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Mechanism of Potentiation of Antitumor Activity of 5-Fluorouracil by Guanine Ribonucleotides against Adenocarcinoma 755*

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Abstract—*The effect of various guanine ribonucleotides on the antitumor activity of 5-fluorouracil (FUra) was investigated by its action on adenocarcinoma 755. 5′-GDP and 5′-GMP were both equally effective in potentiating the antitumor activity of FUra without increasing toxicity. 5′-GTP and 5′-IMP also potentiated the activity but not as much as 5′-GMP. 2′-GMP and 3′-GMP did not enhance the antitumor activity. In contrast, cGMP antagonized the effects of FUra.*

The incorporation of ^{3}H-labeled FUra into RNA or DNA showed there was no obvious association between the incorporation and antitumor activity after any treatment with guanine ribonucleotides. The combination of FUra and 5′-GMP produced the greatest inhibition of RNA synthesis. The combination of FUra and 2′-GMP had no effect on RNA synthesis. The inhibition of RNA synthesis may be the result of decreased pyrimidine pool size and increased incorporation of FUra into RNA. Potentiation of the antitumor activity of FUra by 5′-GMP was reversed by the injection of cytidine. Moreover, the combination of 5-fluorocytidine (FCyd) and 5′-GMP showed greater antitumor activity than FCyd alone. These results indicate that a decreased CTP pool potentiates the antitumor activity of FUra.

Thus, 5′-GMP or 5′-GDP strongly enhanced the antitumor activity of FUra, and the potentiation resulted from the inhibition of RNA synthesis caused by reduction of the CTP and UTP pool sizes and increased incorporation of FUra into RNA.

INTRODUCTION

5-FLUOROURACIL (FUra) is an antitumor agent which is widely used in the treatment of colorectal and breast tumors. The active metabolites responsible for the cytotoxic effects of FUra may be 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), which is a potent inhibitor of thymidylate synthetase [1] and, hence, of DNA synthesis, and 5-fluorouridine-5′-triphosphate (FUTP), which is incorporated into RNA and interferes with the maturation of ribosomal RNA [2–5]. Thus, FUra produces at least two significant biochemical effects, either of which may be sufficient to inhibit cell division. Recently, FUra residues have been detected in eukaryotic DNA [6, 7] and excision of these residues may contribute to the cytotoxicity [8, 9].

Either inosine or deoxyinosine in combination with FUra has been shown to increase the growth-inhibitory potency of FUra against a variety of cell lines *in vitro* [10–14], but in one cell line, mouse T-cell lymphosarcoma S49, inosine and deoxyinosine protected the cells from the effect of FUra [15]. Kessel and Hall [16] reported that glucose and inosine strongly stimulate the conversion of FUra to ribonucleotides both *in vitro* and *in vivo* but this had little effect on the survival time of tumor-bearing mice. We were interested in attempting to use naturally occurring nucleosides to improve the therapeutic efficacy of available antimetabolites. Among them, the combined administration of guanosine and FUra has been shown to increase the therapeutic ratio of FUra in L1210 leukemia [17] and 5′-GMP has been shown to have a similar effect on the action of FUra on a variety of murine leukemias, ascites tumors and solid tumors [18, 19]. Activation of FUra by pretreatment with guanosine is probably due to the increase in FUra nucleotides and incorporation of FUra residues into the RNA of the tumors [20].

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In this investigation, we studied the effect of various guanine ribonucleotides on the antitumor activity of FUra and attempted to determine the mechanism responsible for potentiation of the antitumor activity of FUra by treatment by 5′-GMP.

MATERIALS AND METHODS

Drugs and isotopes

[6-^{3}H]FUra (28 Ci/mmole) and [5-^{3}H]deoxycytidine (25.6 Ci/mmole), [2,8-^{3}H]adenosine (32.2 Ci/mmole) were purchased from Commissariat à l'Energie Atomique, Gif-Sur-Yvette, France, and New England Nuclear, Boston, MA, respectively. [^{3}H]FUra was diluted with its cold carrier, and the specific activity in the solution for injection was 125 μCi/mg.

FUra,2′-GMP,3′-GMP,5′-GMP,3′,5′-cyclicGMP (cGMP) and 5′-IMP were obtained from Sigma Chemical Co., St. Louis, MO. 5′-GDP and 5′-GTP were purchased from Yamasa Biochemicals for Research, Choshi, Japan. 5-Fluorocytidine (FCyd) purchased from Calbiochem-Behring (La Jolla, CA). Solutions of these agents were prepared in saline.

