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EFFECTS OF 8-AZAADENOSINE AND FORMYCIN ON CELL LETHALITY AND THE SYNTHESIS AND METHYLATION OF NUCLEIC ACIDS IN HUMAN COLON CARCINOMA CELLS IN CULTURE

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Abstract—The cytocidal and biochemical effects of formycin and 8-azaadenosine in the presence and absence of the adenosine deaminase inhibitor, 2′-deoxycoformycin, were studied in human colon carcinoma (HT-29) cells in culture. Logarithmically growing cells were unaffected by 24-hr exposure to either 10^{-6} M formycin or 8-azaadenosine, but 1 to 1.4 log reductions in colony formation were produced by 10^{-5} M of each analog. In the presence of 10^{-6} M 2′-deoxycoformycin, a 3- and 30-fold potentiation of the cytocidal activity of 8-azaadenosine and formycin, respectively, was produced. Inhibition of DNA synthesis but not RNA synthesis by 8-azaadenosine paralleled its cytocidal activity; however, neither variable correlated closely with the cytotoxic effects of formycin. In addition, the methylation of nuclear RNA was unaffected by both drugs while the methylation of 5-methyl-deoxycytidine in DNA was inhibited to a lesser extent than DNA synthesis. Measurements of the incorporation of [^{3}H]formycin and [^{3}H]8-azaadenosine into nuclear RNA and DNA in the presence and absence of 2′-deoxycorformycin indicated that formycin substitution in RNA and DNA was enhanced 10- and 20-fold, respectively, while [^{3}H]8-azaadenosine incorporation into both nucleic acids was increased 6- to 7-fold. These results suggest that the incorporation of formycin into nucleic acids, particularly DNA, correlates closely with its lethal effect on cell viability. On the other hand, the cytocidal activity of 8-azaadenosine more clearly parallels its inhibitory effect on DNA synthesis rather than its substitution into nucleic acids.

Several adenosine analogs whose antitumor activities are marginal when given alone are greatly potentiated when administered in combination with an inhibitor of adenosine deaminase [1–7]. The most potent inhibitor of this enzyme is dCF†, and its antitumor activity is under clinical investigation [8–12]. The role of dCF, both singly and in combination with adenosine analogs, in cancer chemotherapy has been reviewed recently [13].

Two adenosine analogs which have received recent attention are formycin and 8-azaadenosine [14, 15]. When coadministered with dCF, both drugs were markedly cytotoxic to human colon [14] and pancreatic [15] carcinoma *in vitro*. The combination of 8-azaadenosine and dCF was also synergistic chemotherapeutically in pancreatic carcinoma xenografts [15]. Although the mechanism of this synergism is not known, the cytotoxicities of formycin and 8-azaadenosine correlate with their anabolism to nucleotide metabolites [14] and, hence, presumably are related to the metabolism of nucleic acids.

In the present report, we examined the cytocidal activity of combinations of formycin or 8-azaadenosine with dCF in human colon carcinoma (HT-29) cells *in vitro*. In addition, several biochemical variables related to the synthesis of nRNA and DNA were investigated to relate the enhanced cell lethality of these drug combinations to their mechanisms of action.

MATERIALS AND METHODS

Materials. [*Methyl*-^{14}C]thymidine (53 mCi/mmole), [5-^{3}H]UR (25 Ci/mmole), and L-[*methyl*-^{3}H]methionine (80 Ci/mmole) were purchased from the New England Nuclear Corp., Boston, MA, and [G-^{3}H]formycin (5 Ci/mmole) and [2-^{3}H]8-azaadenosine (7 Ci/mmole) were purchased from Moravek Biochemicals, Brea, CA. Formycin and 8-azaadenosine were obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute. RPMI medium 1640 was purchased from Hem Research, Inc., Rockville, MD; heat-inactivated fetal calf serum and trypsin were obtained from GIBCO Lab-

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† Abbreviations: dCF, 2′-deoxycoformycin [(R)-3-(2-deoxy-β-D-erythropentofuranosyl)-3,6,7,8-tetrahydroimidazo[5,4-*d*][1,3]diazepin-8-ol]; formycin, 7-amino-3-[β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine; SDS, sodium dodecylsulfate; nRNA, nuclear RNA; m^{5}CdR, 5-methyl-2′-deoxycytidine; UR, uridine; TdR, thymidine; AdR, deoxyadenosine; GdR, deoxyguanosine; CdR, deoxycytidine; HPLC, high pressure liquid chromatography; PBS, phosphate-buffered saline (6.6 mM Na_2HPO_4, 0.8 M KH_2PO_4, 0.154 M NaCl, pH 7.4); and Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

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oratories, Grand Island, NY; and gentamycin was purchased from Flow Laboratories, McLean, VA.

