MOLECULAR PHARMACOLOGY, 17, 250–255

The Comparative Effects of 5-Azacytidine and Dihydro-5-azacytidine on 4 S and 5 S Nuclear RNA

ROBERT I. GLAZER AND KATHLEEN D. HARTMAN

Applied Pharmacology Section, Laboratory of Medicinal Chemistry and Biology, National Cancer Institute, Bethesda, Maryland 20205

Received July 2, 1979; Accepted October 29, 1979

SUMMARY

GLAZER, R. I., AND K. D. HARTMAN. The comparative effects of 5-azacytidine and dihydro-5-azacytidine on 4 and 5 S nuclear RNA. *Mol. Pharmacol.* 17: 250–255 (1980).

The effects of 5-azacytidine and its hydrolytically stable analog, 5,6-dihydro-5-azacytidine, on the synthesis of low molecular weight nuclear RNA were studied in L1210 cells *in vitro*. Both drugs inhibited the synthesis of >18 S RNA, 5 S RNA, and 4 S RNA maximally within 1 hr of labeling with [^{3}H]adenosine. Dihydro-5-azacytidine was as potent an inhibitor as the parent drug on the synthesis of high molecular weight RNA, but was slightly more effective in inhibiting the synthesis of 5 and 4 S RNA with respect to both concentration of drug and duration of labeling. 5-[^{14}C]Azacytidine was incorporated into 5 and 4 S nuclear RNA after a delay of 1 hr which coincided with its inhibitory effect on the synthesis of these species of RNA. Both drugs affected the methylation of >18 S RNA and 4 S RNA to a greater extent than their synthesis. Analysis of eight methylated nucleosides in 4 S RNA indicated that 5-azacytidine and 5,6-dihydro-5-azacytidine inhibited the methylation of 2-methylguanosine and N^2,N^2-dimethylguanosine to a greater extent than that of the other nucleosides. These studies demonstrate that the dihydro analog of 5-azacytidine has a mode of action similar to that of the parent drug with respect to its effects on the synthesis and methylation of 5 and 4 S nuclear RNA.

INTRODUCTION

5-Azacytidine (AZC)[1] is an effective anticancer drug with activity against human acute nonlymphoblastic leukemia (1) and murine leukemias L1210 and P388 (2, 3). However, because of its instability in aqueous solutions (4, 5), a hydrolytically stable analog, 5,6-dihydro-5-azacytidine (DHAZC), was developed in this laboratory (2, 3, 6) to circumvent this problem and thereby provide greater therapeutic efficacy.

Preliminary studies have shown that AZC is approximately an order of magnitude more potent than DHAZC to L1210 and HeLa cells in tissue culture (7, 8). A similar order of potency pertains to the survival of mice bearing ascitic L1210 cells (2, 3), although both drugs prolong survival to equal extents.

The mechanism of the toxicity of DHAZC is unknown, although it is presumed to be similar to AZC perhaps by serving as a prodrug. On the other hand, recent evidence suggests that differences between DHAZC and AZC exist with respect to the kinetics of their cytotoxicity to L1210 and bone marrow cells in culture (9). The mechanism of action of AZC has been reported to involve inhibition of RNA and DNA synthesis in L1210 cells in culture (10) and in ascites (11), as well as in Novikoff hepatoma cells in culture (12). However, there is more specific evidence that AZC interferes with the synthesis and function of RNA. Previous studies have shown AZC to inhibit the processing of 45 S nucleolar RNA (13, 14), protein synthesis via the defective synthesis of a low molecular weight RNA (15) and the activity of tRNA in an *in vitro* translation system (16), presumably via its incorporation into RNA (10, 14, 17). Recent evidence also suggests that AZC produces hypomethylation of liver tRNA by interfering with the synthesis of 5-methylcytosine via decreased tRNA:cytosine-5-methyltransferase (18–20). Whether the latter effects also pertain to other species of RNA, as well as to neoplastic cells has not been determined.

To further explore the mechanism of action of DHAZC, its inhibitory activity on the synthesis and methylation of low molecular weight mRNA was evaluated in L1210 cells *in vitro* and compared in parallel with the parent drug, AZC.

[1] The abbreviations used are: AZC, 5-azacytidine; DHAZC, 5,6-dihydro-5-azacytidine; nRNA, nuclear RNA; tRNA, transfer RNA; SDS, sodium dodecyl sulfate; m^1A, 1-methyladenosine; m^3C, 3-methylcytidine; m^5C, 5-methylcytidine; m^1G, 1-methylguanosine; m^2G, 2-methylguanosine; $m_2{}^2G$, N^2,N^2-dimethylguanosine; m^7G, 7-methylguanosine; m^5U, 5-methyluridine; 4 S RNA, tRNA.

