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Computer Modeling of the Pharmacokinetics of Fluorouracil and Thymine and Their Kinetic Interaction in Normal Dogs[5]

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Abstract: The kinetic behavior of thymine and 5-fluorouracil has been shown to be non-linear and mediated largely by saturable metabolic processes. *In vivo* estimates of the Michaelis-Menten parameters V_{max} and K_m were obtained from constant infusion data in normal dogs using a system of balance equations that equate drug input with total output at steady-state. These estimates were then successfully used to simulate both steady-state and post-infusion plasma concentration-time curves for both compounds over a range of saturating and non-saturating conditions. It has been shown previously that estimates of V_{max} and K_m obtained from dynamic data can be incorrect if an inappropriate compartmental model is used in the analysis. Determining the Michaelis-Menten parameters at steady-state eliminates this difficulty. Moreover, the use of steady-state derived values to simulate post-infusion data confirms the validity of this technique. The kinetic interaction between thymine and 5-fluorouracil was investigated as a case of competitive metabolic inhibition *in vivo* by calculating K_i values from data obtained during simultaneous constant infusions of the two compounds. These values were then used in conjunction with a series of differential equations incorporating reciprocal metabolic effects to simulate the effect of thymine on FU plasma concentration.

We have shown previously that the elimination of the pyrimidines thymine (Thy), thymidine (dThd), and fluorouracil (FUra) in normal dogs is mediated in part by a saturable metabolic process (1). Other investigators have presented similar findings for monkeys and man (2–4). In addition, co-administration of dThd and FUra has been shown to result in a competitive metabolic interaction resulting in a prolonged half-life for FUra and increased clinical toxicity (5, 6).

For many compounds, plasma concentration *vs.* time data can be analyzed using one of a number of non-linear regression programs. In conjunction with an appropriate pharmacokinetic model, this procedure can provide valid estimates of the elimination rate constants and zero-time intercepts (7). However, when a compound is eliminated from the body by a saturable process the analysis becomes more complex, and simple first-order kinetics are no longer valid over a wide concentration range. Differential equations of the form:

$$\frac{-dC}{dt} = \frac{V_{max}' \cdot C}{K_m + C} \qquad \text{(Eq. 1)}$$

where $-dC/dt$ is the rate of decline of drug concentration at time t, V_{max} is the maximum velocity (in concentration units) of the saturable process and K_m is the Michaelis constant, can be derived to describe such systems. Unfortunately, Equation 1 cannot be integrated and solved explicitly for C (7). It is difficult to use many non-linear regression programs to analyze this type of data, since they require an explicit expression for C. Some programs can accomodate a system of differential equations directly, but these can be costly of computational time (8), and in any case, a large number of unknown parameters may make a good fit difficult to obtain.

We have obtained estimates of V_{max} and K_m for dThd, Thy and FUra by incorporating these parameters into a series of balance equations describing the steady-state input and elimination of drug from the body during constant *i.v.* infusion (1).

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Sedman and Wagner (9) have shown that when estimates of V_{max} and K_m are made from *in vivo* data, selection of an appropriate compartmental model to describe the system is crucial for obtaining valid results. By calculating these parameters at steady-state we have eliminated the need to determine a compartmental model. Our estimates were shown to allow accurate prediction of the non-linear relationship between infusion rate and steady-state concentration. In addition, the metabolic interactions of FUra with Thy and with dThd were described by calculating inhibitor constant (K_i) values during simultaneous infusions of two compounds.

In this report we demonstrate that estimates of Michaelis-Menten parameters obtained during constant infusion can be used in conjunction with an analog modeling program to accurately simulate the dynamic behavior of these compounds following cessation of the infusion or following *i.v.* bolus injection. In addition, the effect of simultaneous infusion of Thy on FUra plasma levels was successfully simulated.

Materials and Methods

Theoretical

On the basis of data reported previously, a model that describes the pharmacokinetics of dThd, Thy and FUra at any dose was assumed to include the following features: (1): a two-compartment open system, (2) Michaelis-Menten elimination from the central compartment, and (3) a parallel first-order urinary excretion process from the central compartment.

