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The Effect of 5-Azacytidine and Dihydro-5-azacytidine on Nuclear Ribosomal RNA and Poly(A) RNA Synthesis in L1210 Cells *in Vitro*

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SUMMARY

GLAZER, R. I., A. L. PEALE, J. A. BEISLER AND M. M. ABBASI. The effect of 5-azacytidine and dihydro-5-azacytidine on nuclear ribosomal RNA and poly(A) RNA synthesis in L1210 cells *in vitro*. *Mol. Pharmacol.* 17: 111-117 (1979).

The effect of 5-azacytidine (AZC) and 5,6-dihydro-5-azacytidine (DHAZC) on nRNA synthesis was studied in L1210 cells *in vitro*. AZC produced 50% inhibition of the incorporation of [^{14}C]uridine into nuclear rRNA and nonpolyadenylic acid and polyadenylic acid-containing heterogeneous nRNA at concentrations of 0.3 mM, 0.4 mM, and approximately 0.6 mM, respectively. The median inhibitory concentrations of DHAZC on these three species of nRNA were 2 mM, 2 mM and approximately 3 mM, respectively. AZC inhibited 2′-*O*-methylation and DHAZC affected base methylation of rRNA; however, methylation of nonpolyadenylic acid and polyadenylic acid-containing heterogeneous nRNA was not significantly impaired. Measurement of the concentration and specific radioactivity of *S*-adenosyl-L-methionine and UTP showed that AZC and DHAZC inhibited uridine incorporation into RNA by interference with its cellular uptake, but did not impair methionine transport. AZC and DHAZC did not inhibit [^{3}H]adenosine incorporation into nRNA at 0.1 mM, but inhibited rRNA and poly(A) heterogeneous RNA synthesis by 40% at 1 mM. [^{14}C]AZC and [^{14}C]DHAZC were incorporated into all species of nRNA at concentrations that did not impair transcription, with the highest incorporation occurring in polyadenylic acid-containing heterogeneous nRNA. These results suggest that AZC and DHAZC at low concentrations may produce their cytotoxic and antitumor effects via their incorporation into nRNA, and at higher concentrations inhibit the methylation of rRNA, as well as the transcription of rRNA and polyadenylic acid-containing RNA.

INTRODUCTION

5-Azacytidine (AZC)[2] is a potent anticancer agent that has shown activity against L1210 murine leukemia (1, 2) as well as human acute myeloblastic leukemia (3, 4). Its hydrolytically stable analog, 5,6-dihydro-5-azacytidine (DHAZC) (1, 5), was synthesized to circumvent the instability of AZC in aqueous solution (6). DHAZC possesses the same degree of antitumor activity and dose schedule dependency as the parent drug, but its potency is approximately 30-fold lower *in vivo* (1, 2). AZC and DHAZC are also cytotoxic to L1210 cells in tissue culture (7, 8) with AZC being approximately 10-fold more potent than the reduced analog (7, 8). The mechanism of this toxicity is not known, although inhibition of RNA and DNA synthesis has been observed in L1210 cells in culture (7) and in ascites (9).

Evidence that the action of AZC may in part be due to interference with RNA synthesis was first shown by Reichman *et al.* (10) who demonstrated that AZC caused aberrant processing of the 45 S precursor to rRNA. Subsequent studies by Weiss and Pitot (11, 12) confirmed that although transcription of the 45 S precursor was not impaired by AZC, its subsequent processing to 28 and 18 S rRNA was impaired. This effect was believed to result from the extensive incorporation of AZC into total cellular RNA (7, 13). In this regard, AZC-modified tRNA was less active in supporting protein synthesis *in vitro* in a reticulocyte lysate system (14). These results imply that incorporation of AZC into various species of nRNA may be more important for its biological activity than its weak effect on inhibiting transcription per se (15). Moreover, inhibition by AZC of the methylation of rRNA and

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[2] The abbreviations used are: AZC, 5-azacytidine; DHAZC, 5,6-dihydro-5-azacytidine; hnRNA, heterogeneous nuclear RNA; poly(A), polyadenylic acid; SAM, *S*-adenosyl-L-methionine.

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tRNA in HeLa cells (16), as well as of 5-methylcytidine in hepatic tRNA of mice administered the drug (17) suggest that interference with specific RNA methyltransferases may also play a role in the impaired functioning and possibly the processing as well of certain species of RNA.

To assess the effect of AZC and its reduced congener, DHAZC, on the synthesis of nRNA, the action of these drugs was measured in L1210 cells *in vitro*. In addition, the incorporation of these agents into different species of nRNA was characterized.