Animals and tumor cells

Groups of six male BDF_1 mice (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan) weighing 20–23 g were used. Solid tumor adenocarcinoma 755 cells (5×10^5/mouse) were implanted s.c. on day 0 into the right thigh.

All mice were housed in plastic cages in an air-conditioned room (25°C) kept in the light for 12 h each day and received CA-1 pellets (CLEA Japan, Inc., Tokyo, Japan) and water *ad libitum*.

Biological assay of antitumor activity

Six mice per group were used. Twenty-four hours after implantation, the mice were given FUra (20 mg/kg/day) or FUra plus one of the guanine ribonucleotides (100 mg/kg/day) i.v. The treatment was repeated on four successive days. The tumors of treated and untreated mice were weighed on day 12.

Uptake of [^{3}H]FUra and incorporation into DNA and RNA

The effects of the guanine ribonucleotide derivatives on the incorporation of [^{3}H]FUra into DNA and RNA were studied. Mice with 7-day tumors were used. Each guanine derivative was injected i.v. at 100 mg/kg together with [^{3}H]FUra at 2.5 or 5 mCi/20 mg/kg for studying the incorporation into RNA or DNA, respectively. The mice were killed by decapitation either at 30 min, 24 h (total incorporation and incorporation into RNA) or 3 h (incorporation into DNA). The tumors were carefully dissected free of hemorrhagic, necrotic and non-tumor tissues, then weighed and chilled as quickly as possible.

For studies of RNA incorporation of [^{3}H]FUra, the tumor (about 400 mg) was minced and homogenized in 3 ml of 0.02 M sodium acetate (pH 5.0), 0.14 M sodium chloride, 10 μg of polyvinyl sulfate per ml, and 0.2% sodium laurylsulfate with a Polytron homogenizer (Kinematica, Amlehnhalde, Switzerland), and 300 μl of this solution was used to determine total radioactivity. The supernatant was separated by centrifugation (1700 ***g***, 10 min). The total RNA fraction was extracted as described previously [21]. For determining incorporation of [^{3}H]FUra into the DNA fraction of the tumor, the fraction was extracted by the procedure described previously [22].

DNA and RNA synthesis

FUra (20 mg/kg), alone or in combination with a guanine ribonucleotide (100 mg/kg), was injected i.v. into male BDF_1 mice bearing solid adenocarcinoma 755 (tumor weight, about 400 mg). The tumor-bearing mice were also pulse labeled for 30 min with 2.5 mCi of [^{3}H]deoxycytidine or [^{3}H]adenosine per kg at the time of or 3 h after administration of the drugs to measure DNA or RNA synthesis, respectively. Labeled DNA and RNA were extracted as described previously [21, 22].

The results in the mice treated with FUra were compared to the incorporation of labeled precursor into DNA or RNA of untreated animals 7 days after tumor implantation. The results were expressed as a percentage of the appropriate control.

Ribonucleoside triphosphate pool size

To measure ribonucleoside triphosphate pool sizes, FUra (20 mg/kg), alone or in combination with guanine ribonucleotide (100 mg/kg), was injected i.v. to male BDF_1 mice bearing a 7-day-old adenocarcinoma 755 (about 400 mg). Groups of four to six mice were killed by cervical dislocation 10 min after the treatment and the tumors were minced and homogenized with a Polytron homogenizer. The acid-soluble extracts were neutralized by extraction with 2 vol. of 0.5 M tri-*n*-octylamine in trichlorotrifluoroethane as described as Khym [23] and then concentrated *in vacuo*. For ribonucleoside triphosphate determination, the neutralized extracts were directly analyzed by a high pressure liquid chromatograph (Model SP8750, Toyo Soda, Tokyo, Japan), equipped with a 4 mm × 30 cm TSKgel DEAE-2SW column (Toyo Soda, Tokyo). Samples were eluted with 55 mM potassium phosphate buffer (pH 6.9) containing acetonitrile (20%) at a flow rate of 1.0 ml/min. Absorption peaks were

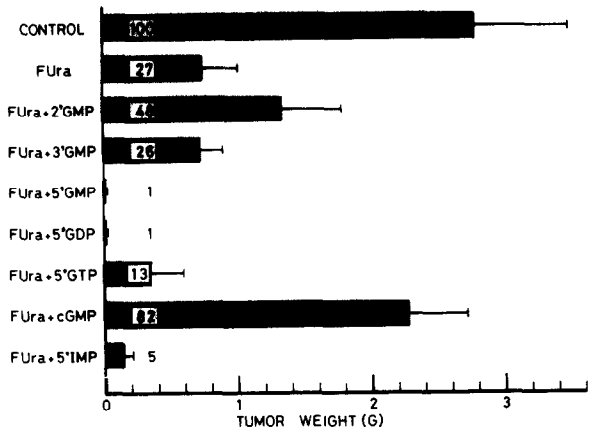

Fig. 1. Effect of guanine ribonucleotides on antitumor activity of FUra. Numbers are mean percentage of control. Bars, S.E.

