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Selective protection of tubercidin toxicity by nitrobenzyl thioinosine in normal tissues but not in human neuroblastoma cells*

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Summary. Tubercidin, an adenosine analogue, is toxic to human neuroblastoma cell lines, to peripheral blood mononuclear cells (PBMCs), and to myeloid colony-forming cells (CFU-C) as tested by a short-term labeled precursor uptake and by a clonogenic assay. When it was co-administered with a potent purine transport inhibitor, nitrobenzyl thioinosine (NBTI), the cytotoxic effect of tubercidin was abolished in PBMCs but not in neuroblastoma cells. Studies of nucleoside transport in neuroblastoma cells demonstrate that although [^{3}H]NBTI binds to the plasma membrane of these cells, the transport of thymidine into the cells is only partially inhibited in the presence of excess NBTI. These data imply that neuroblastoma cells contain a nucleoside transport mechanism which is insensitive to NBTI. "Host protection" with a nucleoside transport inhibitor such as NBTI, may allow effective therapy with otherwise toxic dosages of tubercidin and other cytotoxic nucleosides in patients with neuroblastoma.

Introduction

Neuroblastoma is the second most common malignant solid tumor of childhood. The disease may be localized to its site of origin or disseminated widely. Despite aggressive treatment regimens based on cell cycle kinetics and pharmacokinetic considerations, the prognosis for children with disseminated disease has not changed over the last 30 years. Among the many published studies of combined-agent chemotherapy in neuroblastoma, the role of purine analogues has been neglected [18]. We studied the effect of an adenosine analogue, tubercidin, in combination with a potent nucleoside transport inhibitor, nitrobenzyl thioinosine (NBTI), on three human neuroblastoma cell lines, on peripheral blood mononuclear cells, and on myeloid colony formation representative of host tissues, with the object of developing a rational combination chemotherapy for neuroblastoma.

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Abbreviations used: NBTI, nitrobenzyl thioinosine; PHA, phytohemagglutinin; PBMC, peripheral blood mononuclear cells; tubercidin, 4-amino-7 (β-D-ribofuranosyl)-pyrol[2,3-1] pyrimidine; VTL, valine, threonine, leucine mixture

Tubercidin is an adenosine analogue that follows some of the metabolic pathways of adenosine and is a substrate for the same nucleoside transport system as adenosine. The expression of tubercidin toxicity requires its transport and phosphorylation by adenosine-kinase to mono-, di-, and triphosphate esters [19] and subsequent incorporation into DNA and RNA [23].

NBTI is a specific, high-affinity inhibitor of nucleoside transport; it acts on a wide variety of cells through interference with the transport step of the uptake process of a wide spectrum of nucleosides. Various types of cultured cells, including HeLa colon carcinoma [14], L51784 mouse lymphoblasts, and RPMI 6410 human lymphoblastoid cells [20], possess sites on the plasma membrane to which NBTI binds reversibly but with high affinity. Occupancy of such sites cause competitive inhibition of nucleoside uptake, suggesting direct association with the transporter protein [14, 20]. NBTI also inhibits the transport of a wide range of cytotoxic nucleoside analogues, including nebularine and tubercidin, leading to protection against the cytotoxic effects of these drugs [20, 21]. However, recent observations have shown that the sensitivities of various cell lines to inhibition of nucleoside transport differs substantially [3, 22]. These selective differences in sensitivities to NBTI are potentially useful for selective cancer chemotherapy. Lynch et al. [15, 16] have shown that co-administration of NBTI in optimal dosages with lethal dosages of tubercidin to mice selectively protect host tissue but eliminate mouse leukemia cells from the ascitic fluid.

In the present study we demonstrate a selective protection by NBTI against tubercidin cytotoxicity to normal tissue but not to neurblastoma cells and discuss the possible use of cytotoxic nucleoside analogues in combination with selective host protection in chemotherapy.

Materials and methods

Chemicals. Tubercidin, 4-amino-7 (β-D-ribofuranosyl)-pyrol[2,3-a]pyrimidine, and tritiated nitrobenzyl thioinosine-[^{3}H]NBTI (16 Ci/mmol) were purchased from Moravek Biochemical (Brea, Calif), NBTI from Sigma Chemicals, and [^{3}H]thymidine (7 Ci/mmol) [^{14}C]valine (10 Ci/mmol), [^{14}C]threonine (20 Ci/mmol), and [^{14}C]leucine (50 Ci/mmol) from ICN (Irvine, Calif).

