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POTENTIATION OF 1-β-D-ARABINOFURANOSYLCYTOSINE IN HEPATOMA CELLS BY 2′-DEOXYADENOSINE OR 2′-DEOXYGUANOSINE*

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Abstract—A 2-hr pretreatment with 2′-deoxyadenosine (AdR), 2′-deoxyguanosine (GdR), or thymidine (TdR) synergized the growth-inhibitory effect of subsequent arabinosylcytosine (Ara-C) treatment in rat hepatoma cell lines N_1S_1 and 3924A and in the rat fibroblast line BF5. In addition, the sequence of 100 μM AdR or GdR for 2 hr followed by 15 μM Ara-C for 6 hr resulted in reduction of N_1S_1 and 3924A colony formation to 13 per cent of control values, representing synergistic killing when compared with treatment by any of these agents alone. Measurements in N_1S_1 hepatoma cells showed that AdR caused increased dATP (221 per cent) and decreased dCTP and dTTP (23 and 41 per cent respectively) in comparison with untreated controls. GdR caused increases in dGTP (351 per cent control) and dATP (191 per cent control) and decreased dCTP (36 per cent control) and dTTP (37 per cent control). Pretreatment with AdR (but not GdR) caused increased cellular Ara-CTP concentration. Pretreatment with AdR (100 μM) for 2 hr increased the incorporation of [^{3}H]Ara-C into DNA by 2-fold, and 2-hr pretreatment with GdR (100 μM) increased [^{3}H]Ara-C incorporation into DNA 3-fold. AdR also increased incorporation of [^{3}H]Ara-C into RNA, but GdR did not have this effect. In all three cell lines, AdR treatment decreased the concentrations of cellular UTP and CTP. Of the three deoxynucleosides, AdR, GdR and TdR, the most potent synergism with Ara-C was given by GdR. It was concluded that for all three nucleosides the potentiation was primarily a consequence of decreased cellular pools of dCTP.

Arabinosylcytosine (Ara-C)‡ is an antineoplastic drug of clinical importance and has been widely used in treatment of leukemia. The inhibitory and cytotoxic effects of Ara-C have been attributed to the inhibition of DNA polymerase by Ara-CTP and to the incorporation of Ara-CTP into nucleic acids [1–4]. Inhibition of DNA synthesis by Ara-CTP *in vitro* is competitively antagonized by dCTP [1, 4]. Moreover, dCTP inhibits the intracellular phosphorylation of Ara-C by feedback inhibition of deoxycytidine kinase, the rate-limiting step in conversion of Ara-C to Ara-CTP [5].

In studies of deoxyribonucleoside triphosphate (dNTP) pools in several cell lines, a close correlation with the rate of DNA synthesis was established for the dCTP pool. When cells were incubated with thymidine, the depletion of the dCTP pool was sufficient to inhibit DNA synthesis, although dCTP pools remained large in comparison with the other dNTP pools [6]. In other recent studies, reduction in the cellular content of dCTP by various agents has been shown to increase cellular sensitivity to Ara-C inhibition [7]. Incorporation of Ara-C into DNA was increased 16-fold after a 1-hr pretreatment with 20 μM pyrazofurin. 3-Deazauridine treatment had no effect on Ara-C incorporation into DNA during a 1-hr incubation, but after a 4-hr treatment with 3-deazauridine, Ara-C incorporation into DNA was twice that of controls [7]. Biosynthesis of dCTP in rat hepatoma cells in regulated primarily by feedback inhibition of ribonucleotide reductase by dATP, dTTP and dGTP [8]. Consequently, inhibition of ribonucleotide reductase by antimetabolites has been a target for potentiation of Ara-C. The use of hydroxyurea, imidazopyrazole, or 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone in combination with Ara-C was shown to increase the total cell content of Ara-C metabolites, but Ara-CTP incorporation into DNA was less than in untreated controls [7]. In the same study, Plagemann and coworkers demonstrated that reduction of the dCTP pool size in Novikoff hepatoma cells by thymidine treatment had little effect on total Ara-C uptake into the cells, but the amount of Ara-CTP incorporated into DNA after a 1-hr thymidine treatment was 2.5-fold the untreated control. The synergism of thymidine and Ara-C *in vitro* has been confirmed by Harris *et al.* [9] and by Grant *et al.* [10].