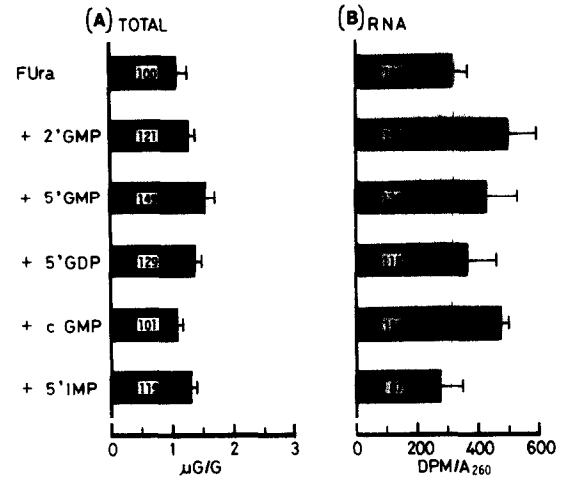

Fig. 2. Total concentration of [³H]FUra in tumors and [³H]FUra incorporation into tumor RNA 30 min after administration of [³H]FUra alone or in combination with guanine ribonucleotides. Each value is the mean of three mice. Bars, S.E.; numbers in columns, percentage of control.

detected at 254 nm with a UV-8 model-II Detector (Toyo Soda, Tokyo). Peak heights were determined by manual measurement and normalized with internal standards. The results are given as percentages of the 0-min values observed in the tumor.

Statistical analysis

The *t*-test for small samples was used to test the statistical significance of the results, $P \leq 0.05$ was considered significant.

RESULTS

Effect of guanine ribonucleotide derivatives on antitumor activity of FUra against adenocarcinoma 755

FUra at 20 mg/kg is the maximum nontoxic dose in this schedule [19, 23] producing a moderate inhibition of tumor growth (T/C value was 27%). 5′-GMP at 100 mg/kg alone had no effect on the tumor growth [19], but it enhanced the antitumor activity of FUra without increasing its toxicity for the host as reported previously [19]. The combination of FUra and 5′-GDP, as well as FUra and 5′-GMP, markedly inhibited the tumor growth as shown in Fig. 1 (T/C values for both were 1%). 5′-IMP and 5′-GTP moderately potentiated the antitumor activity of FUra, but 2′-GMP and 3′-GMP had no effect. By contrast, cGMP antagonized the effects of FUra (T/C value was 82%).

Uptake of [³H]FUra into tumor and tumor RNA

To investigate the mechanism of the synergistic interaction between FUra and the guanine ribonucleotides, 5′-GMP, 5′-GDP and 2′-GMP we determined the incorporation patterns of FUra 30 min and 24 h after administration of the drugs.

Total [³H]FUra incorporation into the tumor was increased by 49, 29 and 21% by the addition of 5′-GMP, 5′-GDP and 2′-GMP, respectively, 30 min after treatment (Fig. 2A), but these guanine ribonucleotides made no difference in the incorporation of [³H]FUra into the tumor at 24 h. The incorporation of [³H]FUra into the tumor RNA was increased by 58, 50 and 34% by the addition of 2′-GMP, cGMP and 5′-GMP, respectively, at 30 min after drug administration (Fig. 2B). After 24 h there was no difference in the incorporation of [³H]FUra into tumor RNA for when it was given with any guanine ribonucleotide compared with FUra alone. Thus, there was a difference in FUra incorporation into RNA at 30 min, but there was no obvious relationship between FUra incorporation into RNA and antitumor activity.

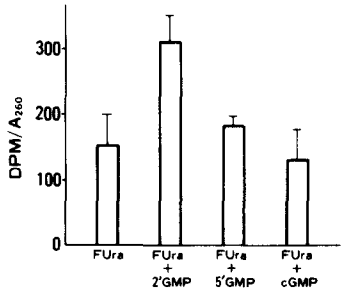

Fig. 3. Effect of guanine ribonucleotides on [³H]FUra incorporation into DNA of adenocarcinoma 755. Each value is the mean of three mice. Bars, S.E.