Cell lines. The two cell lines, NUB-6 and EW-2, were newly established from surgically resected tumors from a pa-

tient with recurrent stage IV neuroblastoma (NUB-6) and from a patient with a brain metastasis (EW-2). Histological and ultrastructural examination confirmed the diagnosis of neuroblastoma. The cell lines were established and maintained in alpha medium (Ontario Cancer Institute, Toronto, Ont) supplemented with 20% heat-inactivated fetal bovine serum (FBS) (Hyclone, Logan, Utah) and antibiotics in 75-cm^2 flasks (Corning) at 37 °C in 5% CO_2 humidified air. The cultured NUB-6 cells were mainly neuroblast-like cells with a small percentage of epitheloid-like and spindle-like cells, while EW-2 cells appeared uniformly as neuroblasts. For these studies NUB-6 and EW-2 were used between passages 18 and 76. Adherent cells were grown to subconfluency before subcloning and feeding. Cells were removed from the surface with citrate-saline, followed by trypsinization (0.25%) for 3 min and by repeated pipetting to ensure a single-cell suspension. In some experiments cells were removed without trypsinization, using glass beads, with no effect on nucleoside transport rates. Cell viability was determined either by trypan blue exclusion or with propidium iodide staining. Cell number was determined by counting with a Coulter Counter (Coulter, Hialeah, Fla). The human neuroblastoma cell line IMR-32 was obtained through ATCC and maintained as above.

Tubercidin cytotoxicity and NBTI effect. To determine the sensitivity of neuroblastoma cell lines and PBMCs to tubercidin, $1-2 \times 10^5$ neuroblastoma cells/well were placed in a microtiter plate in 0.2 ml α-medium free of nucleosides, and PHA-stimulated PBMCs (1×10^5/well) were also plated in RPMI medium supplemented with 10% FCS. Neuroblastoma cells were allowed to adhere for 24 h prior to further experimentation. Graded concentrations of tubercidin in the presence of absence of NBTI were added for another 24 h. Thereafter, the medium was replaced with valine – threonine – leucine (VTL)-free medium, and the cells were pulsed with [^{14}C]valine – threonine – leucine (0.1 μCi/ml) for 4 h. Cells were harvested with a semi-automated cell harvester (Cambridge Technology, Inc.), and the radioactivity was measured in a Beckman liquid scinitillation counter (model LS2800). The results were expressed as mean counts per minute. To assay the effect of the drug on DNA synthesis [^{3}H]thymidine uptake was also determined in the presence or absence of tubercidin and measured as described above.

Stem cell colony formation assay. The clonogenic assay reported is a modification of the basic two-stage system substituting a low-gelling-temperature agarose for agar. Essentially, the system consists of a 1-ml bottom layer of 1.5% low-gelling-temperature agarose (SeaPrep 15/45, FMC colloids, Rockland, Mass) in McCoy's medium with 10% FBS and 5 μg/ml insulin in a gridded 35-mm dish (Lux, Miles Laboratories, Naperville, Ill). The top layer consisted of 1 ml 1% agarose in the same medium containing the cell fraction. For determination of cloning efficiency (CE) and subsequent drug sensitivity studies, monolayer cultures of NUB-6 and EW-2 cells were first dispersed into a single-cell suspension with citrate saline and 0.25% trypsin-EDTA (Gibco laboratories, Grand Island, NY). The trypsin was neutralized with FBS, and the cells were resuspended in complete cloning medium. Cell viability, as determined by trypan blue exclusion, was >95% in all experiments. A range of 10^3 to 10^6 (2.5 or 5×10^4 for most assays) cells per ml was plated in triplicate. The cultures were maintained in 5% CO_2/95% air at 37 °C in high humidity. Cultures were fixed with 1% glutaraldehyde and 4% formaldehyde after 1–3 weeks, and colonies containing a minimum of 30 cells were enumerated.

