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CORRELATION BETWEEN RIBOSOMAL RNA PRODUCTION AND RNA-DIRECTED FLUOROPYRIMIDINE CYTOTOXICITY*

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Abstract—The relationship between cytotoxicity and fluoropyrimidine effects on the production of mature cytoplasmic 28S and 18S ribosomal RNA was studied in S-180 cells for the fluoropyrimidines: 5-fluorouracil (FUra), 5-fluorouridine (FUrd), 5-fluorodeoxyuridine (FdUrd), and 5′-deoxy-5-fluorouridine (5′-dFUrd). After a 6-hr drug exposure, the total cytotoxicity in the absence of added thymidine (dThd) was determined by soft-agar cloning and resulted in LC_{90} (lethal concentration to 90% of cells) values of 0.6 μM FdUrd, 0.7 μM FUrd, 5.3 μM FUra and 93 μM 5′-dFUrd. The RNA-directed (dThd-nonreversible) cytotoxicity was assessed by cloning the cells in the presence of 10 μM dThd. This resulted in an altered order of potency and increased LC_{90} values to 5.5 μM FUrd, 20 μM FUra, 265 μM FdUrd and 870 μM 5′-dFUrd. The production of mature cytoplasmic rRNA was determined by measuring the amount of [^{3}H]cytidine incorporated into the 28S and 18S rANA species following their separation by agarose gel electrophoresis, compared with the level of [^{3}H]cytidine incorporated into the nuclear rRNA. When all four fluoropyrimidines were compared together, the degree of inhibition of cytoplasmic rRNA production was poorly predictive of the total cytotoxicity in the absence of dThd (correlation coefficient, $r = 0.77$). FdUrd, in particular, had a very minor effect on rRNA production even at very toxic drug concentrations. When toxicity was assessed in the presence of dThd, however, there was a strong and significant correlation between rRNA production and RNA-directed cytotoxicity ($r = 0.95$, $P < 0.001$), for all the fluoropyrimidines tested, including FdUrd. Thus, when the inhibition of thymidylate formation was eliminated as a site of drug action and only RNA-directed cytotoxicity was assessed, the impaired production of cytoplasmic rRNA was strongly associated with cytotoxicity. These results demonstrate that the inhibition of mature cytoplasmic rRNA production may be an important common mechanism of RNA-directed cytotoxicity for all the fluoropyrimidines, and not limited to FUrd or FUra.

The incorporation of fluoropyrimidines into RNA may cause potentially lethal aberrations in cellular metabolism; however, a precise mechanism for this RNA-directed or thymidine (dThd‡)-nonreversible fluoropyrimidine cytotoxicity has not been elucidated [1–3]. Previous experiments in our laboratory found marked differences in the patterns of RNA incorporation in murine tumor cells when different fluoropyrimidine precursors were compared [4, 5]. These variations in RNA incorporation are caused primarily by differences between the fluoropyrimidines in their abilities to label rRNA, which has also been found to be associated with resulting RNA-directed cytotoxicity. These observations were consistent with the impaired processing of rRNA being a possible cause of RNA-directed fluoropyrimidine cytotoxicity. Other laboratories have also suggested that the impaired processing of rRNA by 5-fluorouracil (FUra) and 5-fluorouridine (FUrd) may be their primary mechanism of cytotoxic action [6, 7]. Whether this can also be considered a general mechanism of RNA-directed or dThd-nonreversible cytotoxicity for the entire group of fluoropyrimidines is not clear.

Each of the four fluoropyrimidines, FUra, FUrd, 5-fluorodeoxyuridine (FdUrd), and 5′-deoxy-5-fluorouridine (5′-dFUrd), must first be converted to the same active metabolite, 5-fluorouridine-triphosphate (FUTP), prior to incorporation into RNA. These drugs differ, however, in several

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‡ Abbreviations: FUra, 5-fluorouracil; 5′-dFUrd, 5′-deoxy-5-fluorouridine; FUrd, 5-fluorouridine; FdUrd, 5-fluorodeoxyuridine; FdUMP, 5-fluorodeoxyuridine-monophosphate; FUTP, 5-fluorouridine-triphosphate; dThd, thymidine; rRNA, ribosomal RNA; tRNA, transfer RNA; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline; VRC, vanadyl-ribonucleotide complex; and LC, lethal concentration.

