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Effects of Hydroxyurea and Thymidine Derivatives on the Uptake and Metabolism of Deoxycytidine and Arabinofuranosylcytosine in Log Phase and Contact-Inhibited Human Diploid Fibroblasts

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SUMMARY

Hydroxyurea and pyrimidine analogs have been shown to enhance the chemotherapeutic efficacy and the DNA excision repair-inhibitory capacity of arabinofuranosylcytosine (ara-C). Since various cell types are expected to respond differently to these combination treatments and since little is known about the nature of their antiproliferative effects, we have investigated the metabolism and uptake into acid-soluble pools of deoxycytidine (dCyd) and ara-C in cycling and non-cycling human diploid fibroblasts. A substantial fraction of dCyd is converted through deamination to deoxyuridine and thymidine nucleotides, and this occurs to a greater degree in log phase cultures. ara-C is more resistant to deamination and is metabolized primarily to ara-CTP. Hydroxyurea decreases the proportion of dCyd and ara-C which is deaminated under both growth conditions, leading to higher levels of ara-CTP and dCTP. Trifluorothymidine causes an accumulation of dUMP and decreases the formation of dCTP in log phase and confluent cells. Thymidine inhibits deamination in log phase cells but stimulates this pathway in non-cycling cells. Dideoxythymidine did not appreciably alter the spectrum of metabolites of dCyd formed in log or confluent phase cells but was shown to inhibit the transport of dCyd and thymidine across the membrane. These studies provide information regarding the nature of the enhancement of the antiproliferative activity of ara-C by commonly used drugs and indicate that the cycling state of the target cell plays a major role in determining the metabolism of the nucleoside and the efficacy of chemotherapeutic treatments.

INTRODUCTION

The dCyd[1] analog, ara-C, has long been recognized as an important drug in the treatment of acute leukemia (1), and its efficacy has been shown to be greatly improved by a number of agents including dThd (2–4), FdUrd (5), and HU (6, 7). ara-C is also gaining wide use as an inhibitor of the DNA excision repair process in mammalian cells (8–11), and HU has also been shown to enhance this activity (10, 12–14).

ara-CTP is undoubtedly the active metabolite of ara-C in both of these systems, owing either to its inhibition of DNA polymerase (15, 16) or its incorporation into DNA as ara-CMP (17). However, despite much work in the area, it is still not completely known how and in what manner the metabolism of dCyd and ara-C is affected by drugs such as HU or dThd and how this relates to the antiproliferative effects of ara-C. For example, it has been pointed out that the activity of HU in elevating cellular ara-CTP (or dCTP) levels could be due to depletion of dCTP pools through inhibition of ribonucleotide reductase (thereby reducing competition for DNA polymerase binding and the feedback inhibition of dCyd kinase), elevation of pools of cellular dTTP (which serves to activate dCyd kinase), or inhibition at other steps such as the *de novo* synthesis of UMP, the conversion of UTP to CTP, or the reduction of CDP to dCDP (18). Similarly, the effects of dThd in enhancing the efficacy of ara-C could be due to dTTP activation of dCyd kinase, depression of dCTP pools through inhibition of the reductase, or synchronization of cells in S phase (4). Any of these possible mechanisms would be expected to be of some degree of importance in rapidly dividing or quiescent cells. Since we have recently shown that HU and dThd affected uptake of radiolabeled dCyd into the acid-soluble pools of human fibroblasts, it became of interest to examine the effects on the uptake[2] and metabolism under

[1] The abbreviations used are: dCyd, deoxycytidine; ara-C, 1-β-D-arabinofuranosylcytosine; dThd, thymidine; HU, hydroxyurea; FdUrd, 5-fluoro-2′-deoxyuridine; F_3dThd, trifluorothymidine; ddThd, 2′,3′-dideoxythymidine; HPLC, high performance liquid chromatography.

[2] "Uptake" as used throughout this paper refers solely to uptake of nucleoside into acid soluble pools and not specifically to the transport step of this process.

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different growth conditions of dCyd and ara-C by various metabolic inhibitors.

MATERIALS AND METHODS

Cell culturing. Normal human foreskin fibroblasts (HSBP; kindly supplied by Dr. James D. Regan, Oak Ridge, TN) were grown in modified Eagle's medium supplemented with 10% fetal bovine serum and were maintained at 37° in humidity- and carbon dioxide-controlled incubators. All experiments were initiated by seeding 5×10^4 cells in 60-mm tissue culture dishes. Cells were checked for mycoplasma contamination.