NBTI binding assay. Binding of labeled NBTI was performed as described by Cass et al. [4] (see also [5]). Cells (2×10^6) were incubated in a final volume of 0.5 ml serum-free $NaHCO_3$-free medium containing 20 m*M* Hepes (pH 7.4) in the presence of different concentrations of [^{3}H]NBTI, for 30 min at 22 °C. At the end of the incubation period, cells were layered over 0.8 ml silicon oil paraffin mixture (specific gravity 1.03 g/ml) in 1.5-ml microcentrifuge tubes and immediately centrifuged for 30 s (Eppendorf microcentrifuge, model 5412). The supernatant was discarded and the cell pellet was dissolved in 5% Triton-X100, after which the radioactivity was assayed by liquid scintillation counter (Beckman). The difference between the amount of [^{3}H]NBTI bound in the presence and absence of NBTI was calculated as the specific NBTI binding.

Effect of NBTI on nucleoside transport. Transport rates were measured at 22 °C by an initial rate method as described by Cass et al. [4] (see also [11]). Briefly, 0.4 ml transport medium consisting of $NaHCO_3$-free Fisher's medium containing 20 m*M* Hepes (pH 7.4), [^{3}H]thymidine (7 Ci/mmol), and graded concentrations of NBTI were placed in glass tubes. Transport of thymidine was started by rapid addition of 0.1 ml transport medium containing 2×10^6 cells, for exactly 15 s. The reaction was stopped by the addition of 10 m*M* cold thymidine followed by layering over a 0.8 ml silicon oil – paraffin (specific gravity 1.03 g/ml) mixture in 1.5-ml microcentrifuge tubes and immediate centrifugation for 1 min. Background values were determined by simultaneous addition of labeled and excess cold thymidine to the cells. Brief trypsinization did not affect nucleoside transport rates in neuroblastoma cells.

CFU-C colony formation assay. The CFU-C assay was performed as described by Iscove et al. [10]. Briefly, nucleated BM cells (10^5) were cultured in 0.8% methylcellulose, 30% FBS, 20% colony-stimulating activity (CSA) (which was prepared from peripheral blood leukocytes of a normal volunteer as previously described [2] and nucleoside-free alpha medium. The culture mixture was placed in 35-mm petri dishes in duplicate and maintained at 37 °C, with 5% CO_2 in air, in a humidified atmosphere. Colonies were counted after 14 days using an inverted microscope. A CFU-C colony was defined as a cluster of 20 or more cells consisting of granulocytes, monocyte-macrophages, or both. Microscopically, granulocytic colonies could be easily distinguished from macrophage colonies because of the smaller cells and tighter clustering. Individual colonies were picked and spread on glass slides, and cells were analyzed using Wright stain and nonspecific (alpha naphthylbutyrate) esterase staining [24].

Results

Tubercidin toxicity and NBTI effect on neuroblastoma cells and on peripheral blood lymphocytes

We examined the effect of tubercidin on amino acid incorporation into proteins in neuroblastoma cells and in PHA-

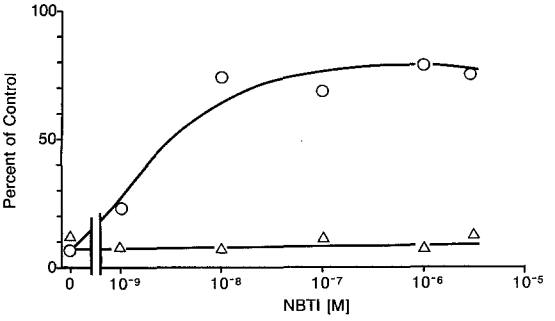

Fig. 1. Effects of tubercidin and NBTI on protein synthesis in PHA-stimulated peripheral blood lymphocytes and in EW-2 neuroblastoma cells. Neuroblastoma cells or PHA-stimulated PBMC were incubated in the presence of 10^{-6} *M* tubercidin (a concentration that inhibited over 90% of lymphocyte or EW-2 growth) in the presence of the indicated NBTI concentrations; after 3 days the rate of protein synthesis was measured using a 4-h pulse of radioactive valine, threonine, and leucine as described in the *Methods* section. O———O PHA-stimulated lymphocytes; (▲———▲, EW-2 neuroblastoma cells. Results are presented as percentages of incorporation into control cultures without any additions (16 625 cpm for PBMCs and 14 800 cpm for EW-2 cells)