It was assumed that each compound studied would follow first-order kinetics at plasma levels well below the apparent K_m for its metabolism. Estimates of such plasma levels were obtained from preliminary experiments and literature reports (10–12). Classical first-order pharmacokinetic parameters were determined using a two compartment model as described below. However, these are valid only when plasma levels of FUra and Thy are well below the point of significant metabolic saturation. To investigate the behavior of each compound at or near its K_m, plasma concentration *vs.* time and urinary data were obtained during steady-state infusions. Using the balance equations described below, the apparent K_m and V_{max} of each compound were calculated *in vivo*. Moreover, by conducting simultaneous FUra+dThd and FUra+Thy infusion studies under steady-state conditions, estimates of apparent K_i's for metabolic inhibition were obtained. While this protocol produces equations and constants which allow the prediction of steady-state levels at given infusion rates (k_o) (or the converse) for each compound, there are limitations to its usefulness. Only steady-state levels can be estimated; nothing can be predicted about the dynamic behavior of the compounds. This is a potentially serious limitation since both dThd and FUra have been administered clinically by routes other than constant *i.v.* infusion.

The Continuous Systems Modeling Program (CSMP) (13) provides a way to obtain dynamic information. This program simulates any continuous process which can be described by a series of differential equations. For the pharmacokinetic model describing the pyrimidines under investigation, equations in the form of Eq. 2 and 3 were used:

$$dC_1/dt = Q_{in} - (k_{12} + k' + V_{max}'/(K_m + C_1))\,C_1 + k_{21}\,C_2 \quad \text{(Eq. 2)}$$

$$dC_2/dt = k_{12}\,C_1 - k_{21}\,C_2 \quad \text{(Eq. 3)}$$

where C_1 and C_2 are drug concentrations in the central and peripheral compartment respectively, Q_{in} is the input function or infusion rate into C_1, k_{12} and k_{21} are the intercompartmental rate constants, and k' is the rate constant for the first-order urinary elimination process. As determined at steady-state, V_{max} is a fundamentally different parameter from that required for use with CSMP. In the former case, V_{max} represents the rate of metabolism of drug mass and has units in the form: μmoles/min. In contrast, Eq. 1 and 2 describe the rate of change in *concentration*, not total drug in each compartment. Thus V_{max} must have units such as μmoles/ml/min. To obtain V_{max} in the appropriate units, values as determined at steady-state were divided by the central compartment volume (V_c) for each compound. The new value is designated V_{max}'. Q_{in} is related to k_o in the same way. The CSMP output provides concentration *vs.* time data for both C_1 and C_2 at any time interval desired. Eqs. 2 and 3 can provide simulations of the approach to steady-state, the steady-state period, and the post-infusion decay. The program does not fit experimental data, rather it provides simulated concentration *vs.* time data when given the appropriate equations and parameter estimates.

Using CSMP, a number of experimental conditions were simulated, including saturating and non-saturating infusions of both Thy and FUra. dThd kinetics were not simulated because of difficulties in obtaining reliable estimates of pharmacokinetic parameters for this compound. The kinetic interaction between Thy and FUra was modeled by incorporating the Michaelis-Menten function for competitive inhibition into Eq. 2.

FUra:

$$dC_1/dt = Q_{in} - (k_{12} + k' + (V_{max}'/(K_m(1 + C_{T1}/K_{iT}) + C_1)))\,C_1 + k_{21}C_2 \quad \text{(Eq. 4)}$$

$$dC_2/dt = k_{12}C_1 - k_{21}C_2 \quad \text{(Eq. 5)}$$

Thy:

$$dC_{T1}/dt = Q_{Tin} - (k_{T12} + k_T' + (V_{maxT}'/(K_{mT}(1 + C_1/K_{IFU}) + C_{T1})))\,C_{T1} + k_{T21}C_{T2} \quad \text{(Eq. 6)}$$

$$dC_{T2}/dt = k_{T12}C_{T1} - k_{T21}C_{T2} \quad \text{(Eq. 7)}$$

where K_{iFU} is the inhibitor constant for FUra on Thy metabolism and K_{iT} is the inhibitor constant for Thy on FUra metabolism. Symbols containing a "T" subscript refer to values for Thy while those without refer to FUra. Simulations were conducted by "infusing" FUra alone for 3 h ($Q_{Tin} = 0$), followed by a 4 h period of co-infusion of FUra and Thy.

