Comparison of Pharmacokinetics of 5-Fluorouracil and 5-Fluorouracil with Concurrent Thymidine Infusions in a Phase I Trial

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ABSTRACT

The serum half-life of 5-fluorouracil (5-FUra) in humans is best described as a biexponential decay function, with $t_{1/2} = 7.8 \pm 2.6$ (S.E.) min and $t_{1/2} = 36.8 \pm 13.5$ min during initial courses of this drug alone. Pharmacokinetics of 5-FUra during courses of daily therapy (for 5 days) revealed prolongation of $t_{1/2}$ in both components of the decay curve, which has not been previously reported. Despite the efficacy of thymidine (dThd) given as a continuous i.v. infusion of 8 g/sq m/day in prevention of high-dose methotrexate toxicity, continuous infusion of dThd at this dose does not prevent the toxicity of 5-FUra or reverse inhibition of DNA and RNA synthesis by 5-FUra. On the contrary, continuous infusion of dThd appears to increase the toxicity of 5-FUra in all respects. Pharmacokinetic studies of 5-FUra during continuous dThd infusion revealed prolongation of the 5-FUra $t_{1/2}$ which remained stable through the course of 5 days of 5-FUra with dThd. This prolonged $t_{1/2}$ is believed to account at least in part for the increased toxicity of 5-FUra with dThd. Dose-limiting mucositis, myelosuppression, and gastrointestinal toxicity were observed at 5-FUra doses ranging from one-half to two-thirds the customarily tolerated dose of 5-FUra alone in similar courses of daily bolus therapy (for 5 days).

INTRODUCTION

The fluoropyrimidines have had a major role in human cancer chemotherapy since their introduction more than a generation ago. 5-FUra has been the most widely used fluoropyrimidine, mainly due to its lower cost and greater availability. It has experimental antineoplastic activity comparable to the other fluoropyrimidines (e.g., 5-fluoro-2'-deoxyuridine and 5-fluorouridine) at equitoxic dosage. The mechanism of action of 5-FUra is uncertain. It is primarily effective against cells that are progressing through the cell cycle; cells in all phases of the cell cycle are susceptible to the cytotoxic effects of 5-FUra (4, 9, 10, 14). The antimetabolic action of 5-FUra against some cells in culture is mitigated by concurrent presence of dThd (24) or uridine (25). However, the earliest described effects of dThd in vivo were fluoropyrimidine potentiation in murine systems (5). More recently, Nayack et al. (19) have reported that the efficacy of 5-FUra against spontaneous murine mammary and colon carcinoma can be improved when immediately preceded by bolus doses of dThd. In vivo experiments suggest a significantly greater incorporation of [14C]-5-FUra in the tumor of the dThd-treated host, but not in the normal gastrointestinal tract and other potential targets of toxicity (19).

dThd has been shown to be capable of preventing the toxicity of high doses of methotrexate when appropriately administered in humans (3, 7) as well as in mice (23). Exogenously administered dThd can bypass the methotrexate-induced block in endogenous TMP biosynthesis; however, antitumor effects are still seen. In murine leukemias, improved antitumor effect can be achieved when dThd is given with methotrexate (22, 23). We have been interested in determining whether the fluoropyrimidine interference with TMP biosynthesis might be also circumvented by exogenous dThd. If such protection were found, dThd might protect the host against inhibition of thymidylate synthetase byFdUMP (Chart 1), thereby achieving a selective enhancement of antitumor effects which are dependent upon inhibition of RNA and protein synthesis. To test this hypothesis, we have investigated the effect of continuous infusions of dThd at doses previously shown to be sufficient to achieve end product reversal of the effect of methotrexate on dThd and DNA synthesis (7, 12). We have investigated the toxicity of 5-FUra given in the customary daily fashion for 5 days, with and without continuous infusion of dThd. To determine whether the toxicity and drug exposure were related, we have studied the pharmacokinetics of 5-FUra during courses of this drug alone, as well as during courses of 5-FUra with dThd.