distinct ways. First, they have individual enzymatic pathways of metabolic activation [1]. Second, they differ in their relative rates of metabolism and in the relative levels produced of the two major active metabolites, FUTP and 5-fluoro-2'-deoxyuridine-monophosphate (FdUMP) [8]. Third, they have different dThd-reversible and dThd-nonreversible cytotoxic potencies in various cell lines [8]. And finally, they can exhibit preferential patterns of incorporation into different types of RNA [4, 5]. Thus, although all four fluoropyrimidines produce the same intracellular metabolites, many differences exist in their intracellular pharmacology. The possibility, therefore, remains that each of these fluoropyrimidines may cause unique aberrations in RNA and may produce dThd-nonreversible or RNA-directed cytotoxicity by different mechanisms. A previous study by Dolnick and Pink [9] concluded that rRNA is not associated with FUra cytotoxicity; rather, changes in mRNA were reported to be responsible.

The following experiments compared the four different drugs, FUra, FUrd, FdUrd, and 5'-dFUrd, in order to test whether the impaired production of rRNA is a general result under conditions which result in dThd-nonreversible cytotoxicity for all the fluoropyrimidines. The degree of inhibition of the production of mature cytoplasmic 18S and 28S ribosomal RNA was measured and correlated with the cytotoxicity produced by each of the fluoropyrimidines. If the inhibition of rRNA production is a general mechanism for fluoropyrimidine activity, then the degree of inhibition should correlate with the same degree of cytotoxicity for each of the fluoropyrimidines. A strong correlation between cytotoxicity and rRNA produced was found to exist for the four fluoropyrimidines, but only in the presence of dThd. In the absence of dThd, when cells were sensitive to an inhibition of thymidylate and DNA synthesis, the association was less strong, suggesting that other actions of the fluoropyrimidines, besides the inhibition of rRNA, were responsible for cell death.

MATERIALS AND METHODS

Chemicals. 5'-dFUrd was obtained from Hoffmann-LaRoche, Inc. (Nutley, NJ). FUra, FUrd and FdUrd were purchased from the Calbiochem-Behring Corp. (La Jolla, CA). [5-^{3}H]Cytidine (22 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA). Thymidine and all other biochemicals, unless otherwise designated, were purchased from the Sigma Chemical Co. (St. Louis, MO).

Cell culture. Murine Sarcoma-180 cells were maintained as suspension cultures in RPMI-1640 medium supplemented with 10% heat-inactivated horse serum plus streptomycin (100 μg/ml) and penicillin (100 units/ml). Tissue culture supplies were all purchased from GIBCO (Grand Island, NY). The concentration of thymidine (dThd) in 10% horse serum is less than 1 μM.

Clonogenic survival. Exponentially growing cells were treated with various concentrations of fluoropyrimidines and then were washed once with sterile phosphate-buffered saline (PBS: 100 mg $CaCl_2$, 200 mg KH_2PO_4, 59 mg $MgSO_4$, 800 mg NaCl, and 1150 mg NaH_2PO_4 in a 1 liter sterile aqueous solution) and counted with a model ZBI Coulter counter (Hialeah, FL). Drug toxicity was assessed using a soft-agar cloning procedure described previously [10]. When specified, the cloning medium contained 10 μM dThd to circumvent any inhibition of thymidylate synthetase. Viability was assessed by measuring the ability of a cell to produce progeny that were visible as distinct individual colonies or clones. Percent clonal growth was calculated by determining the ratio of the clonal growth of treated cells compared with control cells and multiplying by 100. The plating efficiency of the control cells (100 cells per tube) ranged from 60 to 75%.

