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Influence of Molar Ratio on the Combination Effect of 5-Fluorouracil with Guanosine 5′-Monophosphate on P388 and L1210 Leukemias

MASAAKI IIGO* and AKIO HOSHI

Pharmacology Division, National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo 104, Japan

Abstract—*To obtain more effective treatment with the combination of 5-fluorouracil (FUra) and guanosine 5′-monophosphate (GMP), the influence of the time interval between FUra and GMP administration and of the molar ratio of GMP to FUra on the effect on P388 murine leukemia were investigated. The antitumor activity of FUra was significantly potentiated when GMP was administered either 0–60 min before or 5 min after FUra. The potentiated increase in lifespan (ILS) was almost the same as after simultaneous injection of the two agents. Coadministration of FUra and GMP increased the antitumor activity as compared with the respective dose of FUra alone in a treatment schedule of either day 1 only or days 1–9. The multiple-dose regimen (days 1–9) was more effective than a single high-dose regimen, and a GMP/FUra molar ratio of 4 seems to achieve the best therapeutic results against P388 leukemia. Daily simultaneous administration of FUra and GMP on days 1–9 also resulted in a significant increase in the antitumor activity against L1210 Leukemia as compared with FUra alone.*

INTRODUCTION

THERAPY with a combination of antitumor agents has been studied [1, 2], but only limited attention has been paid to the use of natural metabolites in combination with 5-fluorouracil (FUra). The following nucleic acid analogs in combination with FUra enhance the action of FUra: orotic acid [3], uracil [4], thymine [5], thymidine [6], deoxyuridine [7], guanosine [8] and cytidine [8]. Recently, we found that only the combination of FUra with guanosine enhanced the antitumor activity without increasing its toxicity [9], and that guanosine 5′-monophosfate (GMP), which is easily soluble in water, also potentiates the antitumor activity of FUra against subcutaneously implanted adenocarcinoma 755 and Lewis lung carcinoma by simultaneous intravenous injection [10]. In this study, to find a more effective treatment schedule for the combination of FUra and GMP, the influence of the time interval between FUra and GMP administration and of the molar ratio of GMP to FUra on the activity against P388 leukemia were investigated. Furthermore, the effect of GMP on the antitumor activity of FUra against L1210 leukemia was studied.

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*To whom all correspondence should be addressed.

MATERIALS AND METHODS

Drugs

FUra was kindly supplied by Mitsui Pharmaceuticals, Inc., Tokyo, Japan. GMP was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. FUra and GMP were dissolved in 0.9% saline solution and administered i.p. at 0.1 ml/20 g of body weight.

Animals

Groups of six specific pathogen-free male BDF_1 mice with a body weight of 21–23 g (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan) were housed in plastic cages with woodchip bedding and received CA-1 pellet diets (CLEA Japan, Inc., Tokyo, Japan) and water *ad libitum*. All experiments were performed in an animal laboratory with controlled temperature (24°C),

Implantation and treatment of P388 and L1210 leukemias

P388 (10^6) or L1210 (10^5) leukemia cells, which were maintained by weekly passage in BDF_1 mice,

were implanted i.p. into mice. All mice were weighed individually, and assigned to groups of 5–7 mice with a weight range of ≤3 g. Treatment was started on day 1. Each separate experiment included a control group of untreated mice, whose survival ranged from 11.2 ± 1.3 to 12.2 ± 1.3 days. The experiments were terminated when all mice had died or 60 days after implantation.

The average lifespan, that is, the average number of days that mice in each group survived after tumor implantation, was determined and the T/C value, the ratio of the lifespan of the experimental group to that of the control group, was calculated. The increase in lifespan (ILS), representing the percentage increase in lifespan of each group relative to that of the control, was determined from the T/C.

The statistical significance of the difference between FUra alone and in combination with GMP was determined by Student's *t* test.

RESULTS

Effect of time interval between the administration of FUra and GMP on the antitumor activity against P388 leukemia

Coadministration of FUra with GMP resulted in a significant increase in survival over that of mice treated with FUra alone [10], like the combination of FUra and guanosine [9]. Since GMP is thought to be a release form of guanosine, allowing a time interval between administration of FUra and GMP might improve the therapeutic efficacy of the combination. To determine the most effective treatment schedule, the effect of different time intervals between the administration of FUra and GMP was determined. FUra (100 mg/kg) and GMP (1000 mg/kg) were administered i.p. at definite intervals via separate syringes on day 1 only. Data on the effect of the time interval between the two drugs are summarized in Table 1. GMP was not toxic and did not show any antitumor activity at this dose. The antitumor activity of FUra against P388 leukemia was potentiated when GMP was administered at 0–60 min before ($P < 0.01$) or at 5 min after FUra ($P < 0.05$). However, these ILS percentages were approximately the same as for the two drugs injected simultaneously. No significant benefit in survival was obtained by allowing a time interval.

