The Physiological Disposition of 5-Fluorouracil in Mice Bearing Solid L1210 Lymphocytic Leukemia

Marjory Chadwick and William I. Rogers

Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts 02140

SUMMARY

The physiological disposition of 5-fluorouracil (FU) and its metabolites, in particular 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP), has been studied in BDF1 mice bearing 6-day solid L1210 lymphocytic leukemia up to 72 hr after a single i.v. dose of FU-2-14C, 200 mg/kg, 300 to 1000 µCi/kg. The drug persisted in all tissues, particularly in tumor, for hours after its elimination from blood. The anabolics of FU similarly persisted in both acid-soluble and acid-insoluble fractions of tissues. In most tissues, the major portion of the 72-hr exposure to FU, 5-fluorouridine, 5-fluorouridine 5'-monophosphate, and the di- and triphosphates of 5-fluorouridine, based on areas under the concentration-time curves, occurred during the first 24 hr after dosing. In some tissues, including tumor and small intestine, the major portion of the 72-hr exposure to the di- and triphosphates occurred between 24 and 72 hr after dosing. In particular, FdUMP, which is believed to be the active metabolite, persisted in significant concentrations in all tissues for 72 hr. In most tissues, over 50% of the 72-hr exposure to FdUMP occurred between 24 and 72 hr after dosing. In tumor, where the highest overall exposure to FdUMP was observed, and in small intestine, 70% of the exposure occurred after 24 hr. The significance of the persistence of the active metabolite is discussed.

INTRODUCTION

The fluorinated pyrimidine FU2 (NSC 19893) is widely used in the clinic for the palliative therapy of advanced colorectal and breast tumors. Common side effects are gastrointestinal toxicity and bone marrow depression. It is believed (19) that the cytotoxic effects of FU are due to inhibition of DNA synthesis (5, 15) by its nucleotide derivative, FdUMP, which, by its potent, competitive inhibition of thymidylate synthetase, produces a thymidylic acid deficiency (14, 18, 38). FU is converted via the riboside intermediate 5-fluorouridine to FUMP by the sequential action of uridine phosphorylase and uridine kinase (41). FU is converted directly to FUMP by a phosphoribosyltransferase (37). An alternative pathway for 5-fluorouridine formation is by the action of phosphatases on FUMP formed by phosphoribosyltransferase activity. The pathway by which FUMP is converted to FdUMP has not yet been established. It is likely that FdUMP is formed by reduction of FUDP to 5-fluoro-UDP by a ribonucleotide reductase and by subsequent dephosphorylation. FU may also be converted via FdUr to FdUMP by the sequential action of deoxyuridine phosphorylase (41) and deoxythymidylate kinase (6). However, the equilibrium of the reactions catalyzed by nucleoside phosphorylases favors nucleoside degradation (39), and there is evidence that FdUr is rapidly degraded by deoxyuridine phosphorylase (4). FUDP and FUTP are also formed, and the drug is incorporated into RNA (10, 19). The degradation of FU has been described, one of the major catabolites being FUPA (11, 32). Some of the pathways involved in FU metabolism are summarized in Chart I.

The deoxyribonucleotide (FdUMP) has been shown to be present in relatively minor concentrations in Ehrlich ascites tumor cells both in vitro (5) and in vivo 1 hr after FU administration (21). Its presence has not been demonstrated in other tissues, and changes in its distribution with time have not been determined. The present study was initiated to determine the rates of appearance and disappearance of FU and its metabolites, in particular FdUMP, in selected tissues. The overall purpose was to increase our knowledge of the biochemical pharmacology of FU in animals, with a view to exploiting any significant findings to improve the clinical effectiveness of FU in man. A preliminary report of this study has been presented (9).

MATERIALS AND METHODS

FU and 5-fluorouridine were obtained from Drug Research and Development, National Cancer Institute, Bethesda, Md. FU-2-14C (22 mCi/mmol) was purchased from Calatonic, Los Angeles, Calif. CMP, UMP, dUMP, and UDP-glucose were purchased from Calbiochem, Los Angeles, Calif.

Female BDF1 mice weighing 20 g were implanted with approximately 40-mg fragments of solid L1210 lymphocytic leukemia s.c. in the axillary region. On the 6th day of tumor growth, the mice were given single i.v. injections, via the caudal vein, of FU-2-14C in water adjusted to pH 11 with KOH, at 200 mg/kg (600 mg/sq m). Animals to be sacrificed at early time periods after dosing received approximately 300 µCi/kg; animals to be sacrificed at late time periods after dosing received approximately 1000 µCi/kg. Groups of 5 to 10
mice were housed in metabolism cages and allowed food and water ad libitum. They were sacrificed at 8, 16, and 30 min, and at 1, 2, 24, 48, and 72 hr after dosing. Groups of nontumor-bearing mice were similarly treated. The animals were anesthetized with ether, and blood was removed by heart puncture and was transferred to an heparinized tube. Liver, kidney, spleen, small intestine, brain, and tumor were removed and were frozen on Dry Ice. No attempt was made to separate small intestine into mucosal and muscular portions. The brains from 24-hr tumor-bearing animals and from 24-hr, 48-hr, and 72-hr normal animals were separated into a cerebellar-medullar portion, and the remainder and the 2 portions were frozen separately. The hind limbs were removed for bone marrow extraction. The bone marrow cells were flushed from the femurs and tibias with 0.9% NaCl solution, and the total number of cells was determined with a hemocytometer slide. Urine was removed from the bladders of the animals, collected from the metabolism cages, and pooled. The tissues and urine were stored at −20°.

Homogenates of blood and tissues, 20%, were prepared in distilled water. Aliquots of the homogenates were solubilized with Nuclear-Chicago Solubilizer and decolorized with benzoyl peroxide (17). The radioactivity was determined in toluene-Liquifluor scintillation solution by use of a Nuclear-Chicago Solubilizer and decolorized with benzoyl peroxide (17). The radioactivity was determined in toluene-Liquifluor scintillation solution by use of a Nuclear-Chicago Mark I scintillation spectrometer with an external standard channel-ratio correction with a 133Ba source. The remainder of the homogenates were adjusted to a concentration of 0.4 M perchloric acid to extract the acid-soluble components. The insoluble residues were reextracted twice with 0.2 M perchloric acid. The acid extracts were combined and adjusted to pH 11 with KOH, and the insoluble potassium perchlorate was removed. Urine samples were also adjusted to pH 11 with KOH. Radioactivity in the tissue acid extracts and in urine was determined in TEN scintillation solution developed in this laboratory (43).

