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ABSENCE OF BINDING SITES FOR THE TRANSPORT INHIBITOR NITROBENZYLTHIOINOSINE ON NUCLEOSIDE TRANSPORT-DEFICIENT MOUSE LYMPHOMA CELLS

CAROL E. CASS, NORBERT KOLASSA *, YOSHIMASA UEHARA **, EWA DAHLIG-HARLEY, ERIC R. HARLEY and ALAN R.P. PATERSON

Cancer Research Unit and Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7 (Canada)

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Cells of an adenosine-resistant clone (AE_1) of S49 mouse lymphoma cells were compared with cells of the parental line with respect to (a) characteristics of nucleoside transport, (b) high affinity binding of the inhibitor of nucleoside transport, nitrobenzylthionisine (NBMPR), and (c) the antiproliferative effects of the nucleoside antibiotics, tubercidin, arabinosyladenine and showdomycin. Rates of inward transport of uridine, thymidine, adenosine, 2′-deoxyadenosine, tubercidin, showdomycin, and arabinosyladenine in AE_1 cells were less than 1% of those in cells of the parental S49 line. The inhibitor of nucleoside transport, NBMPR, reduced rates of inward nucleoside transport in S49 cells to levels comparable to those seen in the transport-defective mutant. S49 cells possessed high affinity sites that bound NBMPR ($6.6 \cdot 10^4$ sites/cell, K_d = 0.2 nM), whereas site-specific binding of NBMPR to AE_1 cells was not demonstrable, indicating that loss of nucleoside transport activity in AE_1 cells was accompanied by loss of the high affinity NBMPR binding sites. Relative to S49 cells, AE_1 cells were resistant to the antiproliferative effects of tubercidin and showdomycin, but differences between the two cell lines in sensitivity toward arabinosyladenine were minor, suggesting that nucleoside transport activity was required for cytotoxicity of tubercidin and showdomycin, but not for that of arabinosyladenine.

Introduction

Permeation of physiological nucleosides through the plasma membrane of animal cells occurs mainly by a nucleoside-specific, mediated process that is independent of the subsequent, intracellular metabolic transformations to which nucleosides are subject. The latter are determinants of rates of nucleoside uptake * by cells under some conditions [1–3]. The independence of nucleoside transport and subsequent enzymatic events is evident in demonstrations that cells with impaired ability to metabolize nucleosides transport nucleosides at rates similar to those in cells without the metabolic impairment [4–6]. In such cells, nucleoside permeation has the kinetic characteristics of a facilitated diffusion process [3]. The independence of nucleoside transport

* Present address: Department of Pharmacology, University of Vienna Medical School, Vienna, Austria.
** Present Address: Institute of Microbial Chemistry, 14–23, Kamiosaki 3-chome, Shinagawa-ku, Tokyo, 141, Japan.

Abbreviations: NBMPR (nitrobenzylthioinosine), 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine; NBTGR, 2-amino-6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine; dipyridamole, 2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido-[5,4-d]pyrimidine; arabinosyladenine, 9-β-D-arabinofuranosyladenine; tubercidin, 4-amino-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine; showdomycin, 3-β-D-ribofuranosylmaleimide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

* 'Uptake' refers to the cellular accumulation of a nucleoside permeant and its metabolic products. 'Transport' refers to the transporter-mediated passage of nucleoside molecules across the plasma membrane of cells.

and metabolic events is also evident in the properties of a mutant line of mouse lymphoma cells (AE_1) in which nucleoside uptake activity was reduced without loss of nucleoside kinase activities, presumably because of a defect in the nucleoside transport mechanism [7]. The AE_1 clone was isolated [7] in a single-step selection for adenosine resistance from a mutagenized population of cells of the S49 mouse T-cell lymphoma line [8]. AE_1 cells were shown to be resistant to a variety of cytotoxic nucleoside analogs and to have a reduced capacity for uptake of adenosine and thymidine relative to S49 cells [7]. These findings were consistent with a nucleoside-transporter defect in AE_1 cells; the present study demonstrates this defect with a definitive assay for nucleoside transport.

Potent inhibitors of nucleoside transport, such as NBMPR or dipyridamole, have afforded a means of identifying and exploring the nucleoside transport mechanism. These agents impede transporter-mediated uptake of nucleosides in cells or tissues [9–13] and protect cells against the antiproliferative effects of various cytotoxic nucleosides by reducing access of the latter to cells [14,15].

