Nonlinear Pharmacokinetics of Thymidine, Thymine, and Fluorouracil and Their Kinetic Interactions in Normal Dogs

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ABSTRACT

We have investigated the pharmacokinetics of thymidine (dThd), thymine, and fluorouracil (FUra) over a range of doses in normal dogs. Evidence was obtained to show that the metabolic elimination of these pyrimidines is saturable, resulting in nonlinear pharmacokinetic behavior. Additionally, dThd and thymine were shown to inhibit the catabolism of FUra. Following i.v. infusion to low-μM steady-state plasma levels (Css = 7.6 to 12 μM), each compound alone demonstrated an elimination half-life (t1/2) between 2 and 20 min. When Css was increased to near 1000 μM, the elimination of dThd, thymine, and FUra was markedly slower and no longer followed first-order kinetics. Over the same concentration range, plasma clearance of each compound decreased about 90%, while urinary clearance was increased in each case. The relationship between infusion rate and Css was nonlinear. Using equations based on Michaelis-Menten kinetics, in vivo values of Km and Vmax were determined: Km (in μM): dThd, 45.5; thymine, 82.5; FUra, 39.8; Vmax (in μmol/min/kg): dThd, 2.45; Thymine, 2.55; FUra, 2.16.

When FUra and thymine or FUra and dThd were infused simultaneously following a base-line infusion of FUra alone, the Css of FUra increased in proportion to the plasma level of thymine achieved. Plasma clearance and metabolism decreased markedly in a nonlinear manner with thymine concentration, while there was little effect on the urinary clearance of FUra. Assuming competitive enzymatic inhibition, in vivo K values for dThd and thymine effects on FUra metabolism were 24.2 and 17.4 μM, respectively.

INTRODUCTION

The pyrimidine nucleoside dThd has recently undergone extensive preclinical and clinical investigation for its potential use in cancer chemotherapy. It has been used in combination with methotrexate (9, 12), FUra (21, 30, 31), or 1-β-d-arabinofuranosylcytosine (28). dThd has also been used alone in high doses (2). In each of these therapies, the biochemical rationale for using dThd appears to be different (19), and consequently the plasma levels of dThd required to achieve the desired therapeutic effect also appear to be different. Therefore, in designing rational protocols using dThd, it will be necessary to have an understanding of the pharmacokinetics of this compound and its kinetic interactions with other agents.

dThd is rapidly catabolized to the base thymine (4), and saturation of this process at high concentrations results in a nonlinear relation between dose and plasma level of dThd achieved. When a compound displays saturation kinetics, the classical pharmacokinetic equations (11) which normally allow predictions of plasma levels become invalid. Models which incorporate a capacity-limited process are an appropriate way to describe the behavior of such compounds over a wide range of doses. With respect to the use of FUra and dThd in combination, evidence exists for significant pharmacokinetic interactions between these compounds, resulting in a prolonged t1/2 of FUra and subsequent clinical toxicity (15, 30, 31). Since these drug interactions may play an important role in determining the efficacy of the combination, an understanding of their extent and mechanism is important.

While the in vivo metabolism of dThd is a saturable process (32, 33) which may obey Michaelis-Menten kinetics, no detailed pharmacokinetic analysis incorporating this concept has been reported for dThd. Moreover, while the use of dThd in combination with FUra may result in kinetic and metabolic drug interactions of clinical significance, a quantitative analysis of this possibility has not been carried out. For these reasons, we have investigated the complex pharmacokinetics of dThd and its kinetic interactions with FUra in normal dogs. The kinetic behaviors of dThd, of its initial catabolite thymine, and of FUra were defined assuming Michaelis-Menten kinetics. Values for Vmax and Km of each compound were obtained in vivo. The effects of dThd and thymine on FUra metabolism were quantitated using equations incorporating competitive enzyme inhibition. Numerical values (K) representing the extent of inhibition of FUra metabolism were determined in vivo.

MATERIALS AND METHODS

Chemicals. Unlabeled dThd and FUra were obtained from Dr. V. Narayanan, Drug Synthesis and Chemistry Branch, National Cancer Institute. Unlabeled thymine and 5-chlorouracil were purchased from Sigma Chemical Co., St. Louis, Mo. [methyl-3H]dThd, [methyl-3H]thymine, and [carboxyl-14C]inulin were purchased from Amersham-Searle Corp., Arlington Heights, Ill. [methyl-14C]dThd and [methyl-14C]thymine were purchased from New England Nuclear, Boston, Mass. HPLC-grade methanol was obtained from Fisher Scientific, Silver Spring, Md.

General Protocol. The general design of the experiments consisted of individual 3- to 4-hr infusions of dThd, thymine, or FUra at 2 or more doses (saturating and nonsaturating) in a series of 13 normal mongrel dogs weighing 10 to 20 kg. Subsequently, FUra-dThd and FUra-thymine interaction studies were done in the same animals. Several dogs received the complete protocol of experiments which helped minimize interindividual variation.