0026-895X/80/020250-06$02.00/0

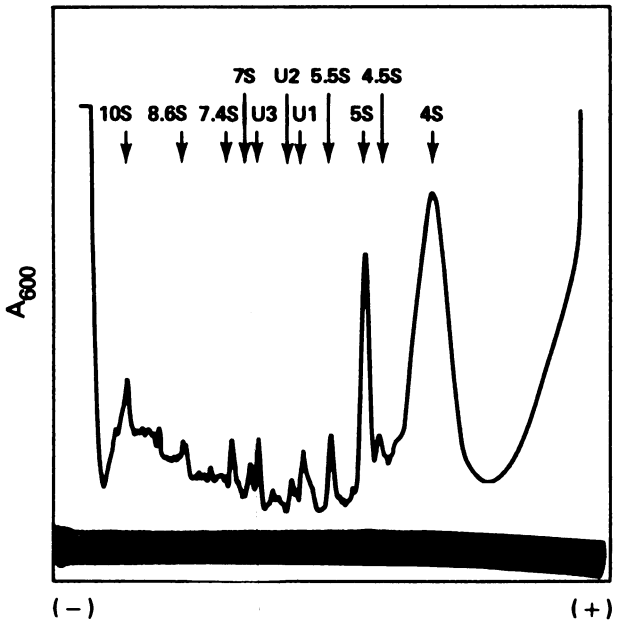

FIG. 1. *nRNA from L1210 cells*
RNA was extracted from isolated nuclei and electrophoresed in 8% polyacrylamide gels. RNA was stained with methylene blue as described under Materials and Methods.

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 $NaBH_4$ (3, 6). [4-^{14}C]AZC (53 mCi/mmole) was purchased from Monsanto Research Corporation, Dayton, Ohio. L-[*methyl*-^{3}H]Methionine (80 Ci/mmole) and [2,8-^{3}H]adenosine (31 Ci/mmole) were purchased from New England Nuclear Corporation, Boston, Massachusetts. m^1A, m^3C, m^5C, m^1G, m^2G, m^2_2G, m^7G and m^5U were obtained from P-L Biochemicals, Milwaukee, Wisconsin.

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 and were further diluted with incubation 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: (1) 25 ml of RPMI 1630 medium, 50 μCi [^{3}H]adenosine (31 Ci/mmole), and 5×10^7 cells; or (2) 25 ml Dulbecco's medium, 0.25% glucose, 250 μCi [*methyl*-^{3}H]methionine (80 Ci/mmole) and 5×10^7 cells. AZC or DHAZC (neutralized to pH 7.0) at the indicated concentrations were incubated with the cells at 37° for 30 min before addition of isotopes. The period of labeling was varied as indicated in the text.

RNA extraction. After incubation, cells were centrifuged at 400*g* for 20 min and washed once with incubation medium. Nuclei were prepared according to the method of Daskal *et al.* (21) 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 nRNA before extraction. nRNA was extracted from nuclei with 3 ml of 0.1% SDS:0.14 M NaCl:0.025 M sodium acetate (pH 5.1) and 3 ml of phenol mixture (phenol:*m*-cresol:water; 7:2:2, v/v, containing 0.1% 8-hydroxyquinoline) by vortexing

