Pharmacokinetics of Fluorouracil in Humans

William E. MacMillian, William H. Wolberg, and Peter G. Welling

Centre for Health Sciences, Department of Human Oncology [W. H. B.], School of Medicine, and School of Pharmacy [W. E. M., P. G. W.], University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

A high-pressure liquid chromatography method has been used to investigate the pharmacokinetics of fluorouracil after single i.v. doses to patients. The method is simple and is specific for fluorouracil. No interference was observed in fluorouracil determinations due to other administered medication. Plasma levels of fluorouracil declined rapidly after dosing. The mean half-life was 11.4 min, and drug was essentially cleared from plasma in 1 hr. Individual differences in plasma fluorouracil levels, and also in derived pharmacokinetic constants, were considerably less than those reported previously.

INTRODUCTION

The study of fluorouracil pharmacokinetics has until recently been impeded by the lack of a sensitive and specific assay. Methods for analyzing fluorouracil in biological fluids with gas chromatography (1), high-pressure liquid chromatography (4), and a microbial kinetic assay (2) have now been described.

Two reports have described the pharmacokinetics of fluorouracil following i.v. doses. One (4) utilized a 1-compartment kinetic model and reported considerable variation in fluorouracil disposition in different patients. The other study (2) suggested that fluorouracil kinetics may be described by the 1-compartment or 2-compartment model. In the latter study, conducted in 3 patients, some dose dependency in fluorouracil elimination kinetics was observed, elimination rates decreasing with increased dose. The small number of patients precluded interindividual comparisons.

In this study the pharmacokinetics of fluorouracil was examined in detail in 8 patients receiving fluorouracil therapy, with the use of a simple and specific method involving high-pressure liquid chromatography. The method is suitable for routine laboratory use.

MATERIALS AND METHODS

Subjects and Protocols. Subjects were 8 patients in the clinical oncology wards of the University Hospitals, Madison, Wis. Subject statistics and diagnostic data are described in Table 1. Subjects were ambulatory and were taking solid food. Fluorouracil was administered by rapid (30-sec) i.v. injection. Dose size was determined by the attending physician and was not influenced by the study. In every case blood samples were taken following the initial dose of a repeated dose regimen. No fluorouracil therapy had been received previously. Blood samples (5 ml) were taken from a different forearm vein from that used for dosing and were placed in heparinized tubes (Vacutainer; Becton-Dickinson, Rutherford, N. J.). Samples were drawn immediately before and at 5, 10, 20, 30, 60, 120, and 180 min after dosing. Plasmas were promptly separated and stored at -20° until assayed.

Patients 1, 2, and 8 were on no other medication. Patients 3 and 4 were receiving Diabinese. Patient 5 also received methylcholoroethylcyclohexylnitrosourea. Patient 6 was receiving Bactrim and Patient 7 was receiving tamoxifen. None of these medications interfered with the assay for fluorouracil.

Assay. Fluorouracil was extracted from plasma by the method of Cohen et al. (1) except that ammonium sulfate was used to saturate plasma in place of sodium sulfate. Ten ml of the organic phase, 16% n-propyl alcohol in ether, were evaporated to dryness at 50° under nitrogen. The residue was redissolved in 50 μl 10⁻² M potassium phosphate buffer, pH 5.5, containing 10⁻³ M thymidine as external standard.

Chromatography was carried out with a μBondapak C₁₈ 4-mm x 30-cm column (Waters Associates, Milford, Mass.) preceded by a guard column (CO: Pell ODS; Whatman, Inc., Clifton, N. J.). The chromatograph consisted of 2 Waters Associates M6000 pumps and a Model M660 solvent programmer, a Waters U6K injector, and a Laboratory Data Control Model 1201 UV-VIS spectrophotometer and chart recorder.

After sample injection the column was eluted for 11 min with 10⁻² M phosphate buffer, pH 5.5. The solvent was then changed in a single step to 8% methanol in phosphate buffer, pH 5.5. The solvent flow rate was 1.2 ml/min. Fluorouracil was measured by the peak height ratio method against thymidine. The calibration curve for fluorouracil was linear between 0.1 and 130 μg/ml in plasma, and assay accuracy was routinely 8%. Commonly occurring nucleosides did not interfere with chromatographic peaks of either fluorouracil or thymidine. Typical chromatograms are given in Chart 1.

Fluorouracil standard (Lot 578103) was obtained from Roche Laboratories, Nutley, N. J., and thymidine (A. R.) was from Sigma Chemical Co., St. Louis, Mo. All other chemicals and reagents were of the highest grade available and were used as received.

