MOLECULAR PHARMACOLOGY, 20:644–648

The Comparative Effects of 5-Azacytidine and Dihydro-5-azacytidine on Polysomal RNA in Ehrlich Ascites Cells *in Vitro*

HSIN-LI LIN AND ROBERT I. GLAZER

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

Received January 27, 1981; Accepted June 12, 1981

SUMMARY

The comparative effects of 5-azacytidine (AZC) and dihydro-5-azacytidine (DHAZC) on the synthesis of polysomal RNA, as well as the translational activity of drug-modified poly(A)RNA were studied in Ehrlich ascites cells *in vitro*. AZC inhibited the incorporation of [^{3}H]adenosine into non-poly(A)RNA in a concentration-dependent manner, but did not affect the synthesis of poly(A)RNA. In contrast, neither RNA fraction was significantly affected by equimolar concentrations of DHAZC except at 10^{-3} M, where 40% inhibition of the synthesis of only non-poly(A)RNA was achieved. Electrophoretic characterization of non-poly(A)RNA revealed that the synthesis of 28 S rRNA was inhibited to a greater extent than was 18 S rRNA by 10^{-5} M AZC, but to equal extents at 10^{-4} M drug. Significant inhibition of 28 S and 18 S rRNA occurred only with 10^{-3} M DHAZC, where approximately 50% inhibition of both rRNA species occurred. Both cytidine analogues were ineffective in inhibiting tRNA synthesis. AZC and DHAZC were incorporated into all species of polysomal RNA, but the amount of drug substitution into a particular species of RNA was not proportional to its effect on the synthesis or function of that RNA. There was more analogue incorporated into poly(A)RNA than into non-poly(A)RNA, but drug-substituted poly(A)RNA showed neither an impairment of its coding efficiency nor qualitative changes in the translation products synthesized in a rabbit reticulocyte translation system *in vitro*. From these results, it appears that the relative differences in the inhibitory effects by the two cytidine analogues on rRNA synthesis, as well as their incorporation into rRNA, correlate closely with their relative potencies as antitumor agents.

INTRODUCTION

AZC,[1] an analogue of cytidine, is an effective antimetabolite against human acute myeloblastic leukemia (1) and acute lymphoblastic leukemia (2, 3), as well as several experimental murine tumor systems (4, 5). In both prokaryotic and eukaryotic systems, AZC is phosphorylated, and incorporated into DNA and RNA (5, 6). The mechanism of action of AZC is not completely understood, but various studies have shown that it inhibits DNA, RNA, and protein syntheses (see review in Ref. 7). However, the accumulated evidence thus far has shown that AZC primarily interferes with the synthesis and function of RNA. Several studies by Weiss and Pitot (8–10) revealed that AZC inhibited the processing of 45 S nucleolar RNA at the 32 S RNA level in Novikoff hepatoma cells. Both 45 S and 32 S RNA from AZC-treated cells showed altered electrophoretic mobilities which were not the consequence of undermethylation. In contrast, Reichman *et al.* (11) found aberrant processing of 45 S RNA in HeLa cells after AZC treatment which resulted in either complete degradation of the 45 S precursor RNA or its alteration to an unrecognizable form. AZC inhibited nuclear rRNA and poly(A)RNA to equal extents in L1210 ascites cells, and affected methylation of nuclear rRNA to a lesser extent than its synthesis (12). The methylation and synthesis of nuclear 4 S RNA were also inhibited to equal degrees by AZC (13), and a dose-response relationship similar to that for nuclear rRNA and poly(A)RNA was observed (12, 13). Analysis of the effects of AZC on cytoplasmic species of RNA has revealed that the synthesis of 28 S and 18 S rRNA (14), tRNA acceptor activity (15), tRNA methylation (16, 17), and the ability of tRNA to support protein synthesis (18) are inhibited; however, the synthesis of cytoplasmic poly(A)RNA was not affected (14). Inhibition of protein synthesis by AZC was thought to occur at the initiation level, possibly as a result of the defective synthesis of a low molecular weight RNA (19).

