ABSTRACT

The antitumor activity of 5-fluorouracil (FUra) against ascites Sarcoma 180 was significantly enhanced by coadministration of guanosine, and slightly by adenosine, but not by cytidine or uridine. In advanced ascites Sarcoma 180, guanosine also enhanced the action of FUra, but adenosine, uridine, and cytidine did not. The potentiation of antitumor activity by guanosine was reversed by addition of cytidine. The antitumor activity of FUra was significantly potentiated when guanosine was administered either 0 to 15 min before or 5 min after FUra.

Changes in metabolites of FUra after potentiation by guanosine were investigated. Total radioactivity in the plasma was significantly decreased 10 min after the combined administration of [6-14C]FUra (3 mg/kg i.p.) and guanosine (100 mg/kg i.p.) in comparison with that of [6-14C]FUra alone and was slightly decreased by coadministration of [6-14C]FUra and adenosine. Conversely, it was significantly increased by uridine or cytidine. The decrease in total radioactivity in the plasma caused by guanosine was completely reversed by addition of cytidine. FUra, 5-fluorouridine, α-fluoro-β-ureidopropionic acid, and α-fluoro-β-alanine were found in the plasma. Intact FUra accounted for about 55% of the total radioactivity. The proportion of metabolites of [6-14C]FUra was not changed by coadministration of [6-14C]FUra and guanosine, adenosine, or cytidine, but the proportion of FUrd was increased by uridine.

In the ascitic fluid, the total radioactivity derived from [6-14C]FUra was decreased by its combined administration with guanosine, and it was reversed by addition of cytidine. This pattern was similar to that in the plasma. The main FUra compound was intact FUra itself (90%), and 5-fluorouridine accounted for 1% of the total radioactivity in the ascitic fluid.

On the other hand, total radioactivity of [6-14C]FUra in the tumor cells was significantly and slightly increased by guanosine and adenosine, respectively. Total radioactivity after [6-14C]FUra in combination with uridine or cytidine was less than that after [6-14C]FUra alone. Incorporation of [6-14C]FUra into RNA was increased about 3.7 times by its combination with guanosine in comparison with FUra alone, and it was increased 2.0, 0.6, and 0.7 times by adenosine, uridine, and cytidine, respectively. Moreover, FUra-nucleotides were significantly increased by guanosine. The increased radioactivity in RNA and FUra-nucleotides of tumor cells caused by guanosine was completely reversed by cytidine. These changes in incorporation into tumor cells were comparable to those in antitumor activity against ascites Sarcoma 180. The potentiation of antitumor activity of FUra by guanosine was considered to be due to an increase in incorporation of FUra into FUra-nucleotides and RNA in the tumor cells.

INTRODUCTION

FUra has been used widely in the treatment of various tumors, particularly carcinoma of the stomach, colon, pancreas, breast, and ovary (8, 9, 36, 50). However, the rate of response to treatment is 10 to 30% with FUra, and the duration of remission is generally short (1, 9, 20, 37). In order to enhance the antitumor activity of FUra, many combinations with FUra have been examined (12, 14–16, 24, 28, 39, 42). Recently, we found that the antitumor activity of FUra is markedly potentiated by guanosine in comparison with FUra monotherapy for L1210 leukemia without increasing the toxicity to the host (24). The FUra-guanosine combination produces the optimal chemotherapeutic effect of FUra only by simultaneous injection (24). Moreover, the combination of FUra and GMP produces marked antitumor effects in the P388 and L1210 leukemia, ascites Sarcoma 180, and Ehrlich ascites carcino ma systems (26), as does the combination of FUra and guanosine. This combination also produces marked antitumor effects in solid tumor systems when given by i.v. injection (26). Thus, the therapeutic effect of FUra on various murine tumors is markedly potentiated by guanosine or GMP. In our study reported previously (24), guanosine was a greater potentiator of antitumor activity of FUra than was either adenosine or inosine. However, the potentiation by guanosine (24) or GMP was prevented by addition of cytidine or uridine.

The purpose of the present study is to clarify the pharmacological basis of the potentiation of the antitumor activity of FUra by guanosine, especially with regard to metabolites in the plasma, ascitic fluid, and tumor cells.