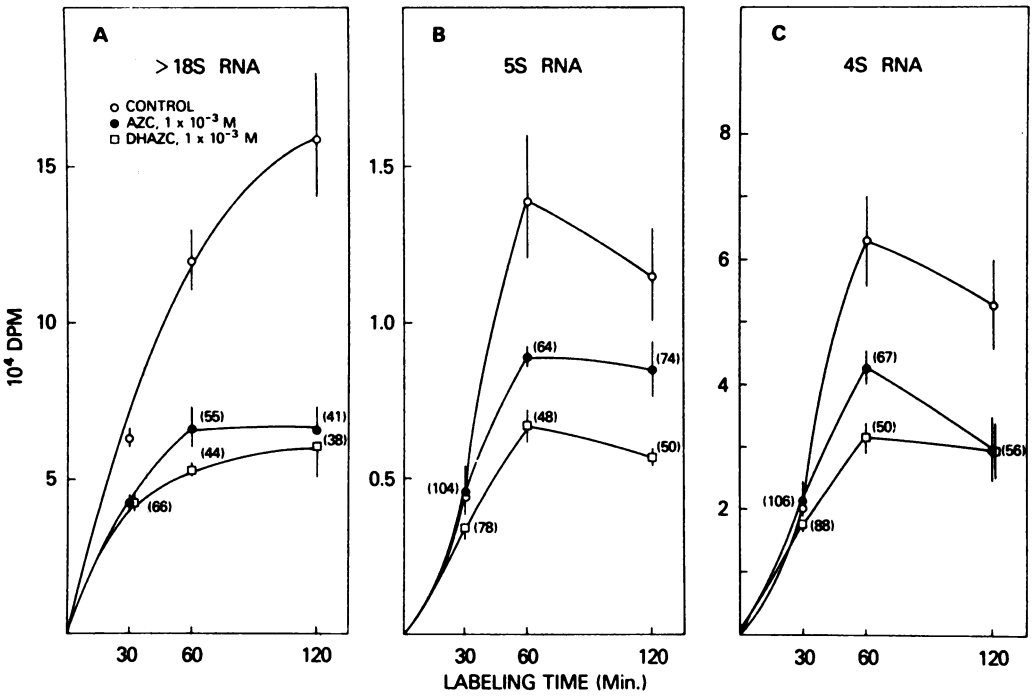

FIG. 2. *Time course of nRNA synthesis in L1210 cells treated with AZC and DHAZC*
Cells were treated with AZC or DHAZC for 30 min and labeled with [^{3}H]adenosine for 30 min, 1 hr and 2 hr. The radioactivity in each RNA fraction was determined from its position after polyacrylamide gel electrophoresis as shown in Fig. 3. Each value is the mean ± SE of three determinations.

vigorously for 2 min. The emulsion was clarified by centrifugation at 12,000*g* for 10 min and the upper aqueous phase removed and precipitated with 3 vol of 2% potassium acetate in 95% ethanol at −20° overnight.

Electrophoresis. nRNA was resolved by electrophoresis in cylindrical polyacrylamide gels (0.4 × 7 cm) containing 8% (w/v) acrylamide, 0.32% (w/v) diallytartardiamide, 6 M urea, 0.1% (w/v) SDS, 0.2% (w/v) ammonium persulfate, 0.04% (v/v) *N,N,N′N′*-tetramethylenediamine, 0.4 M Tris–acetic acid (pH 7.2), 0.02 M sodium acetate and 0.002 M EDTA. RNA samples containing one A_{260} unit were mixed with sample buffer to give a final concentration of: 0.04 M Tris–acetic acid (pH 7.2), 0.02 M sodium acetate, 0.002 M EDTA, 0.02% bromphenol blue and 20% (w/v) sucrose (RNase-free). Gels were electrophoresed at 4 mA per gel at 4°. Gels were sectioned into 2-mm slices, dissolved in 2% (w/v) periodic acid at 37° for 15 min, mixed with 10 ml of Aquasol (New England Nuclear, Boston, Mass.) and counted in a Searle Mark III liquid scintillation spectrometer. Gels were stained with 2% methylene blue dissolved in 15% acetic acid for 1 hr and destained by diffusion in 15% acetic acid.

Thin-layer chromatography. RNA (4 S) was isolated by polyacrylamide gel electrophoresis, sliced from the appropriate section of the gel and sectioned into 2-mm slices. RNA was extracted from four gel slices with 1 ml of RNA extraction buffer (0.1% SDS:0.014 M NaCl:0.025 M sodium acetate, pH 5.1) and continuous vortexing at room temperature for 1 hr. The gel was removed by centrifugation at 16,000*g* for 2 min in an Eppendorf centrifuge, and the RNA was precipitated at −20° for 2 hr. Enzymatic digestion was carried out for 18 hr at 37° in 20 μl of 0.05 M Tris–HCl (pH 8.0):5 mM $MgCl_2$ containing 6 μg RNase A, 5 μg calf intestine alkaline phosphatase (1000 units/mg) and 10 μg snake venom phosphodiesterase. Samples were freeze-dried and reconstituted with 20 μl of a standard mixture containing the eight methylated nucleosides at a concentration of 0.4 mg/ml. An aliquot of 5 μl was spotted on 0.25-mm silica gel plates containing fluorescent indicator (EM Laboratories, Elmsford, N.Y.) and the eight methylated nucleosides were separated by two-dimensional thin-layer chromatography with acetonitrile:concentrated NH_4OH (4:1, v/v) for the first dimension and acetonitrile:2 N HCOOH (10:1, v/v) for the second dimension (19).