Uptake studies. Rapidly dividing log phase (day 3 after seeding, 7–10% S phase) or confluent, quiescent cultures of cells (day 13 after seeding, 0.01% S phase) were incubated in the presence of labeled DNA precursors for various periods of time in fresh modified Eagle's medium to avoid possible effects from conditioned medium (19). They were then washed free of excess label with five changes of cold phosphate-buffered saline, and 1 ml of ice-cold 5% trichloroacetic acid was added to the dishes. Extraction was allowed to proceed for 2 hr at 4°. Aliquots of the trichloroacetic acid were removed and counted in Beckman Ready Solv for acid-soluble radioactivity. The remainder of the trichloroacetic acid was neutralized with KOH and subjected to HPLC analysis.

HPLC analysis. HPLC analysis was performed on radiolabeled acid-soluble extracts using a Waters M45 solvent delivery system, a Waters model 440 absorbance detector, and two Waters μBondapak C18 reverse phase columns in series at a flow rate of 1 ml/min. The solvent system was isocratic 0.2 M ammonium phosphate, pH 5. Fractions were collected and counted for radioactivity. Peak identification was by co-chromatography of known standards or, in cases where compounds could not be resolved in this HPLC system, peak fractions were applied to DEAE-cellulose paper (which had been prerun with solvent) and developed in one dimension with 0.04–0.06 N HCl or distilled water. Comigration of radioactivity with known standards confirmed compound identity.

Chemicals. HU, ara-C, dThd, dCyd, FdUrd, F_3dThd, and all marker compounds for chromatography were purchased from Sigma Chemical Co. ddThd was from Cal-biochem. [5-^{3}H]dCyd (20 Ci/mmol) was obtained from ICN Pharmaceuticals. [6-^{3}H]dCyd (10 Ci/mmol) and [5,6-^{3}H]ara-C (20 Ci/mmol) were from Moravek Biochemicals, Inc.

RESULTS

We reported recently (20) that HU treatment of human fibroblasts resulted in anomalous uptake and incorporation into DNA of radiolabeled dThd and [5-^{3}H]dCyd which made measurements of DNA synthesis difficult. In order to study this more closely, we examined the uptake of [5-^{3}H]dCyd into acid-soluble pools of log phase and non-cycling fibroblasts treated with 2 μM HU, 500 μM dThd, 500 μM F_3dThd, 500 μM FdUrd, or 500 μM ddThd. We used these high doses because they gave optimal DNA repair-inhibitory responses (20).[3] Some of the same results as reported below were also obtained with doses as low as 10 μM (F_3dThd, FdUrd) or 100 μM (dThd, ddThd). Since these data were qualitatively similar to those reported herein, they are not included in this communication. In log phase cells all agents except ddThd increased the recovery of label in the acid-soluble pools (Table 1). ddThd caused a very significant reduction of dCyd uptake. In contrast, uptake in confluent cells was quite different. Only HU increased uptake of [5-^{3}H]dCyd and not nearly as markedly as in log phase cells. FdUrd, F_3dThd, dThd, and ddThd were inhibitory to uptake in confluent cells. These dramatic differences in cycling and non-cycling cells and the different responses of the agents tested indicated that metabolism of dCyd was dependent on the cycling state of the cell. It was of obvious interest to determine whether these effects would also be seen with [6-^{3}H]dCyd.

This same pattern was seen, although to a lesser degree, when [6-^{3}H]dCyd was used instead of the 5-^{3}H-labeled compound (see Table 2). That is, HU enhanced uptake under both growth conditions, whereas dThd and F_3dThd enhanced uptake in log phase and inhibited uptake in quiescent cultures. ddThd was, again, inhibitory under both conditions. Furthermore, an examination of the uptake of ara-C labeled at both the 5- and 6-positions demonstrated that the effects of the agents were similar to those seen with dCyd except that dThd only marginally increased ara-C uptake (see Table 3).

In order to ascertain the nature of these uptake differences, acid-soluble extracts were analyzed by HPLC. Peak identification was accomplished by chromatography of known standards in either the HPLC system or by DEAE-cellulose paper chromatography as detailed in "Materials and Methods."

Data derived from experiments utilizing [5-^{3}H]dCyd are summarized in Table 1. The notable features are as follows. In untreated log phase cultures a considerable amount of dCyd is converted through deamination and phosphorylation to dUMP. HU treatment has the same effect of reducing dUMP levels to zero and increasing dCTP/dCDP about 2-fold. F_3dThd almost completely blocks the formation of dCTP/dCDP and causes dUMP to accumulate. FdUrd has the same effect (data not shown). ddThd treatment of log phase cells did not appreciably alter the spectrum of metabolites formed.

