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Cytostatic Effects of 2′,3′-Dideoxyribonucleosides on Transformed Human Hemopoietic Cell Lines

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SUMMARY

The 2′,3′-dideoxy analogues of cytidine, guanosine, adenosine, inosine, and thymidine have been compared for their cytostatic effects on 14 cell lines that include B lymphoblastic, T lymphoblastic, and myeloblastic lines. In all cases 2′,3′-dideoxycytidine (ddCyd) was the most toxic nucleoside, with dideoxyguanosine (ddGuo) next, and little cytostatic action by the analogues of thymidine, adenosine, or inosine. The cytostatic action of ddCyd was examined in more detail. The concentration for 50% inhibition of growth over 4 days (IC_{50}) was 0.2 to 3 μM for five T lymphoblastic lines. Although most B lymphoblastic and myeloblastic lines were less sensitive (IC_{50}, 16–70 μM), some were as sensitive as the T lymphoblastic lines. The four lines most sensitive to ddGuo (three T lymphoblastic and one B lymphoblastic) had IC_{50} values of 47–80 μM. Two lines with high sensitivity to ddCyd had levels of ddCyd triphosphate about 4–7 times higher than those found in the two least sensitive after 1-, 3-, or 24-hr exposure to 3 μM [^{3}H]ddCyd. This was associated with a much higher ratio of the diphosphate to the triphosphate in the least sensitive cells, an observation suggesting slow nucleoside diphosphate kinase action on the diphosphate of ddCyd in the resistant cells. Catabolism of the mono-, di-, and triphosphate was very slow in all cell lines, and in lines of low sensitivity disappearance of the triphosphate had a half life (~19 hr) about twice as long as in sensitive lines. This may be due to continuing slow conversion of diphosphate to triphosphate after removal of nucleoside from the medium.

In the last few years, 2′3′-dideoxyribonucleosides have been under active investigation as potential antiviral agents. In particular, they have been shown (1) to inhibit the *in vitro* infectivity and cytopathic effect on human cells of human immunodeficiency virus, the lymphotropic retrovirus and etiologic agent of acquired immunodeficiency syndrome and related diseases. The initial results with ATH8 cells indicated that the concentrations of 2′,3′-dideoxy analogues of adenosine, guanosine, cytidine, and inosine exhibited a strong antiviral effect at concentrations that were 10- to 20-fold lower than those that inhibited growth of the target cells when no virus was present (1). The concentration of 2′,3′-dideoxyribonucleosides that was required to inhibit growth of ATH8 cells by 50% was about 20 μM for ddCyd and 200 to 2000 μM for the other analogues (1).

More recently, the cytostatic action of these nucleosides on other cell lines has been investigated, and growth inhibition has been found to differ greatly from one cell line to another and in many cases to be much greater than for ATH8 (2–4). In particular, ddCyd appears to be much more toxic for some human T and B lymphoblastic lines than for cells of other origins (2, 3, 5). It was, therefore, of interest to investigate the cytostatic effect of all five 2′,3′-dideoxyribonucleosides that are commercially available on a number of transformed human cell lines. We have also found a considerable range of sensitivity among the 14 cell lines that we have examined.

These different sensitivities of human cell lines to dideoxyribonucleosides could be the result of differences between the cell lines with regard to several parameters, nucleoside transport into cells, activity of various nucleoside and nucleotide kinases, activity of catabolic enzymes such as phosphatases, sensitivity of DNA polymerases to 2′,3′-dideoxyribonucleoside triphosphates, or activity of deoxynucleotidyl transferase. Spigelman *et al.* (4) have pointed out that, of cell lines they examined, those containing deoxynucleotidyl transferase were much more sensitive to ddAdo. Ullman *et al.* (5) have shown that transport-deficient mutants of the CCRF-CEM human lymphoblastic line are relatively resistant to ddCyd. Similarly, sublines deficient in deoxycytidine kinase were much less sensitive to ddCyd (5), although sublines deficient in both deoxy-