MATERIALS AND METHODS

Materials. AZC was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, and the chloride salt of DHAZC was synthesized by reduction of AZC with $NaBH_4$ (2, 5). [4-^{14}C]AZC (53 mCi/mmol) was purchased from Monsanto Research Corporation, Dayton, Ohio, and [4-^{14}C]DHAZC (0.4 mCi/mmol) was synthesized from labeled AZC by $NaBH_4$ reduction. L-[*methyl*-^{3}H]Methionine (80 Ci/mmol), [2,8-^{3}H]adenosine (31 Ci/mmol), and [U-^{14}C]uridine (462 mCi/mmol) were purchased from New England Nuclear Corporation, Boston, Massachusetts.

Animals. L1210 cells were inoculated i.p. into BALB/c × DBA/2 F_1 mice at an inoculum of 10^5 cells/0.1 ml Hanks' balanced salt solution. Cells were harvested 6 days after inoculation, washed once in Dulbecco's medium, and further diluted with the same medium to 2×10^7 cells/ml.

Incubations. Incubations of L1210 cells were carried out at 37° in a shaking water bath at 100 rpm and consisted of: 25 ml Dulbecco's medium, 0.25% glucose, either 50 μCi [*methyl*-^{3}H]methionine (80 Ci/mmol), 5 μCi [^{14}C]uridine (462 mCi/mmol), or 50 μCi [^{3}H]adenosine (200 mCi/mmol), and 5×10^7 cells. Either AZC or DHAZC (neutralized to pH 7) at the indicated concentrations was incubated with the cells for 30 min before addition of isotopes. Labeling was carried out for 30 min unless indicated otherwise.

RNA extraction. After incubation, cells were centrifuged at 400*g* for 20 min and washed once with Dulbecco's medium. Nuclei were prepared according to the method of Daskal *et al.* (18) with Triton X-100 as the detergent, except that cells were swelled in 10 mM magnesium acetate (pH 5.1). This change in swelling medium was found to markedly arrest the activity of intracellular RNase, and thereby minimize hydrolysis of mRNA before extraction. Nuclear rRNA, non-poly(A) hnRNA, and poly(A) hnRNA were extracted using the sodium dodecyl sulfate:phenol procedure previously described (19). Poly(A) hnRNA was isolated on polyuridylic acid–Sepharose as described (20).

Electrophoresis. Disc gel electrophoresis of nRNA or poly(A) was carried out by the method of Dudov *et al.* (21) with 1.75 or 2.5% agarose, respectively, in 6 M urea: 25 mM Na_2HPO_4:0.63 mM citric acid (pH 8.0) with 25 mM Na_2HPO_4:0.63 mM citric acid (pH 8.0) as the running buffer. Gels were dissolved in 0.3 ml of 60% perchloric acid, mixed with 10 ml of Aquasol, and counted in a Searle Mark III liquid scintillation spectrometer.

DEAE-Sephadex chromatography. Alkaline hydrolysates of nuclear rRNA were prepared and chromatographed on DEAE-Sephadex equilibrated with 7 M urea: 20 mM Tris–HCl (pH 7.6) as previously described (22).

Concentration and specific radioactivity of SAM and UTP. Incubations of L1210 cells with AZC and DHAZC, [*methyl*-^{3}H]methionine, and [^{14}C]uridine were carried out as described. Following incubation, cells were centrifuged at 400*g* for 5 min at 4° and washed once with Dulbecco's medium. Cells were then vortexed with 1 ml of ice-cold 1.0 N perchloric acid and the levels and specific radioactivity of SAM and UTP were measured as described (23, 24).

RESULTS

Initial experiments of the effects of AZC and DHAZC on the incorporation of [^{3}H]uridine and [^{3}H]thymidine into total cellular RNA and DNA, respectively, indicated that these agents produced maximum inhibition of [^{3}H]uridine incorporation into RNA within 30 min, but did not affect [^{3}H]thymidine incorporation even after 2 hr of incubation (results not shown). Therefore, further experiments were designed to assess the effect of AZC and DHAZC on nRNA in order to see if they produced a differential effect on a specific species of RNA.

Dose–response experiments of the effect of AZC and DHAZC on nuclear rRNA, non-poly(A)hnRNA, and poly(A)hnRNA labeled with [*methyl*-^{3}H]methionine and [^{14}C]uridine are shown in Fig. 1. Inhibition of [^{14}C]uridine incorporation into all types of nRNA was pronounced in the presence of AZC and DHAZC with DHAZC being 10-fold less potent. Methylation of rRNA was impaired to an equal extent as [^{14}C]uridine incorporation by DHAZC and to a lesser degree by AZC (Fig. 1A). In contrast, methylation of non-poly(A) hnRNA was affected less than uridine incorporation by both drugs (Fig. 1B), while methylation of poly(A) hnRNA was unaffected (Fig. 1C).