FU and its metabolites in the acid extracts and urine were separated on Dowex 1-formate anion-exchange resin columns (12 x 0.5 cm) by the stepwise elution technique of Chaudhuri et al. (11). The Dowex 1-formate was generated from Dowex 1–X10 chloride, 200 to 400 mesh. The acid-soluble extracts, adjusted to pH 11, were loaded onto the columns. Twenty-five ml of water, adjusted to pH 11 with KOH, were used to elute urea, followed by 50 ml of 0.05 M formic acid to elute FU and 5-fluourouridine. Variable amounts of FCMP were also eluted in the latter solvent system. FUPA, together with the remaining FCMP, was eluted with 50 ml of 1.5 M formic acid. FUMP, FdUMP, and FUDP were eluted with 70 ml of 2.5 M formic acid. Nucleoside di- and triphosphates were eluted with 50 ml of 2.2 M ammonium formate. Fractions of 3 to 4 ml were collected every 20 min, and the radioactivity in aliquots of each fraction was determined in TEN scintillation solution. The recovery of radioactivity from the columns averaged 97%.

Fractions comprising each major peak of radioactivity were pooled and, if necessary, concentrated by evaporating them to dryness at room temperature and redissolving them in water. The 0.05 M formic acid fraction was dissolved in water adjusted to pH 11. Components of the individual peaks were separated by thin-layer chromatography on precoated cellulose plates, 0.1 mm thick, containing fluorescent indicator purchased from Brinkmann Instruments, Inc, Westbury, N.Y. Radioautograms of the plates were made. Cellulose scrapings were placed in scintillation vials, radioactive components were eluted by shaking with water, and the radioactivity was determined in TEN scintillation solution. The only fluorinated standards available were FU and 5-fluorouridine. Nonfluorinated compounds were therefore used as standards, except for FUPA, which was identified by comparison of its RF with that reported in the literature (11). Components in the 0.05 M formic acid fraction were separated by the system, n-butyl alcohol:water:formic acid (77:13:10, v/v/v) (11). The RF values for FU and 5-fluorouridine were approximately 0.45 and 0.31, respectively, and the CMP standard remained at the origin. FCMP seemed to cochromatograph with CMP. Components in the 1.5 M formic acid fraction were separated by the same system. FCMP remained at the origin, and the RF of FUPA was 0.42. An unknown with an RF value of 0.18 was also found in most tissues and in urine. FCMP formed such a minor percentage of the total equivalents that it was not quantitated. Components of the 2.5 M formic acid fraction were separated by the following solvent systems: Solvent System A, 5 M ammonium acetate (pH 9.0):saturated sodium tetraborate:95% ethanol:0.5 M Versene (40:160:360:1, v/v/v/v) (35), and Solvent System B, tert-amyl alcohol:formic acid:water (3:2:1, v/v/v) (34). In Solvent System A, UMP remained at the origin, and the RF of dUMP was approximately 0.25. FUMP appeared to correspond to UMP, and FdUMP (RF 0.21) moved slightly behind dUMP. In this system, the UDP-glucose standard and, presumably, FUDPG behaved similarly to UMP and remained close to the origin. In Solvent System B, the RF values of the standards were UDP-glucose, 0.05; UMP, 0.21; and dUMP, 0.30. A similar separation was obtained for the fluorinated compounds. FUDPG was not quantitated. FUDP and FUTP in the 2.2 M ammonium formate fraction were not confirmed and separated by thin-layer chromatography but were quantitated as an aggregate.

Extensive replicate experiments did not seem necessary, as conclusions were based on changes in the concentrations of metabolites with time rather than on the concentrations at a particular time period. Furthermore, each data point represents the result of pooling tissues from 5 to 10 mice. Whenever replicate studies were carried out, good agreement was obtained between the results. The concentrations of acid-extractable drug equivalents in the tissues of tumor-bearing and normal mice at 24, 48, and 72 hr after FU administration were very similar, with the exception of the concentrations in spleen and liver, for reasons discussed later (Table 1). Furthermore, anion-exchange chromatographic
Table 1

Concentrations of FU equivalents in the acid-soluble fractions of tissues from normal BDF₁ mice and from those bearing solid L1210 lymphocytic leukemia

FU-2-¹⁴C was administered i.v. at 200 mg/kg and approximately 300 to 1000 μCi/kg 6 days after tumor implantation. At each time period after injection, tissues from 6 to 10 mice were pooled and extracted with perchloric acid as described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentrations (nmoles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hr</td>
</tr>
<tr>
<td>Blood</td>
<td>2</td>
</tr>
<tr>
<td>Tumor</td>
<td>240</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>20</td>
</tr>
<tr>
<td>Small intestine</td>
<td>82</td>
</tr>
<tr>
<td>Kidney</td>
<td>62</td>
</tr>
<tr>
<td>Liver</td>
<td>57</td>
</tr>
<tr>
<td>Spleen</td>
<td>85</td>
</tr>
<tr>
<td>Cerebellum and medulla</td>
<td>24</td>
</tr>
<tr>
<td>Remainder of brain</td>
<td>19</td>
</tr>
</tbody>
</table>

* The concentration of FU equivalents in bone marrow were expressed in nmoles/10⁹ cells, which approximates nmoles/g.

RESULTS

The dose of FU used in this study, 200 mg/kg i.v., is equivalent on a mg/sq m basis to administration of 15 mg/kg i.v. to a 70-kg man, which is one of the doses used clinically (20). FU at 200 mg/kg produced no deaths when administered i.v. to normal female BDF₁ mice (I. Wodinsky, unpublished data). Advanced, solid L1210 lymphocytic leukemia was chosen for study, as it is known to be sensitive to FU treatment (26). Furthermore, it somewhat resembles the FU-sensitive human tumors in that the primary tumor is solid, has become systemic by 6 days, and is widely disseminated within the host. Unlike many solid human tumors, however, it is rapidly growing.

Urinary Excretion of FU. The cumulative excretion of total radioactivity and of FU and its metabolites in the urine from tumor-bearing mice after i.v. administration of FU-2-¹⁴C is shown in Chart 2. Approximately 60% of the dose was excreted by this route in 24 hr; 40% was excreted during the 1st hr. Approximately 30% of the dose was excreted as FU, taking place during the 1st 30 min after dosing. By 1 hr, FUPA became a major urinary metabolite, and approximately 20% of the dose was excreted in this form. The remainder of the dose excreted consisted of urea, 5-fluorouridine, FCMP and other nucleotides, and unknowns. Urea was not identified by thin-layer chromatography but represents radioactivity eluted in the water fraction. Similarly, nucleotides other than FCMP represent radioactivity eluted in the 2.5 M formic acid and 2.2 M ammonium formate fractions. Exhalation as ¹⁴CO₂ was not determined; however, it is reported that 35% of an i.p. dose of FU-2-¹⁴C was eliminated by this route (10).