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ABBREVIATIONS: ddCyd, 2′,3′-dideoxycytidine; ddGuo, 2′,3′-dideoxyguanosine; ddCMP, 2′,3′-dideoxycytidine-5′-monophosphate; ddCDP, 2′,3′-dideoxycytidine-5′-diphosphate; ddCTP, 2′,3′-dideoxycytidine-5′-triphosphate; ddCDP-choline, 2′,3′-dideoxycytidine 5′-diphosphocholine; ddIno, 2′,3′-dideoxyinosine; ddThd, 2′,3′-dideoxythymidine; ddAdo, 2′,3′-dideoxyadenosine; HPLC, high pressure liquid chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

cytidine kinase and adenosine kinase were able to form as much ddATP as the parental line (6) because of alternative routes to the triphosphate. Hao *et al.* (7) have concluded that factors determining the intracellular concentration of dideoxyribonucleoside triphosphate are more important for antiretroviral activity than relative inhibitory activity of the triphosphate for viral reverse transcriptase. However, because it is unknown whether the triphosphate level similarly determines cytostatic activity and if so whether rates of formation of the triphosphate or its catabolism or both are important, we have endeavored to obtain data relevant to these questions.

Experimental Procedures

Materials

Chemicals. ddCyd and CDP-choline were obtained from Sigma (St. Louis, MO); ddGuo, ddThd, and ddCDP from Pharmacia (Piscataway, NJ); ddAdo and ddIno from United States Biochemical Corp. (Cleveland, OH), and ddCTP from Bethesda Research Laboratories (Gaithersburg, MD). [5,6-^{3}H]ddCyd (6 Ci/mmol) was obtained from Moravek Biochemicals (Brea, CA). Alkaline phosphatase (2800 units/mg) was from Boehringer Mannheim (Indianapolis, IN), and phosphodiesterase I (2-6 units/mg) from *Crotalus durissus* was obtained from Sigma. ddCMP was obtained by incubating ddCTP (0.1 μmol) with 0.013 units of phosphodiesterase I in 50 mM Tris·HCl buffer, pH 8.0, at 30° for 30 min.

Cells. All cell lines (with the exceptions that follow), were grown in RPMI 1640 medium supplemented with 10% dialyzed fetal bovine serum and 1% L-glutamine. MOLT-4 and 8392 cells were grown in RPMI 1640 supplemented with 10% newborn bovine serum and 1% L-glutamine. CCRF-HS-B2 cells were grown in minimum essential medium Eagle supplemented with spinner salts, 10% dialyzed fetal bovine serum, and 1% L-glutamine. Cells were maintained at 37° in an atmosphere of 95% air/5% CO_2 at 97% humidity and were verified to be in logarithmic growth at the time of use. The cell density of cultures was determined with a Coulter counter fitted with a Channelyzer. All cultures were free of *Mycoplasma*.

Methods

Determination of inhibition of cell growth. Cells were cultured at an initial density of 3×10^4 cells/ml in 16-mm Costar cluster well plates. Each well received 100 μl of inhibitor solution in normal saline, followed by 2 ml of cell suspension. After mixing, plates were incubated for 4 days, then the contents of each well were mixed by gentle pipetting, and samples were removed for counting of cells.

Determination of nucleotide pools and rate of nucleotide catabolism. Triplicate 120-ml cultures of logarithmically growing cells at a density of 2×10^5 cells/ml were incubated with 3.0 μM [5,6-^{3}H] ddCyd (7.7×10^7 dpm) in growth medium that was modified by decreasing the serum supplement to 5% and the L-glutamine supplement to 0.5% and by the presence of 25 mM HEPES buffer, pH 7.4. After incubation for 1 hr and for 3 hr, or for 3 hr and 24 hr, 1-ml samples were removed from each flask for determination of cell density and volume and 20-ml samples for determination of nucleotide pools. The latter samples were centrifuged for 3 min at 1000 × *g* and the cell pellets were extracted at 0° with 0.5 N perchloric acid for 10 min. After centrifugation, the supernatant was neutralized with 5 N KOH/0.5 M potassium phosphate. After 10 min on ice, $KClO_4$ was centrifuged off. The supernatants were kept at −70° until analysis by HPLC on a Whatman Partisil 10 SAX column (4.6 × 250 mm) at a flow rate of 1 ml/min. Nucleotides were eluted by a gradient formed with 2.5 mM ammonium phosphate, pH 3.5 (Solvent A) and 1 M ammonium phosphate, pH 3.5 (Solvent B). The column was equilibrated with Solvent A before sample (250 μl) injection. A linear gradient was commenced 5 min after sample injection, to 100% Solvent B over 30 min. Elution times (min) for ddCyd, ddCMP, ddCDP-choline, ddCDP, and ddCTP were 3.6–4.8, 7.2–8.4, 14.4–15.6, 24.0–25.2, and 33.6–34.8 min, respectively. The eluate was collected in 1.2-ml fractions and mixed with 10 ml of ACS and the radioactivity was determined. Intracellular concentrations were calculated from the amount of radiolabeled nucleotide and total cell volume (cell number × volume/cell).