The time course of inhibition by 1 mM AZC and DHAZC revealed that maximum inhibition of [*methyl*-^{3}H]methionine and [^{14}C]uridine incorporation into different species of nRNA occurred rapidly with little difference in these effects throughout 15–60 min of preincubation (Fig. 2).

Since these effects suggested that the processing of rRNA might be affected by the two drugs, the extent of methylation of rRNA was analyzed by DEAE-Sephadex–urea chromatography of alkaline hydrolysates of rRNA. Under these chromatographic conditions, mononucleotides elute at pH 7.6 with a net charge of −2 due to one terminal phosphate, while dinucleotides generated as a result of the protection from alkaline hydrolysis afforded by 2′-*O*-methylated ribose elute with a net charge of −3 (terminal phosphate with a net charge of −2 plus one internal phosphodiester bond with a net charge of −1). A representative experiment is shown in Fig. 3 and the summary of three experiments is presented in Table 1. AZC and DHAZC at 1 mM inhibited the incorporation of [^{14}C]uridine into the mononucleotide (−2 charge) fraction of rRNA by 45 to 60%, while only AZC affected the incorporation of uridine into the dinucleotide (−3 charge)

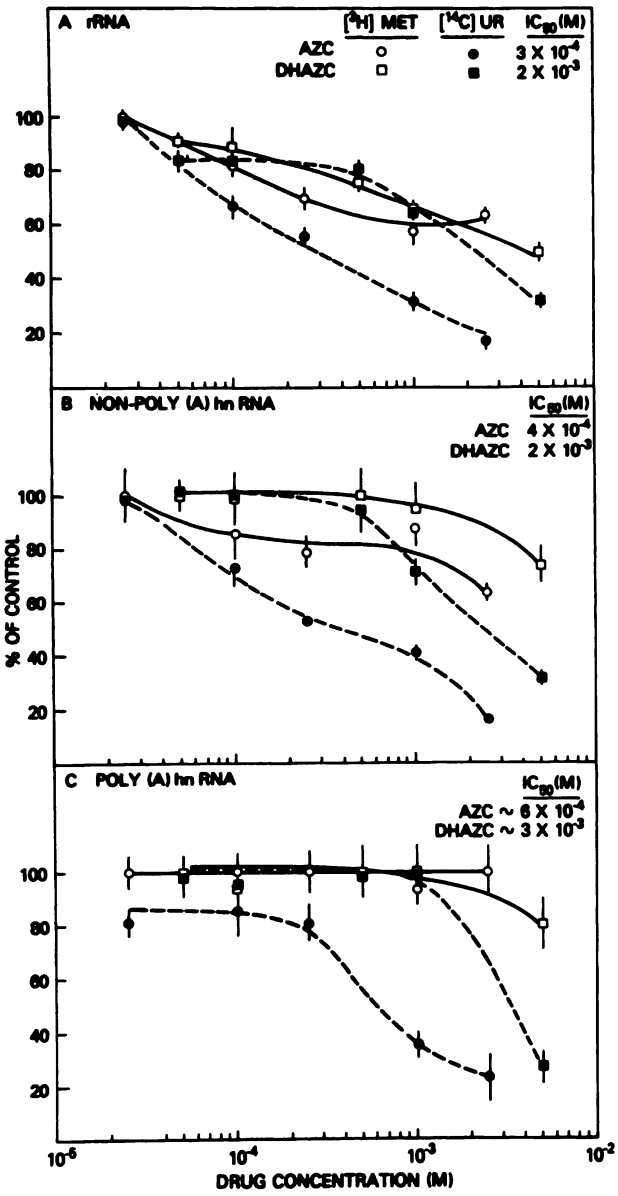

FIG. 1. *Dose response of AZC and DHAZC on incorporation of [methyl-^{3}H]methionine and [^{14}C]uridine into nuclear rRNA, non-poly(A) hnRNA, and poly(A) hnRNA*

L1210 cells were incubated for 30 min with varying concentrations of either AZC or DHAZC followed by incubation for 30 min with 50 μCi of [*methyl*-^{3}H]methionine and 5 μCi of [^{14}C]uridine. Nuclear RNA fractions were extracted from isolated nuclei as described under MATERIALS AND METHODS. The IC_{50} values refer to the concentrations of AZC and DHAZC producing 50% inhibition of [^{14}C]uridine incorporation into RNA. The specific activities (dpm/A_{260}/30 min) for incorporation of [*methyl*-^{3}H]methionine and [^{14}C]uridine, respectively, were: *rRNA*, 276,800 ± 16,600 and 338,500 ± 38,300; *non-poly(A) hnRNA*, 151,600 ± 19,000 and 186,400 ± 19,600; *poly(A) hnRNA*, 494,000 ± 50,000 and 253,500 ± 26,000. Each value is the mean ± SE of six determinations.

fraction. In contrast, AZC significantly reduced only 2′-*O*-methylation in the dinucleotide fraction by 40%, while DHAZC significantly impaired only base methylation in the mononucleotide fraction of rRNA.

