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Transport of 2′-deoxycoformycin in human leukemic and lymphoma cells

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The nucleoside analog 2′-deoxycoformycin (dCF)* has an established place in the treatment of indolent lymphoid malignancies such as hairy cell leukemia [1–3], T-cell lymphoid malignancy [4] and Lennert's lymphoma [5]. Deoxycoformycin acts by inhibiting adenosine deaminase, thus elevating plasma adenosine and deoxyadenosine levels [6]; it is thought that deoxyadenosine mediates the lympholytic action of the drug. The initial step in the action of dCF is its transport into the cell, a process which is rate-limiting in the inactivation of human erythrocyte adenosine deaminase [7]. In red cells, the transport of dCF can be largely inhibited by the nucleoside analog nitrobenzylthioinosine (NBMPR), which suggests that dCF is a substrate for the equilibrative nucleoside transporter. In this study, the transport and accumulation of the nucleoside dCF have been studied in cells from patients with both acute and chronic leukemia, as well as in lymphocytes of thymic origin against which this drug is active.

Materials and Methods

Materials. 2′-Deoxycoformycin was obtained from the National Cancer Institute (Bethesda, MD) and NBMPR from the Sigma Chemical Co. (St. Louis, MO). Nucleosides were tritiated by Moravek Biochemicals (Brea, CA) and stored at −30° in 50% ethanolic solution. [^{3}H]NBMPR was purified on HPLC by Dr. Alan Paterson, University of Alberta, Canada.

Stability and purification of [^{3}H]*dCF.* Unlabeled dCF ran as a single peak (retention time = 11–13 min) on reverse phase HPLC (Waters C18 μBondapak Rad Pak column) with a mobile phase of 13% methanol in 10 mM sodium phosphate buffer, pH 7.0, and a flow rate of 1 mL/min. Because [^{3}H]dCF showed breakdown on storage it was repurified by HPLC on the day of each experiment. Concentrations of [^{3}H]dCF were measured spectrophotometrically using a molar extinction coefficient of 8.1×10^3 at a wavelength of 283 nm [8]. Specific activity of [^{3}H]dCF was about 5000 cpm/pmol.

Source of cells. Thymocytes were isolated from fresh thymuses as previously described [9], while leukemic and lymphoma cells were separated from peripheral blood using Ficoll–Paque. Human myelomonocytic RC2a leukemic cells were cultured using RPMI 1640 medium with 2 mM glutamine, 20 mM HEPES, 20 mg/L gentamycin sulfate and 10% fetal bovine serum.

Measurement of [^{3}H]*dCF uptake rates.* Cells were resuspended to between 1 and 2×10^7 cells/mL in RPMI 1640 medium with 20 mM HEPES (pH 7.4) alone or with concentrations of NBMPR between 0.5 and 30 μM and preincubated at 37° for 15 min. Measurement of dCF uptake rates was commenced by adding [^{3}H]dCF to cells to give a final concentration of 1 μM. After incubations of between 10 and 120 min, uptake was terminated by spinning cells in 1-mL aliquots of suspension through 300 μL of phthalate oil. Cell pellets were processed and counted as previously described [10]. Nonenzymatic breakdown of [^{3}H]dCF was 1–2%/hr at 37°.

Statistics. Results are summarized as mean ± 1 SD unless otherwise indicated.

Results

Influx of dCF into leukemic myeloblasts. Two components of equilibrative nucleoside transport have been described in cultured cells [11, 12]. The first is sensitive to inhibition by nanomolar NBMPR ("NBMPR-sensitive") while the other is only inhibited by micromolar concentrations of NBMPR ("NBMPR-insensitive"). The highest uptake of [^{3}H]dCF (1 μM) was into cultured RC2a myeloblasts at a rate which was linear over 2 hr at 37° at 8.2 ± 2.2 (N = 3) pmol/10^7 cells/hr (Fig. 1). Transport was inhibited 75% (N = 2) by 0.5 μM NBMPR and 86 ± 7% (N = 3) by 15 μM NBMPR. Uptake of [^{3}H]dCF (1 μM) was also measured in thymocytes from fresh thymus and blast cells from patients with leukemias and lymphomas. In all cell types, [^{3}H]dCF uptake was linear with time up to 2 hr and was largely (73 ± 12%, N = 10) inhibited by 15 μM NBMPR (Table 1). The lowest rates of [^{3}H]dCF uptake were by lymphocytes from patients with B-cell CLL which took up the analog at only 0.16 ± 0.07 (N = 3) pmol/10^7 cells/hr (Table 1).

