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Pharmacokinetics of 5-fluorouracil infusions in the rat: Comparison with man and other species

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Summary. *Saturable elimination of 5-FU is exhibited in rats during constant infusions. Over the range of 3–480 mg/m²/h, total-body clearance of 5-FU decreases from 600 ml/min/m² to less than 90 ml/min/m². Previously published values for catabolism of 5-FU by rat hepatocytes can be used to simulate the plasma concentrations of 5-FU that were measured over this range of infusions. The qualitative pattern of nonlinear pharmacokinetics for 5-FU is similar in rats, dogs, monkeys, and humans. However, there are major quantitative differences in total-body clearance values, expressed in terms of surface area (ml/min/m²) or as a fraction of cardiac output. Rat and monkey have substantially lower 5-FU clearance values than dog and man. Although 5-FU clearance values were similar in dogs and humans, total body clearance values for thymidine are 18-fold higher in humans than in dogs. The selection of an appropriate animal model to pursue the clinical observations of high total body clearance of pyrimidines remains uncertain.*

Introduction

5-Fluorouracil (5-FU) is an established agent for the treatment of several neoplastic diseases, either as a single agent or in combination chemotherapy regimens [2]. 5-FU is administered by bolus IV injections for 5 consecutive days or as a continuous 5-day IV infusion. There is no consensus regarding the antitumor efficacy of the two schedules. Furthermore, the toxicities of the two schedules are both qualitatively and quantitatively different. The standard bolus dose is 550 mg/m²/day, which produces myelosuppression as the most severe toxicity. On the other hand, mucositis is dose-limiting for infusions at 1,100 mg/m²/day [2].

The pharmacokinetics of 5-FU elimination from the body are highly schedule-dependent [3, 5]. After a bolus dose, elimination processes are initially saturated by the high drug concentrations achieved immediately after drug delivery. During a continuous infusion, the 5-FU concentration is maintained below saturating levels. Thus, the quantitative differences between doses for the two schedules are partly explained by the higher total-body clearance (CL_{TB}) of drug at low concentrations. For equal total drug exposure ($C \times t$, concentration-time product), a larger dose of 5-FU must be given as a constant infusion. The qualitative differences in toxicity may relate to time-dependent effects, since 5-FU attains high peak concentrations following a bolus, but duration of exposure is short since the plasma $t_{1/2}$ is only 10 min.

In humans, the CL_{TB} of 5-FU during continuous infusions is of the same magnitude as cardiac output, 5 l/min [5, 8]. Although the liver has an abundant supply of the enzymes known to metabolize 5-FU, the maximum contribution of the liver to 5-FU clearance is liver blood flow, about 1.5 l/min. Thus, extrahepatic elimination of 5-FU dominates its clearance from the human body. In an earlier analysis of 5-FU clinical pharmacokinetics [5] it was speculated that the lungs could be the principal site of 5-FU clearance.

Due to the difficulties of clinical experimentation, we wished to develop an animal system to further explore the saturable pharmacokinetics of 5-FU and to directly test the hypothesis of extensive pulmonary clearance. The rat was chosen for two reasons: (a) its extensive use in the preclinical testing of anticancer drugs; and (b) our previous experience using the rat as a model for exploring the delivery of 5-FU via peritoneal dialysis [6].

Materials and methods

Experimental. Female Sprague-Dawley rats (200–250 g) were anesthetized with 50 mg/kg pentobarbital IP. Supplemental doses of 15 mg/kg IM were given as required. Rectal temperature was maintained at 37 ± 0.2° C by means of a heat lamp and temperature controller. 5-FU and 2-[^{14}C]5-fluorouracil (100 μCi/mg, 98% radiochemical purity) were obtained from the Pharmaceutical Resources Branch, National Cancer Institute. Radiolabeled and cold 5-FU were dissolved in Ringer's lactate solution and infused into a jugular vein via a polyethylene catheter. The concentration of 5-FU in the dose solution was adjusted so that 5-FU infusion rates ranged from 3 to 480 mg/m²/h (0.5–80 mg/kg/h) while the Ringer's lactate was infused at a fixed rate of 2 ml/h. The total radioactivity delivered was 50–200 μCi per animal.