In order to examine whether or not the apparent synthesis and methylation of nRNA was a result of interference with the synthesis of the immediate precursor metabolites, the concentrations and specific radioactivity of UTP and SAM were measured (Table 2). AZC and DHAZC did not affect the concentration of UTP, but rather reduced the specific radioactivity of UTP in a dose-dependent manner, and to the same extent as the incorporation of [^{14}C]uridine into nRNA (Fig. 1). These data suggest that AZC and DHAZC inhibited uridine transport and not transcriptions per se. However, the concentration and specific radioactivity of SAM were not affected by either drug.

To test if transcription was truly unaffected by AZC and DHAZC, the incorporation of [^{3}H]adenosine into different species of nRNA was measured (Table 3). It is evident that reduction of incorporation of [^{3}H]adenosine occurred only at 1 mM AZC and DHAZC, and to a lesser

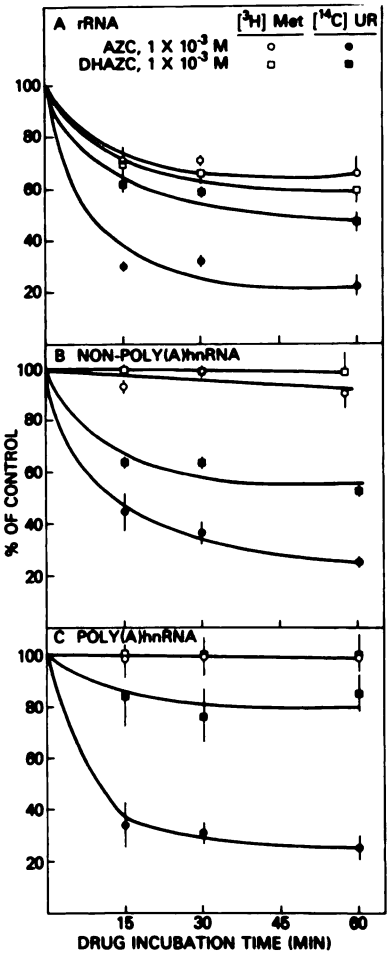

FIG. 2. *Effect of drug preincubation time on the incorporation of [methyl-^{3}H]methionine and [^{14}C]uridine into nuclear rRNA, non-poly(A) hnRNA, and poly(A) hnRNA*

L1210 cells were incubated for 15, 30, and 60 min with either 1 mM AZC or 1 mM DHAZC followed by incubation for 30 min with 50 μCi [*methyl*-^{3}H]methionine and 5 μCi [^{14}C]uridine. Nuclear RNA fractions were extracted from isolated nuclei as described under MATERIALS AND METHODS. The specific activities (dpm/A_{260}/30 min) for the incorporation of [*methyl*-^{3}H]methionine and [^{14}C]uridine, respectively, were: *rRNA*, 291,600 ± 29,500 and 342,700 ± 26,900; *non-poly(A) hnRNA*, 141,000 ± 27,600 and 172,000 ± 10,400; *poly(A) hnRNA*, 518,000 ± 60,000 and 220,900 ± 28,300. Each value is the mean ± SE of three determinations.