Incorporation of [³H]FUra into DNA of the tumor after administration of [³H]FUra alone or in combination with guanine ribonucleotides

Recently FUra incorporation into DNA has been demonstrated [6, 7]. We therefore measured the amounts of FUra residues in the tumor DNA. Small amounts of the available [³H]FUra were incorporated into the DNA, but this was not correlated with the antitumor activity (Fig. 3).

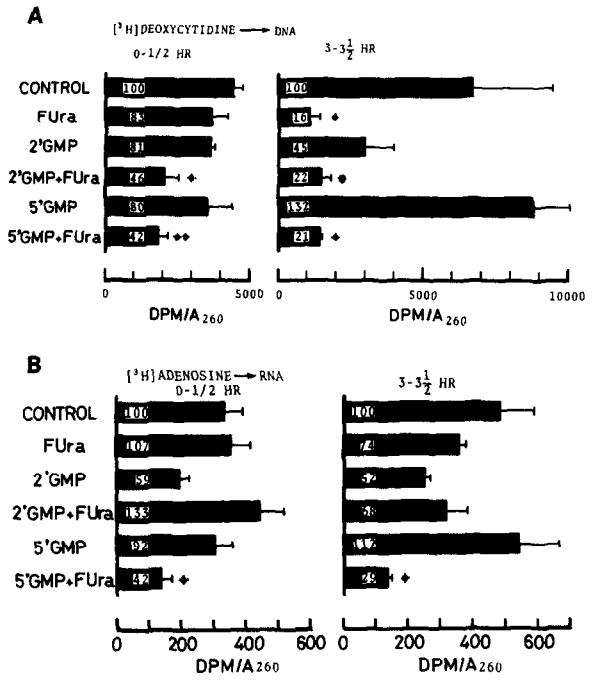

Fig. 4. Effect of FUra and guanine ribonucleotide on DNA (A) or RNA (B) synthesis in tumors. Each value is the mean of three (A) or four (B) mice. Bars, S.E.; numbers in columns, percentage of control. $^{*}P < 0.05$; $^{**}P < 0.01$.

DNA and RNA synthesis

To determine the effect of FUra on total DNA and RNA synthesis, tumors were pulse-labeled with [^{3}H]deoxycytidine and [^{3}H]adenosine, respectively. DNA synthesis was significantly inhibited by FUra alone and in combination with 2'-GMP or 5'-GMP (Fig. 4A). On the other hand, RNA synthesis was markedly inhibited only by the combination of FUra and 5'-GMP; FUra alone and in combination with 2'-GMP had little effect (Fig. 4B). Moreover, we obtained almost the same results by using [^{14}C]cytidine as a label of RNA synthesis: FUra, 0%; 2'-GMP + FUra, 0%; and 5'-GMP + FUra, 58% inhibition of RNA synthesis during the 30 min after administration.

Ribonucleotide pool

The effect of guanine ribonucleotides on the ribonucleotide pool in the tumor during the first 30 min could be an important factor in the inhibition of RNA synthesis. Changes in the nucleoside triphosphate concentrations in tumors after a 10 min exposure to FUra and/or guanine ribonucleotide derivatives are shown in Table 1. 5'-GMP was associated with a decrease in pyrimidine nucleotide pool size. The CTP and UTP levels fell to less than 60% of control values when the animals were treated with the combination of FUra and 5'-GMP. Moreover, 5'-GDP plus FUra produced a significant decrease in CTP and UTP levels, but 2'-GMP, 3'-GMP and 5'-IMP did not. No consistent change was observed in ATP and GTP pool sizes. Thus, there is a marked decrease in the concentrations of CTP and UTP after treatment with the combinations that potentiated the antitumor activity of FUra.

Effect of 5'-GMP on the antitumor activity of FCyd

Since 5'-GMP caused a decrease in CTP and pool sizes, we studied the effect of the combination of FCyd or FUrd with 5'-GMP. The results are given in Fig. 5. 5'-GMP at 300 mg/kg/day enhanced the antitumor activity of FCyd at 3 mg/kg/day, but did not enhance the antitumor activity of FUrd. A dose of 3 mg/kg/day is the maximum nontoxic dose of FCyd and FUrd in this schedule.

In addition, cytidine (100 mg/kg/day) gave considerable protection to tumor cells from the antitumor effect of FUra and 5'-GMP (Table 2).

These results suggest that a decrease in CTP and, to a lesser extent, UTP caused by 5'-GMP is very important for potentiating the antitumor effect of FUra.