Disposition of FU in Blood and Plasma. The disposition of FU and its metabolites in blood from tumor-bearing mice and the disposition of FU alone in plasma from normal mice are shown in Chart 3. The FU concentration in blood and plasma decreased rapidly during the 1st hr, corresponding to the appearance of the drug in the urine and to its conversion to other metabolites. The higher FU concentration in plasma than that in blood over the 1st 30 min suggests that FU may...
have diffused from plasma into red blood cells and by 30 min had reached equilibrium. The drug concentration in blood decreased slowly between 1 and 2 hr and by 24 hr was approximately 0.3 nmole/g. The half-life of FU in mouse plasma is about 15 min, which corresponds to its rapid appearance in urine. A similar value was obtained for the plasma half-life in humans by us and other workers (12). A half-life of about 29 min was calculated for the drug in mouse blood. A total integrated $C \times t$ value for FU of 46 $\mu$moles min/g was calculated for blood, of which 60% was achieved in the 1st hr. The FUPA concentration in blood increased rapidly to a maximum at 1 hr, the time at which FUPA reached its maximum concentration in liver (Table 7) and became a major constituent of urine (Chart 2). Over the next hour, the FUPA concentration in blood declined rapidly, corresponding to the excretion of FUPA in the urine and/or to its further catabolism, and by 24 hr was approximately 0.3 nmole/g. 5-Fluorouridin was also detected in blood, together with low levels of nucleotides.

Comparison of the Concentrations of Drug Equivalents in the Acid-soluble Fractions of Tissues from Tumor-bearing and Normal Mice. By 6 days, solid L1210 lymphocytic leukemia becomes systemic and tumor cells infiltrate normal tissues particularly spleen, liver, and bone marrow (16). In order to determine whether infiltration was sufficiently extensive to result in changes in drug disposition, the concentrations of drug equivalents in the acid-soluble fractions of tissues from normal and tumor-bearing animals were compared (Table 1). The concentrations of drug equivalents in the acid-soluble fractions of tumor are high; therefore, it would be predicted that the infiltration of tumor cells into the tissues would raise the total drug-equivalent concentrations.

At 24 hr (7-day tumor), the drug-equivalent concentrations in the acid-soluble fractions of the tissues from tumor-bearing animals equaled or were less than the concentrations in tissues from normal animals. By 48 hr (8-day tumor), the concentrations of acid-soluble drug equivalents in liver and spleen from tumor-bearing animals were higher than those from normal animals and had increased over the 24-hr concentrations, indicating that tumor cells had infiltrated these organs. By 72 hr (9-day tumor), higher drug-equivalent concentrations were observed in liver, spleen, and bone marrow from tumor-bearing animals, possibly indicating that tumor infiltration of the latter organ had become sufficient to affect drug disposition; however, drug-equivalent concentrations were too low for this conclusion to be definite.

At 48 and 72 hr, the acid-soluble, drug-equivalent concentrations in the tumor-infiltrated spleen were approximately equal to those in the 8-day and 9-day tumor. A possible explanation is that the systemic tumor cells originate from the peripheral layer of the solid tumor mass, which is known, from whole-body radioautographic studies (R. H. Liss, unpublished data), to contain a higher concentration of drug equivalents than the center of the solid tumor. This unequal distribution is probably a result of the poor blood supply at the center of the advanced solid tumor.

Since, by 48 hr and 72 hr after dosing, tumor infiltration can affect the physiological disposition of FU in liver and particularly in spleen, analyses of liver and spleen from normal animals were used in the disposition study at these time periods. Although data for liver and spleen from tumor-bearing and normal animals were not strictly comparable, they were integrated in $C \times t$ determinations, since the concentrations of acid-extractable drug equivalents in liver and spleen at 24 hr, and in all other tissues at 24, 48, and 72 hr, were very similar from both groups of animals (Table 1).

Disposition of FU in Tissues. The concentrations of FU in tissues are summarized in Table 2. In all tissues, as seen in blood and plasma (Chart 3), FU decreased rapidly during the 1st hr as a result of urinary excretion of the drug and its conversion to other metabolites. By 24 hr, drug concentrations were considerably lower than at 2 hr in all tissues except tumor. By 72 hr, FU concentrations were approximately 10 nmole/g or less in most tissues, with less than 1 nmole/g remaining in liver and brain. In tumor, however, the drug apparently persisted longer, for by 72 hr, FU was still present at a concentration of approximately 20 nmole/g. The FU concentrations were not corrected for the presence of drug in the blood of the organs, since for up to 1 hr the FU concentration in blood and highly vascular organs such as liver and spleen were similar and after 1 hr the FU concentration in blood was low. The highest 0- to 72-hr integrated $C \times t$ values were calculated for tumor (240 $\mu$moles min/g) and small intestine (290 $\mu$moles min/g). In kidney, approximately 40% of the 72-hr exposure to FU occurred in the 1st hr, representing the initial output of FU in the urine. In all other tissues, despite the initial marked decline in FU concentrations, the 1-hr $C \times t$ values represented only 15 to 25% of the 72-hr values. By 24 hr, between 80 to 90% of the 72-hr exposure to FU had occurred in all tissues except tumor,
Physiological Disposition of 5-Fluorouracil

Table 2
Concentrations of FU in the tissues of BDF, mice bearing solid L1210 lymphocytic leukemia

FU-2-14C was administered i.v. at 200 mg/kg and approximately 300 to 1000 µCi/kg 6 days after tumor implantation. At each time period after injection, tissues from 5 to 10 mice were pooled and analyzed for FU as described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Tissue</th>
<th>8 min</th>
<th>16 min</th>
<th>30 min</th>
<th>1 hr</th>
<th>2 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>810</td>
<td>840</td>
<td>580</td>
<td>110</td>
<td>93</td>
<td>64</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>1000</td>
<td>900</td>
<td>570</td>
<td>210</td>
<td>240</td>
<td>29</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Kidney</td>
<td>2300</td>
<td>1800</td>
<td>950</td>
<td>98</td>
<td>59</td>
<td>15</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Liver</td>
<td>730</td>
<td>610</td>
<td>290</td>
<td>94</td>
<td>130</td>
<td>7</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>960</td>
<td>700</td>
<td>480</td>
<td>180</td>
<td>70</td>
<td>17</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Brain</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) The 48-hr and 72-hr liver and spleen samples were obtained from normal animals because at these time periods there was significant infiltration of leukemic cells into these tissues.

\(b\) The concentrations of FU in bone marrow were expressed in nmoles/10^6 cells, which approximates nmoles/g.