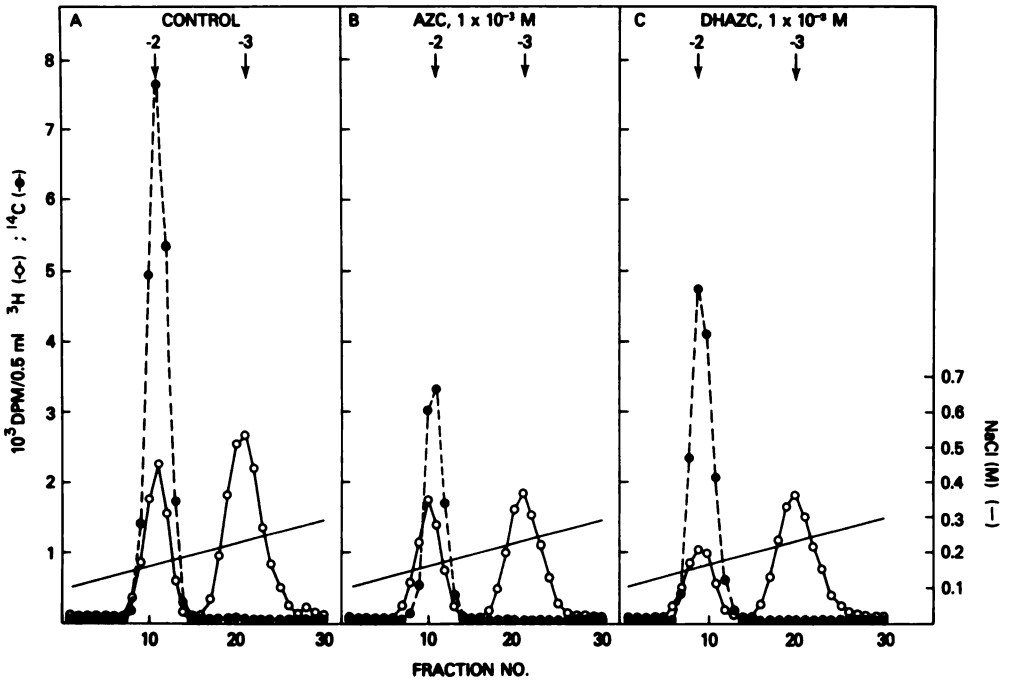

FIG. 3. *DEAE-Sephadex chromatography of alkaline hydrolysates of nuclear rRNA following treatment with AZC and DHAZC*
L1210 cells were incubated for 30 min with either AZC or DHAZC followed by incubation for 30 min with 50 μCi of [*methyl*-^{3}H]-methionine and 5 μCi [^{14}C]uridine. Nuclear rRNA was extracted from isolated nuclei, hydrolyzed with KOH, and chromatographed on DEAE-Sephadex as described under MATERIALS AND METHODS.

TABLE 1

Effect of AZC and DHAZC on the incorporation of [^{3}H]methionine and [^{14}C]uridine into alkaline hydrolysates of nuclear rRNA

L1210 cells were incubated with either AZC or DHAZC for 30 min followed by incubation for 30 min with 50 μCi of [*methyl*-^{3}H]methionine and 5 μCi [^{14}C]uridine. Nuclear rRNA was extracted from isolated nuclei, hydrolyzed with KOH, and chromatographed on DEAE-Sephadex as described under MATERIALS AND METHODS and in Fig. 3. The designations of "−2 charge" and "−3 charge" refer to Fig. 3.

Treatment	RNA fraction			
	−2 Charge		−3 Charge	
	^{3}H	^{14}C	^{3}H	^{14}C
	(10^3 dpm/5 × 10^7 cells/30 min)			
Control	171.2 ± 19.9[a]	398.2 ± 13.6	290.0 ± 26.9	2.25 ± 3.0
	(% of control)			
AZC, 1 mM	77 ± 8	39 ± 3[b]	59 ± 2[b]	46 ± 4[b]
DHAZC, 1 mM	63 ± 4[b]	54 ± 5[b]	75 ± 5	97 ± 6

[a] Mean ± SE of three experiments.
[b] Statistically significant difference ($p < 0.05$) *versus* control.

TABLE 2

Effect of AZC and DHAZC on the concentration and specific radioactivity of SAM and UTP

L1210 cells were incubated with either AZC or DHAZC for 30 min followed by incubation for 30 min with 50 μCi [^{3}H]methionine and 5 μCi [^{14}C]uridine. SAM and UTP were measured as described under MATERIALS AND METHODS.

Treatment	nmol UTP/5 × 10^7 cells	10^3 dpm/nmol UTP	nmol SAM/5 × 10^7 cells	10^3 dpm/nmol SAM
Control	80.7 ± 8.0[a]	14.3 ± 1.1	13.8 ± 1.2	122.2 ± 6.7
		(% of control)		
AZC				
1 mM	101 ± 6	29 ± 3[b]	88 ± 4	88 ± 7
0.5 mM	106 ± 6	35 ± 2[b]	106 ± 7	91 ± 4
DHAZC				
1 mM	111 ± 7	64 ± 8[b]	97 ± 5	91 ± 4
0.5 mM	92 ± 12	99 ± 10	108 ± 8	112 ± 5

[a] Mean ± SE of six determinations.
[b] Statistically significant difference ($p < 0.05$) *versus* control.

extent than uridine incorporation into nRNA (Fig. 1). However, only rRNA and poly(A) hnRNA were inhibited by AZC and DHAZC, whereas non-poly(A)hnRNA was unaffected even at the highest concentration of each drug.