Periodic blood samples (up to 6 h) were obtained from a carotid artery via an indwelling polyethylene catheter. Plasma was separated by centrifugation and 20 μl was spotted directly on silica gel thin-layer chromatography plates. The plates were developed with the upper phase of a mixture of 60% ethyl acetate, 5% formic acid, and 35% water. The R_f of 5-FU was above 0.45, while all metabolites have a lower R_f. Dihydrofluorouracil is unstable in this system, and is measured along with the polar metabolites, fluoroureidopropionic acid, and urea [11].

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Modeling. Steady-state infusion data can be analyzed by a one-compartment model:

$$V\frac{dC}{dt} = G - CL_{TB}C\,, \tag{1}$$

where V is the volume of distribution and G is the infusion rate. CL_{TB} may have saturable and nonsaturable terms:

$$CL_{TB} = \frac{V_{\max}}{K_m + C} + CL_R\,. \tag{2}$$

$V_{\max}$ is the maximum elimination rate for the process, which is half-saturated at a concentration equal to K_m. CL_R is a nonsaturable elimination process, presumably dominated by renal clearance of 5-FU.

When elimination processes are not saturated, the concentrations of 5-FU in some tissues may be lower than the plasma concentration owing to high tissue extraction. For this case, multicompartment models must be considered. For a two-compartment model in which the liver is separate from the rest of the body:

$$V\frac{dC}{dt} = G - CL_RC + Q_L(C_L - C) \tag{3}$$

$$V_L\frac{dC_L}{dt} = Q_L(C - C_L) - \frac{V_{\max,L}C_L}{K_{m,L} + C_L}\,. \tag{4}$$

C_L is the 5-FU concentration in the liver, Q_L is the hepatic blood flow, and V_L is hepatic volume. $V_{\max,L}$ is the maximum 5-FU elimination rate for the liver and $K_{m,L}$ is the concentration of 5-FU in the liver at which this elimination process is half-saturated. It is assumed that the hepatic artery and portal vein can be considered as a single input and that the 5-FU concentration in the hepatic vein is in equilibrium with the concentration in liver tissue. If the concentration of 5-FU in the liver is in excess of $K_{m,L}$, liver extraction decreases and the liver concentration approaches the plasma concentration.

If the lung eliminates 5-FU from the body, its contribution to CL_{TB} is [4, 5]:

$$CL_{TB} = \frac{Q_{co}E_{lung}}{1 - E_{lung}} \tag{5}$$

and pulmonary extraction (E_{lung}, the arteriovenous concentration difference divided by the arterial concentration) is:

$$E_{lung} = \frac{(CL_{TB}/Q_{co})}{1 + (CL_{TB}/Q_{co})}\,. \tag{6}$$

Q_{co} is the cardiac output. Unlike the liver, the lungs belong in the central compartment, since the effluent from the lungs is arterial blood and it is assumed that the 5-FU concentration in effluent blood is in equilibrium with the concentration in tissue.

Parameter estimation. 1. CL_R for 5-FU in rats was reported [1] to be 7 ml/min/kg or 42 ml/min/m^2. A similar value of 1.5 ml/min/kg or 30 ml/min/m^2 has been reported [7] for dogs.

2. $K_{m,L}$ has been determined in rat liver hepatocytes [17] to be 3.3 μg/ml or 25 μM. For the one-compartment model K_m should be higher than $K_{m,L}$, since the liver concentration is lowered by hepatic elimination.

3. $V_{\max,L}$ for rat liver hepatocytes has been reported [17] to be 1,098 ng/30 min/500,000 cells or 0.56 nmol/min/10^6 cells. Lin et al. [15] assumed that 10^6 cells is equivalent to 0.33 mg microsomal protein and 50 mg microsomal protein per gram liver, or 1.5×10^8 cells per gram liver (1.5×10^9 cells in a 10-g liver from a 200-g rat). Thus, $V_{\max,L}$ is 0.85 μmol/min. Weibel et al. [16] also calculated 1.5×10^8 cells/g liver, based on microscopic observations. Siliciano et al. [15] suggest that 10^6 cells is equivalent to only 0.16 mg microsomal protein, which corresponds to 3×10^9 cells per liver or V_{max} of 1.7 μmol/min. For the two-compartment model, simulations were made for both $V_{\max,L}$ values. For the one-compartment model, $V_{\max} = 1.7$ μmol/min was used for all simulations.

