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Deoxycytidine kinase-mediated toxicity of deoxyadenosine analogs toward malignant human lymphoblasts *in vitro* and toward murine L1210 leukemia *in vivo*

(adenosine deaminase/chemotherapy)

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ABSTRACT An inherited deficiency of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) produces selective lymphopenia and immunodeficiency disease in humans. Previous experiments have suggested that lymphospecific toxicity in this condition might result from the selective accumulation of toxic deoxyadenosine nucleotides by lymphocytes with high deoxycytidine kinase levels and low deoxynucleotide dephosphorylating activity. The present experiments were designed to determine if deoxyadenosine analogs which are not substrates for adenosine deaminase might similarly be toxic toward lymphocytes and lymphoid tumors. Two such compounds, 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine, at concentrations of 3 nM and 0.15 μM, respectively, inhibited by 50% the growth of human CCRF-CEM malignant lymphoblasts *in vitro*. Each was phosphorylated in intact cells by deoxycytidine kinase, accumulated as the nucleoside triphosphate, and inhibited DNA synthesis more than RNA synthesis. Both deoxynucleosides had significant chemotherapeutic activity against lymphoid leukemia L1210 in mice.

In humans, a genetic deficiency of the enzyme adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), referred to henceforth as "deaminase," impairs the development of the immune system with resulting immunodeficiency disease (1). Although the biochemical mechanisms mediating lymphospecific toxicity in deaminase deficiency are not precisely understood, deoxyadenosine nucleotides have been implicated by several studies (2, 3). It has been proposed that lymphospecific toxicity in deaminase deficiency might result from the selective phosphorylation and "trapping" of deoxyadenosine by lymphoblasts with high deoxycytidine kinase levels and low deoxynucleotide dephosphorylating activity (4–8). It might be possible to apply current hypotheses concerning lymphospecific toxicity in deaminase deficiency in the design of agents active against human lymphoid neoplasms. There are at least two ways in which the deaminase-deficient state might be pharmacologically mimicked—through the administration of synthetic deaminase inhibitors with or without exogenous deoxyadenosine, or by giving deoxyadenosine antimetabolites that are poor substrates for deaminase but are phosphorylated by deoxycytidine kinase. The feasibility of the first approach has been demonstrated in preliminary trials in which the synthetic deaminase inhibitor deoxycoformycin produced profound and reversible lymphopenia in man (9, 10). Deaminase-resistant deoxyadenosine analogs, however, have not yet been evaluated fully for their lymphocytotoxic potential.

In the present investigations, we studied the toxic effects toward malignant human lymphoblasts of several deoxyadenosine analogs that are poor substrates for deaminase but are phosphorylated by deoxycytidine kinase. Two of the nucleosides, 2-chlorodeoxyadenosine, and 2-fluorodeoxyadenosine, inhibited the growth of a malignant human T lymphoblastoid cell line by 50% at concentrations of 3 nM and 0.15 μM, respectively. In an initial *in vivo* trial, both deoxynucleosides also had significant chemotherapeutic activity against L1210 leukemia in mice.

MATERIALS AND METHODS

Nucleosides. Initial samples of 2-chlorodeoxyadenosine, 9-β-D-arabinofuranosyl-2-chloroadenine (2-chloro-araA), 8-bromodeoxyadenosine, 8-methoxydeoxyadenosine, 2-fluorodeoxyadenosine, and 9-β-D-arabinofuranosyl-2-fluoroadenine (2-fluoro-araA) were synthesized chemically (11–13). Subsequent samples of 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine as well as the deoxynucleoside analogs modified in the C-6 position were prepared enzymatically from the respective bases by using a transdeoxyribosylase from *Lactobacillus helveticus* (ATCC 8018) and thymidine as the deoxyribose donor, as described by Cardinaud (14). The products were isolated by chromatography on AG 1-X8 (acetate form, Bio-Rad) in 10 mM NH_4OH/20% acetonitrile. In some cases, where indicated, analogs radiolabeled in the deoxyribose moiety were synthesized similarly by using deoxy[U-^{14}C]cytidine (Amersham; 470 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels) as the donor. Each product was more than 95% pure as determined by reverse-phase high-performance liquid chromatography (HPLC) (15) and yielded a single UV-absorbing spot when analyzed by thin-layer chromatography on cellulose plates in 1 M ammonium acetate or in butanol/methanol/water/ammonia, 60:20:20:1, (vol/vol). Deoxycoformycin (Pentostatin) was donated by H. W. Dion (Warner–Lambert/Parke–Davis, Detroit, MI). *Erythro*-9-[3-(2-hydroxynonyl)]adenine hydrochloride (EHNA) came from Burroughs Wellcome (Research Triangle Park, NC). Deoxytubercidin was a gift from Morris Robins (University of Alberta).