Table 3
Concentrations of 5-fluorouridine in the tissues of BDF, mice bearing solid L1210 lymphocytic leukemia

FU-2-14C was administered i.v. at 200 mg/kg and approximately 300 to 1000 µCi/kg 6 days after tumor implantation. At each time period after injection, tissues from 5 to 10 mice were pooled and analyzed for 5-fluorouridine as described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Tissue</th>
<th>8 min</th>
<th>16 min</th>
<th>30 min</th>
<th>1 hr</th>
<th>2 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>86</td>
<td>110</td>
<td>220</td>
<td>150</td>
<td>180</td>
<td>46</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>170</td>
<td>150</td>
<td>66</td>
<td>29</td>
<td>13</td>
<td>14</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>180</td>
<td>210</td>
<td>170</td>
<td>100</td>
<td>88</td>
<td>19</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Liver</td>
<td>160</td>
<td>260</td>
<td>320</td>
<td>280</td>
<td>380</td>
<td>12</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Spleen</td>
<td>67</td>
<td>85</td>
<td>170</td>
<td>120</td>
<td>190</td>
<td>16</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Brain</td>
<td>19</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) The 48-hr and 72-hr liver and spleen samples were obtained from normal animals because at these time periods there was significant infiltration of leukemic cells into these tissues.

\(b\) The concentrations of 5-fluorouridine in bone marrow were expressed in nmoles/10^6 cells, which approximates nmoles/g.

where only 60% of the 72-hr exposure had occurred, as a result of the persistence of FU in this tissue. In all tissues, therefore, but particularly in tumor, FU persisted for hours after its elimination from blood.

The concentrations of 5-fluorouridine in tissues are summarized in Table 3. 5-Fluorouridine is formed from FU by uridine phosphorylase (41) and from FUMP by phosphatases. In tumor, bone marrow, liver, and spleen, the riboside concentration increased over the 1st 2 hr and appeared to be approaching maximum. In small intestine and kidney, however, the concentration decreased over the 1st 2 hr, and in the small intestine, by 2 hr, the 5-fluorouridine concentration was less than 20 nmoles/g, which is considerably lower than in all other tissues except brain. By 24 hr, the concentration of 5-fluorouridine was less than 20 nmoles/g in all tissues except tumor, where approximately 50 nmoles/g of 5-fluorouridine remained, falling to below 20 nmoles/g by 72 hr. The riboside of FU, therefore, also persisted in the tissues for hours after dosing. The highest 0- to 72-hr integrated \(C X t\) values were calculated for tumor (250 μmole min/g) and liver (310 μmole min/g). One of the lowest values was that of small intestine (40 μmole min/g), which resulted from the initial rapid decline in riboside levels in this organ. In all tissues, the major portion of the 72-hr exposure to 5-fluorouridine occurred during the 1st 24 hr after dosing.

The concentrations of FUMP in tissues are summarized in Table 4. FUMP is formed from 5-fluorouridine by uridine kinase (41) and from FU by a phosphoribosyltransferase (37). By far, the highest concentrations of this metabolite were observed in spleen. The concentration of FUMP increased during the 1st 2 hr in tumor, bone marrow, small intestine, liver, and spleen, and in all except bone marrow and spleen it appeared to have approached its maximum. In kidney, the concentration of FUMP appeared to decline from a maximum at 8 min. By 24 hr, the concentrations of FUMP were low in all tissues and continued to fall over the next 2 days. In tumor, FUMP persisted at significant concentrations for longer than in the other tissues. The highest 0- to 72-hr integrated \(C X t\) values were calculated for tumor (94 μmole min/g) and spleen (200 μmole min/g). The true \(C X t\) value for spleen may be higher than calculated, as the concentration of FUMP in this tissue was still rising at 2 hr. In tumor, bone marrow, kidney,
liver, and spleen, 80 to 95% of the 72-hr \( C \times t \) value was reached by 24 hr. In small intestine, 70% of the 72-hr \( C \times t \) value was reached by 24 hr. In all tissues, therefore, the major portion of the 72-hr exposure to FUMP occurred during the 1st 24 hr after dosing.

The concentrations of FdUMP in tissues are summarized in Table 5. FdUMP is formed from FUMP by an unestablished pathway that probably involves a ribonucleotide reductase, or possibly, FdUMP is formed from FU, via FUdR, by the sequential action of deoxycytidine phosphorylase (41) and deoxthyridine kinase (6). FdUMP is reported to be the active metabolite of FU (14, 18, 38). In tumor, the FdUMP concentration rose slowly over the 1st 2 hr; by 24 hr, however, it had increased 9-fold. At 48 hr, it decreased to 50% of the 24-hr concentration, and this concentration was maintained at 72 hr. In the tumor, therefore, the maximum FdUMP concentration occurred at some time between 2 and 48 hr after dosing, probably between 2 and 24 hr, since the concentration of its precursor, FUMP, sharply decreased during this time period. FdUMP reached a maximum concentration at 1 or 2 hr after dosing in all the other tissues except spleen, where the maximum concentration occurred between 2 and 24 hr after dosing. In kidney, and particularly in small intestine, FdUMP remained at a relatively constant concentration over the next 3 days. In bone marrow and brain, the FdUMP concentration remained relatively constant over the 1st 24 hr and then declined. Further investigation demonstrated that the FdUMP concentration in bone marrow did not increase over the 1st 24 hr, as was previously reported (9). In liver and spleen, the 24-hr concentration was less than the 2-hr concentration, and the level continued to decline over the next 2 days. The active metabolite therefore persisted in all tissues for 72 hr after the dose, particularly in the tumor.

The highest 0- to 72-hr integrated \( C \times t \) values were calculated for tumor (210 μmole/min/g) and spleen (140 μmole/min/g). The true values may be considerably higher than calculated, as it is not known if the maximum concentration of FdUMP was reached in these tissues at the time periods studied. The \( C \times t \) method of expressing the results further emphasized the persistence of FdUMP in the tissues. In tumor and small intestine, as calculated from the data available, 70% of the 72-hr exposure to FdUMP occurred between 24 and 72 hr.

Table 4

<table>
<thead>
<tr>
<th>Concentrations of FUMP in the tissues of BDF, mice bearing solid L1210 lymphocytic leukemia⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU-2-¹⁴C was administered i.v. at 200 mg/kg and approximately 300 to 1000 μCi/kg, 6 days after tumor implantation. At each time period after injection, tissues from 5 to 10 mice were pooled and analyzed for FUMP as described under &quot;Materials and Methods.&quot;</td>
</tr>
<tr>
<td>Tissue</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Tumor</td>
</tr>
<tr>
<td>Bone marrow</td>
</tr>
<tr>
<td>Small intestine</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Spleen</td>
</tr>
<tr>
<td>Brain</td>
</tr>
</tbody>
</table>

⁹ The 48-hr and 72-hr liver and spleen samples were obtained from normal animals because at these time periods there was significant infiltration of leukemic cells into these tissues.

