Simultaneous Evaluation of Intestinal Absorption and Hepatic Extraction of 5-Fluorouracil Using Portal-Systemic Concentration Difference by Short-Period Double Dosing in a Single Conscious Rat

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The intestinal local absorption and the hepatic local disposition of 5-fluorouracil (5-FU) in a single conscious rat was investigated by the simultaneous sampling of portal and systemic bloods (PS method). The portal blood flow rate, measured using a compact electromagnetic flow-meter, was estimated to be 15.3±2.2 ml/min per body weight (250 g). The portal vein and the femoral artery of the rat were cannulated to simultaneously obtain blood samples from two sites. 5-FU (30 mg/kg) was administered first intravenously, and subsequently orally 90 min after intraarterial administration to a single conscious rat (short-period double dosing; DD). Concentrations of 5-FU in the portal and arterial bloods were determined by HPLC. The local absorption ratio ($F_p$) and the absolute bioavailability ($F$) were 71.2±15.4 and 25.1±13.2%, respectively. Consequently, the hepatic extraction ratio ($F_{H}$) was estimated to be 34.9±14.4%. The mean local absorption time ($t_a$) and the mean absorption time ($MAT$) were 37.5±15.5 and 31.4±13.7 min, respectively and they were statistically the same. In conclusion, a PS method by short-period double dosing (PS-DD method) has been developed to evaluate the first-pass effect, separating the intestinal absorption and hepatic elimination of a drug in a single conscious rat. It was demonstrated by applying PS-DD method that the low bioavailability of 5-FU can be explained by the large hepatic first-pass extraction, and that the large inter-individual variation in bioavailability of 5-FU is caused mainly by a large variation in the hepatic first-pass effect. The large variation in $t_a$ or $MAT$ was predicted to be due to a variation in the gastric emptying time.

Key words 5-fluorouracil; intestinal absorption; hepatic extraction; first-pass effect

5-Fluorouracil (5-FU), which has been widely used for the treatment of solid tumors, is clinically available in an oral dosage form.1–3) The estimated bioavailability of 5-FU was low and fluctuated dramatically in humans (0–74%)4) and in rats.5) 5-FU completely disappeared from the intestinal lumen in rats and the absorption rate was large, which suggested carrier-mediated transport of 5-FU through the intestinal wall.6) 5-FU is metabolized mainly to dihydroflourouracil by dihydropirimidine dehydrogenase in the liver.7) 5-FU was extracted 19 to 51% through a single hepatic pass by hepatic arterial infusion,7–8) and 23 to 89% by peritoneal dialysis in human.9) In an intravenous infusion study using rat, the hepatic extraction ratio was estimated to be 23 to 75%.10) Therefore, the large range in the bioavailability of 5-FU can also reflect a fluctuation in the hepatic first-pass extraction. However, there has been no investigation which separately evaluates the intestinal absorption kinetics and the hepatic first-pass elimination of 5-FU in a conscious animal.

An in vivo experiment method was developed to determine the extent and time of intestinal absorption of drugs into the portal system using the concentration difference between portal and systemic bloods (PS method).11–13) Since an absolute bioavailability was calculated by comparing the averaged values of two groups, parenteral and oral administration, the hepatic recovery ratio ($F_{H}$) was calculated without variance by comparing the absolute bioavailability ($F$) and the local absorption ratio ($F_p$) into the portal system. Consequently, it was difficult to estimate inter-individual variations in $F$ and $F_{H}$.

In the present study, PS method was applied to separately estimating the local absorption kinetics and hepatic first-pass effect of a drug by means of an intraarterial dose followed by an oral dose to a single conscious rat in a short time period (PS-DD method). PS-DD method provides both the absolute bioavailability and the hepatic recovery ratio of drug in a single conscious animal, in addition to providing the local absorption ratio and time required to pass from the intestinal tract into the portal system. Thus, it was attempted in this investigation to determine the contribution of gastrointestinal absorption and hepatic first-pass extraction to the extent and rate of bioavailability of 5-FU, taking into consideration inter-individual variations.

MATERIALS AND METHODS

Chemicals 5-FU and 5-bromouracil (internal standard) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium pentobarbital solution (Nembutal for animal injection; Abbot Laboratories, North Chicago, IL) was used to anesthetize the rats for measurement of the flow rate in portal vein. Heparin was obtained from Novo Industries (Denmark). All other chemicals and reagents used were of analytical or HPLC grade.