Initial Parameter Estimates

Thy and/or FUra were administered to normal dogs by constant *i.v.* infusion, with, where appropriate, an initial bolus loading dose. The procedures for drug administration, collecting and processing blood and urine samples, and for determining concentrations of these pyrimidines in plasma and urine using high pressure liquid chromatography have been described previously (1). The plasma concentration *vs.* time data for low dose experiments were analyzed using a digital computer-based nonlinear regression program (14). Post-infusion data points were fit to the equation

$C = R\,EXP(-\alpha t) + S\,EXP(-\beta t)$,

where α and β are the distributional and elimination rate constants respectively, and R and S are the corresponding end-infusion intercepts. Best-fit values for R, S, α, and β were obtained, and the zero-time intercepts (A and B) calculated as:

$A = RT\alpha$ and $B = ST\beta$, where T is the duration of infusion. Intercompartmental rate constants were derived from these parameters as described by Gibaldi and Perrier (7). Volume of the central compartment (V_c) was calculated as: $V_c = X_o/A+B$ where X_o is the total administered dose and is equivalent to k_oT. Urinary clearance was determined using the formula: $Cl_u = UV/C$, where U is urinary concentration, V is volume of urine, and C is plasma concentration. The apparent first-order rate constant (k') for urinary excretion was calculated as $k' = Cl_u/V_c$.

The Michaelis-Menten parameters V_{max} and K_m were determined *in vivo* for Thy 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 (C_{ss}) achieved by infusion at a constant rate (k_o), the input-output balance equation is:

$$k_o = C_{ss}(Cl_u + V_{max}/(K_m + C_{ss})) \quad \text{(Eq. 8)}$$

At C_{ss} where metabolism is saturated, enzyme velocity will approach V_{max}, and since $C_{ss} \times Cl_u$ = urinary excretion, Eq. 8 simplifies to:

$$k_o = V_{max} + \text{urinary excretion} \quad \text{(Eq. 9)}$$

At low rates of infusion when $C_{ss} << K_m$,

$$k_o = C_{ss}(Cl_u + V_{max}/K_m) \quad \text{(Eq. 10)}$$

Thus, urinary excretion was measured at a saturating C_{ss}, and Eq. 9 solved for V_{max}. At low rates of infusion where C_{ss} is well below K_m, C_{ss} and Cl_u were determined and Eq. 10 was solved for V_{max}/K_m. Using this information, K_m could also be calculated. For use with CSMP, V_{max}' was derived from V_{max} and Q_{in} was derived from k_o as described above.

K_i values for the interaction between FUra and Thy were estimated *in vivo* by incorporating the appropriate Michaelis-Menten function into Eq. 8:

$$k_o = (Cl_u + V_{max}/(K_m(1 + I/K_i) + C_{ss}))C_{ss} \quad \text{(Eq. 11)}$$

FUra was infused to a nonsaturating C_{ss}; then a second infusion of dThd or Thy was begun, and the two compounds were infused simultaneously for 4 h. By determining C_{ss} and Cl_u for FUra and C_{ss} for Thy (I) during the interaction period, and using previously determined values of V_{max} and K_m for FUra, Eq. 11 could be solved for K_{iT}, a measure of Thy inhibition of FUra metabolism.

From the data obtained during simultaneous infusion, Eq. 11 was also used to determine the K_i for FU (K_{iFU}) as an inhibitor of Thy metabolism. C_{ss} and Cl_u for Thy were measured during the interaction period. V_{max} and K_m for Thy had been determined by previous experiments with that compound alone. Now the inhibitor concentration (I) equals the C_{ss} of FU. Thus Eq. 11 could be solved for K_{iFU}.

Initial parameter values were adjusted as required in order to obtain the best fit to experimental data, as determined by visual inspection. Initial and best-fit values are listed in the tables accompanying the simulations described below.

Results

Computer simulations of two low dose Thy infusions are shown in Figure 1A (dog 80–30) and 1B (dog 80–64). The experimental data were closely simulated using a two compartment model. The fit to the post-infusion data was excellent, although the initial phase was so rapid only a few experimental points were obtained in this area. The approach to steady-state was simulated well for dog 80–30, but with some error in dog 80–64. The initial parameter estimates for these two experiments were altered somewhat to produce the best-fit simulation (Table I).

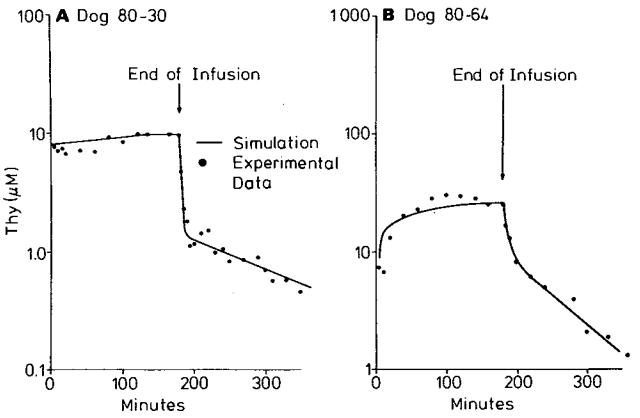

Fig. 1 Plasma concentration-time data for low dose Thy infusion. A and B, k_o = 0.396 μmoles/kg/min. Parameter values for CSMP simulation are listed in Table I.