AZC is unstable in aqueous solutions. To circumvent this chemical instability, a biologically active and hydrolytically stable analogue, DHAZC, was synthesized by Beisler and co-workers (20, 21). DHAZC possesses the

[1] The abbreviations used are: AZC, 5-azacytidine; DHAZC, 5,6-dihydro-5-azacytidine; SDS, sodium dodecyl sulfate; AR, adenosine; UR, uridine.

0026-895X/81/030644-05$02.00/0

same degree of antitumor activity and dose schedule dependency as the parent drug, AZC, but its potency is approximately 30-fold lower *in vivo* (21) and 10-fold lower *in vitro* (22). The mechanism of cytotoxicity of DHAZC is unknown. Although it was originally presumed to be a product of AZC (21), recent metabolic and biochemical studies suggest that this is not the case. DHAZC is a better substrate for cytidine deaminase but a poorer substrate for cytidine kinase than is AZC in HeLa cells (23) and L1210 cells (22). In cultured mouse embryo cells, AZC inhibits DNA methylation and induces muscle cell differentiation, whereas DHAZC is virtually inactive (24). Metabolic studies in rats showed no evidence of formation of AZC or its principal hydrolysis product in urine following the administration of DHAZC (25).

In our laboratory, we previously compared the effects of AZC and DHAZC on the synthesis of nuclear RNA in L1210 cells *in vitro* (12, 13). Although both drugs inhibited the methylation and synthesis of 18 S RNA, 5 S RNA, and 4 S RNA to equal degrees (13), the relative activities of the drugs did not correlate with their markedly different potencies as antitumor agents (21, 22). Thus, it seemed plausible that the differences in cytotoxicity between AZC and DHAZC resulted from their effects on the posttranscriptional processing of nRNA or to modification of the translational activity of drug-substituted mRNA. To investigate these possibilities, the synthesis of polysomal rRNA, tRNA, and mRNA [poly(A)RNA], and the translational activity of AZC- and DHAZC-modified poly(A)RNA were determined in Ehrlich ascites cells *in vitro*.

MATERIALS AND METHODS

Materials. AZC and DHAZC were obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute. [4-^{14}C]AZC (45 mCi/mmole) was purchased from Monsanto Research Corporation (Dayton, Ohio), and [4-^{14}C]DHAZC (50 mCi/mmole) from Moravek Biochemicals (City of Industry, Calif.). [3,4,5-^{3}H]Leucine (120 Ci/mmole), [2,8-^{3}H]adenosine (36.2 Ci/mmole), [5,6-^{3}H]uridine (37.4 Ci/mmole) and L-[^{35}S]methionine (1117 Ci/mmole), were obtained from New England Nuclear Corporation (Boston, Mass.).

Cells. Ehrlich ascites cells (10^5 cells in 0.1 ml of Hanks' balanced salt solution) were inoculated i.p. into Swiss mice. After 7 days the cells were harvested, washed once in RPMI 1630 medium, and resuspended in the same medium.

Incubations. Incubations were carried out at 37° in a shaking water bath and consisted of 5×10^8 cells in 250 ml of RPMI 1630 medium with 10% newborn calf serum, various concentrations of either AZC or DHAZC, and the indicated amounts of [^{3}H]adenosine or [^{3}H]uridine when labeling RNA.

RNA extraction. After incubation, the cells were centrifuged at 400 × *g* for 10 min and washed once with RPMI 1630 medium. The cell pellet was homogenized mechanically in a buffer of 25 mM Tris-HCl (pH 7.5)/25 mM NaCl/5 mM $MgCl_2$/2% Triton X-100/heparin (1 mg/ml). The homogenate was centrifuged at 27,000 × *g* for 5 min and the polysomes were precipitated from the supernatant at 4° for 1 hr by adjusting the concentration of $MgCl_2$ to 0.2 M. Polysomes were isolated by centrifugation at 27,000 × *g* for 5 min, and total polysomal RNA was extracted by the SDS/phenol method (26). RNA was precipitated with 3 volumes of 2% potassium acetate in 95% ethanol at −20° for 1 hr. Total polysomal RNA was further fractionated into poly(A)RNA and non-poly(A)RNA by poly(U)-Sepharose 4B affinity chromatography (27), and the respective RNA fractions were precipitated with 95% ethanol at −20° overnight.

