Evaluation of Capacity-Limited First-Pass Effect through Liver by Three-Points Sampling in Portal and Hepatic Veins and Systemic Artery

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Received December 3, 2001; accepted February 14, 2002

Purpose. The three-points method was newly developed by sampling the portal and hepatic veins and systemic artery. A model of hepatic local disposition with the Michaelis-Menten elimination was proposed to explain the concentration dependency of the hepatic recovery ratio (F_H).

Methods. 5-fluorouracil (5-FU) was selected as a model drug. 5-FU was administered orally 90 min after its intraarterial dose. Blood specimens in both femoral artery and hepatic vein were sampled after intraarterial dose, and blood specimens in both femoral artery and portal vein were taken after oral administration.

Results. It was shown that F_H increased with an increase in the input drug concentration into the liver. The mean absorption time (*MAT*) estimated by nonlinear analysis agreed with the mean local absorption time ($\bar{t}_a$) whereas *MAT* by linear analysis was significantly smaller than $\bar{t}_a$.

Conclusions. The three-points method was newly developed, and the proposed nonlinear model explained well the capacity-limited elimination of 5-FU through the liver. *MAT* by the nonlinear analysis was in good agreement with $\bar{t}_a$.

KEY WORDS: capacity-limited first-pass effect; hepatic vein sampling; local disposition; nonlinear pharmacokinetics.

INTRODUCTION

The extent and rate of bioavailability are the measures to determine the dosage of a drug and to predict the concentration of the drug in the blood after oral administration. They are often defined as the amount ratio (*F*) and the mean absorption time (*MAT*) of administered drug from the intestinal tract into the circulatory system, respectively. These values are generally calculated as the ratio of area under the curve (*AUC*) and the difference of mean resident time (*MRT*) after oral and intravenous administration, respectively. The calculation of *F* and *MAT* is based on the assumption that the hepatic first-pass effect is linear and the total clearance (*Cl*) is always constant. However, many drugs show nonlinear pharmacokinetics after administration at high dose, and the capacity-limited disposition can occur at the early stage when the drug is instantaneously administered into the systemic circulation. The capacity-limited disposition can also occur in case of intoxication (1), drug-drug interaction (2–4), and inhibition of specific enzyme (5–7) or lack of transporter (8). In nonlinear pharmacokinetics, *Cl* is not constant and is dependent on drug concentration (9,10). *F* and *MAT* cannot be calculated by simple division and subtraction, respectively, and extended discussion is required when the disposition of drug is saturated.

An *in vivo* experimental method was developed to determine the extent and rate of intestinal absorption of drugs into the portal system using concentration difference between portal and systemic bloods (PS method) (11–14). This method provides the pharmacokinetic parameters F_a and $\bar{t}_a$, where F_a is the absorption ratio from the intestinal tract to the portal system and $\bar{t}_a$ is the mean local absorption time from the intestinal tract to the portal system. As an extension of the PS method, the intestinal and hepatic first-pass effects of the drug were evaluated by means of an intraarterial dose followed by an oral dose to a single rat in a short time interval (PS-DD method) (15–17). For a single animal, the PS-DD method provides many pharmacokinetic parameters (i.e., *F, MAT,* F_a, $\bar{t}_a$) and the hepatic recovery ratio of drug (F_H). The local moment analysis in the PS-DD method, which is model-independent, has been applied to evaluate the intestinal and hepatic first-pass effects. This method, however, is based on the assumption that the absorption, metabolism, and elimination are linear, and consequently the PS-DD method is not applicable to the analysis of the nonlinear first-pass effect.

In the current study, the three-points method was proposed to separately estimate the local absorption kinetics and hepatic first-pass metabolism by sampling the hepatic vein in addition to the previous PS-DD method. The proposed method clarifies the relationship between F_H and the concentration in the input blood into the liver by constructing Michaelis-Menten model. 5-Fluorouracil (5-FU), used as a model drug that is mainly metabolized in the liver, shows the capacity-limited elimination (18,19). 5-FU remains the first line of therapy for patients with advanced colorectal cancer. 5-FU is metabolized exclusively by dihydropyrimidine dehydrogenase (DPD), which is extensively inhibited by coadministration with sorivudine (6,7). By using the blood concentration difference between input and output bloods of the liver, the local intestinal absorption and hepatic metabolism of 5-FU were simultaneously evaluated in a single rat under the nonlinear condition.