Animals were anesthetized with either pentobarbital or halothane, and...
concentrations between 514 and 1526 μM. In some experiments, the infusion also contained [14C]inulin sufficient to provide a dose of 0.01 μCi/kg/min, with unlabeled inulin added to produce a specific activity of 0.62 μCi/mg. The final solution was infused into a foreleg vein at a rate between 0.233 and 2.0 ml/min using a Harvard infusion pump or a Laboratory Data Control Mini-Pump. To rapidly achieve steady state during high-dose infusions, a loading dose equal to Cm/Vd (where Cm is the desired steady-state level and Vd is the steady-state volume of distribution) was administered via the jugular catheter prior to starting the infusion. Estimates of Vd were obtained from the low-dose experiments and literature data as described below.

**Blood and Urine Samples.** Samples (5 ml) of blood were collected in heparinized tubes at various times during and after each infusion and centrifuged in the cold to obtain plasma. For samples containing thymine and dThd, plasma proteins were precipitated with 10% perchloric acid. The resultant supernatant was neutralized with 4 N KOH and stored at 4° for at least 24 hr. Following removal of the precipitated potassium perchlorate, the supernatant was analyzed by HPLC. When known amounts of [3H]Fura and [3H]thymine were added to control plasma, the recoveries of the added radioactivity were between 82 and 98%. For samples containing Fura, plasma was prepared according to the procedure of Cohen and Brown (5). One ml of saturated ammonium sulfate and 6 ml of ether:n-propyl alcohol (80:20) were added to 1 ml of plasma. 5-Chlorouracil was added as an internal standard. The mixture was shaken for 30 min and then centrifuged. The ether layer was removed and evaporated to dryness at 70–80° under a stream of filtered air. The residue was redissolved in 0.3 ml of 5% phosphate buffer, pH 6.1, and subsequently analyzed by HPLC. Addition of [3H]Fura to control plasma indicated that the final recovery of Fura was approximately 30%. An internal standard correction factor was applied to all calculations.

Urine was collected in 30- to 60-min periods via the urethral cannula. To minimize lag time in urine recovery from the bladder, a 50-ml flush of distilled water was administered 10 min prior to the end of each collection period. Urine containing dThd and thymine was analyzed directly by HPLC without prior treatment. Urine containing Fura was prepared as described above for Fura plasma samples.

**Double-radiolabeled Experiments.** When the infusion contained both 3H and 14C compounds, aliquots of plasma and urine samples were placed in formaldehyde-treated gelatin capsules and evaporated to dryness on a hot plate. The residue was combusted in an oxygen atmosphere, 3H2O and 14CCO2 were collected separately, and the radioactivity was measured by liquid scintillation counting. Aliquots of infused drugs were also combusted to allow calculation of specific activity. This procedure generally provided data on total radioactivity in plasma and urine derived from dThd or thymine and a measure of inulin concentration in these fluids.

**HPLC.** The HPLC separation of thymine and dThd was performed using a modification of the technique described by Rustum (26). Waters Associates (Milford, Mass.) Model 6000A and M45 solvent delivery systems were used in conjunction with a Model 330 solvent programmer. The column was an Altex Ultral-ODS (10 μm; 4.6 mm x 25 cm) (Beckman Instruments, Fullerton, Calif.) in series with a Whatman pre-column filled with CO-Pell ODS (Whatman, Inc., Clifton, N. J.). Isocratic elution at 1 ml/min was with methanol:water (5 to 20% methanol) which had been separately filtered and degassed. Samples were injected using a Waters WISP automatic injector. The column eluate was passed through a Waters 440 UV detector equipped with a 254-nm filter. For low-dose experiments, standard solutions of thymine and dThd were coinjected with unknown samples to allow peak visualization. Eulation volumes corresponding to the peaks were collected in scintillation vials, 10 ml of Liquiscint (National Diagnostics, Sommerville, N. J.) were added, and the radioactivity was determined by liquid scintillation counting. The concentrations of dThd and thymine were calculated using the specific activity of the infused compound. In high-dose experiments, levels of drug were high enough to allow peak detection without addition of standard. Quantitation was by the external standard method and peak area integration using a Waters Data Module. The lower limit of sensitivity for thymine and dThd was 0.5 μM, with a coefficient of variation near 6%.

Samples containing Fura were analyzed with the same system, except that the mobile phase was 5% methanol in 0.001 M acetate buffer, pH 3.8, at a flow rate of 1 ml/min. Samples were prepared as described above, and Fura was quantitated by peak area integration with internal standard calibration. The lower limit of sensitivity was 3 μM, and the coefficient of variation was 14%.