MATERIALS AND METHODS

Seven patients were studied whose age, sex, neoplastic disease, and prior therapy are listed in Table 1. All except Patient 1 had received extensive prior chemotherapy or radiotherapy. All patients had histologically proven metastatic disease which was refractory to standard therapies, and all patients gave written informed consent to this Phase I study. Patients were initially treated at 5-FUra dosage of 460 to 525 mg/sq m/day for 5 days. dThd was administered by continuous i.v. infusion at 8 g/sq m/day by constant infusion pumps (IMED Corp., San Diego, Calif.) for 1 hr commencing 2 hr before the initial dose of 5-FUra in each course. During courses with dThd infusion, 5-FUra dosage was reduced one-third to one-half. Courses were repeated every 4 to 5 weeks.

The experimental plan is outlined in Chart 2. Each patient was studied with respect to 5-FUra pharmacokinetics, the effects of 5-FUra on marrow cytokinetics, and clinical toxicology. The first course of daily 5-FUra consisted of 460 to 525 mg/sq m/day for 5 days. Serum samples were obtained at 0, 5, 10, 15, 20, 30, 40, 60, and 120 min on Days 1 and 5 of therapy. Bone marrow samples were obtained prior to and at 5 hr following 5-FUra on Days 1 and 5 for cytofluorimetry and nucleoside incorporation studies as detailed below.
Serum Pharmacokinetics. Serum samples were deproteinized by precipitation at 4°C with 5% (v/v) perchloric acid and centrifugation. The pH of each sample was adjusted to 7.0 with 4 M potassium hydroxide, and, following overnight storage at 4°C, the potassium perchlorate was removed by centrifugation. The resulting supernatant was filtered at 4°C through a 0.22-μm Millipore filter. HPLC was carried out on a Waters Model ALC 202 liquid chromatograph equipped with a U6K injector, dual Model 6000 pumps, Model 660 solvent programmer, and a standard 254 nm UV absorbance detector. A Schoeffel Model SF770 spectroflow monitor was connected to the system to allow the absorbance to be monitored simultaneously at 270 nm. A Waters μBondapak CN column (3.9 mm inner diameter) x 30 cm) was used in the analysis. BrdUrd was added to a final concentration of 2 × 10⁻⁴ M (internal standard). Elution was performed with 0.005 M ammonium acetate at a flow rate of 1 ml/min and an ambient room temperature of 17°C. Reagents were all of USP grade, and buffers were prepared daily in deionized water (Filterite Corp., Timonium, Md.), degassed by boiling, and filtered through a 0.2-μm cellulose membrane (Gelman Metrical CA-8).

A typical separation of 5-FUra and BrdUrd is presented in Chart 3. Calibration curves were generated by use of standard solutions of 5-FUra and BrdUrd (1, 2, 3, and 4 × 10⁻⁴ M) and 2 × 10⁻⁴ M, respectively) in deproteinized serum. Each standard solution was run 9 times, and peak height ratios of 5-FUra and BrdUrd were processed with a programmable calculator (Texas Instruments Model 59) to obtain a least-squares linear regression equation of the form y = b + mx, with the additional constraints: y intercept < 0.01 and r > 0.95.

For the analysis of patient samples, the calculator was programmed to utilize the constants b and m to convert observed peak height ratios into FUra concentrations directly.

The logarithms of concentrations were plotted directly as a function of time of sample collection for each patient, and first approximations suggested a 2-compartment model of the form:

\[ y = b_1e^{m_1t} + b_2e^{m_2t} \]

Table 1
Profiles of 7 patients studied during 5-FUra and 5-FUra with dThd administration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Neoplastic disease</th>
<th>Prior therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. C.</td>
<td>42</td>
<td>M</td>
<td>Renal cell carcinoma metastatic to cervical spine and lung</td>
<td>Methotrexate-dThd</td>
</tr>
<tr>
<td>E. D.</td>
<td>55</td>
<td>F</td>
<td>Renal cell carcinoma metastatic to lung</td>
<td>Pelvic radiotherapy</td>
</tr>
<tr>
<td>D. L.</td>
<td>45</td>
<td>F</td>
<td>Colonic adenocarcinoma metastatic to liver and peritoneum</td>
<td>Intraarterial fluorouracil deoxyriboside</td>
</tr>
<tr>
<td>T. L.</td>
<td>52</td>
<td>F</td>
<td>Adenocarcinoma of unknown primary metastatic to liver</td>
<td>Cytoxan, Adriamycin, DTIC, methotrexate-Leucovorin</td>
</tr>
<tr>
<td>G. S.</td>
<td>50</td>
<td>M</td>
<td>Mesothelioma en cuirasse</td>
<td>Intraarterial fluorouracil deoxyriboside, methotrexate-dThd</td>
</tr>
<tr>
<td>P. B.</td>
<td>50</td>
<td>F</td>
<td>Colonic adenocarcinoma metastatic to liver</td>
<td>5-FUra, 2-trans-2-chloroethyl-3-(4-hydroxy-2- methylcyclohexyl)-1-nitrosourea, cytarabine, methotrexate-dThd</td>
</tr>
</tbody>
</table>
| A. T.   | 52       | F   | Colonic adenocarcinoma metastatic to paraaortic lymph nodes and lungs | 1

