Use of Uridine Rescue to Enhance the Antitumor Selectivity of 5-Fluorouracil1

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ABSTRACT

We examined the ability of uridine to increase the therapeutic index of 5-fluorouracil (FUra) against C57BL/6 x DBA/2 F1 mice bearing a Day 1 B16 melanoma or L1210 leukemia. FUra (400, 600, or 800 mg/kg, i.p.) followed in 24 hr by a 5-day s.c. infusion with uridine (5 g/kg/day, s.c.) was compared with the maximum tolerated dose of FUra (200 mg/kg, i.p.) plus a 5-day infusion with 0.9% NaCl solution. High-dose FUra plus delayed infusion with uridine was more effective than FUra (200 mg/kg) in inhibiting the growth of the B16 melanoma. High-dose FUra plus uridine rescue was, however, no more effective than FUra (200 mg/kg) in increasing the survival times of mice bearing the L1210 leukemia.

To see if uridine rescue from FUra toxicity correlated with effects against a sensitive normal tissue, bone marrow nucleated cellularity of normal, non-tumor-bearing mice was monitored after drug treatment. In mice treated with FUra (200 mg/kg) followed in 24 hr by a 5-day infusion with either uridine (5 g/kg/day) or 0.9% NaCl solution, there was not as great a decrease in cellularity at the nadir with uridine, and, in addition, uridine accelerated recovery as compared to 0.9% NaCl solution. Furthermore, uridine (5 g/kg/day), but not thymidine (dThd) (5 g/kg/day) or 2'-deoxyuridine (dUrd) (5 g/kg/day), had a sparing effect on the depression in bone marrow nucleated cellularity seen at the nadir on Day 4 after FUra (200 mg/kg).

The specificity of uridine to rescue mice from the lethal toxicity of the related fluorinated pyrimidines, 5-fluorouridine and 5-fluoro-2'-deoxyuridine, was also examined. Mice were treated with 5-fluorouridine (250 mg/kg, i.p.) followed in 24 hr by a 5-day infusion with uridine (1, 5, or 10 g/kg/day), dThd (1, 5, or 10 g/kg/day), or dUrd (1 or 5 g/kg/day). Uridine (1, 5, or 10 g/kg/day) rescued mice from the lethal toxicity of 5-fluorouridine, whereas dThd or dUrd was ineffective. Similarly, a 5-day infusion with uridine, but not dThd or dUrd, rescued mice from the lethal toxicity of 5-fluoro-2'-deoxyuridine (1800 mg/kg, i.p.).

INTRODUCTION

Major disruptions in DNA and RNA synthesis following treatment with FUra2 are due to the anabolism of the drug toFdUMP and FUTP (10). In different tumor cell lines, either the DNA- or RNA-directed actions of FUra may be the principal determinants of its cytotoxicity (5, 21, 25). Furthermore, there is a specificity for dThd, uridine, or dUrd for reversing the cytotoxicity of FUra (5, 23, 26). As for normal host tissues, the importance of the RNA-directed actions of FUra, FdUrd, or FdUrd to their dose-limiting gastrointestinal toxicity in CBA/J mice correlates best with the incorporation of FUTP, derived from these fluorinated pyrimidines, into intestinal RNA rather than with levels ofFdUMP (11).

Our observation that delayed administration of a continuous s.c. infusion of uridine, but not dThd or dUrd, rescues mice from the lethal toxicity of FUra is consistent with FUra/RNA and not inhibition of thymidylate synthetase as a determinant of the toxicity of FUra (14, 15). It seemed possible, therefore, that if differences existed in the RNA- and DNA-directed actions of FUra in tumor as compared to host normal tissues, a selective rescue of normal tissues with uridine could then increase the therapeutic index of FUra.

