Clinical Pharmacological Studies of Concurrent Infusion of 5-Fluorouracil and Thymidine in Treatment of Colorectal Carcinomas

J. Lai-Sim Au, Youcef M. Rustum, E. J. Ledesma, Arnold Mittelman, and Patrick J. Creaven

ABSTRACT

The effects of thymidine (dThd) coadministration on the pharmacokinetics and metabolism of 5-fluorouracil (FUra) were investigated in 29 colorectal cancer patients. Five patients received 5-day i.v. infusion of FUra at 15 mg/kg/day and 24 patients received infusion of FUra (7.5 mg/kg/day, 5 days) and dThd (216 mg/kg/day, 6 days) preceded by a bolus dose of dThd (405 mg/kg). Plasma and urine concentrations of FUra, 5-fluorodeoxyuridine (FdUrd), thymine, and dThd were quantitated by a high-pressure liquid chromatographic assay. Concurrent dThd administration reduced the plasma clearance of FUra at steady state from 389.1 ± 153.5 (S.D.) to 56.0 ± 36.4 liters/kg/day. The mean steady-state plasma concentration of FUra in patients receiving FUra alone was 0.38 μM and was significantly lower than the 1.30 μM in patients receiving FUra-dThd. Plasma concentrations of FUra were linearly dependent on those of thymine. Furthermore, the metabolic and renal clearances of FUra decreased inversely with thymine concentrations indicating that the elimination of FUra was reduced by thymine. In contrast to the absence of FdUrd as a circulating metabolite in patients treated with FUra alone, μM concentrations of FdUrd were detected in plasma of most of the patients treated with FUra-dThd. This together with the linear correlation of FdUrd and dThd concentrations indicates that the interconversion of FUra to FdUrd was enhanced by dThd.

The incidence of dose-limiting leukopenia in the FUra-dThd combination therapy was 40%. There is an inverse correlation between the plasma clearance of FUra at steady state and hematological toxicity. The plasma clearance of FUra in the toxic population was 32.0 ± 16.8 liters/kg/day and was significantly lower than the clearance of 72.0 ± 37.3 liters/kg/day in the nontoxic population (p < 0.001). The corresponding critical toxic steady-state FUra plasma concentration was 1.5 μM.

The biochemical effects of dThd on the incorporation of FUra and FdUrd into RNA and into acid-soluble 5-fluorodeoxyuridylate monophosphate (FdUMP) in human colon tumor cells were studied in vitro. At 100 μM, dThd increased the incorporation of FUra into RNA up to 4-fold but diminished the acid-soluble FdUMP pool. Similarly, the incorporation of FdUrd into acid-soluble FdUMP was inhibited by dThd. The response rate of colorectal carcinoma to FUra was not improved by coadministration of dThd; only one of the 11 patients who had no prior FUra therapy achieved partial remission. The lack of clinical response in these patients may be partly due to the inhibition of anabolism of FUra and FdUrd to FdUMP by dThd.

INTRODUCTION

FUra3 is one of the most widely used antitumor agents in the palliative treatment of solid tumors. In recent years, studies were undertaken to evaluate the effect of normal metabolites on the therapeutic activity of FUra. Several investigators showed that dThd at high doses improves the therapeutic index of FUra in murine tumors (14, 15, 17). Subsequent biochemical studies indicate that pretreatment with dThd preferentially increases the incorporation of FUra into RNA in these tumor cells (14, 15, 17). The pharmacokinetic interaction between FUra and dThd in laboratory animals and patients have since been reported (10, 12, 24). Following bolus administration, dThd was found to prolong the elimination half-life of FUra in patients, and the magnitude of this effect is dependent on the dose of FUra and dThd (10, 24).

In this study, the pharmacokinetics of FUra and the reduction of FUra metabolism by dThd were investigated in 2 groups of patients with colorectal cancer. Five patients received 5-day infusion of FUra and 24 patients were treated under the Phase I and II protocol of FUra-dThd infusion preceded by a bolus administration of dThd. Since these patients were infused to a steady state with respect to the FUra plasma concentration, the plasma clearance of FUra, a pharmacokinetic parameter which is quantitatively more descriptive than the plasma half-life, was established. The large sample size of patients included in this study enables us to correlate the plasma clearance and concentration of FUra at steady state to its dose-limiting bone marrow toxicity. In addition, the biochemical effects of dThd on the metabolism of FUra and FdUrd were examined in disaggregated human colorectal tumor cells in suspension.