Tissue culture. HT-29 cells, which were originally derived from a human colon carcinoma [16], were grown under 5% CO_2:air in RPMI medium 1640 supplemented with 10% fetal calf serum and 50 μg/ml gentamicin. Cell inoculums were 0.83×10^5 cells/10 ml medium in 25 cm^2 plastic flasks (Costar, Cambridge, MA) or increased 10-fold in 150 cm^2 flasks for nRNA analyses.

Drug treatment. Log phase (3 day) cells were treated for 24 hr in the presence or absence of 10^{-6} M dCF administered simultaneously with the indicated concentrations of formycin or 8-azaadenosine. After drug treatment, cells were harvested by treatment with 0.05% trypsin in Hanks' balanced salt solution without Ca^{2+} and Mg^{2+} and containing 0.02 M EDTA. The growth medium was decanted and cell monolayers in 25 cm^2 flasks were first rinsed with 3 ml of trypsin solution followed by incubation for 10 min at 37° with 0.4 ml of trypsin solution. Treatment with trypsin was terminated by the addition of 10 ml of RPMI medium 1640 containing 10% fetal calf serum and 50 μg/ml gentamicin.

Cell viability determinations. Soft agar cloning was performed as described previously [17]. Duplicate 6 cm plastic Petri dishes were plated with 200, 2,000 or 20,000 cells for control and drug-treated flasks. After 14 days, colonies were fixed with 95% ethanol and stained with 0.01% gentian violet in 1% acetic acid. Cell viability is expressed as the number of colonies produced by drug-treated cells divided by the number of colonies produced by control cells (corrected for cloning efficiency) × 100. Cloning efficiency ranged from 50 to 65%.

DNA and RNA determinations. Following addition of formycin or 8-azaadenosine, cells (grown in 25 cm^2 flasks) were pulse-labeled during the last hour of drug treatment with 0.5 μCi each of [5-^{3}H]UR and 0.5 μCi [^{14}C]TdR per 10 ml of media. After treatment with trypsin, the cells were centrifuged at 400 *g* for 10 min at 4° and washed once with 15 ml of ice-cold PBS. DNA and RNA were co-extracted by the addition of 2 ml of 1% SDS:0.1 M Tris–HCl (pH 8.0):0.01 M EDTA followed by 1.0 ml of phenol mixture (phenol:*m*-cresol:water, 7:2:2, by vol., containing 0.1% 8-hydroxyquinoline) and 1.0 ml chloroform. After mixing on a vortex shaker for 5 min, the emulsion was clarified by centrifugation at 10,000 *g* for 10 min. Total nucleic acids were precipitated with 2 vol. of 2% (w/v) potassium acetate in 95% ethanol at −20° overnight. After centrifugation at 10,000 *g* for 10 min, DNA and RNA were dissolved in 1.0 ml of water and radioactivity was determined after mixing with Aquasol (New England Nuclear Corp.) in a Searle Mark III liquid scintillation specrometer. Incorporation of radioactive precursors into RNA and DNA was normalized per 10^6 cells.

Extraction and Agarose electrophoresis of nRNA. Cells were grown at 10-fold the number used in the cell viability experiments (8.3×10^5 cells/100 ml medium in 150 cm^2 flasks) and were pulse-labeled for either 1 hr with 200 μCi [^{3}H]UR or 200 μCi L-[*methyl*-^{3}H]methionine, or for 24 hr with 200 μCi [^{3}H]formycin or [^{3}H]8-azaadenosine (100, 1,000, or 10,000 dpm/pmole). In experiments utilizing [^{3}H]methionine, cells were incubated for 1 hr in L-methionine-free RPMI medium 1640 containing 10% fetal calf serum and 50 μg/ml gentamicin. Cells were harvested by treatment with trypsin and washed once with 200 ml of ice-cold PBS. Drug incorporation into RNA and DNA [17] and isolation of nRNA [18] were as previously described. RNA was separated electrophoretically in 2% agarose:urea:iodoacetate gels as described by Locker [19]. Gels were sliced into 2 mm sections, dissolved in 0.2 ml of 70% perchloric acid, and mixed with 10 ml Aquasol, and the radioactivity was determined. Approximately 1.0 A_{260} unit of RNA was applied per gel.

Measurement of m^{5}CdR. DNA was extracted from nuclei of HT-29 cells by the SDS–phenol and RNase procedure described previously [17]. DNA (10 μg) was dissolved in 40 mM Hepes (pH 7.5):5 mM $MgCl_2$ and digested sequentially at 37° with 100 μg/10 μl DNase I for 30 min, 20 μg/20 μl snake venom phosphodiesterase for 30 min, and 60 μg/20 μl bacterial alkaline phosphatase for 30 min. Aliquots of 25 μl were injected into an Altex high pressure liquid chromatography apparatus equipped with a Brownlee RP-18 3 cm precolumn and RP-18 10 cm analytical column connected in tandem. m^{5}CdR was eluted with 10 mM KH_2PO_4 (pH 3.8):5% methanol at a flow rate of 2 ml/min. The elution times for CdR, m^{5}CdR, GdR, TdR and AdR were 1.7, 3.0, 3.8, 4.5 and 8.6 min respectively. m^{5}CdR was monitored by its absorbance at 268 nm and its associated radioactivity, and concentration was measured by liquid scintillation counting and integration of the area of its absorbance peak with a Hewlett–Packard 3390A integrator, respectively. Results were calculated as dpm/nmole m^{5}CdR.