These studies showed that conditions that reduced the size of the cellular dCTP pool often potentiated Ara-C. Conversely, an L5178Y mouse lymphoma

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‡ Abbreviations: Ara-C, 1-β-D-arabinofuranosylcytosine (arabinosylcytosine); Ara-CTP, 5′-triphosphate of Ara-C; AdR, 2′-deoxyadenosine; GdR, 2′-deoxyguanosine; TdR, thymidine; and dNTP. deoxyribonucleoside triphosphate.

subline with an elevated dCTP pool exhibited enhanced resistance to Ara-C [11]. Because AdR and GdR, after conversion to their 5'-triphosphates, are more potent inhibitors of ribonucleotide reductase than thymidine, we considered using these deoxynucleosides to potentiate Ara-C. The present report compares the use of the deoxynucleosides AdR, GdR and TdR as potentiators of the growth-inhibitory and cytotoxic effects of Ara-C in established rat hepatoma and fibroblast cell lines.

MATERIALS AND METHODS

Chemicals. AdR, GdR, TdR, Ara-C, poly(dA-dT), and DNA (calf thymus) were purchased from the Sigma Chemical Co., St. Louis, MO. [^{3}H]Ara-C was obtained from Amersham/Searle, Arlington Heights, IL. Tissue culture supplies were from the Grand Island Biological Co., Grand Island, NY. DNA polymerase from *Escherichia coli* was a product of the Boehringer Mannheim Corp. Indianapolis, IN. High pressure liquid chromatography (h.p.l.c.) columns were from Whatman, Inc., Fair Lawn, NJ.

Cell culture. All cell lines were maintained in McCoy's medium 5A supplemented with 5% dialyzed horse serum, penicillin (100 units/ml) and streptomycin (100 μg/ml). For growth inhibition experiments, 10 ml cultures were initiated in plastic flasks (Falcon Plastics, Oxnard, CA) at an initial cell density of 5×10^4/ml. Novikoff hepatoma cells of line N_1S_1-67 were grown in stationary suspension culture; their mean log-phase doubling time under these conditions was 11 hr, and the limiting culture density was 1.7×10^7 cells/flask. For cloning studies, a monolayer variant of the N_1S_1-67 was used. Morris hepatoma cells of line 3924A were grown in monolayer cultures, with a mean log-phase doubling time of 15 hr and a limiting density of 4×10^6 cells/flask. BF5 rat skin fibroblast cells were grown as monolayers; the mean doubling time was 28 hr, and the limiting density was 3.5×10^6 cells/flask. The origins and properties of these cell lines are documented in a previous paper [12]. For cloning studies, 500 cells in 10 ml of growth medium were seeded in a 25 cm^2 culture flask and maintained in a 95% air–5% CO_2 atmosphere in a humid incubator. After 7–10 days, the growth medium was decanted, and the cells were rinsed in isotonic phosphate buffered saline, and then stained for 5 min in saturated crystal violet solution in isotonic saline. Colonies were counted visually. Cloning efficiency of untreated cells under these conditions was 70 per cent for N_1S_1 and 60 per cent for 3924A. Cultures were tested and found to be free of mycoplasma contamination using a test kit obtained from Flow Laboratories, Rockville, MD.

Deoxyribonucleotide assays. Deoxyribonucleoside triphosphates were assayed in 60% methanol extracts prepared by the method of Tyrsted [13] as modified by Harrap and Paine [14]. dGTP and dCTP were measured in these extracts by the DNA polymerase method of Solter and Handschumacher [15], using native calf thymus DNA as template/primer. dTTP and dATP were measured in the same extracts by the modified DNA polymerase procedure of Lindberg and Skoog [16] using poly(dA-dT) as template/primer. Standards were run with all assays.