Chart 2. Experimental design for 5-FUra (5-FU) and 5-FUra with dThd (TdR) pharmacokinetic and bone marrow studies.
where \( y \) is the molar 5-FUra concentration and \( t \) is the time measured from the moment of injection (11). Because the data extend only to 120 min, the equation is accurate only out to this time.

The parameters were evaluated by a direct grid search program (2) with several modifications. The least-squares criterion was applied:

\[
S_i = \sum_i [F_{m}(b, m; t) - y_i]
\]

where \( F_i(b, m; t) \) is the value of the function \( y = b_1e^{-mt} + b_2e^{-mt} \) with parameters \( b_j, m_j \) at the \( j \)th iteration. The summation is over all data points \( i \).

Initial estimates of the constants were obtained by solving \( y = b_1e^{-mt} \) from the first 2 data points and \( y = b_2e^{-mt} \) from the last 2 data points. Initial step size was one-tenth of the value of the parameter with grid points \( b_1 \pm k_1 b, m_1 \pm k_1 m \) for \( k_1 = 1, 0, 1, \) and \( j = 2 \) or 0.5). A condition for termination of the analysis is either that \( S_i \) is no longer changing or that the parameters have been determined to an acceptable accuracy. The Gauss criterion of goodness of fit is acceptable accuracy. The Gauss criterion of goodness of fit is

\[
S_i = \sum_i [F_{m}(b, m; t) - y_i]
\]

Table 2 illustrates the pharmacokinetic data obtained in 7 patients during initial courses of 5-FUra without dThd. The baseline serum half-life (\( t_{1/2a} \)) of 5-FUra in these 7 patients on Day 1 was found to be best described by computer plots in 2 phases, \( \alpha \) and \( \beta \), where \( \alpha = 7.8 \pm 2.6 \) (S.E.) min and \( \beta = 36.8 \pm 15.5 \) min. The \( \alpha \) component during initial 5-FUra courses proved to be prolonged appreciably in 3 patients and minimally in 2 patients and to decrease in 2 patients over the span of 5 consecutive days of therapy. There were more uniform prolongations of the \( \beta \) component assessed on Day 5 in 5 of 7 patients studied. At Day 5, mean \( t_{1/2\beta} = 19.5 \pm 6.3 \) min, while \( t_{1/2\beta} = 43.2 \pm 12.7 \) min. The differences in \( t_{1/2\alpha} \) and \( \beta \) on Days 1 and 5 were statistically significant (F test). Bone marrow cytoxicity analyses of nucleoside incorporation, expressed as the percentage of suppression of basal pretherapy counts per minute of 4 precursors, are summarized in Table 3. Although digestion experiments were carried out with all 4 precursors, only representative data for dThd and deoxycytidine alkali stable incorporation (DNA) and for adenosine and uridine alkali labile incorporation (RNA) are portrayed. Suppression of marrow incorporation of these precursors was equal in DNA and RNA fractions. Suppression was progressive during courses of 5 days and maximal at Hr 5 of Day 5.

Clinical toxicity observed following 5-FUra courses without dThd was in most cases minimal (Table 4). Myelosuppression in the range of 1000 to 2000 WBC/cu mm was observed in one of 4 patients treated at 525 mg/sq m and one of 4 patients treated at 460 mg/sq m. No other significant or dose-limiting toxicity was observed.