MATERIALS AND METHODS

Chemicals. [6-14C]FUra (55 mCi/mmol) and ACS-II liquid scintillation counting cocktail were purchased from The Radiochemical Centre, Amersham, Buckinghamshire, England. FUra, FUrd, FdUrd, FUMP, FdUMP, FBAL, and DSAL were supplied by Mitsui Pharmaceuticals, Inc., Tokyo, Japan. Calf intestine type I alkaline phosphatase, guanosine, adenosine, uridine, cytidine, and other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. [6-14C]FUra was diluted with its cold carrier, and the specific activity of the solution for injection was 83.3 μCi/mg. FUra, uridine, and cytidine were dissolved in 0.9% NaCl solution, and guanosine and adenosine were homogenized with 0.5% carboxymethyl cellulose in 0.9% NaCl solution.

3 The abbreviations used are: FUra, 5-fluorouracil; FUrd, 5-fluorouridine; FdUrd, 5-fluoro-2'-deoxyuridine; FUMP, 5-fluorouridine 5'-monophosphate; FdUMP, 5-fluoro-2'-deoxyuridine 5'-monophosphate; FBAL, α-fluoro-β-ureidopropionic acid; FBAL, α-fluoro-β-alanine; PCA, perchloric acid; FUDP, 5-fluorouridine 5'-diphosphate; FUTP, 5-fluorouridine 5'-triphosphate.
Tumor and Animals. Ascites Sarcoma 180 was used for the studies reported in this paper. The tumor was maintained i.p. in female ddY mice (Shizuoka Agricultural Cooperation Association for Laboratory Animals, Hamamatsu, Japan), and 6 mice in each group were housed in plastic cages and received CA-1 pellet diets (Clea Japan, Inc., Tokyo, Japan) and water ad libitum.

Antitumor Activity. Ascites Sarcoma 180 cells prepared from 7-day-old tumors were injected i.p. at a concentration of 1 × 10^7 cells/mouse. Twenty-four hr after implantation, the mice were given various doses of FUra or FUra plus guanosine (100 mg/kg) i.p. for 5 consecutive days. Moreover, FUra plus guanosine or other nucleosides (adenosine, uridine, and cytidine) were tested by early i.p. treatment (Days 1 to 5) and delayed i.p. treatment (Days 5 to 9). The doses of FUra and nucleosides were 3 and 100 mg/kg, respectively. Survivors were counted daily for 90 days after the first treatment. Mean survival time or cure rate relative to that of the untreated control group was determined for evaluation of drug activity.

Analysis of FUra and Its Metabolites in Plasma. Ascites Sarcoma 180 (1 × 10^7 cells/mouse) was implanted i.p. On the seventh day of tumor growth, the mice (body weight, 29 to 31 g) were given i.p. a single injection of [6-3H]FUra alone (3 mg/kg; 7.5 μCi/mouse) or in combination with guanosine, adenosine, uridine, or cytidine (100 mg/kg). The mice were killed 10 or 30 min later under ether anesthesia by exsanguination from the axillary artery and vein. Blood was aspirated into syringes that had been wet with a heparin solution. Total radioactivity in the plasma was determined after blood cells were removed by centrifugation. A sample of plasma was dissolved with 1 ml of Soluene 350 (Packard Instrument Co., La Grange, Ill.). ACS-II complete liquid scintillation cocktail was used for all radioactivity assays, and the radioactivity was determined by use of a Packard Model 3320 scintillation spectrometer. The remaining plasma was mixed with 2 volumes of concentrated HCl:methanol (1:100). Denatured proteins were removed by centrifugation, and 25 μl of supernatant were chromatographed on silica gel chromatography plates containing a fluorescent indicator (Merck and Co., Inc., Darmstadt, Federal Republic of Germany) to separate FUra and metabolites. The plates were developed with the use of a solvent system consisting of chloroform:methanol:acetic acid (30:10:5). The Rf values of the various metabolites were: FUra, 0.70; FUrđ, 0.39; FdUrd, 0.55; 5,6-dihydro-5-fluorouracil, 0.74; FUPA, 0.13; FBAL, 0.01; and FUMP, 0.00.

The plates were scanned with an Aloka thin-layer chromatogram scanner (Aloka Co., Ltd., Mitaka, Japan) to locate the zones containing radioactivity. The presence of metabolites in each radioactive zone was confirmed by autoradiography with an LKB Ultrascan, LKB-Produkter AB, Bromma, Sweden.