Isolation of RNA. Cells were washed once with ice-cold PBS and suspended in 8 vol. of lysis buffer A which consisted of 10 mM Tris–HCl (pH 7.0), 10 mM NaCl, 1% Triton X-100, 1% ethanol and 50 μM vanadyl–ribonucleotide complex (VRC) (BRL, Bethesda, MD). This mixture was vortexed and stored on ice for 10 min. Nuclei were collected by spinning at 700 *g* for 10 min and the pellet was washed once with 5 vol. of lysis buffer A. The supernatant fractions were combined to form the cytoplasmic RNA fraction. The pellet containing the nuclear RNA was set aside. The cytoplasmic RNA fraction was extracted three times with an equal volume of phenol : chloroform (1 : 1) and three times with an equal volume of chloroform : isoamyl alcohol (24 : 1). Three volumes of absolute ethanol and 1/20 volume of 5 M NaCl were added, and the nucleic acids were precipitated overnight at −20°. The precipitated RNA was spun down at 5000 *g* for 15 min, resuspended in sterile water, reextracted with phenol/chloroform, and precipitated in ethanol as before.

Gel electrophoresis of RNA. Cytoplasmic and nuclear RNA were fractionated on vertical 1.5% agarose slab gels containing 6 M urea and 0.015 M iodoacetate with a Tris-phosphate running buffer (0.04 M Tris, 0.036 M NaH_2PO_4, 0.001 M EDTA, pH 7.4). RNA bands were visualized under u.v. light after staining with ethidium bromide. Incorporation of [^{3}H]cytidine into the different size classes of RNA was determined by slicing the RNA bands out of the gel and incubating the slices in 1 M NaOH overnight to hydrolyze the RNA. The gel slices were neutralized with concentrated HCl, and the radioactivity was assayed by liquid scintillation counting. Using the optical density from the scanning of the gel and the corresponding amount of radioactivity, a specific activity of [^{3}H] incorporation can be obtained.

Ribosomal RNA production. S-180 cells were treated for 6 hr concurrently with fluoropyrimidines and 0.33 μCi/ml [^{3}H]cytidine in order to label the newly synthesized rRNA. Cytoplasmic and nuclear RNA were isolated and analyzed by agarose/urea gel electrophoresis as described above. The amounts of radioactivity contained in the 28S and 18S rRNA in drug-treated cells were compared with control cells to determine the amount of cytoplasmic rRNA production occurring during the 6-hr exposure period. The amount of radiolabeled cytoplasmic

rRNA produced was expressed as a percent of the control cells (control = 100%).

Statistical analysis. The degree of correlation between the inhibition of rRNA production and cytotoxicity was examined using a linear least squares fitting analysis. The Pearson correlation coefficient, r, and the P value (null hypothesis: slope equals zero) were also determined [11].

RESULTS

Fluoropyrimidine cytotoxicity in S-180 cells. Following a 6-hr treatment with FUra, FUrd, FdUrd, or 5'-dFUrd, S-180 cells were cloned in soft-agar. Overall fluoropyrimidine cytotoxicity was measured by determining the percent clonal growth in the absence of dThd (Fig. 1), whereas the RNA-directed cytotoxicity was assessed by adding 10 μM dThd to the cloning medium after the 6-hr drug exposure (Fig. 1). This concentration of dThd did not alter cell viability and had the maximal potency at reversing FdUrd toxicity.

The different fluoropyrimidines exhibited very individual cytotoxic profiles. Without dThd, the overall LC_{90} values were, in order of decreasing potency, 0.6 μM FdUrd, 0.7 μM FUrd, 5.3 μM FUra and 93 μM 5'-dFUrd. The addition of dThd did not affect clonal growth by itself; however, it did decrease the cytotoxicity produced by each of the fluoropyrimidines and it changed their order of potency. In the presence of dThd, the RNA-directed, dThd-nonreversible LC_{90} values increased to 5.5 μM FUrd, 20 μM FUra, 265 μM FdUrd, and 870 μM 5'-dFUrd. Although generally reported to be exclusively cytotoxic via inhibition of thymidylate synthetase, FdUrd also exhibited dThd-nonreversible toxicity. However, FdUrd cytotoxicity was reduced the most markedly by the addition of dThd to the cloning medium.