Pharmacokinetics of bolus 5-FUra given with dThd as a continuous infusion at a rate of 8 g/sq m/day were different from those observed when 5-FUra was given alone, as is illustrated in Table 5. For Day 1, the \( t_{1/2\alpha} \) rose from 7.8 \pm 3.3 to 11.2 \pm 2.0 min and \( t_{1/2\beta} \) rose from 36.8 \pm 15.5 to 76.7 \pm 25.2 min. These changes did not reach statistical significance at the level of \( p < 0.05 \) in paired tests of values for \( t_{1/2\alpha} \) and \( \beta \) with and without dThd for each patient. Serum \( t_{1/2\beta} \) values for separate courses in each patient were generally consistent.
Table 3

Inhibition of nucleoside incorporation after each course of treatment

Percentage of inhibition of nucleoside incorporation in bone marrow cells of patients after 5-day courses of 5-FUra or 5-FUra with dThd.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of inhibition from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNA (alkali-stable counts)</td>
</tr>
<tr>
<td></td>
<td>Deoxycytidyl</td>
</tr>
<tr>
<td>Bolus 5-FUra (460-525 mg/sq m/ day)</td>
<td>47.7 ± 27.9</td>
</tr>
<tr>
<td>Bolus 5-FUra + continuous dThd (277-460 mg/sq m/day + 8 g/sq m/day)</td>
<td>74.9 ± 10.1</td>
</tr>
</tbody>
</table>

* Day 5, Hr 5 versus Day 1, Hr 0.
+ Mean ± S.E. for 6 patients.

Table 4

Phase I results of daily bolus 5-FUra with and without continuous infusion of dThd

Clinical toxicity of 5-FUra with and without dThd in 7 patients; grading of mucosal and gastrointestinal toxicity follows Eastern Cooperative Oncology Group scale, while central nervous system toxicity consisting of vertigo is simply designated + or 0.

<table>
<thead>
<tr>
<th>Patient</th>
<th>5-FUra bolus (mg/sq m/day for 5 days)</th>
<th>dThd continuous infusion (8 g/sq m/day)</th>
<th>Platelet nadir (x 10⁹/l)</th>
<th>WBC nadir (x 10⁹/l)</th>
<th>Oral mucositis</th>
<th>Emesis and/or diarrhea</th>
<th>Other (central nervous system)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. C.</td>
<td>460</td>
<td>+</td>
<td>183 (5)</td>
<td>6.6 (4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>266</td>
<td>+</td>
<td>141 (5)</td>
<td>5.5 (11)</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>460</td>
<td>+</td>
<td>140 (20)</td>
<td>3.2 (7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E. D.</td>
<td>460</td>
<td>-</td>
<td>317 (17)</td>
<td>4.9 (17)</td>
<td>+ (9-11)</td>
<td>+ (10-14)</td>
<td>+ (21-23)</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>+</td>
<td>159 (8)</td>
<td>4.4 (23)</td>
<td>+ (9-15)</td>
<td>+ (4-14)</td>
<td>+ (15-22)</td>
</tr>
<tr>
<td></td>
<td>460</td>
<td>-</td>
<td>294 (4)</td>
<td>3.6 (18)</td>
<td>+ (12)</td>
<td>+ (6-8)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>460</td>
<td>+</td>
<td>180 (4)</td>
<td>2.5 (23)</td>
<td>+ (7-12)</td>
<td>+ (7-14)</td>
<td>+ (19-22)</td>
</tr>
<tr>
<td></td>
<td>525</td>
<td>+</td>
<td>154 (14)</td>
<td>2.5 (21)</td>
<td>+ + (6-10)</td>
<td>+ + (6-12)</td>
<td>+ (20-30)</td>
</tr>
<tr>
<td>D. L.</td>
<td>525</td>
<td>-</td>
<td>256 (16)</td>
<td>1.3 (16)</td>
<td>+ (5-7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>+</td>
<td>8 (12+)</td>
<td>0.1 (14+)</td>
<td>+ + + (7+)</td>
<td>+ + + (8+)</td>
<td>+ Rash, sepsis fatal (21)</td>
</tr>
<tr>
<td>T. L.</td>
<td>460</td>
<td>-</td>
<td>147 (5)</td>
<td>1.3 (14)</td>
<td>+ (9-12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>+</td>
<td>123 (6)</td>
<td>1.2 (14)</td>
<td>0</td>
<td>+ (7-15)</td>
<td>0</td>
</tr>
<tr>
<td>G. S.</td>
<td>525</td>
<td>-</td>
<td>157 (10)</td>
<td>4.4 (5)</td>
<td>+ (4-8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>460</td>
<td>+</td>
<td>63 (15)</td>
<td>5.4 (5)</td>
<td>+ + (4-8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>460</td>
<td>+</td>
<td>15 (11)</td>
<td>5.0 (4)</td>
<td>+ + (5-10)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P. B.</td>
<td>525</td>
<td>-</td>
<td>112 (21)</td>
<td>3.6 (21)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>+</td>
<td>98 (12)</td>
<td>3.7 (20)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. T.</td>
<td>460</td>
<td>-</td>
<td>180 (21)</td>
<td>3.9 (21)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>+</td>
<td>160 (11)</td>
<td>4.7 (11)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>+</td>
<td>89 (12)</td>
<td>2.6 (19)</td>
<td>+ + (7-10)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, days from beginning of treatment.