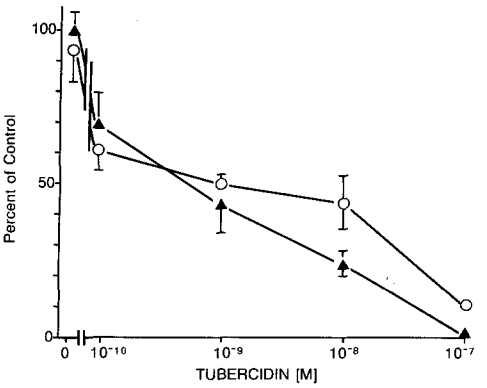

Fig. 2. Effect of tubercidin and NBTI on neuroblastoma colony formation. Neuroblastoma cells (2.5×10^4/plate) were grown in the presence of 10^{-10} – 10^{-7} *M* tubercidin with 10 μ*M* NBTI (▲———▲), or without NBTI (O———O). Colony formation in agarose was assessed as described in *Materials and methods*. Data are expressed as percentages of growth in control dishes. NBTI by itself had a minimal effect on colony formation. Control values for neuroblastoma colony formation were 3345 colonies/-5×10^{-4} cells (+NBTI) and 3586 colonies/5×10^4 cells (−NBTI)

stimulated PBMCs, and determined the protective effect of the nucleoside transport inhibitor NBTI on these cells. Because tubercidin may affect the thymidine pool and NBTI may alter the transport of thymidine into the cells, we used radiolabeled valine, threonine, and leucine to measure protein synthesis rather than [^{3}H]thymidine incorporation into DNA, as index of cell growth. Control experiments using cell numbers and cell viability gave similar results. Tubercidin (10^{-6} *M*) completely inhibited protein synthesis in neuroblastoma NUB-6 cells, and the addition of NBTI over a wide range of concentrations did not protect neuroblastoma cells against tubercidin toxicity (Fig. 1). In contrast, PBMCs treated with tubercidin were rescued by 10^{-8} *M* NBTI to a highly significant degree. Higher concentrations of NBTI caused a small increase in the rescue of PBMC.

Effects of tubercidin and NBTI on neuroblastoma colony formation

Addition of tubercidin to neuroblastoma cells grown in agarose caused inhibition of colony formation over a wide range of concentrations (50% inhibition observed at 10^{-9} *M* tubercidin; Fig. 2). A high NBTI concentration (10^{-5} *M*) did not protect neuroblastoma colony formation against tubericidin cytotoxicity (similar results were observed with two other neuroblastoma cell lines).

NBTI protection against tubercidin inhibition of CFU-C colony formation

There is a clear dose-related effect of tubercidin on CFU-C growth of bone marrow cells (Fig. 3). In contrast to neuroblastoma cells, the addition of NBTI completely protects CFU-C colony formation at low tubercidin concentrations and partially protects against high tubercidin concentrations.

Nucleoside transport and binding assays. To determine whether the lack of sensitivity of neuroblastoma cells to NBTI is the result of an altered nucleoside transport system, we assayed both binding of NBTI and transport inhibition by NBTI. Analysis of the binding of radioactive NBTI to neuroblastoma cells have shown that EW-2 and IMR-32 cells have about 54 200 and 120 450 binding sites per cell, respectively (Fig. 4). In the neuroblastoma cells tested, about 60% inhibition of [H^3]thymidine transport was noted at a concentration of 10^{-5} *M* NBTI (Fig. 5), indicating the existence of a nucleoside transport component insensitive to NBTI inhibition. In comparison, about 90% of thymidine transport into HeLa cells was inhibited at a concentration of 10^{-9} *M* NBTI [14].

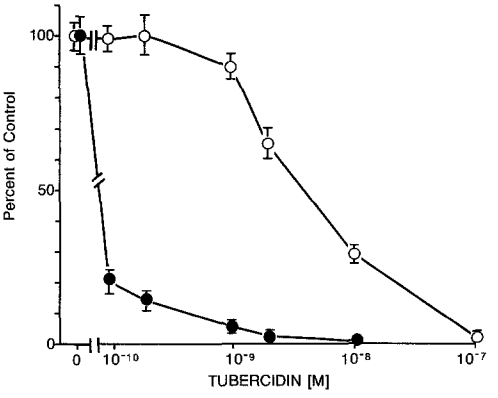

Fig. 3. Protection by NBTI against inhibition of CFU-C colony growth by tubercidin. Bone marrow cells (10^5/plate) were grown in the presence of 30 μ*M* NBTI (O———O) or without NBTI (●———●) and the indicated concentrations of tubercidin. The formation of CFU-C colonies was determined as described in *Materials and methods*. Data are expressed as percentages of growth in control dishes without tubercidin. Control values for CFU-C colony formation were 60 colonies/10^5 cells