**Pharmacokinetic Calculations.** The Michaelis-Menten parameters Vmax and Km were determined in vivo for dThd, thymine, and Fura by independently infusing each compound to steady state, measuring the resultant levels in plasma and urine, and applying the following equations. At steady state (Cm) achieved by infusion at a constant rate (k0), the input-output balance equation is:

\[ k_0 = \frac{V_{\text{max}} + \text{urinary excretion}}{C_m} \]  

where Cm is urinary clearance, Vmax is maximum enzyme velocity, and Km is Michaelis constant. At a Cm where metabolism is saturated, enzyme velocity will approach Vmax, and since Cm x C/Cl = urinary excretion, Equation A simplifies to:

\[ k_0 = V_{\text{max}} + \text{urinary excretion} \]  

At low rates of infusion when Cm << Km, Equation B solved for Vmax. At low rates of infusion where Cm is well below Km, Cm and Cl were determined, and Equation C was solved for Vmax/Km. Using this information, Km could also be calculated. Appropriate plasma levels of each compound to produce saturating or nonsaturating conditions were estimated from preliminary experiments and literature data (32, 33).

The interaction between Fura and dThd (and between Fura and thymine) was investigated as competitive enzyme inhibition in vivo by incorporating the appropriate Michaelis-Menten function into Equation A:

\[ k_0 = \frac{V_{\text{max}} + \text{urinary excretion}}{C_m(1 + I/K_0 + C_m)} \]  

Fura was infused to a nonsaturating Cm, and then a second infusion of dThd or thymine was begun, and the 2 compounds infused simultaneously for 4 hr. By determining Cm and Cl for Fura and Cl for the inhibitor (I) during the interaction period, and using previously determined values of Vmax and Km for Fura, Equation D could be solved for K0, a measure of thymine and dThd inhibition of Fura metabolism.

Plasma clearance (Cl/p) was calculated during steady-state periods as k0/Cm. Urinary clearance was determined using the formula: Clu = UV/P, where U is urinary concentration, V is volume of urine, and P is plasma concentration. The plasma concentration versus time data for some low-dose experiments were analyzed using a digital computer-based nonlinear regression program (24). Postinfusion data points were fit to the equation.
C = R EXP(−αt) + S EXP(−βt)

where α and β are the distributional and elimination rate constants, respectively, and R and S are the corresponding end-infusion intercepts (11). Best-fit values for R, S, α, and β were obtained, and the zero-time intercepts (A and B) calculated as A = RTα and B = STβ, where T is the duration of infusion (11). Steady-state volumes of distribution (Vd) were calculated from low-dose data as Vd = k0/C0/β.

RESULTS

Plasma Concentration versus Time Data. The plasma pharmacokinetics of dThd, thymine, and FUra were determined both during and following a series of constant i.v. infusions of each compound over a wide range of infusion rates. The behavior of thymine following i.v. bolus injection was also investigated. Results of the bolus experiments are shown in Chart 1. After a dose of 141 µmol/kg, thymine levels in plasma declined in an apparent monoexponential manner with a τ1/2 of 18 min. When the dose was increased 5-fold (in the same animal), the resultant plasma decay was described by a convex curve. Such behavior is characteristic of compounds eliminated, at least in part, by a capacity-limited process (11). Moreover, it is clear from Chart 1 that the overall decay of thymine is slower at the higher dose.

Following low dose infusion (C0 = 4.5 to 28 µM), the plasma disappearance curves for dThd and thymine were similar with an apparent first-order elimination τ1/2 between 2 and 12 min, although the α and β phases were poorly resolved. In several experiments, a slower terminal phase was also seen (τ1/2 = 34 to 156 min). This may represent elimination from a third compartment consisting of more slowly perfused tissues.

The postinfusion behavior of dThd and thymine was markedly different when C0 values near 1000 µM were attained. Results of typical low- and high-dose dThd infusions are shown in Chart 2. The data indicate a concentration-dependent decrease in dThd elimination similar to that previously reported in humans (32). Following infusion to a plasma concentration of 24 µM, levels of dThd declined rapidly with a first-order τ1/2 of 11.2 min. When plasma levels were increased to 781 µM, the postinfusion decay had an apparent τ1/2 of 80 min. However, since there is considerable evidence that dThd metabolism is saturated at the higher concentration, elimination probably follows Michaelis-Menten kinetics. Thus, calculation of a first-order rate constant for clearance is not a technically valid procedure in this situation.

As seen in Chart 2, thymine was formed in vivo during dThd infusions and achieved levels in plasma which varied with dThd concentration. The thymine:dThd concentration ratio in plasma at the end of the low-dose infusion was 0.879, while for the high-dose infusion it was 0.382. This relative decrease in thymine levels at higher dThd concentrations is suggestive of metabolic saturation. Since it is difficult to analyze the kinetic behavior of a metabolite when synthetic and elimination processes occur simultaneously at varying rates, exogenous infusions of thymine were also performed. The plasma disappearance of thymine was markedly slowed at higher doses (mean C0, 1246 µM), and high-dose decay curves demonstrated the convex shape described earlier for thymine bolus injection. These results suggest that
thymine catabolism also approaches saturation near mM plasma levels.