Analysis of FUra and Its Metabolites in Ascitic Fluid and Tumor Cells. After 10 or 30 min of isotope incorporation, the mice were killed, and the peritoneum were carefully dissected. The whole ascitic fluid (3 to 7 ml) from each animal was transferred as quickly as possible to heparinized test tubes and immediately chilled. Tumor cells in the fluid (approximately 8 × 10^7 cells) were collected by centrifugation at 500 × g for 15 min at 0°. Samples of the ascitic fluid were dissolved with Soluene 350, and radioactivity was counted by a liquid scintillation spectrometer. The remaining ascitic fluid was mixed with 2 volumes of HCl:methanol (1:100), and the resulting precipitate was removed by centrifugation. FUra and metabolites in the HCl:methanol extract of the ascitic fluid were separated by thin-layer chromatography similar to that used for the plasma.

The cell pellets were washed twice with cold 0.9% NaCl solution and homogenized in 20 ml of HCl:methanol with a Polytron homogenizer (Kinematica, Amlehnhalde, Switzerland), and a sample of this solution was used to determine total radioactivity. The insoluble residues were separated by centrifugation at 1000 × g for 20 min and reextracted with 20 ml of HCl:methanol. The supernatants were combined and dried in a vacuum at 30°. The dried supernatant was dissolved in 300 μl of methanol, and 50 μl were subjected to thin-layer chromatography pating, similar to that used for the plasma. Furthermore, the HCl:methanol-insoluble residue of the cells was extracted twice with 15 ml of 0.2 N PCA followed by centrifugation. The PCA extracts were combined and adjusted to pH 11 with KOH to precipitate potassium perchlorate. The supernatant contained FUra-nucleotides. The samples were then examined for intracellular acid-soluble fluoropyrimidine nucleotides by extended gradient elution chromatography on Dowex 1-formate, a modified method of Hurbert et al. (23); the gradient elution with 500 ml of mixer (H2O) is started with gradually increasing amounts of formic acid (8 μl up to 250 ml and later continued with a mixture of formic acid (8 μl) plus ammonium formate (1.6 μl) up to 500 ml. FUDP, FUDP-glucose, FUDP-galactose, and FUTP were not available as markers, but their identities were ascertained by their mobilities relative to UDP, UDP-glucose, UDP-galactose, and UTP, respectively.

The acid-insoluble precipitate remaining after 0.2 n PCA extraction was dissolved in 10 ml of 0.3 n KOH and incubated overnight at 37° to ensure complete hydrolysis of RNA. The KOH digest was cooled to 0°, and 0.5 ml of 0.6 n PCA was added. The precipitate was removed by centrifugation and washed twice by resuspension in 10 ml of cold 0.2 n PCA followed by centrifugation. Aliquots of the supernatant were used to determine total activity incorporated into RNA. The pellet contained the DNA fractions.

Statistical Analysis. The statistical significance of differences between means was determined by Student's t test.

RESULTS

Antitumor Activity. The effect of guanosine on the antitumor activity of FUra against ascites Sarcoma 180 was examined. The combination of FUra at 0.5 to 20 mg/kg and guanosine at 100 mg/kg showed a significantly better cure rate than did FUra alone (Chart 1), inasmuch as the combination of FUra and GMP potentiates the antitumor activity (26).

The influence of time interval between administration of FUra and guanosine is shown in Chart 2. The antitumor activity of FUra was significantly potentiated only when guanosine was administered at 0 to 15 min before or at 5 min after FUra.

Effect of pyrimidine and purine nucleosides on antitumor activity of FUra is shown in Chart 3. Four of the 6 mice were tumor free on Day 45 when given daily simultaneous treatment with FUra and guanosine. The antitumor activity of FUra was slightly enhanced by coadministration of adenosine, but not at all by cytidine or uridine. The potentiation of antitumor activity by
Antitumor activity of FUra in combination with pyrimidine or purine nucleosides against advanced ascites Sarcoma 180