4. Q_L is 24 ml/min for a 200-g rat with a 10-g liver [14].

5. Q_{co} values are listed in Table 1.

6. V for 5-FU is 600 ml/kg [5], or 120 ml for a 200-g rat.

7. V_L was assumed to be 8 ml for a 10-g liver.

Table 1. 5-FU CL_{TB} and cardiac output in four species

Species	CL_{TB} (ml/min/m^2)	Q_{co} (ml/min/m^2)	Hypothetical E_{lung}
Man	2,800 [8]	2,600[b]	0.52[c]
Rat	600[a]	1,500	0.29
Monkey	347 [10]	1,600	0.18
Dog	1,200 [7]	2,100	0.36

[a] This study
[b] Calculated from Lindstedt [13]
[c] Assume all CL_{TB} in lungs, use Eq. (6)

Results

Figure 1 is a plot of the plasma concentration vs time data for infusion rates ranging from 3 to 480 mg/m^2/h. At infusion rates of 3 and 32 mg/m^2/h, steady-state concentrations (0.6 μM and 9 μM) are reached by 1 h. The maximum 5-FU CL_{TB} determined from these experiments is 20 ml/min. This corresponds to 100 ml/min/kg or 600 ml/min/m^2. At an infusion rate of 480 mg/m^2/h, plasma 5-FU concentrations of 800 μM are observed and steady state, if achieved, requires at least 3 h of infusion. CL_{TB} has decreased to less than 3 ml/min or 90 ml/min/m^2.

The solid lines in Fig. 1 are simulations for the one-compartment model (Eq. 1). The parameters $V_{\max}$ and CL_R were estimated as described in *Methods,* and K_m was empirically adjusted to 80 μM to fit the lower rates of infusion. The simulations are consistent with all five data sets shown in Fig. 1. The experimental data for the second highest infusion rate, 220 mg/m^2/h, seems to drift higher throughout the infusion, but this behavior is not predicted by the model except at higher infusion rates, such as 480 mg/m^2/h. It is not known whether the discrepancy is a model failure or a spurious data set.

Figure 2 presents simulations for the 5-FU concentration in the central compartment of the two-compartment model (Eqs. 3 and 4). For each infusion rate, simulations were made for both the lower (1.5×10^9) and upper (3×10^9) estimates of the number of cells per liver. The overall agreement between the simulations and data is similar to that for the one-compartment model, but *none* of the parameters in these two-compartment model simulations were adjusted to fit the data. Since the

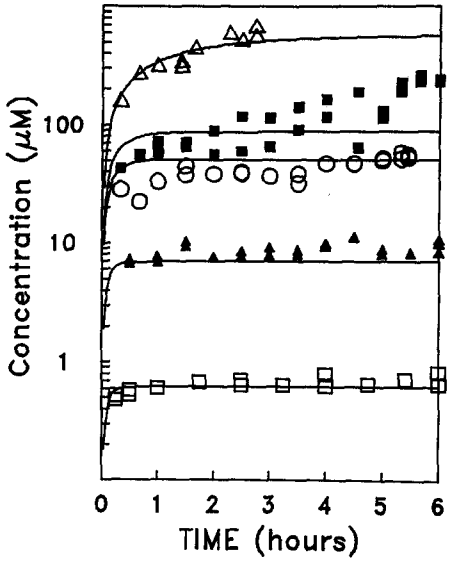

Fig. 1. Experimental data and model simulations for 5-FU infusions in the rat. Infusion rates (mg/m^2/h) are: 3 (□), 32 (▲), 160 (○), 220 (■), 480 (△). *Solid lines* are one-compartment model simulations