Agarose-urea gel electrophoresis. Disc gel electrophoresis of poly(A)RNA and non-poly(A)RNA was carried out by the method of Locker (28) with 2% agarose/6 M urea. Gels were run in a 0.4 M Tris/0.02 M sodium acetate/0.033 M acetic acid/0.001 M EDTA (buffer (pH 7.4) for about 3 hr at 5° at the field strength of 6.7 V/cm, and contained up to 1 A_{260} unit per gel. After electrophoresis, gels were sliced into 2-mm sections, dissolved in 60% perchloric acid, mixed with 10 ml of Aquasol, and counted in a Searle Mark III liquid scintillation spectrometer.

Rabbit reticulocyte lysate translation system. The translational activity of AZC and DHAZC-substituted poly(A)RNA was measured in an *in vitro* rabbit reticulocyte lysate translation system (Bethesda Research Laboratories, Rockville, Md.). The assay was carried out as previously described (29) and contained either [^{3}H] leucine (5 μCi) or [^{35}S]methionine (5 μCi) as the radioactive tracer. To compare the differences in translation products between the control and drug-treated poly(A)RNA-directed protein synthesis, a 10% polyacrylamide slab gel (14 × 11 cm) with 5% stacking gel (14 × 2.5 cm) was employed with the SDS buffer system de-

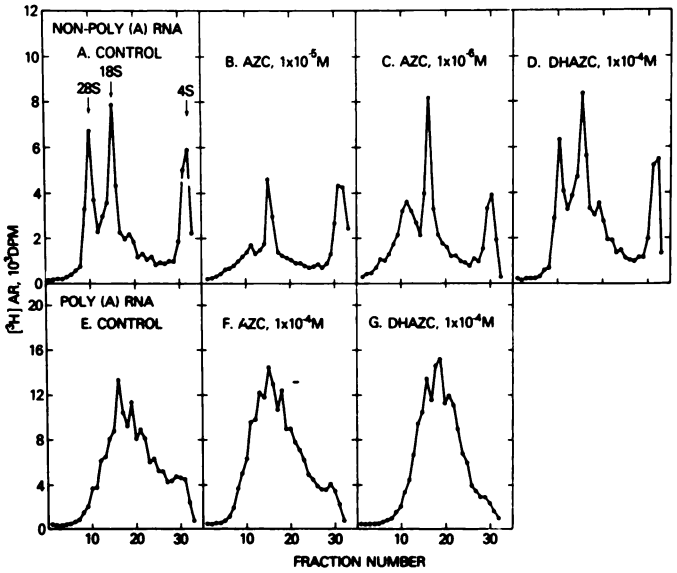

FIG. 1. *Agarose gel electrophoresis of non-poly(A)RNA and poly(A)RNA from Ehrlich cells treated with AZC and DHAZC*

Ehrlich ascites cells were incubated at 37° with or without AZC or DHAZC. After 1 hr, 250 Ci of [^{3}H]AR were added to each flask and incubation was continued for 1 hr before the cells were harvested. Polysomal non-poly(A)RNA and poly(A)RNA were extracted as described under Materials and Methods. Control values averaged 0.4 pmole of [^{3}H]AR incorporated per A_{260} of non-poly(A)RNA, and 2.5 pmoles per A_{260} of poly(A)RNA.

TABLE 1

Effects of AZC and DHAZC on the synthesis of non-poly(A)RNA

Non-poly(A)RNA was isolated and electrophoresed in 2% agarose-urea gels as described in Fig. 1. The radioactivity in 28 S, 18 S, and 4 S RNA fractions was determined and the results are expressed as percentages of control = 100%. Each value represents the mean ± standard error of three to five experiments or the mean of two experiments. Total radioactivity values (disintegrations per minute) in control 28 S, 18 S, and 4 S RNA were 8,200 ± 1,000, 11,700 ± 1,100, and 11,400 ± 1,400, respectively, for seven determinations.