Groups of ten mice each, as indicated, were given i.p. injections for 5 consecutive days beginning on the fifth day after tumor implantation. Survivors were checked daily.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>MSD±</th>
<th>ILS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUra (3 mg/kg/day)</td>
<td>17.1 ± 2.0</td>
<td>10</td>
</tr>
<tr>
<td>FUra (3 mg/kg/day) + guanosine (100 mg/kg/day)</td>
<td>21.0 ± 4.9</td>
<td>37</td>
</tr>
<tr>
<td>FUra (3 mg/kg/day) + guanosine (100 mg/kg/day) + cytidine (100 mg/kg/day)</td>
<td>16.7 ± 2.7</td>
<td>8</td>
</tr>
<tr>
<td>FUra (3 mg/kg/day) + adenosine (100 mg/kg/day)</td>
<td>15.3 ± 2.0</td>
<td>-1</td>
</tr>
<tr>
<td>FUra (3 mg/kg/day) + uridine (100 mg/kg/day)</td>
<td>15.6 ± 2.5</td>
<td>1</td>
</tr>
<tr>
<td>FUra (3 mg/kg/day) + cytidine (100 mg/kg/day)</td>
<td>17.3 ± 4.2</td>
<td>12</td>
</tr>
</tbody>
</table>

a MSD, mean survival day. MSD of the untreated control mice was 15.5 ± 2.6 days. ILS, increase in life span.
b Mean ± S.D.
c Difference between the 2 groups significant (p < 0.05) by 2-tailed Student's t test.

FUra and Its Metabolites in Plasma. Influence of guanosine on the metabolism of FUra may occur in the short period after administration. Therefore, metabolites of [6-14C]FUra 10 min after simultaneous injection of [6-14C]FUra and nucleosides were investigated. Moreover, the effect of guanosine on the incorporation of [6-14C]FUra was ascertained 30 min after administration.

Total radioactivity and the percentage of FUra and its metabolites in the plasma after [6-14C]FUra administration were determined. The total radioactivity in the plasma 10 min after administration of [6-14C]FUra plus guanosine was significantly lower than that after [6-14C]FUra alone (p < 0.05), but not after [6-14C]FUra plus adenosine (Table 2). On the other hand, the radioactivity 10 min after administration of [6-14C]FUra plus the other pyrimidines, uridine and cytidine, was significantly higher than after [6-14C]FUra alone (p < 0.01). The decrease in total radioactivity 10 min after coadministration of [6-14C]FUra and guanosine was overcome by the addition of cytidine. Moreover, total radioactivity 30 min after administration of [6-14C]FUra plus guanosine was significantly less than that after [6-14C]FUra alone (p < 0.05).

The metabolites of [6-14C]FUra in the plasma were FDUrd, FUPA, and FBAL. FdUrd was not detected (Chart 4). FUra accounted for approximately 60% of the radioactivity 10 min after the administration of [6-14C]FUra either alone or in combination with nucleosides (Table 2A). FUra accounted for approximately 1% in all experiments except in the combination of [6-14C]FUra with uridine (about 9%). The degraded metabolites FUPA and FBAL were also detected in the plasma. Thirty min after injection of [6-14C]FUra or [6-14C]FUra plus guanosine, unchanged FUra remained at 34.6 and 21.7%, respectively. The percentage of FBAL was increased after injection of the combination of [6-14C]FUra and guanosine.

Effect of Guanosine on the Metabolism of 5-FUra

Antitumor activity of FUra in combination with pyrimidine or purine nucleosides against advanced ascites Sarcoma 180 (Table 1).
Table 2

Total concentrations and percentage of FUra and its metabolites in plasma, ascitic fluid, and tumor cells after administration of [6-14C]FUra alone or in combination with a nucleoside

On Day 7 after ascites Sarcoma 180 (1 x 10⁶ cells) was implanted i.p., the mice were given a single injection of [6-14C]FUra alone (3 mg/kg; 7.5 μCi/mouse) or in combination with a nucleoside (100 mg/kg). Plasma and ascitic fluid levels and cellular uptake of [6-14C]FUra and its metabolites were analyzed as described in "Materials and Methods." Experiment 1, FUra and metabolites 10 min after each treatment; Experiment 2, FUra and metabolites 30 min after each treatment.