Growth Curves. The effects of deoxyadenosine analogs on the growth of the human malignant T-cell line CCRF-CEM, the B-cell line WI-L2, and variants of the latter deficient in deoxycytidine kinase and adenosine kinase (16) were performed as described with growth measured after 72 hr (5). Some cul-

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Abbreviations: deaminase, adenosine deaminase; 2-chloro-araA, 9-β-D-arabinofuranosyl-2-chloroadenine; 2-fluoro-araA, 9-β-D-arabinofuranosyl-2-fluoroadenine; araA, 9-β-D-arabinofuranosyladenine; HPLC, high-performance liquid chromatography; EHNA, *erythro*-9-[3-(2-hydroxynonyl)]adenine; ID_{50}, 50% inhibitory concentration.

tures were supplemented with either 5 μM deoxycoformycin or 10 μM EHNA; these additions had no effect on the 50% inhibitory concentrations (ID_{50}) of any of the compounds reported here.

Deaminase. The susceptibility of each analog to metabolism by deaminase was determined by incubating 100 μl of 1 mM nucleoside with either 25 units (1 unit = 1 μmol of adenosine deaminated per min) of calf spleen enzyme (Sigma) or a lymphocyte extract containing 1 unit of enzyme for up to 24 hr. The reaction mixtures were chromatographed on cellulose thin-layer plates as described above. Those compounds yielding a single UV-absorbing spot idential to the starting material after 3 hr of incubation with the deaminase were considered to be resistant to deamination.

Nucleotide Formation. CCRF-CEM or WI-L2 cells at a density of 1×10^6/ml in RPMI-1640 medium containing 10% heat-inactivated, dialyzed, fetal calf serum were incubated with 20 μM 2-fluorodeoxyadenosine for 4 hr. The cells were then centrifuged and extracted and the nucleotides were separated by HPLC on a Whatman Partisil SAX column with a gradient as described (5) or by isocratic HPLC in 0.25 M potassium phosphate, pH 3.6/0.5 M KCl before and after selective destruction of the ribonucleotides by treatment with sodium periodate (17).

In similar experiments using cells cultured with 2-chlorodeoxyadenosine we were unable to identify unequivocally a new UV absorbance peak in the triphosphate region. To maximize nucleotide detection, CCRF-CEM cells at a density of 2×10^8/ml were incubated with 2-chlorodeoxy[^{14}C] adenosine (specific activity, 270 mCi/mmol) at a concentration of 0.4 μM for 2 hr and then extracted, neutralized, and fractionated by HPLC as described above. Additionally, 1-ml fractions were collected and monitored for radioactivity. The ^{14}C-containing peak in the triphosphate region was diluted to a salt concentration of 0.1 M and applied to a 1-ml DEAE-cellulose column equilibrated with 10 mM triethanolamine at pH 4.7. After the column was washed with this buffer, the nucleotides were eluted with 1 M triethanolamine, lyophilized, and dephosphorylated enzymatically as described by Moore and Hurlbert (18). The resulting nucleosides were reanalyzed by reverse-phase HPLC on a Waters μBondapak C_{18} column (Waters Associates) in the presence of authentic unlabeled 2-chlorodeoxyadenosine (15).

Effects on DNA and RNA Synthesis. The effects of 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine on DNA and RNA synthesis in the CEM malignant T-cell line was performed as described for deoxyadenosine, using [6-^{3}H]thymidine and [5-^{3}H]uridine incorporation into acid-precipitable material as a measure of DNA and RNA synthesis, respectively (5). In one experiment the effect of 2-chlorodeoxyadenosine on DNA synthesis in mice was also assayed. Six-week-old BALB/c mice were injected with nucleoside (100 mg/kg) via the intraperitoneal route; control mice received saline only. Either 1 or 5 hr later they were injected again with 10 μCi of [6-^{3}H]thymidine. Ten minutes later they were sacrificed, and the thymus, spleen, and intestine were removed, weighed, homogenized in water, and extraced with trichloroacetic acid at a final concentration of 10%. After centrifugation and washing with 10% trichloroacetic acid, the precipitate was boiled for 60 min in 10% trichloroacetic acid, and the supernatant was assayed for radioactivity in a liquid scintillation counter.

