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Elevation of plasma levels of fluorinated pyrimidines by guanosine 5'-monophosphate*

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Summary. The plasma concentration of 5-fluorouracil (FUra) following the i.v. administration of FUra and guanosine 5'-monophosphate (GMP) or guanosine 5'-triphosphate (GTP) was markedly elevated. These values were more than 5-fold higher than those obtained with FUra alone over 60 min after administration. The elevation of plasma levels corresponded to the dose of GMP. Higher levels of FUra were maintained in the plasma after injection of inosine or inosine 5'-monophosphate in combination with FUra than after FUra alone, but they were lower than those induced by GMP or GTP.

Moreover, plasma levels of two other fluorinated pyrimidines, 5'-deoxy-5-fluorouridine (DFUR) and 5-fluoro--2'-deoxycytidine (FdCyd), were also elevated by GMP. The combination of DFUR and GMP resulted in higher plasma levels of DFUR itself and FUra (12- and 10-fold, respectively, 30 min after treatment). After administration of FdCyd plus GMP, the plasma levels of FdCyd, 5-fluoro-2'-deoxyuridine, which is converted from FdCyd by cytidine deaminase, and FUra were 2-, 6-, and 7-fold higher, respectively, than those after FdCyd alone 30 min after treatment. Thus, GMP is the most effective compound for the maintenance of high plasma levels of fluorinated pyrimidines.

Introduction

5-Fluorouracil (FUra), which has been used clinically for the treatment of gastrointestinal cancer, is known to degrade rapidly into inactive metabolites [2]. To improve the chemotherapeutic effect of FUra, its use in combination with various nucleosides and pyrimidine bases has been studied [3–6, 8, 15]; after our own studies we reported that guanosine and guanosine 5'-monophosphate (GMP) potentiated the antitumor activity of FUra in solid and ascites tumor systems [6, 10]. The mechanism of potentiation of the antitumor activity of FUra by guanosine or GMP is considered to be increased incorporation of FUra into FUra nucleotides and RNA in the tumor cells [9]. Furthermore, we now show that GMP can elevate the plasma levels of FUra and its derivatives if given by i.v. injection with the fluorinated pyrimidines. We anticipate that the elevated plasma levels of fluorinated pyrimidines following administration with GMP will cause enhanced tumor levels of the drugs and potentiate their antitumor activity.

Materials and methods

Drugs. FUra, 5-fluoro-2'-deoxyuridine (FdUrd), GMP, 2'-GMP, 3'-GMP, inosine, inosine 5'-monophosphate and 3',5'-cyclic GMP were obtained from Sigma Chemical Co. (St. Louis, Mo). 5-Fluoro-2'-deoxycytidine (FdCyd) was obtained from Calbiochem-Behring (La Jolla, Calif). Guanosine 5'-triphosphate (GTP) was purchased from Yamasa Biochemicals for Research (Choshi, Japan). 5'-Deoxy-5-fluorouridine (DFUR) was kindly supplied by Nippon Roche (Tokyo, Japan). These drugs were dissolved in 0.9% saline solution and administered i.v.

Animals. Male BDF_1 mice with body weight 20–23 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were housed in plastic cages with wood-chip bedding and received CA-1 food pellets (CLEA Japan, Inc., Tokyo, Japan). All experiments were performed in an animal laboratory with controlled temperature (25° C).

HPLC assay of fluorinated pyrimidine concentrations in plasma. Groups of two to six mice were used. FUra at 20 mg/kg (maximum nontoxic dose for four i.v. injections [7]), DFUR (100 mg/kg), and FdCyd (100 mg/kg) were administered i.v. Blood samples were collected from the descending vena cava under light anesthesia with ether at specified time intervals after administration of the drugs. Plasma samples (0.3–0.5 ml) were adjusted with distilled water to a total volume of 1 ml, and 0.1 ml 0.5 *M* NaH_2PO_4 buffer and 8 ml ethyl acetate were added as recommended by Wu et al. [16]. After extraction and centrifugation the organic layer was collected and dried in vacuo at 37° C. The residue was dissolved with 10 m*M* sodium acetate buffer (pH 4.0) in the same volume of plasma, and 30 µl was injected on the HPLC column, for which a Hibar prepacked column, LiChrosorb RP-18 (5 µm; Cica-Merck, Tokyo, Japan) was used. FUra, FdUrd, and FdCyd were separated by using 2% methanol-sodium acetate buffer (10 m*M*, pH 4.0). The retention times of FUra, FdCyd, and FdUrd were 7, 17, and 21 min, respectively. For determination of DFUR, separation by means of 2% methanol

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– sodium acetate buffer (10 min) followed by a linear gradient from 2% to 15% methanol – sodium acetate buffer (15 min). The retention time of DFUR was 20 min.