<table>
<thead>
<tr>
<th>Metabolites (%)</th>
<th>Treatment</th>
<th>Total FUra and its metabolites (nmol/ml or 10⁶ cells)</th>
<th>FBAL</th>
<th>FUFA</th>
<th>FUnJ</th>
<th>F Urda</th>
<th>Acid-insoluble</th>
<th>FUra (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Plasma</td>
<td>FUra</td>
<td>13.1 ± 1.0 (4)</td>
<td>23.7</td>
<td>20.5</td>
<td>0.6</td>
<td>&lt;0.1</td>
<td>0</td>
<td>55.2</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine</td>
<td>10.5 ± 1.8 (4)</td>
<td>17.5</td>
<td>12.9</td>
<td>1.3</td>
<td>&lt;0.1</td>
<td>0</td>
<td>56.1</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine + cytidine</td>
<td>15.1 ± 1.6 (3)</td>
<td>19.3</td>
<td>12.9</td>
<td>0.7</td>
<td>&lt;0.1</td>
<td>0</td>
<td>67.2</td>
</tr>
<tr>
<td></td>
<td>FUra + adenosine</td>
<td>14.3 ± 2.5 (3)</td>
<td>37.0</td>
<td>16.0</td>
<td>1.0</td>
<td>&lt;0.1</td>
<td>0</td>
<td>46.0</td>
</tr>
<tr>
<td></td>
<td>FUra + uridine</td>
<td>16.5 ± 0.5 (4)</td>
<td>14.2</td>
<td>14.9</td>
<td>8.9</td>
<td>&lt;0.1</td>
<td>0</td>
<td>62.0</td>
</tr>
<tr>
<td></td>
<td>FUra + cytidine</td>
<td>19.1 ± 2.2 (3)</td>
<td>11.7</td>
<td>23.1</td>
<td>0.9</td>
<td>&lt;0.1</td>
<td>0</td>
<td>64.4</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>FUra</td>
<td>18.1 ± 3.2 (4)</td>
<td>24.0</td>
<td>40.7</td>
<td>0.7</td>
<td>&lt;0.1</td>
<td>0</td>
<td>34.6</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine</td>
<td>12.4 ± 2.5 (4)</td>
<td>44.2</td>
<td>34.1</td>
<td>&lt;0.1</td>
<td>0</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td>B. Ascitic fluid</td>
<td>FUra</td>
<td>40.2 ± 10.0 (4)</td>
<td>0.4</td>
<td>3.6</td>
<td>0.9</td>
<td>&lt;0.1</td>
<td>0</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine</td>
<td>29.4 ± 2.5 (4)</td>
<td>2.0</td>
<td>4.0</td>
<td>1.9</td>
<td>&lt;0.1</td>
<td>0</td>
<td>92.1</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine + cytidine</td>
<td>49.1 ± 4.1 (3)</td>
<td>3.5</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0</td>
<td>96.5</td>
</tr>
<tr>
<td></td>
<td>FUra + adenosine</td>
<td>29.8 ± 8.5 (3)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>FUra + uridine</td>
<td>53.5 ± 9.5 (4)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>10.4</td>
<td>&lt;0.1</td>
<td>0</td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td>FUra + cytidine</td>
<td>56.8 ± 9.1 (3)</td>
<td>0.9</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0</td>
<td>99.1</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>FUra</td>
<td>22.3 ± 2.7 (4)</td>
<td>7.5</td>
<td>29.4</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0</td>
<td>63.1</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine</td>
<td>20.7 ± 3.1 (4)</td>
<td>8.1</td>
<td>25.7</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0</td>
<td>66.2</td>
</tr>
<tr>
<td>C. Tumor cells</td>
<td>FUra</td>
<td>51.9 ± 8.9 (4)</td>
<td>2.3</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>7.2</td>
<td>19.8</td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine</td>
<td>149.4 ± 56.5 (4)</td>
<td>9.9</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>8.4</td>
<td>25.0</td>
<td>56.5</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine + cytidine</td>
<td>35.9 ± 13.0 (3)</td>
<td>2.8</td>
<td>3.4</td>
<td>2.0</td>
<td>4.3</td>
<td>17.0</td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td>FUra + adenosine</td>
<td>93.2 ± 47.5 (3)</td>
<td>14.3</td>
<td>&lt;0.1</td>
<td>1.2</td>
<td>6.3</td>
<td>22.4</td>
<td>55.6</td>
</tr>
<tr>
<td></td>
<td>FUra + uridine</td>
<td>43.4 ± 5.2 (4)</td>
<td>7.0</td>
<td>1.7</td>
<td>1.5</td>
<td>4.3</td>
<td>14.1</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td>FUra + cytidine</td>
<td>38.1 ± 4.8 (3)</td>
<td>6.2</td>
<td>&lt;0.1</td>
<td>0.1</td>
<td>7.7</td>
<td>20.0</td>
<td>66.1</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>FUra</td>
<td>44.5 ± 12.9 (4)</td>
<td>13.2</td>
<td>1.0</td>
<td>0.5</td>
<td>9.7</td>
<td>40.3</td>
<td>35.3</td>
</tr>
<tr>
<td></td>
<td>FUra + guanosine</td>
<td>143.1 ± 14.6 (4)</td>
<td>6.5</td>
<td>3.5</td>
<td>0.4</td>
<td>6.7</td>
<td>43.9</td>
<td>39.0</td>
</tr>
</tbody>
</table>