During infusions of labeled dThd and thymine, there was a rapid appearance in plasma of unidentified radioactivity. Upon HPLC analysis, most of this material eluted near the solvent front, well away from the positions of dThd, thymine, and dihydrothymine standards. While the nature of these compounds was not determined, they probably represent further pyrimidine catabolites such as β-aminoisobutyric acid and water (25). The ratio of thymine or dThd to total radioactivity in plasma at the end of each infusion was dose dependent. At low μM levels of dThd, the mean dThd:total ratio was 0.052 ± 0.009 (S.E.). This ratio increased over 10-fold to 0.544 ± 0.11 when plasma dThd was raised to 853 ± 190 μM. Similar results were obtained during thymine infusions. The relative decrease in metabolite levels at higher doses is additional evidence for metabolic saturation.

Infusion of FUra to a range of concentrations demonstrated the dose-dependent elimination of this compound (Chart 3).

There was a clear trend for a decreased rate of elimination as the infusion rate (and C∞) was increased. Two of the decay curves in Chart 3 showed the characteristic convex shape expected for a compound eliminated by a saturable process. The higher dose curves (Dogs 81-120 and 81-108) would probably have also shown this effect had later time points been obtained, while the lowest curve (Dog 81-100) may not have achieved a sufficient degree of saturation to deviate from first-order behavior.

Urinary and Plasma Clearance. Clu and Clp were determined at steady state for dThd, thymine, and FUra during both low- and high-dose infusions. The results are shown in Table 1. In these animals, the mean inulin clearance, an estimate of the glomerular filtration rate, was 2.78 ± 0.47 ml/min/kg. At low doses, dThd, thymine, and FUra each demonstrated a Clu less than inulin clearance. During high-dose infusions, which produced plasma concentrations near 1 mM, contrasting changes were observed in the Clp of the 3 compounds. The Clp for thymine and FUra increased 99 and 50.4%, respectively, compared to the low-dose results (although the latter change did not achieve statistical significance), but both values remained below inulin clearance. In contrast, the Clu of dThd increased 2518% over the dosage range studied, and during high-dose infusions was 1.47 times greater than inulin clearance.

The values for plasma clearance of the 3 compounds are also listed in Table 1. Each compound showed an extensive (approximately 90%) decrease in Clp when steady-state plasma levels were increased from μM to mM concentrations. Since urinary clearance did not decrease at high dose, the change in Clu must reflect metabolic saturation for each compound. A comparison of urinary and plasma clearance at low dose demonstrates that renal processes contribute only a small percentage to the overall elimination of these pyrimidines. For dThd, the urinary contribution to total plasma clearance is 0.234%, meaning that over 99% of the compound is eliminated by metabolism when plasma levels are in the low-μM range. As plasma concentrations are increased and metabolic elimination approaches saturation, the relative importance of urinary excretion increases. At mM plasma concentrations, Clu represents between 31.6 and 58.8% of the total clearance of the 3 compounds. dThd demonstrated the most dramatic change over the dosage range studied, with the fraction of clearance due to urinary processes increasing over 250-fold.

Michaelis-Menten Parameters. Vmax and Km were calculated for dThd, thymine, and FUra metabolism using the combined results of low- and high-dose experiments as described above. The results are shown in Table 2. Vmax was similar for each

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Urinary clearance (Clu) and plasma clearance (Clp) of dThd, thymine, and FUra</th>
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<tr>
<td>Dose</td>
<td>Clu (ml/min/kg)</td>
</tr>
<tr>
<td></td>
<td>Thymine</td>
</tr>
<tr>
<td>Low</td>
<td>0.970 ± 0.23 (6)</td>
</tr>
<tr>
<td>High</td>
<td>1.93 ± 0.15 (6)</td>
</tr>
<tr>
<td>% of change</td>
<td>99.0%</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
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* Low-dose infusions produced a mean C∞ between 7.66 and 12.2 μM. High dose infusions produced a mean C∞ between 743 and 1246 μM.
* Inulin clearance averaged 2.78 ± 0.47 ml/min/kg.
* Mean ± S.E.
* Numbers in parentheses, number of determinations of each parameter.
* High dose compared with low dose.
* Probability of no difference between high and low dose as determined by 2-tailed paired t test for thymine and dThd and 2-tailed unpaired t test for FUra.
compound, ranging from 2.16 to 2.55 μmol/min/kg. These values represent true in vivo maximum metabolic velocities only if the metabolism of each compound was essentially saturated during the high-dose infusions. The plasma and clearance data presented above have provided substantial evidence that this is in fact the case. Additional evidence for metabolic saturation was provided by the consistency of the V_max calculation over a range of plasma levels. For example, during a dThd infusion to a C_m of 587 μM, the calculated V_max was 2.61 μmol/min/kg. In a second experiment in which the C_m was 1711 μM, the calculated V_max was 2.79 μmol/min/kg. Since V_max remained essentially constant over a near 3-fold concentration range, metabolism must have been saturated at the lower plasma level. Similar results were found for FUra and thymine.