Activity of 2-Chlorodeoxyadenosine and 2-Fluorodeoxyadenosine Against Leukemia L1210 *in Vivo*. Increasing numbers of L1210 ascites cells were implanted intraperitoneally in BALB/c × DBA/2 female mice (hereafter referred to as CDF1 mice). Groups of mice inoculated with 10^5 cells also received intraperitoneal injections of the nucleosides dissolved in isotonic saline, either once a day for 6 days beginning on day 1 or at 3-hr intervals on days 1, 5, and 9 (19). Surviving mice were observed for 60 days. Dying animals were examined for ascites and splenomegaly to determine the number of deaths not associated with leukemia (20).

RESULTS

Effect of Deaminase. Thirteen deoxynucleosides were screened: 2-chlorodeoxyadenosine, 2-fluorodeoxyadenosine, 2-chloro-araA, 2-fluoro-araA, 6-methylmercaptopurine deoxyriboside, 6-methylpurine deoxyriboside, purine deoxyriboside, N^6-allyldeoxyadenosine, N^6-(Δ^2-isopentenyl)deoxyadenosine, N^6-dimethyldeoxyadenosine, 8-bromodeoxyadenosine, 8-methoxydeoxyadenosine, and deoxytubercidin.

Only 2-fluorodeoxyadenosine, 2-fluoro-araA, N^6-(Δ^2-isopentenyl)deoxyadenosine, and N^6-allyldeoxyadenosine yielded new UV-absorbing spots when analyzed by thin-layer chromatography after incubation with either calf spleen deaminase or human lymphocyte extract. However, even with these analogs, prolonged incubation periods were required and the rate of product formation was estimated to be $\simeq$0.1% of the rate with deoxyadenosine. Similar results have been reported with 2-fluoroadenosine and N^6-(Δ-isopentenyl)adenosine (21, 22).

Toxicity Toward Human Lymphoblasts. Six of the 13 deoxynucleosides inhibited the growth of human lymphoblastoid cell lines by 50% or more at concentrations of 10 μM or less (Table 1). The two most toxic compounds, 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine, were more potent than

Table 1. Toxicity of deaminase-resistant deoxyadenosine analogs toward lymphoblasts

	ID_{50}, μM				
Compound	T lymphoblasts	B lymphoblasts	AKase-deficient B lymphoblasts	dCKase-deficient B lymphoblasts	AKase-, dCKase-deficient B lymphoblasts
2-Chlorodeoxyadenosine	0.003	0.035	0.035	>2	>2
2-Fluorodeoxyadenosine	0.15	0.12–0.35	0.12	8	12
2-Fluoro-araA	0.42	2.5	ND	ND	ND
6-Methylmercaptopurine deoxyriboside	2	1	1	>40	>40
N^6-Allyldeoxyadenosine	3	2.5	2.5	>40	>40
N^6-(Δ^2-Isopentenyl)deoxyadenosine	10	5	5	>30	>30

The human T lymphoblastoid cell line CCRF-CEM at a density of 2×10^5 cells per ml and the B lymphoblastoid cell line WI-L2 and its enzyme deficient variants at a density of 1×10^5 cells per ml were seeded in RPMI-1640 medium containing 10% fetal calf serum with or without nucleosides at varying concentrations. The number of live cells after 72 hr was plotted on semilogarithmic paper, and the concentration of nucleoside yielding 50% inhibition of growth (ID_{50}) was approximated. In similar experiments, the ID_{50} for deoxyadenosine and araA toward CCRF-CEM cells cultured with 5 μM deoxycoformycin or EHNA averaged 5 and 0.5 μM, respectively. ND, not done; AKase, adenosine kinase; dCKase, deoxycytidine kinase.