Nucleoside triphosphate and drug triphosphate levels. HT-29 cells were grown in 150 cm^2 flasks and treated with formycin, 8-azaadenosine and dCF as described under "*Drug treatment*". In some experiments, either [^{14}C]TdR and [5-^{3}H]UR were added at concentrations of 5 μCi/100 ml media or [^{3}H]formycin and [^{3}H]8-azaadenosine were added in the amounts described under "*Extraction and Agarose electrophoresis of nRNA*". After treatment, cells were trypsinized and centrifuged at 400 *g* for 10 min. Cells were washed with ice-cold PBS and transferred to a 1.5 ml Eppendorf centrifuge tube. The cells were centrifuged for a few seconds in an Eppendorf centrifuge, and the cell pellet was immediately mixed with 0.2 ml of ice-cold 1.0 N perchloric acid. After mixing on a vortex shaker for 1 min, the extract was centrifuged and neutralized with KOH using phenol red as an internal pH indicator. Aliquots of 25 μl of the perchloric acid extract were chromatographed using 3 cm and 10 cm Brownlee amino columns connected in tandem and an Altex HPLC apparatus. Elution of triphosphate metabolites was performed isocratically with 0.4 M KH_2PO_4 (pH 3.8):5% (v/v) acetonitrile at a flow rate of 4 ml/min, and absorbance was monitored at 268 nm. The elution times of CTP, UTP, ATP and GTP were 3.8, 4.4, 5.7 and 9.2 min respectively. Formycin triphosphate co-eluted with CTP, and 8-azaadenosine triphosphate eluted in 7.2 min.

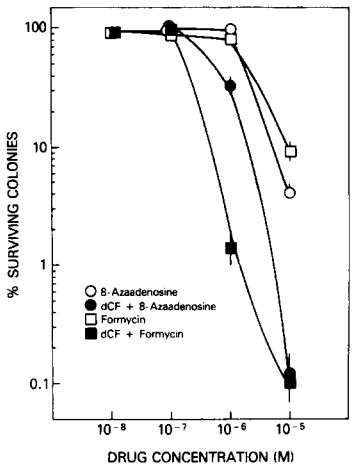

Fig. 1. Viability of HT-29 cells following exposure to formycin or 8-azaadenosine in the presence and absence of dCF. HT-29 cells were incubated for 24 hr with (●, ■) or without (○, □) 10^{-6} M dCF and 8-azaadenosine (○, ●) or formycin (□, ■). Cell viability was determined by soft agar cloning as described under Materials and Methods. Results are expressed as the percentage of surviving cell colonies versus control colonies (corrected for cloning efficiency). Values are the means ± S.E. of six to eight determinations.

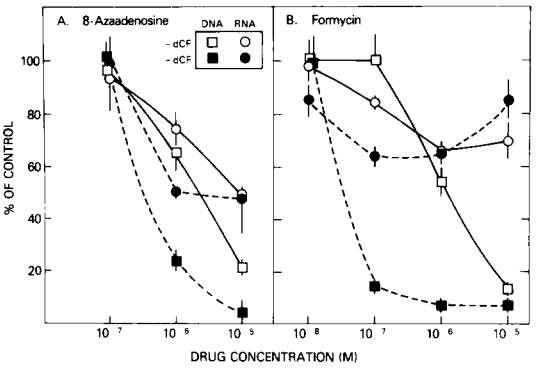

Fig. 2. Total RNA and DNA syntheses following exposure to formycin or 8-azaadenosine in the presence and absence of dCF. HT-29 cells were incubated for 24 hr with (●, ■) or without (○, □) 10^{-6} M dCF and 8-azaadenosine or formycin followed by labeling for 1 hr with 0.5 μCi each of [5-*methyl*-^{14}C]thymidine and [5-^{3}H]uridine. Total RNA (○, ●) and DNA (□, ■) were extracted as described under Materials and Methods. Values are the means ± S.E. of four to five determinations.