Antitumor activity of single administration

Coadministration of FUra and GMP resulted in a significant increase in survival time over that for mice treated with the respective doses of FUra alone. A single administration of 100 mg of FUra per kg was weakly active (29% ILS) against P388 leukemia (Table 2); simultaneous treatment with 157 mg of GMP per kg (GMP/FUra molar ratio = 0.5) enhanced the antitumor activity of FUra (48% ILS, $P < 0.01$). The survival time was increased with increasing doses of GMP until the dose reached 1565 mg/kg (GMP/FUra molar ratio = 5.0). Antitumor activity of 200 mg of FUra per kg alone was moderately high (38% ILS), and simultaneous treatment with 626 mg of GMP per kg (GMP/FUra molar ratio = 1.0) enhanced the antitumor activity of FUra (66% ILS, $P < 0.05$). However, survival time after coadministration of FUra with GMP at 1879 mg/kg (GMP/FUra molar ratio = 3.0) did not show a significant

Table 1. Effect of the time interval between the drugs on the antitumor activity of the FUra–GMP combination against P388 leukemia

Sequence of administration	Time interval (min)	Survival Mean ± S.D. (days)	ILS (%)
FUra*		13.8 ± 0.8	23
FUra + GMP*	0	17.2 ± 1.6†	54
GMP → FUra	5	18.0 ± 1.6†	61
GMP → FUra	30	16.4 ± 1.3†	46
GMP → FUra	60	17.0 ± 1.9†	52
GMP → FUra	180	15.0 ± 1.9	34
FUra → GMP	5	16.8 ± 2.6‡	50
FUra → GMP	30	14.4 ± 1.5	29
FUra → GMP	60	15.0 ± 1.6	34
FUra → GMP	180	14.6 ± 1.1	30
GMP		12.3 ± 1.0	10

*Drugs were given i.p. on day 1 to groups of 5 BDF_1 mice. Doses of FUra and GMP were 100 and 1000 mg/kg/day respectively. Mean survival time of untreated control mice was 11.2 ± 1.3 days.

†$P < 0.01$, significantly greater than FUra monotherapy.

‡$P < 0.05$, significantly greater than FUra monotherapy.

Table 2. Antitumor activity of FUra in combination with various molar ratios of GMP against P388 leukemia (day 1 only)

Dose (mg/kg)*		GMP/FUra	Survival		
FUra	GMP	molar ratio	Mean ± S.D. (days)	ILS (%)	60-day survivors
100	0	0.0	15.0 ± 0.7	29	0/5
100	157	0.5	17.2 ± 0.8†	48	0/5
100	313	1.0	17.0 ± 0.7†	47	0/5
100	626	2.0	18.4 ± 1.1‡	59	0/5
100	939	3.0	20.4 ± 3.3†	76	0/5
100	1252	4.0	20.5 ± 0.9‡	77	0/5
100	1565	5.0	20.8 ± 1.1‡	79	0/5
200	0	0.0	16.0 ± 1.7	38	0/5
200	313	0.5	16.8 ± 0.4	45	0/5
200	626	1.0	19.3 ± 1.0§	66	0/5
200	1252	2.0	18.2 ± 1.1§	57	0/5
200	1879	3.0	18.0 ± 1.4	55	0/5
0	1879	—	12.8 ± 1.3	10	0/5

*FUra and GMP were administered simultaneously i.p. via separate syringes on day 1 after i.p. implantation of 1×10^6 cells. The untreated control mice survived 11.6 ± 1.1 days.

†$P < 0.01$, significantly greater than FUra monotherapy.

‡$P < 0.001$, significantly greater than FUra monotherapy.

§$P < 0.05$, significantly greater than FUra monotherapy.

difference in comparison with FUra monotherapy ($P > 0.05$).

Antitumor activity of multiple-dose regimen

FUra was more active against P388 leukemia when administered alone in a multiple-dose regimen (days 1–9). Simultaneous administration of GMP potentiated the antitumor activity of FUra, and the survival time was increased with increasing GMP/FUra molar ratios when FUra was given at doses of 1 and 3 mg/kg; however, when the GMP/FUra molar ratio was increased to 30, the ILS remained at the maximum (Table 3). The antitumor activity resulting from co-administration of FUra at 3 mg/kg with GMP at 47 mg/kg was comparable to that of FUra alone at 10 mg/kg (123% ILS). Moreover, after co-administration of 10 mg of FUra per kg and 63–125 mg of GMP per kg for 9 consecutive days, there were some 60-day survivors and the ILS was more than 280%. The best molar ratio of GMP to FUra at 10 mg/kg in this schedule was 4. Coadministration of 20 mg of FUra per kg and more than 63 mg of GMP per kg in a multiple-dose schedule resulted in a significant decrease in survival time as compared with that after FUra monotherapy and synergism of toxicity was shown when more than the optimal dose (10 mg/kg) was given in a multiple-dose regimen.