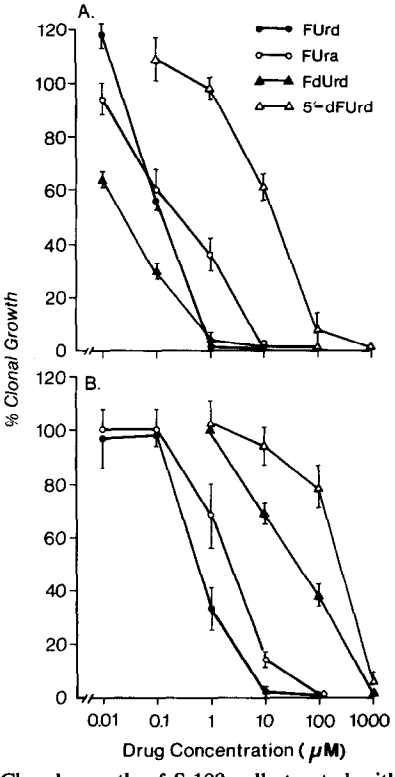

Fig. 1. Clonal growth of S-180 cells treated with fluoropyrimidines. S-180 cells were treated for 6 hr with different concentrations of fluoropyrimidines and then cloned in the absence (A) and presence (B) of 10 μM thymidine as described under Materials and Methods. Bar, ± standard error of the mean; 100% was 60 to 75 clones.

Effects of fluoropyrimidines on nuclear and cytoplasmic rRNA production. The effects of various concentrations of fluoropyrimidines on the production of mature cytoplasmic 28S and 18S rRNA and tRNA and on nuclear RNA were examined. S-180 cells were exposed simultaneously for 6 hr to fluoropyrimidines and [^{3}H]cytidine. Following electrophoresis of the extracted cytoplasmic or nuclear RNA, the levels of [^{3}H]cytidine incorporation in mature 28S and 18S rRNA and in 4S tRNA were measured, as was the level of incorporation into the nuclear RNA species. Figure 2 (A and B) shows a representative profile for the control cells compared with those treated with either 0.5 or 10 μM FUrd.

Comparison of the level of [^{3}H]cytidine incorporation in drug-treated and control cells allowed for the accurate assessment of the effects of the fluoropyrimidines on rRNA production. All four fluoropyrimidines decreased the amount of newly synthesized labeled 28S and 18S rRNA appearing in the cytoplasm; however, their relative potencies for this event were quite different (Table 1). FUrd was the most potent inhibitor, followed by FUra, FdUrd,

Table 1. Fluoropyrimidine effect on ribosomal RNA

Treatment		
Drug	Concentration (μM)	rRNA Production* (% of control)
FUra	1	77
	10	38
	100	23
FUrd	0.5	49
	2	23
	10	15
FdUrd	2.5	79
	25	50
	250	20
5'-dFUrd	10	100
	100	64
	1000	29

S-180 cells were incubated for 6 hr concurrently with [^{3}H]cytidine and different concentrations of fluoropyrimidines. The production of cytoplasmic rRNA was determined as described under Materials and Methods. Control rRNA (see Fig. 2) production = 100%.

* Determined as [^{3}H]cytidine incorporated in 28S and 18S rRNA from treated cells ÷ the same in control cells × 100. Results are from one comparative experiment in which all drugs were examined in the same experiment with the same cell batch. Each drug was examined individually in at least three other experiments, the results of which were similar to those presented.