For measurement of the catabolism of nucleotides, cells were incubated with [5,6-^{3}H]ddCyd for 3 hr, as described above. At the end of the incubation period, cells were harvested and resuspended to the original volume in fresh medium of the same composition but lacking [5,6-^{3}H]ddCyd. The suspension was divided between three flasks and incubation continued at 37°. At 0, 5, and 10 hr, 1-ml and 20-ml samples were processed from each flask, as described previously.

Identification of nucleotides. Preliminary identification of ddCMP, ddCDP, and ddCTP was based on comparison of their elution times with those of authentic compounds. Because authentic ddCDP-choline was not available, the elution time of the peak believed to be due to this compound was compared with that of CDP-choline (8.5–9.5 min). To confirm the assignments, replicate neutralized extracts were treated with 1 mM $MgCl_2$ and 1 mM $ZnCl_2$ (final concentrations) and brought to pH 10.4 with 1 N NaOH and alkaline phosphatase (28 units) was added. After incubation for 30 min at 30°, the pH was lowered to 7.8 with 1 N HCl before HPLC. ddCMP, ddCDP, and ddCTP were converted to ddCyd but ddCDP-choline was unaffected. This procedure was used for all samples used in Table 4. A representative sample was also treated with phosphodiesterase I. The sample was adjusted to pH 8.9 with 1 N NaOH and 0.1 unit of phosphodiesterase was added. After incubation for 30 min at 30°, the pH was readjusted to 7.8. HPLC indicated that fractions identified as ddCyd and ddCMP were unchanged but ddCTP, ddCDP, and ddCDP-choline were completely converted to ddCMP. For further confirmation of these conclusions, the metabolites formed in a large batch of cells were partially separated by ion exchange chromatography as follows.

A 460-ml incubation of the B lymphoblastic cell line 6410 (4.45×10^5 cells/ml) with 0.3 μM [5,6-^{3}H]ddCyd (specific activity, 1.1×10^5 cpm/nmol) was harvested after 24 hr, and cell extract was prepared as described. Extract (1 ml) was chromatographed at 4° on a Whatman DE52 column (1 × 10 cm) previously equilibrated with 20 mM triethylammonium bicarbonate, pH 6.8. Fractions of 2.5 ml were collected during washing with 50 ml of the same buffer and subsequent elution by a linear gradient from 20 mM to 0.5 M triethylammonium bicarbonate, pH 6.8. Radioactivity determinations indicated elution of four peaks appearing, respectively, in fractions 3, 6–9, 42–44, and 47–50. Pooled fractions were freeze-dried and the residues were each dissolved in a small volume of cold water. Residues obtained from these peaks were found by HPLC to contain, respectively, I, ddCyd; II, ddCMP and ddCDP-choline; III, ddCDP; IV, ddCTP together with some ddCMP and ddCyd. Treatment of each of these fractions with alkaline phosphatase and with phosphodiesterase I produced the same transformations as in the case of the crude extract.

Results

Cytostatic effects of 2′,3′-dideoxyribonucleosides. Growth inhibition of four human cell lines by 200 μM dideoxyribonucleosides over a 4-day period is shown in Fig. 1. Growth of every cell line was inhibited to some extent by one or more of the nucleoside analogues. ddCyd was the most inhibitory nucleoside in every case, with ddGuo apparently equally inhibitory in a few cases and a close second in the remainder. These two nucleosides were most consistently inhibitory for the T lymphoblastic lines, but some B lymphoblastic and nonlymphoblastic lines were quite sensitive also. ddThd was the least inhibitory nucleoside, having very little cytostatic effect on several lines in each group and no major effect on any. ddIno and ddAdo were also weakly cytostatic, with 50% inhibition of growth at 200 μM in only four cases.

