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Potentiation of the chemotherapeutic action of 5'-deoxy-5-fluorouridine in combination with guanosine and related compounds

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Summary. The effect of inosine, guanosine, and guanosine 5'-monophosphate (GMP) on the antitumor activity of 5'-deoxy-5-fluorouridine (5'-DFUR) was investigated using P388 leukemia and P815 mastocytoma.

The antitumor activity of 5'-DFUR was markedly enhanced by coadministration of inosine or guanosine. The increase in lifespan (ILS) of mice treated with 5'-DFUR was augmented by the combination with guanosine or inosine in a dose-dependent fashion, and the maximum ILS was about 160% with the combination, while that in the case of 5'-DFUR alone was only 48% in the P388 leukemia system. The therapeutic ratio (dose at ILS_{max}/dose at ILS_{30}) of the combination with guanosine or inosine was 333 and 136, respectively, whereas that of 5'-DFUR alone was 3.6. GMP also markedly potentiated the antitumor activity of 5'-DFUR in both P388 leukemia and P815 mastocytoma systems, just as it potentiated the activity of 5-fluorouracil in the latter system.

The uric acid level in the serum was elevated after IP injection of guanosine or inosine but the value was much lower in the case of guanosine than in inosine.

Introduction

5-Fluorouracil (5-FU) is widely used in the palliative treatment of solid tumors, but its severe host toxicity presents a major difficulty. Therefore, extensive research efforts have been directed toward the development of a 5-FU prodrug, and some 5-FU derivatives with less toxicity than 5-FU have been found [1, 9, 13, 16, 22]. Among them, 5'-deoxy--5-fluorouridine (5'-DFUR), synthesized by Cook et al. [7], has a better therapeutic index in several animal tumors [16, 27] and is less immunosuppresive [23] than 5-FU.

Recently, we found that guanosine [10, 14] or guanosine 5'-monophosphate (GMP) [12, 15] markedly enhances the antitumor activity of 5-FU without increasing toxicity to the host in various murine tumor systems, including solid tumors. This potentiation by guanosine may be due to an increase in 5-FU nucleotides induced by both pyrimidine phosphorylase and uridine-cytidine kinase with increased ribose-l-phosphate derived from guanosine [8, 14, 17].

5'-DFUR is also metabolized to 5-FU by pyrimidine phosphorylase [3, 6, 16], and 5-FU was found to accumulate more selectively in tumor tissue when 5'-DFUR was administered than when 5-FU was given to mice and rats [25]. Moreover, the activity of pyrimidine nucleoside phosphorylase is higher in tumor tissues than in normal tissues [21]. Therefore, we assumed that guanosine in combination with 5'-DFUR would produce marked potentiation of the antitumor effect of 5'-DFUR alone. In this study, the effect of guanosine and GMP on the antitumor activity of 5'-DFUR was determined in the P388 leukemia and P815 mastocytoma systems.

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Materials and methods

Drugs. 5'-DFUR was kindly supplied by Hoffman-La Roche (Tokyo, Japan). Guanosine, inosine and GMP were obtained from Sigma Chemical Co. (St. Louis, Mo). Guanosine was suspended homogeneously in a 0.5% solution of carboxymethyl cellulose in physiological saline, other compounds were dissolved in 0.9% saline solution, and the drugs were administered intraperitoneally (IP).

Animals. Groups of five or six male BDF_1 or CDF_1 mice with body weight of 21–23 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were housed in plastic cages with wood chip bedding and received a CA-1 pellet diet (CLEA Japan, Inc., Tokyo, Japan) with water ad libitum. All experiments were performed in an animal laboratory with controlled temperature (25 °C).

Implantation and treatment of P388 leukemia and P815 Mastocytoma. P388 leukemia (1×10^6 cells/mouse) and P815 mastocytoma (2×10^6 cells/mouse) were implanted IP on day 0. The tumors had been maintained by weekly IP transfer in syngeneic mice in the laboratory of the National Cancer Center Research Institute, Tokyo. Beginning 24 h after implantation, the mice were given consecutive daily IP treatments for 5 days. The survival times of the treated and untreated mice were determined.