RESULTS

Cell viability. The cytocidal effects of formycin and 8-azaadenosine in the presence and absence of dCF were assessed by soft agar cloning of HT-29 cells following 24 hr of treatment with the drugs (Fig. 1). Neither formycin nor 8-azaadenosine alone was toxic except at 10^{-5} M concentrations where a 1 and 1.4 log reduction, respectively, in colony formation was observed. Simultaneous addition of 10^{-6} M dCF with either analog markedly potentiated the cytotoxicity. Cell lethality by the combination of dCF + formycin or dCF + 8-azaadenosine was increased by 30- and 3-fold, respectively, compared to treatment with either drug alone when estimated by the concentration of formycin or 8-azaadenosine necessary to produce a 1 or 2 log reduction in colony formation. Exposure of cells for 2 hr with either drug in the presence or absence of dCF did not produce a significant reduction in colony formation (data not shown).

DNA and RNA syntheses. DNA and RNA syntheses were measured by the incorporation of [^{14}C]TdR and [^{3}H]UR, respectively, under the same conditions that were used to assess cell viability (Fig. 2). In the absence of dCF, DNA and RNA syntheses were inhibited equally at 10^{-6} M formycin or 8-azaadenosine, but DNA synthesis was affected to a greater extent than RNA synthesis at a 10^{-5} M concentration of each drug. In the presence of dCF, inhibition of DNA synthesis by 10^{-6} M 8-azaadenosine (Fig. 2A) was enhanced 3-fold, but RNA synthesis was virtually unaffected. A similar differential effect was produced by dCF + formycin (Fig. 2B). An 8-fold increase in inhibition of DNA synthesis was produced by dCF + 10^{-7} to 10^{-6} M formycin versus formycin alone, but little or no potentiation of the inhibition of RNA synthesis occurred. Thus, the potentiation by dCF of the inhibitory effect of

Table 1. Effects of formycin and 8-azaadenosine on the methylation and synthesis of nRNA*

Treatment	Methylation	Synthesis
	(dpm/A_{260})	
Control	9,500 ± 400	196,200 ± 18,000
	(% of control)	
dCF, 10^{-6} M	98 ± 2	103 ± 3
8-Azaadenosine, 10^{-5} M	89 ± 7	55 ± 3
dCF + 8-azaadenosine, 10^{-6} M	97 ± 2	59 ± 1
Formycin, 10^{-5} M	115 ± 15	75 ± 2
dCF + formycin, 10^{-6} M	103 ± 7	69 ± 7

* Values are the means ± S.E. of three determinations, HT-29 cells were incubated for 24 hr with dCF, formycin, dCF + formycin, 8-azaadenosine or dCF + 8-azaadenosine followed by labeling for 1 hr with either 200 μCi L-[*methyl*-^{3}H]methionine in L-methionine-free medium or with 200 μCi [5-^{3}H]uridine. nRNA was extracted as described under Materials and Methods.

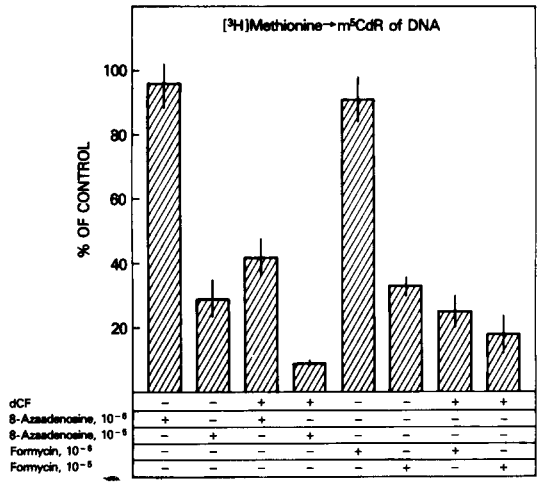

Fig. 3. Effect of formycin or 8-azaadenosine in the presence and absence of dCF on the methylation of m^5CdR in DNA. HT-29 cells were incubated as described in Table 1. The radioactivity present in m^5CdR of DNA was measured as described under Materials and Methods. Values are the means ± S.E. of three determinations.

8-azaadenosine on DNA synthesis appeared to correlate quantitatively with the synergism produced by this drug combination on cell lethality. On the other hand, the dose–response relationship and degree of potentiation by dCF + formycin versus formycin alone did not completely parallel the effects on cell lethality.

Methylation of nRNA and DNA. Previous studies indicated that formycin and 8-azaadenosine in the presence of dCF were effective inhibitors of nRNA methylation in L1210 cells [20]. To see if methylation of nRNA or DNA was affected under conditions which produced cell lethality, the incorporation of L-[*methyl*-^{3}H]methionine into nRNA and DNA was measured. The methylation of nRNA was not affected by either formycin or 8-azaadenosine in the presence or absence of dCF despite inhibition of [^{3}H]UR incorporation into RNA (Table 1). In contrast, methylation of DNA was inhibited significantly by formycin and 8-azaadenosine in the presence and absence of dCF (Fig. 3). Incorporation of L-[*methyl*-^{3}H]methionine into m^5CdR of DNA was inhibited markedly by the combination of dCF + adenosine analog, particularly at 10^{-6} M drug concentration. However, drug inhibition of the labeling of m^5CdR with [^{3}H]methionine was less than the inhibition of DNA synthesis in all instances (Fig. 2). dCF alone did not affect DNA methylation.