Agarose gel electrophoresis of rRNA labeled with [^{3}H]adenosine indicated that 1 mM AZC and DHAZC did not alter the distribution of labeling of 32S, 28S and 18S rRNA (Fig. 4). No evidence was observed of destructive processing or accumulation of precursor rRNA.

Incorporation of AZC and DHAZC into nRNA. Since AZC and DHAZC inhibited transcription of nRNA only at 1 mM, the possibility that the cytotoxicity produced by these drugs might also be related to their incorporation into nRNA at lower drug concentrations was examined. To ascertain this possibility, [^{14}C]AZC and [^{14}C]-DHAZC were incubated with L1210 cells and the incorporation into the three species of nRNA was monitored (Table 4). [^{14}C]AZC and [^{14}C]DHAZC were incorporated into poly(A)hnRNA to a much greater extent in comparison with either rRNA or non-poly(A)hnRNA. [^{14}C]AZC reached a maximum level of incorporation into nRNA at 1 mM. Only one concentration of [^{14}C]DHAZC could be tested due to its low specific radioactivity. Interestingly,

TABLE 3

The effect of AZC and DHAZC on nRNA synthesis

L1210 cells were incubated with either AZC or DHAZC for 30 min followed by incubation for 30 min with 50 μCi of [^{3}H]adenosine (200 mCi/mmol). Nuclear rRNA, non-poly(A) hnRNA, and poly(A) hnRNA were isolated as described under MATERIALS AND METHODS.

Treatment	rRNA	Non-poly(A) hnRNA	poly(A) hnRNA
	(dpm/30 min/5 × 10^7 cells)		
Control	434,570 ± 27,310[a]	433,160 ± 17,810	57,550 ± 3,860
	(% of control)		
AZC			
0.1 mM	84 ± 2	91 ± 6	89 ± 6
1 mM	58 ± 3[b]	93 ± 3	62 ± 4[b]
DHAZC			
0.1 mM	82 ± 2	107 ± 7	98 ± 13
1 mM	54 ± 4[b]	98 ± 5	67 ± 7[b]

[a] Mean ± SE of three assays.

[b] Statistically significant difference ($p < 0.05$) *versus* control.

0.5 mM [^{14}C]DHAZC was incorporated 16 times more effectively into poly(A)hnRNA, 3 times more extensively into non-poly(A)hnRNA, but only one half as much into rRNA in comparison with an equimolar concentration of [^{14}C]AZC.

The distribution of [^{14}C]AZC and [^{14}C]DHAZC in poly(A)hnRNA (Fig. 5) was similar to the labeling of this RNA species with [^{14}C]uridine (Fig. 6). However, the diminution of the incorporation of [^{14}C]uridine into the leading 6–8 S poly(A) hnRNA peak produced by 1 mM AZC was also observed with the incorporation of the same concentration of [^{14}C]AZC as precursor (Fig. 5).

Alkaline hydrolysis and DEAE-Sephadex chromatography of poly(A) hnRNA labeled with [^{14}C]AZC and [^{14}C]DHAZC indicated that 100% of the radioactivity was associated with the mononucleotide (−2 charge) fraction with no label being present in the oligonucleotide 5′-"cap" region (−5 to −6 charge) ((22), results not shown).

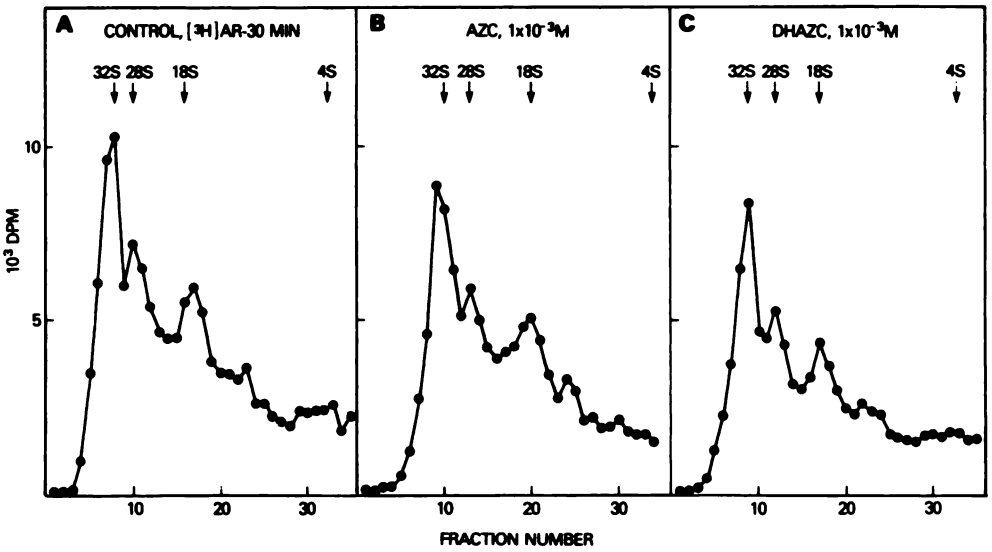

FIG. 4. *Agarose gel electrophoresis of nuclear rRNA following treatment with AZC or DHAZC*