The inhibition of nucleoside transport by NBMPR and congeners is related to the tight, but reversible, binding of these compounds to specific, plasma membrane sites which are evidently on, or part of, functional nucleoside transporter elements in the cell membrane [16–18]. NBMPR bound at these sites is displaceable by congeners of NBMPR that are also inhibitors of nucleoside transport. NBMPR is bound tightly at these sites (K_d, 10^{-9}–10^{-10} M) which number about 10^4 per human erythrocyte and $(1–3) \cdot 10^5$ per cell for several lines of cultured neoplastic cells [14,16,19]. Although binding of NBMPR to the high affinity sites correlates with inhibition of the transporter function [15,16,19], NBMPR-transporter interactions, in addition to simple occupancy of high affinity sites, appear to be required for complete inhibition of nucleoside transport in HeLa cells [15,20]. Jarvis and Young [17,21] have reported that NBMPR sites are present on sheep erythrocytes capable of transporting nucleosides, but are absent from erythrocytes lacking that capability *, and have concluded that NBMPR binding took place at functional transporter sites. The molecular weight of the NBMPR-binding component of the nucleoside transport mechanism in membrane preparations from human erythrocytes has been estimated to be about 122 000 by radiation-inactivation analysis with high-energy electrons [22]. Jarvis and Young [23] have reported a 15-fold purification of the NBMPR-binding activity from detergent-solubilized fractions of human erythrocyte membranes.

The current work was undertaken to further characterize the nucleoside transport-deficient clone (AE_1) of cells isolated by Cohen et al. [7] from the S49 line of mouse lymphoma cells. A procedure based on the use of NBMPR as a 'stopper' to end nucleoside uptake during intervals of a few seconds [24] enabled determination of definitive time courses of nucleoside uptake by S49 and AE_1 cells from which initial rates of uptake were obtained. With the latter measure of transport, the transport-deficient character of AE_1 cells was clearly evident. Comparisons of site-specific binding of NBMPR in S49 and AE_1 cells indicates that loss of transport activity was accompanied by loss of transport inhibitory sites.

Materials and Methods

Cell culture. Cell stocks were grown in antibiotic-free Dulbecco's modified Eagle's medium containing 2.2 g/l of $NaHCO_3$ and 3 g/l D-glucose supplemented with heat-inactivated horse serum (10%). Cultures were kept at 37°C in an atmosphere of 5% CO_2/air.

In experiments which determined rates of cell proliferation, cells were cultured in loosely capped 50-ml bottles containing 20 ml medium supplemented with antibiotics (penicillin G_1, 100 units/ml; streptomycin, 100 µg/ml). Cultures at an initial density of $(0.5–1.0) \cdot 10^5$ cells/ml were incubated for 72 h with various concentrations of nucleosides. Cell numbers in such cultures were determined at 24-h intervals with an electronic particle counter. Proliferation rates were defined as the number of cell doublings during specified intervals of culture.

Nucleoside uptake experiments. Cells for use in transport experiments were grown in antibiotic-containing medium. For the assay of nucleoside uptake during short intervals, cells were suspended in 'transport medium' consisting $NaHCO_3$-free Fischer's me-

* In sheep erythrocytes, nucleoside transport is under control of two allelomorphic genes [21].

dium containing 20 mM Hepes (pH 7.4), and uptake measurements were performed at 22°C within 30 min after cells were transferred to transport medium. A detailed description of the uptake assay is given elsewhere [24]. Briefly, 100 μl transport medium containing the nucleoside permeant (25 μCi/ml) was layered over 150 μl silicone oil/paraffin oil mixture (specific gravity, 1.03 g/ml) in 1.5-ml polypropylene microcentrifuge tubes. Intervals of nucleoside uptake were started by rapid addition of 100 μl transport medium containing $(2–6) \cdot 10^6$ cells. Uptake intervals were stopped by the addition of 200 μl transport medium containing NBMPR (Usually 20 μM; exceptions are noted), followed immediately by centrifugation for 30 s (Eppendorf microcentrifuge, model 5412). 'Zero-time uptake' values were determined with incubation mixtures completed by the addition of cells to 100-μl portions of medium containing both ^{3}H-labelled permeant and 10 μM NBMPR; transport blockade is essentially instantaneous under these circumstances [24]. Immediately after the completion of such mixtures, cells were pelleted. The water space in cell pellet was determined by using $[^3H]H_2O$ in place of nucleoside permeant in the uptake assay.