RESULTS

Individual plasma levels of fluorouracil are given in Table 2. Means ± S.E. are shown both for the original data and for plasma levels expressed as a percentage of the dose.
Table 1
Subject statistics and diagnostic data

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Wt (kg)</th>
<th>WBC 10^6/cu mm</th>
<th>RBC 10^6/cu mm</th>
<th>BUN (mg%)</th>
<th>SGOT units/ml</th>
<th>SGPT units/ml</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>40</td>
<td>178</td>
<td>78</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>64</td>
<td>170</td>
<td>71</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>44</td>
<td>40</td>
<td>Colon carcinoma, liver metastases</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>72</td>
<td>173</td>
<td>73</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>20</td>
<td>10</td>
<td>Colon carcinoma</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>48</td>
<td>170</td>
<td>115</td>
<td>7.4</td>
<td>5.0</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>Carcinoma of rectum, liver metastases</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>52</td>
<td>178</td>
<td>68</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>-</td>
<td>Adenocarcinoma of stomach</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>69</td>
<td>172</td>
<td>65</td>
<td>4.7</td>
<td>3.3</td>
<td>21</td>
<td>26</td>
<td>-</td>
<td>Carcinoma of prostate</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>36</td>
<td>167</td>
<td>87</td>
<td>9.7</td>
<td>4.8</td>
<td>7</td>
<td>16</td>
<td>-</td>
<td>Right radical mastectomy</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>67</td>
<td>175</td>
<td>70</td>
<td>7.2</td>
<td>3.9</td>
<td>14</td>
<td>23</td>
<td>23</td>
<td>Adenocarcinoma of sigmoid colon</td>
</tr>
</tbody>
</table>

Mean ± S.E. 56 ± 5 173 ± 1 78 ± 6

a BUN, blood urea nitrogen; SGOT, aspartate aminotransferase; SGPT, alanine aminotransferase.
b - , not reported.

Chart 1. Result of high-pressure liquid chromatography of extracted human plasma containing (A) only endogenous thymidine and (B) 3.64 μg fluorouracil (5-FU) and 1.21 μg thymidine, as external standard.

The latter values are summarized on a semilogarithmic scale in Chart 2.

Plasma drug levels declined rapidly after dosing, and the mean elimination half-life was 11.4 min. Although plasma samples were taken at 120 and 180 min postdosing, any fluorouracil present at those times was generally below the detection limit of the assay (<0.1 μg/ml).

In most cases plasma fluorouracil profiles dropped more sharply during the 5- to 10-min period than at later times. This was at first interpreted in terms of a distribution phase and an elimination phase, typically described in terms of the pharmacokinetic 2-compartment open model (2). Analysis of individual data in terms of this model, however, frequently yielded unrealistically small drug distribution volumes. In some cases the volume of the central drug compartment was calculated to be less than that of plasma water. These anomalous distribution values suggest that mixing of drug was incomplete during at least part of the early blood sampling period and that the apparent "distribution" phase was either complicated by or due solely to a drug-mixing phenomenon.

Because the 5-min data point was thus unreliable, individual fluorouracil profiles were analyzed in terms of Equation A (5) which is appropriate to the pharmacokinetic 1-compartment open model.

\[
C = \frac{k_d}{V_{tr}} [1 - e^{-k_{el}T} e^{-k_{el} (T - t)}] \tag{A}
\]
Table 2

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose (mg/kg)</th>
<th>0 min</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.9</td>
<td>0</td>
<td>123</td>
<td>22</td>
<td>13</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>11.9</td>
<td>0</td>
<td>47</td>
<td>29</td>
<td>8.7</td>
<td>2.6</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>12.0</td>
<td>0</td>
<td>59</td>
<td>43</td>
<td>20</td>
<td>16</td>
<td>6.3</td>
</tr>
<tr>
<td>4</td>
<td>8.7</td>
<td>0</td>
<td>26</td>
<td>17</td>
<td>4.8</td>
<td>3.2</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>8.5</td>
<td>0</td>
<td>73</td>
<td>19</td>
<td>6.6</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>12.0</td>
<td>0</td>
<td>77</td>
<td>34</td>
<td>17</td>
<td>6.8</td>
<td>0.1</td>
</tr>
<tr>
<td>7</td>
<td>11.5</td>
<td>0</td>
<td>51</td>
<td>31</td>
<td>26</td>
<td>19</td>
<td>2.9</td>
</tr>
<tr>
<td>8</td>
<td>11.5</td>
<td>0</td>
<td>31</td>
<td>18</td>
<td>8.2</td>
<td>3.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Mean ± S.E. 10.9 ± 0.1 0 ± 0 61 ± 11 27 ± 3.3 13 ± 2.6 7.5 ± 2.2 1.7 ± 0.8

Mean ± S.E.a 0 ± 0 76 ± 15 32 ± 3.7 15 ± 2.6 8.7 ± 2.3 1.6 ± 0.9

a Mean ± S.E. of plasma fluorouracil levels expressed as 10^4 × percentage of dose per ml of plasma.