In this report, we describe our studies with transplantable mouse tumors which indicate the potential of high-dose FUra plus uridine rescue to increase the therapeutic index of FUra. Furthermore, we present evidence that FUra-induced toxicity to a sensitive host tissue can be selectively reversed with uridine but not with either dThd or dUrd. Finally, we have demonstrated that uridine, but not dThd or dUrd, also rescues normal, non-tumor-bearing mice from the lethal toxicity of FdUrd or FdUrd.

MATERIALS AND METHODS

Drugs. FUra and dThd were obtained from Dr. V. L. Narayanan, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Uridine and dUrd were obtained from Sigma Chemical Co. (St. Louis, Mo.). FdUrd and FdUrd were obtained as a gift from Hoffmann-La Roche Inc. (Nutley, N. J.). Sodium pentobarbital was obtained from Veterinary Laboratories, Inc. (Lenexa, Kans.).

FUra was dissolved in 2% NaHCO3 (w/v) just prior to use. FdUrd and FdUrd was dissolved in 0.9% NaCl solution (w/v) just prior to use. All drugs were administered i.p. in volumes of 0.01 ml/g of mouse body weight. Uridine, dThd, and dUrd were each prepared in 0.9% NaCl solution, and all solutions were sterilized by passage through 0.22-µm Millipore filters just prior to administration by continuous s.c. infusion. In all experiments, mice which did not receive drug(s) were given an equivalent volume of 0.9% NaCl solution.

Animals and Tumors. Male C57BL/6 x DBA/2 F1 (hereafter called B6D2F1) mice, weighing 20 to 23 g, were obtained from Harlan-Sprague-Dawley Laboratories (Madison, Wis.). Male C57BL/6 mice, weighing 20 to 23 g, were obtained from Microbiological Associates (Bethesda, Md.). Male DBA/2 mice, weighing 18 to 23 g, were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Animals were caged in an air-conditioned room lighted from 8 a.m. to 8 p.m. and had free access to standard Purina laboratory chow diet and tap water. The L1210 lymphocytic leukemia in the ascites form was passed weekly in DBA/2 mice. The solid B16 melanoma was passaged every 10 to 14 days in C57BL/6 mice by s.c. implantation of 0.25 ml of a 1/5 (w/v) tumor brei. The tumors were obtained from E. G. and G. Mason Research Institute (Worcester, Mass.).
Continuous s.c. Infusion. Mice were anesthetized with a single s.c. injection of sodium pentobarbital (75 mg/kg) prior to insertion of a s.c. cannula. The technique for continuous s.c. infusion with uridine, dThd, or dUrd has been described (13, 15).

Antitumor Activity of FUra plus Uridine Rescue. All chemotherapy experiments were routinely begun between 8 and 9 a.m. For experiments with the solid B16 melanoma, B6D2F, mice were anesthetized on Day 0 with a single s.c. injection of sodium pentobarbital (75 mg/kg), and 0.25 ml of a 1/5 (w/v) tissue brei was injected into the axillary region with puncture in the inguinal region. On Day 1, the mice were randomly distributed into groups of 8 each, and they were then each given a single i.p. injection of either 0.9% NaCl solution or FUra (200, 400, 600, or 800 mg/kg). Twenty-four hr later, a s.c. infusion with either 0.9% NaCl solution or uridine (5 g/kg/day) was begun and continued for 5 days. On Day 16 after tumor inoculation, the mice were sacrificed by cervical dislocation. The tumors were excised surgically, and the weight of each tumor was determined. For experiments with the L1210 tumor, B6D2F, mice were given a single i.p. injection of 1 x 10^7 L1210 ascites cells on Day 0. On Day 1, the mice were randomly distributed into groups of 8 each, and they were then each given a single i.p. injection of either 0.9% NaCl solution or FUra (200, 400, 600, or 800 mg/kg). Twenty-four hr later, a s.c. infusion with either 0.9% NaCl solution or uridine (5 g/kg/day) was begun and continued for 5 days. Animals were observed for individual days of death, and the median survival time and percentage of ILS were determined as described previously (8).