Evaluation of antitumor effect. Survival times of the treated aminals were compared with those of untreated controls in both the tumor systems, and the increase in lifespan (ILS) was calculated.

Measurement of uric acid in serum. Male BDF_1 mice were used in groups of four. Blood samples were collected by heart puncture under light ether anesthesia at specified

time intervals after IP injection of guanosine or inosine at 1000 mg/kg, and the individual sera were obtained by centrifugation (Eppendorf Gerätebau, Hamburg, FRG). The serum was assayed for uric acid with the Uric Acid B-Test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan).

Data were analyzed for significance by means of the two-tailed Student's *t*-test.

Results

P388 leukemia mice were treated IP with various doses of 5'-DFUR plus guanosine or inosine (100 mg/kg/day). The addition of guanosine or inosine markedly enhanced the antitumor activity compared with of 5'-DFUR alone. The survival time of animals treated with 5'-DFUR alone was slightly increased until the dose reached 300 mg/kg, and in those treated with 5'-DFUR in combination with guanosine or inosine it was markedly increased until dose of 5'-DFUR reached 300 mg/kg/day. The therapeutic effect of the combination of guanosine (100 mg/kg/day) or inosine (100 mg/kg/day) and 5'-DFUR at 3–300 mg/kg/day or 10–300 mg/kg/day respectively was higher than the maximum effect of 5'-DFUR monotherapy, which was attained at 300 mg/kg/day (Fig. 1). The maximum ILS with the combination of 5'-DFUR and guanosine or inosine was 164% or 155% respectively, but that with 5'-DFUR alone was 48%. The minimum effective dose of 5'-DFUR at which the ILS was 30% was 83 mg/kg/day in the case of 5'-DFUR alone, 2.2 mg/kg/day for 5'-DFUR + inosine, and 0.9 mg/kg/day for 5'-DFUR + guanosine. The maximum effective doses were the same (300 mg/kg/day). Therefore, the therapeutic ratios (dose at $ILS_{maximum}$/dose at $ILS_{30\%}$) of 5'-DFUR, 5'-DFUR + inosine, and 5'-DFUR + guanosine were 3.6, 136, and 333 respectively.

The influence of the dose of guanosine or inosine on the therapeutic activity in combination with 5'-DFUR (100 mg/kg/day) is shown in Fig. 2. The ILS was enhanced with increasing doses of guanosine or inosine, and 5'-DFUR + guanosine was somewhat better than 5'-DFUR + inosine. The maximum ILS with added guanosine or inosine was 148% and 120% respectively. A high dose (1000 mg/kg/day) of guanosine alone showed slight activity, but inosine at 1000 mg/kg/day had no antitumor activity at all. High doses of these compounds had no toxicity for the host.

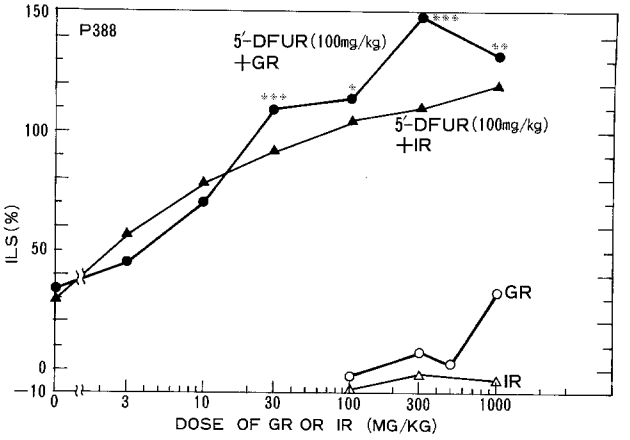

Fig. 2. Antitumor activity of 5'-DFUR (100 mg/kg/day) in combination with various doses of guanosine *(GR)* or inosine *(IR)* against P388 leukemia. The drugs were given IP for 5 consecutive days to groups of six BDF_1 mice. The mean survival times (±SD) of the untreated control mice were 10.3±1.2 and 10.0±0.9 days, respectively. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to 5'-DFUR + IR

GMP also potentiated the antitumor activity of 5'-DFUR in the P388 leukemia (Fig. 3) and P815 mastocytoma (Fig. 4B) systems, just as it potentiated the activity of 5-FU in the latter system (Fig. 4A).