After pelleting the cells, the supernatant medium was removed from microcentrifuge tubes and 1.4 ml water was added to each as a rinse. After removal of oil and water by aspiration, cell pellets were solubilized in 0.2 ml 5% Triton X-100 in water. Each microcentrifuge tube was then placed in a polyethylene scintillation vial and its contents were mixed with 8 ml xylene/Triton X-100 scintillant for assay of radioactivity by liquid-scintillation counting [25].

Initial rates of nucleoside uptake were estimated by fitting parabolas to the uptake data by the method of least-squares [24]. Initial rates were derived from coefficients of the first order terms of the parabolas. In presenting results from uridine uptake studies, pellet contents of ^{3}H-labelled permeant have been expressed as the ratio of the observed content of ^{3}H-labelled permeant to that present in a volume of medium equal to the water content of the pellet ('pellet/medium ratio'). Ratios larger than unity would indicate accumulation of permeant and/or its metabolites in the pellet and, if cellular uptake of a non-metabolized permeant was nonconcentrative, the ratios would approach unity as a maximum value. With respect to ^{3}H-labelled permeant that became associated with cells during uptake intervals of 0 s (i.e., the 'zero-time' cellular content of permeant), pellet/medium ratios were 0.1–0.2 and were independent of permeant concentration.

Binding of NBMPR. Binding of NBMPR to cells was measured by a modification of a previously described procedure [15] in which cells were incubated with graded concentrations of [G-^{3}H]NBMPR in the presence or absence of concentrations of NBTGR that would displace the former from specific cellular binding sites. The difference between the amounts of [G-^{3}H]NBMPR that became associated with cells in the presence and absence of 6.3 μM NBTGR was considered to measure specifically bound NBMPR [19]. Duplicate incubation mixtures were prepared by placing 1.0-ml portions of cell suspension ($7 \cdot 10^6$ cells/ml in serum-free Fischer's medium supplemented with 20 mM Hepes (pH 7.4)) on 100-μl portions of a silicone oil/paraffin oil solution (specific gravity, 1.03 g/ml) contained in 1.5-ml polypropylene microcentrifuge tubes. Incubation mixtures were completed by the addition of 20 μl [G-^{3}H]NBMPR in an isotonic, phosphate-buffered salts medium, and the resulting mixtures were incubated at 22°C for 30 min to permit equilibration of the labeled ligand with cellular binding sites. Cell pellets were prepared and processed for the determination of radioactive content as described above with these modifications. Supernatants were sampled for determination of free [G-^{3}H]NBMPR concentration, and cell pellets were solubilized by the addition of 0.5 ml 10% Triton X-100 and mixed with 9 ml xylene/Triton scintillant [25]. The ^{3}H activity in supernatant samples was assayed with the same scintillant.

Chemicals. Radioactive compounds were obtained from Moravek Biochemicals (City of Industry, CA), except for 2′-deoxy[8-^{3}H]adenosine and [U-^{14}C]-sucrose, which were purchased from ICN Pharmaceuticals (Irvine, CA), $[^3H]H_2O$, which was purchased from New England Nuclear (Boston, MA), and [1-^{14}C]showdomycin, which was prepared by CEA (Gif-sur-Yvette, France) and was generously provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The latter compound was repurified by thin-layer chromatography and when necessary, labelled nucleosides were repurified by high performance liquid chromatography. Cell culture materials were obtained from GIBCO (Canada), Burlington, Ontario.

Results and Discussion

In the permeation of nucleoside molecules in cultured cells, equilibration between extracellular and intracellular compartments is rapid. With several cell-permeant combinations, transporter-mediated fluxes of permeant in both directions across the plasma membrane are established within a few seconds of exposure of cells to nucleoside-containing medium, and intracellular metabolism (principally conversion to nucleotides) becomes the major determinant of nucleoside uptake rate [3]. In the studies reported here, definition of time courses of nucleoside uptake by cells required measurement of uptake during intervals as short as a few seconds. Techniques by which cells can be exposed to nucleosides for such brief intervals include (a) rapid separation of cells from permeant-containing medium, (b) rapid addition of potent inhibitors of nucleoside transport to reaction mixtures, and (c) combinations of (a) and (b) to achieve intervals of uptake as short as a few seconds. Application of this technology allows measurement of definitive time courses of cellular uptake of nucleosides, and from these, determination of initial rates of transport.