Table I. CSMP Simulation – Low Dose Thy Infusions (Dogs 80–30 and 80–64)

Model Equations:

$dC_1/dt = Q_{in} - (k' + k_{12} + (V_{max}'/(K_m + C_1)))\,C_1 + k_{21}C_2$

$dC_2/dt = k_{12}C_1 - k_{21}C_2$

Parameter Values:	A. Dog 80–30		B. Dog 80–64	
	Initial	Best-fit	Initial	Best-fit
Q_{in} (μmoles/l/min)	3.5	3.6	2.3	2.8
k' (min^{-1})	0.013	0.013	0.011	0.011
k_{12} (min^{-1})	0.062	0.10	0.058	0.058
k_{21} (min^{-1})	0.0071	0.007	0.018	0.018
V_{max}' (μmoles/l/min)	23.4	26.0	15.4	20.0
k_m (μM)	69.5	69.0	200	200
C_1 (start) (μM)	0	0	0	0
C_2 (start) (μM)	0	0	0	0

Only one change in the intercompartmental rate constants was made (k_{12} increased in dog 80–30), indicating good initial estimates. A good fit in the transition area between distributional and elimination phases is additional evidence that appropriate values of the intercompartmental rate constants have been used. Q_{in} and V_{max}' had to be altered somewhat, but these parameters are dependent on an estimate of V_c which introduces additional variability in the initial estimate. At low plasma concentrations ($C << K_m$), variation in K_m and V_{max} will have a major effect on the metabolic rate. Although the estimates of K_m were quite different in the two animals, no changes were necessary to produce the best-fit, suggesting that the initial values were accurate.

To simulate a high dose Thy infusion, the best-fit estimates obtained at low dose were used as a starting point for most parameter values. The loading dose was divided by V_c to provide an initial estimate of the instantaneous concentration in C_1, and k' was estimated experimentally. A simulation was achieved which matched all phases of plasma-time curve (Figure 2). To produce this fit, the initial parameter estimates were altered somewhat (Table II). The intercompartmental

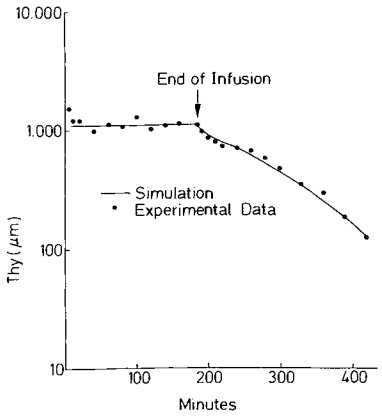

Fig. 2 Plasma concentration-time data for high dose Thy infusion. k_o = 4.80 μmoles/kg/min; bolus loading dose = 783 μmoles/kg. Parameter values for CSMP simulation are listed in Table II.

Table II. CSMP Simulation – High Dose Thy Infusion (Dog 80–30)
Model Equations:
$dC_1/dt = Q_{in} - (k' + k_{12} + (V_{max}'/K_m + C_1))) C_1 + k_{21}C_2$
$dC_2/dt = k_{12}C_1 - k_{21}C_2$

Parameter Values:	Initial	Best-fit
Q_{in} (μmoles/l/min)	43	43
k' (min^{-1})	0.018	0.018
k_{12} (min^{-1})	0.100	0.200
k_{21} (min^{-1})	0.007	0.03
V_{max}' (μmoles/l/min)	26	22
K_m (μM)	69	100
C_1 (start) (μM)	7000	7000
C_2 (start) (μM)	0	0

rate constants had to be substantially increased to produce the required 'break' in the decay curve, and V_{max}' and K_m were varied slightly to make final adjustments in the fit. Simulation of a high dose Thy bolus administration was also performed (data not shown). A good fit to experimental data was achieved with only slight modification of the best-fit infusion estimates shown in Table II.