Administration of guanosine or inosine had a marked effect on serum uric acid levels. Statistically significant increases in serum uric acid levels occurred at 30–60 and 15–60 min after IP injection of guanosine and inosine respectively at 1000 mg/kg. The maximum concentration of uric acid after injection of guanosine was 5.3 mg/100 ml at 60 min after administration, and that after inosine injection was 13.7 mg/100 ml at 15 min after administration. These values were respectively 2.8 and 7.2 times higher than the normal value of 1.9 mg/100 ml (Fig. 5).

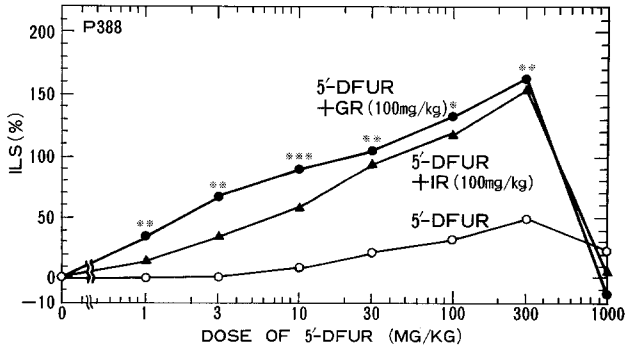

Fig. 1. Effect of guanosine *(GR)* and inosine *(IR)* on the antitumor activity of 5'-DFUR against P388 leukemia. The drugs were given IP for 5 consecutive days to groups of five BDF_1 mice. The mean survival time (±SD) of the untreated control mice was 10.3±1.2 days. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to 5'-DFUR + IR

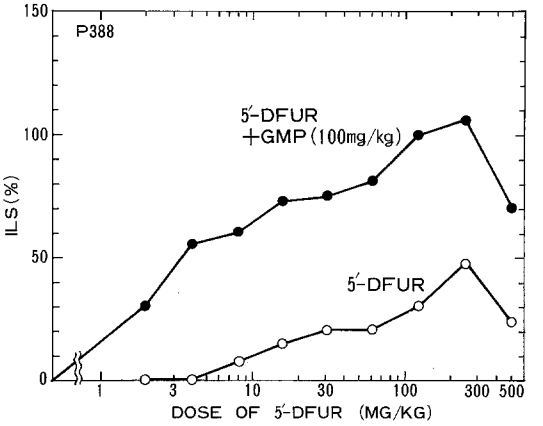

Fig. 3. Effect of guanosine 5'-monophosphate *(GMP)* on the antitumor activity of 5'-DFUR against P388 leukemia. 5'-DFUR and GMP (100 mg/kg/day) were given IP for 5 consecutive days to groups of six BDF_1 mice. The mean survival time (± SD) of the untreated control mice was 10.7±0.8 days

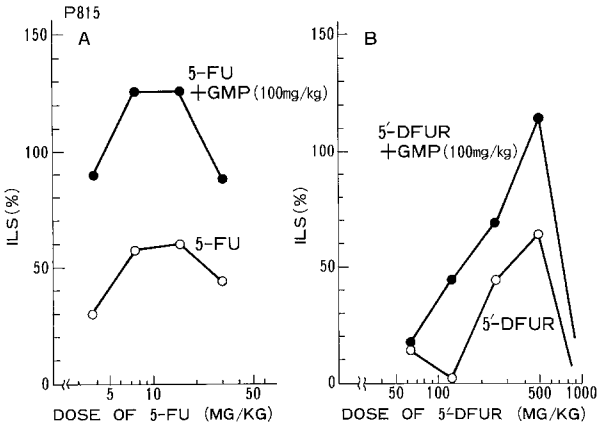

Fig. 4A, B. Effect of guanosine 5'-monophosphate (GMP) on the antitumor activity of 5-FU **(A)** and 5'-DFUR **(B)** against P815 mastocytoma. The drugs were given IP for 5 consecutive days to groups of six CDF_1 mice. The survival time (± SD) of the untreated control mice was 11.7 ± 3.4 days