The effect of GMP on the antitumor activity of FUra was also studied with ascites L1210 murine leukemia, with the multiple treatment schedule and 10 mg of FUra per kg (Table 4). The antitumor activity of FUra against L1210 leukemia was moderately improved by simultaneous treatment with GMP at 125 mg/kg (82 vs 48%, $P < 0.01$).

DISCUSSION

The ability of guanosine to increase the antitumor activity of FUra has been reported [8, 9], and the therapeutic ratio of the combination was significantly increased in L1210 leukemia [9]. However, guanosine shows low solubility in water and GMP, which is easily soluble in water, is selected as a good potentiator instead of guanosine [10]. GMP is thought to be a release form of guanosine; however, no significant benefit in ILS was obtained by allowing a time interval.

The potentiation of antitumor activity of FUra by GMP against ascites P388 murine leukemia was demonstrated in both single and multiple treatment schedules. A multiple-dose regimen (daily, days 1–9) was more effective than a single high-dose regimen against P388 leukemia. In particular, when GMP at 125 mg/kg was administered simultaneously with FUra at 10 mg/kg in daily treatments on days 1–9, there were a number of 60-day survivors. A multiple-dose regimen and a dose of GMP high enough to give a GMP/FUra molar ratio of 4.0 is necessary to achieve the best therapeutic effect against P388 leukemia.

Simultaneous administration of FUra with GMP also increased the antitumor activity against ascites L1210 leukemia over that of FUra alone. Enhancement by GMP was more effective against P388 leukemia than L1210 leukemia. The difference in enhancement by GMP in P388 and

Table 3. Antitumor activity of FUra in combination with various molar ratios of GMP against P388 leukemia (days 1–9)

	Dose (mg/kg)*		GMP/FUra	Survival		
	FUra	GMP	molar ratio	Mean ± S.D. (days)	ILS (%)	60-day survivors
A	1.0	0.0	0.0	16.0 ± 1.9	36	0/5
	1.0	9.4	3.0	21.8 ± 1.6†	85	0/5
	1.0	12.5	4.0	22.0 ± 2.3†	86	0/5
	1.0	15.7	5.0	22.6 ± 0.9†	92	0/5
	1.0	31.3	10.0	23.0 ± 1.4†	95	0/5
	1.0	93.9	30.0	23.4 ± 1.7†	98	0/5
	3.0	0.0	0.0	19.8 ± 1.3	68	0/5
	3.0	9.4	1.0	23.2 ± 2.2‡	97	0/5
	3.0	18.8	2.0	24.0 ± 1.9§	103	0/5
	3.0	28.2	3.0	24.8 ± 2.6§	110	0/5
	3.0	37.6	4.0	25.0 ± 1.0†	112	0/5
	3.0	47.0	5.0	26.4 ± 1.5†	124	0/5
	3.0	94.1	10.0	26.4 ± 3.1§	124	0/5
B	10	0.0	0.0	25.2 ± 4.1	123	0/5
	10	31.3	1.0	28.0 ± 2.5	148	0/5
	10	62.6	2.0	43.2 ± 16.2‡	>282‖	2/5
	10	93.9	3.0	44.8 ± 14.1‡	>296‖	2/5
	10	125.3	4.0	43.0 ± 16.2‡	>281‖	2/5
	10	156.6	5.0	33.8 ± 4.9	199	0/5
	20	0.0	0.0	22.0 ± 10.3	95	0/5
	20	62.6	1.0	12.4 ± 0.5	10	0/5
	20	125.3	2.0	13.4 ± 0.5	19	0/5
	20	187.9	3.0	12.2 ± 0.4	8	0/5
C	10	0.0	0.0	22.8 ± 0.8	87	0/7
	10	62.6	2.0	30.7 ± 7.3‡	152	0/7
	10	93.9	3.0	39.3 ± 10.9§	>222‖	1/7
	10	125.3	4.0	43.6 ± 15.7§	>257‖	3/7
	20	0.0	0.0	21.3 ± 7.8	75	0/6
	20	62.6	1.0	15.3 ± 8.3	25	0/6

*FUra and GMP were administered simultaneously i.p. via separate syringes on days 1–9. The untreated control mice in experiments A, B and C survived 11.8 ± 1.0, 11.3 ± 0.5 and 12.2 ± 1.3 days respectively.
†$P < 0.001$, significantly greater than FUra monotherapy.
‡$P < 0.05$, significantly greater than FUra monotherapy.
§$P < 0.01$, significantly greater than FUra monotherapy.
‖Calculated with survival time of 60-day survivors as 60.