DISCUSSION

Guanosine and 5'-GMP potentiate the antitumor activity of FUra against various tumors including the solid adenocarcinoma 755 [17, 19]. In the present study, various guanine ribonucleotides were tested for their capacity to enhance the antitumor activity of FUra by using mice bearing adenocarcinoma 755 when treated with the maximum effective dose of FUra. 5'-GDP and 5'-GMP markedly enhanced the antitumor activity of FUra. 5'-GTP and 5'-IMP moderately enhanced it, but 2'-GMP and 3'-GMP showed no potentiation. By contrast, cGMP diminished the antitumor activity of FUra. Light *et al.* [24] and Wood *et al.* [25] reported that the concentration of cGMP in the blood is raised in animals bearing tumors as well as in patients with malignant diseases [26]. Moreover, Dickens *et al.* [27] reported that cGMP induces TMP synthetase activity in growing cells. Therefore, cGMP may be involved in the transmission of a chemical signal for the proliferation of cells.

The activation of FUra to FUMP is via the pyrimidine phosphoribosyltransferase pathway with PRPP and/or the two-step route utilizing uridine phosphorylase (ribose-1-phosphate) and uridine kinase. Inosine is a good source of ribose-1-phosphate [10, 28] and it has been shown that when the FUra-resistant cell line of Novikoff hepatoma is treated with FUra and inosine, it is almost as responsive as the parent cell line treated with FUra alone, to both interference with rRNA maturation and inhibition of cell growth by FUra [10]. However, Kessel and Hall [16] reported that inosine does not increase the therapeutic efficacy of FUra in murine leukemias *in vivo*. Inosine is converted to

Table 1. *Nucleoside triphosphate levels in adenocarcinoma* 755

	No. of animals	nmoles/100 mg of tumor tissue UTP	CTP	GTP	ATP
Control	6	12.00 ± 1.17* (100)†	4.73 ± 0.50 (100)	6.45 ± 0.45 (100)	30.66 ± 1.84 (100)
FUra	6	8.97 ± 1.07 (75)	3.41 ± 0.31 (72)	5.56 ± 0.52 (86)	25.28 ± 3.59 (82)
2′-GMP	4	8.88 ± 0.69 (74)	3.46 ± 0.13 (73)	5.22 ± 0.35 (81)	22.86 ± 1.72‡ (75)
2′-GMP + FUra	4	11.37 ± 1.58 (95)	4.20 ± 0.63 (89)	6.56 ± 0.74 (102)	29.78 ± 3.79 (97)
3′-GMP	4	11.58 ± 0.96 (97)	4.29 ± 0.46 (91)	5.83 ± 0.55 (90)	31.71 ± 7.95 (103)
3′-GMP + FUra	4	9.31 ± 0.60 (78)	3.73 ± 0.39 (79)	5.14 ± 0.52 (80)	22.82 ± 2.72‡ (74)
5′-GMP	6	7.60 ± 1.25‡ (63)	2.65 ± 0.47‡ (56)	5.48 ± 0.54 (85)	24.60 ± 2.16 (80)
5′-GMP + FUra	4	6.30 ± 0.90§ (53)	2.24 ± 0.34§ (47)	5.08 ± 0.81 (79)	22.63 ± 3.16‡ (74)
5′-GDP	4	7.88 ± 0.59‡ (66)	2.98 ± 0.23‡ (63)	4.88 ± 0.23 (76)	20.74 ± 0.89§ (68)
5′-GDP + FUra	4	7.30 ± 0.50‡ (61)	3.17 ± 0.21‡ (67)	5.05 ± 0.14 (76)	20.14 ± 0.70§ (66)
5′-IMP	4	10.62 ± 0.23 (88)	3.90 ± 0.05 (82)	6.65 ± 0.18 (103)	32.56 ± 1.99 (106)
5′-IMP + FUra	4	8.62 ± 0.60 (72)	3.44 ± 0.26 (73)	5.28 ± 0.44 (82)	24.94 ± 1.29 (81)

*Values are means ± S.E.
†Numbers in parentheses, percentage of control.
‡Different from the control group, $P < 0.05$.
§Different from the control group, $P < 0.01$.