The experimental post-infusion data obtained at low-dose for FUra were limited because plasma concentrations rapidly fell below detectable levels. In the experiment shown in Figure 3, only 4 post-infusion data points were obtained. Under such conditions the fitting of data to a biexponential equation and the calculation of intercompartmental rate constants may be inaccurate. Nevertheless, such estimates were used to produce the CSMP simulation shown in Figure 3. Surprisingly, an adequate fit was obtained with only slight modification of the initial estimates (Table III). The stimulation approaches steady-state somewhat too rapidly, but the C_{ss} was intentionally fit to the 240 min point rather than an average concentration. Considering the limited experimental data, the overall fit of the simulation is good. This is important, since the simulation of low dose FUra infusion was subsequently required for modeling the FUra/Thy interaction.

The results of simulating a high-dose FUra infusion are shown in Figure 4. A good fit was obtained to the post-infusion data, which demonstrated the convex shape expected for a saturating Michaelis-Menten process (7). Initial estimation of C_1 (start) was based on the loading dose given and V_c, while k'

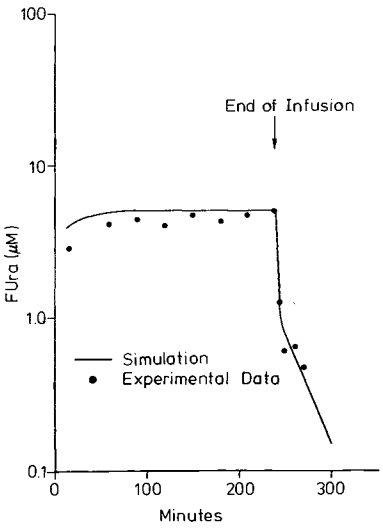

Fig. 3 Plasma concentration-time data for low dose FUra infusion. k_o = 0.396 μmoles/kg/min. Parameter values for CSMP simulation are listed in Table III.

Table III. CSMP Simulation – Low Dose FUra Infusion (Dog 80–116) Model Equations:
$dC_1/dt = Q_{in} - (k' + k_{12} + (V_{max}'/(K_m + C_1))) C_1 + k_{21}C_2$
$dC_2/dt = k_{12}C_1\ k_{21}C_2$

Parameter Values:	Initial	Best-fit
Q_{in} (μmoles/l/min)	2.0	1.9
k' (min^{-1})	0.0049	0.0049
k_{12} (min^{-1})	0.10	0.10
k_{21} (min^{-1})	0.040	0.040
V_{max}' (μmoles/l/min)	8.0	8.0
K_m (μM)	17.2	17.0
C_1 (start) (μM)	0	0
C_2 (start) (μM)	0	0

was estimated experimentally. Other initial parameter values (Table IV) were those producing the best low dose simulation (Table III). Although the high and low dose infusions were done in different animals, good fits to the data were obtained with similar parameter values. This is in contrast to the high dose Thy experiments which required rather extensive changes of the low dose estimates (which were obtained in the same

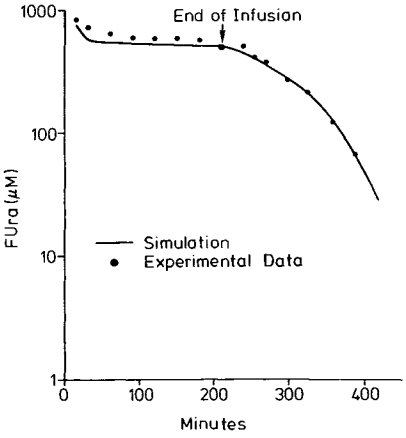

Fig. 4 Plasma concentration-time data for high-dose FUra infusion. k_o = 2.93 μmoles/kg/min; bolus loading dose = 635 μmoles/kg. Parameter values for CSMP simulation are listed in Table IV.

Table IV. CSMP Simulation – High Dose FUra Infusion (Dog 81–112)
Model Equations:
$dC_1/dt = Q_{in} - (k' + k_{12} + (V_{max}'/(K_m + C_1))) C_1 + k_{21}C_2$
$dC_2/dt = k_{12}C_1 + k_{21}C_2$

Parameter Values:	Initial	Best-fit
Q_{in} (μmoles/l/min)	11.5	12
k' (min^{-1})	0.0082	0.0082
k_{12} (min^{-1})	0.10	0.10
k_{21}(min^{-1})	0.04	0.037
V_{max}' (μmoles/l/min)	8	8.7
K_m (μM)	17	17
C_1 (start) (μM)	2240	2240
C_2 (start) (μM)	0	0

dog). A major difference between Thy and FUra was the low dose derived estimate of k_{21} which was 5.7 times greater for FUra. To simulate high dose post-infusion data, k_{21} had to be increased 4-fold for Thy, but the initial estimate for FUra required only slight adjustment. It is not clear if these results reflect differences in the relative intercompartmental transfer of Thy and FUra at low and high dose or simply result from the lack of more extensive low dose data.