Table 5

Half-life of serum 5-FUra during therapy with 5-FUra plus dThd

Serum pharmacokinetics of 5-FUra on Days 1 and 5 in patients receiving daily boluses of 5-FUra (for 5 days) with concurrent dThd. t₁/₂ α and β derived from HPLC peak height ratios according to biexponential decay function $y = b_1 e^{-\alpha t} + b_2 e^{-\beta t}$ (see text).

<table>
<thead>
<tr>
<th>Patient</th>
<th>t₁/₂ α (Day 1)</th>
<th>t₁/₂ β (Day 1)</th>
<th>t₁/₂ α (Day 5)</th>
<th>t₁/₂ β (Day 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. C.</td>
<td>9.1 (2)</td>
<td>47.5 (2)</td>
<td>7.1 (2)</td>
<td>47.5 (2)</td>
</tr>
<tr>
<td>T. L.</td>
<td>20.8</td>
<td>216.2</td>
<td>11.5</td>
<td>31.4</td>
</tr>
<tr>
<td>A. T.</td>
<td>9.1</td>
<td>46.7</td>
<td>9.7</td>
<td>83.1</td>
</tr>
<tr>
<td>P. B.</td>
<td>12.4</td>
<td>46.0</td>
<td>8.4</td>
<td>29.8</td>
</tr>
<tr>
<td>D. L.</td>
<td>9.8</td>
<td>109.3</td>
<td>3.5</td>
<td>96.7</td>
</tr>
<tr>
<td>G. S.</td>
<td>3.7 (3)</td>
<td>29.2 (3)</td>
<td>4.2 (3)</td>
<td>43.7 (3)</td>
</tr>
<tr>
<td>E. D.</td>
<td>13.4 (3)</td>
<td>42.2 (3)</td>
<td>27.9 (3)</td>
<td>79.9 (3)</td>
</tr>
</tbody>
</table>

Mean ± S.E. 11.2 ± 2.0 76.7 ± 25.2 10.3 ± 3.1 58.9 ± 10.2

* Number in parentheses, number of courses; inasmuch as no differences related to dosage were observed, these are represented as mean values for patients who received more than one course of 5-FUra with dThd.

with one another, and so are represented with mean values. After initial prolongations observed on Day 1 of 5-FUra with dThd compared to Day 1 of 5-FUra alone, there was no further increase in $t_{1/2}$ α or $t_{1/2}$ β over the 5-day course of bolus 5-FUra therapy with dThd infusion. Charts 4 and 5 illustrate raw data of HPLC-determined serum 5-FUra levels in Patients T. C. and D. L., illustrating changes in patients with and without toxicity.

To establish whether our sequence of therapy might have influenced the results, owing to cumulative effects of antecedent therapy, 2 patients were restudied during treatment with 5-FUra alone after 2 to 3 courses of 5-FUra with and without dThd. There was no significant difference between $t_{1/2}$ values on Day 1 obtained initially and during later retreatment of these 2 patients (Patients E. D. and G. S.).

Bone marrow studies summarized in Table 3 reveal that the inhibition of precursor incorporation was similar in TCA-precipitable alkali-labile fractions representing RNA and in alkali-stable DNA incorporation. Despite an 11- to 27% increase in...
inhibition of alkali-stable (DNA) incorporation, small sample size and large standard errors make this difference nonsignificant.