MATERIALS AND METHODS

Chemicals and Reagents. All chemicals and reagents used are of analytical or spectroquality grade. FUra was obtained from Hoffman-LaRoche, Inc. (Nutley, N. J.) and dThd was obtained from the National Cancer Institute (Bethesda, Md.). Scintillation counting cocktail (ACS) was purchased from Amersham Corp. (Arlington Heights, Ill.) and [6-3H]FUra and [6-H3]FdUrd were purchased from Moravek Biochemicals (City of Industry, Calif.).

Patient Protocol. A total of 29 colorectal cancer patients was included in the study. Five patients received a total of 7 treatments of 5-day i.v. infusion of FUra at 15 mg/kg/day; all 5 patients had received prior FUra treatment. Twenty-four patients, most of them having pulmonary and/or liver metastasis, were studied under the Phase I and II FUra-dThd combination chemotherapy protocol. Among the latter group, 13 patients had received prior FUra treatment while the other 11 patients had not. All patients had initial platelet count >150,000, WBC >4000, bilirubin level <2.5 mg/100 ml, serum creatinine <1.5

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2 To whom requests for reprints should be addressed.
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mg/100 ml, and a creatinine clearance >60 ml/min. Each FUra-dThd
treatment course consists of a preloading dose of 405 mg dThd per kg
by i.v. bolus over 30 min to provide high levels of thymine and
deoxyribose 1-phosphate, followed by continuous infusions of FUra
(7.5 mg/kg/day, 5 days) and dThd (216 mg/kg/day, 6 days), re-
peated every 4 weeks. The infusion was initiated 2 hr after the bolus
dThd and was administered by a volumetric infusion pump (McGaw).
FUra dose was escalated or deescalated by 20 to 50%. Each patient
received a minimum of 2 treatments or was treated until disease
progressed. In several patients, the FUra dose during the second and
third treatments was decreased due to the drug-related toxicity ob-
served during the first treatment. Serial blood samples were collected
in heparinized tubes, and the plasma fractions were stored at -20°
for subsequent analysis. Urine samples were collected at 24-hr intervals
and stored frozen. The platelet and WBC counts were determined at
weekly intervals after completion of each course.

Analysis of FUra, FdUrd, Thymine, and dThd in Plasma. All glass-
wares used in the extraction procedures were silanized with siliclad
(Clay Adams, Parsippany, N. J.). dino (10 μg) was added to 1 ml of
plasma as an internal standard. The plasma was buffered to pH 6.0
with 0.1 ml of 2 M NaH₂PO₄ buffer and extracted twice with 10 ml ethyl
acetate. The organic layers were pooled, dried under a stream of
nitrogen at 70°, reconstituted, and transferred with methanol. Since
dThd phosphorylase activity has been reported in human plasma (18),
experiments were performed to rule out the phosphorylation of FdUrd
and dThd in situ. No degradation was detected at the end of the
extraction procedures when FdUrd and dThd were added to either
freshly obtained or previously frozen plasma from volunteers. The
overall recoveries of FUra, FdUrd, thymine, and dThd ranged from 80
to 95%. Urine samples were diluted 10-fold with distilled water and
extracted under the same conditions. The extracts were analyzed by
HPLC using a 4- x 30-mm C₁₈-reverse-phase-column
(Water Associates, Inc., Milford, Mass.) and an HPLC unit completed
with a Model 6000A solvent delivery system, a Model U-6K injector,
and a Model 440 dual-wavelength UV detector at 254 and 280 nm
(Waters Associates). The separation of FUra, thymine, FdUrd, dino,
dThd from endogenous interferences were obtained by isocratic
elution with 0.1% acetonitrile in 2.5 mw ammonium acetate buffer
(pH 3.8) at a flow rate of 2 ml/min, and their elution volumes were 5.3,
9.2, 14.0, 19.6, and 23.6 ml, respectively. Peak identification was
further ensured by the corresponding 254/280 nm UV absorbance
ratios of 1.5, 1.8, 1.0, and 1.05 for FUra, thymine, FdUrd, and dThd,
respectively. Calibration curves of FUra, thymine, and dThd were
constructed with ratios of the respective UV absorbance peak height
to that of dino at 254 nm. The UV absorbance of the endogenous
interferences eluting in close proximity to FdUrd were minimized at
280 nm; therefore, the calibration curve of FdUrd was constructed with
the peak height ratio of its absorbance at 280 nm to that of dino at 254
nm. The lower sensitivity of this assay are 0.1 μM for FUra and
thymine and 0.3 μM for FdUrd and dThd, with a coefficient of variation
ranging from 3 to 10%.