Table 5

<table>
<thead>
<tr>
<th>Concentrations of FdUMP in the tissues of BDF, mice bearing solid L1210 lymphocytic leukemia⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU-2-¹⁴C was administered i.v. at 200 mg/kg and approximately 300 to 1000 μCi/kg, 6 days after tumor implantation. At each time period after injection, tissues from 5 to 10 mice were pooled and analyzed for FdUMP as described under &quot;Materials and Methods.&quot;</td>
</tr>
<tr>
<td>Tissue</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Tumor</td>
</tr>
<tr>
<td>Bone marrow</td>
</tr>
<tr>
<td>Small intestine</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Spleen</td>
</tr>
<tr>
<td>Brain</td>
</tr>
</tbody>
</table>

⁹ The 48-hr and 72-hr liver and spleen samples were obtained from normal animals because at these time periods there was significant infiltration of leukemic cells into these tissues.

\[ a \] The concentrations of FdUMP in bone marrow were expressed in nmoles/10⁹ cells, which approximates nmoles/g.
after dosing. In bone marrow during the same period, 50% of the 72-hr exposure occurred. In kidney, liver, and spleen, 60, 50, and 40%, respectively, of the 72-hr exposure to FdUMP occurred between 24 and 72 hr after injection. In most tissues, therefore, the major portion of the 72-hr exposure to FdUMP occurred between 48 and 72 hr after dosing.

The concentrations of FUDP + FUTP in tissues are summarized in Table 6. The di- and triphosphates are presumably formed by nucleotide kinases from FUMP and may be incorporated into RNA (10, 19). The FUDP + FUTP concentration increased during the 1st 2 hr in tumor, bone marrow, small intestine, liver, and spleen and in each, except tumor and probably liver, it appeared to have approached its maximum. In kidney, the concentration seemed to decline from a maximum at 8 min. By 24 hr in tumor, the FUDP + FUTP concentration had decreased to approximately one-half the 2-hr value and remained at that level, which was significantly higher than in the other tissues, for the next 2 days. In small intestine and particularly in kidney, FUDP + FUTP remained at a relatively constant concentration for 3 days, declining somewhat by 72 hr. In bone marrow, liver, and spleen, the concentration declined relatively rapidly between 2 and 24 hr and continued to decline slowly over the next 2 days. In brain, the FUDP + FUTP concentration remained relatively constant over 48 hr, falling to low levels at 72 hr. The nucleotide di- and triphosphates, therefore, persisted in all tissues, particularly in tumor, for 72 hr after the dose. The highest 0- to 72-hr integrated C x t value was calculated for tumor (130 n mole/min/g). The true value may be higher than that calculated because the maximum concentrations of FUDP + FUTP may not have been attained at the time periods studied. In tumor, small intestine, and kidney, as calculated from the data available, 60% of the 72-hr exposure to FUDP + FUTP occurred between 24 and 72 hr after dosing. In bone marrow, liver, and spleen, 20 to 30% of the 72-hr exposure occurred during the same time period.

The concentrations of FUPA in tissues are summarized in Table 7. In all tissues except kidney, changes in FUPA concentration with time were similar to those observed in blood (Chart 3). The concentrations of FUPA increased rapidly to maxima at 1 hr and declined rapidly over the next hour, corresponding to the excretion of FUPA in the urine and/or to the further catabolism of FUPA, and by 24 hr, the concentrations were very low. In the kidney, the FUPA concentration increased rapidly over the 1 st 30 min, then more slowly over the next 1.5 hr, and had not reached a maximum at 2 hr. The change in rate of FUPA accumulation at 30 min corresponded with the appearance of FUPA as a major urinary catabolite. By 24 hr, the concentration of FUPA in the kidney was similar to that in the other tissues. The highest concentrations of FUPA were found in liver, which is the main site of FU degradation (11). There is no degradation of FU by spleen, as the concentration of catabolite found could be accounted for by the FUPA known to be present in the blood of spleen (2). This finding is in agreement with the work of others (11). There appeared to be degradation of FU in small intestine and brain, as the concentrations of FUPA were greater than that present in the blood in these organs. The concentrations of FUPA found in tumor were relatively low. Since solid LI210 is a highly vascular tumor, it is possible that the FUPA was present in blood rather than in tissue and that FU was not degraded by tumor. Other workers demonstrated that Ehrlich ascites cells are unable to degrade FU and that Sarcoma 180 may also lack this ability (11).

The concentrations of drug equivalents in the acid-insoluble fractions of the tissues, between 2 and 72 hr after FU administration are summarized in Table 8. They represent the differences between concentrations of total drug equivalents and acid-soluble drug equivalents in the tissues. The concentrations of drug equivalents in acid-soluble fractions are included for comparison in the same table. In this study, we did not confirm, by separating the RNA nucleotides from the acid-insoluble fractions, that all the insoluble drug equivalents consisted of FU incorporated into RNA. Particularly at time periods earlier than 24 hr, differences between concentrations of total and acid-soluble drug equivalents could also represent 14CO2 released during acid extraction (10). The minority of the total FU equivalents in the tissues were acid-insoluble,
Table 7
Concentrations of FUPA in the tissues of BDF, mice bearing solid L1210 lymphocytic leukemia

FU-2-14C was administered i.v. at 200 mg/kg and approximately 300 to 1000 μCi/kg 6 days after tumor implantation. At each time period after injection, tissues from 5 to 10 mice were pooled and analyzed for FUPA as described under “Materials and Methods.”

<table>
<thead>
<tr>
<th>Tissue</th>
<th>8 min</th>
<th>16 min</th>
<th>30 min</th>
<th>1 hr</th>
<th>2 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>8</td>
<td>10</td>
<td>21</td>
<td>46</td>
<td>38</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>6</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Small intestine</td>
<td>29</td>
<td>34</td>
<td>41</td>
<td>120</td>
<td>82</td>
<td>2</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>120</td>
<td>210</td>
<td>320</td>
<td>390</td>
<td>440</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Liver</td>
<td>410</td>
<td>420</td>
<td>610</td>
<td>920</td>
<td>340</td>
<td>3</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>7</td>
<td>11</td>
<td>16</td>
<td>28</td>
<td>23</td>
<td>1</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

a The 48-hr and 72-hr liver and spleen samples were obtained from normal animals because at these time periods there was significant infiltration of leukemic cells into these tissues.

Table 8
Concentrations of FU equivalents in the acid-insoluble and acid-soluble fractions of tissues from BDF, mice bearing solid L1210 lymphocytic leukemia

FU-2-14C was administered i.v. at 200 mg/kg and approximately 300 to 1000 μCi/kg 6 days after tumor implantation. At each time period after injection, tissues from 5 to 10 mice were pooled and extracted with perchloric acid as described under “Materials and Methods.”