Mean values for the in vivo K_m of each compound are also listed in Table 2. K_m is the theoretical substrate concentration which results in half-maximal enzyme velocity. While there was some variability in the determination of this parameter, the results fell in a "biologically reasonable" micromolar range.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>V_max (μmol/min/kg)</th>
<th>K_m (μM)</th>
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<tr>
<td>Thymine</td>
<td>2.55 ± 0.16 a</td>
<td>82.5 ± 24 (5)</td>
</tr>
<tr>
<td>dThd</td>
<td>2.45 ± 0.15 b</td>
<td>45.5 ± 12 (4)</td>
</tr>
<tr>
<td>FUra</td>
<td>2.16 ± 0.18</td>
<td>39.8 ± 7.3 (16)</td>
</tr>
</tbody>
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a Mean ± S.E.

b Numbers in parentheses, number of determinations of each parameter.

A major benefit of knowing the whole-body values of V_max and K_m is that they can be substituted, along with an estimate of Cl_p, into Equation A, which can then be solved for either k_0 or C_m. In this way, plasma levels can be related to infusion rate over a wide range of concentrations, as shown for dThd in Chart 4. The relationship is clearly nonlinear, as would be expected for a compound in which plasma clearance depends on a Michaelis-Menten process. The change in slope is indicative of metabolic saturation as plasma levels increase. Resumption of linearity above 500 μM is a result of urinary excretion, an inherently first-order process (ignoring tubular effects) which contributes an increasing fraction of total elimination as metabolism becomes saturated. A good correlation between the theoretical curve and individual infusion experiments can be seen. Using the data in Tables 1 and 2, similar curves were generated for thymine and FUra. A curve such as that shown in Chart 4 allows an approximate k_0 to be determined for any desired C_m, and remains valid under conditions where first-order equations are not applicable.

FUra-Thymine and FUra-dThd Interaction Experiments. The effect of thymine on the C_m of FUra is shown in Chart 5 for 2 experiments done using different infusion rates of thymine (0.71 and 2.00 μmol/min/kg). During the infusion of FUra alone (k_0 = 0.396 μmol/min/kg for both experiments), plasma FUra rapidly achieved steady-state levels of 6.06 and 4.38 μM, respectively. Upon initiation of the thymine infusion, plasma FUra immediately began to rise in both experiments and approach a new steady state. The magnitude of this effect was directly related to the C_m of thymine; in Chart 5A, 14.5 μM thymine produced a 102% increase in the C_m of FUra while in Chart 5B 266 μM thymine produced at least a 1562% increase in FUra levels. Additional experiments confirmed the dose-dependent effect of thymine on plasma FUra concentrations during simultaneous infusion of the 2 compounds. A similar effect was seen when dThd was coinfused with FUra, the magnitude of which correlated with the concentration of thymine produced in vivo from dThd.

Thymine was shown to affect the disposition of FUra in a dose-dependent manner, leading to an increase in plasma levels of the latter compound. Since equilibrium dialysis studies performed according to the method of Ehrnebo et al. (8) demonstrated that FUra was not significantly bound to plasma proteins, the possibility of displacement by thymine can be ruled out. Therefore, it is concluded that thymine inhibits the elimination of FUra. This is demonstrated in Chart 6, which shows the effect of thymine on FUra plasma clearance. Cl_p was measured for FUra at steady state before and during each thymine or dThd infusion, and the percentage decrease in Cl_p was related to the C_m of thymine achieved. A theoretical curve derived from data produced by computer modeling (14) of the thymine/FUra interaction is also shown in Chart 6. The relationship appears biphasic with an initial rapid fall in Cl_p, reaching about 65% inhibition as plasma thymine increases to 25 μM. Thereafter, Cl_p continues to decrease, but at a smaller rate, reaching 94% inhibition at 266 μM thymine. Measurements of urinary excretion before and during the interaction period demonstrated that neither thymine nor dThd decreased the urinary clearance of FUra (data not shown). Thus, the effect of thymine and dThd on Cl_p (FUra) must be due to metabolic inhibition. A more direct confirmation of this hypothesis was provided by determining the effect of thymine on the apparent V_max/K_m for FUra. V_max/K_m is a measure of metabolic clearance, which for FUra equals plasma clearance minus urinary...
clearance. Since thymine has little effect on the urinary clearance of FUra, a plot of apparent $V_{\text{max}}/K_m$ versus thymine (not shown) is similar to the plot of $C_{\text{p}}$ versus thymine (Chart 6), demonstrating a rapid initial inhibition of FUra metabolism as thymine increases, followed by a slower decrease to essentially 100% inhibition.