MATERIALS AND METHODS

Chemicals

5-FU was purchased from Sigma Chemical Co. (St. Louis, Missouri). Sodium pentobarbital solution (Nembutal) was purchased from Abbott Laboratories (North Chicago, Illinois). Heparin was obtained from Shimizu Pharmaceutical

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ABBREVIATIONS: Global pharmacokinetic parameters: *AUC,* area under the curve; *MRT,* mean resident time; *MAT,* mean absorption time; *F,* oral bioavailability; *Cl,* total clearance in the body; F_a, absorption ratio of drug from intestinal tract into the portal system; F_H, hepatic recovery ratio on linear analysis; Q_p, blood flow rate of portal vein; $dA(t)/dt$, absorption rate of drug from intestinal tract into the portal system; $\bar{t}_a$, local mean absorption time; *X,* total amount of the drug that reaches systemic circulation; $dX(t)/dt$, absorption rate of drug into systemic circulation. Local hepatic disposition parameters: *V,* distribution volume of liver; Q_H, blood flow rate of hepatic vein; K_m, Michaelis constant; V_{max}, maximum elimination rate.

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Co., Ltd. (Shizuoka, Japan). All other chemicals and reagents used were of analytical or HPLC grade.

Animal Experiment

All procedures were performed in compliance with *Principles of Laboratory Animal Care* (National Institutes of Health Publication No. 85-23, revised 1985).

Healthy male Wistar rats, weighting 211–269 g, were purchased from Shimizu Experimental Materials Company, Ltd. (Kyoto, Japan) and maintained on standard chow and water *ad libitum.* All animals were starved 16 h, with free access to water, prior to the experiments. The animals were anesthetized with pentobarbital (50 mg/kg body weight), and a midline incision was made to open the abdomen. The duodenum, drawn out from the incision, was fixed to expose the portal vein. Intramedic polyethylene tubing (PE10, Becton Dickinson Co., Ltd., Sparks, Maryland), as a cannula for the portal vein, was inserted into the junction of the portal vein and the inferior pancreaticoduodenal vein, and the tip of the catheter was placed close to the liver. The catheter was secured to the mesentery with a drop of cyanoacrylate adhesive (Aron Alpha, Sankyo Co.. Ltd., Japan). The free end of the catheter was exteriorized through a small puncture using an 18-gauge needle in the side abdominal wall. The cannulation to the hepatic vein is operated by modifying the reported method (20,21). The hepatic vein junction of the left and the central lobe was easily identified. For a cannula, a pair of indwelling catheter and needle (22-gauge, Terumo Co., Ltd., Tokyo, Japan) was inserted upward about 3 mm into the junction of the hepatic vein. The catheter was fixed at the inside of the hepatic vein using a cardiovascular surgical suture (BEAR surgical sutures, Kyowa Precision Instruments Co., Ltd., Chiba, Japan). The catheter was connected to a polyethylene tube (PE10, Becton Dickinson Co. Ltd.), and the free end of the catheter was exteriorized through a small puncture using an 18-gauge needle in the side abdominal wall. The right femoral artery of each rat was also cannulated, and the free side of the catheter was subcutaneously conducted and exteriorized at the back of the leg. These three cannulas were filled with heparinized saline solution (100 U/ml) and connected to a 1-ml syringe (PLASTIPAK; Becton Dickinson Co., Ltd.). Each rat was held in an animal cage (Bollman cage; Natsume Co., Ltd., Tokyo, Japan) and was allowed to recover from the pentobarbital anesthesia for more than 6 h. To all the operated rats, 5-FU dissolved in saline solution (10 mg/ml) was administered orally 90 min after its intraarterial administration (10 mg/ml) at the same dosage (30 mg/kg) (17). Blood specimens (50 μl) in both the femoral artery and hepatic vein were sampled at 5, 15, 30, 60, and 90 min after an intraarterial dose, and blood specimens (50 μl) in both the femoral artery and the portal vein were taken at 5, 15, 30, 60, 90, 120, and 180 min after oral administration.