Ribonucleotide analysis. Approximately 2×10^7 cells were harvested by centrifugation at 1500 *g* for 5 min, and cell pellets were extracted in 0.6 ml of ice-cold 0.7 N perchloric acid. Extracts were centrifuged at 20,000 *g* for 20 min to remove precipitated protein. Perchlorate was removed from the supernatant fraction by addition of 45 mg potassium bicarbonate, followed by centrifugation at 1500 *g* for 5 min. The extracts were then brought to pH 2.8 by addition of 20 μl of 4 N HCl. Two hundred microliter aliquots were analyzed by h.p.l.c. on a Whatman Partisil PSX-10/25 SAX strong anion exchange column, 25 × 0.46 cm, using buffer conditions described by Lui *et al.* [17]. Peak areas were integrated using a Varian model CDS-111L integrator, from detector response factors obtained with known amounts of standard nucleotides.

Analysis of Ara-C metabolites. Cells were incubated for the appropriate time in medium containing 10^{-7} M [^{3}H]Ara-C (0.5 Ci/mmole), and this incubation was preceded, where appropriate, with a 2-hr preincubation with 100 μM AdR or GdR. Cells were then extracted and analyzed on anion-exchange columns as described for ribonucleotide analysis. On this column, the Ara-C nucleotides co-eluted with their ribosyl analogues, e.g. Ara-CTP eluted with CTP. The column eluent was collected in a fraction collector loaded with miniature scintillation vials containing 5 ml of high water-capacity liquid scintillation mixture (Aquasure, New England Nuclear Corp., Boston, MA), and 1-min fractions were collected and counted.

Macromolecular incorporation of Ara-CTP. The incorporation of [^{3}H]Ara-C into RNA and DNA was measured by the method of Schmidt and Thannhauser [18] as modified by Fleck and Munro [19]. Labeled cells were extracted in cold perchloric acid and the acid-soluble pools were counted in Aquasol (New England Nuclear Corp.). Cellular RNA was hydrolyzed in 0.5 M NaOH, and DNA was hydrolyzed in 1.5 M perchloric acid; 1-ml samples of the hydrolysates were added to scintillation vials containing 10 ml of Aquasol, and tritium was measured in a liquid scintillation counter.

RESULTS

The effect of AdR on Ara-C-induced growth inhibition in two hepatoma cell lines and a fibroblast line is summarized in Table 1. In this experiment we measured growth over 72 hr in the continuous presence of AdR and Ara-C. Addition of AdR (100 μM) to the cultures 2 hr prior to Ara-C treatment resulted in synergistic inhibition of growth in all the cell lines examined. Addition of AdR 2 hr after Ara-C was in all cases less effective than Ara-C pretreatment, although greater than additive inhibition was still obtained. Table 1 also shows data which indicate that the potentiation by AdR was not a nonspecific purine effect. Pretreatment with hypoxanthine had no effect on Ara-C growth inhibition. Adenosine alone was slightly toxic, and the toxicities of adenosine and Ara-C were additive. 2'-Deoxyinosine slightly increased the growth-inhibitory effect of

Table 1. Inhibition of growth of rat hepatoma cells and of fibroblasts by combinations of Ara-C plus purines or thymidine*

Treatment		Cell count at 72 hr as % control		
First agent	Second agent	BF5	3924A	N_1S_1
Ara-C (0.3 μM)		22 ± 3	82 ± 6	77 ± 5
AdR (100 μM)		59 ± 5	86 ± 8	83 ± 3
AdR (100 μM)	Ara-C (0.3 μM)	9 ± 1 (13)†	33 ± 6 (71)	33 ± 3 (64)
Ara-C (0.3 μM)	AdR (100 μM)	12 ± 2 (13)	53 ± 4 (71)	58 ± 5 (64)
GdR (100 μM)		81 ± 3	89 ± 6	87 ± 2
GdR (100 μM)	Ara-C (0.3 μM)	12 ± 2 (18)	58 ± 7 (73)	50 ± 4 (67)
TdR (100 μM)		54 ± 3	82 ± 3	86 ± 2
TdR (100 μM)	Ara-C (0.3 μM)	8 ± 1 (12)	63 ± 2 (67)	49 ± 4 (66)
Hypoxanthine (100 μM)		103 ± 4	91 ± 8	94 ± 4
Hypoxanthine (100 μM)	Ara-C (0.3 μM)	25 ± 2 (22)	74 ± 4 (75)	85 ± 5 (72)
Adenosine (100 μM)		70 ± 5	76 ± 7	72 ± 6
Adenosine (100 μM)	Ara-C (0.3 μM)	15 ± 1 (15)	63 ± 4 (62)	52 ± 3 (55)
2′-Deoxyinosine (100 μM)		85 ± 4	95 ± 5	99 ± 3
2′-Deoxyinosine (100 μM)	Ara-C (0.3 μM)	19 ± 2 (19)	52 ± 5 (78)	52 ± 2 (76)

