Vol. 87, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 13, 1979 Pages 664-670

AUGMENTATION BY THYMIDINE OF THE INCORPORATION AND DISTRIBUTION OF 5-FLUOROURACIL IN RIBOSOMAL RNA

Christine K. Carrico[1] and Robert I. Glazer

Applied Pharmacology Section
Laboratory of Medicinal Chemistry
National Cancer Institute
Bethesda, Maryland 20014

Received February 15,1979

SUMMARY

The effect of high dose thymidine (TdR) pretreatment on the action of 5-fluorouracil (5-FU) on rRNA was measured in regenerating liver after partial hepatectomy. TdR did not enhance the inhibitory or non-inhibitory effects of 5-FU on the synthesis of rRNA and poly(A)RNA, respectively. However, TdR pretreatment intensified the effect of 5-FU on interference with the processing of rRNA from precursor rRNA. The distribution of [^{3}H]5FU, as well as [^{3}H]-orotic acid was enhanced in the 2'-O-methylated sequences of rRNA following pretreatment with TdR. Thus, the combination regimen of TdR plus 5-FU resulted in enhanced interference with the processing of rRNA.

INTRODUCTION

Thymidine has been variously reported to protect against (1) or enhance (2) the cytotoxicity of 5-FU. High doses of TdR[2] have been shown to increase the incorporation of 5-FU into RNA (3,4), and a correlation has been reported between the increased incorporation of 5-FU into RNA and the enhancement of antitumor activity by this drug combination (4). The effect of 5-FU on RNA synthesis appears to be an important part of its cytotoxic action since this antimetabolite impairs the processing of rRNA (5,6). Our studies with 5-FU employing the regenerating liver system have also shown that 5-FU is incorporated into, and impairs the synthesis of rRNA (7).

---

[1]Fellow of the Pharmacology-Toxicology Research Associate Program, NIGMS, NIH.

[2]The abbreviations used are: 5-FU, 5-fluorouracil; poly(A)RNA, polyadenylic acid-containing RNA; TdR, thymidine.

0006-291X/79/070664-07$01.00/0

The experiments described in this report were initiated to investigate whether the administration of high doses of TdR would alter the effect of 5-FU on rRNA. Our results show that in regenerating liver, large doses of TdR augment and alter the incorporation of 5-FU into rRNA.

MATERIALS AND METHODS

Sepharose 4B was obtained from Pharmacia Fine Chemicals, Piscataway, NJ; poly(U) and TdR were purchased from Sigma Chemical Co., St. Louis, MO: 5-Fluoro-[6-^{3}H]uracil (2 Ci/mmole) was obtained from Amersham Corp., Arlington Heights, IL. Aquasol and [5-^{3}H]orotic acid (20 Ci/mmole) were obtained from New England Nuclear Corp, Boston, MA. 5-FU was obtained from Dr. Harry B. Wood, Jr., Drug Synthesis and Development Branch, NCI, NIH.

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 75 to 100 g were maintained under conditions of alternating 12 hr periods of light and darkness. Food and water were available prior to surgery, but food was withheld following surgery. Partial hepatectomies were performed under ether anesthesia according to the method of Higgins and Anderson (8). 5-FU was dissolved in 0.9% NaCl solution and injected i.p. at a dose of 20 mg/kg at 1 hr and 16 hr after partial hepatectomy. TdR was dissolved in 0.9% NaCl solution and injected i.p. at a dose of 500 mg/kg 30 min prior to each 5-FU injection. Control animals received an equal volume of 0.9% NaCl solution.

The incorporation of 5-FU into RNA was measured in rats given 500 mg/kg of TdR 30 min prior to 20 mg/kg of [^{3}H]5-FU (16 mCi/mmole) at 1 hr and 16 hr after partial hepatectomy. RNA synthesis was measured in animals injected i.p. with [^{3}H]orotic acid (500 µCi/kg, 20 Ci/mmole) 15 min after the 16 hr dose of 5-FU.