Treatment	% Control		
	28 S RNA	18 S RNA	4 S RNA
AZC			
10^{-4} M	30 ± 4	29 ± 4	100 ± 14
10^{-5} M	34 ± 6	66 ± 7	95 ± 9
10^{-6} M	100	112	106
DHAZC			
10^{-3} M	48 ± 5	59 ± 5	97 ± 17
10^{-4} M	113 ± 10	122 ± 2	100 ± 3
10^{-5} M	118 ± 17	119 ± 5	90 ± 10

scribed by Laemmli and Favre (30). Samples were prepared as previously described (29), and assays were quantitated by densitometric scanning of the fluorographs of the electrophoresed [^{35}S]methionine-labeled proteins.

RESULTS

Effect of AZC and DHAZC on polysomal RNA synthesis. Representative agarose gel electrophoretic profiles of non-poly(A)RNA and poly(A)RNA from cells treated with AZC and DHAZC are shown in Fig. 1. AZC at 10^{-5}–10^{-4} M concentrations produced 25% and 40% inhibition of non-poly(A)RNA, but had no effect on the incorporation of [^{3}H]AR into poly(A)RNA. DHAZAC did not inhibit either class of RNA at concentrations up to 10^{-4} M, but did inhibit non-poly(A)RNA by 40% at 10^{-3} M concentration (data not shown). AZC at 10^{-3} M inhibited non-poly(A)RNA by 70% but still did not produce inhibition of poly(A)RNA synthesis (data not shown).

On the basis of the electrophoretic separation of non-poly(A)RNA, the differential effects of AZC and DHAZC on 28 S and 18 S rRNA and 4 S RNA (tRNA) were tabulated (Table 1). AZC at 10^{-5} M inhibited 28 S rRNA by 66% and 18 S rRNA by 34%. At 10^{-4} M AZC, not much further inhibition of 28 S rRNA was achieved, whereas 18 S rRNA was reduced by 71%. On the other hand, DHAZC was inhibitory to 28 S and 18 S rRNA only at 10^{-3} M concentration, where 52% and 41% inhibition was produced, respectively; 4 S RNA was not affected by either drug at concentrations which inhibited rRNA.

Incorporation of AZC or DHAZC into polysomal RNA. In order to correlate the amount of AZC or DHAZC incorporated into polysomal RNA with their inhibitory effects on RNA synthesis, experiments were carried out with ^{14}C-labeled AZC or DHAZC as radioactive precursors. As summarized in Table 2, at 10^{-4} M concentration of each drug, 1500 and 120 pmoles of [^{14}C]

TABLE 2

Incorporation of [^{14}C]AZC and [^{14}C]DHAZC into polysomal RNA

Ehrlich ascites cells were incubated at 37° for 2 hr with varying concentrations of either [^{14}C]AZC (45 mCi/mmole) or [^{14}C]DHAZC (50 mCi/mmole) before the cells were harvested. Polysomal poly(A)RNA and non-poly(A)RNA were extracted as described under Materials and Methods. Under these experimental conditions, the synthesis of non-poly(A)RNA as measured by [^{3}H]AR incorporation was inhibited 40, 25, and 0% by 10^{-4}, 10^{-5}, and 10^{-6} M AZC, respectively, and was unaffected by DHAZC; neither AZC nor DHAZC affected poly(A)RNA synthesis. Each value represents the mean ± standard error of three experiments.

Treatment	Drug incorporated into	
	Poly(A)RNA	Non-poly(A)RNA
	pmoles/A_{260}	
[^{14}C]AZC		
10^{-4} M	1500 ± 410	120 ± 10
10^{-5} M	420 ± 20	90 ± 5
10^{-6} M	50 ± 10	10 ± 2
[^{14}C]DHAZC		
10^{-4} M	400 ± 170	9 ± 2
10^{-5} M	60 ± 20	1.4 ± 0.3
10^{-6} M	40 ± 20	0.6 ± 0.1