Two simulations of the FUra/Thy interaction, differing only in the Thy infusion rate, are depicted in Figure 5A and 5B. The C_1 (plasma) concentration *vs.* time plots of both experimental and simulated data are shown for both FUra and Thy. Parameter values and model equations are listed in Table V. In panel A, as FUra was infused alone for the first 180 min, the simulation produced a C_{ss} of 4.75 μM, which is in general

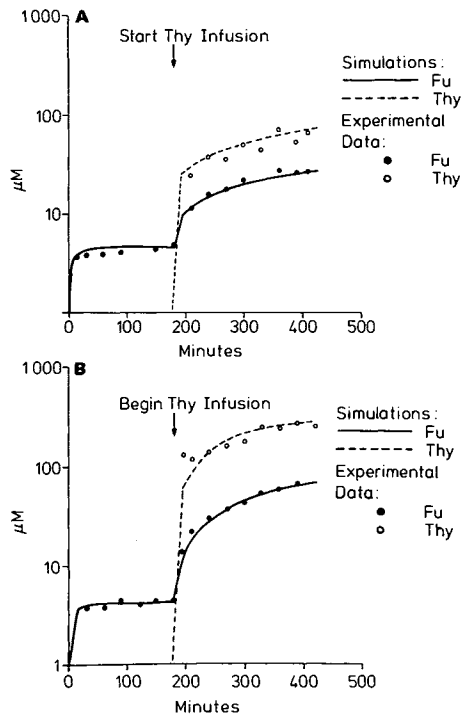

Fig. 5 Plasma concentration-time data for FUra + Thy Experiments
A. k_o(FUra) = 0.396 μmoles/kg/min (0–420 min); k_o(Thy) = 1.42 μmoles/kg/min (180–420 min)
B. k_o(FUra) = 0.396 μmoles/kg/min (0–420 min); k_o(Thy) = 2.0 μmoles/kg/min (180–420 min)
Parameter values for CSMP simulations are listed in Table V.

Table V. CSMP Simulations – FUra/Thy Interaction
Model Equations:
FU:
$dC_1/dt = Q_{in} - (k_{12} + k' + (V_{max}'/K_m(1 + C_{T1}/K_{iT}) + C_1))) C_1 + k_{21}C_2$
$dC_2/dt = k_{12}C_1 - k_{21}C_2$
Thy:
$dC_{T1}/dt = Q_{Tin} - (k_{T12} + k_T' + (V_{max}'_T/K_{mT}(1 + C_1/K_{iFU}) + C_{T1}))) C_{T1} + k_{T21}C_{T2}$
$dC_{T2}/dt = k_{T12}C_{T1} - k_{T21}C_{T2}$

Parameter Values	Expt. A.: Dog 80–116 Initial	Best-fit	Expt. B: Dog 80–116 Initial	Best-fit
Q_{in} (μmoles/l/min)	2	2	2	1.85
k' (min^{-1})	0.007	0.007	0.007	0.007
k_{12} (min^{-1})	0.10	0.10	0.10	0.10
k_{21} (min^{-1})	0.040	0.040	0.040	0.070
V_{max}' (μmoles/l/min)	9	9	9	9
K_m (μM)	17	17	17	17
C_1 (initial (μM)	0	0	0	0
C_2 (initial) (μM)	0	0	0	0
K_{iT} (μM)	11.2	11.2	11.2	9
*Q_{Tin} (μmoles/l/min)	7.55	9	11	12.5
k'_T (min^{-1})	0.009	0.009	0.009	0.009
k_{T12} (min^{-1})	0.10	0.20	0.20	0.20
k_{T21} (min^{-1})	0.007	0.02	0.02	0.02
$V_{max}'_T$	12	12	12	12
K_{mT} (μM)	38	38	38	38
C_{T1} (initial) (μM)	0	0	0	0
C_{T2} (initial) (μM)	0	0	0	0
K_{iFU} (μM)	36	36	36	36

*Q_{Tin} = 0 from 0 to 180 min.

agreement with the experimental data. When the Thy infusion was started, levels of that compound rose rapidly in plasma. Although the experimental data were somewhat variable, a good simulation of Thy levels was achieved. During this time, FUra levels also rose, although the infusion rate of FUra was constant. The simulation of this metabolic interaction gave an excellent fit to the experimental results. Table V shows that few modifications of the initial parameter estimates were required. Only slight adjustments to Q_{Tin}, K_{T12} and K_{T21} were made. It is remarkable that this complex system required so little adjustment. The changes needed in several Thy parameters may have been due to the fact that the initial estimates were derived from a low dose Thy experiment in a different dog. The most important result of this simulation is that the estimated values of K_{iT} and K_{iFU} appeared to be correct. That the initial estimates of these parameters did not need to be altered supports their validity and the method of calculation.