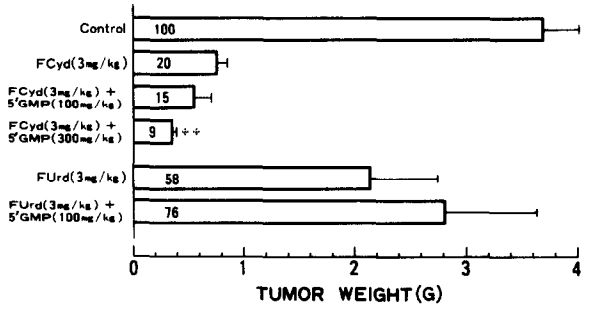

Fig. 5. *Effect of* 5′*-GMP on the antitumor activity of* 5*-fluorocytidine (FCyd) or* 5*-fluorouridine (FUrd) against adenocarcinoma* 755. *Numbers in columns, percentage of control.* **P < 0.01.

hypoxanthine. Hypoxanthine and FUra require the same cosubstrate (PRPP) for conversion to nucleotides and also share the same mechanism of transport through the plasma membrane. Thus, the combination of FUra and inosine shows an inconsistency. On the other hand, guanosine and 5′-GMP strongly potentiate the antitumor activity of FUra in various tumors *in vivo* [17, 19, 29]. 5′-GDP as well as 5′-GMP potentiated the antitumor activity of FUra in the present study. 5′-GDP and 5′-GMP increased the incorporation of FUra into RNA, but 2′-GMP also increased it, though the latter showed no effect on the antitumor activity of FUra. These compounds serve as ribose donors; therefore, it is unlikely that only the increase in incorporation of FUra into RNA accounts for the large increase in FUra efficacy.

We measured the DNA and RNA synthesis in tumors to determine the mechanism of potentiation of the antitumor activity of FUra by 5′-GMP or 5′-GDP. The combination of FUra and 5′-GMP or 2′-GMP caused marked inhibition of DNA synthesis. This inhibition may result from the inhibition of thymidylate synthetase, which provides the only *de novo* source of thymidylate for DNA synthesis. Thymidylate synthetase is known to be inactivated rapidly in the presence of the FUra metabolite, FdUMP [1, 30]. However it is apparent the antitumor activity and the effect on DNA synthesis were not correlated quantitatively. On the other hand, inhibition of RNA synthesis by the combination of FUra and 5′-GMP or 2′-GMP showed good correlation with antitumor activity. The combination of 5′-GMP and FUra caused a marked inhibition of

Table 2. Effect of cytidine on antitumor activity of FUra plus 5'-GMP

	Tumor weight (g) (mean ± S.E.)	T/C (%)
Control	3.49 ± 0.43	—
FUra (20)*	0.90 ± 0.17 ††	26
FUra (20) + 5'-GMP (100)	0.11 ± 0.03 ††	3
FUra (20) + 5'-GMP (100) + cytidine (100)	0.43 ± 0.08	12
FUra (20) + 5'-GMP (100) + cytidine (30)	0.21 ± 0.04	6

*The numbers in parentheses indicate the dose of the compound (mg/kg/day).
†$P < 0.01$.

RNA synthesis. Measurement of the incorporation of [^{3}H]adenosine or [^{14}C]cytidine into RNA showed that a significant correspondence existed between the inhibition of RNA synthesis and antitumor activity. In studies on the effect of FUra alone on total RNA in Ehrlich ascites tumor cells FUra is found to replace uracil in RNA [31, 32] but not to effect the incorporation of [^{32}P]phosphate into RNA [33]. Our data provide strong evidence that the synergistic cytocidal action of FUra plus 5'-GMP against adenocarcinoma 755 is caused by inhibition of RNA synthesis, which may be mediated through the diminished pyrimidine nucleotide pool size and increased incorporation of FUra into RNA. It is not clear how 5'-GMP effects the size of the pyrimidine nucleotide pool in tumors. Since it is unlikely that 5'-GMP can enter the cells directly, cell entry may be mediated by metabolism of the nucleotide to a nucleoside or other form prior to entering into the cells. Otherwise, 5'-GMP may exert its effect at the level of the cell membrane. There were marked decreases in the CTP and UTP pool sizes in tumors treated with the combinations that potentiated the antitumor activity of FUra. A rapid and preferential reduction of CTP synthesis, which is caused by cyclopentenyl cytidine [34], occurs concomitantly with the reduction of CTP levels in the inhibition of the transcription of rRNA, and inhibits cell growth. By contrast, an increased CTP level is associated with rapid growth of tumor cells [35, 36]. Moreover, resistance to FUra in some cell lines is correlated with an increased CTP level [37]. Thus the CTP level in a tumor may be very important in the proliferation of the cells, though the terminal product of pyrimidine ribonucleotide biosynthesis, CTP, regulates pyrimidine biosynthesis by acting as a specific inhibitor of the first step of the *de novo* pyrimidine pathway [38] as well as the salvage pathway [39]. In addition, the fact that cytidine reversed the antitumor activity of FUra and that 5'-GMP potentiated the antitumor activity of FCyd suggest a decreased CTP level, and a low level of the CTP pool and an increased incorporation of FUra into RNA produced marked inhibition of RNA synthesis after treatment with the combination of FUra and 5'-GMP, thus enhancing the antitumor activity of FUra.