Animals were sacrificed by decapitation at 18 hr after partial hepatectomy, and their livers were minced and homogenized in 9 volumes of buffer containing: 25 mM Tris-HCl (pH 7.5)-25 mM NaCl-5 mM $MgCl_2$-2% (v/v) Triton X-100-0.1% (w/v) heparin. Polysomes were isolated by the Mg ++ precipitation method of Palmiter (9). RNA was extracted from the polysomes as described (10) and separated into rRNA and poly(A)RNA by poly(U)Sepharose affinity chromatography (11). Electrophoresis of rRNA was carried out in agarose-urea gels (10) and alkaline hydrolysates of rRNA were chromatographed on DEAE Sephadex (11) as previously described.

RESULTS AND DISCUSSION

In regenerating liver, the administration of large doses of TdR (500 mg/kg) increased the incorporation of 5-FU into polysomal RNA (TABLE 1). Both rRNA and poly(A)RNA exhibited approximately a three-fold increase in the degree of incorporation of 5-FU in the presence of TdR. Other laboratories have reported a 2-4 fold stimulation by TdR of incorporation of the drug into the total RNA of normal or tumor tissues (3,4).

Experiments designed to measure the effect of 5-FU or 5-FU plus TdR regimens are presented in TABLE 2. While the administration of 20 mg/kg of 5-FU significantly inhibited the incorporation of [^{3}H]orotic acid into rRNA, the addition

TABLE 1

The Effect of TdR on the Incorporation of 5-FU into Polysomal RNA

RNA Species	pmoles 5-FU/μg RNA			
	-TdR	%	+TdR	%
rRNA	0.86 ± 0.16	100	2.36 ± 0.23	274
Poly(A)RNA	2.94 ± 1.58	100	10.34 ± 3.00	352

Rats were given 20 mg/kg of [^{3}H]5-FU (16 mCi/mmole) i.p. at 1 hr and 16 hr after partial hepatectomy. Either 0.9% NaCl or TdR (500 mg/kg) was administered prior to 5-FU treatment. Each value is the mean ± S.E. of 5 determinations.

TABLE 2

The Effect of TdR Plus 5-FU on the Incorporation of [^{3}H]Orotic Acid into Polysomal RNA

RNA Species	DPM/μg RNA							
	Control				5-FU			
	-TdR	%	+TdR	%	-TdR	%	+TdR	%
rRNA	336 ± 49	100	416 ± 40	124	121 ± 19	36	87 ± 21	21
Poly(A)RNA	1451 ± 245	100	1626 ± 256	112	1594 ± 475	110	1190 ± 344	73

Rats were given 20 mg/kg of 5-FU i.p. at 1 hr and at 16 hr after partial hepatectomy. Either 0.9% NaCl or TdR (500 mg/kg) was administered 30 min prior to 5-FU treatment. [^{3}H]orotic acid (500 μCi/kg) was injected i.p. 15 min after the 16 hr injection of 5-FU and animals were killed at 18 hr after partial hepatectomy. Each value is the mean ± S.E. of 5 determinations. Percentages for rats treated with 5-FU or 5-FU + TdR were calculated relative to 0.9% NaCl- or TdR-treated controls, respectively.

of TdR did not significantly enhance this effect. 5-FU alone had no significant effect on the incorporation of [^{3}H]orotic acid into poly(A)RNA. With the addition of TdR, a minimal degree of inhibition was observed (27%) but this did not appear to be statistically significant ($p > 0.1$). It should be noted that TdR alone had no significant effect on the incorporation of labelled precursor into RNA.

Agarose-urea gel electrophoresis of the rRNA fraction indicated that the distributional anomalies observed in the presence of 5-FU alone were also present in rRNA fractions from rats treated with 5-FU plus TdR (Figure 1).