Assay Procedure

The concentration of 5-FU in blood was determined by modifying the reported methods (17). The blood sample (50 μl) was added to 250 μl of internal standard solution (1 μg/ml of 5-bromouracil) dissolved in 0.1 M phosphate buffer at pH 2.5. The mixture was extracted three times with 750 μl of ethyl acetate. The combined organic layers were evaporated under a nitrogen stream at 50°C, and the residue was reconstituted with 250 μl of mobile phase. A 100-μl portion was injected into an HPLC system (800 series, Japan Spectroscopic Co., Ltd., Tokyo, Japan) and a Chemcosorb 5-ODS-H reverse-phase column (5 μm, 150 × 4.6 mm i.d., Chemco Scientific, Osaka, Japan). The detector wavelength, flow rate, and column temperature were set at 260 nm, 1.0 ml/min, and 40°C, respectively. The mobile phase consisted of 10 mM sodium acetate buffer (pH 4.0):methanol (100:1, v/v). The chromatographic peaks were integrated with an electric integrator (Chromatopac C-R6A, Shimadzu Co., Ltd., Kyoto, Japan). Using the peak area or height ratio to an internal standard ranging from 0.1 to 62.5 μg/ml of 5-FU, calibration parameters were calculated. All correlation coefficients were above 0.999.

In the preliminary experiment, 5-FU concentrations were compared between portal and systemic bloods from 0 to 90 min after intraarterial administration. It was found that the concentration difference between portal and arterial bloods was negligibly small.

Data Analysis

The absorption rate of 5-FU from the intestinal tract into the portal system, $dA(t)/dt$, was calculated by Eq. (1),

$$\frac{dA(t)}{dt} = Q_p[C^{por}_{p.o.}(t) - C^{sys}_{p.o.}(t)] \tag{1}$$

where Q_p is blood flow rate through the portal vein, $C(t)$ is the time course of 5-FU concentration in the blood, and t is the time after oral administration. The superscripts, por and sys, specify portal and systemic vein, respectively. The subscript p.o. specifies oral administration. The value of Q_p (15.3 ml/min) per 250 g rat was adopted from the literature (15). The local moments for the absorption rate-time curve for 5-FU are defined by the following Eqs. (2) and (3),

$$F_a = \int_0^\infty \frac{dA(t)}{dt}\,dt/D = Q_p(AUC^{por}_{p.o.} - AUC^{sys}_{p.o.})/D \tag{2}$$

$$\bar{t}_a = \int_0^\infty t\frac{dA(t)}{dt}\,dt \Big/ \int_0^\infty \frac{dA(t)}{dt}\,dt = \frac{MRT^{por}_{p.o.}/AUC^{por}_{p.o.} - MRT^{sys}_{p.o.}/AUC^{sys}_{p.o.}}{AUC^{por}_{p.o.} - AUC^{sys}_{p.o.}} \tag{3}$$

where F_a is the local absorption ratio from the intestinal tract into the portal system, $\bar{t}_a$ is the mean local absorption time from the gastrointestinal tract into the portal system, and D is the dose of administration.

F, F_H, and MAT are calculated by two methods: linear analysis and nonlinear analysis.

Linear Analysis

This method is based on the assumption that F_H is constant and independent of the concentration of 5-FU in the blood. In the linear system, the absorption process is expressed by convolution integral (22),

$$C_{p.o.} = \int_0^t f(\tau)\, C_{i.v.}(t-\tau)\, d\tau \tag{4}$$

where the subscripts p.o. and i.v. specify oral and intravenous administration, respectively, and $f(\tau)$ is a weight function that

expresses the absorption process from the intestinal tract into the systemic circulation. On this assumption, F and the mean absorption time MAT are calculated according to Eqs. (5) and (6) by comparing moment parameters, AUC and MRT, of the time course of 5-FU in the systemic blood after intraarterial and oral administration.