The second FUra/Thy interaction simulation is shown in Panel B. In this experiment, the same infusion rate of FUra (Q_{in}) used in Panel A was combined with a 1.5-fold higher rate for Thy (Q_{Tin}). The initial and best-fit parameter estimates are listed in Table V. Again an excellent match between experimental and simulated data was obtained for both FUra and Thy. Starting from the best-fit estimates for Expt. A, only a few additional changes were required (other than Q_{Tin}). K_{iT} was lowered by 20 % to increase the inhibitory effect of Thy on FUra somewhat. The final value was within the range of experimental estimates in this dog. k_{21} was increased to adjust the shape of the FUra curve. These results provide additional support for the validity of the experimental determinations of K_{iT} and K_iFU, and demonstrate their applicability in the dynamic case over at least 4-fold range of Thy concentrations.

Discussion

In each of the computer simulations presented in this report, a reasonably close fit to the actual experimental data was obtained. For the low dose experiments, the initial parameter estimates produced good simulations with only minimal alterations. For the high dose experiments, more extensive changes were required. Since the calculations of intercompartmental rate constants were made only at low dose, it had to be assumed that the same values would apply at high (saturating) doses. From the current experiments it is not possible to ascertain if this is true. The calculated values of the Michaelis-Menten parameters required relatively little alteration.

Although some parameters had to be changed in order to fit high dose data, it is important to realize that there may be more than one combination of values that could fit the experimental data. These simulations were done by trial and error, and given the large number of parameters, the 'biologically correct' solution may not have been found. Indeed, the model system used probably does not completely reflect the true biochemical and physiological properties of pyrimidine metabolism *in vivo*. For example, metabolic and renal elimination were limited to the central compartment. This is a reasonable approximation since the major sites of elimination (blood, liver, kidney) are well perfused tissues and thus are components of that compartment. Nevertheless, there are many slowly perfused tissues in the body which are therefore components of the peripheral compartment and which metabolize these compounds (15, 16). This discrepancy may have resulted in simulated peripheral concentrations (C_2) many fold higher than those actually present in tissues.

In addition, the alterations in k_{12} and k_{21} were probably required to shift the thermodynamic balance of material towards C_1 where it could be metabolized. An alternate approach would have been to add peripheral metabolism to the system. However, this would create new difficulties, since the experimental data provided no basis for estimating these parameters. Likewise, although concentrations in the peripheral compartment appeared high, no experimental value for tissue concentration was obtained. Collins et al. (17) have demonstrated that arbitrary changes in the distribution of metabolism between compartments do not affect the ability of their model to predict the plasma concentration-time behavior of FUra.

We have demonstrated that the kinetic interaction between Thy and FUra is a case of competitive metabolic inhibition *in vivo*. This is shown in Figure 5 in which the differential equations incorporating this concept produce an excellent stimulation of the effects of Thy on plasma levels of FUra when both compounds are infused simultaneously. While the data are too limited to conclude that the parameter estimates are numerically valid, the presented model system has been shown to be useful for predicting the reciprocal interaction between Thy and FUra. The interaction between dThd and FUra, while more clinically relevant, was not simulated since some parameter estimates for dThd were not obtained. When such estimates are available, the model system can be easily adapted to study this interaction. Since Thy is the proximal inhibitor of FUra metabolism, the simulations presented in this report provide useful information.

In conclusion, the simulations described here represent one possible system that provides an adequate fit to the experimental data available. Experimental estimates of Michaelis-Menten parameters required only minimal adjustment which suggests that the steady-state system used for their determination is valid. Moreover, since the values determined at steady-state are shown to be applicable to dynamic data, the presented system should prove useful for predicting the behavior of these compounds in situations where a specific compartmental model cannot be applied. It is not certain if the results obtained in dogs apply to human pharmacokinetics. Nevertheless, the model systems and experimental techniques used and validated in this project provide a rational framework for future investigations in man. This applies not only to FUra and related pyrimidines, but to any drug that displays saturation kinetics *in vivo*.