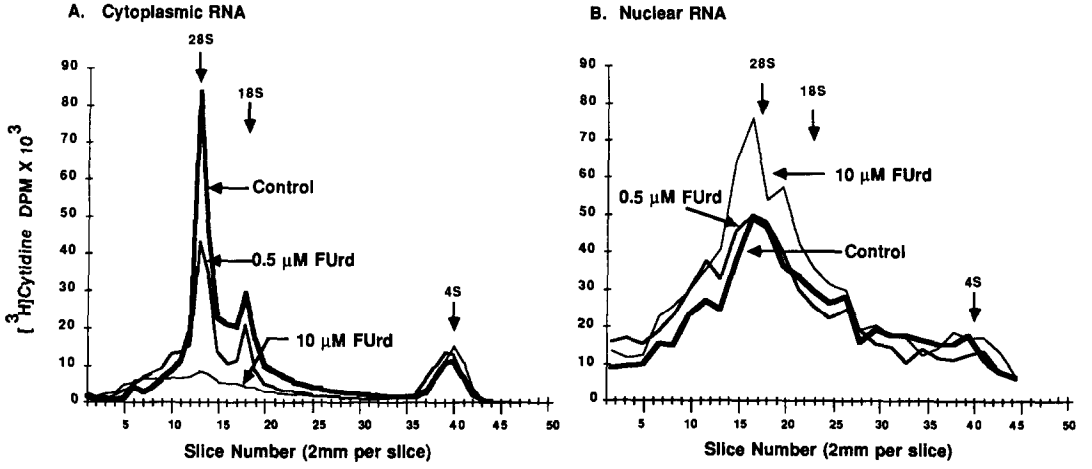

Fig. 2. RNA production in S-180 cells treated with fluorouridine and [^{3}H]cytidine. S-180 cells were concurrently incubated with fluoropyrimidines and 0.33 μCi/ml of [^{3}H]cytidine for 6 hr. The nuclear and cytoplasmic RNA were isolated and 30 μg of RNA per lane was electrophoresed on 1.5% agarose/6 M urea vertical slab gels. Individual lanes were scanned to quantitate the RNA and then cut into 2 mm slices, and the radioactivity in each was determined as described under Materials and Methods. Results are shown for the control cells and cells treated with 0.5 or 10 μM FUrd. Analogous results were observed with the other fluoropyrimidines. The scanning and cutting procedure allowed for accurate determination of the specific activity of the tRNA, which was identical (4010 ± 287 dpm/μg RNA) for both treated and control. The mature cytoplasmic rRNA production was determined by measuring the total radioactivity contained in the 28S and 18S rRNA peaks (control = 100%).

and 5'-dFUrd. The 10 μM FUrd treatment, as observed in Fig. 2, essentially abolished the formation of mature cytoplasmic 28S and 18S ribosomal RNA, whereas there was no inhibition in the formation of the nuclear RNA species.

The incorporation of [^{3}H]cytidine into newly synthesized tRNA was unaffected by fluoropyrimidine treatment even under conditions where rRNA labeling was maximally inhibited. The specific activity, determined as described in the legend and in Materials and Methods, of the tRNA from all treatment conditions was found to be identical. This observation, along with the absence of any reduction in the nuclear RNA labeling from the drug concentrations that completely suppress the formation of cytoplasmic rRNA, supports previous reports suggesting that fluoropyrimidines selectively inhibit rRNA processing events but do not impair the general production of other RNA [12], and that the observed alterations in rRNA labeling were not artifacts due to changes in the cytidine nucleotide pool sizes. The total amount of each type of cytoplasmic RNA isolated per cell also remained the same in all experiments following the 6-hr exposure period.

Correlation between cytoplasmic rRNA production and cytotoxicity. The inhibition of cytoplasmic rRNA production was compared with the overall cytotoxicity of all four fluoropyrimidines in the absence of dThd by correlating the degree of rRNA produced with the percent clonal growth (Fig. 3). In general, higher concentrations of fluoropyrimidines resulted in greater cytotoxicity and a greater inhibition of rRNA production. However, when the relationship between cytotoxicity and rRNA production for all four fluoropyrimidines was analyzed by linear regression, the correlation was poor ($r = 0.77$).

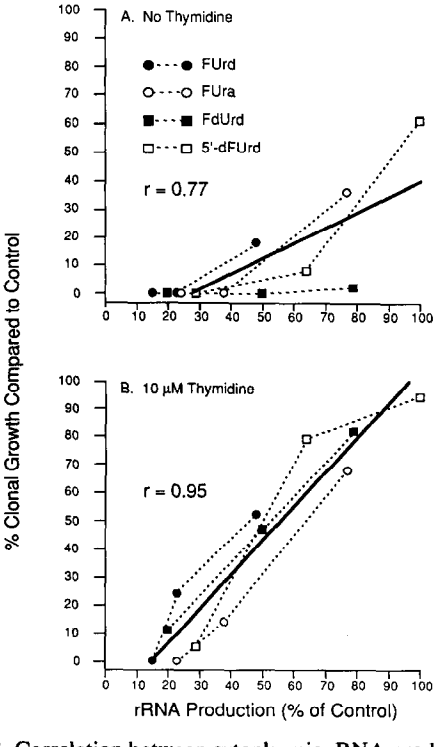

Fig. 3. Correlation between cytoplasmic rRNA production and clonal growth. S-180 cells were exposed to fluoropyrimidines for 6 hr and the synthesis of rRNA and the clonal growth in the absence (A) and presence (B) of 10 μM thymidine were determined as described under Materials and Methods. Key: (●) 0.5, 2, 10 μM FUrd; (○) 1, 10, 100 μM FUra; (■) 2.5, 25, 250 μM FdUrd; and (□) 10, 100, 1000 μM 5'-dFUrd. Linear correlation was analyzed by least squares fitting analysis.