Table 4. Antitumor activity of FUra in combination with GMP against L1210 leukemia

Dose (mg/kg)*		Survival	
FUra	GMP	Mean ± S.D. (days)	ILS (%)
0	0.0	8.8 ± 0.8	
10	0.0	13.0 ± 0.6	48
10	62.6	15.2 ± 1.6†	72
10	125.3	16.0 ± 1.7‡	82

*FUra and GMP were administered simultaneously i.p. via separate syringes daily on days 1–9 to groups of 6 BDF_1 mice.
†$P < 0.05$, significantly greater than FUra monotherapy.
‡$P < 0.01$, significantly greater than FUra monotherapy.

L1210 leukemia cells may be due to a difference in the activity of pyrimidine nucleoside phosphorylase or pyrimidine 5′-phosphoribosyl transferase. Nucleotide formation by FUra in L1210 leukemia cells is less than that in P388 leukemia cells [11].

It is quite likely that GMP at the high dose levels required for maximum enhancement of FUra activity acts by increasing PRPP pools with resultant enhanced rates of phosphoribosylation of FUra or increases the pools of ribose 1-phosphate with resultant enhanced anabolism of FUra by uridine phosphorylase [12].

REFERENCES

1. Corbett TH, Griswold DP Jr, Roberts BJ, Peckham JC, Schabel FM Jr. Evaluation of single agents and combinations of chemotherapeutic agents in mouse colon carcinoma. *Cancer* 1977, **40**, 2660–2680.

2. DAVIS HL JR, VON HOFF DD, ROZENCWEIG M *et al.* Gastrointestinal cancer: esophagus, stomach, small bowel, colorectum, pancreas, liver, gallbladder and extrahepatic ducts. In: STAQUET MJ, ed. *Randomized Trials in Cancer: a Critical Review by Sites.* New York, Raven Press, 1978, 147–230.
3. KANZAWA F, HOSHI A, KURETANI K. Improvement of therapeutic effect of 5-fluorouracil by orotic acid. *Journal Pharmacobiodyn* 1979, **2**, 257–259.
4. FUJII S, IKENAKA K, FUKUSHIMA M, SHIRASAKA T. Effect of uracil and its derivatives on antitumor activity of 5-fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil. *Gann* 1978, **69**, 763–772.
5. FUJII S, KITANO S, IKENAKA K *et al.* Effect of coadministration of thymine or thymidine on the antitumor activity of 1-(2-tetrahydrofuryl)-5-fluorouracil and 5-fluorouracil. *Gann* 1980, **71**, 100–106.
6. SANTELLI G, VALERIOTE F. *In vivo* enhancement of 5-fluorouracil cytotoxicity to AKR leukemia cells by thymidine in mice. *JNCI* 1978, **61**, 843–847.
7. JATO J, WINDHEUSER JJ. 5-Fluorouracil and derivatives in cancer chemotherapy III: *in vivo* enhancement of antitumor activity of 5-fluorouracil (FU) and 5-fluoro-2'-deoxyuridine (FUDR). *J Pharm Sci* 1973, **62**, 1975–1978.
8. OSSWALD H, YOUSSEF M. Potentiation of the chemotherapeutic action of 5-fluorouracil by combination with cytidine or guanosine on HRS-sarcoma. *J Cancer Res Clin Oncol* 1979, **93**, 241–244.
9. IIGO M, ANDO N, HOSHI A, KURETANI K. Effect of pyrimidines, purines and their nucleosides on antitumor activity of 5-fluorouracil against L-1210 leukemia. *J Pharmacobiodyn* 1982, **5**, 515–520.
10. 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.
11. KESSEL D, HALL TC. Nucleotide formation as a determinant of 5-fluorouracil response in mouse leukemias. *Science* 1966, **154**, 911–913.
12. TAMEMASA O, TEZUKA M. Additive formation of antineoplastic 5-fluorouracil nucleosides from 5-fluorouracil by Ehrlich ascites tumor extracts in the presence of ribose 1-phosphate/uridine or deoxyribose 1-phosphate/deoxyuridine. *J Pharmacobiodyn* 1982, **5**, 720–726.