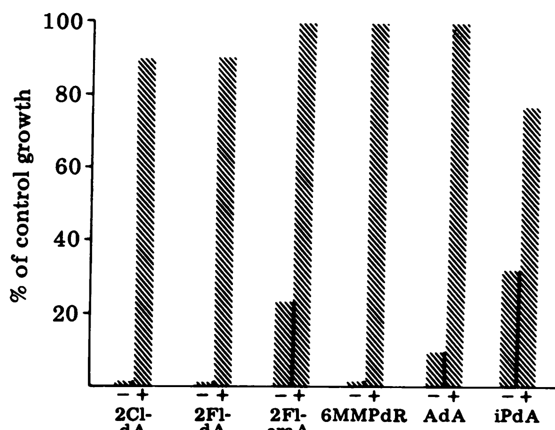

FIG. 1. Prevention of deoxynucleoside toxicity in CCRF-CEM lymphoblasts by deoxycytidine. CCRF-CEM cells were cultured at a density of 2×10^5 cells per ml in RPMI-1640 medium supplemented with 1 μM 2-chlorodeoxyadenosine (2Cl-dA), 1 μM 2-fluorodeoxyadenosine (2Fl-dA), 1 μM 2-fluoro-araA (2Fl-araA), 10 μM 6-methylmercaptopurine deoxyriboside (6-MMPdR), 10 μM N^6-allyldeoxyadenosine (AdA), or 10 μM N^6-(Δ^2-isopentenyl)deoxyadenosine (iPdA) either with (+) or without (–) 10 μM deoxycytidine. After 72 hr the growth was determined as a percentage of control growth: (live cells with nucleosides/live cells without nucleosides) × 100.

either deoxyadenosine or araA assayed similarly in cultures supplemented with 5 μM deoxycoformycin or EHNA (5, 23).

Human B lymphoblasts deficient in deoxycytidine kinase, but not adenosine kinase, were resistant to the toxic effects of the deoxynucleosides (Table 1). Similarly, human T or B lymphoblasts cultured in a medium supplemented with 10 μM deoxycytidine were protected from deoxynucleoside toxicity (Fig. 1).

Nucleotide Formation. When the human T-cell line CCRF-CEM was incubated with 20 μM 2-fluorodeoxyadenosine for 4 hr and the resulting cellular extracts were analyzed by HPLC, a new periodate-resistant UV-absorbing peak eluted in the triphosphate region, averaging approximately 75–100 pmol of nucleotide per 10^6 cells in several experiments (Fig. 2, curve a). Under similar conditions, the WI-L2 B lymphocyte line accumulated 12–30 pmol per 10^6 cells (not shown). In each case, the addition of 10 μM deoxycytidine to identically treated cultures markedly diminished nucleotide formation (Fig. 2, curve c). Moreover, the deoxycytidine kinase-deficient variant of WI-L2 failed to form detectable 2-fluorodeoxyadenosine triphosphate (<1 pmol/10^6 cells).

When CCRF-CEM cells, but not WI-L2 cells, were incubated with 2-chlorodeoxy[^{14}C]adenosine labeled in the deoxyribose moiety, radioactive peaks appeared in the mono-, di-, and triphosphate regions eluting after the respective guanine nucleotides. The peak in the triphosphate region was resistant to sodium periodate treatment and was not detectable when the lymphoblasts were identically cultured in the deoxycytidine-containing medium (Fig. 3). When the radioactive triphosphate peak was dephosphorylated enzymatically and reanalyzed by reverse-phase HPLC in two experiments, 70% of the radioactivity cochromatographed with authentic 2-chlorodeoxyadenosine, none was with deoxyguanosine or deoxycytidine, and 30% eluted in the void volume. These results suggest that small quantities (approximately 40 pmol/10^8 cells) of 2-chlorodeoxyadenosine were converted to the nucleoside triphosphate.