$$F = AUC^{sys}_{p.o.}/AUC^{sys}_{i.a.} = F_a F_H \quad (5)$$

$$MAT = MRT^{sys}_{p.o.} - MRT^{sys}_{i.a.} \quad (6)$$

F_H is calculated by Eq. (7).

$$F_H = F/F_a \quad (7)$$

Nonlinear Analysis

This method is based on the assumption that F_H depends on the input concentration of 5-FU in the blood and 5-FU is eliminated in the liver according to the Michaelis-Menten equation. In this assumption,

$$V\frac{dC}{dt} = Q_H(C_{in} - C_{out}) - \frac{V_{max}}{K_m + C_{out}}C_{out} \quad (8)$$

where V is the distribution volume of liver, Q_H is the blood flow rate of hepatic vein, C_{in} is the concentration of 5-FU in the blood entering the liver, C_{out} is the concentration of 5-FU in the hepatic vein, K_m is the Michaelis constant, and V_{max} is the maximum elimination rate. The relation between blood flow rates of portal vein and hepatic vein is adopted from the literature; that is, portal vein:hepatic artery = 4:1 (23). C_{in} is equal to the drug concentration in systemic vein after intraarterial administration whereas C_{out} presents the concentration in the mixture of the portal vein and hepatic artery after oral administration. The concentration in the systemic vein is assumed to be equal to that in the systemic artery. The change of blood concentration in the global process is slow compared with that in the single-pass process through the liver. Thus, the transient difference of the concentration of 5-FU is approximated to be zero at short time interval in the liver, and Eq. (8) becomes

$$Q_H(C_{in} - C_{out}) - \frac{V_{max}}{K_m + C_{out}}C_{out} = 0 \quad (9)$$

Equation 10 is derived from Eq. (9),

$$\frac{C_{out}}{C_{in}} = \frac{\left(C_{in} - K_m - \frac{V_{max}}{Q_H}\right) + \sqrt{\left(C_{in} - K_m - \frac{V_{max}}{Q_H}\right)^2 + 4C_{in}K_m}}{2C_{in}} \quad (10)$$

where C_{out}/C_{in} is hepatic recovery ratio (F_H) and depends on the concentration of drugs in the blood. Because C_{out}/C_{in} is the function of C_{in} Eq. (9), C_{out} can be determined by Eq. (10). The time courses of C_{in} and C_{out} obtained according to Eq. (10) were fitted to the concentration of 5-FU in hepatic and systemic bloods by MULTI (24).

The transfer rate of drug $dX(t)/dt$, which reaches systemic circulation, is described in Eqs. (11) and (12).

$$\frac{dX(t)}{dt} = Q_p[C^{por}(t) - C^{sys}(t)]\frac{C_{out}(t)}{C_{in}(t)} \quad (11)$$