Discussion

The new fluorinated pyrimidine, 5'-DFUR, which is activated by nucleoside phosphorylase, has a differential selectivity different from that of 5-FU; the basis for this is initial phosphorolysis to 5-FU [5]. Since 5'-DFUR itself is not directly cytotoxic, its superior therapeutic index compared to other fluoropyrimidines [16] may largely reflect the selective activation of 5'-DFUR by sensitive tumor cells rather than by bone marrow cells, which can activate 5-FU, 5-fluorouridine, and 5-fluoro-2'-deoxyuridine [4], or liver cells. Recently we found that guanosine [10, 14] or GMP [12, 15] markedly enhances the antitumor activity of 5-FU without increasing toxicity to the host in various murine tumor systems, including solid tumors. Tezuka and Tamemasa also reported the potentiation by various purine nucleosides and nucleotides, including guanosine and guanosine 5'-triphosphate, of the antitumor activity of 5-FU against Ehrlich ascites carcinoma [26]. Guanosine is easily degraded by purine nucleoside phosphorylase into guanine and ribose l-phosphate. Therefore, the addition of guanosine may well enrich the ribose l-phosphate pools in the cells, resulting in the enhanced production of 5-fluorouridine 5'-monophosphate by nucleoside phosphorylase and kinase, and accordingly in increase in total intracellular 5-FU accumulation as nucleotides and incorporation into RNA [8]. The incorporation of the fluorinated pyrimidine into RNA disrupts the process of RNA maturation [2, 20] and then has cytotoxic effects [18, 19]. This effect of 5-FU + guanosine is maintained even in the presence of excess thymidine, which prevents the effect of 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) on DNA synthesis [11].

Rustum et al. [24] showed that in some tumor cell lines the initial levels and the retention of intracellular FdUMP pools might be more important than the incorporation of FUTP into RNA for the antitumor activity revealed by 5-FU. In contrast, our present study suggests that in P388 leukemia the incorporation into RNA may be more critical for therapeutic response than the FdUMP pools, because the potentiation of the antitumor activity of 5-FU + guanosine against P388 was not reversed by thymidine. According to Maehara et al., the activity of nucleoside phosphorylase is generally higher in tumor tissues than in normal tissues as far as they tested [21], and DFUR also is metabolized to 5-FU by this enzyme. Therefore, DFUR + guanosine may give better results than DFUR alone. In fact, the combination of DFUR with guanosine or GMP markedly enhanced the antitumor activity.

Serum uric acid level was rapidly elevated, reaching 13.7 mg/100 ml by 15 min after injection of 1000 mg inosine/kg, while, in the case of guanosine, it was gradually increased to attain the maximum of 5.3 mg/100 ml by 60 min after the injection. These data indicate that guanosine is degraded more slowly than inosine; therefore, the combination of 5'-DFUR with guanosine gave a better therapeutic ratio than that with inosine. The lesser increase in serum uric acid seen after the administration of guanosine may be more favorable for the prevention of gout than the greater increase with inosine. The marked enhancement of antitumor activity shown by 5'-DFUR in combination with guanosine or its monophosphate, unaccompanied by increased toxicity, lead us to be optimistic concerning the clinical usefulness of this therapy.

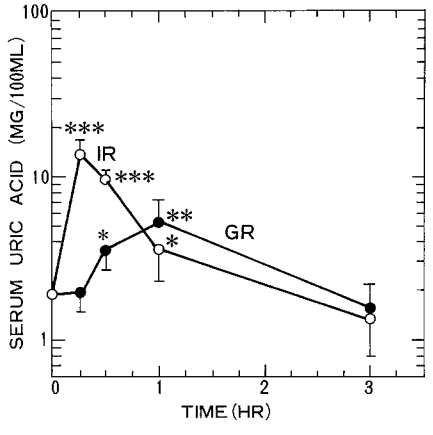

Fig. 5. Changes in uric acid serum levels with time after IP injection of guanosine *(GR)* or inosine *(IR)* at 1000 mg/kg. The data points represent the means of values obtained from four mice. The *bars* indicate SD. The *asterisks* indicate significant differences in the serum level of uric acid between the treated and the nontreated groups: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

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