Animal Experiment Male Wistar rats, weighing 210–260 g, were purchased from Shimizu Experimental Materials Co., Ltd. (Kyoto, Japan) and maintained with standard chow and water ad libitum. Rats were starved overnight with free access to water prior to the experiments. Under light ether anesthesia, the abdomen was opened through a midline incision. A polyethylene catheter (PE10) filled with heparinized saline (100 IU/ml) was inserted into the portal vein from the junction of the

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portal vein and inferior pancreaticoduodenal vein,\textsuperscript{14} and the tip of the catheter was placed just close to the liver. The catheter was secured to the mesentery with the adhesive. The free end of the catheter was exteriorized to the right side of the abdominal wall. The right femoral artery of the same rat was cannulated, and the free end of the cannula filled with heparinized saline was subcutaneously conducted and exteriorized at the back of the leg. Each rat held in the Bollman cage was allowed to recover from ether anesthesia for more than 2 h. 5-FU (30 mg/kg) in saline (10 mg/ml) was administered, first intraarterially and subsequently orally 90 min after the intraarterial dose. Portal and arterial blood samples (60 μl each) were simultaneously collected at proper intervals. The reduced blood volume was supplemented with an equal volume of saline after each sampling.

The blood flow rate in the portal vein was measured substantially by the method mentioned above. Seven rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). After insertion of the catheter into the portal vein, a perivascular flow sensor (internal diameter: 1.5 mm; Skalar Medical, Delft, the Netherlands) was set around the portal vein. The portal blood flow rate was measured using a compact electromagnetic flowmeter (MDL1401; Skalar Medical).

**Analytical Procedure** The concentration of 5-FU in blood was determined by modifying the reported methods.\textsuperscript{15,16} The blood sample (50 μl) was added to 0.25 ml of internal standard solution (1 μg/ml of 5-bromouracil) dissolved in 50 mm phosphate buffer at pH 2.5. The mixture was extracted three times with 0.75 ml 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 phosphate buffer (50 mm; pH 2.5). A 90 μl portion was injected into an HPLC system (800 series, Japan Spectroscopic Co., Ltd., Tokyo, Japan) equipped with a variable wavelength detector (825-UV), automatic sampling system (850-AS), and a Chemosorb 5-ODS-H reverse-phase column (5 μm, 150 x 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 (99:1, v/v). The chromatographic peaks were integrated with an electric integrator (Chromatopac C-R6A, Shimadzu Co., Kyoto, Japan). Using the peak area ratio to an internal standard ranging from 0.05 to 50 μg/ml of 5-FU, calibration parameters were calculated by the variance-stabilizing transformation method.\textsuperscript{17} Accuracy and precision were within 10% at all concentrations.

**Data Analysis** The absorption rate of 5-FU, \( \frac{dA(t)}{dt} \), from the intestinal tract into the portal system was calculated by Eq. 1.\textsuperscript{12,13}

\[
\frac{dA(t)}{dt} = Q_s(C_b(t) - C_{b<s}(t))
\]

(1)

where \( Q_s \) is blood flow rate in the portal system, and \( C_b(t) \) is the time course of 5-FU concentration in the blood. The superscripts, por and art, specify portal and arterial, respectively. The local moments for the absorption rate-time curve for 5-FU are defined by the following Eqs. 2 and 3.

\[
F_a = \int_0^\infty \frac{dA(t)}{dt} \frac{dt}{Dose} = Q_s(AUC_{por} - AUC_{art})/Dose
\]

(2)

\[
\bar{t}_a = \int_0^\infty \frac{dA(t)}{dt} \frac{dt}{\int_0^\infty dA(t) dt}
\]

\[
= \frac{MRT_{por} * AUC_{por} - MRT_{art} * AUC_{art}}{AUC_{por} - AUC_{art}}
\]

(3)

where \( F_a \) is the local absorption ratio from the intestinal tract into the portal system, and \( \bar{t}_a \) is the mean local absorption time from the gastrointestinal tract into the portal system.

The extent of bioavailability (\( F \)) and the mean absorption time (\( MAT \)) are calculated by comparing moments of the time courses of 5-FU in the artery after intraarterial and oral administration as follows.\textsuperscript{18}

\[
F = \frac{AUC_{por}}{AUC_{art}} = F_a \cdot F_h
\]

(4)

\[
MAT = MRT_{por} - MRT_{art} = \bar{t}_a
\]

(5)

where \( F_h \) is the hepatic recovery ratio and subscripts p.o. and i.a. mean oral and intraarterial administration, respectively.

The area under the curve (\( AUC \)) and the mean residence time (\( MRT \)) of the front portion due to the first intraarterial dose were calculated using a linear trapezoidal method with an extrapolation to infinite time. The predicted plasma concentration in the artery due to the first intraarterial dose was subtracted from the arterial and portal blood concentrations in the rear portion after the second oral dose. \( AUC \) and \( MRT \) of the time course after the second oral dose were calculated using the subtracted time course data in the rear portion, adjusting the second dosing time to the origin. Statistical analysis was performed by two-way analysis of variance (ANOVA) at a 5% level of significance.