For each interaction experiment, $K_i$ for the in vivo inhibition of FUra metabolism was calculated using Equation D as described above. The mean $K_i$ for thymine as an inhibitor of FUra metabolism in vivo was $17.4 \pm 7.81 \mu M$. This value represents the plasma level of thymine required to produce a 50% inhibition of FUra metabolism and applies to exogenously administered thymine and thymine formed in vivo from dThd. Inspection of Chart 6 reveals that the $C_{\text{ss}}$ of thymine which caused a 50% fall in $C_{\text{p}}$ of FUra (around 15 $\mu M$) is similar to the calculated $K_i$ for thymine. The close agreement between $K_i$ and the 50% inhibitory level for $C_{\text{p}}$ (and $V_{\text{max}}/K_m$) supports the validity of the model used to describe the interaction between these 2 compounds.

In 5 interaction experiments, dThd was infused as the inhibitor compound. The mean apparent $K_i$ for dThd inhibition of FUra metabolism was $24.2 \pm 7.83 \mu M$. However, dThd probably functions as an inhibitor indirectly through the production of thymine, so this value represents the $K_i$ for thymine as modulated by the relationship between $C_{\text{ss}}$ dThd and $C_{\text{ss}}$ thymine. That is, inhibition of FUra metabolism is directly related to the thymine concentration, so when a $K_i$ is calculated using measured dThd levels, it must be considered an apparent value, not a true $K_i$. Moreover, since the conversion of dThd to thymine is a saturable process, the ratio of thymine to dThd in plasma varies with the $C_{\text{ss}}$ of dThd. Thus, the apparent $K_i$ for dThd as an inhibitor of FUra should depend on the dThd concentration. Although the present experiments were performed with a limited range of dThd concentrations so that the trend was not noticed, this factor would need to be considered if $K_i$ determinations were done near concentrations where dThd catabolism is saturated.

**DISCUSSION**

We have obtained evidence demonstrating that dThd, thymine, and FUra are each eliminated, in part, by a saturable metabolic process. For this reason, the concept of capacity-limited (Michaelis-Menten) kinetics was incorporated into the design and interpretation of the present experiments. $V_{\text{max}}$ and $K_m$ were determined for each compound in vivo and used to describe the relationship between infusion rate and steady-state plasma concentration over a wide range of doses. In addition, the pharmacokinetic interactions between FUra and dThd and between FUra and thymine were characterized as a case of competitive enzyme inhibition in vivo and quantitated by the calculation of $K_i$ values for each compound.

The dose dependence of dThd, thymine, and FUra $t_{\text{1/2}}$ has been shown (Charts 1 to 3). When $C_{\text{ss}}$ was increased from low $\mu M$ to near mm levels, the postinfusion plasma decay was markedly slowed. Two infusions of FUra ($0.396 \mu \text{mol/kg/min}$) to plasma levels near 10 $\mu M$ yielded a $t_{\text{1/2}}$ of about 20 min. As
shown in Chart 3, following infusion to C∞ between 245 and 1526 μM, there was a progressive slowing of the plasma clearance of FUra but, since the decay is not a first-order process, it is difficult to quantitate this effect.

Conventional therapeutic protocols for FUra involve the administration of low doses by either i.v. bolus injection (77 to 115 μmol/kg) or constant i.v. infusion (0.05 to 0.93 μmol/kg/min) (20). In a series of clinical studies, the t½ of FUra following bolus injection was between 2.2 and 20 min, and the C∞ during constant infusion ranged between 0.8 and 71 μM (6, 20). Our low-dose results are consistent with these findings, a fact which suggests that the pharmacokinetic behavior of FUra in dogs and humans is similar. Garrett et al. (10) have reported a dose dependence for FUra t½ following i.v. bolus administration of low doses (39 to 172 μmol/kg). The increase in elimination t½ seen in that study was fairly small (1.58- to 2.35-fold). Since high doses of FUra (approaching mM plasma levels) are not used clinically, the large decrease in elimination observed in our experiments has not been previously reported.

Many of the plasma decay curves seen following high doses of thymine or FUra demonstrated the characteristic convex shape on a semilogarithmic plot (11) expected of a compound eliminated by a saturable process (Charts 1 and 3). In addition, the slope of the high-dose thymine curve was always less than that of the low-dose decay (Chart 1), even within an identical range of plasma concentrations. This phenomenon may be related to a shift in the equilibrium of catabolic processes acting on thymine, as metabolites build up after administration of a high (saturating) dose (10). Published FUra decay curves do not show the expected convex form (20). Collins et al. (8) have devised a 2-compartment physiological model for FUra, incorporating saturable metabolic elimination, which was able to simulate clinical decay data without producing a convex curve. Based on our observations, these findings probably result from the use of doses too low to produce the expected-shape curves. Not until plasma levels reached about 500 μM in our experiments did the decay curve demonstrate a convex shape and an increased t½. Thus, while there is evidence that the elimination of FUra is saturable, it may be of little relevance with respect to traditional clinical dosing protocols (20). More recently, however, Speyer et al. (27) have demonstrated nonlinear elimination of FUra following i.p. administration, as evidenced by a dose-dependent decrease in total body clearance.