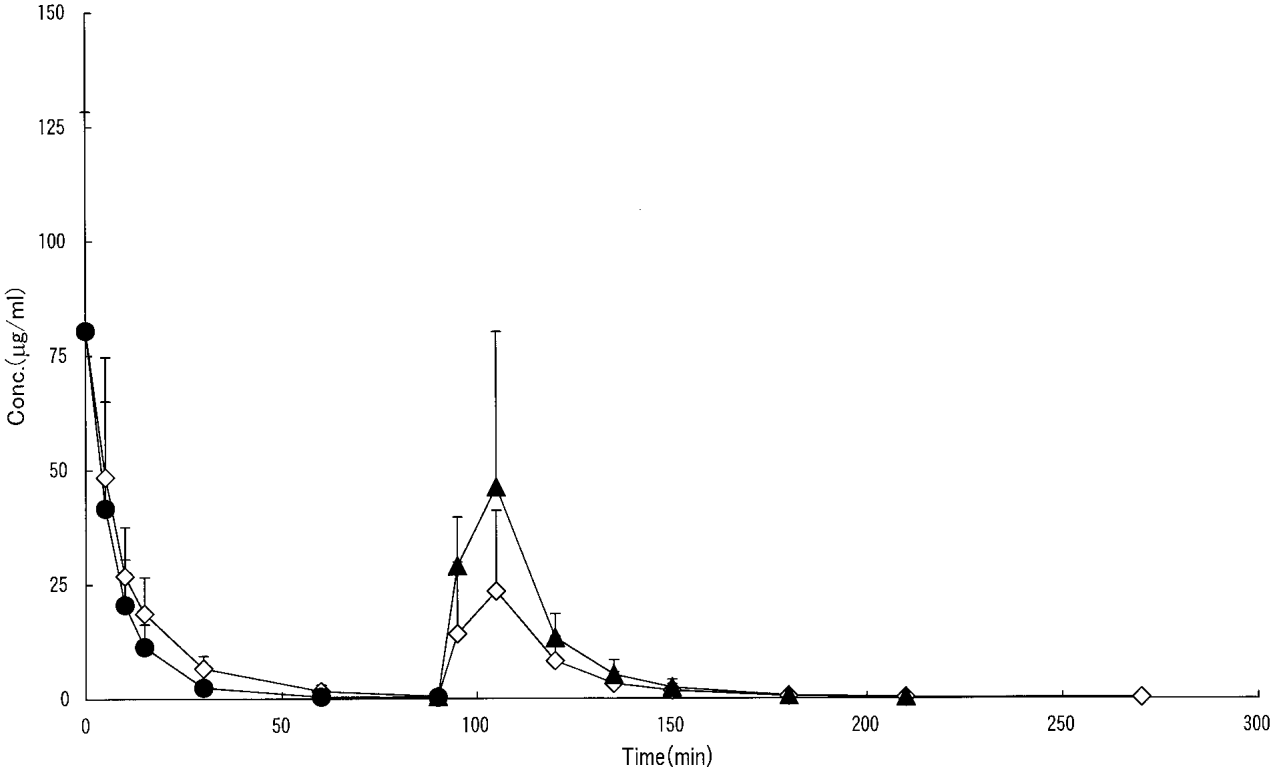

Fig. 1. The time courses of portal (▲), systemic (◇), and hepatic (●) blood concentration of 5-FU in rats. Each point represents the mean ± SD (n = 4).

In Eq. (11), $C^{por}(t)$ and $C^{sys}(t)$ are the observed concentrations after oral administration whereas $C_{out}(t)/C_{in}(t)$ is the predicted concentration ratio using Eq. (10), where t is the time after oral administration. By integrating Eq. (11), the total amount of transferred drug (X) is given by

$$X = F \cdot D = \int_0^\infty Q_p[C^{por}(t) - C^{sys}(t)] \frac{C_{out}(t)}{C_{in}(t)} dt \qquad (12)$$

F is calculated by X/D. MAT is defined by Eq. (13).

$$MAT = \int_0^\infty t \frac{dX(t)}{dt} dt \Big/ \int_0^\infty \frac{dX(t)}{dt} dt \qquad (13)$$

The AUC and the MRT of the front portion due to the first intraarterial dose were calculated using a linear trapezoidal method with an extrapolation to infinite time. The AUC and MRT of the time course after the oral dose were calculated using the subtracted time course data in the rear portion, adjusting the second dosing time to the origin. All estimated parameters were expressed as arithmetic means and standard deviations of those from four rats.

RESULTS AND DISCUSSION

Figure 1 presents the time courses of portal, systemic, and hepatic blood concentrations of 5-FU of rats (n = 4). After intraarterial administration, the blood concentration of 5-FU in the systemic vein was always higher than that in the hepatic vein. After oral administration, the blood concentration of 5-FU in the portal vein was always higher than that in the systemic vein. Figure 2 presents the F_H against the concentration of 5-FU in the input blood (C_{in}). The lines in Fig. 2 are F_H predicted according to Eq. (10). The predicted F_H are in good agreement with the experimental values. In Eq. (10), F_H approaches unity as C_{in} increases. The relation between $C^{hep}_{i.a.}/C^{sys}_{i.a.}$ ($= C_{out}/C_{in}$) and $C^{sys}_{i.a.}$($= C_{in}$) are plotted in individual rats (A, B, C, D). The value of $C^{hep}_{i.a.}/C^{sys}_{i.a.}$ ($= C_{out}/C_{in}$) increases with $C^{sys}_{i.a.}$($= C_{in}$). F_H took values from 0.2 to 0.3 at the lowest input concentration. Table I shows pharmacokinetic parameters of 5-FU of each rat. The absorption ratio from intestinal tract to the portal system (F_a) and local mean absorption time from the intestinal tract to the portal system ($\bar{t}_a$) were 0.761 ± 0.207 and 18.5 ± 2.9 min, respectively. According to linear analysis, F, F_H, and MAT were 0.606 ± 0.091, 0.870 ± 0.382, and 11.6 ± 3.3 min, respectively. According to nonlinear analysis, F and MAT were 0.487 ± 0.118 and 17.2 ± 3.7 min, respectively. According to nonlinear method, F_H was not a constant because F_H was dependent on the concentration of 5-FU in the input blood. Michaelis constant (K_m) and the maximum elimination rate (V_{max}) were 2.26 ± 0.72 μg/ml and 185 ± 43 μg/min, respectively.