The uptake of uridine by S49 and AE_1 cells was compared in the experiments summarized in Fig. 1. Time courses of nucleoside uptake by both cell lines extrapolated through zero-time values and clearly measured initial rates. As noted above, initial rates of nucleoside uptake measure rates of membrane transport, the first step in the nucleoside uptake process. Uridine transport rates were saturable for S49 cells, and the apparent kinetic constants obtained in the experiment of Fig. 1 were: K_m, 103 μM; and V, 3.9 pmol/μl pellet water per s. Rates of uridine uptake in AE_1 cells were less than 1% of those in S49 cells and required uptake intervals of 0.5–4 min for definitive rate measurements, in agreement with the earlier conclusion that AE_1 cells are deficient in their ability to transport nucleosides [7]. Experiments of similar design showed that transport of adenosine

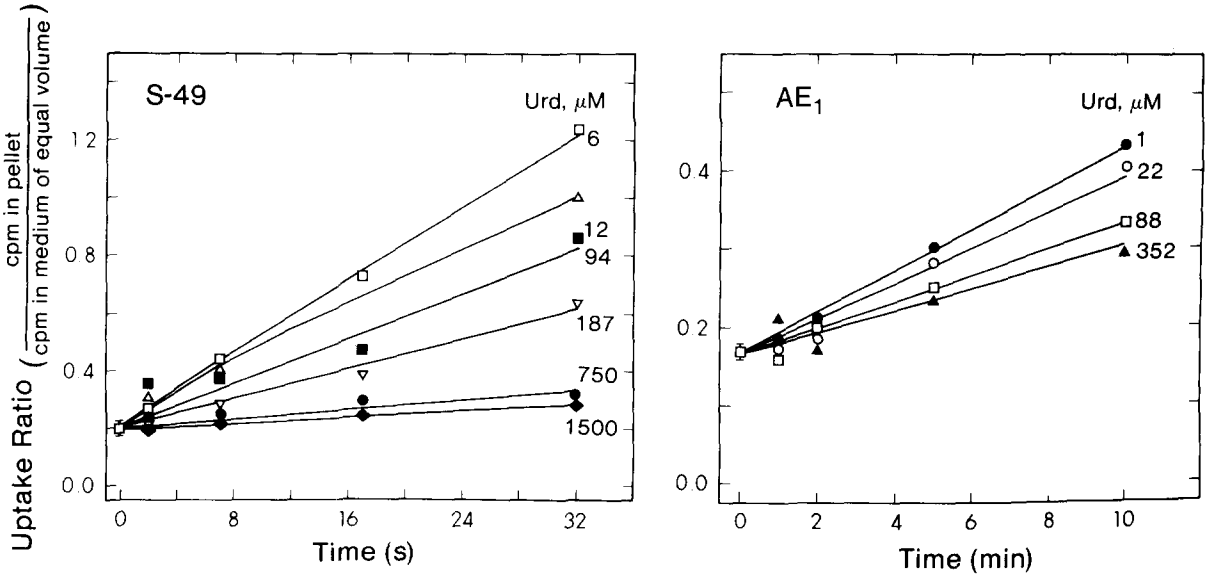

Fig. 1. Transport of uridine by S49 and AE_1 mouse lymphoma cells. Intervals of uptake were started by rapid addition of cell suspension to equal volumes of transport medium containing [5-^{3}H]uridine (Urd), as described in Materials and Methods; final concentrations are indicated. Intervals of uptake by S49 cells were ended by addition of medium containing NBMPR (final conc., 10 μM), immediately followed by centrifugation. Intervals of uptake by AE_1 cells, being much longer, were ended simply by centrifugation through oil. The ^{3}H content of cell pellets was determined as described in Materials and Methods. Presented are 'uptake ratios', that is, the ^{3}H content of the pellet (cpm) divided by the ^{3}H content of a volume of medium equal to the pellet water space. Zero-times values for uptake ratios are means (±S.D.) of those obtained with six uridine concentrations.

TABLE I

KINETIC CONSTANTS FOR TRANSPORT OF NUCLEOSIDES BY S49 LYMPHOMA CELLS

The values listed below are means (±S.D.) derived from experiments conducted and analyzed as described in the legend of Fig. 1.