Table 3

<table>
<thead>
<tr>
<th>Subject</th>
<th>k_{el} (min^-1)</th>
<th>T_{1/2} (min)</th>
<th>V (% body wt)</th>
<th>V_kel (ml/min)</th>
<th>AUC^b (µg • min/ml)</th>
<th>r^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.063</td>
<td>11.0</td>
<td>25.0</td>
<td>1229</td>
<td>2130</td>
<td>-1.0</td>
</tr>
<tr>
<td>2</td>
<td>0.083</td>
<td>8.3</td>
<td>23.8</td>
<td>1403</td>
<td>860</td>
<td>-0.98</td>
</tr>
<tr>
<td>3</td>
<td>0.036</td>
<td>19.4</td>
<td>23.8</td>
<td>626</td>
<td>1530</td>
<td>-0.98</td>
</tr>
<tr>
<td>4</td>
<td>0.063</td>
<td>11.1</td>
<td>36.9</td>
<td>2671</td>
<td>480</td>
<td>-0.98</td>
</tr>
<tr>
<td>5</td>
<td>0.091</td>
<td>7.6</td>
<td>18.5</td>
<td>1147</td>
<td>1650</td>
<td>-1.0</td>
</tr>
<tr>
<td>6</td>
<td>0.120</td>
<td>5.8</td>
<td>7.1</td>
<td>552</td>
<td>1640</td>
<td>-0.99</td>
</tr>
<tr>
<td>7</td>
<td>0.050</td>
<td>14.0</td>
<td>17.7</td>
<td>770</td>
<td>1380</td>
<td>-0.98</td>
</tr>
<tr>
<td>8</td>
<td>0.051</td>
<td>13.7</td>
<td>48.1</td>
<td>1719</td>
<td>610</td>
<td>-0.98</td>
</tr>
</tbody>
</table>

Mean ± S.E. 0.070 ± 0.010 11.4 ± 1.5 25.1 ± 4.4 1265 ± 246 1285 ± 204

a Plasma clearance.

b Area under plasma fluorouracil level versus time curve, calculated by trapezoidal rule.

c Correlation coefficient from linear regression of 10- to 60- min log fluorouracil levels versus time.

C is the concentration of fluorouracil in plasma, k_{el} is the drug infusion rate, T is the duration of infusion (30 sec), t is the total time since the start of infusion, and V is the apparent distribution volume of fluorouracil in the body.

The value of k_{el} was obtained by linear regression analysis of 10- to 60-min plasma levels, the value of C at 30 sec was obtained by extrapolation from the terminal elimination phase, and V was calculated by substituting known values of other constants into Equation A and rearranging.

Values of pharmacokinetic constants thus obtained are given in Table 3. Fluorouracil distributes into an apparent volume equivalent to 25% body weight and has a total plasma clearance of approximately 1300 ml/min, which is greater than normal hepatic plasma flow.

The elimination half-life of fluorouracil ranged from 5.8 to 19.4 min, although there was no evidence of dose-dependent elimination kinetics over the rather narrow dosage range used.

DISCUSSION

The assay used in this study is simple and is specific for fluorouracil. It has the advantage over previous methods in incorporating an external standard for accuracy and is not influenced by other medication, at least not those taken by our subjects. The retention time of thymidine during liquid chromatography was rate limiting during the assay procedure, and substitution of an external standard with a smaller retention volume would decrease the overall assay time.

One major advantage of the liquid chromatography procedure is its potential for adaptation to investigate the kinetics and disposition of fluorouracil metabolites.

The plasma levels of fluorouracil are consistent with those reported by Garrett et al. (2). Despite the different models used in data interpretation, numerical values of pharmacokinetic constants are also similar in the 2 studies.

The mean plasma drug half-life (ln 2/k_{el}), distribution volume, and plasma clearances of 11.4 min, 25% body weight, and 1265 ml/min, respectively, obtained in the 1-compartment model interpretation are in close agreement with equivalent values of 10.9 min (ln 2/β), 33% body weight, and 1441 ml/min obtained by 2-compartment model analysis (2).

Whether the 1-compartment or the 2-compartment model is more appropriate in the analysis of fluorouracil data is probably an academic question, particularly in view of the rapid clearance of the drug. However, interference by drug mixing with drug distribution calculations is a real problem and may lead to erroneous interpretation of blood level data, particularly for rapidly cleared drugs where data points are ideally required at very early times after dosing.

The pharmacokinetic values obtained from the 2 studies differ from those of Sitar et al. (4), who reported a mean plasma half-life for fluorouracil of 20.3 min and a mean overall distribution volume of 89% body weight. There was
also considerably less variation in plasma levels and pharmacokinetic parameters in the present study compared to those reported by Sitar et al. The different extent of individual variation observed in the 2 studies may be due to the analytical methods or to the patients used.

The results we have obtained do not, however, detract from the hypothesis that differences in fluorouracil kinetics may account for some of the variation in patient response to therapy (4). This hypothesis, although intriguing, must be examined in a large number of patients and must consider the complex metabolism and pharmacology of fluorouracil (3, 6).

**ACKNOWLEDGMENTS**

We thank the staff nurses in the clinical oncology wards of University Hospitals for their help in carrying out this study.

**REFERENCES**

Pharmacokinetics of Fluorouracil in Humans

William E. MacMillan, William H. Wolberg and Peter G. Welling


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/10/3479

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, use this link
http://cancerres.aacrjournals.org/content/38/10/3479.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.