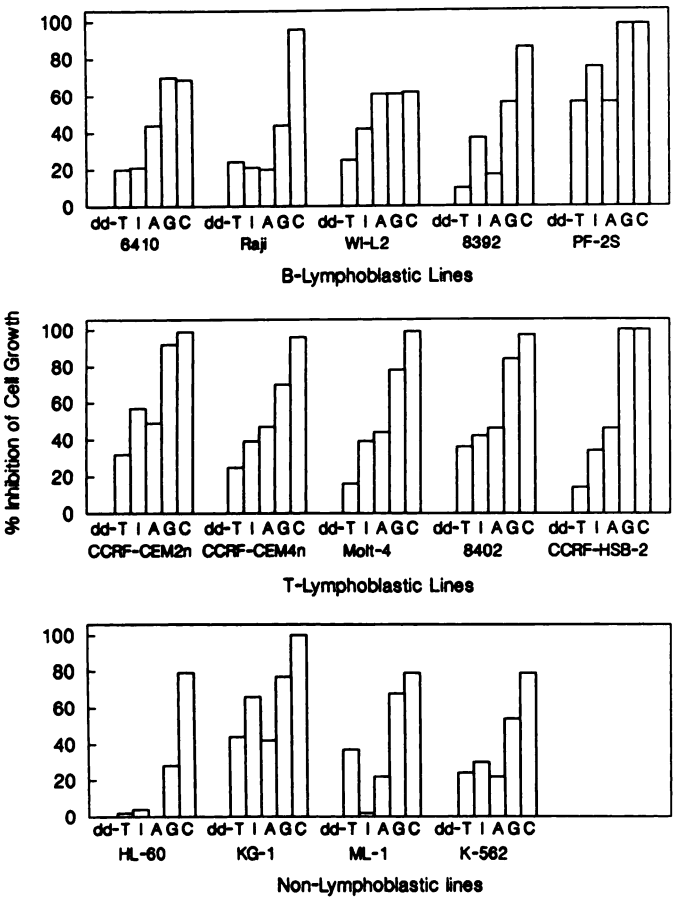

Fig. 1. Percentage of inhibition of the growth of cells of the various lines exposed over a 4-day period to 200 μM 2′,3′-dideoxynucleosides.

TABLE 1

Growth inhibition of human transformed cell lines by ddCyd

Assays were performed as described in Experimental Procedures.

	IC_{50}
	μM
B lymphoblastic lines	
6410	63
Raji	5
WI-L2	70
8392	26
PF-2S	2
T lymphoblastic lines	
CCRF-CEM (2*n*)	0.2
CCRF-CEM (4*n*)	3
MOLT-4	1.7
8402	3
CCRF-HSB-2	1
Nonlymphoblastic lines	
HL-60	25
KG-1	1.8
ML-1	35
K562	16

IC_{50} values for ddCyd and ddGuo. To obtain a more precise indication of the cytostatic effect of ddCyd and ddGuo, these nucleosides were examined for IC_{50} values with cell lines on which they produced a major inhibition of growth. Results for ddCyd are shown in Table 1. All the T lymphoblastic lines examined are very sensitive to ddCyd, with 50% inhibition of growth at concentrations in the range 0.2 to 3 μM. It was of interest that a tetraploid variant of the CCRF-CEM line was significantly and reproducibly 10-fold less sensitive than the parental line. High sensitivity is not confirmed to T lymphoblastic lines; the growth of two B lymphoblastic lines (Raji and PF-S2) and one nonlymphoblastic line (KG-1) was also inhibited 50% at concentrations in the low micromolar range. Furthermore, the growth of all the other cell lines examined was inhibited 50% at concentrations in the range 16 to 70 μM.