* Mean ± S.D. (nmol/ml).
* Numbers in parentheses, numbers of animals.
* Ratio of radioactivity of the component to the total radioactivity (%).
* Difference between the two groups significant (p < 0.05) by 2-tailed Student's t test.
* Difference between the 2 groups significant (p < 0.01) by 2-tailed Student's t test.

The percentage of unchanged FUra in the tumor cells 10 min after administration of all combinations was of roughly equal magnitude (60 to 70%), and FUrda accounted for less than 2% of the total radioactivity (Table 2C). Moreover, FUra-nucleotides and acid-insoluble fractions accounted for about 7 and 20% of the radioactivity, respectively, except after injection of the combination of [6-14C]FUra with uridine (4 and 14%, respectively). Thirty min after administration of either [6-14C]FUra or [6-14C] FUra plus guanosine, the percentages of FUra, FUrda, FUra-nucleotides, and the acid-insoluble fractions were about 35, 0.5, 10, and 40%, respectively. FUra was decreased and the acid-insoluble fraction of [6-14C]FUra into tumor cells due to guanosine was significantly overcome by the addition of cytidine (p < 0.05); total radioactivity in the cells was less than that after [6-14C]FUra alone. Thirty min after administration of [6-14C]FUra plus guanosine, total radioactivity in the cells remained at a higher level than that after [6-14C]FUra alone (p < 0.001).

The percentage of unchanged FUra in the tumor cells 10 min after administration of all combinations was of roughly equal magnitude (60 to 70%), and FUrda accounted for less than 2% of the total radioactivity (Table 2C). Moreover, FUra-nucleotides and acid-insoluble fractions accounted for about 7 and 20% of the radioactivity, respectively, except after injection of the combination of [6-14C]FUra with uridine (4 and 14%, respectively). Thirty min after administration of either [6-14C]FUra or [6-14C] FUra plus guanosine, the percentages of FUra, FUrda, FUra-nucleotides, and the acid-insoluble fractions were about 35, 0.5, 10, and 40%, respectively. FUra was decreased and the acid-insoluble fraction of [6-14C]FUra into tumor cells due to guanosine was significantly overcome by the addition of cytidine (p < 0.05); total radioactivity in the cells was less than that after [6-14C]FUra alone. Thirty min after administration of [6-14C]FUra plus guanosine, total radioactivity in the cells remained at a higher level than that after [6-14C]FUra alone (p < 0.001).
Effect of Guanosine on the Metabolism of 5-FUra

![Chart 5. Representative profile of intracellular FURA-nucleotides obtained from a PCA extract of Sarcoma 180 tumor cells 30 min after coadministration of [6-14C]FURA (3 mg/kg i.p.; 7.5 µCi/mouse) and guanosine (100 mg/kg, i.p.). The PCA extract after HCl: methanol extraction was analyzed by Dowex 1-formate column chromatography as described in “Materials and Methods.” FUDP, FUDP-sugar, and FUTP were not clearly available as markers, but their labeled compounds were ascertained by UDP, UDP-sugar, and UTP. FUMP fraction may contain FdUMP, and the FUDP-sugar fraction may contain FdUMP-glucose and FUDP-galactose. The sugar was not identified.