Results

When FUra was administered i.v. to BDF_1 mice at 20 mg/kg, it rapidly disappeared from the plasma. However, the FUra levels in the plasma after administration of FUra (20 mg/kg) and GMP (100 mg/kg) were more than five times higher than those after FUra alone (Fig. 1). The elevation of FUra in the plasma corresponded to the dose of GMP. The dose of GMP required to potentiate the antitumor activity of FUra was 30 mg/kg or more. GMP at 300 mg/kg induced a markedly high level of FUra in plasma, nine times higher than after FUra alone (Fig. 2). GTP showed almost the same effect as GMP, but 2'-GMP and 3'-GMP were only moderately effective (Table 1).

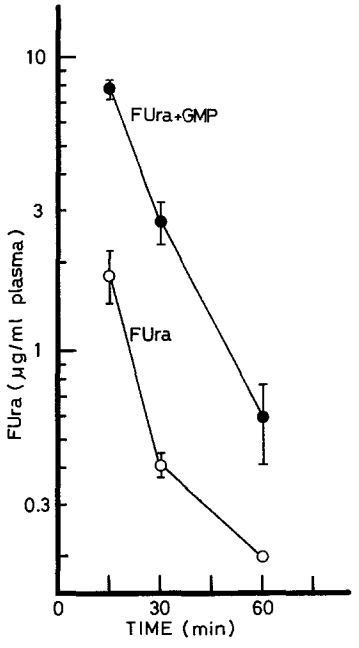

Fig. 1. Plasma concentration-vs-time curves of FUra in BDF_1 mice after i.v. administration of FUra (20 mg/kg) and GMP (100 mg/kg). Means ± SE of 3–6 mice

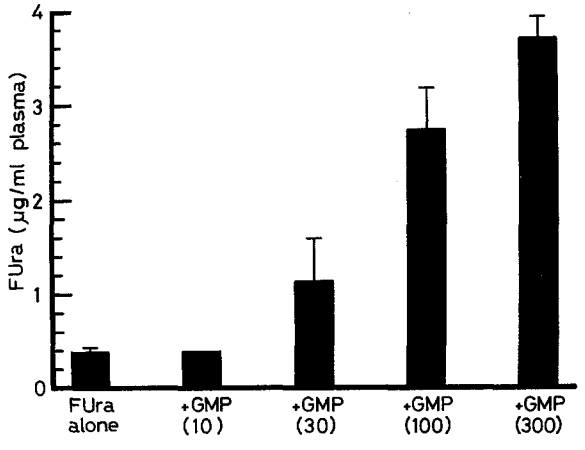

Fig. 2. Plasma concentration of FUra 30 min after i.v. administration of FUra (20 mg/kg) and various doses of GMP. Means ± SE of 2–6 mice

Table 1. Plasma concentrations of FUra in BDF_1 mice 30 min after i.v. administration of FUra (20 mg/kg) and GMP or its related compounds (100 mg/kg)

Treatment	Plasma concentration (μg/ml)[a] of FUra (no. of animals)	%
FUra alone	0.41 ± 0.04 (5)	100
FUra + GMP	2.75 ± 0.44 (6)	671
FUra + GTP	2.55 ± 0.31 (4)	622
FUra + 3'-GMP	1.48 ± 0.32 (2)	361
FUra + 2'-GMP	1.02 ± 0.21 (2)	249
FUra + 3', 5'-cyclic GMP	0.35 ± 0.10 (2)	85
FUra + inosine 5'-monophosphate	1.45 ± 0.05 (2)	354
FUra + inosine	1.56 ± 0.16 (2)	380

[a] Means ± SE

3',5'-Cyclic GMP did not affect the plasma level of FUra. Inosine and inosine 5'-monophosphate (IMP) also enhanced FUra levels in the plasma, but less markedly than did GMP. Thus, GMP and GTP are the most effective compounds for maintaining high plasma FUra levels.

DFUR is a prodrug of FUra, and it shows a better therapeutic index in several animal tumors, with less toxicity to the host, than FUra [12]. GMP also potentiates the antitumor activity of DFUR, just as it potentiates the activity of FUra [11]. When DFUR was administered i.v. to BDF_1 mice at 100 mg/kg, the plasma DFUR level rapidly decreased and the level of FUra derived from DFUR was very low in the plasma. On the other hand, when DFUR was administered in combination with GMP (300 mg/kg), high levels of DFUR (5- to 17-fold) and FUra (4- to 10-fold) derived from DFUR were detected during the first 60 min after treatment (Fig. 3).

Another fluorinated pyrimidine, FdCyd, is more active than FdUrd against certain experimental tumors [1, 14].