Calculation of Plasma Clearance. The plasma clearances (CLs)
of FUra and dThd were estimated by Equations A and B.

\[
CL_{FUra} = \frac{D_{bolus} + D_{infused}}{AUC_{FUra}} \quad \text{(A)}
\]

\[
CL_{dThd} = \frac{\text{Rate of infusion}}{C_{PAS}} \quad \text{(B)}
\]

where \(D_{bolus}\) is the amount of drug given by bolus dose, \(D_{infused}\) is
the amount of drug infused from Time zero to \(t\), \(AUC\) is the area under
the plasma concentration-time curve, and \(C_{PAS}\) is the steady-state plasma
concentration from \(t_1\) to \(t_2\). Equation A describes the relationship
between the CL and the AUC of dThd before the steady state is
reached. When the bolus dThd dose is quantitatively eliminated and
the dThd plasma concentration reaches a plateau, Equation A can be
simplified to Equation B which describes the relationship of CI and AUC
under steady-state conditions. The CLs of FUra and dThd during the
first 48 hr of infusion were estimated by Equation A and from 48 to
120 hr by Equation B. The AUC used in Equation A represents the
truncated AUC from Time zero to \(t\), and is not corrected for the AUC
from \(t\) to infinity. Based on the rate of decline of the concentrations of
dThd and FUra from 24 to 48 hr, the AUC from 48 hr to infinity is
estimated to be less than 5% of the AUC from Time zero to 48 hr. The
AUC of dThd during the first 2 hr was estimated based on the extrap-
olated dThd plasma concentration at zero time and did not take into
account a distribution phase; thus, the clearance calculated by Equa-

RESULTS

Plasma Concentrations of dThd, Thymine, FUra, and
FdUrd. HPLC profiles of extracts of patient plasma samples
obtained before treatment and at 2 hr during the FUra-dThd
infusion (or 4 hr after the bolus dThd dose) are illustrated in

In Vitro Metabolism of FUra and FdUrd. Tumor specimens were
obtained from 4 patients prior to the initiation of chemotherapy. The
pathology of the specimens was confirmed independently. Single-cell
suspensions of the tumors were prepared using an enzymatic digestion
method described by Slocum et al. (21). Viability of cells obtained
by this method was above 80%, as determined by the ability to exclude
the trypan blue dye. Cell incubation was carried out in a complete
medium which consisted of 10% Mycoplasma-tested horse serum in
Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand
Island Biological Co., Grand Island, N. Y.) and a buffer system of 8 mm
4-2-(hydroxyethyl)-1-piperazineethanesulfonic acid and 16 mm 3-N-
morpholinopropanesulfonic acid (Sigma Chemical Co., St. Louis, Mo.)
in 0.7 N sodium hydroxide, pH adjusted to 7 with hydrochloric acid.
Five to 10 million cells were incubated with 100 μM dThd at 37° for 3
hr, [6-H³]FUra or [6-H³]FdUrd was then added at a concentration of 1
μg/ml (specific activity, 1 μCi/μg), and cells were incubated for an
additional 3 hr. Similar incubations without dThd were used as controls.
At the end of the incubation period, cells were washed and extracted
with 100 μl perchloric acid in 10 mM formate buffer and immediately
neutralized with 45 μl of 2 N potassium hydroxide. The radioactivity
in the acid-soluble fraction was counted in 10 ml ACS in a Packard 3330
counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The acid-
soluble FdUMP was separated by paper chromatography on Polygram
Cel 300 PEI papers (Brinkmann Instruments, Inc., Westbury, N. Y.)
using a solvent system of 1 M lithium chloride in saturated borate
buffer, pH 4.5:95% ethanol (50:50). FdUMP was separated from 5-
fluorouridine monophosphate under these conditions with a R₅ value of
0.28. The acid-insoluble RNA fraction was digested with 300 μl 1 N
sodium hydroxide at 4° for 20 hr. Radioactivity in the RNA digest was
counted in 10 ml ACS. Quenching was corrected by an external
standard method.
Chart 1. The corresponding concentrations of FUra, thymine, FdUrd, and dThd at 2 hr were 42.39, 678.46, 2.32, and 44.77 μM, respectively. Results obtained from 24 patients receiving a total of 50 treatments of FUra-dThd are summarized in Table 1. There is a large intersubject variability as indicated by the wide ranges of concentrations. The mean plasma concentration-time profiles of FUra, FdUrd, thymine, and dThd in patients treated with FUra-dThd are represented in Chart 2a. The mean plasma concentration-time curve of FUra in 5 patients receiving a total of 7 treatments of FUra alone is illustrated in Chart 2b. Following a bolus dose, plasma concentrations of dThd were sustained at 30 to 600 μM for up to 24 hr, then dropped to a steady state at 48 hr, and was maintained at a level of 2 to 3 μM by the continuous infusion. dThd was rapidly converted to thymine in vivo; the plasma concentration-time profile of thymine was similar to that of dThd. In humans, the elimination of thymine is slower than that of dThd (25, 26), this may have accounted for the higher plasma concentrations of thymine when compared to dThd (Chart 2a).