Abbreviations

A	zero-time intercept of the distributional phase in a two-compartment model following an i. v. bolus dose
B	zero-time intercept of the elimination phase in a two-compartment model following an i. v. bolus dose
C	concentration
C_1	concentration in the central compartment of a two-compartment model
C_2	concentration in the peripheral compartment of a two-compartment model
Cl_u	urinary clearance
CSMP	continuous systems modeling program
C_{ss}	steady-state concentration
dThd	thymidine
EXP	exponent
FUra	5-fluorouracil
I	inhibitor concentration
i. v.	intravenous
k_o	infusion rate (μmoles/min)
k'	apparent first-order rate constant for urinary excretion
k_{12}	apparent first-order intercompartmental transfer rate
k_{21}	apparent first-order intercompartmental transfer rate
K_i	inhibitor constant
K_{iT}	inhibitor constant for thymine
K_{iFU}	inhibitor constant for fluorouracil
K_m	Michaelis constant
Q_{in}	input rate for computer simulations (μmoles/l/min)
R	end-infusion intercept of the distributional phase in a two-compartment model following a constant infusion
S	end-infusion intercept of the elimination phase in a two-compartment model following a constant infusion
t	time
T	duration of infusion
Thy	thymine
U	concentration in urine
V	volume
V_c	apparent volume of the central compartment
V_{max}	maximum enzyme velocity (μmoles/min)
V_{max}'	maximum enzyme velocity (μmoles/l/min)
X_o	loading dose or total administered dose
α	apparent first-order distributional rate constant in a multicompartment model
β	apparent first-order elimination rate constant in a multicompartment model

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Inosine Analogs as Anti-Leishmanial Agents

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Abstract: Several criteria were used to select a number of inosine analogs as potential growth inhibitors of the protozoan parasite *Leishmania tropica*. Of nine compounds tested, seven showed a high degree of selective toxicity towards *L. tropica* promastigotes as compared to mouse L1210 cells; these include analogs of formycin B, 7-substituted analogs of 7-deazainosine and analogs of inosine in which the sugar moiety has been modified to confer metabolic stability. The metabolism of 7-deazainosine in *L. tropica* promastigotes was shown to involve conversion to cytotoxic adenosine nucleotide analogs (tubercidin derivatives) that become incorporated into RNA. The results suggest several new classes of compounds which have potential as anti-leishmanial agents.

Inosine analogs such as allopurinol riboside (1–3), thiopurinol riboside (2, 4), formycin B (2, 3, 5–8) and 3'-deoxyinosine (9) inhibit the growth of the pathogenic protozoan *Leishmania* at concentrations that have little effect on mammalian cells. Accordingly, these and related compounds offer promise as chemotherapeutic agents for the treatment of leishmaniasis. Metabolic studies of the aforementioned analogs have revealed features important for their apparent selective toxicity. First, these analogs possess modified sugar or base moieties that confer resistance towards catabolism. Second, the selectivity appears to result from differences in purine salvage pathways in the host and parasite. *Leishmania* has a nucleoside phosphotransferase that effectively converts these inosine analogs to their corresponding nucleoside 5'-monophosphates (1, 4, 6–9), whereas mammalian cells are either ineffective or less effective in phosphorylation of these compounds (8, 10). Third, the 5'-monophosphates of these analogs are either directly cytotoxic to *Leishmania* or are further converted to other cytotoxic metabolites in these organisms. Thiopurinol riboside-5'-monophosphate is believed to be the cytotoxic metabolite of thiopurinol in *Leishmania* (4), whereas the effects of allopurinol riboside and formycin B appear to result from conversion of their 5'-monophosphates to corresponding cytotoxic adenosine nucleotide metabolites and their subsequent incorporation into RNA (1, 6–8). 3'-Deoxyinosine is converted to adenosine nucleotide analogs, but is not incorporated into RNA (9).

The aforementioned findings provide a rationale for the design of additional potentially useful anti-leishmanial agents. In the present work, we describe the growth inhibitory effects of a number of inosine analogs towards *L. tropica* promastigotes and L1210 mouse leukemia cells. In addition, we have examined the metabolism of one of these analogs, 7-deazainosine, and found it to be similar to that reported for allopurinol riboside and formycin B.

Materials and Methods

L. tropica promastigotes (Clone POJ2 of Iran strain 252, obtained from B. Ullman) were grown at 26°C in room air supplemented to 9 % CO_2 using a defined medium consisting of Dulbecco's modified Eagle's medium containing 1 % glucose, 0.3 % bovine serum albumin, 5 mg/l hemin and 10 μM hypoxanthine. Stock cultures were maintained by reseeding

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