Table 3
Formation of total nucleotides and nucleoside mono-, di-, and triphosphate from [6-14C]FURA in tumor cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phosphorylated products (nmol/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>FUrA</td>
<td>3.7 ± 1.7a</td>
</tr>
<tr>
<td>FUrA + guanosine</td>
<td>12.5 ± 4.4</td>
</tr>
<tr>
<td>FUrA + guanosine + cytidine</td>
<td>1.5 ± 0.8</td>
</tr>
<tr>
<td>FUrA + adenosine</td>
<td>5.9 ± 1.9</td>
</tr>
<tr>
<td>FUrA + uridine</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>FUrA + cytidine</td>
<td>2.9 ± 0.2</td>
</tr>
</tbody>
</table>

Phosphorylated products (nmol/10⁶ cells)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>FUMP</th>
<th>FUDP-sugar</th>
<th>FUDP</th>
<th>FUTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUrA</td>
<td>4.3 ± 1.3</td>
<td>2.3</td>
<td>0.6</td>
<td>1.3</td>
<td>0.1</td>
</tr>
<tr>
<td>FUrA + guanosine</td>
<td>9.6 ± 4.0</td>
<td>3.8</td>
<td>1.3</td>
<td>4.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Phosphorylated products (nmol/10⁶ cells)

- ^a^ Sugar was not identified.
- ^b^ Mean ± S.D. (N = 3 or 4).
- ^c^ Values shown are the means of 3 or 4 determinations.
- ^d^ Difference between the 2 groups significant (p < 0.01) by 2-tailed Student’s t-test.
- ^e^ Numbers in parentheses, ratios to total nucleotides (%).
- ^f^ Difference between the 2 groups significant (p < 0.05) by 2-tailed Student’s t-test.

**DISCUSSION**

Many attempts have been made to synthesize analogues of FUrA to increase either the antitumor activity or the therapeutic
In ascitic fluid, FURA was the major component (more than 100 mg/kg). The mice were sacrificed, and their RNA was isolated as described in "Materials and Methods."

The total radioactivity derived from [6-14C]FURA in the plasma was significantly decreased when the mice were inoculated with a combination of guanosine and [6-14C]FURA (p < 0.05), but in contrast it was increased when the FURA was combined with uridine or cytidine (p < 0.01). The decrease in incorporation of [6-14C]FURA into the plasma when it was combined with guanosine was completely reversed by the addition of cytidine. These changes in total radioactivity in the plasma were similar to those in ascitic fluid. Conversely, incorporation of [6-14C]FURA into tumor cells was significantly increased by guanosine in comparison with [6-14C]FURA alone (p < 0.05). On the other hand, after treatment with the combination of [6-14C]FURA and uridine or cytidine, the incorporation was decreased. Moreover, the increase in the incorporation of [6-14C]FURA when it was combined with guanosine was completely reversed by the addition of cytidine (p < 0.05). Thus, the incorporation of [6-14C]FURA into tumor cells was markedly enhanced by coadministration of guanosine and [6-14C]FURA. The increase in incorporation of [6-14C]FURA by guanosine into the tumor cells produced significant increase in FURA-nucleotide levels and incorporation into RNA.

The metabolites of [6-14C]FURA, FURd, FUPA, and FBAL, as well as intact FURA, were found in the plasma, and the percent-agreement was similar in all groups (FLA, 50 to 60%; FURd, 1%; FUPA, 15 to 25%; FBAL, 10 to 40%). FURd levels were low in all groups except those given [6-14C]FURA combined with uridine (9%). In ascitic fluid, FURA was the major component (more than 90%), and FURd was found in a small amount (less than 2%) except after coadministration with uridine. Uridine, guanosine, adenosine, and inosine yield their ribose moiety as ribose-1-phosphate, through the reaction catalyzed by nucleoside phosphorylase. Tamemasa and Tezuka (45) and Yano and Tamemasa (49) reported that the formation of FURd and FURA was at the highest level with uridine and that the amounts of product formed from adenosine, guanosine, and inosine were one-half of that formed from uridine in the Ehrlich ascites tumor extract system. However, excess uridine antagonized the antitumor activity of FURA in vivo (24) because of the competitive pyrimidine of FURd. On the other hand, the main radioactive component in the tumor cells was FURA (55 to 70%) followed by the RNA fraction (15 to 25%) in all groups 10 min after administration, but radioactivity incorporated into the RNA fraction was similar to that of FURA (about 40%) 30 min after administration. The percentage of each [6-14C]FURA, and its metabolite was scarcely affected by the addition of guanosine.