<table>
<thead>
<tr>
<th>Tissue</th>
<th>2 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AIF</td>
<td>ASF</td>
<td>AIF</td>
<td>ASF</td>
</tr>
<tr>
<td>Blood</td>
<td>23</td>
<td>140</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Tumor</td>
<td>180</td>
<td>520</td>
<td>7</td>
<td>240</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>-c</td>
<td>180</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Small intestine</td>
<td>110</td>
<td>410</td>
<td>24</td>
<td>82</td>
</tr>
<tr>
<td>Kidney</td>
<td>180</td>
<td>760</td>
<td>-c</td>
<td>62</td>
</tr>
<tr>
<td>Liver</td>
<td>310</td>
<td>1080</td>
<td>30</td>
<td>57</td>
</tr>
<tr>
<td>Spleen</td>
<td>200</td>
<td>700</td>
<td>9</td>
<td>85</td>
</tr>
<tr>
<td>Brain</td>
<td>7</td>
<td>85</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

a The concentrations of FU equivalents in bone marrow were expressed in nmoles/10⁹ cells, which approximates nmoles/g.
b AIF, acid-insoluble fraction; ASF, acid-soluble fraction.
c The concentration of total drug equivalents was not determined in these tissues at these time periods.
d The 48-hr and 72-hr liver and spleen samples were obtained from normal animals because at these time periods there was significant infiltration of leukemic cells into these tissues.

with the exception of the equivalents in liver at 72 hr and in blood at 24, 48, and 72 hr. The acid-insoluble drug equivalents appeared to reach maximum concentrations in all tissues during the 1st 2 hr after FU administration. By 24 hr, the concentrations were considerably lower than at 2 hr, and they remained relatively constant or declined over the next 48 hr. Liver contained the highest concentration of acid-insoluble FU equivalents at all the time periods studied. At 1 and 2 hr, liver also contained the highest concentrations of acid-soluble equivalents. Prior to 1 hr, the concentration of acid-soluble equivalents was higher in kidney than in liver, as a result of the initial output of FU in the urine. After 24 hr, tumor contained the highest concentration of acid-soluble equivalents. The concentrations of total drug equivalents in liver at these late time periods were lower than those in several other tissues; however, a relatively high percentage was acid insoluble. Tumor, on the other hand, contained the highest concentration of total equivalents, of which a relatively low percentage was acid insoluble. At any time period, however, apart from these noteworthy exceptions, the relative tissue concentrations of acid-insoluble FU equivalents roughly paralleled the concentrations of the acid-soluble equivalents. FU equivalents, therefore, persisted in the RNA-containing acid-insoluble fractions of the tissues for 72 hr after drug administration.

Whole-body radioautographic studies demonstrated the localization of radioactivity in the Purkinje and granular layers of the cerebellum (27). Biochemical studies indicated differences in the concentration and distribution of FU equivalents between the cerebellar-medullar portion of the
Methods.

animals. At each time period after injection, tissues from 5 to 10 mice were pooled and acid-extracted as described under "Materials and Methods."

Concentrations of FU equivalents in the cerebellum + medulla portion and the remainder of the brain from normal BDF1 mice and from those bearing solid L1210 lymphocytic leukemia

FU-2-14C was administered i.v. at 200 mg/kg and approximately 300 to 1000 μCi/kg to both normal animals and 6-day tumor-bearing animals. At each time period after injection, tissues from 5 to 10 mice were pooled and acid-extracted as described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Brain tissue</th>
<th>Time after injection (hr)</th>
<th>Total FU equivalents (nmoles/g)</th>
<th>Acid-extractable</th>
<th>% acid-extractable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal animals</td>
<td>Cerebellum + medulla</td>
<td>24</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Remainder</td>
<td>48</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Cerebellum + medulla</td>
<td>72</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Remainder</td>
<td>72</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Tumor-bearing animals</td>
<td>Cerebellum + medulla</td>
<td>24</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Remainder</td>
<td>21</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

brain and the remainder of the brain. This comparison was made only for the brains from normal animals sacrificed at 24, 48, and 72 hr after drug administration and from tumor-bearing animals sacrificed at 24 hr after drug administration (Table 9). The concentration of total drug equivalents was marginally higher in the cerebellar-medullar portion of the brain than in the remainder of the brain. The concentration of acid-extractable drug equivalents was also higher in the cerebellar-medullar portion than in the remainder. The acid-soluble fractions of brain samples from the tumor-bearing animals of one experiment were analyzed for FU and its metabolites, and it was found that the concentrations of 5-fluorouridine, FUMP, andFdUMP were marginally higher in the cerebellar-medullar fraction than in the remainder of the brain.

DISCUSSION

The kinetics of disappearance of FU from the tissues was similar for all the tissues studied. In all tissues, except kidney, 25% or less of the 72-hr exposure to the drug occurred in the 1st hr, whereas in blood, 60% of the total exposure to FU occurred during this time period. By 24 hr, 80% or more of the 72-hr exposure to FU had occurred in all tissues, except tumor (where only 60% had occurred), whereas in blood, approximately 100% of the total exposure to the drug had occurred. The drug persisted in significant concentrations in all tissues, and particularly in tumor, for hours after its elimination from blood. Retention of FU by the tissues, despite low concentrations in blood, may be due to solubility of the drug in nonaqueous cell compartments or to binding to intracellular structures from which it is acid extractable. FU does not bind to plasma proteins (W. I. Rogers, unpublished data), but binding to other structures cannot be ruled out. If the drug is bound intracellularly, the C X t values calculated for FU would not be based entirely on drug molecules that are available for metabolism.

The anabolites of FU also persisted in significant concentrations for several days after drug administration. The greater part of the 72-hr exposure of tissues to 5-fluorouridine and FUMP, the precursors of the active metabolite, FdUMP, occurred during the 1st 24 hr. Similarly, in bone marrow, liver, and spleen, the greater exposure to FUDP + FUTP also occurred during the 1st 24 hr; however, in tumor, small intestine, and kidney, 60% of the 72-hr exposure occurred after 24 hr. The di- and triphosphates may be incorporated into RNA. FU equivalents were found in the RNA-containing, acid-insoluble fractions of all tissues at all time periods. FU-containing RNA, therefore, persisted in the tissues for 72 hr after drug administration. The incorporation of FU into RNA has various biochemical effects (30), however, the biological and pharmacological consequences of this incorporation are not clear (8, 19).