Correlation of [^{3}H]*dCF uptake with density of nucleoside transport sites.* Equilibrium binding of the ligand [^{3}H] NBMPR was used to estimate the maximal number of nucleoside transport sites per cell [10], and these values were compared to rates of [^{3}H]dCF uptake measured in parallel. The maximal number of nucleoside transport sites varied from 100,800 ± 19,700 (N = 10) in cultured RC2a cells to 1,190 ± 125 (N = 3) sites/cell in lymphocytes from patients with CLL. Figure 2 shows that the rate of [^{3}H] dCF uptake correlated closely with the density of nucleoside transport sites (N = 12; $r = 0.99$; $P < 0.001$). NBMPR-

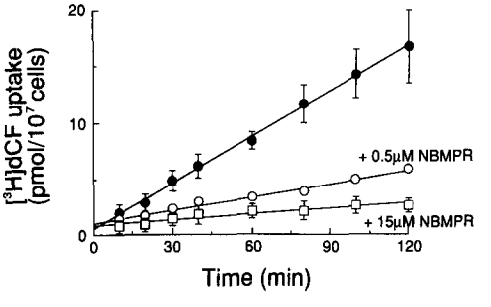

Fig. 1. Time course of [^{3}H]dCF uptake by cultured leukemic RC2a myeloblasts at 37°. Cells were preincubated in the absence (●) and presence of either 0.5 μM (○) or 15 μM (□) NBMPR before adding [^{3}H]dCF to give a final concentration of 1 μM. Means ± 1 SEM of three separate experiments are shown except for the data in the presence of 0.5 μM NBMPR which are the mean of two separate experiments.

* Abbreviations: dCF, 2′-deoxycoformycin; NBMPR, nitrobenzylthioinosine; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkins lymphoma; and CLL, chronic lymphocytic leukemia.

Table 1. Comparison in human leukemic and lymphoma cells of the number of nucleoside transport sites per cell with the rates of uptake of 1 μM [^{3}H]dCF (with and without 15 μM NBMPR)

Cell type	NBMPR binding (sites/cell)	dCF uptake rate (pmol/10^7 cells/hr)		
		−NBMPR	+NBMPR	% Inhibition
AML	27,600	2.10	0.72	65.7
AML	8,280	0.60	0.17	72.0
AML	500	0.16	0.07	58.8
Thymocytes (Thymus)	26,070	1.56	0.21	86.5
T-ALL	16,300	0.72	0.24	66.7
T-ALL	9,900	0.65	0.25	62.2
B-ALL (Burkitt's)	26,500	1.56	0.50	67.7
B-NHL	6,600	0.77	0.22	71.2
CLL	1,260	0.13	ND*	
CLL	1,050	0.24	0.00	100
CLL	1,270	0.12	0.03	77.5

* Not determined.

sensitive [^{3}H]dCF uptake rates were also highly correlated with nucleoside transport site numbers ($N = 11$, $r = 0.98$; $P < 0.001$). Influx of [^{3}H]dCF into lymphoid blast cells of T-phenotype fitted closely on the correlation line established for all hemopoietic cell types (Fig. 2). A comparison of [^{3}H]dCF uptake rate with [^{3}H]cytosine arabinoside influx at 1 μM of each permeant was performed in cultured RC2a cells. [^{3}H]dCF influx was only 0.1% of the concurrent [^{3}H] cytosine arabinoside influx.

Discussion

The characteristics of [^{3}H]dCF transport have been reported only in human erythrocytes which take up this analog by the equilibrative, nucleoside transporter which is inhibited by nanomolar NBMPR [7]. Influx of dCF into erythrocytes is slow and consistent with the low affinity of dCF demonstrated for its erythrocyte transporter. Deoxycoformycin uptake has been measured indirectly in cultured leukemia cell lines by monitoring the inactivation rate of intracellular adenosine deaminase [13, 14], and dCF uptake rates were reported as 0.2% of the rate observed with adenosine. These studies also showed that in these cell lines dCF uptake was inhibited by both NBMPR and dipyridamole.