Effect of Chemotherapy on Bone Marrow Nucleated Cellularity. Normal, non-tumor-bearing B6D2F, mice were given a single i.p. injection of FUra (200 mg/kg) on Day 0. Twenty-four hr later, the mice were randomly distributed into groups of 20 each, and a s.c. infusion with either 0.9% NaCl solution or uridine (5 g/kg/day) was begun and continued for 5 days. Mice in groups of 4 each per treatment were sacrificed by cervical dislocation on Days 3, 4, 6, and 8 after FUra, and the number of nucleated cells per femur was determined as described (7). In another experiment, normal, non-tumor-bearing B6D2F, mice were given a single i.p. injection of FUra (200 mg/kg) on Day 0. On Day 1, the mice were randomly distributed into groups of 4 each, and a s.c. infusion with 0.9% NaCl solution, uridine (5 g/kg/day), dThd (5 g/kg/day), or uridine (5 g/kg/day) was begun. Seventy-two hr after the infusion was begun, the animals were sacrificed by cervical dislocation, and the number of nucleated cells per femur was determined. Preliminary experiments showed that the nadir in bone marrow nucleated cellularity occurred on Day 4 after FUra (200 mg/kg) treatment.

Effect of Either Uridine, dThd, or dUrd Infusion to Rescue Mice from the Lethal Toxicity of FUrd or FdUrd. In preliminary experiments with normal, non-tumor-bearing B6D2F, mice, we determined that the approximate 90% lethal dose for a single i.p. dose of either FUrd or FdUrd was 250 and 1800 mg/kg, respectively. For experiments with FUrd, mice were given a single i.p. injection of FUrd (250 mg/kg). Twenty-four hr later, the mice were randomly distributed into groups of 6 each, and a s.c. infusion of either 0.9% NaCl solution or uridine (1.5 or 10 g/kg/day) was immediately begun and continued for 5 days. Survivors were determined daily for 30 days after FUrd. Similar experiments were also done in which mice received a single i.p. injection of FUrd (250 mg/kg) followed in 24 hr by a 5-day infusion with 0.9% NaCl solution, dThd (1, 5, or 10 g/kg/day), or dUrd (1 or 5 g/kg/day). Experiments similar to those with FUrd were also done in which mice received a single i.p. injection of FdUrd (1800 mg/kg) followed in 24 hr by a 5-day infusion with 0.9% NaCl solution, uridine (1.5, or 10 g/kg/day), dThd (1, 5, or 10 g/kg/day), or dUrd (1 or 5 g/kg/day). The dosage schedules of uridine, dThd, or dUrd which were used did not cause any marked gross toxicity (15).

RESULTS

Effect of High-Dose FUra plus Uridine Rescue on the Growth of Mouse B16 Melanoma. In previous studies from this laboratory, we have found that FUra at 200 mg/kg was the maximum tolerated dose when given as a single i.p. injection against either normal or L1210 tumor-bearing B6D2F, mice (6, 15, 16). In order to confirm that FUra (200 mg/kg) was also the maximum tolerated dose against mice bearing a Day 1 B16 melanoma implanted s.c., we compared the effect of either 0.9% NaCl solution or FUra (200, 400, or 600 mg/kg) plus 0.9% NaCl solution infusion on tumor growth and survival. Table 1 (Experiment 1) shows the results obtained when tumor weights were determined from control and FUra-treated mice on Day 16 after tumor implantation. As can be seen, FUra (400 or 600 mg/kg) plus 0.9% NaCl solution infusion inhibited tumor growth as compared to FUra (200 mg/kg) plus 0.9% NaCl solution infusion, the latter of which was ineffective as compared to control. However, FUra (200 mg/kg) plus 0.9% NaCl solution infusion resulted in 8 of 8 Day 16 survivors, whereas FUra (400 or 600 mg/kg) plus 0.9% NaCl solution infusion resulted in 5 of 8 and 3 of 8 Day 16 survivors, respectively. These results, which are consistent with our previous studies on FUra toxicity, indicate that FUra (200 mg/kg) was the maximum tolerated dose tested against the mouse B16 melanoma. In addition, a 5-day infusion with 0.9% NaCl solution did not appear to alter FUra toxicity.