FdUrd, in particular, showed very little inhibition of rRNA production even at highly toxic drug concentrations. At a FdUrd concentration which resulted in a 97% cell kill, the amount of cytoplasmic rRNA produced was decreased only slightly (20% inhibition) (Fig. 3). In contrast, at a concentration producing the same degree of toxicity as FdUrd, FUrd produced a much larger inhibition of cytoplasmic rRNA production (75% inhibition). Thus, when different drugs were compared, the inhibition of cytoplasmic rRNA production was poorly predictive of the overall final fluoropyrimidine cytotoxicity measured in the absence of added dThd.

A much stronger correlation was observed between rRNA production and dThd-nonreversible (i.e. RNA directed) cytotoxicity (Fig. 3). Linear regression analysis comparing cytotoxicity with rRNA production demonstrated a strong correlation for all four fluoropyrimidines ($r = 0.95$, $P < 0.001$). When dThd was present, equally toxic treatments of FUrd and FdUrd now resulted in the same degree of impaired cytoplasmic rRNA production. Thus, under conditions where the addition of dThd abolished the block in thymidylate and DNA synthesis, the degree of rRNA inhibition was highly predictive and significantly associated with the final RNA-directed cytotoxicity, independent of the fluoropyrimidine used. This observation is consistent with the hypothesis that all the fluoropyrimidines may share the inhibition of rRNA production as a possible mechanism of action for producing RNA-directed or dThd-nonreversible cytotoxicity. The results obtained in the absence of dThd also demonstrate that significant cytostasis or cytotoxicity can be produced in cells at a time when rRNA processing remains unaltered.

DISCUSSION

These studies compared the relationship between cytoplasmic 28S and 18S rRNA production and cytotoxicity for the four different fluoropyrimidines, FUra, FUrd, FdUrd, and 5′-dFUrd, in S-180 cells. When cytotoxicity was assessed in the absence of dThd, the level of fluoropyrimidine inhibition of rRNA production poorly correlated with cell lethality (Fig. 3). Without added dThd, S-180 cells are known to be sensitive to an inhibition of thymidylate synthesis and DNA synthesis [4]. All of the fluoropyrimidines produced an inhibition of rRNA production in S-180 cells, although FdUrd and 5′-dFUrd were much less potent than FUra and FUrd and required much higher concentrations to produce similar levels of rRNA inhibition. FdUrd, in particular, was the most toxic fluoropyrimidine in the absence of dThd, yet it inhibited the production of rRNA only slightly. This observation indicates that the inhibition of rRNA was not merely a general response to cytostasis, since FdUrd toxicity occurred at drug concentrations that only minimally impaired ribosomal RNA production. When comparing all four fluoropyrimidines together, the level of rRNA production was poorly indicative of the overall toxicity. Thus, the inhibition of rRNA could not explain completely the total cytotoxicity of these agents in the absence of dThd, suggesting that other actions of the fluoropyrimidines were responsible for cell death.

When cytotoxicity was assessed in the presence of dThd, fluoropyrimidine effects on rRNA production were significantly correlated with cell death (Fig. 3). Under these conditions, by definition, only RNA-directed cytotoxicity was measured, and cells were no longer sensitive to FdUMP-mediated actions, such as the inhibition of thymidylate synthetase [13]. The addition of dThd changed the order of potency from FdUrd > FUrd > FUra > 5′-dFUrd to FUrd > FUra > FdUrd > 5′-dFUrd. When all four fluoropyrimidines were examined together, a similar significant correlation between toxicity and rRNA production ($r = 0.95$, $P < 0.001$) was observed. Thus, when DNA-related effects were eliminated as a site of drug action, the level of rRNA inhibition was highly predictive of the RNA-directed toxicity of the fluoropyrimidines for all four drugs including FdUrd. These results suggest that the fluoropyrimidines do not cause unique aberrations in RNA and, instead, they may produce dThd-nonreversible cytotoxicity by a similar mechanism: the inhibition of rRNA production.