Four cell lines were examined for IC_{50} of ddGuo, with the results shown in Table 2. In all cases ddGuo was much less inhibitory than ddCyd, with IC_{50} values for ddGuo 20 to 400 times higher than for ddCyd. However, inhibition of growth was still quite significant at moderate concentrations of ddGuo in the case of some cell lines; for example, the IC_{50} for the PF-2S line was 47 μM.

ddCyd nucleotide pools in lines of different sensitivity. It is clear from the preceding results that there is a considerable range of sensitivity of cells to dideoxyribonucleosides among the lines examined. In the case of ddCyd, the IC_{50} values covered a 350-fold range. Intracellular concentrations of nucleotides formed from ddCyd were, therefore, examined in the two most sensitive and the two least sensitive lines in order to determine whether sensitivity correlated with intracellular ddCTP concentration after relatively short term exposures to ddCyd. The results are shown in Table 3.

It may be seen that the two sensitive lines, CCRF-CEM (2*n*) and CCRF-HSB-2, did have substantially higher intracellular concentrations of ddCTP than the two more resistant lines, WI-L2 and 6410, especially at the shorter time period. However, the ratio of ddCTP levels in the most sensitive and least sensitive lines was only from 4 to 7 whereas the ratio of IC_{50} values was from 63 to 350.

Cooney *et al.* (8) have reported that ddCDP-choline is a major metabolite of ddCyd in certain cell lines and have speculated that it may play a role in the clinically observed neurotoxicity of ddCyd. For some cell lines the intracellular concentration of ddCDP-choline in cells exposed to 1 μM ddCyd for 24 hr was as high as that of ddCDP. In our HPLC analyses of nucleotides in cells exposed for 1 or 3 hr, we did not see another metabolite peak as large as that of ddCDP. However, in a further study with cells exposed for 3 and 24 hr, we did identify ddCDP-choline and determined its concentration (Table 4). For three of the four cell lines, the intracellular level of ddCDP-choline was 20–50% that of ddCDP at both time intervals. Only in the case of CCRF-HSB-2 were the concentrations similar.

Rates of ddCyd nucleotide catabolism in most sensitive and least sensitive lines. A potential factor in determining cell sensitivity to ddCyd is the rate at which the nucleotides formed from it are broken down in the cell. This contributes to determination of the degree of exposure of the cells to ddCyd nucleotides, especially when ddCyd is present in the external milieu for a limited period. Estimates of the rate of catabolism of ddCyd nucleotides are given in Table 5. It may be seen that

TABLE 2

Growth inhibition of transformed human cell lines by ddGuo

Assays were performed as described in Experimental Procedures.

Cell line	Type	IC_{50}
		μM
CCRF-CEM (2*n*)	T lymphoblastic	80
CCRF-HSB-2	T lymphoblastic	56
8402	T lymphoblastic	75
PF-2S	B lymphoblastic	47

TABLE 3

Concentrations of intracellular ddCyd nucleotides in cells of various lines incubated with ddCyd for 1 and 3 hr

Concentration of ddCyd in the medium was 3 μM except as indicated.

Cell line	ddCMP		ddCDP		ddCTP	
	1 hr	3 hr	1 hr	3 hr	1 hr	3 hr
			μM			
CCRF-CEM (2n)[a]	0.039 ± 0.007	0.054 ± 0.000	0.52 ± 0.04	0.93 ± 0.03	0.093 ± 0.003	0.18 ± 0.01
CCRF-HSB-2[a]	ND[b]	ND	0.28 ± 0.04	0.70 ± 0.17	0.22 ± 0.03	0.43 ± 0.03
CCRF-CEM (2n)	0.09 ± 0.03	0.16 ± 0.09	1.20 ± 0.06	3.43 ± 0.14	0.59 ± 0.29	1.71 ± 0.05
CCRF-HSB-2	ND	0.34 ± 0.10	0.69 ± 0.13	4.01 ± 1.02	0.74 ± 0.14	1.51 ± 0.24
WI-L2	0.020 ± 0.014	0.42 ± 0.01	2.32 ± 0.26	4.84 ± 0.10	0.10 ± 0.07	0.41 ± 0.14
6410	0.131 ± 0.021	0.38 ± 0.05	2.37 ± 0.16	5.22 ± 0.33	0.13 ± 0.07	0.38 ± 0.20

[a] Concentration of ddCyd in the medium, 1 μM.
[b] ND, not detectable.