To determine if uridine rescue could increase the therapeutic index of FUra, we compared the effect of high-dose FUra plus uridine rescue with the maximum tolerated dose of FUra alone on the growth of a Day 1 B16 melanoma, implanted s.c. Table 1 shows the results obtained when tumor weights were determined from control and drug-treated mice on Day 16 after tumor implantation. As can be seen (Experiments 2 and 3), FUra (400 or 600 mg/kg) plus uridine rescue were both more effective than was FUra (200 mg/kg) alone in inhibiting tumor growth. Similarly in Experiment 4, FUra (600 or 800 mg/kg) plus uridine rescue were both more effective than was FUra (200 mg/kg) alone in inhibiting tumor growth. The fact that all animals treated with the higher doses of FUra remained alive at Day 16 indicated that uridine had prevented the anticipated host toxicity. These results with the mouse B16 melanoma indicated that a single i.p. injection of high-dose FUra followed in 24 hr by a 5-day infusion with uridine was more tumor inhibitory than the maximum tolerated dose of FUra alone.

Effect of High-Dose FUra plus Uridine Rescue on the Survival of Mice Bearing the Ascitic L1210 Leukemia. To determine if uridine rescue could increase the therapeutic index of FUra against a mouse leukemia, we compared the effect of high-dose FUra plus uridine rescue with the optimal dose of FUra alone on the survival times of mice bearing a Day-1 L1210 leukemia, implanted i.p. As shown in Table 2 (Experiment 1), FUra (400 mg/kg) plus uridine rescue (5 g/kg/day) was no more effective than was FUra (200 mg/kg) alone in that the ILS was 63 and 69%, respectively. Similarly in Experiments 2 and 3, FUra (600 or 800 mg/kg) resulted in an ILS which was not greater than that seen with FUra (200 mg/kg) alone. These results with the mouse L1210 leukemia indicated that a single i.p. injection of high-dose FUra followed in 24 hr by a 5-day infusion with uridine did not produce an ILS greater than that which occurred with the optimal dose of FUra alone.

Effect of Uridine, dThd, or dUrd Infusion on the FUra-induced Depression of Bone Marrow Nucleated Cellularity. To see if uridine rescue from the toxicity of a FUra correlated with effects against a sensitive normal tissue in mice, we monitored bone marrow nucleated cellularity after drug treatment. As can be seen (Chart 1), in mice treated with a single i.p. injection
Table 1

Effect of FUra plus uridine rescue on the growth of B16 melanoma in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days 2–6</th>
<th>Individual days of death</th>
<th>Day-16 survivors/totals</th>
<th>Tumor wt (mg)</th>
<th>Tumor wt (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days2–6</td>
<td>0.9% NaCl solution infusion</td>
<td>Uridine infusion (5 g/kg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td>1</td>
<td>200</td>
<td>+</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>+</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td>+</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>200</td>
<td>–</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>–</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td>–</td>
<td>+</td>
<td>8</td>
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<td>3</td>
<td>200</td>
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<td>+</td>
<td>8</td>
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<td>+</td>
<td>8</td>
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<td>8</td>
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<td></td>
<td>400</td>
<td>–</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>800</td>
<td>–</td>
<td>+</td>
<td>8</td>
</tr>
</tbody>
</table>

*Groups of 8 mice were inoculated with 0.25 ml of a 1/5 (w/v) B16 melanoma brei by s.c. injection on Day 0. On Day 1, mice received a single i.p. injection of FUra, as indicated. Twenty-four hr later, a s.c. infusion of either 0.9% NaCl solution or uridine (5 g/kg/day), as indicated, was begun and continued for 5 days. Survivors were checked daily. Survivors were sacrificed on Day 16 after tumor implantation, and the tumors were dissected free and weighed.