Drug incorporation into nRNA and DNA. HT-29 cells were incubated with both nontoxic and lethal concentrations of [^{3}H]formycin or [^{3}H]8-azaadenosine under conditions identical to those used for cloning. The incorporation of labeled drug into nRNA and DNA was measured in the presence and absence of 10^{-6} M dCF (Fig. 4). [^{3}H]Formycin and [^{3}H]8-azaadenosine were both incorporated into nRNA to a greater degree than into DNA, and [^{3}H]8-azaadenosine was incorporated more extensively than [^{3}H]formycin into both nucleic acid fractions. The presence of dCF in the growth medium produced a 10- and 20-fold increase in the incorporation of [^{3}H]formycin into nRNA and DNA,

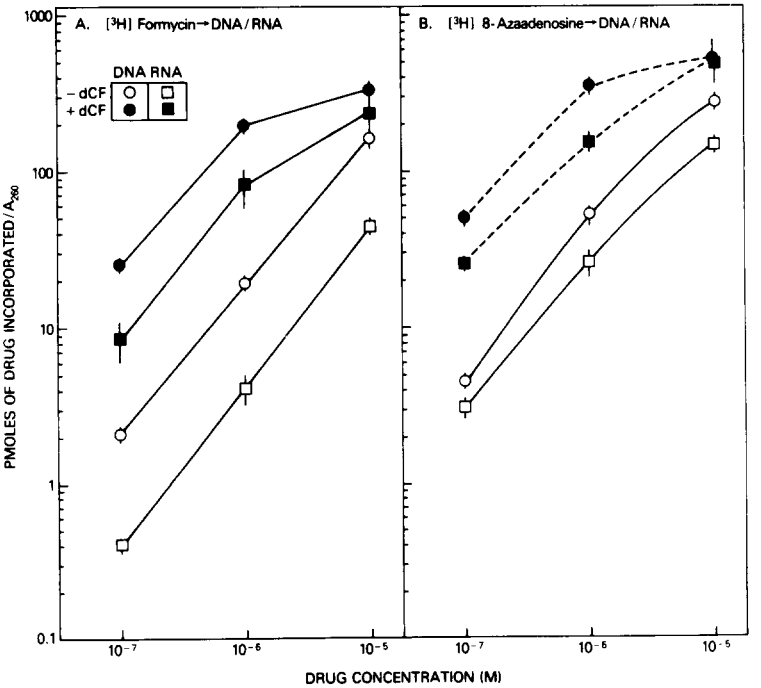

Fig. 4. Incorpoation of [^{3}H]formycin or [^{3}H]8-azaadenosine into nRNA and DNA in the presence and absence of dCF. HT-29 cells were incubated for 24 hr with (●, ■) or without (○, □) 10^{-6} M dCF and [^{3}H]8-azaadenosine or [^{3}H]formycin. nRNA and DNA were extracted as described under Materials and Methods. Values are the means ± S.E. of five to six determinations.