In untreated confluent cultures a larger amount of dCTP is formed relative to dUMP than was seen in log phase cells. HU treatment has little effect except to increase slightly the amount of dCTP formed. dThd, however, dramatically blocks dCTP formation and enhances accumulation of dUMP and dUrd. Again, ddThd treatment does not change the types or relative quantity of any of the products formed.

Metabolism of [5-^{3}H]dCyd proceeding through dUMP to dTMP leads to the loss of the labeled hydrogen. For this reason, one cannot follow the formation of dThd nucleosides derived from dCyd treatment with this labeled compound. It was thus necessary to use dCyd labeled at the 6 position. Fig. 1 shows that, as in the case with [5-^{3}H]dCyd, the metabolism of this compound is different in cycling and non-cycling cells. In log phase cells, nearly all of the radioactivity is found in dTTP and dTMP whereas in confluent cells, although a great deal of dTTP is formed, a substantial amount of dCTP is also produced. This is essentially the same pattern as seen in the experiments utilizing [5-^{3}H]dCyd. Table 2 summarizes the results obtained with [6-^{3}H]dCyd. In log phase cells both HU and dThd treatment reduce the amount of dTTP formed and increase the amount of dCyd converted to dCTP. As expected, F_3dThd treatment leads to an accumulation of dUMP and as seen in the studies with [5-^{3}H]dCyd, ddThd treatment does not significantly

[3] R. D. Snyder, manuscript in preparation.

TABLE 1

Uptake and metabolism of [5-^{3}H]dCyd

Most numbers are the average of three determinations and are derived from HPLC analysis as detailed in "Materials and Methods." All extracts were prepared after 1 hr of labeling.

Growth condition/treatment	Percentage of total acid-soluble radioactivity						Uptake[a]
	dCyd	dCMP	dCDP/dCTP	dUrd	dUMP	Unknown	
Log phase cells							
Untreated	12	12	30	9	24	7	
2 mM HU	14	11	70	2	<1	1	300
500 μM dThd	16	11	59	5	<1	5	289
500 μM F_3dThd	2	2	4	18	71	<1	230
500 μM ddThd	14	12	36	8	21	7	41
500 μM FdUrd							225
Confluent cells							
Untreated	8	11	54	4	6	8	
2 mM HU	6	14	61	4	7	2	129
500 μM dThd	4	14	20	27	28	3	18
500 μM F_3dThd	<1	16	20	31	25	<1	50
500 μM ddThd	6	9	52	7	7	9	15
500 μM FdUrd							25

[a] Percentage of control at 1 hr.

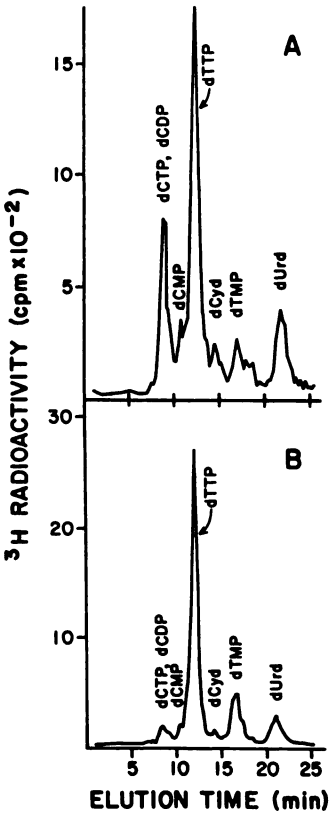

FIG. 1. *HPLC profiles of radiolabeled metabolites of [6-^{3}H]dCyd formed in log phase and non-cycling cells*

A, Confluent cultures; B, log phase cells.

change the profile of metabolites formed. In resting cells, HU treatment has an effect similar to that seen in log cultures except that the degree of effect is much smaller. F_3dThd and ddThd treatment had the same effect as in log phase cultures, whereas dThd increased the formation of dTTP and reduced dCTP levels.

Fig. 2 demonstrates that in untreated log phase cultures ara-C is metabolized primarily to ara-CTP, although some ara-UTP/TTP are also formed. HU treatment significantly enhances the formation of ara-CTP with a concomitant reduction in the amounts of unreacted ara-C and ara-UTP. In confluent cells, ara-CTP formation exceeds that in log phase cells and HU has a reduced effect. Table 3 summarizes these results and those obtained with dThd treatment. Under both growth conditions the conversion of ara-C to ara-CTP is favored over the deamination reaction and HU treatment enhances this conversion. dThd treatment tended to reduce slightly the deamination of ara-C in cycling cells while enhancing the process in quiescent cells.