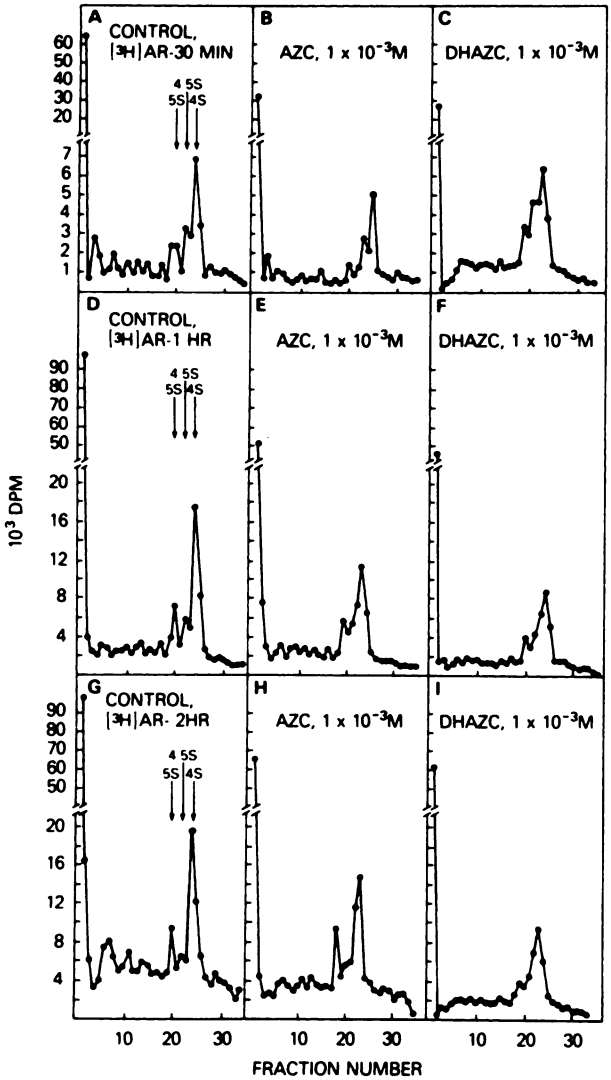

FIG. 3. *Polyacrylamide gel electrophoresis of nRNA from L1210 cells treated with AZC and DHAZC*

Cells were incubated for 30 min with 1 × 10^{-3} M AZC or DHAZC followed by labeling with 50 μCi of [^{3}H]adenosine for 30 min (A–C), 1 hr (D–F) or 2 hr (G–I).

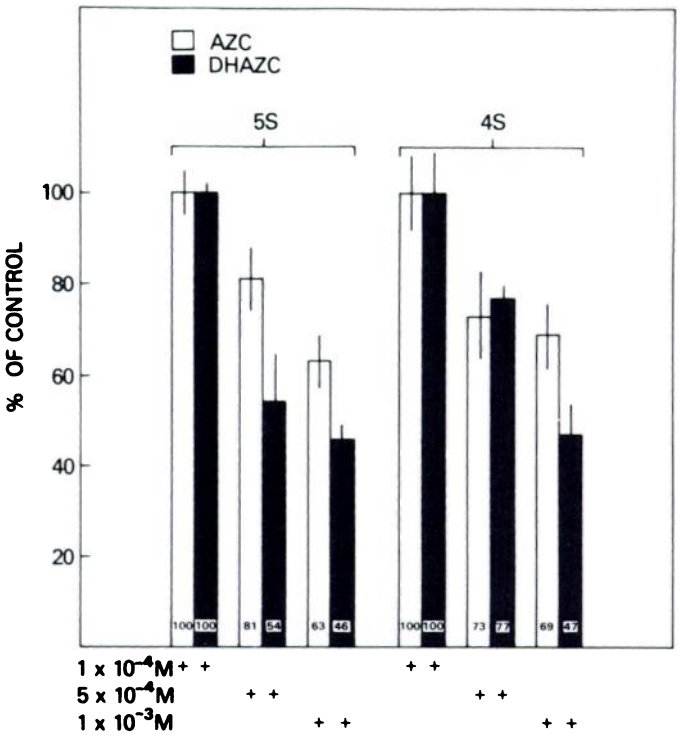

FIG. 4. *Dose response of 5 and 4 S RNA synthesis in L1210 cells treated with AZC and DHAZC*

Cells were treated for 30 min with varying concentrations of AZC or DHAZC followed by labeling for 1 hr with [^{3}H]adenosine. The radioactivity present in each RNA fraction was determined after polyacrylamide gel electrophoresis of 1 A_{260} unit of total RNA as shown in Fig. 3. Each value is the mean ± SE of three determinations. Control values (dpm) for 5 and 4 S RNA were 9060 ± 1200 and 36,000 ± 4200, respectively, for four determinations.

RESULTS

Effect on nRNA synthesis. RNA extracted from isolated nuclei and electrophoresed in denaturing 8% polyacrylamide gels was stained with methylene blue (Fig. 1). The major low molecular weight species of nRNA present were 4 S and 5 S RNA with lesser amounts of 4.5, U1 (5.9 S), U2 (6.2 S) and U3 (6.8 S) RNA.

Initial studies were carried out by incubating L1210 cells with AZC or DHAZC for 30 min followed by pulse-labeling with [^{3}H]adenosine for varying periods of time (Fig. 2). The major species of RNA that were labeled under the incubation conditions were >18 S, 5 S, and 4 S RNA. Both antimetabolites at 1×10^{-3} M reduced the labeling of >18 S nRNA by approximately 50% with inhibition remaining essentially unchanged throughout 1–2 hr of labeling (Fig. 2A). The 5 S and 4 S nRNA were inhibited by both drugs to the same extent as >18 S nRNA with DHAZC producing a moderately greater effect than AZC (Figs. 2B, C). Electrophoretic profiles of low molecular weight nRNA in 8% polyacrylamide–urea–SDS gels revealed time-dependent labeling with [^{3}H]-adenosine of mainly 5 S and 4 S nRNA (Figs. 3A, D, G) in control incubations. AZC and DHAZC inhibited the labeling of these species of nRNA but did not appear to affect their distribution pattern (Figs. 3B, C, E, F, H, I).