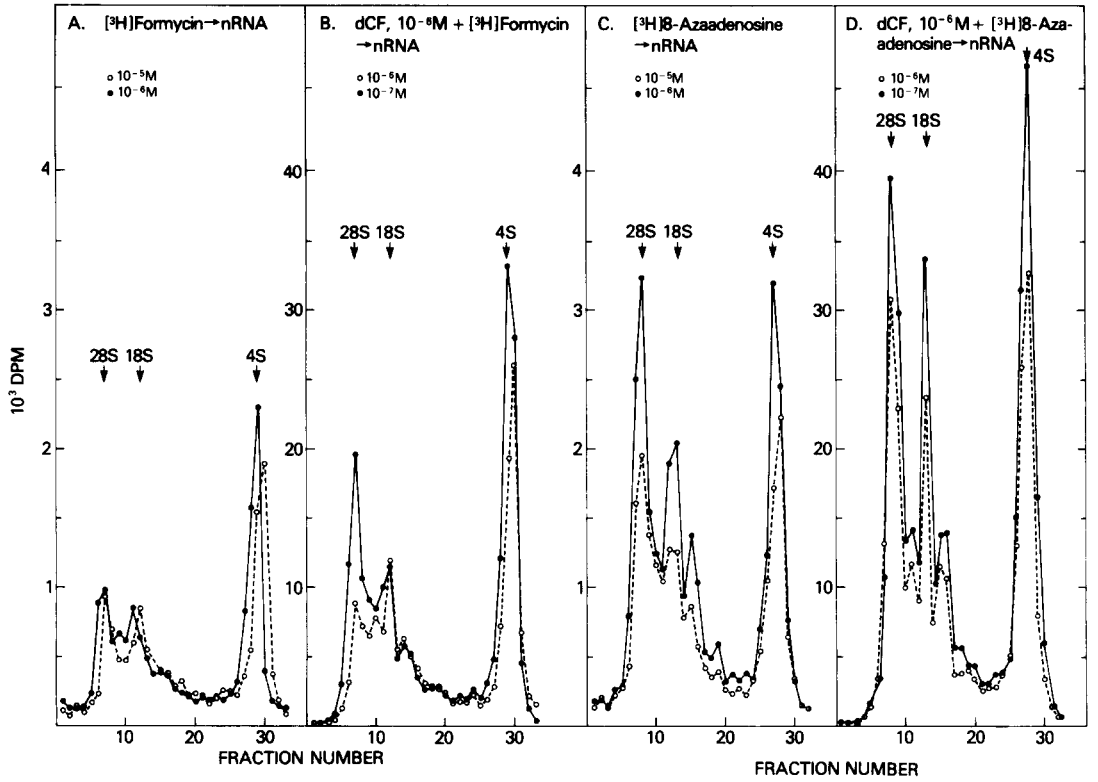

Fig. 5. Agarose gel electrophoresis of nRNA labeled with [^{3}H]formycin or [^{3}H]8-azaadenosine in the presence and absence of dCF. HT-29 cells were incubated as described in the legend of Fig. 4.

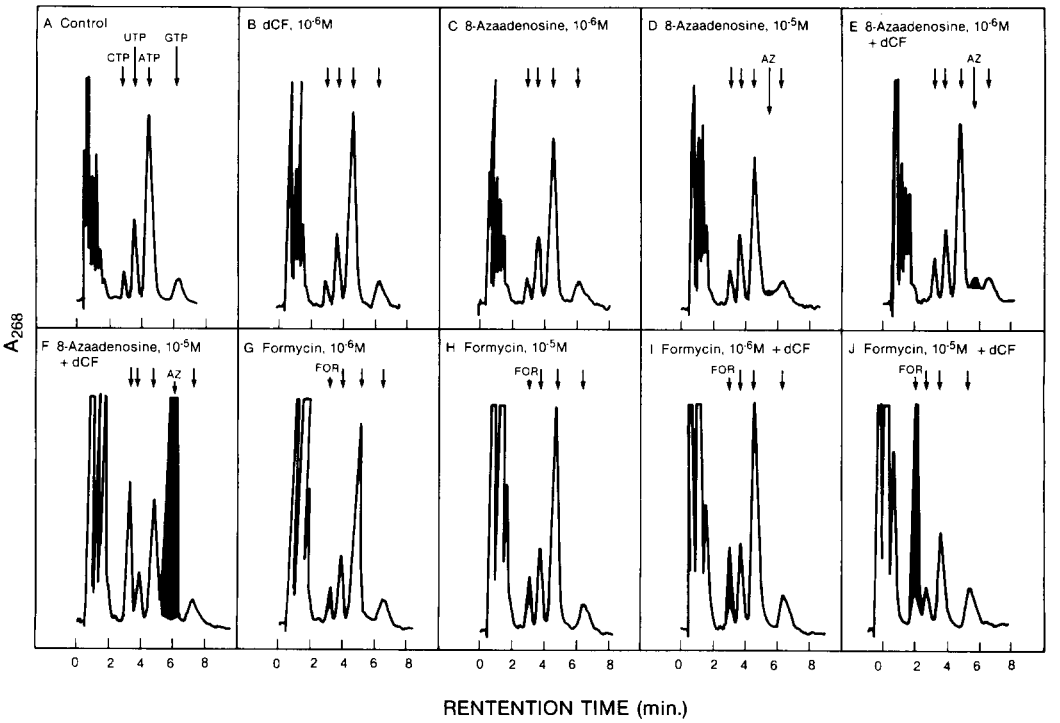

Fig. 6. Analysis by HPLC of nucleoside triphosphate and drug triphosphate levels. Perchloric acid extracts were chromatographed by anion-exchange HPLC as described under Materials and Methods. AZ and FOR denote 8-azaadenosine triphosphate and formycin triphosphate respectively.

Table 2. Ribonucleoside triphosphate and drug triphosphate levels after formycin or 8-azaadenosine treatment*