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REFERENCES

1. Heidelberger C. Fluorinated pyrimidines. *Prog Nucleic Acid Res Mol Biol* 1965, **4**, 2–50.
2. Glazer RI, Hartman K. The effect of 5-fluorouracil on the synthesis and methylation of low molecular weight nuclear RNA in L1210 cells. *Mol Pharmacol* 1980, **17**, 245–249.
3. Glazer RI, Legraverend M. The effect of 5-fluorouridine 5'-triphosphate on RNA transcribed in isolated nuclei *in vitro*. *Mol Pharmacol* 1980, **17**, 279–282.
4. Tseng W, Medina D, Randerath K. Specific inhibition of transfer RNA methylation and modification of mice treated with 5-fluorouracil. *Cancer Res* 1978, **38**, 1250–1257.
5. Wilkinson D, Tlsty T, Hanas RJ. The inhibition of ribosomal RNA synthesis and maturation in Novikoff hepatoma cells by 5-fluorouridine. *Cancer Res* 1975, **35**, 3014–3020.
6. Ingraham HA, Tseng BY, Goulian M. Nucleotide levels and incorporation of 5-fluorouracil and uracil into DNA of cells treated with 5-fluorodeoxyuridine. *Mol Pharmacol* 1981, **21**, 211–216.
7. Major P, Egan EM, Herrick D, Kufe D. 5-Fluorouracil incorporation in DNA of human breast carcinoma cells. *Cancer Res* 1982, **42**, 3005–3009.
8. Herrick D, Major P, Kufe D. Effect of methotrexate on incorporation and excision of 5-fluorouracil residues in human breast carcinoma DNA. *Cancer Res* 1982, **42**, 5015–5017.
9. Kufe D, Scott P, Fram R, Major D. Biological effect of 5-fluoro-2'-deoxyuridine incorporation in L1210 deoxyribonucleic acid. *Biochem Pharmacol* 1983, **32**, 1337–1340.
10. Cory JG, Breland JC, Carter GL. Effect of 5-fluorouracil on RNA metabolism in Novikoff hepatoma cells. *Cancer Res* 1979, **39**, 4905–4913.