When administered by zero-order infusion, the plasma concentration of a drug which is eliminated by linear kinetics will rise up to a plateau steady-state level. This was observed in patients treated with FUra alone; their steady-state FUra concentrations ranged from 0.1 to 1.0 μM. However, in patients treated with FUra-dThd, the FUra plasma concentrations during the first 24 hr were the highest, reaching 20 μM, and subsequently declined along with the thymine and dThd concentrations to a plateau of 1 to 2 μM by 48 hr (Chart 2a). This extraordinary behavior in FUra plasma concentrations indicates nonlinear kinetics in FUra disposition in the presence of high concentrations of thymine and dThd at early time points. In patients treated with FUra alone, the circulating FdUrd was below the detection limit of 0.3 μM. However, in patients treated with FUra-dThd, μM concentrations of FdUrd were observed.

**Plasma Clearances of FUra and dThd.** The plasma clearances of FUra and dThd during different time intervals following the initiation of FUra-dThd therapy are summarized in Table 2. Following a combination of bolus dose of 405 mg/kg and daily infusion at 216 mg/kg, the plasma clearance of dThd during the first 48 hr was 10 liters/kg/day. After the bolus dose was eliminated and when the steady-state plasma concentration of 2 to 3 μM was achieved by the infusion, the clearance of dThd was increased to 325 liters/kg/day. Similar nonlinear kinetics of FUra was observed. During the first 48 hr, FUra was eliminated with a clearance of 6 liters/kg/day. From 48 to 120 hr when FUra concentrations were at a steady state, the FUra clearance was 56 liters/kg/day.

The effects of dThd coadministration and prior FUra treatment on the FUra clearance at steady state were compared in Table 3. When administered alone, FUra was cleared at a rate of 389 liters/kg/day. With concurrent dThd infusion, the FUra clearance was reduced about 6-fold to 56 liters/kg/day. This indicates that dThd coadministration significantly reduced the
elimination of FUra (p < 0.001). Chronic and repeated FUra treatments were associated with decreased hepatic metabolism (11) and reduced FUra clearance (10). However, when the clearances of FUra during FUra-dThd infusion were compared in patients with or without prior FUra therapy, there was no significant difference among the 2 patient groups.

Renal Elimination of FUra and dThd. Results of urinary excretion of FUra and dThd in 6 patients are summarized in Table 4. Renal excretion of these 2 drugs varied at different time intervals. During the first 48 hr, about 15% of the daily FUra dose was excreted unchanged. After 48 hr when the plasma concentrations were at a steady state, the daily excretion of FUra decreased to 5%. In the case of dThd, there was a greater than 20-fold variation, from 10 to 20% daily excretion in the first 48 hr to about 0.5% in 48 to 120 hr.

The correlation between the renal clearances of FUra from 0 to 48 and 48 to 120 hr and the averaged plasma concentrations during these time intervals are illustrated in Chart 3. The renal clearance of FUra was dependent on its plasma concentrations. At concentrations below 2 μM, the renal clearance of FUra was 3.5 ± 1.4 (S.D.) liters/kg/day (n = 6) and decreased to 0.92 ± 0.3 liters/kg/day (n = 4) at 10 to 25 μM concentrations.