The three-points method was newly developed to separately estimate the local absorption kinetics and hepatic first-pass metabolism by sampling the hepatic vein in addition to the previous PS-DD method. The three-points method offers the hepatic recovery ratio at each sampling time by measuring the concentration of 5-FU in portal and hepatic bloods. In general, it is considered to be difficult to determine the local pharmacokinetic parameters F_a and F_H *in vivo*. Weiss (25,26) proposed a recirculatory concept based on the network transport theory using the Laplace transport where the drug disposition is regarded as linear. Kwan presented the experimen-

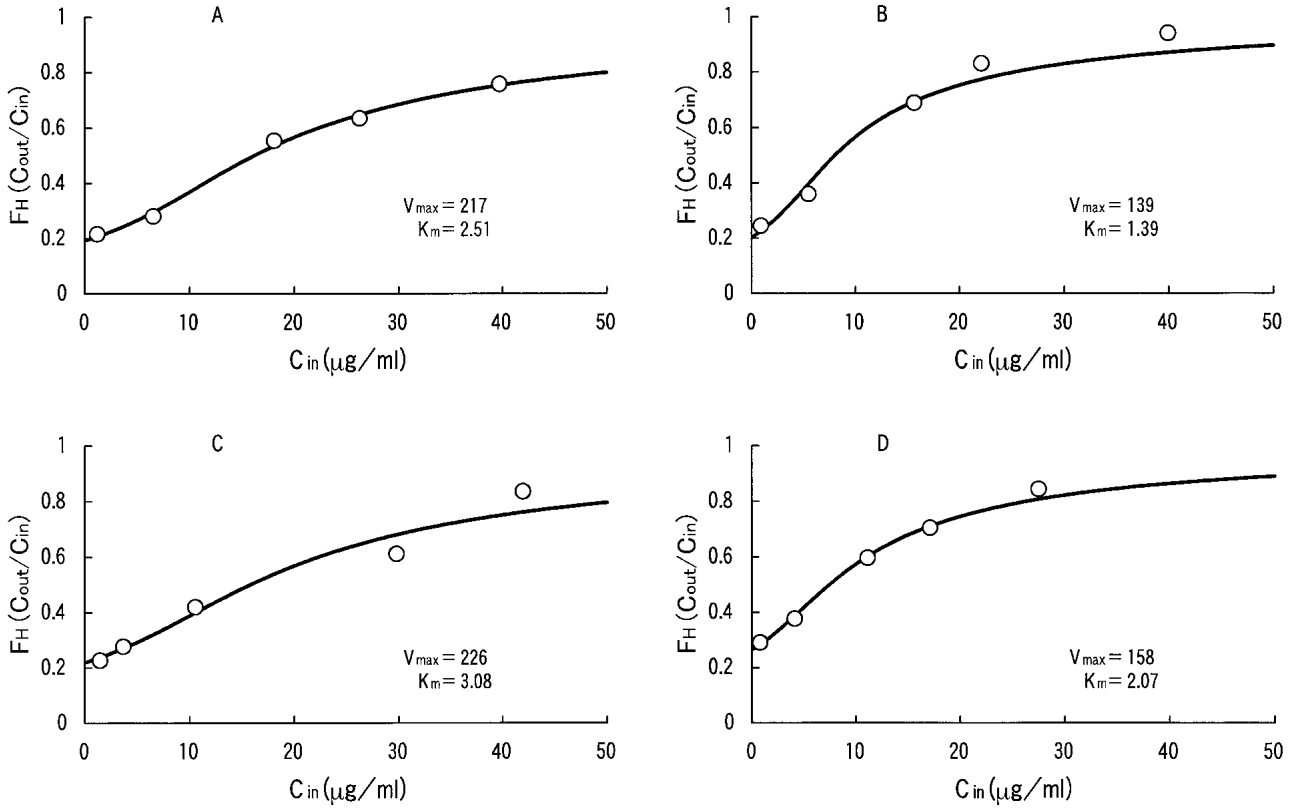

Fig. 2. The relationship between C_{out}/C_{in} ($= F_H$) and C_{in} in the individual rat (A–D). Open circles indicate the experimental data points, and the lines are those predicted by MULTI according to single-line fitting.

Table I. Pharmacokinetic Parameters of 5-FU by Linear Analysis and Nonlinear Analysis in Individual Rat

Rat	A	B	C	D	Mean ± SD
Body weight (g)	217	269	211	263	240 ± 30
F_a	0.857	0.995	0.528	0.661	0.761 ± 0.207
$\bar{t}_a$ (min)	18.8	18.7	21.8	14.8	18.5 ± 2.9
Linear analysis					
F	0.552	0.548	0.741	0.584	0.606 ± 0.091
F_H	0.645	0.550	1.40	0.883	0.870 ± 0.382
MAT (min)	11.6	13.5	6.90	14.3	11.6 ± 3.3[a]
Nonlinear analysis					
F	0.492	0.650	0.384	0.420	0.487 ± 0.118
MAT (min)	12.5	17.2	17.4	21.6	17.2 ± 3.7
V_{max} (μg/min)	217	139	226	158	185 ± 43
K_m (μg/ml)	2.51	1.39	3.08	2.07	2.26 ± 0.72

[a] Significant at 5% between $\bar{t}_a$ and MAT in linear analysis by ANOVA.

tal strategies *in vivo* for evaluating many factors affecting the oral bioavailability. In the report, Kwan (27) stressed the capability of evaluating the individual contributions to absorption, losses in the gut lumen, and first-pass elimination in the gut wall and the liver, though limited to the assumption that the absorption and elimination of drug are linear.