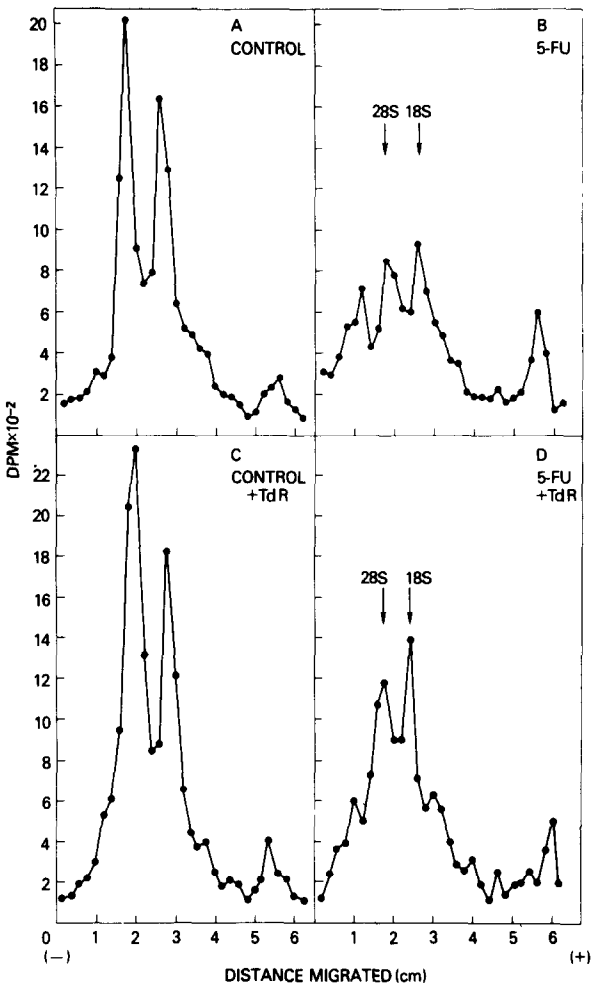

Figure 1. Agarose-urea gel electrophoresis of rRNA from 0.9% NaCl- and 5-FU-treated animals in the absence and presence of TdR pretreatment. See TABLE 2 and MATERIALS AND METHODS for experimental details.

These results suggest that the three-fold increase in the incorporation of 5-FU due to TdR pretreatment might be further affecting the processing of rRNA.

Since the processing of precursor rRNA is dependent on 2'-O-methylation, experiments were designed to assess the effect of TdR on the distribution of [^{3}H]orotic acid in 2'-O-methylated sequences of rRNA (TABLE 3). When the distribution of radioactivity was measured in alkaline hydrolysates of rRNA from 0.9% NaCl, TdR or 5-FU-treated rats, approximately 95% of the label was found in the mononucleotide (-2) fraction, and approximately 3% was found in the

TABLE 3

Effect of TdR Plus 5-FU on the Distribution of [^{3}H]Orotic Acid in Alkaline Hydrolysates of rRNA

Treatment	RNA Fraction		
	Flow-through	-2	-3
	(% of Distribution of Radioactivity)		
0.9% NaCl	1.9	94.3	3.8
0.9% NaCl + TdR	1.4	95.3	3.3
5-FU	1.6	95.6	2.7
5-FU + TdR	3.1	93.8	6.2

Rats were treated as described in TABLE 2. Alkaline hydrolysates of rRNA were chromatographed on DEAE Sephadex as previously described (7).

dinucleotide (-3) fraction arising from 2'-O-methylation (TABLE 3). In rRNA from rats treated with 5-FU plus TdR, the distribution of radioactivity was altered relative to treatment with 5-FU alone; hence, more radioactivity was present in the 3'-termini (flow-through) and dinucleotide (-3) fractions (TABLE 3).

When the distribution of radioactivity from [^{3}H]5-FU was measured in alkaline hydrolysates of rRNA, a markedly different chromatographic profile was obtained (Figure 2). Most (86.5%) of the total radioactivity from [^{3}H]5-FU treatment alone was located in the flow-through fraction, indicating its incorporation in 3'-termini of rRNA; however, the distribution of [^{3}H]5-FU in this fraction was increased to 95.6% following TdR pretreatment. In addition, there was a concomitant decrease from 11.8% to 3.0% in the distribution of [^{3}H]5-FU located in the mononucleotide (-2) fraction of rRNA from rats pretreated with TdR. In contrast, approximately 1.5% of the incorporated [^{3}H]5-FU was found in the dinucleotide (-3) peak of rRNA from rats treated with 5-FU irrespective of TdR pretreatment. However, in the presence of TdR treatment, the "dinucleotide" peak was shifted to a charge of -3.5, implying a non-stoichiometric distribution of 2'-O-methylated sequences. Therefore, it would appear that TdR pretreatment facilitates the incorporation of 5-FU