Interrelationship of the Plasma Concentrations of FUra, FdUrd, Thymine, and dThd. The linear regression analysis of the correlations of FUra, FdUrd, thymine, and dThd concentrations is shown in Chart 4. In spite of the large intersubject variability, there is a good linear correlation between the plasma concentrations of FUra and thymine at 24 hr (Chart 4a, r² = 0.73). A similar correlation is observed between the steady-state concentrations of FUra and thymine at 120 hr (r² = 0.80; data not shown). When either the FUra or dThd concentrations were below 4 μM, FdUrd was not detected in plasma, indicating that the interconversion of FUra to FdUrd is dependent on dThd.

Comparison of the Steady-State Plasma Clearance-Concentration of FUra with the Incidence of Leukopenia. Among the 2 subgroups of patients treated with FUra-dThd including 13 patients with and 11 patients without prior FUra treatment, one patient in the second group achieved partial remission. Partial remission is defined as 50% or greater decrease in the sum of the product of the diameters of the measured lesions; simultaneous increase in the size of any lesion or appearance of new lesions may occur. The dose-limiting toxicity of the FUra-dThd combination infusion therapy was myelotoxicity and was mainly leukopenia. Leukopenia (WBC count of less than 3000) was observed in 22 of 50 treatment courses. There is an inverse correlation between the hematological toxicity and steady-state plasma clearance of FUra. The plasma clearances of FUra at steady state in 24 patients received a total of 50 treatments in patients are summarized and compared in Table 5. Patients who experienced toxicity were eliminating FUra at a statistically significant lower rate than the patients with no toxicity (p < 0.001). The toxicity was best correlated with the steady-state plasma concentrations of FUra. Chart 5 illustrates the plasma concentrations of FUra at 24 and 120 hr during infusion in individual patients. In general, the plasma concentrations of FUra in the nontoxic populations were lower than those in the toxic populations. At 120 hr when FUra concentrations had plateaued, 19 of 21 treatment courses with FUra concentrations above 1.5 μM resulted in leukopenia. The plasma concentration-time curves of FUra in 2 representative patients with and without leukopenia are shown in Chart 6. The FUra concentrations at 24 hr are similar in both patients; however, the patients with toxicity achieved a higher steady-state FUra concentration than the nontoxic patient.

Effects of dThd on the Metabolism of FUra and FdUrd in
Table 4

<table>
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<tr>
<th>Patient</th>
<th>0–24 hr</th>
<th>24–48 hr</th>
<th>48–72 hr</th>
<th>72–96 hr</th>
<th>96–120 hr</th>
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<td>FUrA</td>
<td>P. A.</td>
<td>8.5</td>
<td>23.3</td>
<td>4.1</td>
<td>6.5</td>
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<td></td>
<td>A. G.</td>
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<td>6.6</td>
<td>5.1</td>
<td>9.6</td>
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<td></td>
<td>P. S.</td>
<td>20.8</td>
<td>6.3</td>
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<td></td>
<td>J. M.</td>
<td>14.6</td>
<td>18.0</td>
<td>3.5</td>
<td>3.8</td>
</tr>
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<td></td>
<td>E. F.</td>
<td>4.8</td>
<td>12.6</td>
<td>a</td>
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<tr>
<td></td>
<td>W. E.</td>
<td>27.5</td>
<td>17.3</td>
<td>8.2</td>
<td>10.1</td>
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<td>Mean ± S.D.</td>
<td>16.4 ± 8.6</td>
<td>14.0 ± 16.8</td>
<td>5.2 ± 2.1</td>
<td>6.8 ± 2.5</td>
<td>5.3 ± 1.9</td>
</tr>
<tr>
<td>dThd</td>
<td>P. A.</td>
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<td>3.8</td>
<td>0.1</td>
<td>0.2</td>
</tr>
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<td>A. G.</td>
<td>28.6</td>
<td>1.0</td>
<td>0.6</td>
<td>0.4</td>
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<td>P. S.</td>
<td>24.8</td>
<td>0.4</td>
<td>a</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>J. M.</td>
<td>24.4</td>
<td>3.1</td>
<td>1.4</td>
<td>2.0</td>
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<tr>
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<td>E. F.</td>
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<td>54.2</td>
<td>a</td>
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<td></td>
<td>W. E.</td>
<td>29.4</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>21.5 ± 10.9</td>
<td>10.4 ± 21.5</td>
<td>0.5 ± 0.6</td>
<td>0.6 ± 0.8</td>
<td>0.5 ± 0.7</td>
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* Not available.