* Cells were grown in the presence of the drugs for 72 hr, then counted on a Coulter counter. Where pairs of agents were used, the interval between addition of the first and second drugs was 2 hr. Values are means of triplicates (+ S.E.M.) expressed as percentages of quadruplicate controls.

† Values in parentheses are counts predicted if the effects of the two agents showed simple summation.

Ara-C. GdR, however, strongly potentiated Ara-C, as did pretreatment with TdR (Table 1). Effects of AdR and GdR were transient; a 24-hr pretreatment did not potentiate Ara-C (results not shown), and measurements showed that the nucleosides were almost completely removed from the medium after 24 hr.

The data of Table 1 were measurements of growth inhibition. Subsequent experiments examined cell killing, rather than inhibition, and used a lethal, rather than a growth-inhibitory, concentration of Ara-C. Results of an AdR plus Ara-C combination study are shown in Table 2. In this experiment, following the 2-hr pretreatment with AdR, Ara-C was added to the culture for 6 hr. Then 500 treated cells were transferred to flasks containing 10 ml of drug-free medium for cloning efficiency determination. The cytotoxic effects of AdR and Ara-C were markedly synergistic in all three lines. To compare the relative efficacy of AdR, GdR and TdR in potentiating the cytotoxicity of Ara-C, a parallel experiment was performed with hepatoma 3924A cells, in which a 2-hr pretreatment with deoxynucleoside preceded a 2-hr Ara-C treatment, followed by a colony assay as described previously. Results are shown in Table 2. All three deoxynucleoside plus Ara-C combinations gave a synergistic cytotoxic effect, with GdR plus Ara-C giving the highest degree of synergism.

The mechanism of potentiation of Ara-C by TdR in L1210 mouse leukemia cells has been postulated to be a reduction in cellular dCTP pools [9]. This would have the dual effect of decreasing the inhibition of deoxycytidine kinase by dCTP, thus

Table 2. Potentiation of Ara-C cytotoxicity by AdR, GdR and TdR in rat hepatoma cells and fibroblasts*

Treatment	Number of colonies (% of untreated control)		
	BF5	3924A	N_1S_1
Ara-C (15 μM)	54 ± 4	51 ± 7	29 ± 2
AdR (100 μM)	67 ± 3	75 ± 9	84 ± 5
AdR (100 μM) + Ara-C (15 μM)	24 ± 2 (36)†	13 ± 1 (38)	12 ± 2 (24)
GdR (100 μM)	77 ± 6	98 ± 3	91 ± 2
GdR (100 μM) + Ara-C (15 μM)	29 ± 2 (42)	8 ± 3 (60)	11 ± 1 (26)
TdR (100 μM)	84 ± 4	95 ± 5	93 ± 5
TdR (100 μM) + Ara-C (15 μM)	30 ± 2 (45)	15 ± 2 (48)	12 ± 1 (27)

* Pretreatment with AdR, GdR or TdR was for 2 hr, and Ara-C treatment was for 6 hr. Values are means for triplicate cultures, expressed as percentages of quadruplicate controls. Following treatment, 500 cells were transferred to drug-free medium, and colonies were counted after 1 week, as described in Materials and Methods.