In the current study, we attempted two calculation methods; that is, linear analysis and nonlinear analysis. Linear analysis is based on linear pharmacokinetics whereas nonlinear analysis is not only limited to linear pharmacokinetics, but also is applicable to nonlinear pharmacokinetics. When a drug shows linear absorption and disposition, MAT should agree with $\bar{t}_a$. In the current study, however, MAT estimated by linear analysis was significantly smaller than $\bar{t}_a$ whereas MAT by nonlinear analysis was in good agreement with $\bar{t}_a$. F estimated by linear analysis was considerably smaller than that by the nonlinear analysis though the difference was statistically insignificant. The hepatic recovery ratio was dependent on 5-FU concentration in the portal blood. The Michaelis-Menten elimination was adopted to describe the relationship between hepatic recovery ratio and concentration of 5-FU in the blood. The theoretical curves (F_H versus C_{in}) predicted according to the proposed nonlinear model were in good agreement with the experimental points as shown in Fig. 2. K_m in the current investigation is a hybrid parameter which is considered to be intimately related to dihydropyrimidine dehydrogenase (DPD) that exclusively metabolize 5-FU (6,7). It is reported in a hepatic perfusion experiment that K_m is estimated to be 2.88 μg/ml (18), which is close to 2.26 μg/ml in the current investigation.

In conclusion, the three-points method was newly developed, and the proposed nonlinear model explained well the capacity-limited elimination of 5-FU through the liver. MAT by nonlinear analysis was in good agreement with $\bar{t}_a$. It was shown that the linear pharmacokinetic analysis can give F and MAT including systemic error when a drug is nonlinearly eliminated through the liver.

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