TABLE 4

Concentrations of intracellular ddCyd nucleotides in cells of sensitive and resistant lines incubated with ddCyd for 3 and 24 hr

Concentration of ddCyd in the medium was 3 μM.

	ddCMP		ddCDP-choline		ddCDP		ddCTP	
	3 hr	24 hr	3 hr	24 hr	3 hr	24 hr	3 hr	24 hr
				μM				
CCRF-CEM (2n)	ND[a]	0.50 ± 0.15	1.45 ± 0.13	5.64 ± 1.05	4.18 ± 0.00	15.28 ± 0.26	1.41 ± 0.21	7.00 ± 1.77
CCRF-HSB-2	0.59 ± 0.00	7.27 ± 0.01	1.42 ± 0.24	8.22 ± 0.26	1.12 ± 0.13	7.16 ± 0.14	0.79 ± 0.13	5.77 ± 1.17
WI-L2	0.16 ± 0.01	3.00 ± 0.11	0.81 ± 0.07	4.22 ± 0.47	1.83 ± 0.13	8.55 ± 2.06	0.15 ± 0.01	0.71 ± 0.07
6410	0.31 ± 0.01	4.35 ± 0.33	0.76 ± 0.01	7.34 ± 1.32	2.02 ± 0.11	18.97 ± 3.78	0.17 ± 0.00	1.48 ± 0.55

[a] ND, not detected.

TABLE 5

Rates of catabolism of ddCyd nucleotides in sensitive and resistant cell lines

Half-lives were calculated from the drop in intracellular concentrations 5 and 10 hr after cells were suspended in fresh medium. For further details see Experimental Procedures.

	$t_{1/2}$		
	ddCMP	ddCDP	ddCTP
		hr	
CCRF-CEM (2n)	10.9	10.0	9.5
CCRF-HSB-2	6.3		8.8
WI-L2	18.5	6.8	18.7
6410	13.5	8.3	19.5

rates of disappearance of ddCTP are remarkably slow in all four cell lines and that they are even slower in the least sensitive lines than in the sensitive ones, so that this does not seem to be a factor contributing directly to sensitivity to growth inhibition.

Discussion

It is clear from the comparison of the effects of the five 2′,3′-dideoxyribonucleosides on various lines that ddCyd is invariably the most toxic of these analogues, no matter whether the cell lines are B lymphoblastic, T lymphoblastic, or of nonlymphoblastic origin. In the four cases where ddGuo appeared to have a cytostatic effect comparable to that of ddCyd when both nucleosides were tested at 200 μM (Fig. 1), determination of IC_{50} values for ddGuo and ddCyd with these four lines showed that in all these cases ddCyd was cytostatic at much lower concentrations than ddGuo (Tables 1 and 2). The other three nucleosides are much less cytostatic and in a few cases 200 μM ddThd and/or ddIno produced negligible inhibition of growth. The pattern of inhibition of the five T lymphoblastic lines is especially uniform. These results are in agreement with a previous report (1, 2) of the relative cytostatic effects of these five nucleosides on ATH8 cells ($OKT4^+$ T lymphoblasts) and with the reported relative effects of ddThd and ddCyd on Raji and Molt/4F cells (2).

Under our experimental conditions, ddCyd is highly cytostatic to T lymphoblastic cells, with IC_{50} values of 0.2 to 3.0 μM. It is of interest that the parental CCRF-CEM line (CCRF-CEM 2n) was 15-fold more sensitive to ddCyd than the tetraploid subline developed during culture in this department. The latter is aneuploid and variable with $2n = 82$, XX inv. del. 9. However, the two lines show no significant differences in sensitivity to 2-chlorodeoxyadenosine (9). Our IC_{50} values for the action of ddCyd on T and B lymphoblastic lines are significantly lower than other reported values (1–3, 5). This is probably explained at least in part by the fact that in our experiments growth was measured over a period of 4 days, so that 4–4.5 cell divisions occurred, and inhibition was readily determined. In some other reports cell growth was measured over periods ranging from 1 to 3 days. Although we have made no direct measurements, it seems likely that inhibition of growth may increase with the length of exposure of cells to the drug.

Although the greatest and most uniform effects of ddCyd on cell growth were observed with T lymphoblastic cells, some B lymphoblastic and nonlymphoblastic lines were very sensitive also. Thus, growth of cells of the Raji and PF-2S B lymphoblastic lines and of the myeloblastoid line KG-1 was 50% inhibited at concentrations of 2–5 μM. However, much less sensitivity was exhibited by other myeloblastoid (HL60, ML-1) and B cell (WI-L2, 6410) lines. Similarly, a B cell (PF-2S) and T cell (CCRF-HSB-2) line were the most sensitive to ddGuo (Table 2).