Inhibition of 5 S and 4 S nRNA by AZC and DHAZC was dose dependent with concentrations of drug greater than 1×10^{-4} M required to impair their synthesis (Fig. 4).

Incorporation of AZC into nRNA. To assess the temporal relationship between inhibition of 5 S and 4 S nRNA by AZC and its incorporation into these species of RNA, gel electrophoresis of nRNA labeled with [^{14}C]AZC was carried out after varying periods of labeling (Fig. 5). [^{14}C]AZC was incorporated into 5 S and 4 S nRNA after 1 hr with clear resolution of these labeled species of nRNA after 2 hr. Although labeled DHAZC was unavailable for comparison, recently completed studies indicate that both drugs are similarly distributed in high molecular weight nRNA from L1210 cells (22).

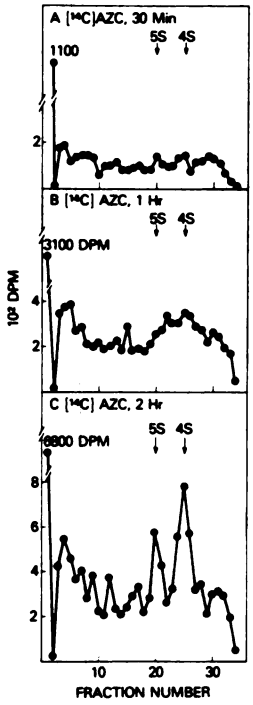

FIG. 5. *Incorporation of [^{14}C]AZC into nRNA in L1210 cells*

Cells were treated with 1×10^{-4} M [^{14}C]AZC (20 mCi/mmole) for 30 min (*A*), 1 hr (*B*) or 2 hr (*C*). Polyacrylamide gel electrophoresis of nRNA was performed as described under Materials and Methods.

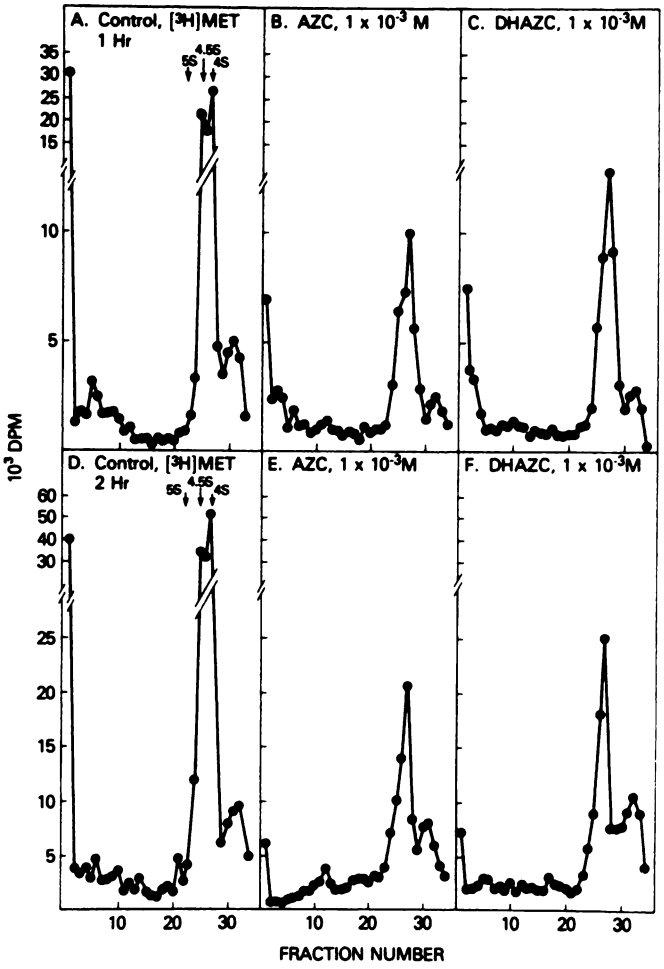

FIG. 6. *Methylation of 4 S RNA in L1210 cells treated with AZC or DHAZC*

Cells were treated with 1×10^{-3} M AZC or DHAZC followed by labeling with [*methyl*-^{3}H]methionine for 1 hr (A–C) or 2 hr (D–F).

Effect of AZC and DHAZC on the methylation of 4 S RNA. Recent studies have suggested that AZC can affect the methylation of liver tRNA (4 S RNA) via inhibition of RNA methyltransferase activity (18–20). To investigate the importance of this process in neoplastic cells, pulse-labeling experiments were carried out using [*methyl*-^{3}H]methionine as precursor (Fig. 6). The predominant species of nRNA that was methylated was 4 S RNA (Figs. 6A, D). AZC and DHAZC at 1×10^{-3} M inhibited methylation of 4 S RNA by 55–60%, while at 1×10^{-4} M of each drug, 10–20% inhibition of methylation was observed after 2 hr of labeling (Fig. 7).