Incorporation of [^{3}H]thymidine into intracellular pools of nucleotide mono-, di-, and triphosphate in neuroblastoma cells was similar in the presence or absence of 30 μ*M* NBTI, regardless of the time interval between NBTI administration and [H^3]thymidine pulsing (data not shown).

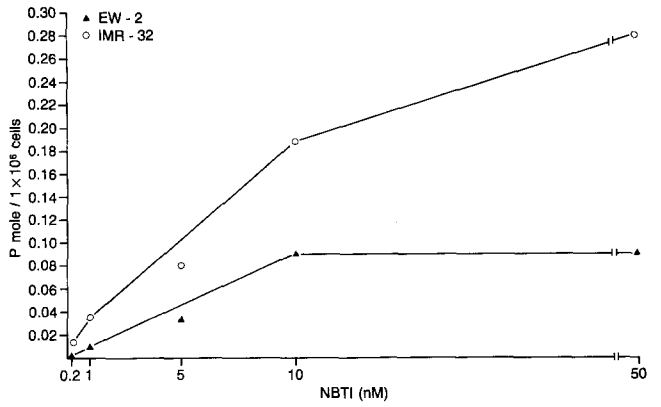

Fig. 4. NBTI binding to neuroblastoma cell-lines EW 2 and IMR-32. Cells (2×10^6) were incubated in Fisher's transport medium for 30 min in the presence of different concentrations of [^{3}H]NBTI. Specific binding was assayed as described in *Materials and methods*. The number of binding sites was estimated from the maximal binding achieved and saturating NBTI concentration (10 μM)

Discussion

NBTI administration in vivo or to cultured cells can protect against the cytotoxicity of various nucleoside analogues by inhibiting their transport across the cell membrane [15, 20–21]. In the present work we show that peripheral blood mononuclear cells and bone marrow CFU-C colonies can be protected against tubercidin toxicity by NBTI (Figs. 1 and 3). In contrast, the three neuroblastoma cell lines were not protected by high concentrations of NBTI, as shown by both the clonogenic assay and the incorporation of amino acids into newly synthesized proteins (Figs. 1 and 2). In all three neuroblastoma lines we demonstrated the existence fo numerous functional nucleoside transport sites, as estimated by site-specific NBTI binding. The number of the nucleoside transport sites on neuroblastoma line was consistent with values reported in other cultured cells [27]: 3117/lymphoblast (ALL), 330000/RPMI (B cell lymphoblastoid line), 150000/HeLa (human cervical carcinoma), and 69800/RC2a (monoblast from AML). However, there are no reports on nucleoside-binding sites in other human solid tumors. In contrast to many other cultured cells, the transport of nucleosides into neuroblastoma cells was only slightly inhibited by NBTI

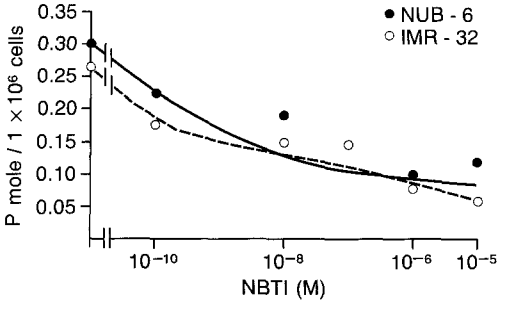

Fig. 5. Effect of NBTI on [H^3]thymidine transport in neuroblastoma cells. Cells (2×10^6) were incubated for 15 s in Fisher's transport medium in the presence of graded concentrations of NBTI, and the rate of [H^3]-thymidine transport determined as described in *Materials and methods*

(Fig. 5). Incorporation of thymidine into nucleotide mono-, di-, and triphosphate was similar in the presence or absence of NBTI, implying that neuroblastoma cells contain a nucleoside transport system which is insensitive to inhibition by NBTI. The existence of a nucleoside transport system partially resistant to inhibition by NBTI has been demonstrated in several cultured cells [3, 5, 21]. Mutant cells with altered sensitivity to inhibition by NBTI but with otherwise normal transport properties have also been described [5]. It has been suggested that NBTI binding occurs at transporter sites different from the substrate binding sites [5–8], and thus loss of NBTI binding may lead to insensitivity to inhibition of nucleoside transport of NBTI.