Following each high-dose dThd infusion, producing C∞ as high as 1711 μM, the plasma decay was log-linear with no evidence of a convex shape (Chart 2). Similar behavior has been reported in man after administration of high-dose dThd (32, 33). These findings are unusual, since there is considerable evidence that dThd metabolism is near saturation in this concentration range (see below). It is possible that a 3- to 4-hr postinfusion sampling period was not long enough to observe the convex nature of the plasma decay curve. In contrast to dThd, thymine disappearance followed the predicted nonlinear pattern after both bolus administration and constant infusion.

Additional evidence for capacity-limited elimination was provided by the nonlinear relationship demonstrated between k0 and C∞ for each compound (Chart 4) and from plasma and urinary clearance measurements. The dose-concentration relationships for dThd (Chart 4) may be clinically useful since plasma levels of this compound between 1 and 4000 μM have been achieved by various protocols (9, 16). Clearance calculations for each compound indicated that, when C∞ was increased from low μM to near mM concentrations, C/l increased markedly. At the same time, C/l increased in each case (Table 1). It can be concluded, therefore, that the decrease in C/l results from saturation of nonurinary (i.e., metabolic) elimination.

As metabolism becomes saturated, the relative importance of renal processes to overall elimination increases. At high plasma concentrations, urinary clearance becomes a major, and in some cases the predominant, route of elimination. Similar results were reported by Zaharko et al. (32, 33) for dThd clearances in humans and monkeys. This change in the pattern of dThd elimination has clinical relevance in that the renal function of patients treated with dThd will be a more important consideration at high dose than at low dose.

During low dose infusions, the C/l for dThd, thymine, and FUra was consistently below inulin clearance. Since equilibrium dialysis studies (8) demonstrated that none of the compounds were significantly protein bound, this suggests that, in the dog at μM plasma levels, these compounds are reabsorbed by the kidney. Reabsorption was particularly extensive for dThd, which had an inulin clearance ratio (C/l:inulin clearance) of only 0.056. Other investigators have reported conflicting findings in man for FUra and dThd. Garrett et al. (10) and Clarkson et al. (3) found FUra urinary clearance to be 50 to 70% greater than glomerular filtration at plasma concentrations around 10 μM. Hughes et al. (13) reported dThd urinary clearance at μM plasma levels to be 4-fold greater than creatinine clearance. This evidence for tubular secretion of FUra and dThd in humans, in contrast to reabsorption seen in the dog at comparable plasma levels, indicates possible species differences in the renal handling of these 2 compounds. At mM plasma levels, thymine and FUra continue to undergo tubular reabsorption, while dThd appears to be secreted (Table 1). Zaharko et al. (32) reported C/l for dThd in humans to be 1.65-fold above creatinine clearance, which is comparable to our inulin clearance ratio for high-dose dThd of 1.47. In addition, our data and that of Zaharko et al. (32) indicate tubular reabsorption of thymine at millimolar plasma levels.

The values for C/l of FUra and dThd found in dogs are generally similar to those reported in humans. Myers (20) has summarized pharmacokinetic data for several series of patients given low-dose infusions of FUra. At plasma levels comparable to those achieved in our experiments, C/l ranged from 3.6 to 7.0 liters/min. Assuming an average body weight of 70 kg, this is equivalent to 51.4 to 100 ml/min/kg and is in agreement with our finding of mean C/l = 60.6 ml/min/kg (Table 1). For dThd, the data of Ensminger and Frei (9) indicates a C/l of 24 liters/min (approximately 342 ml/min/kg) during infusions producing median plasma levels of 1.5 μM. At 7-fold higher plasma levels, we found a mean C/l for dThd of 67.1 ml/min/kg (Table 1). It is not clear if this difference represents a trend toward metabolic saturation, species differences, or both. In contrast, the data of Zaharko et al. (32) for C/l in humans at mM dThd levels (3.1 to 7.7 ml/min/kg) is comparable to our finding of C/l = 6.97 ml/min/kg (Table 1).

The liver plasma flow of normal dogs is around 24 ml/min/kg (17). Inspection of the low-dose plasma clearances listed in Table 1 will reveal that even if hepatic extraction is 100% efficient, each of these compounds must also be metabolized in other body tissues, since C/l exceeds liver plasma flow. Zimmerman and Seidenburg (34) reported the occurrence of dThd phospho-
rylase activity in kidney, intestinal mucosa, and spleen, as well as in liver in a number of mammalian species, while Pauly et al. (22) found enzyme activity in human and dog plasma. Significant "dihydrouracil dehydrogenase" activity (which is probably due to uracil reductase; for review, see Wasternack (29)) occurs in rat liver, intestinal mucosa, spleen, kidney, brain, and skeletal muscle (23). The extensive production of volatile, low-molecular-weight metabolites upon infusion of dThd (25) and thymine and the elimination of 80% of a low dose of [14C]FUra as labeled carbon dioxide (30) indicates the importance of catabolic pathways to the overall metabolism of these pyrimidines.