1 Mean ± S.E. of 8 mice per treatment group on Day 16.
2 p < 0.05 as compared to FUra (200 mg/kg) by Student’s t test analysis.
3 p < 0.005 as compared to FUra (200 mg/kg) by Student’s t test.
4 p < 0.01 as compared to FUra (200 mg/kg) by Student’s t test.
5 p < 0.001 as compared to FUra (200 mg/kg) by Student’s t test.

Table 2

Effect of FUra plus uridine rescue on the survival times of mice bearing L1210 leukemia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days 2–6, uridine (g/kg/day)</th>
<th>Median survival time (days)</th>
<th>Range of individual survival times (days)</th>
<th>Increase in median survival time %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>1</td>
<td>200</td>
<td>5</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>5</td>
<td>13.5</td>
<td>7–15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>200</td>
<td>5</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>5</td>
<td>13.0</td>
<td>9–21</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>200</td>
<td>5</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>5</td>
<td>13.0</td>
<td>8–16</td>
</tr>
</tbody>
</table>

*Groups of 6 mice each were inoculated with 1 x 10⁶ L1210 cells by i.p. injection on Day 0. On Day 1, mice received a single i.p. injection of FUra, as indicated, followed in 24 hr by a s.c. infusion of uridine for 5 days. Animals not receiving drug were given an equivalent volume of 0.9% NaCl solution. Animals were monitored for day of death, and median survival times were determined.

...of FUra (200 mg/kg) followed in 24 hr by a 5-day infusion with uridine (5 g/kg/day), there was not as great a decrease, as compared to 0.9% NaCl solution infusion, in the total number of bone marrow nucleated cells per femur on Days 3, 4, 6, and 8 after FUra. In addition, by Day 8, bone marrow nucleated cellularity had almost recovered in mice given a uridine infusion in contrast to a 0.9% NaCl solution infusion (82 and 49% of Day 0 control, respectively).

To determine the nucleoside specificity of this effect, we measured bone marrow nucleated cellularity at the nadir (Day 4) after FUra in mice treated with a single i.p. injection of FUra (200 mg/kg) followed in 24 hr by an infusion with either uridine (5 g/kg/day), dThd (5 g/kg/day), or dUrd (5 g/kg/day). As shown in Table 3, only uridine had a sparing effect on bone marrow nucleated cell numbers compared to 0.9% NaCl solution (25.0 and 13.6% of Day 0 control, respectively). In contrast, neither dThd nor dUrd prevented the FUra-induced depression in bone marrow cell numbers (13.2 and 16.5% of control, respectively).

...of FUra plus uridine rescue on the survival times of mice bearing L1210 leukemia...
Experiment 1). In contrast, dThd (1, 5, or 10 g/kg/day) or dUrd (1 or 5 g/kg/day) both failed to rescue mice from the lethal toxicity of a single i.p. injection of FdUrd (250 mg/kg) (Table 4, Experiments 2 and 3, respectively). In the next set of experiments, mice were treated with a single i.p. injection of FdUrd (1800 mg/kg) followed in 24 hr by a 5-day infusion with either uridine, dThd, or dUrd. As can be seen (Table 5, Experiment 1), uridine (1 or 5 mg/kg/day) failed to rescue mice from the lethal toxicity of FdUrd. In contrast, neither dThd (1, 5, or 10 g/kg/day) nor dUrd (1 or 5 g/kg/day) (Table 5, Experiments 2 and 3, respectively) rescued mice from the lethal toxicity of FdUrd.

**DISCUSSION**

In a previous report from this laboratory, we demonstrated that mice can be rescued from the lethal toxicity of a single i.p.