The fact that ddThd inhibited dCyd uptake in both log phase and non-cycling cells but did not appear to alter the metabolism of dCyd suggested that ddThd might inhibit transmembrane transport. To test this we looked at the effects of ddThd on [^{3}H]dThd uptake and on the uptake of dCyd in the presence of HU or dThd. Table 4 demonstrates that ddThd completely blocks the enhancement of dCyd uptake by HU or dThd. In addition, ddThd blocks the uptake of dThd and the HU enhancement of that uptake. In experiments not presented here, it was observed that ddThd did not block the phosphorylation of dThd in human fibroblasts confirming previous studies suggesting that ddThd was not an effective substrate for cellular thymidine kinase. It is thus unlikely that the inhibition of dThd uptake seen here is due to interference with its phosphorylation. A more likely explanation might be that ddThd competitively inhibits the binding of the labeled deoxynucleoside to the carrier molecule.

In order to test this hypothesis, we conducted experiments in which uptake of labeled dCyd was measured in the presence of increasing concentrations of non-labeled dCyd. Plagemann and Erbe (21) have suggested that passive diffusion rather than carrier-mediated transport occurs when high levels of exogenous nucleoside are present. Collins *et al.* (9), as well, have shown that the HU enhancement of dThd uptake declines as exogenous

TABLE 2

Metabolism of [6-^{3}H]dCyd

Numbers are derived from only one HPLC determination. Cell extracts were prepared after 1 hr of labeling.

Growth condition/treatment	Percentage of total acid-soluble radioactivity							Uptake[a]
	dCyd	dCMP	dCDP/dCTP	dUrd	dUMP	dTMP	dTTP	
Log phase cells								
Untreated	1	3	3	8	<1	14	70	
2 mM HU	12	15	31	4	<1	2	31	161
500 μM F_3dThd	1	2	7	22	54	3	<1	131
500 μM ddThd	2	5	2	4	<1	16	65	74
500 μM dThd	4	6	19	6	<1	9	45	120
500 μM FdUrd								104
Confluent cells								
Untreated	2	5	13	15	<1	10	50	
2 mM HU	11	9	23	4	<1	7	41	155
500 μM F_3dThd	3	5	6	32	46	2	<1	89
500 μM ddThd	1	9	15	15	<1	7	51	36
500 μM dThd	1	4	7	20	<1	4	66	75
500 μM FdUrd								81

[a] Percentage of control at 1 hr.

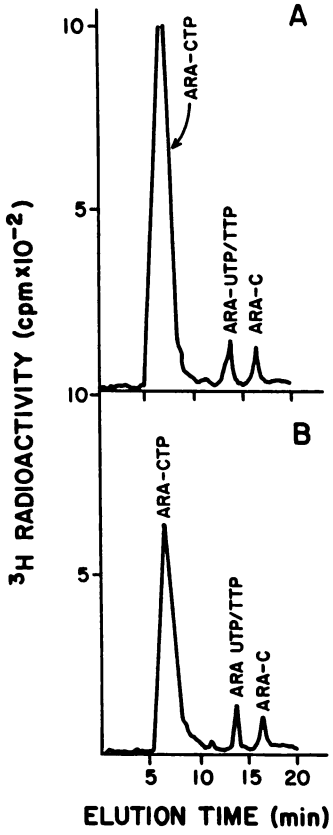

FIG. 2. *Metabolism of [5,6-^{3}H]ara-C in log phase fibroblasts*

A, HU treatment (2 mM); B, untreated. ara-UTP and ara-TTP are not resolved in this solvent system.

TABLE 3

Uptake and metabolism of [5,6-^{3}H]ara-C

Numbers are derived from a single determination by HPLC. All extracts were prepared after 1 hr of labeling.