Clinical toxicity observed during 13 courses of 5-FUra given with dThd are represented in Table 4. Initial 5-FUra doses with dThd were 50 to 75% of 5-FUra doses given without dThd. Serious mucositis was observed at doses of 5-FUra as low as 277 mg/sq m in one patient receiving concurrent radiotherapy to the cervical spine for vertebral metastases (3000 rads in 15 fractions over 20 days). This Grade II to III mucositis was ascribed to the combination of radiotherapy and 5-FUra with dThd. Dose-limiting mucositis with 5-FUra with dThd was variable and occurred over a range of 5-FUra doses, at 370, 375, 460, and 525 mg/sq m. Dose-limiting leukopenic myelosuppression was observed at 370 to 460 mg/sq m. Gastrointestinal toxicity included vomiting and diarrhea of greater than 24 hr in 3 patients at doses of 300 to 525 mg/sq m, although these were observed in each case in conjunction with more serious symptoms of oral mucositis which were felt to be the dose-limiting toxicity. Thrombocytopenia was regularly observed earlier than leukopenia, as has been reported previously for fluoropyrimidines alone. One case of fatal toxicity (Patient D. L.) was observed in a patient with prior pelvic irradiation and poor bone marrow reserve, who also had a markedly prolonged 5-FUra $t_{1/2}$. This patient experienced severe mucositis, myelosuppression, exfoliative dermatitis, and hepatitis. Dramatically suppressed marrow nucleoside incorporation was apparent throughout the course of treatment of this patient; despite anterior crest aspirations, prior bone marrow irradiation may have introduced sampling artefact. Prolongation of Day 1 $t_{1/2}$ $\beta$ to values more than 2-fold those of nontoxic patients was observed in association with severe toxicity in one patient. dThd levels assessed by HPLC in several patients were in the range of 1 to 10 $\mu$M at steady state, 10-fold the endogenous dThd levels measured in these patients during 5-FUra therapy without dThd infusion. Chart 6 illustrates dThd levels assessed by HPLC in 2 patients.

As the patient sample studied was limited, no valid conclusion about the antitumor potential of combinations of dThd and 5-FUra can be drawn. Of 2 patients with renal cell carcinoma, one exhibited stable disease at maximally tolerated 5-FUra plus dThd doses, and one withdrew short of toxicity after 2 cycles of the combination without response. One patient with mesothelioma failed with progressive disease at maximally tolerated doses of 5-FUra. One patient with adenocarcinoma of the colon exhibited progression of disease at maximally tolerated doses of 5-FUra. One patient each with adenocarcinoma of the colon and adenocarcinoma of unknown primary origin exhibited stable disease after 2 to 3 cycles and were withdrawn after reaching dose-limiting toxicity. One patient with colonic adenocarcinoma died too early to allow an adequate assessment of response.

**DISCUSSION**

5-FUra alone or in combination, is widely utilized in current chemotherapy programs for gastrointestinal, mammary, and prostate adenocarcinoma, as well as for certain squamous carcinoma. Systemic chemotherapy with 5-FUra is optimal when administered i.v. monthly (daily for 5 days) or weekly, after loading schedules to mild toxicity (1, 6). Efforts to increase...
the antineoplastic activity and therapeutic index of 5-FUra have focused upon biochemical strategies for averting host toxicity during antitumor treatment.

A major advance in design of antimetabolite therapy was the introduction of antimetabolite reversal with products of the target pathway. Methotrexate with folic acid (Leucovorin) was the first such regimen applied clinically, and methotrexate-thymidine is currently under investigation. Despite the preeminent role of fluoropyrimidines among the antimetabolites, no program of rescue has yet been demonstrated to be effective for these agents.

The mechanism of antineoplastic action of 5-FUra has been debated for a generation and is believed to involve direct incorporation into RNA of sensitive cells, as well as indirect interference with DNA synthesis as a result of the avid binding of FdUMP to thymidylate synthetase (21). The relative role of these competing mechanisms has not been firmly established, although it is widely assumed that the lethal action of 5-FUra involves thymidylate synthetase inhibition by FdUMP, rather than effects on RNA synthesis and function (9, 18). This conclusion rests largely upon observations that dThd mitigates the lethal effect of 5-FUra on cultured cells and the demonstration that thymidylate synthetase in certain murine tumors resistant to 5-FUra has a decreased affinity for FdUMP. Continued RNA and protein biosynthesis in tumor cells at fluoropyrimidine concentrations sufficient to inhibit tumor cell division has been adduced to support this hypothesis, as well as the partial reversal of lethal effects in cultured cells by uridine, presumably acting through expansion of dUMP pools (18, 25).

