Disposition of 5-Fluorouracil after Intravenous Bolus Doses of a Commercial Formulation to Cancer Patients

Daniel S. Sitar, Jr., Douglas H. Shaw, Jr., Michael P. Thirlwell, and John R. Ruedy

Departments of Medicine [D. S. S., D. H. S., M. P. T., J. R. R.], Surgery [M. P. T.], and Pharmacology and Therapeutics [D. S. S., J. R. R.], McGill University, The Montreal General Hospital, 1650 Cedar Ave., Montreal, Quebec, H3G 1A4, Canada

SUMMARY

A high-pressure liquid chromatographic method that is used to determine the pharmacokinetic disposition of 5-fluorouracil from the plasma compartment is presented. The method requires only 0.5 ml of plasma for each determination and is sensitive to 0.1 mg of drug per liter. Novel methodology with the use of an ion-specific electrode technique for the determination of urinary excretion kinetics of 5-fluorouracil and its metabolites is also presented. This study demonstrated a much greater variability for the disposition of 5-fluorouracil by cancer patients than has been reported previously. The apparent volume of distribution for this drug varied more than 37-fold. Its plasma half-life varied more than 19-fold, and its urinary excretion half-life varied almost 400-fold. These data are compatible with the hypothesis that this variation could account, at least in part, for the variable therapeutic and toxic response to 5-fluorouracil. The methodology presented in this study is sufficiently simple and sensitive to allow assessment of this hypothesis by investigating cancer patients who receive therapeutic doses of 5-fluorouracil.

INTRODUCTION

There is a lack of information concerning the correlation of therapeutic and toxic effects with the kinetic disposition of drugs used in the treatment of cancer. This is due largely to the lack of sensitive and specific assays that can be used to determine drug disposition after therapeutic doses. 5-Fluorouracil is an important and commonly used drug in the treatment of many solid tumors (2). The need for further pharmacokinetic studies on its disposition in humans has been recently emphasized (9). Specific gas-liquid chromatographic (3) and mass spectrometric assays (5, 7) have been developed to determine 5-fluorouracil in biological samples. Both methods require that a derivative be formed before the drug can be analyzed. In the present study we report a method for analysis of 5-fluorouracil in plasma by high-pressure liquid chromatography that is sensitive to 0.1 mg/liter, requires only 0.5 ml of plasma for each determination, and does not require that a derivative be formed before the drug can be analyzed. The drug and its metabolites are excreted mainly by the kidney in humans (4). We also describe a method that utilizes an ion-specific electrode technique to follow urinary excretion of 5-fluorouracil and its metabolites. These methods were used to investigate the kinetic disposition of i.v. 5-fluorouracil in 16 cancer patients.

MATERIALS AND METHODS

Criteria for Volunteers and Protocol. Sixteen ambulatory patients with the diagnosis of cancer of the gastrointestinal tract who were to receive weekly doses of i.v. 5-fluorouracil as part of their therapy gave consent to participate in this study after being informed of its nature and purpose. The dose of 5-fluorouracil was chosen by the attending physician and was not influenced by this investigation.

A single i.v. dose of 5-fluorouracil (Hoffmann-La Roche, Ltd., Montreal, Quebec, Canada) was administered over 1 to 1.5 min. Blood samples were taken from an indwelling venous cannula with heparin lock into tubes containing sodium oxalate at the following time intervals: predose and 5, 10, 15, 30, 45, 60, 90, and 120 min after the end of the injection (total volume, 54 ml). Plasma was separated. Complete spontaneously voided urine samples were also obtained from some of these patients predose and at timed intervals until at least 48 hr after drug administration. The urine samples were not pooled, so that the kinetics of elimination of the dose could be investigated. All samples were frozen and stored at −20° until analyzed.

Determination of 5-Fluorouracil by High-Pressure Liquid Chromatography. Plasma (0.5 ml) was mixed with ammonium sulfate (0.2 g) in a round-bottom test tube. Isopropyl alcohol:chloroform (25:75, 10 ml) was added, and the plasma was extracted by shaking for 20 min. The tubes were centrifuged, and 9.0 ml of the organic (lower) layer were removed. The solvent was evaporated with heat in a nitrogen atmosphere, and the residue was dissolved in isopropyl alcohol:chloroform (15:85, 50 μl). An aliquot of this solution was injected on a column for determination of 5-fluorouracil by high-pressure liquid chromatography. The instrument was a Varian Model 4100 with variable wavelength detector (Varian Instruments, Georgetown, Ontario, Canada). The column was a 3-mm x 50-cm stainless steel tube packed with silica gel particles (10 μm in diameter). The column was eluted with isopropyl alcohol:acetic acid:chloroform (15:1:84). The solvent flow rate was 40 ml/
hr at a pressure of $7 \times 10^4$ pascals. Detector wavelength was 272 nm with a 2-nm bandpass. A calibration curve was constructed by adding known amounts of 5-fluorouracil to blank plasma samples and subjecting them to analysis as described above.