![Graph](chart1.png)

**Chart 1.** Serial changes in the numbers of nucleated cells per femur of mice following chemotherapy. Mice were each treated with a single i.p. injection of FdUrd (200 mg/kg). Twenty-four hr later, a s.c. infusion of uridine (5 g/kg/day) (O) or 0.9% NaCl solution (x) was begun and continued for 5 days. Mice in groups of 4 each per treatment were sacrificed on the days indicated, and the number of nucleated cells per femur was determined. Measurements were also made of untreated Day 0 controls (x) and 24 hr after FdUrd (200 mg/kg) (x) groups. The values obtained for the paired femora of each mouse were averaged and compared to an untreated Day 0 control group. Mean ± S.E. of 4 mice/treatment group.

### Table 3

Effect of infusion with uridine, dThd, or dUrd on the FdUrd-induced depression of the numbers of nucleated cells per femur of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>FdUrd (mg/kg)</th>
<th>Drug</th>
<th>g/kg/day</th>
<th>Nucleated cells/femur × 10^9</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>Uridine</td>
<td>5</td>
<td>21.8 ± 0.32</td>
<td>100.0</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>dThd</td>
<td>5</td>
<td>2.97 ± 0.10</td>
<td>13.6</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>Uridine</td>
<td>5</td>
<td>5.45 ± 0.13</td>
<td>25.0</td>
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<tr>
<td>4</td>
<td>200</td>
<td>dThd</td>
<td>5</td>
<td>2.88 ± 0.09</td>
<td>13.2</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>dUrd</td>
<td>5</td>
<td>3.58 ± 0.11</td>
<td>16.5</td>
</tr>
</tbody>
</table>

### Table 4

Effect of infusion with uridine, dThd, or dUrd to rescue mice from the lethal toxicity of FdUrd

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment</th>
<th>Day 0, drug (mg/kg)</th>
<th>Days 1–5, drug (g/kg/day)</th>
<th>Individual days of death</th>
<th>Day 30-survivors/total treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>FdUrd</td>
<td>100</td>
<td>Uridine</td>
<td>6, 7, 7, 10</td>
<td>2/6</td>
<td></td>
</tr>
<tr>
<td>dThd</td>
<td>100</td>
<td>Uridine</td>
<td>7, 8, 8, 11</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td>dUrd</td>
<td>100</td>
<td>Uridine</td>
<td>7, 8, 8, 10</td>
<td>1/6</td>
<td></td>
</tr>
</tbody>
</table>

Groups of 6 mice each were given a single i.p. injection of FdUrd followed in 24 hr by infusion with either 0.9% NaCl solution, uridine, dThd, or dUrd as indicated for 5 days. Survivors were monitored daily for 30 days after FdUrd treatment.

### Table 5

Effect of infusion with uridine, dThd, or dUrd to rescue mice from the lethal toxicity of FdUrd

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment</th>
<th>Day 0, drug (mg/kg)</th>
<th>Days 1–5, drug (g/kg/day)</th>
<th>Individual days of death</th>
<th>Day 30-survivors/total treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>FdUrd</td>
<td>100</td>
<td>Uridine</td>
<td>6, 7, 7, 10</td>
<td>2/6</td>
<td></td>
</tr>
<tr>
<td>dThd</td>
<td>100</td>
<td>Uridine</td>
<td>7, 8, 8, 11</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td>dUrd</td>
<td>100</td>
<td>Uridine</td>
<td>7, 8, 8, 10</td>
<td>1/6</td>
<td></td>
</tr>
</tbody>
</table>

Groups of 6 mice each were given a single i.p. injection of FdUrd followed in 24 hr by infusion with either 0.9% NaCl solution, uridine, dThd, or dUrd as indicated for 5 days. Survivors were monitored daily for 30 days after FdUrd treatment.