† Number in parentheses indicate the predicted cloning efficiency assuming AdR and Ara-C effects were summative, i.e. efficiency (AdR) × efficiency (Ara-C)/100.

Table 3. Deoxyribonucleoside triphosphate pools in N_1S_1 hepatoma cells after a 2-hr treatment with AdR or GdR*

Treatment	Deoxyribonucleoside triphosphate content (nmoles/10^9 cells)			
	dTTP	dCTP	dGTP	dATP
None	75 ± 9	47 ± 4	31 ± 5	42 ± 6
AdR (100 μM)	31 ± 4†	11 ± 2†	20 ± 4	93 ± 14†
	(41)	(23)	(64)	(221)
GdR (100 μM)	28 ± 3†	17 ± 3†	108 ± 17†	80 ± 7†
	(37)	(36)	(351)	(191)

* Extraction and assay are described in Materials and Methods. Values are means ± S.E.M. for triplicate cultures. Numbers in parentheses express results as percentages of the untreated control.

† Significantly different from untreated control ($P < 0.05$ in two-tailed t-test).

allowing Ara-C to be more rapidly phosphorylated, and also of increasing the degree of inhibition of DNA synthesis by Ara-CTP, since dCTP competes with this process. We speculated that the potentiation of Ara-C by AdR and GdR might be mediated by a similar mechanism. The effect of a 2-hr treatment of N_1S_1 hepatoma cells with 100 μM AdR or GdR is shown in Table 3. Addition of AdR doubled the dATP pool size and reduced the dCTP and dTTP pools to 23 and 41 per cent respectively, of controls; smaller reduction was seen in the dGTP pool size. Two-hour treatment of N_1S_1 hepatoma cells with 100 μM GdR resulted in an increase of over 3.5-fold in the dGTP pool size, and the dATP pool size was increased to 1.9 times the control level. The dCTP pool size was reduced to 36 per cent, and the dTTP pool to 37 per cent of control.

The effect of a 4-hr treatment of the three cell lines with 100 μM AdR on the ribonucleotide pools was also examined. Results are summarized in Table 4. No marked effect was seen on the purine pools, but in all cases the pyrimidine ribonucleotide pools were decreased by the deoxyadenosine treatment.

The effect was studied of a 2-hr preincubation with 100 μM AdR or GdR on conversion of Ara-C to its triphosphate, Ara-CTP, in a subsequent 3-hr incubation. To minimize dilution of the limited quantity of [^{3}H]Ara-C available, an Ara-C concentration of 10^{-7} M was used in these experiments. Results are shown in Table 5. All three of these cell lines are comparatively resistant to Ara-C, and in all cases the Ara-CTP pool size was very small. AdR pretreatment gave a marked increase in the Ara-CTP pool, but GdR pretreatment had no significant effect.

Table 6 presents results of studies on the effect of AdR or GdR pretreatment (100 μM for 2 hr) on incorporation of [^{3}H]Ara-C (10^{-7} M) into nucleic acids during a subsequent 3-hr incubation. AdR pretreatment caused an increase in total acid-soluble counts and in both RNA and DNA counts. Following pretreatment with GdR, counts from [^{3}H]Ara-C in the acid-soluble fraction were not increased, and

Table 4. Effect of AdR treatment on ribonucleotide pools in rat fibroblasts and hepatoma cells*

Cell line	Treatment	Cellular ribonucleotides (nmoles/10^9 cells)			
		ATP + ADP	GTP + GDP	UTP + UDP	CTP + CDP
BF5	None	6570	980	1470	197
	AdR (100 μM)	8330	1030	790	90
		(127)†	(105)	(54)	(46)
3924A	None	17,550	5340	4960	880
	AdR (100 μM)	16,560	5890	3770	750
		(94)	(110)	(75)	(85)
N_1S_1	None	17,840	3200	4730	2670
	AdR (100 μM)	19,120	3010	1720	530
		(107)	(94)	(36)	(20)

* Ribonucleotides were extracted and measured as described in Materials and Methods. AdR treatment was for 4 hr. Values are means for triplicate cultures.

† Numbers in parentheses are AdR-treated values expressed as percentages of the untreated control.