To assess the specificity of this effect on RNA methylation, 4 S RNA from drug-treated cells was isolated by electrophoresis, extracted, and enzymatically digested. Recovery of 4 S RNA after extraction amounted to

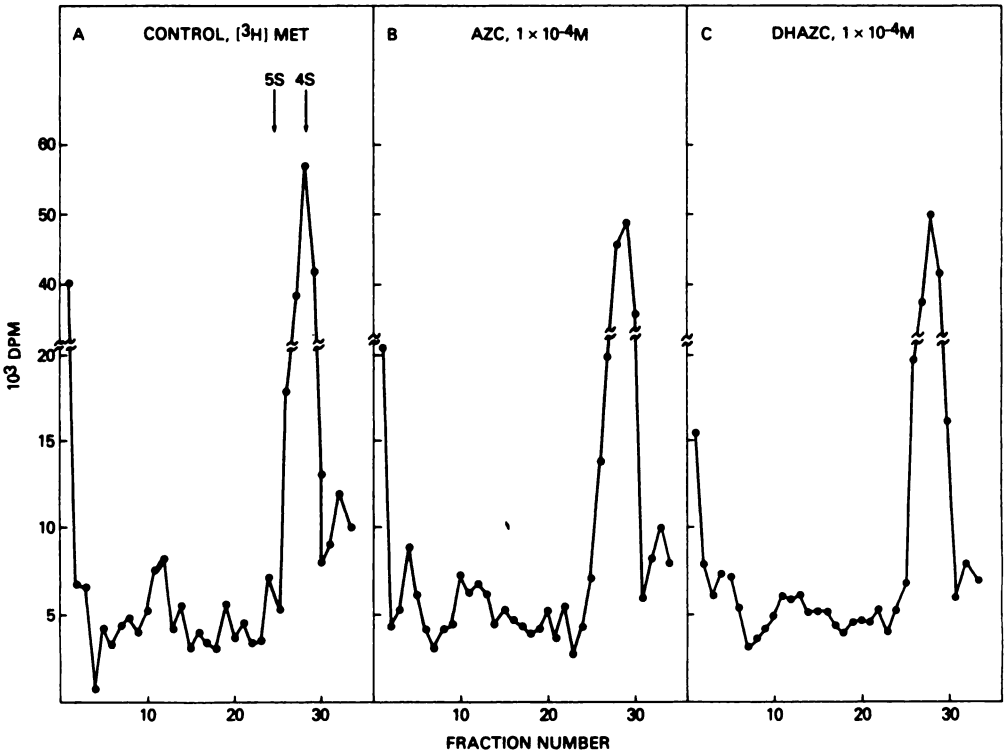

FIG. 7. *Methylation of 4 S RNA in L1210 cells treated with AZC or DHAZC*
Cells were treated with 1×10^{-4} M AZC or DHAZC followed by labeling with [*methyl*-^{3}H]methionine for 2 hr.

approximately 30% of the radioactivity present in the gel. Incorporation of radioactivity into eight methylated nucleosides of 4 S RNA was measured by two-dimensional thin-layer chromatography (Table 1). The labeling of almost all of the base-methylated nucleosides was diminished by 1×10^{-3} M AZC and DHAZC with m^2G and m_2^2G being affected to the greatest extent. At 1×10^{-4} M AZC, methylation of m^2G and m_2^2G was still significantly reduced while the same concentration of DHAZC only affected the labeling of m^1G.

DISCUSSION

The present experiments were designed to investigate the comparative effects of AZC and its congener, DHAZC, on the synthesis and methylation of low molecular weight nRNA. The main species of this class of nRNA showing significant time-dependent labeling *in vitro* under our incubation conditions were 5 S and 4 S RNA. This was not unexpected since other classes of low molecular weight nRNA either have a half-life of 20–30 hr or no measurable turnover rate (23). Thus, we have not ruled out that these drugs might affect other species of low molecular weight nRNA to an equal degree as 5 S and 4 S RNA.

AZC and DHAZC inhibited the synthesis and methylation of high molecular weight, 5 S and 4 S RNA to approximately equal extents at 1×10^{-3} M; however, no inhibition was observed at 1×10^{-4} M. These results are

TABLE 1

Methylation of nucleosides in 4 S RNA from L1210 cells treated with AZC or DHAZC

Cells were treated with either 1×10^{-4} and 1×10^{-3} M AZC or DHAZC for 30 min followed by labeling with [*methyl*-^{3}H]methionine for 2 hr. RNA (4 S) was isolated by polyacrylamide gel electrophoresis, hydrolyzed and methylated nucleosides separated by two-dimensional thin-layer chromatography as described under Materials and Methods. Each value is the mean ± SE of three to five experiments, except the controls which represent the mean ± SE of seven experiments.