Effects on DNA Synthesis. The toxicity of 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine toward human T

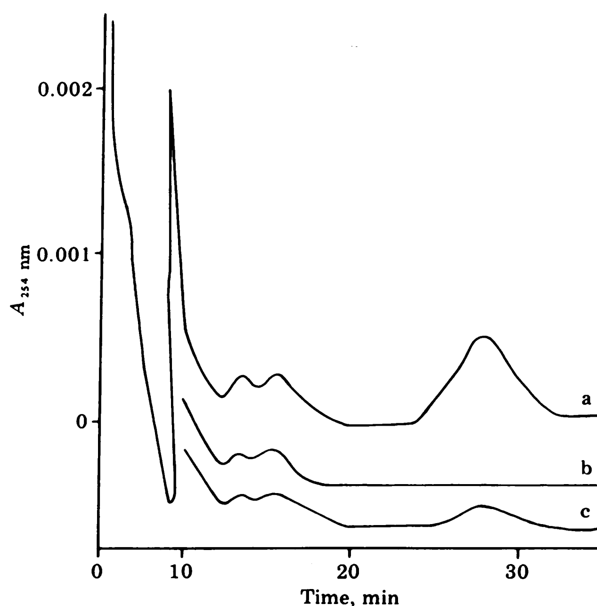

FIG. 2. HPLC of periodate-treated extracts of CCRF-CEM lymphoblasts after 4 hr of culture with 20 μM 2-fluorodeoxyadenosine (curve a), medium alone (curve b), or 20 μM 2-fluorodeoxyadenosine and 10 μM deoxycytidine (curve c). The nucleoside triphosphates from 2×10^6 cells were separated isocratically. The changing periodate-resistant peak to the right presumably represents 2-fluoro-dATP.

lymphoblasts was accompanied by inhibition of DNA synthesis more than RNA synthesis (Fig. 4). Intraperitoneal administration of 2-chlorodeoxyadenosine (100 mg/kg) to BALB/c mice also inhibited thymidine incorporation into acid-precipitable material in the thymus, spleen, and intestinal epithelium by more than 90% after 1 hr. However, the duration of this effect was short-lived, and by 5 hr the inhibition was less than 50% in all three tissues (Fig. 5). Other studies showed little effect of 2-chlorodeoxyadenosine on [5-^{3}H]uridine incorporation into acid-precipitable material in the same mouse tissues.

Chemotherapeutic Activity of 2-Chlorodeoxyadenosine and 2-Fluorodeoxyadenosine Toward L1210 Leukemia. As expected from its short-lived inhibition of DNA synthesis *in vivo*, 2-chlorodeoxyadenosine was most effective when administered via a multiple dosage schedule on days 1, 5, and 9

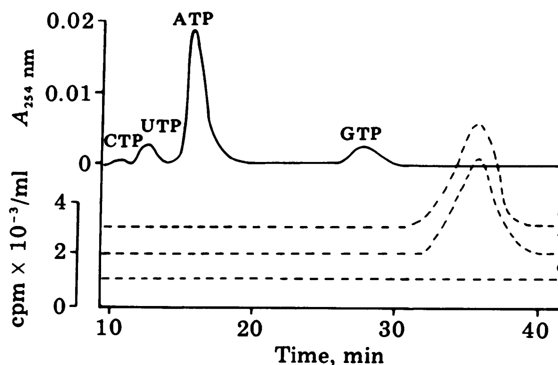

FIG. 3. HPLC of ^{14}C-labeled nucleoside triphosphates from CCRF-CEM lymphoblasts incubated with 2-chlorodeoxy[^{14}C]adenosine. Tracings a and b show the unchanged radioactive peak that appeared in the triphosphate region before and after periodate treatment of the extract. When 10 μM deoxycytidine was added to the lymphocyte incubation medium (tracing c), the new peak failed to appear.

Table 2. Activity of 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine against L1210 leukemia in CDF1 mice

Cells implanted, no.	Treatment: Type*	Treatment: Dosage, mg/kg	Treatment: Schedule	Day of death: ≤5	6	7	8	9	10	11	12	13	14	15	16	17	18	32	35	60-Day survivors/total	Median: Day of death†	Median: % increase in lifespan†
10^6	None	0	0		1	8	1													0/10	7.0	
10^5		0	0				17	3												0/20	8.0	
10^4		0	0					8	2											0/10	9.0	
10^3		0	0					1	9											0/10	10.0	
10^2		0	0							8	2									0/10	11.0	
10^1		0	0							2	7									0/10	12.0	
1		0	0								1	3					1			5/10	13.0	
10^5	2Cl-dA†	9	q3h, days 1,5,9								1	2	2		1			1	1	2/10	14.0	+75
10^5		6								2	1	4	3							0/10	12.0	+50
10^5		4							7	2	1									0/10	10.0	+25
10^5		100	qd, days 1–2	4					1	1										0/6	Toxic	
10^5		50	qd, days 1–6										3	2	1					0/6	14.5	+81
10^5	2-Fl-dA*	20	q3h, days 1,5,9						1		2	2	3		1		1			0/10	13.5	+69
10^5		10						4	5		1									0/10	10.0	+25