**Column Chromatography of Urine Samples.** Urine samples (10 ml) were applied to a 2.5- x 76-cm polyacrylamide gel column (Bio-Gel P-2, 200 to 400 mesh), and the sample was eluted by ascending chromatography at a flow rate of 90 ml/hr (Ismatic Pump; Brinkman Instruments, Toronto, Ontario, Canada) with double-distilled water. The column eluant was monitored at 254 nm. Fractions (6.0 ml) were collected and analyzed for 5-fluorouracil and metabolites as described below.

**Determination of 5-Fluorouracil and Metabolites in Urine and Column Fractions.** The method is a modification of an earlier reported procedure for the measurement of urinary fluoride (1). Urine or column eluant (3.0 ml) and perchloric acid (3.0 ml) were mixed and heated in a sealed tube in a boiling water bath for 30 min. The tube was allowed to cool to room temperature, and sodium hydroxide (3.0 ml) and water (1.0 ml) were added. After mixing, 5.0 ml of this solution were added to 5.0 ml of total ionic strength adjustment buffer containing (1,2-cyclohexene-1,2-dimethanol)tetraacetic acid to complex polyvalent cations, which interfere with fluoride ion determinations (10). Fluoride ion was then measured with an ion-specific electrode (Orion 94-09; Canlab, Montreal, Quebec, Canada). All determinations were corrected for concentration of free fluoride ion present before perchloric acid oxidation. A calibration curve was constructed by adding known amounts of 5-fluorouracil to blank urine specimens or water and subjecting them to fluoride ion analysis as described above.

**Chemicals.** All chemicals used in this investigation were of the highest grade available from commercial suppliers. Pure 5-fluorouracil was provided by Hoffmann-La Roche, Ltd.

**Data Analysis.** Calibration curves for 5-fluorouracil and fluoride ion were derived by linear regression analysis of the data, followed by analysis of variance of the goodness of fit of the data to the calibration line. Pharmacokinetic analyses of the disappearance of the drug from the plasma and the excretion of 5-fluorouracil and metabolites into urine were accomplished by assuming a 1-compartment open model with 1st-order elimination. Urinary excretion $t_{1/2}$ was determined from data points derived by subtraction of the amount of fluorine-containing compounds in urine samples from the total dose administered (11). Pharmacokinetic constants for $t_{1/2}$, $C_{au}$, $V_d$, and $Cl_d$ were compared by an unpaired $t$ test. The minimum level for a significant difference was accepted to be $p = 0.05$, with the use of a 2-tailed test (6).

**RESULTS**

The efficiency of the extraction of 5-fluorouracil from plasma was 71%. Chart 1 illustrates representative chromatograms for the high-pressure liquid chromatography of an extracted plasma blank and a plasma sample that contained 5-fluorouracil. There was no interference by naturally occurring substances in the plasma sample, and there was no interference by vincristine, cyclophosphamide, diphenoxylate, atropine, diethylstilbestrol, pyridium, nystatin, pentobarbital, diazepam, furosemide, multivitamins, or any metabolites of these substances that might have been circulating in the plasma of subjects investigated in this study. A representative calibration curve for the high-pressure liquid chromatographic analysis of 5-fluorouracil is presented in Chart 2. The curve is linear to at least 80 mg/liter for this drug. The lower limit of detection is 0.1 mg/liter. The S.E. of the slope for the calibration line is 1.2%.

In whole blood, values for 5-fluorouracil concentration are 88% of those found in plasma. This could indicate binding to plasma proteins and/or limited distribution into RBC. We could follow plasma concentrations of 5-fluorouracil for at least 2 hr with this methodology.

Table 1 illustrates the characteristics of the patient population in this study. These patients represent an older population. Although female patients weigh less than

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3 The abbreviations used are: $t_{1/2}$, half-life; $C_{au}$, extrapolated plasma concentration to $t = 0; V_d$, apparent volume of distribution; $Cl_d$, plasma clearance.
males, the difference is not statistically significant. The administered dose of 5-fluorouracil was similar for both groups when compared on a mg/kg or mg/sq m basis. Table 2 presents the pharmacokinetic data for the elimination of 5-fluorouracil from the plasma compartment. Elimination was consistent with a 1-compartment open model. The plasma t_{1/2} was very short, but showed a greater than 19-fold variation. The V_d showed a greater than 37-fold variation, which was reduced by approximately 30% when body weight was taken into consideration. C_l was the most variable, approaching 400-fold. There was no indication of dose-dependent elimination from the plasma. There were no statistically significant differences between male and female patients in the elimination of 5-fluorouracil from the plasma.