11. Gotto AM, Belkhode ML, Touster O. Stimulatory effects of inosine and deoxyinosine on the incorporation of uracil-2-^{14}C, 5-fluorouracil-2-^{14}C, and 5-bromouracil-2-^{14}C into nucleic acids by Ehrlich ascites tumor cells *in vitro*. *Cancer Res* 1969, **29**, 807–811.
12. Hakala MT, Rustum YM. The potential value of *in vitro* screening. *Methods Cancer Res* 1979, **16**, 247–287.
13. Laskin JD, Evans RM, Slocum HK, Burke D, Hakala MT. Basis for natural variation in sensitivity to 5-fluorouracil in mouse and human cells in culture. *Cancer Res* 1979, **39**, 383–390.
14. Laskin JD, Hakala MT. Metabolism of 5-fluorouracil (FU) in mouse and human cells as a basis for their differing sensitivity. *Proc Am Assoc Cancer Res* 1977, **18**, 57.
15. Ullman B, Kirsch J. Metabolism of 5-fluorouracil in cultured cells. Protection from 5-fluorouracil cytotoxicity by purines. *Mol Pharmacol* 1979, **15**, 357–366.
16. Kessel D, Hall TC. Influence of ribose donors on the action of 5-fluorouracil. *Cancer Res* 1969, **29**, 1749–1754.
17. Iigo M, Ando N, Hoshi A, Kuretani K. Effect of pyrimidines, purines and their nucleosides on antitumor activity of 5-fluorouracil against L1210 leukemia. *J Pharmacobiodyn* 1982, **5**, 515–520.
18. Iigo M, Hoshi A. Influence of molar ratio on the combination effect of 5-fluorouracil with guanosine 5′-monophosphate on P388 and L1210 leukemias. *Eur J Cancer Clin Oncol* 1984, **20**, 411–415.
19. Iigo M, Nakajima Y, Kuretani K, Hoshi A. Potentiation of the chemotherapeutic effect of 5-fluorouracil by combination with guanosine 5′-monophosphate. *Gann* 1983, **74**, 291–298.
20. Iigo M, Kuretani K, Hoshi A. Relationship between antitumor effect and metabolites of 5-fluorouracil in combination treatment with 5-fluorouracil and guanosine in ascites sarcoma 180 tumor system. *Cancer Res* 1983, **43**, 5687–5694.
21. Chapekar MS, Glazer RI. Effects of human immune interferon on cell viability, (2′,5′)oligoadenylate synthesis, and polyamine-dependent protein phosphorylation in human colon carcinoma cells *in vitro*. *Cancer Res* 1984, **44**, 2144–2149.
22. Iigo M, Hoshi A. Mechanism of potentiation of antitumor activity of 5-fluorouracil against adenocarcinoma 755 by L-cysteine. *Biochem Pharmacol* 1986, **35**, 727–730.
23. Khym JX. An analytical system for rapid separation of tissue nucleotides at low pressure on conventional ion exchangers. *Clin Chem* 1975, **21**, 1245–1252.
24. Light PA, Nowak M, Albano JDM. Alterations in concentration of cAMP and cGMP in guinea pig plasma during development of a transplantable leukemia. *Biochem Soc Trans* 1979, **7**, 1035–1037.
25. Wood PJ, Pao G, Cooper A. Changes in guinea pig plasma cyclic nucleotide levels during the development of a transplantable leukemia. *Cancer* 1984, **53**, 79–82.
26. Peracchi M, Lombardi L, Maiolo AT *et al.* Plasma and urine cyclic nucleotide levels in patients with acute and chronic leukemia. *Blood* 1983, **61**, 429–434.
27. Dickens MS, Lucas-Lenard J, Rot JS. Induction of thymidylate synthetase activity in *Tetrahymena* by cyclic guanosine monophosphate. *Biochem Biophys Res Comm* 1975, **67**, 1319–1325.
28. Cory JG, Crumley J, Wilkinson DS. Evidence for role of purine nucleoside phosphorylase in sensitivity of Novikoff hepatoma cells to 5-fluorouracil. *Adv Enzyme Regul* 1977, **15**, 153–166.
29. Iigo M, Hoshi A. Effect of guanosine on antitumor activity of fluorinated pyrimidines against P388 leukemia. *Cancer Chemother Pharmacol* 1984, **13**, 86–90.
30. Danenberg PV, Langenbach RJ, Heidelberger C. Structures of reversible and irreversible complexes of thymidylate synthetase and fluorinated pyrimidine nucleotides. *Biochemistry* 1974, **13**, 926–933.
31. Danneberg PB, Montag BJ, Heidelberger C. Studies on fluorinated pyrimidines. IV. Effects on nucleic acid metabolism *in vivo*. *Cancer Res* 1958, **18**, 329–334.
32. Harbers E, Chaudhuri NK, Heidelberger C. Studies on fluorinated pyrimidines. VIII. Further biochemical and metabolic investigations. *J Biol Chem* 1959, **234**, 1255–1262.
33. Bosch L, Harbers E, Heidelberger C. Studies on fluorinated pyrimidines. V. Effects on nucleic acid metabolism *in vitro*. *Cancer Res* 1958, **18**, 335–343.
34. Glazer RI, Knode MC, Lim M-I, Marquez VE. Cyclopentenyl cytidine analogue. An inhibitor of cytidine triphosphate synthesis in human colon carcinoma cells. *Biochem Pharmacol* 1985, **34**, 2535–2539.
35. Weber G, Olah E, Jui MS, Tzeng D. Biochemical programs and enzyme-pattern-targeted chemotherapy in cancer cells. *Adv Enzyme Regul* 1978, **17**, 1–22.
36. Williams JC, Kizaki H, Weber G, Morris HP. Increased CTP synthetase activity in cancer cells. *Nature* 1978, **271**, 71–73.
37. Kaufman ER. Resistance to 5-fluorouracil associated with increased cytidine triphosphate levels in V79 Chinese hamster cells. *Cancer Res* 1984, **44**, 3371–3376.
38. Gerhart JC, Pardee AB. The enzymology of control by feedback inhibition. *J Biol Chem* 1962, **237**, 891–896.
39. Drengo A. Feedback regulation in the ribopyrimidine "salvage" pathway. *Exp Cell Res* 1966, **41**, 338–349.