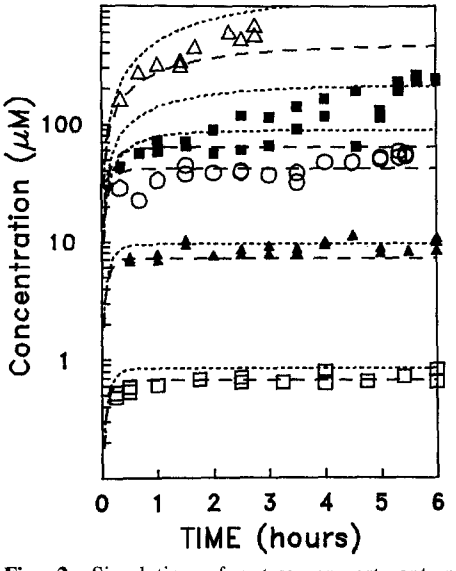

Fig. 2. Simulations for two-compartment model. *Dashed lines* correspond to upper estimate for number of hepatocytes per liver (3×10^9) and *dotted lines* correspond to lower estimate (1.5×10^9). *Symbols* for experimental data as in Fig. 1

parameters for elimination processes are limited to the liver and kidney, it does not appear that elimination from other tissues is necessary to explain 5-FU clearance from rats.

Discussion

The experimental data in this study provide a basis for pharmacokinetic modeling of 5-FU in the rat, and also for comparison with 5-FU pharmacokinetics in other species. The maximum CL_{TB} for 5-FU of 600 ml/m^2/min (at infusion rates of 3–32 mg/m^2/h) is similar to the value of 720 ml/m^2/min (120 ml/kg/min) reported for rats at infusion rates of 6–12 mg/m^2/h [1].

The constant infusion data were collected over a wide enough range (3–480 mg/m^2/min) to fully explore the saturable elimination behavior of 5-FU. This data could be reasonably fit with either a one- or a two-compartment physiological model. For the two-compartment model, all of the parameters were determined a priori. For the one-compartment model, the K_m value was adjusted to match the data. Although it is not possible to discriminate between the two models, the essential information gained from the modeling exercise is that for rats, unlike humans, the liver and kidneys can account for all of the CL_{TB} of 5-FU.

There is qualitative similarity in the saturable elimination of 5-FU exhibited in rats, dogs [7], monkeys [10], and humans [5, 8]. However, as shown in Table 1, the CL_{TB} rates are quantitatively different. When CL_{TB} is expressed on a surface area basis, there is an eight-fold range in the values, from 347 ml/min/m^2 in monkeys to 2,800 ml/min/m^2 in humans.

One of the goals of this study was to determine which animal species might be appropriate for further studies of the high clearance values of 5-FU observed clinically. In Table 1, the maximum values of pulmonary extraction of 5-FU in humans, rats, dogs, and monkeys are listed. These extraction values are an upper limit, since the calculation assumes that the lungs are the sole organ of elimination for each species, as derived in Eq. [6]. Since it is known that there is extensive liver metabolism of 5-FU, the actual pulmonary extractions are lower.

The pulmonary extraction of 5-FU in humans (52%) is substantially higher than for all other species. The 18% extraction for monkeys is too low for reliable experimental determinations, even if there were no extrapulmonary elimination. The maximum pulmonary extraction for the rat (29%) would be large enough to experimentally determine, but it has been shown that liver and kidney clearance is sufficient to account for most of the observed total body clearance in the rat. Thus, neither the rat nor the monkey would be a suitable species in which to test the hypothesis that the lungs are the dominant organ of elimination for 5-FU. The dog is more attractive (36% extraction), but a final judgment would require determination of hepatic clearance.

The high CL_{TB} for 5-FU is also characteristic of other pyrimidines, such as thymidine (TdR) and fluorodeoxyuridine (FUdR). CL_{TB} for FUdR can be calculated to be 25 l/min or 15 l/min/m^2 from the human studies of Ensminger et al. [8]. From the data of Howell et al. [9], CL_{TB} of 23 l/min/m^2 can be calculated for TdR infusions in patients of 8 g/m^2/day. Although the dog seemed promising as a model for 5-FU clearance, CL_{TB} for TdR is far less in the dog than in man. Covey and Straw [7] report CL_{TB} values for TdR of 67 ml/min/kg or 1.3 l/min/m^2 for infusions given to dogs. The high CL_{TB} values exhibited by pyrimidines in humans are of considerable pharmacokinetic interest, but the choice of a suitable animal model which could be used to explore this phenomena is unclear.

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