A representative curve for the elimination of a dose of 5-fluorouracil and its metabolites into the urine is presented in Chart 3. In this population, the mean elimination t_{1/2} as determined from urinary excretion data was about 15 days. The 3-fold difference between male and female patients (Table 3) is not significantly different and is overshadowed by an overall 250-fold variation in elimination t_{1/2} in the total group. All these patients had creatinine clearance values within normal limits.

A representative chromatogram for the gel filtration of a urine sample from a cancer patient receiving 5-fluorouracil is presented in Chart 4. When the urinary content of organically bound fluorine was compared with organically bound fluorine-containing compounds eluted from the column, recovery was calculated to be 96%. It is readily apparent that absorbance determination was not useful for the isolation of 5-fluorouracil and its metabolites. However, analysis of each fraction for organically bound fluorine allowed us to monitor the nature and extent of the excretion of the drug and metabolites into urine. We were unable to detect unchanged drug in the urine of these patients. This was confirmed by freeze-drying the fractions where 5-fluorouracil should have eluted (65 to 70) and subjecting the residue to high-pressure liquid chromatographic analysis. Experiments in which 5-fluorouracil was added to control urine samples before column chromatographic analysis showed no shifting of elution time because of the presence of other substances in the sample. The metabolites of 5-fluorouracil detected by this technique have not yet been characterized in this laboratory.

**DISCUSSION**

Our study demonstrates a feasible methodology for the investigation of 5-fluorouracil disposition after therapeutic administration.

**Table 1. Characteristics of the patient population receiving 5-fluorouracil chemotherapy**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Dose (mg/kg)</th>
<th>Body surface area (sq m)</th>
<th>Dose (mg/sq m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (10)</td>
<td>62 ± 3</td>
<td>62 ± 3</td>
<td>11.6 ± 1.0</td>
<td>1.68 ± 0.05</td>
<td>435 ± 39</td>
</tr>
<tr>
<td>Female (6)</td>
<td>64 ± 4</td>
<td>50 ± 5</td>
<td>11.3 ± 1.1</td>
<td>1.43 ± 0.07</td>
<td>391 ± 26</td>
</tr>
<tr>
<td>Total (16)</td>
<td>61 ± 2</td>
<td>58 ± 3</td>
<td>11.5 ± 0.8</td>
<td>1.60 ± 0.05</td>
<td>421 ± 28</td>
</tr>
<tr>
<td>(49-80)</td>
<td>(37-83)</td>
<td>(7.6-17)</td>
<td>(1.29-2.04)</td>
<td>(311-575)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, range.

**Table 2. Pharmacokinetic constants for the elimination of 5-fluorouracil from the plasma of cancer patients**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Plasma t_{1/2} (min)</th>
<th>C_(l) (mg/liter)</th>
<th>C_l (ml/min)</th>
<th>V_d (liters)</th>
<th>V_d/body wt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (10)</td>
<td>17.8 ± 5.8</td>
<td>30.2 ± 9.6</td>
<td>1.88 ± 0.86</td>
<td>41.8 ± 12.3</td>
<td>66 ± 16</td>
</tr>
<tr>
<td>Female (6)</td>
<td>24.4 ± 8.4</td>
<td>32.0 ± 11.6</td>
<td>3.11 ± 2.36</td>
<td>75.1 ± 40.0</td>
<td>126 ± 62</td>
</tr>
<tr>
<td>Total (16)</td>
<td>20.3 ± 4.7</td>
<td>30.9 ± 7.1</td>
<td>2.34 ± 1.00</td>
<td>54.3 ± 16.6</td>
<td>89 ± 25</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, range.

**Chart 3. Representative curve for the elimination of 5-fluorouracil and its metabolites into urine after an i.v. dose. The ordinate is the portion of the total dose remaining in the body over time that has not yet been excreted in urine.**

**Table 3. Pharmacokinetic constants for the elimination of a dose of 5-fluorouracil into urine by cancer patients**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean ± S.E.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (8)</td>
<td>18.24 ± 10.12</td>
<td>1.08-84.92</td>
</tr>
<tr>
<td>Females (3)</td>
<td>6.15 ± 5.42</td>
<td>0.34-16.98</td>
</tr>
<tr>
<td>Total (11)</td>
<td>14.94 ± 7.53</td>
<td>0.34-84.92</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number in group.
The large variation in elimination from the body suggests a possible mechanism for the accumulation of the drug, with successive doses resulting in toxicity, or, conversely, the rapid elimination of the dose, resulting potentially in failure to demonstrate a therapeutic effect. Our inability to detect unchanged 5-fluorouracil in the urine of these patients is in agreement with a recent report by Cohen et al. (4), but not with the report by Mukherjee et al. (8). Ongoing investigations in our laboratory are assessing the possibility that alteration in urinary excretion of 5-fluorouracil and its metabolites can account for some of the variation in response to therapeutic doses.

ACKNOWLEDGMENTS

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REFERENCES

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