The major growth-inhibitory effect of FURA has been reported to be associated with FdUMP, which in the presence of N5,10-methylenetetrahydrofolate binds covalently to thymidylate (TMP) synthetase, inactivates the enzyme, and thereby blocks DNA synthesis (3, 21, 31, 40, 43). FURA, after its conversion to FUTP, also is incorporated into bacterial and mammalian mRNA, rRNA, and tRNA at a substantial rate and thereby may produce a structurally or a functionally deficient species of RNA (7, 17, 35, 44, 48). In general, it is apparent that anabolism occurred more rapidly than catabolism in the tumor cells. The conversion of FURA to its nucleotides is considered to be a prerequisite for its antimetabolite and antineoplastic activity. In our experiments, FURA was markedly converted into ribonucleotides and incorporated into RNA, especially when it was given in combination with guanosine.

The active metabolite FdUMP was not detected in acid extracts in the study. Kessel et al. (30) and Kessel and Hall (29) were also unable to detect FdUMP in acid extracts of 15 kinds of mouse leukemia cells. FdUMP when bound to TMP synthetase is not acid soluble (33). This is in contrast with the observations of Chadwick and Rogers (11) and Chadwick and Chang (10), who, after injecting [14C]FURA into mice bearing L1210 leukemia, found FdUMP in both normal and tumor tissues. Myers et al. (38) made similar observations in in vivo experiments using mouse leukemia P1534. Bosch et al. (6) also found FdUMP in acid-soluble pools of Ehrlich ascites cells after incubation with [14C]FURA in vitro. These opposing findings could be due to differences in experimental conditions and time.

Adenosine and inosine cause greater incorporation of [14C]FURA into RNA than do guanosine on Novikoff hepatoma cell line (13) and Tanemasa and Tezuka (45) reported that FURd formation from FURA was almost the same after incubation with adenosine, guanosine, or inosine in vitro. Moreover, guanosine does not affect the pyrophosphorobosyl phosphate level in mouse T-cell lymphosarcoma S49 (46). However, in our study reported here, guanosine produced more marked conversion of FURA to ribonucleotides than did adenosine, and the incorporation into RNA was increased. A possible explanation for our results may be related to the fact that administration of guanosine would presumably decrease the intracellular pyrimidine pools in FRAsensitive tumor cells because of feedback mechanisms by guanosine (5). The fact that cytidine prevented this potentiation by guanosine suggests that the potentiation is mediated by a decrease in the size of the endogenous pyrimidine nucleotide pool similar to adenosine-induced pyrimidine starvation in fibroblasts and lymphoid cells (18). Moreover, in our previous report (24),
this potentiation by guanosine is maintained even in the presence of excess thymidine which bypasses the effect of FdUMP on DNA synthesis. These experiments support the concept that RNA rather than the DNA-directed action of FUra is an important determinant of its activity. The effect of FUra plus guanosine on RNA metabolism may contribute to the cytotoxic action of FUra as observed in the responsiveness of several animal tumors (4, 30, 32, 34, 35). rRNA processing is especially sensitive to FUra, and this effect is proportional to the incorporation of FUra into rRNA and the sensitivity of a tumor to FUra (47). Despite these data implicating ribonucleotide formation as an important determinant in the antitumor activity of FUra, increasing the conversion of FUra to ribonucleotides by coadministration of ribose donors, such as glucose or inosine, has little effect on the drug-promoted survival of a tumor-bearing host either in mice (29) or humans (19). However, only guanosine and GMP markedly potentiated the antitumor activity of FUra in various tumor systems (24, 26). In addition to increasing the conversion of FUra to ribonucleotides, GTP increase may perturb 5'-‘cap’ formation in small rRNA species (51) and then affect the function of RNA and protein synthesis.

A unified mechanism for FUra actions has not yet been established and indeed may not exist. The biochemical significance of the incorporation of FUra into RNA is not entirely clear but the incorporation into RNA caused by guanosine may potentiate the antitumor activity of FUra in this tumor system.

REFERENCES


Relationship between Antitumor Effect and Metabolites of 5-Fluorouracil in Combination Treatment with 5-Fluorouracil and Guanosine in Ascites Sarcoma 180 Tumor System

Masaaki Iigo, Kazuo Kuretani and Akio Hoshi


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/12_Part_1/5687

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.