To investigate some of the possible causes of the different sensitivities of cell lines to ddCyd, we examined the formation of nucleotides from ddCyd in two cell lines having the greatest

sensitivity to the nucleoside (CCRF-CEM 2*n* and CCRF-HSB-2) and two having the least (WI-L2 and 6410). Cells were exposed to labeled nucleoside for 1, 3, and 24 hr at 37°. The shorter periods of accumulation more closely approximate a clinical situation in which the drug is administered orally or by intravenous bolus, producing elevated plasma levels for a limited period, and the 24-hr period corresponds better to prolonged infusion of drug.

The concentration of ddCTP was 4–7 times greater in the most sensitive cells than the level in the least sensitive cells at all three time intervals (Tables 3 and 4). This ratio may be compared with the ratio of 112 for the mean IC_{50} for the least sensitive cells divided by the mean IC_{50} for the most sensitive cells. Although the ratio of the ddCTP concentrations is lower, the fact that it is increased in the more sensitive cells suggests that the intracellular level of ddCTP may be an important factor in determining the cytotoxicity. The 3-hr ddCTP concentration found in all four lines was of the same order as reported for L1210, ATH8, and MOLT-4F cells, after 5 hr of incubation with 5 μM ddCyd, by Balzarini *et al.* (2) but much lower than the level (58.6 μM) reported for MOLT-4 cells, incubated with 5 μM ddCyd for 24 hr, by Hao *et al.* (7).

In all four cell lines the intracellular concentration of ddCDP was considerably higher than the concentration of the triphosphate after 3 hr. The ddCDP concentration more than doubled between 1 and 3 hr and further increased several fold by 24 hr. This high level of ddCDP is in agreement with the results of Balzarini *et al.* (2). At all time intervals the ratio [ddCDP]/[ddCTP] was much lower for the sensitive lines than for the resistant lines (Table 6). This suggests that in the resistant lines the nucleoside diphosphate kinase that presumably converts ddCDP to ddCTP is less active than in the sensitive lines. Whether this is due to differences in the amount of this enzyme present in the cells or to different characteristics such as substrate specificity has not been determined.

Although we did not measure intracellular ddGTP formation, the IC_{50} values obtained for four cell lines (Table 2) would lead one to expect that significant concentrations of ddGTP would be formed by these cells. However, for MOLT-4 cells, which exhibited about 80% growth inhibition by 200 μM ddGuo (Fig. 1), Hao *et al.* (7) found very low levels of ddGTP (0.046 μM) after 24-hr exposure to 5 μM ddGuo. A possible reconciliation of these observations can be based on the assumption that intracellular ddGTP levels do not become appreciable until the concentration of nucleoside in the medium approaches the IC_{50} value.

TABLE 6

Ratio of intracellular ddCDP to ddCTP in cell lines incubated with ddCyd

Data are from Tables 3 and 4.

Cell line	[ddCDP]/[ddCTP]		
	1 hr	3 hr	24 hr
CCRF-CEM (2*n*)	2.03	2.00, 2.96	2.18
CCRF-HSB-2	0.93	2.66, 1.42	1.24
WI-L2	23.2	11.8, 12.2	12.0
6410	18.2	13.7, 11.9	12.8

The rates of catabolism of ddCTP in the various cell lines (Table 5) gave no indication that more rapid nucleotide catabolism was associated with resistance. In fact, ddCTP levels dropped very slowly in cells of the resistant lines, with a half-life of about 19 hr, twice that for the sensitive lines. Half-lives for the mono- and diphosphate were also quite long. Such slow catabolism for nucleotides is uncommon but in the second phase of elimination of 2′,2′-difluorocytidine triphosphate from Chinese hamster ovary cells the half-life is over 16 hr (10). The long half-life for ddCTP elimination from the resistant cells may be due in part to continuing formation of ddCTP from ddCDP, which is present at relatively high concentrations (≈5 μM) and only drops slowly ($t_{1/2}$ ≈ 7 hr), rather than to inability of phosphatases to break down ddCTP.

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