Table 5. Effect of pretreatment with AdR or GdR on formation of Ara-CTP in rat fibroblasts and hepatoma cells*

Treatment	Ara-CTP pool (nmoles/10^9 cells)		
	BF5	3924A	N_1S_1
Control	0.060 ± 0.004	0.015 ± 0.003	0.028 ± 0.003
AdR (100 μM), 2 hr	0.102 ± 0.010 (170)†	0.044 ± 0.005 (293)	0.060 ± 0.004 (214)
GdR (100 μM), 2 hr	0.050 ± 0.012 (83)	0.017 ± 0.002 (113)	0.026 ± 0.002 (93)

* Ara-CTP was measured as described in Materials and Methods. Following preincubation, [^{3}H]Ara-C (0.1 μM; 0.5 Ci/mmole) was added for 3 hr. Values are means ± S.E.M. of triplicates.

† Numbers in parentheses give pool sizes in AdR- or GdR-pretreated cultures as percentages of the control.

counts in RNA were only slightly decreased, but a marked increase was seen in the amount of radioactive Ara-C incorporated into DNA.

DISCUSSION

Several recent studies demonstrated that thymidine pretreatment could potentiate Ara-C in various *in vitro* and *in vivo* experimental systems [7, 9, 10, 20, 21]; the synergistic effects of TdR and Ara-C in combination were attributed to the elevated cellular dTTP pool resulting from the presence of TdR. dTTP acts as an inhibitor of CDP reduction by ribonucleotide reductase [8] and, thus, leads to a decreased dCTP pool [22, 23]. The diminished dCTP pool size has two consequences: (1) deoxycytidine kinase (the rate-limiting enzyme in conversion of Ara-C to Ara-CTP) is released from feedback inhibition [24], thus leading to higher cellular Ara-CTP concentrations, and (2) the observed competition of dCTP with inhibition of DNA polymerase by Ara-CTP and with incorporation of Ara-CTP into DNA is reduced.

Consideration of the regulatory properties of the ribonucleotide reductase of rat hepatoma cells indicated that dATP was a more potent inhibitor than dTTP [8]. This suggested that AdR might also be potentiator of Ara-C toxicity in these cells by a mechanism similar to that of TdR. The data of Table 1 show that a brief (2-hr) pretreatment with AdR was indeed potently synergistic with Ara-C in the three cell lines we examined. Administration of AdR after the Ara-C was less effective, but it still gave greater than additive results. A similar time-sequence dependence was seen for the TdR plus Ara-C combination in L1210 cells [9]. Hypoxanthine did not potentiate Ara-C, and additive or slightly supra-additive inhibition was obtained with combinations of adenosine or deoxyinosine with Ara-C (Table 1). The mechanisms of these latter effects are unknown; adenosine causes reduced CTP pools sizes in rat hepatoma cells and fibroblasts [25], so it is possible that a consequent decrease in dCTP occurs. The potent synergism of AdR and Ara-C was observed not only in growth inhibition studies, but also in cytotoxicity, measured by cloning experiments (Table 2). AdR is readily deaminated by adenosine deaminase, an enzyme known to be very active in hepatoma cells [26]. It is possible that the potentiating effect of AdR might be even greater if it were used in combination with an adenosine deaminase inhibitor. This remains to be studied.

Studies with lymphoblasts showed that GdR greatly diminished the dCTP pool size [27]. This may be a result of two factors: (1) dGTP specifically inhibits the CDP reductase activity of ribonucleotide reductase and (2) it activates the ADP reductase activity (thus an increased dGTP pool size causes a secondary increase in dATP, which further blocks CDP reductase) [8, 27, 28]. The present growth inhibition studies show that GdR was synergistic with Ara-C; in 3924A hepatoma cells it was slightly more effective in this regard than TdR, and in N_1S_1 hepatoma cells the two deoxynucleosides equally

Table 6. Incorporation of Ara-C into RNA and DNA of N_1S_1 hepatoma cells following pretreatment with AdR or GdR*