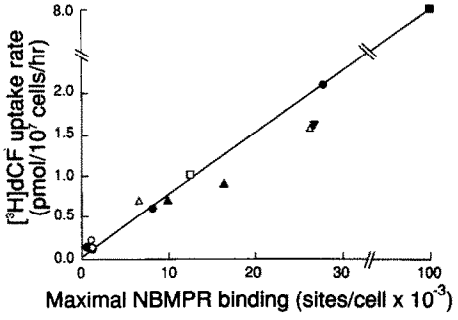

Fig. 2. Correlation between [^{3}H]dCF uptake and nucleoside transport capacity measured by the maximum number of [^{3}H]NBMPR binding sites. [^{3}H]dCF uptake rate was measured during incubation of cells with 1 μM [^{3}H]dCF. Samples were taken at intervals up to 120 min and uptake rate was calculated by linear regression. Cells were obtained from patients with B-cell chronic lymphocytic leukemia (○), non-Hodgkin's lymphoma (△), T-cell acute lymphoblastic leukemia (▲), acute myeloid leukemia (●) as well as thymocytes isolated from human thymus (▼). Values are also shown for RC2a myeloblasts (■) and for human erythrocytes (□) calculated from Ref. 7.

Similar features for dCF transport were seen in this study which measured [^{3}H]dCF uptake into fresh and cultured leukemic cells. First, the inhibitor NBMPR blocked 59–100% of [^{3}H]dCF influx. Second, the uptake of dCF (1 μM) was extremely slow and contrasted with the 800-fold faster uptake of another nucleoside analog, cytosine arabinoside. Third, a close correlation was established between the initial rate of [^{3}H]dCF uptake and the number of equilibrative nucleoside transporter sites per cell (measured by [^{3}H]NBMPR binding) over a two log range of transporter density (1,200 to 101,000 sites/cell). Other studies have reported a correlation between initial transport rate of a nucleoside and the maximum number of [^{3}H]NBMPR binding sites [10, 15]. All the above observations suggest that dCF enters hemopoietic cells predominantly via the equilibrative NBMPR-sensitive nucleoside transporter and that other types of transport such as demonstrated for azidothymidine uptake [16] are minor components of dCF entry. Furthermore some of the NBMPR-insensitive entry of [^{3}H]dCF may represent cellular uptake of nonenzymatic degradation products of [^{3}H]dCF which are formed at significant rates (1–2%/hr). Finally, the sensitivity of certain lymphoid malignancies of T-phenotype to dCF cannot be ascribed to any unique transport capacity of T-cells for this nucleoside analog.

In summary, the present study establishes that dCF enters cells of both lymphoid and myeloid origin predominantly by the NBMPR-sensitive equilibrative nucleoside transporter and that its rate of permeation is extremely slow.

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Protective effects of propylene glycol, a solvent used pharmaceutically, against paracetamol-induced liver injury in mice

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The most widely used antidote against paracetamol-induced liver injury is *N*-acetylcysteine [1], but many other compounds, such as alternative sulphydryl compounds and antioxidants, have been evaluated as potential protective agents [2, 3]. In this study we report the unexpected beneficial effects in mice of a solvent frequently used as a vehicle for water-insoluble compounds and pharmaceutical preparations.

Materials and Methods

Male C57 BL6 mice (19–23 g body wt), obtained from Charles River (Margate, Kent), were fed a standard diet (SDS R&M No. 1, Scientific Diet Service, Witham, Essex) up to 4 hr before the experiment. Access to drinking water only was then allowed until 1 hr after administration of paracetamol, when food was reinstated. Paracetamol (Sigma Chemical Co., Poole, Dorset) was dissolved in warm sterile water (25 mg/mL) by mixing on a heated stirrer and administered to the mice at a dose of 450 mg/kg by stomach tube. Propylene glycol BP (1,2-propanediol, Macarthy Medical, Romford, Essex) was administered at a dose of 4 mL/kg i.p.

Three groups of mice were studied: Group 1, mice were given paracetamol alone; Group 2, mice were given paracetamol and propylene glycol at the same time, i.e. 0 hr; Group 3, mice were given paracetamol followed by propylene glycol 3 hr later. Seven normal mice, without any treatment were used to obtain control samples.

Samples and assays. After 24 hr the mice were anaesthetized with ether and a capillary blood sample (10 μL) was collected from the tail vein. The Normotest blood clotting time (Nyegaard, Birmingham) was determined using a Fibrometer (Becton and Dickinson). The abdomen was opened and a blood sample was taken from the inferior vena cava with a heparinized syringe. The blood was kept on ice, centrifuged at 4° and the plasma