Nucleoside	K_m (μM)	V (pmol/μl pellet water per s)
Uridine [a]	114 ± 19	5.8 ± 0.9
Thymidine [b]	94 ± 14	5.1 ± 0.5
Adenosine [c]	23 ± 1	5.6 ± 0.1

[a] Calculated from three experiments, including the one described in Fig. 1.
[b] Calculated from four experiments.
[c] From Fig. 2A.

and thymidine into S49 cells were also saturable (Fig. 2A and Table I), and that comparable processes were not evident in AE_1 cells (see below). With the assay used in Fig. 1, we have also shown the existence of NBMPR-sensitive adenosine transport processes in HeLa cells, P388 cells and L5178Y cells [24]. In these cells, kinetic constants for adenosine transport were: K_m, 16–21 μM; and V, 7–15 pmol/μl pellet water per s [24].

Kinetic experiments on nucleoside permeation conducted with S49 cells suggested that the nucleoside transport mechanism is of broad specificity. Fig. 2B shows that adenosine inhibited the transport of uridine in a competitive manner. In similar experiments (data not shown), uridine and thymidine were apparently competitive inhibitors of adenosine transport. The apparent K_i values obtained for adenosine, uridine and thymidine in these inhibition studies were similar to their K_m values (Table I) for inward transport, suggesting that in S49 cell these nucleosides are substrates for the same transport mechanism.

In the experiments of Fig. 3, uptake rates of adenosine, tubercidin, 2′-deoxyadenosine, arabinosyladenine, thymidine and showdomycin * into S49 cells greatly exceeded those into AE_1 cells. When the

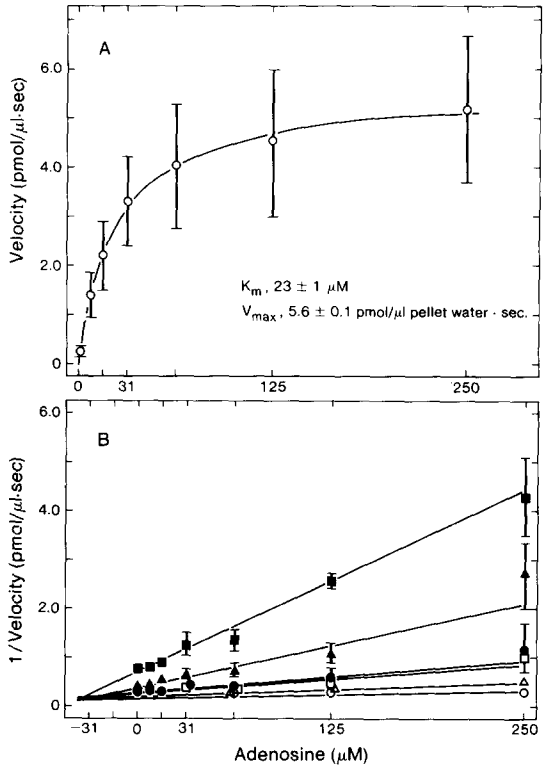

Fig. 2. Transport of adenosine and uridine in S49 mouse lymphoma cells. Panel A, transport of adenosine. Time courses of uptake of [8-^{3}H]adenosine were determined at the concentrations indicated by the procedure described in the legend to Fig. 1. Initial velocities of uptake were derived from such time courses as described in Materials and Methods. Presented are mean values (±S.D.) from six separate experiments, along with the best-fitting Michaelis-Menten curve, obtained by the weighted nonlinear regression algorithm of Cleland [26]. Panel B, inhibition of uridine transport by adenosine. Time courses of cellular uptake of [5-^{3}H]uridine at graded concentrations (31 μM, ■; 63 μM, ▲; 125 μM, ●; 250 μM, □; 500 μM, △; and 1000 μM, ○) by S49 lymphoma cells were determined, as described in the legend of Fig. 1, in the presence of nonradioactive adenosine at the concentrations indicated. Reciprocals of the initial velocities of uridine uptake are plotted vs. inhibitor (adenosine) concentrations in a Dixon plot [27]. These data indicate that adenosine competitively inhibited (K_i, 27 μM) the transport of uridine.

incubation medium contained 22 μM NBMPR, the uptake of each of these nucleosides by S49 cells was greatly reduced and time courses of uptake were indistinguishable from those of AE_1 cells (data not shown). The low rates of nucleoside uptake in AE_1 cells were not perceptibly changed by NBMPR (data

* In the case of [^{14}C]showdomycin, the radioactivity in AE_1 cell pellets descreased with time during incubations longer than 2 s. The reason for this finding is not clear, but chemical instability of showdomycin has been noted [29].