Quantitating the appearance of [^{3}H]cytidine label in mature cytoplasmic RNA provided an easy way to assess the blocked production of rRNA. An important observation was that the specific activity of [^{3}H]cytidine-labeled transfer RNA was unchanged by even the most toxic FUrd treatment (Fig. 2). This is consistent with previous reports suggesting that fluoropyrimidines act selectively to inhibit rRNA processing and do not affect general mRNA or tRNA production [12]. It also shows that the drug-induced differences in the patterns of rRNA labeling were not artifacts due to changes in cytidine nucleotide pool size.

There has also been the recent suggestion that the fluoropyrimidines may alter mRNA, as well as certain SnRNA [9, 14–16] and that these changes may contribute to dThd-nonreversible cytotoxicity. One report [9] suggested that the dThd-nonreversible toxicity in the human cancers examined did not appear to correlate with the suppression of rRNA production. These investigators were able to demonstrate that, under long-term exposure conditions, rRNA processing appeared to recover even though the cells ultimately died. The mRNA studies completed focused on one particular mRNA, that for dihydrofolate reductase (DHFR). It is certainly apparent that the metabolism of this mRNA is altered dramatically in cells treated with FUra. However, other studies are suggesting that these changes are secondary to other cellular events. One study by our laboratory [4] demonstrated that there was no correlation between the actual level of FUra incorporation into hnRNA and resulting toxicity. It was shown that a nontoxic dose of 5′-dFUrd could result in a much greater level of FUra incorporation into hnRNA, compared to a very toxic dose of FUrd. In addition to this work, our laboratory has also demonstrated that different species of mRNA, even though having similar transcription rates, exhibit individual and very different changes in their metabolism in cells exposed to FUra [17]. The combined results imply that mRNA can certainly be altered by

fluoropyrimidines, but this action appears unrelated to the incorporation of FUra into precursor mRNA and is apparently secondary to other cellular changes induced by the fluoropyrimidine.

The relevance of RNA-directed fluoropyrimidine cytotoxicity to the overall antitumor activity of these drugs in humans is unclear. In the present study, S-180 cells were much more sensitive to fluoropyrimidines in the absence of dThd, when DNA-directed effects predominated [4, 13]. RNA-directed cytotoxicity occurred only with high concentrations of fluoropyrimidines in the presence of dThd. FdUrd and 5'-dFUrd, in particular, required high concentrations to cause similar levels of inhibition of rRNA production compared to the other fluoropyrimidines, suggesting that the RNA-directed actions of these two drugs are probably of limited importance in producing their antitumor activity. FUrd and FUra, however, were considerably more potent in their RNA effects, and the difference between their dThd-reversible and dThd-nonreversible activities was small. Therefore, RNA-directed actions may be very important in the activity of these two agents [6, 7]. In agreement with the present studies, Herrick and Kufe [7] reported a strong association between the degree of rRNA processing and cytotoxicity in Friend murine erythroleukemia cells. Thus, under conditions where RNA-directed actions predominate, the inhibition of rRNA production may be an important mechanism of fluoropyrimidine antitumor activity.

In summary, these experiments demonstrate that the inhibition of mature cytoplasmic RNA production can be a general result for RNA-directed, dThd-nonreversible cytotoxicity for each of the fluoropyrimidines. When the inhibition of thymidylate formation and DNA synthesis was eliminated as a site of drug action by supplementing with dThd, the impaired production of cytoplasmic rRNA strongly correlated with toxicity, regardless of the fluoropyrimidine used. In the absence of dThd, when cells were allowed to remain sensitive to an inhibition of thymidylate synthase and DNA synthesis, the correlation was poor. Thus, all the fluoropyrimidines were capable of producing a common effect on RNA metabolism that was significantly associated with RNA-directed cytotoxicity. No other unique aberrations in RNA metabolism were observed. It is concluded that the inhibition of rRNA production may be an important common mechanism of RNA-directed or dThd-nonreversible cytotoxicity for all the fluoropyrimidines.

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