However, the potential role of 5-FUra interference with RNA and protein synthesis has become a recent issue. It has been paradoxical that dThd could not restore the growth and replication of tumor cells in vitro at concentrations that were not observed to alter RNA synthesis. Moreover, in mouse L-cells (16) and in the Novikoff hepatoma cell (25), uridine was able to achieve up to 77% restoration of cell replication in the presence of 5-fluorouridine. In vivo, dThd given to mice treated with 5-FUra increased the toxicity of 5-FUra, in comparison with the drug given alone in earlier studies (5). This phenomenon was ascribed to a common degradative pathway, with competition between 5-FUra and dThd at the level of the rate-limiting enzyme, dihydrouracil dehydrogenase. It has also been found that dThd in a variety of schemes may increase the therapeutic effect of 5-FUra in mice bearing CDF-1 spontaneous mammary carcinoma and a transplantable colon carcinoma. An increase in therapeutic index was not rigorously established through a full dose-response curve in these studies where enhancement of antitumor activity was achieved by administration of dThd in bolus doses ranging from 100 to 500 mg/kg i.p. prior to the administration of 5-FUra, at 50 mg/kg i.p. weekly. Pretreatment with dThd was postulated to spare DNA synthesis inhibition in animals by end product reversal of thymidylate synthetase blockade by FdUMP. Pharmacokinetics of dThd metabolism in humans have been evaluated during clinical trials of methotrexate with dThd reversal; a t\textsubscript{1/2} in the range of 10 to 15 min was found, demonstrating the necessity of continuous dThd infusions if end product reversal is to be attempted. Accordingly, a program of continuous dThd infusion (8 g/sq m/day) which has been shown effective for end product reversal of methotrexate toxicity was adopted for the present studies with 5-FUra. This has previously been shown to be well tolerated and nontoxic in patients treated concurrently with methotrexate (7). We have found that the pharmacokinetics of 5-FUra in the serum follow a biphasic decay function previously reported for methotrexate (11, 13). Similar patterns of serum 5-FUra elimination have recently been suggested by MacMillan et al. (15) who have carried out their analysis omitting time intervals up to 10 min to fit a one-phase model:

\[
C = \frac{k_d}{\text{V}_{\text{rat}}} [1 - e^{-k_a t}] [e^{-k_d t} - t]
\]

These authors have attributed the first observed phase to mixing. It was concluded that incomplete mixing contributed to discrepancy with one monophasic model used or was the sole basis for this discrepancy. In contrast, our data has clearly fit a biphasic decay function of the form \( y = mx + b (r > 0.98) \). These functions were found to accurately describe the pharmacokinetics of 5-FUra throughout 5-day courses of bolus 5-FUra given alone and with concurrent exogenous dThd, incorporating all data points from 5 to 120 min.

We have observed an alteration of 5-FUra pharmacokinetics produced by antecedent 5-FUra. Previous pharmacokinetic studies have been limited to the day of initial treatment with 5-FUra (15). The prolongation of the serum 5-FUra t\textsubscript{1/2} in our 7 patients was clearly evident over a span of 5-day courses given without dThd, regardless of whether analyzed as a single exponential decay function or more accurately as a biexponential decay function. It is notable that this prolongation was observed in both \( \alpha \) and \( \beta \) phases of the decay functions, raising a question that the \( \alpha \) phase described is a simple function of distribution. \( t_{1/2} \) did not appear to be dose related in the range examined during the present study or in the study of MacMillan et al. (15). Intrinsic variability of hepatic 5-FUra clearance may provide an explanation for differences in the \( t_{1/2} \) observed (8). The basis for the protraction of serum 5-FUra \( t_{1/2} \) during 5-day courses of 5-FUra therapy and during concurrent dThd infusion is presumed to be the saturation of catabolic pathways, including dihydrooracil dehydrogenase and competition for excretion. A distribution phase is in all probability a component of the \( \alpha \) phase we have described, although prolongation observed over time and with dThd is difficult to explain in distributive terms alone. Early hepatic clearance functions may well be manifest here.