Pretreatment	Incorporation of radioactivity (% control)		
	Acid-soluble	RNA	DNA
AdR (100 μM), 2 hr	336 ± 23	282 ± 69	202 ± 59
GdR (100 μM), 2 hr	82 ± 29	78 ± 41	312 ± 72

* Methods of extraction and analysis are described in Materials and Methods. Following pretreatment, cells were incubated with [^{3}H]Ara-C (0.1 μM; 0.5 Ci/mmole) for 3 hr. Values are percentages of the radioactivity measured in cultures that were not pretreated (mean ± S.E.M. of triplicates).

potentiated Ara-C (Table 1). The comparative cytotoxicity study (Table 2) shows that all three deoxynucleosides were synergistic with Ara-C. If the "potentiation factor" is expressed as the ratio of the predicted number of clones to the measured number of clones, where the predicted value was calculated by assuming that the individual agents showed summation (as defined by Webb [29]), then the order of effectiveness as potentiators in the two hepatoma lines was GdR > TdR > AdR.

Results of measurements of deoxynucleoside triphosphate pools in AdR- or GdR-treated cells (Table 3) were consistent with the proposed mechanism of potentiation. AdR treatment increased dATP pool size and decreased the pool sizes of the other three dNTPs, especially dCTP, in agreement with the known inhibitory effect of dATP on CDP and GDP reductase activities [8, 28]. GdR treatment increased dGTP pool size, secondarily increased dATP pool size, and decreased dCTP and dTTP pool sizes (Table 3). AdR treatment also changed the ribonucleotide pool sizes (Table 4). The adenine and guanine nucleotide pools were not greatly altered, but consistent reductions were found in the pyrimidine ribonucleotide pool sizes; the mechanism of this effect is unknown. GdR treatment increased cellular GTP and GDP pool sizes and, like AdR, also caused a decrease in the pool sizes of the pyrimidine ribonucleotides (data not shown). It is possible that this antipyrimidine effect, by decreasing CDP, may have contributed to the depletion of cellular dCTP following AdR or GdR treatment.

The effects of Ara-C on DNA synthesis are probably determined by the Ara-CTP/dCTP ratio so that drug toxicity may be increased either by an elevation in Ara-CTP or a decrease in dCTP. The cellular Ara-CTP pools measured in our study (Table 5) were all very small; using identical experimental conditions we found cellular Ara-CTP pools to be about 100-fold larger in the WI-L2 lymphoblast line. In a colony assay following a 6-hr exposure to Ara-C, the LD_{50} values for WI-L2 cells and mouse L1210 cells were much lower than those for fibroblasts and hepatoma cells (BF5 rat fibroblasts, 26 μM; 3924A rat hepatoma, 18 μM; N_1S_1 rat hepatoma, 7 μM; WI-L2 human lymphoblasts, 0.3 μM and L1210 mouse leukemia, 0.2 μM). The reason for the lesser sensitivity to Ara-C of the hepatoma lines has not been studied in detail; unlike many rat tissues they possess cytidine deaminase activity [30]. In all three cell lines, pretreatment with AdR increased formation of Ara-CTP, but GdR had no significant effect. Another difference between the effects of AdR and GdR is seen in the data of Table 6. AdR pretreatment caused increased cellular accumulation of acid-soluble Ara-C derivatives and increased incorporation into both RNA and DNA. One hundred micromolar GdR pretreatment caused a greater stimulation of Ara-C incorporation into DNA than did AdR, but there was no increase in the accumulation of acid-soluble Ara-C derivatives and no significant change in the incorporation into RNA. It is possible that stimulation of Ara-C incorporation into RNA requires an increased Ara-CTP pool and that increased incorporation into DNA occurs by a decrease in the pool size of the competing metabolite, dCTP, without any increase in Ara-CTP.

The TdR plus Ara-C combination has been tested *in vivo* by Danhauser and Rustum [21]. Because AdR and GdR do not appear to be highly toxic, and the exposure time required for potentiation of Ara-C is short, we are planning to assess the feasibility of using the AdR plus Ara-C and GdR plus Ara-C combinations *in vivo*.

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