Growth condition/treatment	Percentage of total acid-soluble radioactivity			Uptake[a]
	ara-C	ara-CTP	ara-UTP/TTP	
Log phase cells				
Untreated	16	51	22	
2 mM HU	10	72	10	155
500 μM F_3dThd				152
500 μM dThd	15	60	15	108
500 μM ddThd				34
Confluent cells				
Untreated	3	79	13	
2 mM HU	1	89	2	113
500 μM F_3dThd				89
500 μM dThd	6	58	29	44
500 μM ddThd				28

[a] Percentage of control at 1 hr.

dThd increases. Fig. 3 demonstrates that, as dCyd levels in the medium increase, the ddThd-induced inhibition of uptake decreases. Moreover, the enhancement of uptake of dCyd by HU and dThd decreases with increasing dCyd concentration. These results suggest that the effects of ddThd, HU, and dThd are all related to the carrier-mediated process and that, when the system is flooded with high levels of exogenous dCyd, the large amount of passive diffusion tends to override the effects seen by these agents. However, the enhancement of dCyd uptake by F_3dThd did not seem to be dependent on exogenous dCyd levels as only a slight decrease in uptake was noted as dCyd concentration increased.

DISCUSSION

We have previously observed in a variety of studies in human fibroblasts that uptake of dCyd differs in dividing and nondividing cells and that cells under these growth conditions respond in various fashions to modulators of dCyd uptake. In particular, whereas uptake of dCyd and ara-C into acid-soluble pools is enhanced by HU, dThd, F_3dThd, and FdUrd in log phase cells, these agents, with the exception of HU, actually inhibited the process in non-cycling cells (Tables 1–3). As will be discussed below, ddThd was very inhibitory to uptake under both growth

TABLE 4

Effect of ddThd on uptake of dThd and dCyd in log phase cells[a]

Treatment	Labeled precursor	Percentage of control uptake into acid-soluble pools
2 mM HU	[5-^{3}H]dCyd	314
500 μM dThd	[5-^{3}H]dCyd	321
500 μM ddThd	[5-^{3}H]dCyd	32
2 mM HU + 500 μM ddThd	[5-^{3}H]dCyd	33
500 μM dThd + 500 μM ddThd	[5-^{3}H]dCyd	42
2 mM HU	[^{3}H]dThd	228
500 μM ddThd	[^{3}H]dThd	14
2 mM HU + 500 μM ddThd	[^{3}H]dThd	20

[a] All determinations were made after 1 hr of incubation at 37°.

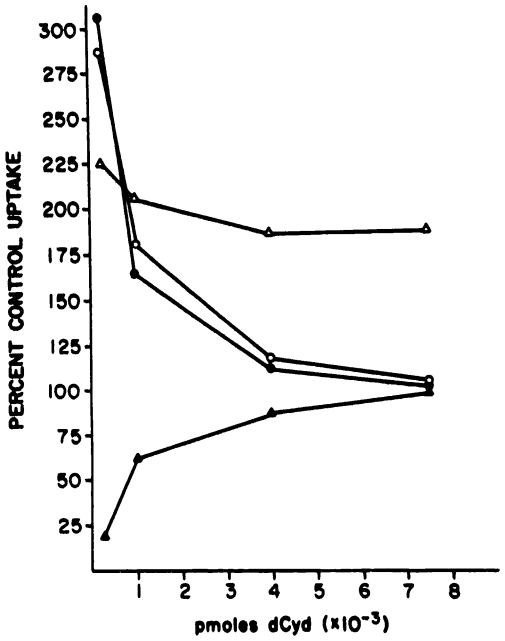

FIG. 3. *Dependence of agent-induced modulation of uptake of dCyd on exogenous nucleoside concentration*

Uptake studies were performed as described in the text. [5-^{3}H]dCyd was used either undiluted (250 pmol/dish) or with appropriate amounts of nonlabeled dCyd up to 7500 pmol/dish. Data are expressed as percentage of control uptake where the control for each point is uptake in cells at a given dCyd concentration in the absence of antimetabolites. ●---●, 2 mM HU; ○---○, 500 μM dThd; ▲---▲, 500 μM ddThd; △---△, 500 μM F$_3$dThd.

conditions. In order to understand these results, it was necessary to evaluate the metabolism of the labeled compounds.

We have demonstrated (Tables 1 and 2) that in human fibroblasts deamination of dCyd is a major pathway of dCyd metabolism consistent with observations in rat (18), chick (22), and mouse (23) cells. We also demonstrated that the deamination process was more substantial in log phase than in non-cycling cells. We have previously shown that log phase cells have 10-fold higher dTTP pools than do confluent cultures and that dCTP pools are only slightly lowered in non-cycling cells (24). From this preferential lowering of dTTP pools, we might predict that dCyd kinase would be more active and would lead to more dCTP formation in log phase cells. This does not, however, appear to be the case, as dCyd is deaminated to a greater extent in log phase cells.