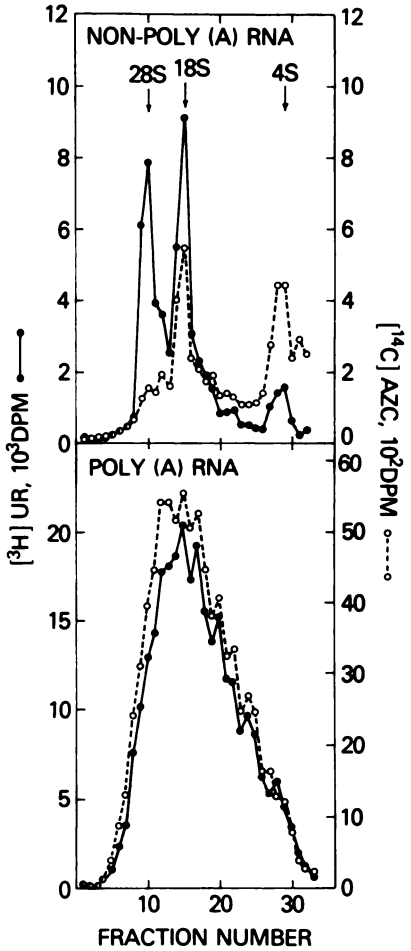

FIG. 2. *Agarose coelectrophoresis of [^{14}C]AZC- and [^{3}H]UR-labeled non-poly(A)RNA and poly(A)RNA*

RNA containing [^{14}C]AZC from cells incubated for 2 hr with 10^{-5} M [^{14}C]AZC, and poly(A)RNA containing [^{3}H]UR from separately incubated control cells were coelectrophoresed in agarose-urea gels as described under Materials and Methods. Note the differences in scale between [^{3}H]UR- and [^{14}C]AZC-labeled RNA.

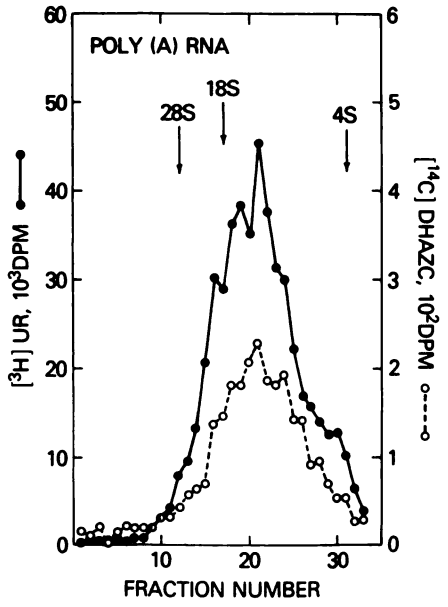

FIG. 3. *Agarose coelectrophoresis of [^{14}C]DHAZC- and [^{3}H]UR-labeled poly(A)RNA*

Poly(A)RNA containing [^{14}C]DHAZC from cells incubated for 2 hr with 10^{-5} M [^{14}C]DHAZC, and poly(A)RNA containing [^{3}H]UR from separately incubated control cells were coelectrophoresed in agarose-urea gels as described under Materials and Methods. Note the differences in scale between [^{3}H]UR- and [^{14}C]DHAZC-labeled RNA.

AZC per A_{260} were incorporated into poly(A)RNA and non-poly(A)RNA, respectively. In comparison, 10^{-4} M [^{14}C]DHAZC was incorporated only 8% as much as AZC into poly(A)RNA and 27% as much as AZC into non-poly(A)RNA. Similar relative effects were noted at 10^{-5} M AZC and DHAZC. Further analysis by coelectrophoresis of [^{14}C]drug-labeled RNA fractions with [^{3}H]uridine-labeled control non-poly(A)RNA and poly(A)RNA did not reveal significantly altered electrophoretic mobilities for either AZC-substituted non-poly(A)RNA and poly(A)RNA (Fig. 2) or DHAZC-substituted poly(A)-RNA (Fig. 3). The incorporation of [^{14}C]AZC into non-poly(A)RNA indicated that drug substitutions of 18 S rRNA and 4 S RNA were equivalent, whereas minimal labeling of 28 S rRNA occurred. The incorporation of [^{14}C]DHAZC into non-poly(A)RNA was so low that characterization by coelectrophoresis was not possible.