* 2Cl-dA, 2-chlorodeoxyadenosine; 2Fl-dA, 2-fluorodeoxyadenosine.
† Dying mice only.

after tumor implantation (Table 2). At concentrations up to 9 mg/kg body weight per injection, a graded dose–response curve was noted. There were no toxic deaths at the highest dose level, suggesting that the maximal tolerated dose had not yet been achieved. When tested via a similar schedule, 2-fluorodeoxyadenosine also had significant chemotherapeutic activity in the L1210 lymphocytic leukemia system, without demonstrable host toxicity.

DISCUSSION

The association of a genetic deficiency of deaminase with immunodeficiency and the possible importance of deoxyadenosine trapping in mediating lymphospecific toxicity in the disease prompted us to examine the toxic effects toward human lymphoblasts of several deoxyadenosine analogs that are poorly metabolized by deaminase. We identified two compounds, 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine, which were more toxic as single agents toward human lymphoblasts in tissue culture than the combination of deoxyadenosine or araA plus a deaminase inhibitor (5, 23). The toxicity of these drugs was not augmented by the addition of deaminase inhibitors to the culture medium.

Intact human lymphoblasts converted 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine to the respective triphosphates with resulting inhibition of DNA synthesis. Human B lymphoblasts deficient in deoxycytidine kinase but not adenosine kinase were resistant to the growth inhibitory effects of the deoxynucleosides and failed to form detectable 2-fluorodeoxyadenosine triphosphate. The addition of deoxycytidine to the culture medium inhibited analog nucleotide formation and permitted the cells to grow normally. These results strongly suggest that the toxicity of the deoxyadenosine analogs requires phosphorylation by deoxycytidine kinase. Because levels of deoxycytidine kinase are higher in normal and malignant lymphoblasts than in other tissues (4, 24), we predict that lymphoid tumors might be more sensitive than most normal cells to the toxic effects of these nucleosides.

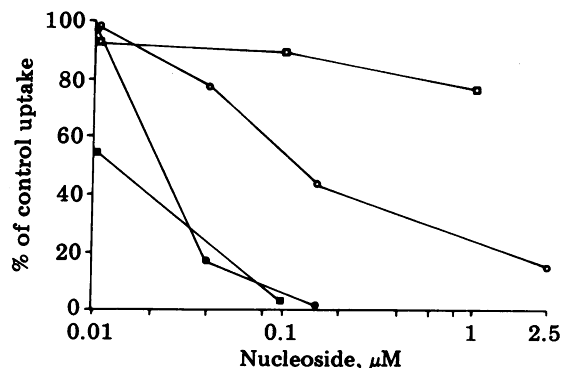

FIG. 4. Effect of nucleosides on DNA and RNA synthesis. CCRF-CEM cells at a density of 1×10^6 cells per ml in RPMI medium with 10% fetal calf serum was grown with or without 2-chlorodeoxyadenosine or 2-fluorodeoxyadenosine. After 16 hr, the incorporation of [6-^{3}H]thymidine or [5-^{3}H]uridine into acid-precipitable material was assayed. ●, 2-Fluorodeoxyadenosine, thymidine uptake; ○, 2-fluorodeoxyadenosine, uridine uptake; ■, 2-chlorodeoxyadenosine, thymidine uptake; □ 2-chlorodeoxyadenosine, uridine uptake. % of control uptake = (cpm with inhibitor/cpm without inhibitor) × 100.