Treatment	Methylated nucleoside							
	m^2G	m_2^2G	m^5C	m^1G	m^7G	m^1A	m^5U	m^3C
	(dpm)							
Control	5850 ± 750	16110 ± 1280	2640 ± 330	4440 ± 510	2990 ± 350	2000 ± 290	3540 ± 410	660 ± 70
	% of control							
AZC, 1×10^{-4} M	53 ± 6[a]	67 ± 10[a]	88 ± 8	73 ± 8	76 ± 5	81 ± 9	86 ± 9	66 ± 9[a]
1×10^{-3} M	34 ± 5[c]	47 ± 3[c]	79 ± 10	48 ± 7[b]	62 ± 5[a]	47 ± 3[b]	52 ± 8[b]	57 ± 6[b]
DHAZC, 1×10^{-4} M	74 ± 7	81 ± 8	98 ± 10	68 ± 8[a]	77 ± 6	101 ± 10	88 ± 11	95 ± 12
1×10^{-3} M	48 ± 7[b]	37 ± 3[c]	69 ± 7[b]	47 ± 5	81 ± 10	55 ± 6[a]	44 ± 3[c]	57 ± 8[b]

[a] $p < 0.05$ vs control.
[b] $p < 0.01$ vs control.
[c] $p < 0.001$ vs control.

in agreement with our previous studies where it was shown that transcription of nuclear rRNA and poly(A)RNA were inhibited only at high concentrations of AZC and DHAZC (22). More importantly, both drugs are incorporated into nRNA at concentrations that do not affect transcription as shown in the present experiments for the incorporation of [^{14}C]AZC into high molecular weight, 5 S and 4 S RNA.

An equally important aspect of the mechanism of action of AZC and DHAZC is their ability to inhibit the methylation of 4 S RNA. Each agent exhibited a qualitatively similar pattern of inhibition of the eight major methylated bases in 4 S RNA. AZC primarily reduced the labeling of m^2G and m_2^2G at 1×10^{-4} M while DHAZC mainly inhibited m^1G at an equivalent concentration. Higher concentrations of each inhibitor produced an additional spectrum of inhibitory activity, although m^2G and m_2^2G remained the methylated nucleosides most affected by both drugs. The methylation of m^5C was unaffected by AZC and DHAZC. Clearly, the specificity previously reported for AZC for inhibiting the methylation of only m^5C in mouse liver 4 S RNA *in vivo* via its methyltransferase (18, 19) does not apply to L1210 leukemia cells *in vitro*. Reduced methylation of m^5C in 4 S RNA did not appear to be essential for aminoacylation via tRNA synthetase (20). These results differ from those reported by Kalousek *et al.* (24) and Lee and Karon (25) where reduced amino acid acceptor activity of AZC-modified tRNA was demonstrated. Thus, it is possible that the primary effect of AZC, and possibly DHAZC as well, could reside in the reduction of synthesis of other methylated bases in tRNA as shown in our studies with L1210 cells, and account for the low rate of protein synthesis in AZC-treated cells (25). In addition, incorporation of AZC and DHAZC into 4 S RNA, 5 S RNA, rRNA and mRNA (23, 25) may also contribute to a reduced fidelity and rate of translation.

ACKNOWLEDGMENT

The authors appreciate the assistance of Mrs. Margaret Green in the preparation of this manuscript.

