temperatures ranging between 20 and 37°C.
[b] The K_i of 2'-deoxycoformycin as a competitive inhibitor of adenosine transport was estimated to be 10 mM[22].
[c] ND, not determined.
[d] This conclusion is based upon the ability of NBMPR to protect human RPMI 6410 cells from the cytotoxicity caused by these nucleoside analogues[38].
[e] Influx into cells was inhibited 75% by 10 μM dipyridamole.
[f] This permeant exhibited nonsaturable kinetics over the concentration range 0.01 to 240 μM.

TABLE 2

Membrane transport properties of nucleoside analogues characterized in our laboratory by rapid sampling techniques with human erythrocytes

Nucleoside analogue	Influx inhibited by			K_m (μM)[a]	Ref.
	NBMPR	Nucleosides	Nucleobases		
5-Iodo-2'-deoxyuridine	+	+	-	73 (20°C) 150 (37°C)	20, 39 b
3'-Azido-3'-deoxythymidine	-	-	-	∞[c] (20°C)	40
2',3'-Dideoxythymidine	-	-	-	∞[c] (20°C)	41
Acyclovir	-	-	+	260 (37°C)	42
Desciclovir	-	-	-	∞[c] (37°C)	b
Ganciclovir	+	+	+	890[d] (37°C) 13,000[e] (37°C)	b
3-Deazaadenosine	+	+	-	450 (37°C)	43[b]
Guanine arabinoside	+	+	ND[f]	3,400 (37°C)	b

[a]These K_m values were determined at the temperatures indicated parenthetically.
[b]Manuscript in preparation.
[c]These permeants exhibited nonsaturable kinetics over the concentration range investigated (up to 10 mM).
[d]This K_m value relates to influx of ganciclovir into erythrocytes via the purine nucleobase carrier and was determined in the presence of 1.0 μM dilazep.
[e]This K_m value relates to influx of ganciclovir into erythrocytes via the nucleoside carrier and was estimated in the presence of 2.0 or 3.0 mM adenine.
[f]ND, not determined.

system. Results of transport studies with acyclovir provided yet a different mechanism of cell permeation; this so-called "acyclic nucleoside" was found to be a co-permeant of the erythrocyte purine nucleobase carrier, with a K_m of 260 μM. Desciclovir, a xanthine oxidase-activable prodrug of acyclovir with greatly improved oral bioavailability[44], exhibited nonsaturable kinetics of influx into human erythrocytes. This observation, together with the finding that its influx rate was only marginally ($\leq 10\%$) inhibited by permeants or inhibitors of the nucleoside and nucleobase carriers, led us to conclude that desciclovir permeates these cells predominantly by nonfacilitated diffusion. Finally, ganciclovir was found to permeate human erythrocytes largely via the same purine nucleobase carrier (K_m = 890 μM) used by acyclovir but also to a minor extent via the NBMPR-sensitive nucleoside transporter (K_m = 13 mM).

OTHER EXPERIMENTAL EVIDENCE DEMONSTRATIVE OF NUCLEOSIDE ANALOGUE TRANSPORT MECHANISMS: In addition to the direct transport studies with radiolabeled permeants described above, nonradioactive nucleoside analogues have been used to obtain two other types of experimental evidence concerning the manner by which they enter cells. First, the observed ability of NBMPR to protect certain cultured cells against the cytotoxicity of many nucleoside analogues indicates strongly that these particular compounds depend largely upon the NBMPR-sensitive, nonconcentrative nucleoside transporter for entry into the cells under study[8,18]. Conversely, lack of NBMPR-protection of these same cells against other nucleoside analogues would suggest that the latter compounds can enter cells by an alternative process. Second, the observed ability of many nucleoside analogues, when added to the external medium, to accelerate the efflux of a radiolabeled nucleoside from cells ("*trans*-acceleration") is considered evidence that these nucleoside analogues are transported across the cell membrane by the same transporter as the radiolabeled permeant[8,18]. However, these two indirect approaches to investigating the transport mechanism of nucleoside analogues do not provide nearly as much information about the cell permeation properties of these agents as do direct kinetic studies of the cellular influx of radiolabeled nucleoside analogues.

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