Table 2 demonstrates using 6-labeled dCyd that, whereas only 7% of the total radioactivity is recovered in dCyd, dCMP, dCDP, and dCTP in log phase cells, 3 times that amount is formed in confluent cells. With ara-C (Table 3) there is only an increase of 67% to 82% in nondeaminated metabolites. ara-C is not as effective a substrate for deamination as dCMP (25) while being nearly as good a substrate as dCyd for dCyd kinase (26). This suggests that the difference in formation of dCTP in log phase and confluent cells may be at the level of the deaminase rather than phosphorylation.

HU treatment enhanced uptake of dCyd and ara-C into acid-soluble pools (Table 1–3). This enhancement was greater in log phase cells and was most pronounced in studies with [5-^{3}H]dCyd. HPLC analysis demonstrated that this was due to increased dCTP production at the expense of the deamination pathway. This increased production of dCTP and ara-CTP by HU has been reported previously (6, 7, 18) but, because detailed analysis of metabolites formed has generally not been undertaken, it has been difficult to determine the nature of the effects seen. However, Walsh *et al.* (6) suggested that transport or effects on dCyd kinase were not involved, and Tyrsted (27) demonstrated that HU apparently did not inhibit dCMP deaminase in stimulated lymphocytes. Moreover, we have shown (24) that HU does not lower dCTP pools in these cells but that it does substantially elevate dTTP pools. Taken together, it seems most likely that HU enhances phosphorylation by elevating dTTP which in turn blocks the deaminase and/or activates dCyd kinase. This effect must be largely specific for log phase cells, however, since non-cycling cells show only a marginal change in the spectrum of metabolites formed and a very minor increase in uptake (Table 1–3).

dThd treatment increased uptake of dCyd and ara-C in log phase cells and HPLC analysis shows that dCTP and ara-CTP formation is favored in those cells (Tables 1–3). However, unlike with HU, dThd markedly depressed uptake of all three labeled compounds in confluent cultures, and HPLC analysis revealed that dCTP and ara-CTP formation were depressed under these conditions (Tables 1–3). It is likely that one contributing factor to the failure of dThd to promote dCTP or ara-CTP formation in confluent cells is that dTTP is not formed to any great extent in these cells. We have shown (24) that treatment of log phase and confluent cells with

500 μM dThd resulted in the formation of 232 and 15 pmol of dTTP/10^6 cells, respectively, most likely reflecting the lower activity of dThd kinase in non-cycling cells. In the absence of high levels of dTTP, deamination would again be favored. The enhancement of ara-CTP formation by dThd has been observed by several authors (2–4).

The inhibitors of thymidylate synthetase, F_3dThd and FdUrd, also apparently enhance uptake of [5-^{3}H]dCyd but not [6-^{3}H]dCyd in log phase cells (Tables 1 and 2). This is due to the "trapping" of label as dUMP, thus preventing loss upon subsequent methylation (Table 1). Since deamination is not the major pathway for ara-C metabolism, the effect of accumulating deaminated metabolites would be reduced from that seen with dCyd. In non-cycling cells, both F_3dThd and FdUrd block uptake of [5-^{3}H]dCyd. dCTP formation is depressed and deamination products accumulate to about the same proportion as in confluent cells treated with dThd. Since FdUrd and F_3dThd exert no real effect on [6-^{3}H]dCyd or ara-C uptake in nondividing cells (Tables 1 and 2), the apparent inhibition of uptake by these agents, as in the case of dThd, must be due to enhancement of the deamination process. At present, it is unclear what factors contribute to this enhanced deamination.

Finally, ddThd proved to be inhibitory to uptake of all labeled compounds under both growth conditions while not altering the spectrum of metabolites formed (Tables 1–3). Table 4 demonstrates that ddThd not only inhibits dCyd uptake but completely abolishes the enhanced uptake of dCyd by HU and dThd in log phase cells. Moreover, ddThd also blocks uptake of labeled dThd and the HU enhancement of that uptake. Since ddThd is apparently not a substrate for dThd kinase in these cells (data not shown), it is not likely that this inhibition is at the level of phosphorylation. A more likely possibility might be that ddThd is a competitive inhibitor of binding to the transport carrier molecule (reviewed in Ref. 28). Fig. 3 demonstrates that if high concentrations of exogenous unlabeled dCyd are added to the medium, the inhibition of uptake by ddThd is abolished, consistent with the notion that passive diffusion rather than carrier-mediated transport obtains under these conditions (21).