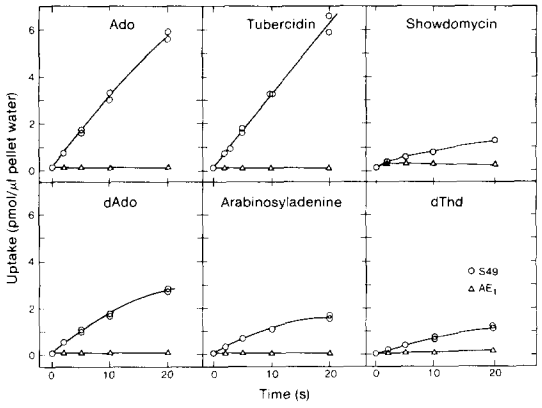

Fig. 3. Nucleoside transport by S49 and AE_1 cells. Intervals of uptake were started by rapid mixing of equal volumes of cell suspension and various radioactive nucleosides (final concentrations 1 μM). Intervals of uptake were ended at the times indicated by addition of NBMPR-containing medium, immediately followed by centrifugation. The radioactive content of cell pellets was determined as described in Materials and Methods. Duplicate experiments are represented by single symbols in instances of superimposing results. Portions of these data were presented in a preliminary report [28]. Ado, adenosine; dAdo, deoxyadenosine; dThd, thymidine.

not shown). Thus, the mutational event in AE_1 cells eliminated transport of a number of nucleosides by the NBMPR-sensitive mechanism. The structural diversity of the nucleoside permeants tested in the experiments of Fig. 3 indicated that the mutationally deleted permeation mechanism is of broad specificity, a characteristic also suggested by the permeant competition experiments described above.

Many types of animal cells possess NBMPR binding sites, evidently located on nucleoside transporter elements of the plasma membrane and accessible to the ligand from the external aspect of the membrane. NBMPR occupancy of these sites correlates with inhibition of nucleoside transport. Panel A of Fig. 4 indicates that [G-^{3}H]NBMPR became associated with S49 cells during exposure to graded concentrations of [G-^{3}H]NBMPR by binding to specific sites and also by binding in a nonspecific manner. Determinations of specifically bound NBMPR were made over a range of NBMPR concentrations, and, for each, the concentration of free NBMPR in the medium was determined. Free NBMPR was presumed to be in equilibrium with that specifically bound. Mass law analysis indicated that for S49 cells, the maximum number of