The mean in vivo estimates of $K_m$ and $V_{max}$ listed in Table 2 fall in a "biologically reasonable" range. $V_{max}$ represents the sum of the rates of all metabolic processes acting on each compound. Since the pyrimidines studied are rapidly and extensively catabolized (4, 30), a large portion of the activity measured is probably due to thymidine phosphorylase (34) in the case of dThd and uracil reductase (29) for thymine and FUra. Nevertheless, anabolic processes are also incorporated in the determination of $V_{max}$, so the values do not represent the activity of a single enzyme or tissue. Similarly, the values obtained for $K_m$ are whole-body averages for all metabolic processes acting on each compound and are also influenced by distributional and uptake factors (11). Thus, direct comparison of the in vivo results with in vitro determinations may not be valid.

A determination of these Michaelis-Menten parameters for dThd and thymine in vivo has not been previously reported, but Collins et al. (6) have published values for FUra. These authors simulated the behavior of FUra for both constant i.v. infusion and bolus administration. By fitting the simulations to literature data, estimates of $V_{max}$ and $K_m$ were obtained (75 µmol/min and 15 µM, respectively). Assuming a mean human body weight of 70 kg, this value of $V_{max}$ is equivalent to 1.07 µmol/min/kg. The close agreement between our determinations for FUra (Table 2) and those of Collins et al. (6) suggest no major species differences in the kinetics of FUra metabolism between humans and dogs.

We have demonstrated that thymine and dThd inhibit the metabolism of FUra, causing a rise in $C_{as}$ of FUra during simultaneous infusion (Chart 5). The dose-dependent inhibitory effect of thymine on $C_{pl}$ of FUra is shown in Chart 6. Our data indicate that the large effect of dThd on FUra metabolism is inhibition induced by thymine formed in vivo, since there was no distinction between the effects of thymine produced in this way and by direct infusion. Our findings are consistent with those of Au et al. (1), who demonstrated in a series of colorectal cancer patients receiving infusions of dThd and FUra that dThd-induced reduction of FUra plasma clearance is inversely related to the thymine plasma concentration. The concept of competitive metabolic inhibition is supported by the overlapping degradatory pathways for FUra and thymine (29) and the importance of catabolic processes to the overall elimination of these compounds. Moreover, Woodcock et al. (30), using radiolabeled FUra, conclusively demonstrated that dThd can almost completely eliminate the catabolism of FUra as measured by labeled carbon dioxide production.

Clinical studies with FUra plus dThd have shown that a major effect of the combination is an inhibition of FUra catabolism, with subsequent prolongation of its plasma $t_m$. This combination has frequently resulted in enhanced FUra toxicity, while therapeutic responses have been minimal (1, 15, 21, 30). It is possible that the effective increase in FUra dose induced by dThd has obscured any benefits of biochemical modulation produced by the combination, such as those originally proposed by Martin and Stoff (18). Future clinical use of FUra plus dThd may be improved if there is a better understanding of the effects on FUra pharmacokinetics. This would allow adjustment in the dosage of FUra to compensate for metabolic inhibition.

Our studies have resulted in quantitation of the kinetic behavior of dThd, thymine, and FUra in dogs using models incorporating Michaelis-Menten kinetics. In addition, the interaction among thymine, dThd, and FUra has been extensively described. Experimental data, including the effect of thymine on FUra plasma clearance (Chart 6), was simulated using an analogue modeling program (14), the results of which will be described in more detail elsewhere. Values for $V_{max}$, $K_m$, and $K_t$ of thymine and FUra required to produce the simulation shown in Chart 6 were within 10% of the experimentally derived estimates, indicating a reasonable agreement between the theoretical model and the experimental data.

It is not certain if the results reported here in dogs will quantitatively apply to human pharmacokinetics. Nevertheless, the model systems and experimental techniques used and validated in these studies will provide a rational framework for future investigations in humans. This applies not only to dThd and related pyrimidines but also to any compound which displays saturation kinetics in vivo. At the very least, these studies have provided an indication of the type of data which might be collected in future investigations of this kind.

ACKNOWLEDGMENTS

We wish to thank Willie Wynn and Danielle Szapary for their expert technical assistance. Thanks are also due to Drs. Daniel Zaharko, Robert Dedrick, and Jerry Collins for reading this manuscript and offering many helpful suggestions.

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Manuscript in preparation.


Nonlinear Pharmacokinetics of Thymidine, Thymine, and Fluorouracil and Their Kinetic Interactions in Normal Dogs

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