Harley et al. [9] examined the inward transport of tubercidin and adenosine in L5178 and HeLa cells and found that these permeants have high affinity (K_m, 38 μM or less) for the nucleoside transport mechanism and that NBTI blocks the transport of both permeants into these cells instantaneously. They have also shown that formation of metabolites from tubercidin is eventually abrogated by co-administration of NBTI.

Earlier reports have indicated that mice are protected against potentially lethal dosages of nebularine and other toxic nucleoside analogues by co-administration of NBTI [16, 21]. Thus, in the case of mice bearing IP implants of Ehrlich ascites carcinoma and leukemia L1210/T08, co-administration of the two drugs in optimal dosages resulted in substantial kill of neoplastic cells and long-term survivals, while administration of tubercidin alone in a single dose of 45 mg/kg caused death in about 90% of BIOD2F1 mice. Co-administration of NBTI prevented these injuries and shifted the LD_{50} of injected tubercidin to higher values [13].

The ability of primary neuroblastomas [1, 26] and derived cell lines to form colonies in agar or agarose with relatively high plating efficiencies has permitted studies of drug sensitivity in this tumor [23]. Johnson and Glaubiger found a reasonable correlation between [^{3}H]thymidine uptake and colony growth in agar for four human neuroblastoma cell lines exposed to a series of therapeutic drugs [12]. Although similar correlations were found with other tumor lines [17, 25, 28], greater differences between primary tumors and cell lines appeared at the ID_{90} level [14]. The use of primary cell lines in this study provides a suitable cell culture model for the study of nucleoside cytotoxicity for potential use in vivo.

The data presented have demonstrated the use of a short-term precursor incorporation assay and a long-term clonogenic assay in the assessment of combined-agent chemotherapy efficacy and the effect on tumorigenicity of neurblastoma cells. It was also shown that in vitro "host protection" with nucleoside transport inhibitors such as NBTI may allow an effective therapy with otherwise toxic dosages of nucleoside analogues such as tubercidin in patients with neuroblastoma. Thus, at the clinically effective dose of 10^{-9} M tubercidin, neuroblastoma growth may be abrogated whilst the normal hematopoietic component remains protected by NBTI.

References

1. Ablett GA, Smith PJ, Sheridan JW, Lihou MG (1984) Limitations of the agar colony forming assay of the assessment of pediatric tumors. Br J Cancer 50: 541