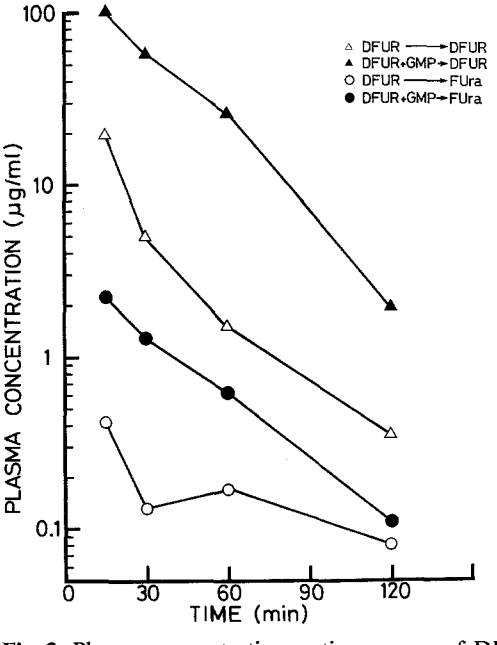

Fig. 3. Plasma concentration-vs-time curves of DFUR and FUra after i.v. administration of DFUR (100 mg/kg) and GMP (300 mg/kg). Means of 2 mice

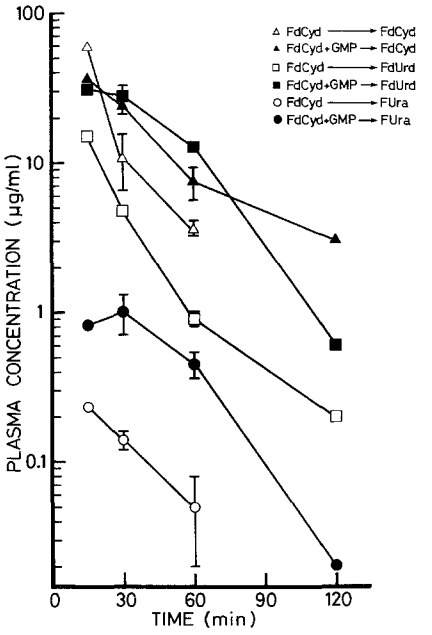

Fig. 4. Plasma concentration-vs-time curves of FdCyd, FdUrd, and FUra after i.v. administration of FdCyd (100 mg/kg) and GMP (300 mg/kg). Means ± SE of 2–4 mice

The effects of FdCyd might be related to the incorporation of FdCyd residues into DNA, and it has a different mechanism from that of FdUrd [13, 17]. The antitumor activity of FdUrd is also potentiated by the addition of guanosine [6]. However, we do not know whether the antitumor activity of FdCyd is potentiated by GMP. Therefore, we measured FdCyd, FdUrd, and FUra levels in plasma after treatment with FdCyd (100 mg/kg) and GMP (300 mg/kg). The plasma level of FdCyd after treatment with the combination of FdCyd and GMP was only twice that after FdCyd alone (Fig. 4). FdCyd has been shown to undergo deamination to FdUrd. In our experiment, large amounts of FdUrd were produced from FdCyd. The plasma levels of FdUrd after injection of the combination of FdCyd and GMP were markedly higher than those after FdCyd alone (2- to 14-fold). The plasma FUra levels after the combination of FdCyd and GMP were also markedly higher than after FdCyd alone (4- to 7-fold).

Discussion

Plasma FUra levels after the injection of FUra in combination with GMP at 100 mg/kg were more than 5-fold higher than those after FUra alone. Higher doses of GMP caused higher levels of FUra in plasma. Inosine and inosine 5'-monophosphate also elevated plasma FUra levels, but less so than GMP. FdUrd and DFUR were also maintained at markedly high levels when the drugs were administered with GMP, but FdCyd was minimally maintained even when given with GMP. In the case of FdUrd plus GMP, FdUrd and FUra in the plasma were also maintained at high levels (data not shown). These results suggest that fluorinated cytidine and fluorinated uridine may differ in the mechanism by which GMP maintains the plasma levels of fluoropyrimidines.

FUra in the combination with GMP markedly potentiates antitumor activity, but barely increases the host toxicity [10]. The antitumor activity of DFUR [11] and FdUrd were also markedly potentiated by GMP. These fluorinated pyrimidines were maintained at high levels in plasma when given in combination with GMP. Therefore, the combination of FdCyd and GMP will also potentiate antitumor activity and may have better antitumor activity than the combination of GMP with other fluoropyrimidines, because not only was FdCyd maintained at a high level in the plasma; FdUrd and FUra, which were derived from FdCyd, were also maintained at markedly higher levels. Now we need to pursue the mechanisms by which plasma levels of fluorinated pyrimidines are elevated by GMP and their toxicity to the host is not increased.

In conclusion, all fluorinated pyrimidines tested (FUra, FdUrd, DFUR and FdCyd) were maintained at higher concentrations in plasma when administered with GMP. Therefore, GMP may potentiate the antitumor activity of all fluorinated pyrimidines.

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