Treatment	CTP	UTP	ATP	GTP	Drug triphosphate
			(nmoles/10^6 cells)		(nmoles/10^6 cells)
Control	0.70 ± 0.07	2.30 ± 0.28	5.34 ± 0.58	1.51 ± 0.05	
			(% of control)		
dCF, 10^{-6} M	109 ± 8	106 ± 3	113 ± 6	107 ± 17	
8-Azaadenosine					
10^{-6} M	109 ± 8	105 ± 9	100 ± 7	89 ± 7	ND†
10^{-5} M	170 ± 8	118 ± 7	110 ± 15	139 ± 17	0.17 ± 0.03
10^{-6} M + dCF 10^{-6} M	140 ± 14	114 ± 11	110 ± 14	115 ± 12	0.25 ± 0.04
10^{-5} M + dCF 10^{-6} M	416 ± 50	54 ± 6	51 ± 8	99 ± 16	4.04 ± 0.50
Formycin					
10^{-6} M	‡	99 ± 18	84 ± 8	96 ± 18	ND†
10^{-5} M	‡	118 ± 11	111 ± 9	128 ± 17	0.44 ± 0.06‡
10^{-6} M + dCF 10^{-6} M	‡	97 ± 5	100 ± 8	102 ± 13	0.73 ± 0.38‡
10^{-5} M + dCF 10^{-6} M	‡	73 ± 12	27 ± 2	83 ± 30	3.02 ± 0.52‡

* Triphosphate metabolites were measured in perchloric acid extracts by anion-exchange HPLC as described under Materials and Methods. Each value is the mean ± S.E. of three determinations.

† ND, none detected.

‡ Formycin triphosphate coeluted with CTP, thus making estimation of CTP concentrations difficult. Formycin triphosphate concentrations were calculated from the absorbance difference between control levels of CTP and the absorbance associated with the CTP peak following formycin treatment. The concentrations of formycin triphosphate based on u.v. absorbance were similar to those determined with [^{3}H]formycin as precursor.

respectively, and a 6- to 7-fold enhancement of the labeling of both nucleic acid fractions by [^{3}H]8-azaadenosine.

Agarose gel electrophoresis of nRNA labeled with either [^{3}H]formycin or [^{3}H]8-azaadenosine revealed that both drugs were distributed into 28S and 18S rRNA and 4S RNA (Fig. 5). No accumulation of rRNA precursors or unusual molecular weight patterns of rRNA were produced by either drug under conditions which caused marked cell lethality.

Nucleoside triphosphate and drug triphosphate levels. Analysis by HPLC of triphosphate metabolites following formycin or 8-azaadenosine treatment is shown in Fig. 6 and Table 2. Neither 10^{-6} M dCF nor 10^{-6} M formycin and 8-azaadenosine in the presence or absence of dCF produced marked changes in nucleoside triphosphate concentrations. 8-Azaadenosine at a 10^{-5} M concentration produced a significant increase in the level of CTP, particularly in the presence of dCF where it was elevated 4-fold. Under the latter condition, UTP and ATP concentrations were reduced 50%. Formycin at a 10^{-5} M concentration + dCF also reduced ATP and UTP levels by 70 and 30% respectively. Thus, only at 10^{-5} M drug + dCF did significant changes in UTP levels occur which would result in an underestimation of RNA synthesis due to an enhanced specific radioactivity of UTP. This indeed was found to be the case using [^{3}H]UR as precursor and measuring the specific radioactivity of [^{3}H]UTP by HPLC analysis (results not shown). The increases in the specific radioactivity of [^{3}H]UTP also explain the somewhat higher rate of RNA synthesis after treatment with 10^{-5} M formycin + dCF and the leveling off of RNA synthesis following treatment with 10^{-5} M 8-azaadenosine + dCF. No differences occurred in the amounts of [^{14}C]dTTP formed/10^6 cells under the various experimental conditions (results not shown).

Drug triphosphate levels could be determined by u.v. absorbance only at 10^{-5} M drug or at 10^{-6} or 10^{-5} M drug + dCF (Fig. 6). At 10^{-5} M drug + dCF, a striking increase in drug triphosphate levels was apparent with a concomitant reduction in ATP concentration. The amount of drug triphosphate correlated qualitatively with its utilization for RNA synthesis.

DISCUSSION

The mode of action of formycin is believed to involve its anabolism to the nucleoside triphosphate, particularly in the presence of adenosine deaminase inhibitors [14, 21, 22], and its subsequent incorporation into RNA [23, 24]. Formycin has been observed previously to impair the processing, but not the transcription, of rRNA 45S precursor and to preferentially inhibit 4S and 5S cytoplasmic RNA [25]. We previously found that formycin and 8-azaadenosine in the presence of dCF were relatively weak inhibitors of nRNA synthesis in L1210 cells but impaired the methylation of 4S nRNA to an equal or greater degree than its synthesis [20]. Both analogs were also more inhibitory to the synthesis of 4S nRNA in comparison to high molecular weight nRNA [20]. In the present investigation, formycin and 8-azaadenosine were also weak inhibitors of nRNA synthesis but neither drug affected nRNA methylation. Impaired processing of rRNA precursor or the preferential inhibition of 4S nRNA also was not observed (unpublished observations). The differences between the previous results using L1210 ascites cells and the present data may reflect the growth conditions and long-term drug exposure used with the HT-29 cells, a differential effect on species of low molecular weight nRNA which are not highly methylated, or both. In contrast, both drugs were effective inhibitors of DNA synthesis, but a correlation between cell lethality and inhibition of DNA