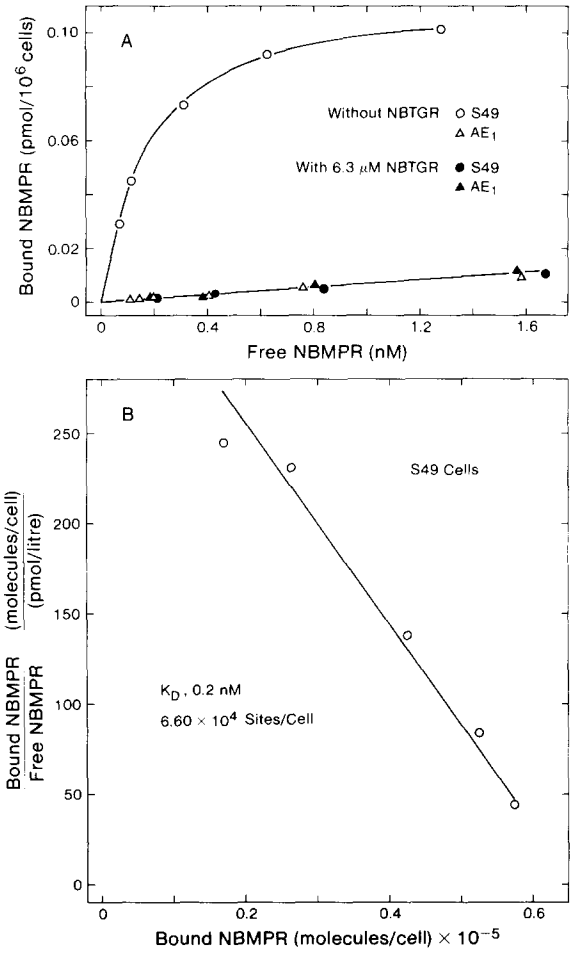

Fig. 4. Absence of NBMPR binding sites on nucleoside transport-deficient AE_1 lymphoma cells. Panel A, S49 (○,●) and AE_1 (△,▲) cells were incubated for 30 min with medium containing various concentrations of [G-^{3}H]NBMPR in the presence (●,▲) or absence (○,△) of 6.3 μM NBTGR. After collection of cells by centrifugation for determination of their ^{3}H content (bound NBMPR), the concentration of free [G-^{3}H]NBMPR in the supernatant was measured. Panel B, Mass law analysis by the method of Scatchard of data for the site-specific binding of NBMPR to S49 cells. In this instance, bound NBMPR represents the difference between cell-associated NBMPR in the presence and absence of NBTGR. The latter, a tightly bound inhibitor of nucleoside transport with potency comparable to NBMPR [19], was used at a concentration that assured almost total occupancy of the inhibitor binding sites (6.3 μM).

NBMPR-specific binding sites and the dissociation constant for site-bound NBMPR were $6.6 \cdot 10^4$ sites/cell and 0.2 nM, respectively (Fig. 4, Panel B). These

values are similar to those previously reported for NBMPR binding to erythrocytes and various cultured cells [15–19]. In experiments with the transport-deficient AE_1 cells, specific binding of NBMPR was not seen. The cell content of NBMPR after incubation of AE_1 cells with graded concentrations of NBMPR was the same whether that incubation was conducted in the presence or absence of 5 μM NBTGR (Fig. 4, Panel A). The absence of such sites on the nucleoside transport-deficient AE_1 cells supports the conclusion of Jarvis and Young [17] that NBMPR binds to functional nucleoside transporter elements.

The effects of tubercidin, showdomycin and arabinosyladenine on the proliferation of S49 and AE_1 cells were compared in the experiments of Fig. 5. Cells were cultured in media containing graded concentrations of the nucleoside analog. Cell concentrations were determined daily, and proliferation rates were estimated from the cell population doublings that occurred during specified intervals. As an analog of adenosine, the metabolic fate of tubercidin is similar to that of adenosine in that mono-, di- and triphosphate esters of tubercidin are formed in cells; however, tubercidin is not a substrate for adenosine deaminase [30]. The concentrations of tubercidin that reduced proliferation rates of S49 and AE_1 cells by 50% were 0.025 and 7 μM, respectively (Table III). The 280-fold resistance of AE_1 cells to tubercidin, relative to the parental S49 cells, is consistent with (a) impairment of nucleoside transporter activity in AE_1 cells, and (b) our earlier demonstration that entry of tubercidin into cells is mediated by the nucleoside transport mechanism [14]. Recent studies in this laboratory have demonstrated that adenosine and tubercidin are competitive substrates for the nucleoside transport mechanism of cultured L5178Y mouse lymphoma cells *. The data of Fig. 5, which deal with the influence of NBMPR on the response of S49 and AE_1 cells to tubercidin, are consistent with the idea that antiproliferative effects of tubercidin at low concentrations (less than 1 μM) signify function of the nucleoside transport mechanism. The