Some of the most significant observations made in this study were on the disposition of FdUMP. The active metabolite persisted in the tissues for 72 hr after dosing. Between 40 and 70% of the 72-hr exposure of tissues to FdUMP occurred after the 1st 24 hr. By 72 hr, except in bone marrow, FdUMP was the metabolite present in the highest concentrations. The persistence of FdUMP was particularly marked in tumor, where some of the highest concentrations were observed and which had the highest overall C X t value. Furthermore, the concentrations of FdUMP found in the tissues were significant, as the nucleotide is a potent competitive inhibitor of thymidylate synthetase. The reported K_m for dUMP is about 1.5 X 10^-5 M, whereas the K_i is 5.2 X 10^-8 M without preincubation and 3.6 X 10^-9 M with preincubation of the enzyme with FdUMP (19, 38).

The reasons for the persistence of FdUMP in the tissues are not known. Data are lacking on the factors affecting the rate of turnover of FdUMP. As was suggested for FU, binding of FdUMP to intracellular structures may prevent turnover of the nucleotide, offering a possible explanation for its persistence. In this case, the C X t values for FdUMP would be based upon concentrations of acid-extractable FdUMP, of which an unknown percentage would not be free and available for metabolism. Further investigation is necessary to determine whether such binding occurs.

The high concentrations of FdUMP observed in tumor cells might be related to the 50% decrease in tumor weight observed at 48 and 72 hr after female BDF1 mice bearing 6-day solid L1210 lymphocytic leukemia were dosed i.v. with FU, 200 mg/kg, and the mean tumor weights for treated and control animals were determined over 72 hr (I. Wodinsky, unpublished data). However, this effect might also reflect the 20% decrease in body weight of the tumor-bearing animals caused by therapy. Despite the decrease in size of the local tumor, this treatment schedule resulted in an increase in life-span of only 30%, as apparently FU had no lasting effect on the systemic development of the tumor. Other workers (26) demonstrated that the systemic phase of advanced L1210, as measured by...
splenic transplantability, persisted even after treatment resulting in disappearance of the local tumor. We did not assay for the presence of the systemic tumor phase. However, the changes in the physiological disposition of FU in liver and spleen of tumor-bearing animals, discussed earlier, provide indirect evidence for the presence of systemic tumor cells.

The above experiment also shows that all $\chi^2$ values calculated for FU and its metabolites in tumor were based on data obtained from a changing cell population. Consequently, interpretation of these values must be undertaken with caution. The reduction in cell number is particularly important in interpreting the significance of the FdUMP concentration in tumor. The postulated active metabolite increased in concentration over the 1st 24 hr, during which time the surviving tumor cell population may have undergone metabolic changes as a result of oncolytic drug action. The striking increase in FdUMP concentration at 24 hr versus 2 hr may be a result of these changes rather than a cause. Although FdUMP may be responsible for the oncolytic action of FU, the high concentrations found in tumor may not be essential for this action. It is also possible, assuming binding of FdUMP to intracellular structures, that, in the surviving tumor cell population, more of these structures were available for trapping and preventing turnover of the nucleotide, resulting in increased concentrations of acid-extractable FdUMP.

As FdUMP persisted in the tumor in significant concentrations for at least 3 days after a single dose of FU, it should be possible to obtain at least an equivalent antitumor effect by intermittent administration of high doses as by chronic administration of lower doses. An equivalent effect was obtained in an experiment in which FU was administered i.p. in various treatment schedules to CDF1 female mice bearing 5-day solid L1210 lymphocytic leukemia and to normal mice (Table 10; I. Wodinsky, unpublished data). For this experiment, the CDF1 and BDF1 mice were interchangeable, and a 5-day tumor was used, since it is not systemic (16) and so increased the probability of obtaining a significant antitumor effect.

The toxic dose of the drug for normal mice is close to the optimal dose for antileukemic activity, so that the therapeutic range is narrow and was not improved by changing the dosage regimen (Table 10). The poor therapeutic indices of intermittent doses at 2- and 4-day intervals may be consistent, assuming similar kinetics of FdUMP disposition, with our finding that the relative concentrations of the active metabolite in tumor and in small intestine, the major site of toxicity in mice, did not change significantly over 3 days. Because of the lethality of the advanced tumor, the effect of intermittent large doses at intervals greater than 4 days could not be determined. With a less advanced tumor and greater intervals between doses, the antitumor effect may be improved without a corresponding increase in toxicity.

The persistence of FdUMP in the small intestine and in the bone marrow may also be correlated with the observed toxic effects of the drug in normal animals. In small intestine, the major site of toxicity in both mice and humans, the maximum FdUMP concentration was reached by 1 hr, and this concentration persisted for at least 3 days after the mice were dosed. This observation correlates with the finding that intermittent administration of high doses and chronic administration of lower doses of FU produced equivalent toxic effects (Table 10). The persistence of FdUMP in the small intestine may also explain the finding of Urano et al. (42) that, after treatment of mice with FU, a single plateau was demonstrated on the average survival time versus FU concentration curve at 6.5 days. Although the animals that died demonstrated symptoms of intestinal toxicity, the authors concluded that this somewhat delayed death might not represent the so-called "intestinal death" because it was not consistent with the rapid clearance of FU itself from the body. However, this finding would be consistent with the persistence of FdUMP in the small intestine.

Only low concentrations of FdUMP were observed in the bone marrow, although this is a site of toxicity in mice and of delayed toxicity in humans. The dose of FU administered, however, was shown to be toxic to mouse bone marrow, as demonstrated by a decrease in the number of spleen colony-forming units. Following an i.p. dose of FU at 200 mg/kg, the number of colony-forming units from normal bone marrow decreased to 0.3% of their normal value by 48 hr after dosing and took 6 days to return to normal, indicating that there was extensive damage to the stem cells (I. Wodinsky, unpublished data). A similar effect should be produced after i.v. administration; however, it may be less severe because FU is known to be more toxic via the i.p. versus the i.v. route (22). FU is less toxic to normal bone marrow cells than to L1210 cells (7). However, this appears to be related largely to the proliferative state of the cells (7, 28) and not to the striking difference in FdUMP concentrations in these 2 groups of cells.