2. Amato D, Freedman MH, Saunders EF (1976) Granulopoiesis in severe congenital neutropenia. Blood 47: 531
3. Beh JA (1983) Nitrobenzylthioinosine insensitive uridine transport in human lymphoblastoid and murine leukemia cells. Biochem Biophys Res Commun 110: 417–423
4. Cass CE, Dahlig E, Lau EY, Lynch TP, Patterson ARP (1979) Fluctuation in nucleoside uptake and binding of the inhibitor of nucleoside transport, nitrobenzylthioinosine, during the replication cycle of HeLa cells. Cancer Res 39: 1245–1252
5. Cohen A, Leung C, Thomson E (1986) Characterization of mouse lymphoma cells with altered nucleoside transport. J Cell Physiol (in press)
6. Dahlig-Harley E, Eilam Y, Petterson ARP, Cass CE (1981) Binding of nitrobenzylthioinosine to high affinity sites: on the nucleoside transport mechanism of HeLa cells. Biochem J 200: 295–305
7. Eilam Y, Cabantchik I (1977) Nucleoside transport in mammalian cell membranes: a specific inhibitor mechanism of high affinity probes. J Cell Physiol 92: 185–202
8. Heichal O, Ish-Shalom D, Koren R, Stein WD (1978) The kinetic dissection of transport from metabolic trapping during substrate uptake by intack cells. Uridine uptake by quiescent and serum activated Nil 8 hamster cells and their murine sarcoma virus-transformed counterparts. Biochim Biophys Acta 551: 169–186
9. Harley ER, Patterson ARP, Cass CE (1982) Initial rate kinetics of the transport of adenosine and 4-amino-7-(β-D-ribofuranosyl) pyrrol [2,3-d]pyrimidine (tubercidin) in cultured cells. Cancer Res 42: 1289–1295
10. Iscove NN, Senn JS, Till JE, McCulloch EA (1971) Colony formation by normal and leukemic human marrow cells in culture: effect of conditioned medium from human leukocytes. Blood 31: 1
11. Jarvis SM, Young JD, Ansay M, Archibald AL, Harkness AR, Simmonds RJ (1980) Is inosine the physiological energy source of pig erythrocytes? Biochim Biophys Acta 597: 183
12. Johnson GE, Glaubiger DC (1983) Correlation of cellular tritiated thymidine incorporation with soft agar clonogenicity in chemosensitivity testing of human neuroblastoma cells. Cancer Treat Reports 67: 163–168
13. Kollassa N, Jakobs ES, Buzzel GR, Patterson ARP (1984) Manipulation of toxicity and tissue distribution of tubercidin in mice by nitrobenzylthioinosine 5'-monophosphate. Biochemical Pharmacology 31(10): 1863–1874
14. Lanzon JG, Patterson ARP (1977) Binding of the nucleoside transport inhibited nitrobenzyl thioinosine to HeLa cells. Mol Pharmacol 13: 833–891
15. Lynch TP, Paran JH, Patterson ARP (1981) Therapy of mouse leukemia L1210 with combination of nebularin and nitrobenzyltheoinozine-5'-monophosphate. Cancer Res 41: 562–565
16. Lynch TP, Jacobs ES, Paran JH, Patterson ARP (1981) Treatment of mouse neoplasm with high doses of tubercidin. Cancer Res 41: 3200–3204
17. Morgan D, Freshney RI, Darling JL, Thomas DGT, Celik F (1983) Assay of anticancer drugs in tissue culture: cell cultures of biopsies from human astrocytoma. Br J Cancer 47: 2054
18. Nitscke R, Caugir A, Crist W, Berry DH (1980) Intensive chemotherapy for metastatic neuroblastoma, a Southwest Oncology Group study. Med Pediatr Oncol 8(3): 281
19. Parker Re, Brown PR (1973) Incorporation of nucleosides into the nucleotide pools of human erythrocytes. Biochemistry 12: 3294–3302
20. Patterson ARP, Yang SF, Lau EY, Cass CE (1977) Low specificity of the nucleoside transport mechanism of RPMI G410 cell. Mol Pharmacol 16: 900–908
21. Patterson ARP, Paran H, Yang SE, Lynch TP (1979) Protection of mice against lethal dosages of nebularin by nitrobenzyl-thionosine. Cancer Res 39: 3607–3611
22. Plagemann PGW, Wohlhucter RA (1984) Nucleoside transport in mamalian cells. Multiple forms with different sensitivity to inhibition by nitrobenzyl thioinosine or hypoxanthine. Biochim Biophys Acta 773: 39–52
23. Suhadolnik RJ (1979) Nucleosides as biological probes. Wiley, New York, pp 158–169
24. Tucker SB, Pierre RV, Jordan Re (1977) Rapid identification of monocytes in a mixed mononuclear cell preparation. J Immunol Method 14: 267
25. Twentyman PR, Walls GA, Wright KA (1984) The response of tumor cells to radiation and cytotoxic drugs – a comparison of clonogenic and isotope uptake assays. Br J Cancer 50: 625–631
26. Von Hoff DD, Casper J, Bradley E, Trent JM, Hodach A, Reichert C, MaKuch R, Altman A (1980) Direct cloning of human neuroblastoma cells in soft agar culture. Cancer Res 40: 3591–3597
27. Wiley JS, Jones SP, Saywer WH, Patterson ARP (1982) Cytosine arabinoside influx and nucleoside transport site in acute leukemia. J Clin Invest 69: 479–489
28. Wilson AP, Ford CHJ, Newman CE, Howell A (1984) A comparison of three assays used for the in vitro chemosensitivity testing of human tumor. Br J Cancer 49: 57

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