Chart 3. Concentration-dependent renal elimination of FUrA. Renal clearance is calculated by Equation C and expressed as liters/kg/day (l/kg-d).

Human Colorectal Tumor Cells. The effects of dThd in the intracellular uptake and metabolism of FUrA and FdUrd in colon tumor cells obtained from 4 patients are summarized in Table 6. The total acid-soluble pool of FUrA equivalents and the incorporation of FUrA equivalents into RNA were both increased about 4-fold by dThd treatment. However, the free FdUMP pool in the acid-soluble extracts of cells was decreased by dThd. A similar inhibitory effect of dThd on the metabolism of FdUrd to FdUMP was observed. In addition, the total acid-soluble pool of dThd equivalents in cells was reduced by dThd.

DISCUSSION

Nonlinear Kinetics of FUrA and dThd. The pharmacokinetics of FUrA and dThd was characterized in 29 colorectal cancer patients. Data in this study indicate that both drugs were eliminated by dose-dependent kinetics.

The mM plasma concentrations of dThd following a bolus dose of 405 mg/kg were comparable to those observed by Zaharko et al. (25, 26) and Woodcock et al. (24). The steady-state plasma concentrations of dThd maintained by infusion of 216 mg/kg/day were 2 to 3 μM, confirming the earlier report of Kirkwood et al. (10). The plasma and renal clearances of dThd were concentration dependent and varied 30- to 40-fold. At μM concentrations, up to 99% of the administered dThd is eliminated by metabolism, and when the metabolism is saturated at mM concentrations, renal excretion becomes a major route of elimination. These data support the previous finding of Zaharko et al. (25, 26) in which an up to 10-fold increase in total body clearance was observed in humans when the dThd dose was decreased from 2.2 g/kg/day by infusion to 24 mg/kg by bolus administration.

Without exogenous dThd, FUrA was cleared at a rate of 389 liters/kg/day with a corresponding mean steady-state plasma concentration of <1 μM. In the presence of 20 to 30 μM thymine concentrations, FUrA was cleared at a rate of 56 liters/kg/day with a steady-state concentration of 1 to 2 μM. The FUrA clearance was further reduced to 6 liters/kg/day in the pres-
ence of 200 to 300 μM thymine concentrations. The renal clearance of FUra changed inversely with its plasma concentrations. The glomerular filtration rate for a standard 70-kg man with normal renal function is 120 ml/min, or 2.5 liters/kg/day. The observed renal clearances of FUra either exceed or are below the glomerular filtration rate, thus indicating that, in addition to being filtered, FUra is actively secreted out of and reabsorbed into the renal tubules.

**Effects of dThd on the Pharmacokinetics of FUra and the Circulating FdUrd.** Alteration of the pharmacokinetics of FUra in humans and animals by concurrent administration of dThd has been reported (10, 12, 24). Data obtained in this investigation are in agreement with previous reports. The metabolism and the renal excretion of FUra were both affected by dThd. The mean plasma clearance of FUra in 5 colorectal patients receiving 5-day infusion of 15 mg/kg/day was 389 liters/kg/day which greatly exceeds the hepatic plasma flow of 18.5 liters/kg/day. Plasma flow is calculated using the assumption that the hepatic blood flow for a standard 70-kg man is 1.5 liters/min and that the hematocrit is 40%. The renal excretion of FUra is a minor pathway of elimination which accounts for less than 5% of the total body clearance during prolonged infusion (4) and during concurrent daily infusion of 7.5 mg FUra per kg and 216 mg dThd per kg as observed in this study. This indicates that, when infused alone, over 90% of the metabolism of FUra occurs outside the liver and the extrahepatic metabolic clearance is about 300 liters/kg/day. FUra enters cells by passive diffusion (9) and does not bind to plasma or tissue proteins (6). Therefore, the alteration in the disposition kinetics of FUra is presumably due to a change in its metabolic and renal clearances and not by a change in its distribution. The concurrent administration of dThd reduced the plasma clearance of FUra at steady state to 56 liters/kg/day, or only 3 times the hepatic plasma flow. Ho et al. (8) reported recently that, in humans, on a g/g basis, the activity of the catabolizing enzyme dihydrouracil dehydrogenase in the liver is 20-fold higher than that in the lung, bone marrow, and colon (8). Since