The enhanced uptake of [5-^{3}H]dCyd by HU and dThd in log phase cells is depressed as exogenous dCyd concentration is increased (Fig. 3). Collins *et al.* (9) have shown this same diminution of HU-enhanced uptake of dThd as exogenous dThd concentration was increased. We interpret our results as indicating that, under conditions of increasing passive diffusion, the phosphorylation process becomes saturated and shunting of additional dCyd from the deamination pathway by HU or dThd has no consequence. F_3dThd enhancement of uptake is not nearly as dependent on dCyd concentration (Fig. 3). This is most likely due to the accumulation of both dCTP, as phosphorylation saturates, and dUMP, thus preserving all of the label in the cells regardless of dCyd concentration in the medium.

These studies have shown that: 1) cycling human fibroblasts channel dCyd and ara-C to a great extent through the deamination pathway whereas in non-cycling cells phosphorylation to dCTP and ara-CTP is favored; 2) HU and dThd enhance the formation of dCTP and ara-CTP in cycling cells probably by elevating dTTP pools which in turn activates dCyd kinase and deactivates dCMP deaminase: 3) in non-cycling cells HU again promotes dCTP and ara-CTP formation, but dThd has the opposite effect, causing increased deamination, indicating that the metabolism of dCyd and ara-C is dependent on the cycling state of the target cells; 4) F_3dThd treatment leads to an accumulation of dUMP in log phase cells as dCyd is deaminated, whereas in confluent cultures F_3dThd has the same effect of promoting deamination as seen with dThd; 5) ddThd inhibits transport of dCyd, and dThd most likely by competing for the binding of the transport carrier molecule; and 6) uptake and metabolism of dCyd and ara-C are stringently regulated in human fibroblasts and it should therefore be no surprise that these nucleosides are metabolized differently in cycling and non-cycling cells. These studies make it apparent that a complete understanding of the metabolism of dCyd and ara-C under any given set of conditions is necessary in order to assess clinical outcomes or evaluate the results of *in vitro* experiments using these nucleosides.

REFERENCES

1. Bodey, G. P., E. J. Freirich, R. W. Monto, and J. S. Hewlett. Cytosine arabinoside (NSC-63878) therapy for acute leukemia in adults. *Cancer Chemother. Rep.* **53**:59–66 (1969).
2. Grant, S., C. Lehman, and E. Cadman. Enhancement of 1-β-D-arabinofuranosylcytosine accumulation within L1210 cells and increased cytotoxicity following thymidine exposure. *Cancer Res.* **40**:1525–1531 (1980).
3. Kinahan, J. J., E. P. Kowal, and G. B. Grindey. Biochemical and antitumor effects of the combination of thymidine and 1-β-D-arabinofuranosylcytosine against leukemia L1210. *Cancer Res.* **41**:445–451 (1981).
4. Blumenreich, M. S., T.-C. Chou, M. Andreeff, K. Vale, B.D. Clarkson, and C. W. Young. Thymidine as a kinetic and biochemical modulator of 1-β-D-arabinofuranosylcytosine in human acute nonlymphocytic leukemia. *Cancer Res.* **44**:825–830 (1984).
5. Grant, S. and E. Cadman. Modulation of 1-β-D-arabinofuranosylcytosine metabolism and cytotoxicity in L1210 cells by fluoropyrimidine pretreatment. *Cancer Res.* **42**:3550–3556 (1982).
6. Walsh, C., R. W. Craig, and R. P. Agarwal. Increased activation of 1-β-D-arabinofuranosylcytosine by hydroxyurea in L1210 cells. *Cancer Res.* **40**:3286–3292 (1980).
7. Rauscher, F., III, and E. Cadman. Biochemical and cytokinetic modulation of L1210 and HL-60 cells by hydroxyurea and effects on 1-β-D-arabinofuranosylcytosine metabolism and cytotoxicity. *Cancer Res.* **43**:2688–2693 (1983).
8. Hiss, E. A., and R. J. Preston. The effect of cytosine arabinoside on the frequency of single-strand breaks in DNA of mammalian cells following irradiation or chemical treatment. *Biochim. Biophys. Acta* **478**:1–8 (1977).
9. Collins, A. R. S., S. L. Schor, and R. T. Johnson. The inhibition of repair in UV-irradiated human cells. *Mutat. Res.* **42**:413–432 (1977).
10. Dunn, W. C., and J. D. Regan. Inhibition of DNA excision repair in human cells by arabinofuranosylcytosine: effects on normal and xeroderma pigmentosum cells. *Mol. Pharmacol.* **15**:367–374 (1979).
11. Snyder, R. D., W. L. Carrier, and J. D. Regan. Application of arabinofuranosylcytosine in the kinetic analysis and quantitation of DNA repair in human cells after ultraviolet irradiation. *Biophys. J.* **35**:339–350 (1981).
12. Collins, A. R. S., S. Squires, and R. T. Johnson. Inhibitors of repair DNA synthesis. *Nucleic Acids Res.* **10**:1203–1213 (1982).
13. Mullinger, A. M., A. R. S. Collins, and R. T. Johnson. Cell growth state determines susceptibility of repair DNA synthesis to inhibition by hydroxyurea and 1-β-D-arabinofuranosylcytosine. *Carcinogenesis* **8**:1039–1043 (1983).
14. Snyder, R. D., B. Van Houten, and J. D. Regan. The inhibition of ultraviolet radiation-induced DNA repair in human diploid fibroblasts by arabinofuranosyl nucleosides. *Chem. Biol. Interact.* **50**:1–14 (1984).
15. Furth, J. J., and S. S. Cohen. Inhibition of mammalian DNA polymerase by the 5′-triphosphate of 1-β-D-arabinofuranosylcytosine and 5′-triphosphate of 1-β-D-arabinofuranosyladenine. *Cancer Res.* **28**:2061–2067 (1968).
16. Graham, F. L., and G. F. Whitmore. Studies in mouse L-cells on the incorporation of 1-β-D-arabinofuranosylcytosine *in vitro* and on inhibition of DNA polymerase by 1-β-D-arabinofuranosylcytosine 5′-triphosphate. *Cancer Res.* **38**:2636–2644 (1970).