Incubation conditions were the same as those described in Fig. 1, except that [^{3}H]adenosine served as labeled precursor. Nuclear rRNA isolated and electrophoresed in agarose–urea gels as described under MATERIALS AND METHODS. Approximately equal amounts of radioactivity were applied to each gel; therefore, the relative dpm do not reflect the specific activities of the RNA samples.

TABLE 4

Incorporation of [^{14}C]AZC and [^{14}C]DHAZC into nuclear rRNA, non-poly(A) hnRNA, and poly(A) hnRNA

L1210 cells were incubated for 30 min with either 100 μCi of [4-^{14}C]AZC (40, 8, and 4 mCi/mmol) or 5 μCi [4-^{14}C]DHAZC (0.4 mCi/mmol). Nuclear rRNA, non-poly(A) hnRNA, and poly(A) hnRNA were extracted as described under MATERIALS AND METHODS.

Treatment	rRNA		Non-poly(A) hnRNA		Poly(A) hnRNA		Total nRNA
	Per A_{260}	Per 5 × 10^7 cells	Per A_{260}	Per 5 × 10^7 cells	Per A_{260}	Per 5 × 10^7 cells	Per 5 × 10^7 cells
				(pmol of drug incorporated)			
[^{14}C]AZC							
0.1 mM	156 ± 15[a]	520 ± 19	171 ± 20	407 ± 30	701 ± 97	427 ± 26	979 ± 131
0.5 mM	499 ± 50	1,178 ± 107	450 ± 43	1,643 ± 152	4,325 ± 400	804 ± 60	3,625 ± 35
1 mM	491 ± 42	1,434 ± 109	915 ± 56	1,679 ± 219	6,665 ± 1,293	2,633 ± 253	5,839 ± 713
[^{14}C]DHAZC							
0.5 mM	305 ± 58	1,073 ± 125	1,965 ± 124	4,904 ± 451	73,956 ± 7,127	17,373 ± 2,023	21,773 ± 851

[a] Mean ± SE of three assays.

DISCUSSION

The present investigation has compared the effect of AZC with its hydrolytically stable analog, DHAZC, on nRNA synthesis in L1210 cells. It appears that with regard to the incorporation of labeled uridine into RNA, the uptake of the nucleoside precursor rather than transcription *per se* is inhibited by both drugs. This was deduced from experiments showing the similarity in the reduction of the specific radioactivity of UTP and the incorporation of uridine into RNA without changes in the concentration of UTP. In addition, the incorporation of labeled adenosine into nRNA was only marginally affected by AZC and DHAZC. In agreement with these results is the recent study by Plagemann (25) who showed that uridine transport in Novikoff hepatoma cells in culture was competitively inhibited by AZC resulting in a reduction in the radiolabeled pool of uridine nucleotides while the adenine nucleotide pool was not affected.

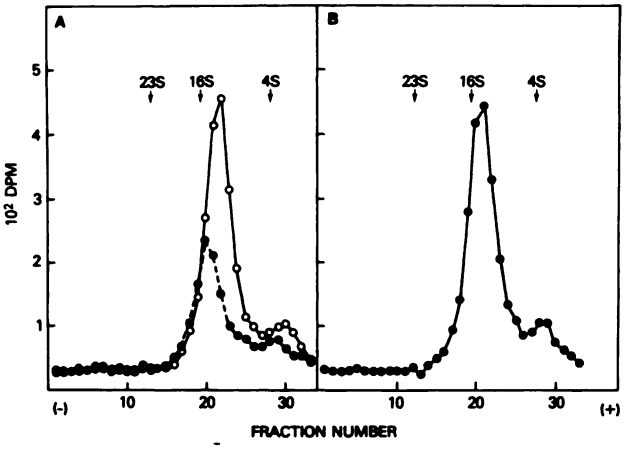

FIG. 5. *Incorporation of [^{14}C]AZC and [^{14}C]DHAZC into poly(A) hnRNA*

L1210 cells were incubated for 30 min with either 100 μCi of [^{14}C]AZC (4 or 40 mCi/mmol) or 5 μCi [^{14}C]DHAZC (0.4 mCi/mmol). Poly(A) hnRNA was extracted from isolated nuclei and electrophoresed in agarose–urea gels as described under MATERIALS AND METHODS. (A) ○, 0.1 mM [^{14}C]AZC (40 mCi/mmol); ●, 1 mM [^{14}C]AZC (4 mCi/mmol). (B) ●, 0.5 mM [^{14}C]DHAZC (0.4 mCi/mmol).