<table>
<thead>
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<th>No. of observations</th>
<th>Range</th>
<th>Median</th>
<th>Mean ± S.D.</th>
<th>p</th>
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<td>With leukopenia</td>
<td>20</td>
<td>5.6–63.1</td>
<td>32.6</td>
<td>32.0 ± 16.8</td>
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<tr>
<td>Without leukopenia</td>
<td>30</td>
<td>28.9–192.3</td>
<td>58.7</td>
<td>72.0 ± 37.3</td>
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</table>

**Effect of dThd on accumulation of FUra and FdUrd by human colon tumor cells**

Cells were pretreated with 100 μM dThd for 3 hr, incubated for 1 hr with 1 μg of [6-3H]FUra or [6-3H]FdUrd per ml (1 μCi/ml), washed, and extracted with perchloric acid. Similar incubations without dThd pretreatment were used as controls. The incorporation of FUra and FdUrd equivalents into RNA was not corrected for the rate of RNA synthesis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Range</th>
<th>Median</th>
<th>FdUMP Range</th>
<th>Median</th>
<th>Incorporation into RNA</th>
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<td>Acid soluble</td>
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</tr>
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<td>FUra control</td>
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<td>60</td>
<td>0.5–2.0</td>
<td>12</td>
<td>6–22</td>
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<tr>
<td>dThd → FUra</td>
<td>60–528</td>
<td>240</td>
<td>&lt;0.3</td>
<td>41–80</td>
<td>50</td>
</tr>
<tr>
<td>dThd → FdUrd</td>
<td>20–80</td>
<td>50</td>
<td>5–62</td>
<td>21</td>
<td>1.4–8.0</td>
</tr>
</tbody>
</table>
the reduction in the plasma clearance of FUra by dThd was only 5-fold, the metabolism of FUra in the extrahepatic tissues would be affected to a much greater extent than that in the liver. The hepatic clearance would now be increased up to 30% of the plasma clearance and the extrahepatic metabolic clearance would be at least 65%, or the equivalence of 36 liters/kg/day. Therefore, the extrahepatic metabolic clearance is reduced by dThd for approximately 10-fold. During the first 48 hr when the circulating thymine and dThd were at the mm concentrations, the mean plasma clearance of FUra was 5.6 liters/kg/day, out of which 15% was accounted for by renal clearance. Thus, the net metabolic clearance is 4.8 liters/kg/day, indicating that the hepatic clearance can be suppressed in the presence of 200 to 300 μM thymine concentrations. The reduction in FUra metabolism is probably due to competitive inhibition of the dihydouracil dehydrogenase by the circulating thymine. This is supported by the observed linear correlation between FUra and thymine concentrations.

The renal clearance of FUra was altered by dThd. Although the amount of FUra excreted in urine increased with thymine-dThd concentrations, the renal clearance of FUra was reduced from 5.5 to 0.9 liters/kg/day. These data indicate that thymine-dThd inhibits the active secretion of FUra from the renal tubules.

The interconversion of FUra toFdUrd was affected by dThd. Using an HPLC assay with a lower limit of 0.3 μM, FdUrd was absent from the plasma samples of patients receiving FUra infusion alone but present in those of patients receiving FUra-dThd at early time points when the FUra and dThd concentrations were abundant. The FdUrd concentrations were in the μM range and are significantly lower than those observed following bolus doses of FUra and dThd as reported by Kirkwood et al. (10) and Woodcock et al. (24). The inhibition of the FUra catabolism by thymine would theoretically spare FUra to the anabolic pathways. In addition, dThd may serve as a source of the circulating deoxyribose 1-phosphate utilized in the metabolism of FUra to FdUrd, as well as protecting FdUrd from catabolism by competing for the dThd phosphorylase (12). Data analysis of the plasma concentrations of FUra, FdUrd, and dThd indicates that the formation of FdUrd is better correlated with the concentration of dThd than of FUra, and the best linear correlations were obtained between the FdUrd concentrations and the product of FUra and dThd concentrations. Thus, the formation of FdUrd may be viewed as a bimolecular reaction depending on the availability of FUra and deoxyribose 1-phosphate generated during the phosphorolysis of dThd to thymine. Since FdUrd is not detected when FUra is infused alone, the metabolism to FdUrd is a minor pathway of FUra which may be rate limited by the presence of deoxyribose 1-phosphate.