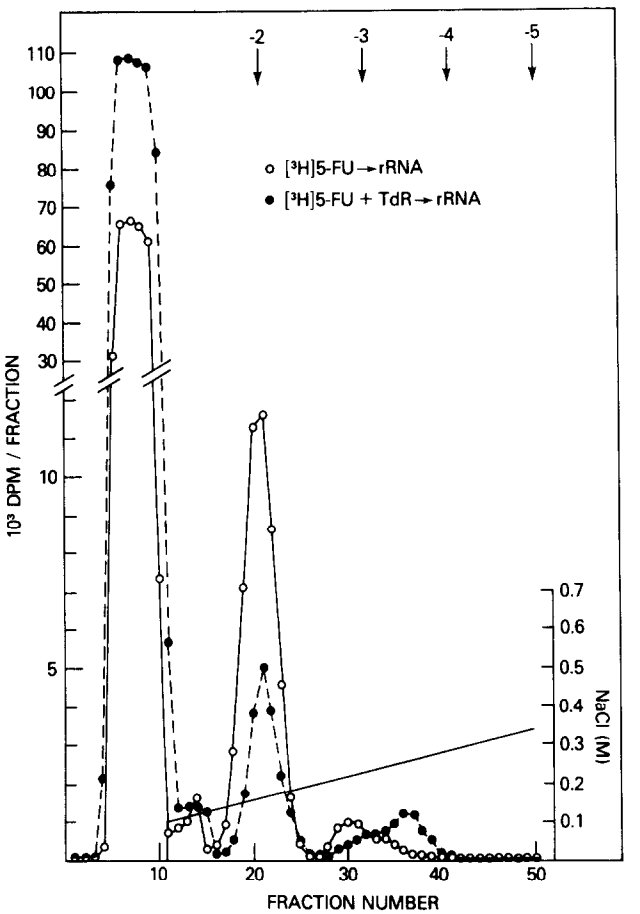

Figure 2. DEAE Sephadex-urea chromatography of alkaline hydrolysates of rRNA labeled with [^{3}H]5-FU. See TABLE 1 and MATERIALS AND METHODS for experimental details.

into terminal positions, as well as alters ribose methylation in a manner resulting in an altered charge distribution of 2'-O-methylated nucleotides.

Thus, it appears that TdR augments the effect of 5-FU on the processing of rRNA without intensifying its action on the synthesis of rRNA and poly(A)RNA per se. The predominant effect of coadministration of TdR and 5-FU on rRNA appears to reside with enhanced interference with its processing. The data suggest that the latter effect may be related to altered 2'-O-methylation and the incorporation of 5-FU within or adjacent to such sequences in rRNA.

REFERENCES

1. Reeves, W.J. and Cailleau, R. (1969) Proc. Soc. Exptl. Biol. Med. 131, 1068-1072.

2. Santelli, G. and Valeriote, F. (1978) J. Natl. Cancer Inst. 61, 843-849.
3. Forsthoefel, P.F., Blend, M.J. and Snow, J.W. (1978) Teratology 18, 269-276.
4. Nayak, R., Martin, D., Stolfi, K., Furth, J. and Spiegelman, S. (1978) Proc. Am. Assoc. Cancer Res. 19, 63.
5. Stenram, U. and Willén, R. (1970) Chem.-Biol. Interactions 2, 79-88.
6. Wilkinson, D.S. and Pitot, H.C. (1973) J. Biol. Chem. 248, 63-68.
7. Carrico, C.K. and Glazer, R.I. (1978) Fed. Proc. 37, 1693.
8. Higgins, G. M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186-202.
9. Palmiter, R.D. (1974) Biochemistry 13, 3606-3615.
10. Glazer, R.I. (1977) Biochim. Biophys. Acta 475, 492-500.
11. Eiden, J.J. and Nichols, J. L. (1973) Biochemistry 12, 3951-3956.
12. Glazer, R.I. and Peale, A.L. (1978) Biochem. Biophys. Res. Commun. 81, 521-526.