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

Jerry M. Collins

Pharmacokinetics Section, Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, Bldg. 10, Room 6N119, Bethesda, MD 20205, USA

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 (CLT) of drug at low concentrations. For equal total drug exposure (C×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 t½ is only 10 min.

In humans, the CLT 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-[14C]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 Rf of 5-FU was above 0.45, while all metabolites have a lower Rf. Dihydrofluorouracil is unstable in this system, and is measured along with the polar metabolites, fluoroureidopropionic acid, and urca [11].
Modeling. Steady-state infusion data can be analyzed by a one-compartment model:

\[
V \frac{dC}{dt} = G - CL_{TB}C,
\]  

(1)

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

\[
CL_{TB} = \frac{V_{max}}{K_m + C} + CL_R.
\]  

(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, multicompartiment models must be considered. For a two-compartment model in which the liver is separate from the rest of the body:

\[
V \frac{dC}{dt} = G - CL_R C + Q_L(C_L - C).
\]  

(3)

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

(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]:

\[
CL_{TB} = \frac{Q_{co} E_{lung}}{1 - E_{lung}}.
\]  

(5)

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

\[
E_{lung} = \frac{(CL_{TB}/Q_{co})}{1 + (CL_{TB}/Q_{co})}.
\]  

(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/m². A similar value of 1.5 ml/min/kg or 30 ml/min/m² 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. \( Q_{lung} \), for rat liver hepatocytes, has been reported [17] to be 1.098 ng/30 min/500,000 cells or 0.56 nmol/min/10⁶ cells. Lin et al. [15] assumed that 10⁶ cells is equivalent to 0.33 mg microsomal protein and 50 mg microsomal protein per gram liver, or 1.5 x 10⁸ cells per gram liver (1.5 x 10⁷ 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 x 10⁸ cells/g liver, based on microscopic observations. Siliciano et al. [15] suggest that 10⁶ cells is equivalent to only 0.16 mg microsomal protein, which corresponds to 3 x 10⁷ 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.

Results

Figure 1 is a plot of the plasma concentration vs time data for infusion rates ranging from 3 to 480 mg/m²/h. At infusion rates of 3 and 32 mg/m²/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/m². At an infusion rate of 480 mg/m²/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/m².

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/m²/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/m²/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 x 10⁸) and upper (3 x 10⁸) 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