Translational activity in vitro of AZC or DHAZC-substituted poly(A)RNA in a rabbit reticulocyte lysate system. To investigate whether the incorporation of AZC or DHAZC into poly(A)RNA had an inhibitory effect on its coding efficiency, poly(A)RNA was isolated from drug-treated cells by poly(U)Sepharose affinity chromatography and translated *in vitro* in a rabbit reticulocyte lysate system. As shown in Table 3, there were no detectable quantitative differences in the *in vitro* translational activity between control and drug-substituted poly(A)RNA isolated from cells incubated for 2 hr with 10^{-5}–10^{-3} M AZC or DHAZC. Electrophoresis in SDS-polyacrylamide gels of *in vitro* translated proteins directed by control and AZC- or DHAZC-substituted poly(A)RNA did not show any new radiolabeled products, although some minor qualitative changes were apparent (Fig. 4).

TABLE 3

In vitro protein synthesis directed by AZC- or DHAZC-substituted poly(A)RNA

Ehrlich ascites cells were incubated at 37° for 2 hr with varying concentrations of AZC or DHAZC. Polysomal poly(A)RNA was isolated and translated *in vitro* in a rabbit reticulocyte lysate system using [^{3}H]leucine (120 Ci/mmole) as radioactive precursor. The efficiency of protein synthesis by drug-treated poly(A)RNA is expressed as a percentage of control = 100%. Control values averaged 12 pmoles of leucine incorporated per microgram of RNA per 30 minutes at 30°. Incorporated radioactivity represents trichloroacetic acid-insoluble proteins chromatographically separated following release of labeled tRNA (28). Each value represents the mean ± standard error of three to five experiments.

Drug concentration	% Control	
	AZC-treated Poly(A)RNA	DHAZC-treated Poly(A)RNA
10^{-3} M	102 ± 13	104 ± 5
10^{-4} M	90 ± 10	93 ± 6
10^{-5} M	92 ± 12	100 ± 5

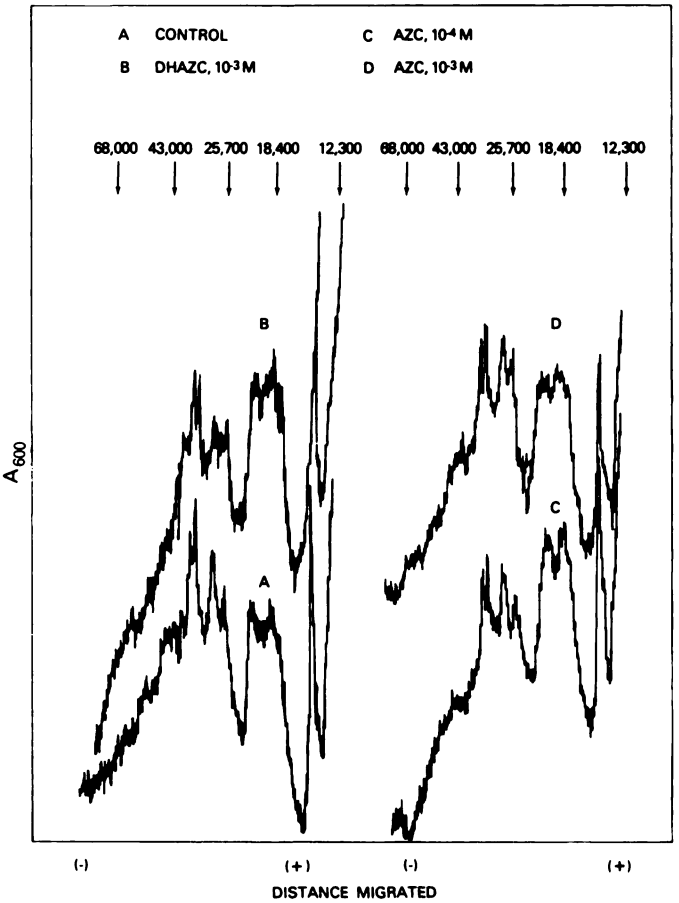

FIG. 4. *Densitometric tracings of autoradiographs of the translation products directed in vitro by AZC- and DHAZC-modified poly(A)RNA*

Translation products directed by control or drug-modified poly(A)RNA in a reticulocyte lipate translation system were electrophoresed in SDS-polyacrylamide gels as described under Materials and Methods. Drug-substituted poly(A)RNA was obtained from Ehrlich cells incubated for 2 hr with 10^{-3} M AZC or 10^{-3} M DHAZC.