**RESULTS AND DISCUSSION**

The blood flow rate (\( Q_b \)) in the portal vein was estimated to be 15.3 ± 2.2 ml/min per body weight (250 g), which was close to that in the literature.\textsuperscript{19,20} The portal blood flow rate was reported to be unaffected by pentobarbital anesthesia or portal vein cannulation.\textsuperscript{19} Thus, the estimated flow rate of portal blood was regarded to be close to that in the conscious rat.

Figure 1 shows the time courses of mean portal and arterial blood concentrations of 5-FU, when 5-FU (30 mg/kg each) was administered first intraarterially and subsequently orally, 90 min after the intraarterial administration in a single rat (n = 6). The time course of 5-FU in the portal vein after intraarterial administration was almost superimposed on the time course in the artery before oral administration, indicating the negligible transfer of 5-FU from the blood vessel to the intestinal lumen after parenteral administration of 5-FU.\textsuperscript{13} After
oral administration, the concentration of 5-FU following the oral dose was always higher in the portal vein than in the artery. Table 1 presents \( AUC \) and \( MRT \) of the time course of 5-FU concentrations in portal and arterial bloods following the intraarterial and oral administration of 5-FU. After oral administration, \( AUC \) (525 ± 155 \( \mu \)g·min/ml) in the portal vein was significantly greater than that (176 ± 98.6 \( \mu \)g·min/ml) in arterial blood. \( MRT \) (39.9 ± 14.2 min) in the portal vein was almost the same as that (44.2 ± 13.9 min) in the artery. \( F_a \) calculated by Eq. 2 was 71.2 ± 15.4\%, whereas \( F \) was evaluated to be 25.1 ± 13.2\%. The present experimental results agreed with previous reports that the bioavailability of 5-FU is low and extensively varies.\(^4,21\) The hepatic recovery ratio, \( F_H = (F_t/F_a) \), was estimated to be 34.9 ± 14.4\%. The coefficients of variation in \( F_a \), \( F_H \) and \( F \) were 21.6\%, 41.3\% and 52.6\%, respectively, which demonstrates that the large variation in \( F \) is explained not by that in \( F_a \) but by that in \( F_H \).

The absorbed percent (%) in the Loo-Riegelman method was converted to the absorbed amount by absolute bioavailability (\( F \)). Each point represents the mean ± S.D. of 6 rats. Figure 2 presents the time courses of the absorption rate from the intestine into the portal system according to Eq. 1 as well as those into systemic circulation according to the Loo-Riegelman method.\(^{24}\) The absorption rate into systemic circulation was calculated by adjusting the absorbed percent (%) of dose by absolute bioavailability (\( F \)). The time profile of absorption rate into the portal system is almost the same as that into systemic circulation. The time profile in systemic circulation shows no time delay to that in portal system (Fig. 2), which reconfirms that the mean transit time of 5-FU through the liver is negligible. It was demonstrated that gastric emptying was a rate determining step in the absorption kinetics of 5-FU.\(^6\) Therefore, the large inter-individual variation in \( t_e \) (or \( MAT \)) was predicted to be caused by the large variation in gastric emptying time. In Table 1, \( t_e \) (37.5 ± 15.5 min) is greater than \( MRT \) (12.8 ± 0.56 min) in the intraarterial dose, which demonstrates that the absorption kinetics of 5-FU is ‘flip-flop.’

In conclusion, PS-DD method has been developed to separate the global absorption kinetics to the intestinal local absorption and hepatic local disposition, and to assess the extent and rate of bioavailability of a drug in a single conscious rat. By applying PS-DD method, it was found that the low bioavailability and the large

![Graph](image1)

**Fig. 1.** Blood Concentration–Time Courses of 5-FU after Intraarterial Administration Followed by Oral Administration of 5-FU (30 mg/kg Each) in a Single Rat

- and ○ represent averaged concentration values in portal vein and artery blood, respectively. Each point represents the mean ± S.D. of 6 rats.

![Graph](image2)

**Fig. 2.** Predicted Time Courses of Averaged Local Absorption Rate into Portal System (\( dA/dt \)) (●) and Averaged Absorption Rate into Systemic Circulation Predicted by Loo-Riegelman Method (○) of 5-FU Following Oral Dosing of 30 mg/kg.

The absorbed percent (%) in the Loo-Riegelman method was converted to the absorbed amount by absolute bioavailability (\( F \)). Each point represents the mean ± S.D. of 6 rats.
range in bioavailability of 5-FU are mainly due to the large extent and the large fluctuation in hepatic first-pass elimination, respectively.

REFERENCES