17. Kufe, D. W., P. P. Major, E. M. Egan, and G. P. Beardsley. Correlation of cytotoxicity with incorporation of ara-C into DNA. *J. Biol. Chem.* **255**:8997–9000 (1980).
18. Plagemann, P. G. W., R. Marz, and R. M. Wohlhueter. Transport and metabolism of deoxycytidine and 1-β-D-arabinofuranosylcytosine into cultured Novikoff rat hepatoma cells, relationship to phosphorylation and regulation of triphosphate synthesis. *Cancer Res.* **38**:978–989 (1978).
19. Downes, C. S., R. J. Johnson, and F. F. Yew. Effects of conditioned medium on nucleoside uptake, cell cycle progression and apparent DNA repair. *J. Cell Sci* **59**:145–158 (1983).
20. Snyder, R. D. The role of deoxynucleoside triphosphates in the inhibition of DNA excision repair and replication in human cells by hydroxyurea. *Mutat. Res.* **131**:163–172 (1984).
21. Plagemann, P. G. W., and J. Erbe. Thymidine transport by cultured Novikoff hepatoma cells and uptake by simple diffusion and relationship to incorporation into deoxyribonucleic acid. *J. Cell Biol.* **55**:161–178 (1972).
22. Maley, G. F., and F. Maley. Nucleotide interconversions. VIII. Thymidine-sparing effect on the utilization of de-cytidine by chick-embryo mince. *Biochem. Biophys.* **68**:293–301 (1963).
23. Karle, J. M., R. M. Hoerauf, and R. L. Cysyk. Labeling of the thymidine and deoxycytidine bases of DNA by [2-^{14}C]deoxycytidine in cultured L1210 cells. *Cancer Lett.* **19**:147–152 (1983).
24. Snyder, R. D. Deoxynucleotide triphosphate pools in human diploid fibroblasts and their modulation by hydroxyurea and deoxynucleosides. *Biochem. Pharmacol.* **33**:1515–1518 (1984).
25. Ellims, P. H., A. Y. Kao, and B. A. Chabner. Kinetic behavior and allosteric regulation of human deoxycytidylate deaminase derived from leukemic cells. *Mol. Cell. Biochem.* **57**:185–190 (1983).
26. Momparler, R. L., and G. A. Fischer. Mammalian deoxynucleoside kinases. I. Deoxycytidine kinase: purification, properties and kinetic studies with cytosine arabinoside. *J. Biol. Chem.* **243**:4298–4304 (1968).
27. Tyrsted, G. Effect of 5-fluoro-2′-deoxyuridine and hydroxyurea on the phytohemaglutinin-induced increase of thymidine kinase, replicative DNA polymerase deoxycytidylate deaminase and CDP reductase activity in human lymphocytes. *Mol. Cell. Biochem.* **62**:165–174 (1984).
28. Plagemann, P. G. W., and R. M. Wohlhueter. Permeation of nucleosides, nucleic acid bases and nucleotides in animal cells. *Curr. Top. Membr. Transp.* **14**:225–330 (1980).

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