injection of FdUrd with a uridine infusion begun 24 hr after FdUrd and continued for 5 days. In contrast, infusions with either dThd or dUrd failed to rescue mice from the lethal toxicity of FdUrd (14, 15). These experiments led us to examine whether delayed infusion with uridine could increase the therapeutic efficacy of FdUrd against tumor-bearing mice. The results reported herein demonstrate that uridine rescue increased the therapeutic index of FdUrd against the mouse B16 melanoma. The use of uridine rescue allowed a potentially lethal dose of FdUrd to be administered, and while host toxicity was substantially reduced, the antitumor action of FdUrd was retained. The therapeutic superiority of high-dose FdUrd plus uridine rescue was related to the fact that the maximum tolerated dose of FdUrd, when given alone, failed to inhibit the growth of the B16 melanoma, whereas high-dose FdUrd plus uridine rescue resulted in significant inhibition of tumor growth. The biochemical and/or pharmacological basis by
which uridine allows for selective rescue of host, normal tissues from FUra with retention of antitumor activity is, however, not clear.

In contrast to the increased therapeutic effect which we achieved with high-dose FUra plus uridine rescue against the B16 melanoma, the same dosage schedules afforded no advantage against the mouse L1210 leukemia. Although the L1210 leukemia responded to high-dose FUra plus uridine rescue, the percentage of ILS was not greater than that which was obtained with the optimal dose of FUra alone. It is not apparent, at this time, as to why high-dose FUra plus uridine rescue does not result in a therapeutic advantage against the mouse L1210 leukemia.

There is considerable evidence that the cytotoxicity of FUra is related to FUra/RNA formation (19). For example, the responsiveness of several animal and human tumors to FUra is related to its incorporation into RNA (4, 12, 17). It is of particular interest that Martin et al. (20) reported recently that treatment of BALB/c × DBA/2 F1 mice bearing the advanced solid colon tumor 26 with FUra plus delayed administration of uridine given (by repeated injection) allows the maximum tolerated dose of FUra to be doubled without increasing host toxicity, thereby resulting in improved antitumor activity. In addition, their biochemical studies indicate that uridine rescue results in a relatively faster clearance of FUra from bone marrow RNA and tumor RNA and a marked increase in the rate of recovery of DNA synthesis only in the bone marrow.

Pyrimidine nucleoside kinases allow both normal and tumor cells to salvage circulating nucleosides such as uridine, dThd, or dUrd (3, 18, 22). The activity of uridine/cytidine kinase (EC 2.7.1.48), the rate-limiting enzyme for the utilization of uridine, varies in different tumor lines (24) and even, to some extent, within a series of human tumors (1, 2). Thus, even in a tumor where the predominant effect of FUra is due to FUra/RNA, differences in the utilization of uridine in tumor as compared to normal host tissues might allow for a selective antitumor effect.

The organ systems most susceptible to FUra toxicity are the gastrointestinal tract and the bone marrow (9). In order to see if uridine rescue from the lethal toxicity of FUra correlated with effects against a sensitive normal tissue, we monitored the effects of FUra on bone marrow nucleated cellularity. We showed that the ability of uridine to rescue mice from the lethal toxicity of FUra correlated with its sparing effect on the extent of the FUra-induced depression in bone marrow cell numbers, and, in addition, there was a more rapid recovery to pretreatment levels. Furthermore, the specificity of uridine, as compared to dThd or dUrd, to rescue mice from the lethal toxicity of FUra correlated with the protective effect on the FUra-induced depression of bone marrow cellularity which occurred with the uridine, but not dThd or dUrd, infusion. Martin et al. (20) reported that the depression in WBC of mice given a single dose of FUra was not as great after uridine rescue.

Our previous observations that uridine, but not dThd or dUrd, rescues mice from the lethal toxicity of FUra (14, 15) have now been extended to the related fluorinated pyrimidines, FUrd and FdUrd. Our studies with FUrd and FdUrd in normal mice suggest that, with FUra, it may be possible to increase the therapeutic effectiveness of these antimeabolite nucleosides against tumor-bearing mice by the use of uridine rescue.

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Use of Uridine Rescue to Enhance the Antitumor Selectivity of 5-Fluorouracil

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