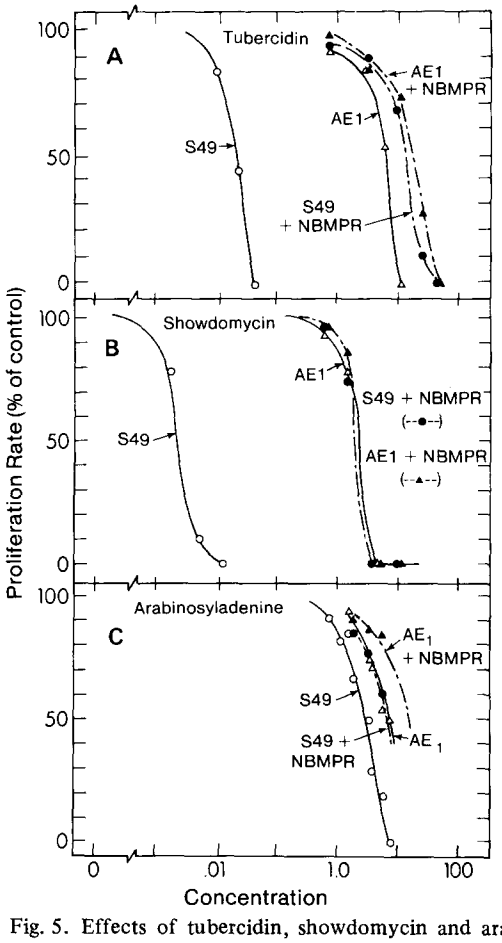

Fig. 5. Effects of tubercidin, showdomycin and arabinosyladenine on cell proliferation. S49 and AE_1 cells were incubated in growth medium containing various concentrations of drug in the presence or absence of 5–8 μM NBMPR. Proliferation rates (number of cell doublings during the incubation period of 0–24 h) under the influence of drug were expressed as percentages of the proliferation rates in the absence of drug. Experiments with arabinosyladenine were conducted in the presence of 1 μM deoxycoformycin. Units of concentration: A and C, μM; B, mM.

presence of 8 μM NBMPR in the culture medium markedly decreased sensitivity of S49 cells to tubercidin, and in the presence of NBMPR, only minor differences were apparent in the antiproliferative effects of tubercidin toward S49 and AE_1 cells.

The cytotoxic activity of the nucleoside antibiotic showdomycin has been linked to the presence of a functional nucleoside transporter in cultured murine leukemia L1210 cells [31] and in *Escherichia coli* [32]. The proliferation of AE_1 cells (Fig. 5) was

* Adenosine and tubercidin were found to be competitive substrates for the nucleoside transport mechanism of L5178Y cells, and K_m and V values for both substrates were 16–26 μM and 6–8 pmol/μl pellet water per s, respectively [24].

TABLE II

IC_{50} VALUES FOR INHIBITION OF CELL PROLIFERATION BY NUCLEOSIDES

S49 and AE_1 cells were incubated in growth medium containing various concentrations of tubercidin, showdomycin or arabinosyladenine in the presence or absence of NBMPR (5–8 μM). In the experiments with arabinosyladenine, 1 μM deoxycoformycin was present throughout the incubation. Nucleoside concentrations (μM) that inhibited proliferation rates (number of cell doublings during the first 24 h of incubation) by 50% are shown.

Nucleoside	S49		AE_1	
	–NMBPR	+NMBPR	–NMBPR	+NMBPR
Tubercidin	0.025	13	7	16
Showdomycin	3	2 200	2 200	2 700
Arabinosyl-adenine	3.5	8	8	17

about 700-fold less sensitive to showdomycin than that of S49 cells, but in the presence of 7.5 μM NBMPR, the responses of the two cell types to graded concentrations of showdomycin were similar (compare IC_{50} values *, Table II). The different sensitivities of S49 and AE_1 cells toward showdomycin indicated that entry of the antibiotic into cells via a NBMPR-sensitive, nucleoside-specific transporter is a necessary first step in expression of the cytotoxic effects of this agent in mammalian cells.

While the sensitivity of S49 and AE_1 cells to tubercidin and showdomycin in the presence and absence of NBMPR correlated well with the functional integrity of the membrane transport system for nucleosides, the response of these cells to arabinosyladenine did not. Arabinosyladenine has been recognized as a substrate of the nucleoside transporter in erythrocytes because cellular uptake of the nucleoside is inhibited by NBMPR [33]. However, NBMPR did not significantly protect L1210 mouse leukemia cells proliferating in culture from inhibition of growth by arabinosyladenine [34]. This apparent discrepancy was found again in the present study. Although NBMPR induced reductions in arabinosyladenine transport rates of more than 100-fold in S49 cells (data not shown), protection of S49 cells by NBMPR against the antiproliferative effects of arabinosyladenine was minimal (Fig. 5). Similarly, relatively small differences in antiproliferative activity of arabinosyladenine toward S49 and AE_1 cells were found (compare IC_{50} values, Table II), although a more than 100-fold difference in transport rates was observed (Fig. 3). The rates of entry of arabinosyladenine (and of adenosine, 2'-deoxyadenosine and tubercidin) in S49 cells treated with nitrobenzylthioinosine were less than 2% of the rates in untreated S49 cells. Apparently, during the prolonged incubations used in proliferation experiments, nonmediated entry of arabinosyladenine is sufficient in both NBMPR-treated S49 cells and the transport-defective mutant to allow intracellular accumulation of growth-inhibitory amounts of arabinosyladenine triphosphates. Thus, it appears that a functional nucleoside transporter is not required for expression of the antiproliferative activity of arabinosyladenine under the conditions of these experiments.

* IC_{50}, nucleoside concentration that inhibited cell proliferation by 50% after a 24-h exposure.

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