DISCUSSION

The present investigation focused on the comparative effects of AZC and DHAZC on the synthesis and function of polysomal RNA. Previously it was found that, in L1210 ascites cells *in vitro*, DHAZC at a concentration of 10^{-3} M was as potent as AZC in inhibiting the synthesis of nuclear RNA, poly(A)RNA, and 5 S and 4 S RNA (12, 13). In the present investigation, a significant difference was observed in the potency of these drugs as exhibited by their relative inhibitory effects on the synthesis of rRNA in Ehrlich ascites cells *in vitro*. At 10^{-5} M AZC, 28 S and 18 S rRNA synthesis was inhibited 65% and 35%, respectively, whereas 10^{-4} M AZC inhibited both 28 S and 18 S rRNA by 70%. In contrast, 50% inhibition of 28 S and 18 S rRNA synthesis was produced only at 10^{-3} M DHAZC.

The amount of cytidine analogue incorporated into a particular species of RNA was not proportional to its effect on the synthesis or function of that RNA. Although rRNA and 4 S RNA contained equivalent amounts of AZC, only the synthesis of rRNA was inhibited.Similarly, poly(A)RNA contained the highest specific radioactivity of labeled AZC and DHAZC, as previously observed for nuclear poly(A)RNA (12), yet drug substitution did not interfere with either its synthesis or translational activity *in vitro*. These results confirm, in part, those of Reuveni and Rosenthal (14), who also observed that AZC inhibited the synthesis of rRNA but had no effect upon the synthesis of poly(A)RNA in BSC-1 cells. From the results of the present study, the primary site of action of AZC and DHAZC appears to be related to its inhibitory effect on rRNA. The relative differences in potency between AZC and DHAZC as antitumor agents (20, 21) correlates closely with their respective inhibitory effects on rRNA synthesis and their incorporation into this species of RNA. In contrast, no such relationship was found in previous studies of the synthesis and methylation of nRNA (12, 13).

AZC has been previously shown to inhibit protein synthesis, and the mechanism for this effect in HeLa cells was assumed to be unrelated to either messenger RNA or ribosomal RNA (19). We have isolated AZC and DHAZC-modified poly(A)RNA and have not found any impairment in their coding efficiency in a rabbit reticulocyte lysate translation system *in vitro*. Drug-modified poly(A)RNA also did not produce any major qualitative changes in translation products, suggesting that miscoding is not related to the mechanism of cytotoxicity produced by these drugs.