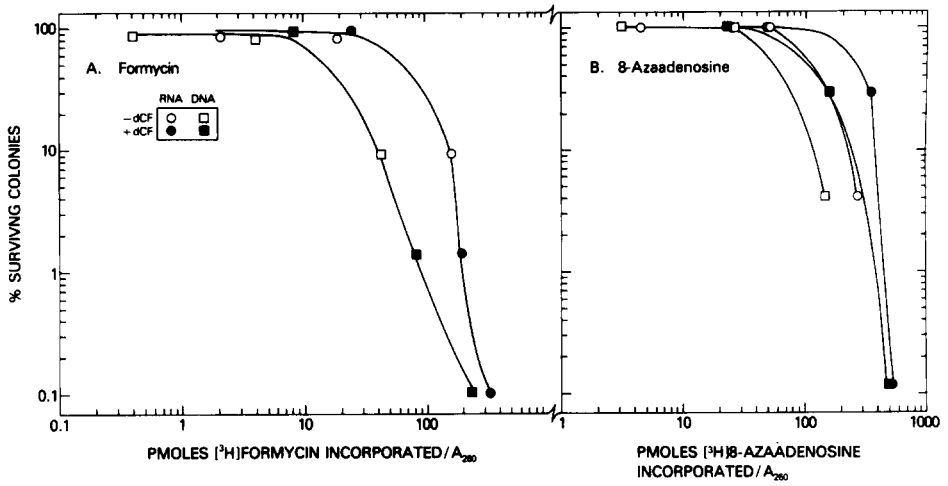

Fig. 7. Relationship between cell viability and the incorporation of [^{3}H]formycin or [^{3}H]8-azaadenosine into nRNA or DNA. Cell viability as determined by soft agar cloning (Fig. 1) is plotted as a function of the amount of [^{3}H]formycin or [^{3}H]8-azaadenosine incorporated into nRNA or DNA (Fig. 4).

synthesis was noted only for 8-azaadenosine, and not for formycin. Although methylation of DNA was also inhibited by both drugs, this effect was less severe than inhibition of DNA synthesis and, thus, could be fully attributable to the latter process. These data further suggest that impairment in the methylation of nucleic acids via conversion to analogs of *S*-adenosylhomocysteine [26] or *S*-adenosylmethionine [27] is inoperative in HT-29 cells.

Potentiation by dCF of the cytotoxicity produced by formycin and 8-azaadenosine has been reported previously for P388 [4], human colon carcinoma [14] and human pancreatic carcinoma [15] cells in culture. In one instance, dCF had about a 7-fold greater effect than 8-azaadenosine in potentiating the antitumor effect of formycin [14]. We have also observed this differential effect in HT-29 cells, and it suggests that the utilization or mechanisms of action of these drugs in the colon carcinoma cells may differ from one another. It is unlikely that these differences are solely due to analog nucleotide formation since 8-azaadenosine was incorporated into nucleic acids to a greater extent than formycin and, at equivalent levels of incorporation, produced less cell lethality (Fig. 7). An excellent correlation was obtained between the incorporation of [^{3}H]formycin into DNA (and to a lesser extent into nRNA) and cell lethality in the presence *and* absence of dCF (Fig. 7A). Enhanced incorporation of [^{3}H]8-azaadenosine into nRNA and DNA also correlated qualitatively with increasing cell lethality, but a continuum between the amount of drug incorporated in the absence and presence of dCF and cell lethality was not present (Fig. 7B). The latter results suggest that the utilization of 8-azaadenosine in the synthesis of nucleic acids to produce its inhibitory effect on cell growth differed depending on whether adenosine deaminase was inhibited. This did not appear to be the case with formycin. The differences in the utilization of 8-azaadenosine in the presence and absence of dCF versus cell lethality may be related to its metabolism to 8-azaguanosine triphosphate following its deamination and phosphorylation to 8-azainosine monophosphate [28]. If incorporation of 8-azaguanosine triphosphate into DNA and RNA is more inhibitory to cell viability than incorporation of an equivalent amount of 8-azaadenosine triphosphate, it would explain why more cell lethality was associated with an equivalent amount of drug incorporation in the absence of dCF than in its presence (Fig. 7).

Thus, these data imply that the incorporation of formycin and 8-azaadenosine drugs into DNA leads to cell lethality which is reflected in a reduced rate of DNA synthesis. On the other hand, drug substitution into nRNA only produced a marginal effect on RNA synthesis. Whether drug-substituted mRNA produces a functional change in its coding activity which subsequently leads to impaired translation is still a possibility. However, we feel that the possibility of this mechanism is remote in view of the lack of effect of drugs such as 5-azacytidine and 5-fluorouracil on the translation of mRNA *in vitro* [29, 30].

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