REFERENCES

1. Saiki, J. H., K. B. McCredie, T. J. Vietti, J. S. Hewlett, F. S. Morrison, J. J. Costanzi, W. J. Stuckey, J. Whitecar and B. Hoogstraten. 5-Azacytidine in acute leukemia. *Cancer* **42**: 2111–2114 (1978).
2. Beisler, J. A., M. M. Abbasi and J. S. Driscoll. Dihydro-5-azacytidine hydrochloride, a biologically active and chemically stable analog of 5-azacytidine. *Cancer Treat. Rep.* **60**: 1671–1674 (1976).
3. Beisler, J. A., M. M. Abbasi, J. A. Kelley and J. S. Driscoll. Synthesis and antitumor activity of dihydro-5-azacytidine, a hydrolytically stable analogue of 5-azacytidine. *J. Med. Chem.* **20**: 806–812 (1977).
4. Notari, R. E. and J. L. DeYoung. Kinetics and mechanisms of degradation of the antileukemic agent 5-azacytidine in aqueous solutions. *J. Pharm. Sci.* **64**: 1148–1157 (1975).
5. Beisler, J. A. Isolation, characterization, and properties of a labile hydrolysis product of the antitumor nucleoside, 5-azacytidine. *J. Med. Chem.* **21**: 204–208 (1978).
6. Beisler, J. A., M. M. Abbasi, J. A. Kelley and J. S. Driscoll. Chemistry of antitumor triazine nucleosides. An improved synthesis of dihydro-5-azacytidine. *J. Carbohyd. Nucleosides Nucleotides* **4**: 281–299 (1977).
7. Voytek, P., J. A. Beisler, M. M. Abbasi and M. K. Wolpert-DeFilippes. Comparative studies of the cytostatic action and metabolism of 5-azacytidine and 5,6-dihydro-5-azacytidine. *Cancer Res.* **37**: 1956–1961 (1977).
8. Futterman, B., J. Derr, J. A. Beisler, M. M. Abbasi and P. Voytek. Studies on the cytostatic action, phosphorylation and deamination of 5-azacytidine and 5,6-dihydro-5-azacytidine in HeLa cells. *Biochem. Pharmacol.* **27**: 907–911 (1978).
9. Presant, C. A. Kinetics of dihydroazacytidine cytotoxicity differ from 5-azacytidine. *Proc. Am. Assoc. Cancer Res.* **20**: 28 (1979).
10. Li, L. H., E. J. Olin, H. H. Buskirk and L. M. Reineke. Cytotoxicity and mode of action of 5-azacytidine on L1210 leukemia. *Cancer Res.* **30**: 2760–2769 (1970).
11. McGuire, W., K. Grotzinger and R. Young. Alterations in [^{3}H]thymidine incorporation into DNA and [^{3}H]uridine incorporation into RNA induced by 5-azacytidine *in vivo*. *Biochem. Pharmacol.* **27**: 745–750 (1978).
12. Plagemann, P. G. W., M. Behrens and D. Abraham. Metabolism and cytotoxicity of 5-azacytidine in cultured Novikoff rat hepatoma and P388 leukemia cells and their enhancement by preincubation with pyrazofurin. *Cancer Res.* **38**: 2458–2466 (1978).
13. Reichman, M., E. Karlan and S. Penman. Destructive processing of the 45 S ribosomal precursor in the presence of 5-azacytidine. *Biochim. Biophys. Acta* **299**: 173–175 (1973).
14. Weiss, J. W. and H. C. Pitot. Effects of 5-azacytidine on nucleolar RNA and the preribosomal particles in Novikoff hepatoma cells. *Biochemistry* **14**: 316–326 (1975).
15. Reichman, M. and S. Penman. The mechanism of inhibition of protein synthesis by 5-azacytidine in HeLa cells. *Biochim. Biophys. Acta* **324**: 282–289 (1973).
16. Momparler, R. L., S. Siegel, F. Avila, T. Lee and M. Karon. Effect of tRNA from 5-azacytidine-treated hamster fibrosarcoma cells on protein synthesis *in vitro* in a cell-free system. *Biochem. Pharmacol.* **25**: 389–392 (1976).
17. Jurovčik, M., K. Raška, Z. Šormova and R. Šorm. Anabolic transformation of a novel antimetabolite, 5-azacytidine, and evidence for its incorporation into ribonucleic acid. *Coll. Czech. Chem. Commun.* **30**: 3370–3376 (1965).
18. Lu, L. W., G. H. Chiang, D. Medina and K. Randerath. Drug effects on nucleic acid modification. I. A specific effect of 5-azacytidine on mammalian transfer RNA methylation *in vivo*. *Biochem. Biophys. Res. Commun.* **68**: 1094–1101 (1976).
19. Lu, L. J. W. and K. Randerath. Effects of 5-azacytidine on transfer RNA methyltransferases. *Cancer Res.* **39**: 940–948 (1979).
20. Harris, J. S. and K. Randerath. Aminoacylation of undermethylated mammalian transfer RNA. *Biochim. Biophys. Acta* **521**: 566–575 (1978).
21. Daskal, I., S. A. Ramirez, R. N. Ballal, W. H. Spohn, B. Wu and H. Busch. Detergent lysis for isolation of intact polysomes of Novikoff hepatoma ascites cells. *Cancer Res.* **36**: 1026–1034 (1976).
22. 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 in L1210 cells *in vitro*. *Mol. Pharmacol.* **17**: 111–117 (1980).
23. Zieve, G. and S. Penman. Small RNA species of the HeLa cells: Metabolism and subcellular localization. *Cell* **8**: 19–31 (1976).
24. Kalousek, F., K. Raška, Jr., M. Jorovčik and F. Šorm. Effect of 5-azacytidine on the acceptor activity of sRNA. *Coll. Czech. Chem. Commun.* **31**: 1421–1424 (1966).
25. Lee, T. T. and M. R. Karon. Inhibition of protein synthesis in 5-azacytidine-treated HeLa cells. *Biochem. Pharmacol.* **25**: 1737–1742 (1976).

Send reprint requests to: Dr. Robert I. Glazer, Laboratory of Medicinal Chemistry and Biology, National Cancer Institute, Building 37, Room 6A01, NIH Bethesda, Md. 20205.