The distribution of FdUMP and the other metabolites in the cerebellar-medullar portion and the remainder of mouse brain between 24 and 72 hr after drug administration was particularly interesting because of the whole-body radioautographic data of Liss et al. (27). In the

<table>
<thead>
<tr>
<th>Therapy regimen</th>
<th>Total daily optimal dose (mg/kg)</th>
<th>Increase in life-span (%) of leukemic mice</th>
<th>Mortality of normal mice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 5 only</td>
<td>200</td>
<td>47</td>
<td>12</td>
</tr>
<tr>
<td>Days 5–10</td>
<td>25</td>
<td>47</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td>Days 5–13</td>
<td>25</td>
<td>41</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>46</td>
<td>100</td>
</tr>
<tr>
<td>Every 2 days on Days 5, 7, 9, 11, 13</td>
<td>100</td>
<td>65</td>
<td>62</td>
</tr>
<tr>
<td>Every 4 days on Days 5, 9, 13</td>
<td>100</td>
<td>57</td>
<td>62</td>
</tr>
</tbody>
</table>

a I. Wodinsky, unpublished data.
cerebellar-medullar portion, there was a lower percentage of
drug incorporation into the acid-insoluble fraction, resulting in
a comparatively high proportion of drug equivalents in the
acid-soluble fraction. The whole-body radioautographic studies
indicated that, within the cerebellum, the radioactivity was
concentrated in the Purkinje and granular cell layers. These
observations are significant in view of reports of an acute
cerebellar syndrome involving cerebellar ataxia that occurred
after FU therapy in 3% of patients and that could be reversed
by withdrawal of the drug, reduction of the dose, or
lengthening of the interval between treatments (31, 36).
Furthermore, loss of neurons in the granular layer of the
cerebellum was observed in 1 patient who died during FU
therapy, and similar neuropathological lesions were observed
in cats that died after FU treatment (36). Assuming that the
localization of FdUMP parallels the localization of
radioactivity, it is possible that the relatively high FdUMP
concentrations in specific areas of the cerebellum may be
correlated with the cerebellar toxicity. Recently, however,
Koenig and Patel (24, 25) have suggested that the cerebellar
syndrome may be a result of the inhibition of the Krebs cycle
by fluorocitrate produced from FU.

The finding that FdUMP persists in mouse tissues after FU
administration is consistent with a number of observations
made by other workers. It was demonstrated in mouse L-cells
in tissue culture that the growth-inhibitory effect of FU
persisted 80 hr or more after exposure to the drug (29).
Furthermore, the inhibitory effect of FU on DNA synthesis in
Ehrlich ascites tumor cells in vivo was found to occur to a
greater degree and for longer duration than in liver cells (15).
Similarly, other workers observed inhibition of DNA synthesis
of long duration in Sarcoma 180 cells in vivo (40). Also,
although cells are most sensitive to FU during the DNA
synthesis phase of the cell cycle (1), exposure to FU during all
phases of the generation cycle results in cell death (7). This
might be explained by the persistence of the active metabolite,
which allows time for cells to enter the sensitive phase. The
lack of correlation observed between the doubling time of
human buffy coat cells growing exponentially in tissue culture
and their sensitivity to FU (3) might be explained by the
persistence of the active metabolite in these cells, resulting in
equal growth inhibition of the more slowly dividing as well as
the more rapidly dividing cells.

Nothing is known of the levels of FdUMP in human tissues
after FU administration, either in sensitive tumors or in those
tissues in which toxicity occurs. There is some indirect
evidence, however, that similarities may exist between the
rates of appearance and disappearance of FdUMP in human
tissues and those found in the mouse. The persistence of
FdUMP in the solid L1210 tumor appears to be consistent
with the clinical effectiveness of FU against some
comparatively slow-growing human tumors and is possibly
consistent with the reported equal responsiveness of human
colorectal tumors to FU administered by single weekly
injection (15 mg/kg) rather than in monthly courses (15
mg/kg/day for 5 days followed by 7.5 mg/kg every other day
until mild toxicity ensues) (23, 33). The persistence of FdUMP
in the mouse small intestine appears to be consistent with the
reports that gastrointestinal toxicity is reduced when FU is
administered to patients by single weekly injection rather than
in intensive monthly courses (23, 33). The extended interval
between weekly doses presumably allows time for the FdUMP
concentration to decrease before the next dose is administered
and for the mucosal cell layer to recover from the toxic effects
of the previous dose. Similarly, the increased interval may
account for the less severe hematopoietic toxicity observed
(23, 33). In mice, at least, there was evidence from the
comparison of FdUMP concentrations for increasing selectivity
of FU for tumor relative to bone marrow with increasing time
after dosage. Our data in mice are consistent with the
preference that some clinicians have for single, weekly doses
over the intensive monthly courses administered by others.

ACKNOWLEDGMENTS

We wish to thank Mr. Isidore Wodinsky for providing us with
experimental therapeutics data, Drs. David W. Yesair and John J. Coffey
for their helpful suggestions during the preparation of this manuscript,
and Dr. Charles J. Kensler for his encouragement and support. The
technical assistance of Miss Charmaine Chang and Mr. Michael Mazrimas
is gratefully acknowledged.

REFERENCES

1. Adams, J. E., Breed, N. L., and Valenti, C. Enhancement of
5-Fluorouracil Cytotoxicity in Synchronized Human Malignant
(ed.), p. 9, Washington: Federation of American Societies for
Experimental Biology, 1961.
3. Aoki, Y., and Moore, G. E. Comparative Sensitivity to Various
Antimetabolites of Several Established Cell Lines Derived from the
Buffy Coat of Normal Humans and Patients with Neoplastic
4. Birnie, G. D., Kroeger, H., and Heidelberger, C. Studies of
Fluorinated Pyrimidines. XVIII. The Degradation of
5-Fluoro-2'-deoxyuridine and Related Compounds by Nucleoside
5. Bosch, L., Harbers, E., and Heidelberger, C. Studies on Fluorinated
Pyrimidines. V. Effects on Nucleic Acid Metabolism in vitro.
Kinase Partially Purified from Animal Tumors. J. Biol. Chem., 240:
7. Bruce, W. R., and Meeker, B. E. Comparison of the Sensitivity of
Hematopoietic Colony-Forming Cells in Different Proliferative
8. Bujard, H., and Heidelberger, C. Fluorinated Pyrimidines. XXVII.
Attempts to Determine Transcription Errors during the Formation
of Fluorouracil-Containing Messenger Ribonucleic Acid.
9. Chadwick, M., and Rogers, W. I. The Distribution of
5-Fluoro-2'-deoxyuridine-5'-monophosphate in Mice after
14, 1970.
10. Chaudhuri, N. K., Montag, B. J., and Heidelberger, C. Studies on
Fluorinated Pyrimidines. III. The Metabolism of 5-Fluorouracil-2-
14C and 5-Fluoroorotic-2-14C Acid in Vivo. Cancer Res., 18:
11. Chaudhuri, N. K., Mukherjee, K. L., and Heidelberger, C. Studies on
Fluorinated Pyrimidines. VII. The Degradative Pathway.


The Physiological Disposition of 5-Fluorouracil in Mice Bearing Solid L1210 Lymphocytic Leukemia

Marjory Chadwick and William I. Rogers


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/32/5/1045

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.