Studies of marrow incorporation of 4 nucleosides during courses of 5-FUra alone revealed progressive suppression over time which was parallel in DNA and RNA and greatest at Hr 5 on Day 5. There was little toxicity during courses of 5-FUra without dThd, and toxicity was correlated with elevated 5-FUra serum \( t_{1/2} \) and suppression of marrow nucleoside incorporation in Patient D. L. only (Table 4). Integration of the biexponential equation \( y = b_1 e^{-\alpha t} + b_2 e^{-\beta t} \) from \( t = 0 \) to \( t = 120 \) min gave the highest value for Patient D. L. as well (1.51 to 1.69 \( \times \) 10\textsuperscript{-2}).

Serum pharmacokinetics of 5-FUra during Day 1 of continuous infusions of dThd yielded \( t_{1/2} \) data which more nearly resembled \( t_{1/2} \) of 5-FUra on Day 5 of previous 5-FUra courses without dThd. During the 5-day course of serial bolus therapy, no significant further change was observed. In order to confirm base-line \( t_{1/2} \) data, one patient was retreated with 5-FUra alone after 2 courses of 5-FUra with dThd. No differences were found, decreasing our concern that the sequence of therapy used in this trial generated partially irreversible effects on 5-
FUra clearance. Serum 5-FUra t₁/₂ in patients receiving 2 or 3 courses of 5-FUra and dThd were similar between courses and are therefore represented as mean values (Table 5).

On the basis of our bone marrow nucleoside incorporation studies, it appears that RNA synthesis is suppressed to the same extent during 5-FUra with dThd therapy as it was with 5-FUra alone, whereas incorporation into DNA was more profoundly suppressed during combined therapy with 5-FUra. Thus, no reversal of marrow target effects was evident in DNA, and no potentiation of marrow target effects was apparent in RNA on addition of dThd to the regimen.

Clinical observations during 13 courses of 5-FUra with dThd therapy consistently indicated an increase in toxicity when 5-FUra was administered with continuous infusions of dThd. Our findings failed to indicate that end product reversal of 5-FUra toxicity might be achieved, as originally suggested in the mouse (17). The dThd dose used here has been well studied as a mode for reversing methotrexate toxicity. Serum dThd levels assessed by HPLC in our patients revealed values in the range previously reported to be nontoxic (7).

No studies of respiratory elimination of [14C]-5-FUra nor any incorporation studies of labeled 5-FUra in marrow and other host organs and/or tumor were possible in this study. Studies of Martin et al. (17) and Nayak et al. (19) suggest that potentiation of fluoropyrimidine incorporation into RNA of tumor or liver might be observed in the absence of overall effects upon in vitro marrow nucleoside incorporation. More definitive studies of such selective pyrimidine nucleosides will be of interest.

Another possibility is that the reported effects of dThd in murine systems require dThd administration in the form of pulse doses rather than continuous infusion. These effects would not be due to restoration of the end product of the target enzyme thymidylate synthetase and would presumably depend upon more complex temporal sequences of interaction. Two patients were studied during bolus infusion of 5-FUra preceded by bolus dThd, according to a schedule similar to that described by Nayak et al. (19). Alteration of 5-FUra serum pharmacokinetics was evident at dThd doses of 1 to 8 g/sq m i.v., 30 min prior to 5-FUra therapy.

Finally, it seems clear to us that pursuit of Phase II potential of 5-FUra with dThd will require evidence that dThd has an influence on the action of 5-FUra beyond alteration of the 5-FUra dose-response curve. In particular, combined studies of labeled 5-FUra incorporation into marrow and tumor are indicated. The adoption of serum sampling at late intervals on Day 1 (e.g., 120 min) may allow earlier prediction and avoidance of profound toxicity. In our experience, the t₁/₂ β of 5-FUra on Day 5 during courses of daily therapy rose to t₁/₂ β = 19.5 ± 6.3 min and t₁/₂ β = 43.2 ± 12.7 min. Two distinct differences in the general pattern of increase with prior therapy occurred in Patients P. B. and D. L., who both suffered hepatic metastases and exhibited signs of response to therapy. It is possible that discrepant t₁/₂ data in these patients may have been due to improvement in liver function (Tables 2 and 5).

REFERENCES

Comparison of Pharmacokinetics of 5-Fluorouracil and 5-Fluorouracil with Concurrent Thymidine Infusions in a Phase I Trial


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