REFERENCES

1. McCredie, K. B., G. P. Bodey, M. A. Burgess, J. U. Gutterman, V. Rodreguez, M. P. Sullivan, and E. J. Freireich. Treatment of acute leukemia with 5-azacytidine. *Cancer Chemother. Rep.* **57**:319–323 (1973).
2. Karon, M., L. Sieger, S. Leimbrock, J. A. Finklestein, M. E. Nesbit, and J. J. Swaney. 5-Azacytidine: a new active agent for the treatment of acute leukemia. *Blood* **42**:359–365 (1973).
3. 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).
4. Li, L. H., E. J. Olin, H. H. Buskivk, and L. M. Reineke. Cytotoxicity and mode of action of 5-azacytidine on L1210 cells. *Cancer Res.* **30**:2760–2769 (1970).
5. Plagemann, P. G. W., M. Behrens, and D. Abraham. Metabolism and cytotoxicity of 5-azacytidine in cultured Novikoff rat hepatoma and P388 mouse leukemia cells and their enhancement by preincubation with pyrazofurin. *Cancer Res.* **38**:2458–2466 (1978).
6. Paces, V., J. Doskocil, and F. Sorm. Incorporation of 5-azacytidine into nucleic acids of *Escherichia coli*. *Biochim. Biophys. Acta* **161**:352–360 (1968).
7. Vesely, J., and A. Cihak. 5-Azacytidine: mechanism of action and biological effects in mammalian cells. *Pharmacol. Ther. Part A Chemother. Toxicol. Metab. Inhibitors* **2**:813–840 (1978).
8. Weiss, J. W., and H. C. Pitot. Inhibition of ribosomal ribonucleic acid maturation by 5-azacytidine and 8-azaguanine in Novikoff hepatoma cells. *Arch. Biochem. Biophys.* **160**:119–129 (1974).
9. Weiss, J. W., and H. C. Pitot. Alteration of ribosomal precursor RNA in Novikoff hepatoma cells by 5-azacytidine—studies on methylation of 45S and 32S RNA. *Arch. Biochem. Biophys.* **165**:588–596 (1974).
10. Weiss, J. W., and H. C. Pitot. Effects of 5-azacytidine on the nucleolar RNA and the preribosomal particles in Novikoff hepatoma cells. *Biochemistry* **14**: 316–326 (1975).
11. Reichman, M., D. Karlan, and S. Penman. Destructive processing of 45S ribosomal precursor in the presence of 5-azacytidine. *Biohim. Biophys. Acta* **299**:173–175 (1973).
12. 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 synthesisis in L1210 cells *in vitro*. *Mol. Pharmacol.* **17**:111–117 (1980).
13. Glazer, R. I., and K. D. Hartman. The comparative effects of 5-azacytidine and dihydro-5-azacytidine on 4 S and 5 S nuclear RNA. *Mol. Pharmacol.* **17**: 250–255 (1980).
14. Reuveni, Y., and L. J. Rosenthal. Effect of 5-azacytidine on cytoplasmic ribosomal and messenger ribonucleic acids in BSC-1 cells. *Antimicrobial. Agents. Chemother.* **15**:235–239 (1979).
15. Lee, T. T., and M. R. Karon. Inhibition of protein synthesis in 5-azacytidine-treated HeLa cells. *Biochem. Pharmacol.* **25**:1737–1742 (1976).
16. Lu, L.-J. W., and K. Randerath. Mechanism of 5-azacytidine induced transfer RNA cytosine-5-methyltransferase deficiency. *Cancer Res.* **40**:2701–2705 (1980).
17. Lu, L.-J. W., G. H. Chiang, and K. Randerath. Effects of 5-azacytidine on transfer RNA modification: comparative study on normal and malignant tissues. *Life Sci.* **27**:577–584 (1980).
18. 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).
19. 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).
20. 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).
21. Beisler, J. A., M. M. Abbasi, J. A. Kelley, and J. S. Driscoll. Synthesis and antitumor activity of dihydro-5-azacytidine, a hydrolytically stable analog of 5-azacytidine. *J. Med. Chem.* **20**:806–812 (1977).
22. 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).
23. 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).
24. Jones, P. A., and S. M. Taylor. Cellular differentiation, cytidine analogues and DNA methylation. *Cell* **20**:85–93 (1980).
25. Malspeis, L., and DeSouze, J. J. V. Metabolism and elimination of 5,6-dihydro-5-azacytidine by the rat. *Proc. Am. Assoc. Cancer Res.* **22**:219 (1981).
26. Glazer, R. I., T. J. Lott, and A. L. Peale. Potentiation by 2′-deoxycoformycin of the inhibitory effect by 3′-deoxyadenosine (cordycepin) on nuclear RNA synthesis in L1210 cells *in vitro*. *Cancer Res.* **38**:2233–2238 (1978).
27. Carrico, C. K., and R. I. Glazer. Effect of 5-fluorouracil on the synthesis and translation of polyadenylic acid-containing RNA from regenerating rat liver. *Cancer Res.* **39**:3694–3701 (1979).
28. Locker, J. Analytical and preparative electrophoresis of RNA in agarose-urea gel. *Anal. Biochem.* **98**:358–367 (1979).
29. Glazer, R. I., and K. D. Hartman. Analysis of the effect of 5-fluorouracil on the synthesis and translation of polysomal poly(A)RNA from Ehrlich ascites cells.*Mol. Pharmacol.* **19**:117–121 (1981).
30. Laemmli, U. K., and M. Favre. Maturation of the head of bacteriophage T4: I. DNA packaging events. *J. Mol. Biol.* **80**:575–599 (1973).

Send reprint requests to: Dr.Robert I. Glazer, National Cancer Institute, Building 37, Room 6D28, Bethesda, Md. 20205.