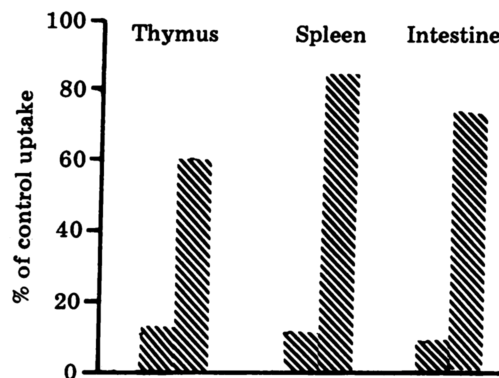

FIG. 5. Effect of 2-chlorodeoxyadenosine on *in vivo* DNA synthesis. Pairs of 6-week-old BALB/c mice received 2-chlorodeoxyadenosine (100 mg/kg) or saline alone by the intraperitoneal route. One and 5 hr later (left and right bars, respectively), each was injected with 10 μCi of [6-^{3}H]thymidine. At 10 min after that the animals were sacrificed and the incorporation of radioactivity into acid-precipitable material in various tissues was assayed. % of control uptake = (cpm/g tissue in treated animal/cpm/g tissue in control animal) × 100.

Recently, Plunkett *et al.* (25) reported that 20 μM 2-chlorodeoxyadenosine decreased dCTP formation in CCRF-CEM cells. This nucleoside concentration, however, is 10^3 times greater than that required to inhibit growth. In preliminary experiments, we have noted the incorporation into DNA of 2-chlorodeoxy[^{14}C]adenosine. The exact importance of each effect in mediating the toxic effects of the nucleoside remains to be established. It is possible, however, that one means by which deoxycytidine prevents the toxicity of this and other deoxyadenosine analogs is by restoring deoxycytidylate pools, as well as through inhibition of nucleotide formation at the level of deoxycytidine kinase.

Similar to previous results with deoxyadenosine, human malignant CCRF-CEM T lymphoblasts were approximately 10-fold more sensitive to the toxic effects of 2-chlorodeoxyadenosine than were WI-L2 B lymphoblasts (5, 6). On the contrary, the sensitivity of the two cell types to 2-fluorodeoxyadenosine differed by not more than 2-fold. Although at a nucleoside concentration of 20 μM, the T cells did accumulate more 2-fluorodeoxyadenosine triphosphate than the B cells, the sensitivity of the HPLC assay did not permit the measurement of cellular nucleotides at the actual concentrations of 2-fluorodeoxyadenosine that inhibited growth.

Initial *in vivo* studies in mice with 2-chlorodeoxyadenosine showed that inhibition of DNA synthesis in proliferating tissues after a large single intraperitoneal dose lasted less than 5 hr. Therefore, it seemed likely that the chemotherapeutic activity of this compound would require its administration at frequent intervals. In the L1210 leukemia system, the maximal tolerated dose of 2-chlorodeoxyadenosine given daily for 6 days produced an 80% increase in life-span, corresponding to a reduction by 1 order of magnitude in tumor cell burden by the end of treatment (20). When the nucleoside was given every 3 hr on days 1, 5, and 9 after tumor implantation, 4 of 10 animals survived more than 30 days without demonstrable host toxicity. The administration of 2-fluorodeoxyadenosine to tumor-bearing mice via a similar multiple dosage regimen also produced a significant, albeit lesser, chemotherapeutic response.

Unlike the toxic deoxyadenosine analogs studied here, both deoxyadenosine and araA at high concentration probably can be phosphorylated in human and mouse cells by adenosine kinase (20, 26–28). Thus, among the deoxyadenosine cogeners, a loss of reactivity with the deaminase was accompanied by a reduced reactivity with adenosine kinase in intact cells. One would therefore expect that tumors resistant to araC by virtue of a loss of deoxycytidine kinase would be crossresistant to these deoxynucleosides but not to deoxyadenosine or araA used in combination with an ADA inhibitor. Results of initial *in vivo* studies with 2-fluoro-araA in araC-resistant L1210 leukemia are consistent with this interpretation (20, 29).

In summary, we have identified several deoxyadenosine analogs that are poor substrates for adenosine deaminase but are converted to nucleoside phosphates by the lymphospecific enzyme deoxycytidine kinase, with resulting inhibition of DNA synthesis. The demonstrated activity of 2-chlorodeoxyadenosine and 2-fluorodeoxyadenosine toward L1210 leukemia *in vivo* warrants further investigation of their chemotherapeutic potential and mechanism of action.

Note Added in Proof. In a second trial, 2-chlorodeoxyadenosine at 15 mg/kg, and 2-fluorodeoxyadenosine at 40 mg/kg, each given q3h on days 1, 5, and 9, produced 5/10 and 3/10 60-day survivors, respectively, in L1210 leukemic mice.

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