It was previously found that the potency of AZC *versus* DHAZC against L1210 leukemia *in vivo* differed by a factor of 30 (1, 2). This is in contrast to the present data which demonstrate that only minor differences exist between the two drugs in their inhibitory effects on transcription and methylation of nRNA. This suggests that other factors such as the metabolism of the drugs may be responsible for the lower potency of DHAZC. It was previously shown that DHAZC was deaminated by cytidine deaminase to a lesser extent than AZC (8) indicating that differences in metabolic activation rather than inactivation were probably responsible for the disparity in their antitumor activities. Evidence supporting this assumption was recently provided by Futterman *et al.* (26) who showed that the V_m of cytidine kinase with DHAZC as substrate was 25% of that with AZC as substrate. In the present study, a measure of the activation of AZC and DHAZC to their triphosphate form was assessed by their incorporation into nRNA. However, in this instance, DHAZC was incorporated into nRNA to an equal or greater extent than AZC. Thus, the anabolism of AZC and DHAZC does not appear to account for their differences in potency against L1210 cells.

The specific effect of AZC and DHAZC that is responsible for their cytotoxic activities is not known. AZC and DHAZC inhibited 2′-*O*-methylation and base methylation of nuclear rRNA, respectively, without altering the specific radioactivity of SAM. Interference by AZC with the methylation of rRNA and tRNA in HeLa cells (16) and of tRNA in mouse liver (17) suggests that the impaired processing of nucleolar RNA (10–12) and inhibition of protein synthesis (12, 14, 16, 27) by AZC may be attributed to this phenomenon. Indeed, inhibition of protein synthesis in HeLa cells by AZC was found to be dependent on rRNA or some low molecular weight species of RNA (27). In addition, we have also demonstrated that the transcription of nuclear rRNA and poly(A) hnRNA is inhibited by high concentrations of AZC and

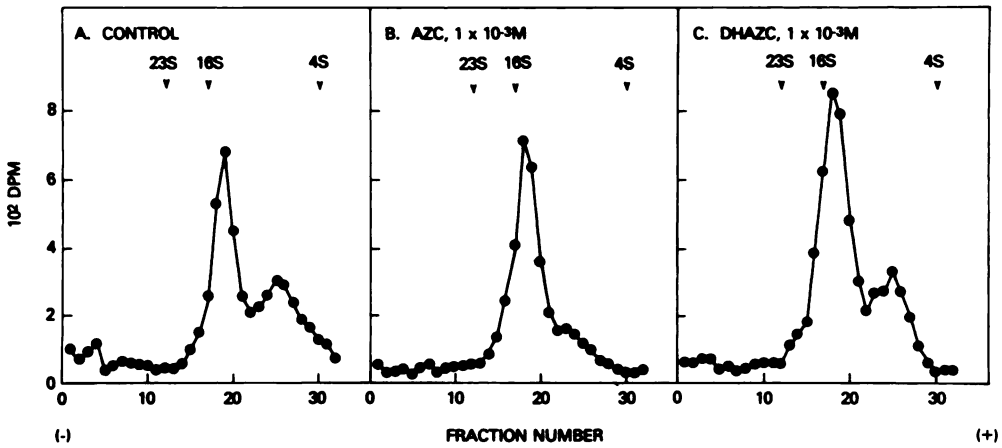

FIG. 6. *Agarose gel electrophoresis of poly(A) hnRNA following treatment with AZC and DHAZC*

L1210 cells were incubated for 30 min with either AZC or DHAZC followed by incubation for 30 min with [^{14}C]uridine. Poly(A) hnRNA was extracted from isolated nuclei by poly(U)–Sepharose chromatography and electrophoresed in agarose–urea gels as described under MATERIALS AND METHODS.

DHAZC. Although this effect does not appear to be one of the more sensitive metabolic processes affected by these agents (15), it could also contribute to impaired translation and inhibition of cell growth.

Thus, the cytotoxic effects of AZC and DHAZC can be attributed to at least three phenomena: incorporation into nRNA, and at higher concentrations inhibition of methylation of rRNA and tRNA, as well as inhibition of transcription of rRNA and poly(A) hnRNA.

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