Effects of dThd on the Accumulation of [6-3H]FUra and [6- 3H]FdUrd in Human Colon Tumor Cells. Pretreatment with 100 μM dThd increased the intracellular accumulation of FUra but decreased that of FdUrd in tumor cells. The incorporation of FUra into RNA was increased, similar to the observations made in murine tumor system (15, 17). However, dThd had no effect on the incorporation of FdUrd into RNA. The metabolism of FUra and FdUrd to FdUMP was diminished. Bowen et al. (2) reported recently that, in Ehrlich ascites cells, the inhibitory effect of dThd on the total intracellular accumulation of FdUrd was primarily the intracellular FdUrd anabolism and minimally on the transport of FdUrd. FdUrd has been shown to share the same metabolic pathway as dThd (16); the decrease in the formation of FdUMP from FUra or FdUrd is probably due to a competitive inhibition of one or more of the 3 enzymes involved in the FdUMP formation, i.e., dThd kinase, phosphoribosyltransferase, and ribonucleotide reductase. The net effect of the decrease in the FdUMP pool and the increase in FUra incorporation into RNA by dThd on the antitumor activity of FUra is unclear.

In summary, dThd alters the pharmacokinetics of FUra. The metabolic and renal clearances of FUra were reduced by concurrent administration of dThd. The most pronounced effect of dThd was the reduction of extrahepatic metabolic clearance by up to 10-fold. Consequently, the tissue exposures to FUra would be increased proportionally. On the other hand, dThd suppressed the formation of FdUMP but increased the incorporation of FUra into RNA in colon tumor cells. The incidence of bone marrow toxicity observed in the 24 patients receiving infusion of dThd and FUra at 7.5 mg/kg/day in this study was similar to that seen with FUra infusion at 30 mg/kg/day (13, 22). The bone marrow toxicity of FUra has been shown to follow a steep dose-response relationship. When the 5-day infusion dose of FUra was increased from 15 to 30 mg/kg/day, a significant increase in leukopenia was observed (13, 22). The absence of a drastic increase in bone marrow toxicity in spite of a 10-fold increase in tissue exposures to FUra as observed in this study suggests that the net effect of the pharmacokinetic and biochemical interactions of FUra and dThd was minimal on the bone marrow cells. The lack of clinical response in 23 of 24 patients suggests that the 4-fold increase in FUra incorporation into RNA did not significantly improve the antitumor activity of FUra in colorectal carcinoma.

Correlation between the Pharmacokinetics of FUra and the Incidence of Leukopenia. At present, little is known about the clinical pharmacology of FUra in terms of predicting response or toxicity to the drug. The rapid clearance, the large intersubject variability in the pharmacokinetics, and the route- and dose-dependent kinetics of FUra (5) have contributed to the difficulty in establishing a thorough clinical pharmacological evaluation. Recently, studies describing the relationship between the response rate to FUra and the total body clearance of FUra in cancer patients have been reported (3, 7). A dose-related effect of FUra on the circulating platelets was observed in 8 patients who received intrahepatic arterial infusion of FUra (20). The incidence of leukopenia in the present study is similar to that reported in Vogel et al. (23). Furthermore, our data suggest that in the presence of 1 to 2 μM dThd and 20 to 30 μM thymine concentrations, the plasma clearance and the concentration of FUra at steady state may be determinants of its dose-limiting myelosuppression and that 1.5 μM appears to be the critical toxic steady-state FUra concentration. This information and the quantitative alteration of FUra pharmacokinetics by dThd are now being utilized in the design and the individualized dosage adjustment of FUra in a Phase I trial of methotrexate, FUra, and dThd by intrahepatic arterial infusion.

REFERENCES

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Clinical Pharmacological Studies of Concurrent Infusion of 5-Fluorouracil and Thymidine in Treatment of Colorectal Carcinomas


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