# Effect of Coadministered Uridine on Intestinal First-Pass Metabolism of 5′-Deoxy-5-Fluorouridine in Conscious Rats—An Evaluation by Method of Portal-Systemic Concentration Difference

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***Purpose.*** The effect of uridine (UR) coadministration on the intestinal metabolism from 5′-deoxy-5-fluorouridine (5′-DFUR) to 5-fluorouracil (5-FU) was evaluated by a method of concentration difference between portal and systemic bloods in conscious rats (PS method).

***Methods.*** 5′-DFUR (100 mg/kg) alone (Group A), or 5′-DFUR + UR (100 mg/kg each) (Group B) was orally administered to conscious rats. The portal and arterial bloods were simultaneously withdrawn from two canulas at appropriate time intervals, and blood concentrations of 5′-DFUR, 5-FU, UR and uracil (U) were assayed by HPLC. The concentration-time profiles of these drugs and its metabolites were analyzed by local moment analysis.

***Results.*** UR coadministration made the local absorption ratio ($F_a$) of 5′-DFUR decrease significantly from 60.1 ± 10.5% to 38.0 ± 18.6% of dose. Though the local absorption ratios ($F_a^m$) of the metabolite (5-FU) were the same between Group A and Group B (8.3 ± 1.9 and 8.7 ± 4.0% of 5′-DFUR, respectively), AUC of arterial 5-FU in Group B was 5 times greater than that in Group A. UR was not detected in the portal blood, and $F_a^m$ of U was estimated to be 41.9 ± 26.8% of UR in Group B.

***Conclusions.*** It is predicted that a large portion of 5-FU generated from 5′-DFUR is further degraded in the intestine in Group A, and U generated from UR blocks 5-FU degradation in the intestine and the systemic circulation in Group B.

**KEY WORDS:** portal-systemic difference method; intestinal metabolism; 5′-deoxy-5-fiuorouridine; uridine; coadministration.

## INTRODUCTION

5′-Deoxy-5-fluorouridine (5′-DFUR) is a metabolic prodrug of 5-fluorouracil (5-FU), the representative anti-tumor drug of fluoropyrimidine, and is used orally in the treatment of solid tumors (1,2). 5′-DFUR is converted to 5-FU exclusively by pyrimidine nucleoside phosphorylases (PyNPase) (3,4) which presents in tumors and in various normal tissues (3,5). The activity of this enzyme in tumors converts 5′-DFUR to 5-FU more effectively than that in normal tissues. PyNPase, however, is much more active in the intestine than in other normal tissues (6), which indicates that 5′-DFUR, orally administered, can be converted to 5-FU in the intestine before it reaches the tumors. The first-pass metabolism from 5′-DFUR to 5-FU through the intestinal wall has been considered to induce an intestinal toxicity after oral administration higher than after intravenous dose of 5′-DFUR (7,8) because 5-FU is much more toxic than its prodrug. It was expected that the inhibition of intestinal PyNPase activity and the block of 5-FU generation from 5′-DFUR reduces its intestinal toxicity. Several attempts have been made to decrease 5-FU generation in the intestine by applying inhibitors against PyNPase (9–12). Uridine (UR), a metabolic substrate to PyNPase, inhibited 5′-DFUR elimination in the homogenized small intestine of rat (9). Therefore, it was predicted that UR coadministration would decrease the intestinal generation of 5-FU. In spite of this expectation, the coadministration of 5′-DFUR with UR invoked both an increase in blood concentration of 5-FU and a decrease in its elimination rate from the systemic circulation (13). They concluded that 5-FU elimination was inhibited by uracil (U) generated from coadministered UR, and 5-FU blood concentration was increased (13). However, they offered no experimental evidence that UR coadministration decreased the generation of 5-FU from 5′-DFUR through the intestinal wall and consequently decreased 5-FU concentration in the portal blood.

Recently, a new method based on local moment analysis has been developed to directly evaluate the intestinal generation of metabolites in a single conscious rat (14). This method was originally developed to separately evaluate an intestinal and hepatic first-pass effects of parent drugs based on the simultaneous determination of portal and systemic blood concentrations (PS method) (15–17). It was shown in the preceding study (14) that about 70% and 10% of dosed amount were detected as intact form and as 5-FU, respectively, in the portal system after oral administration of 5′-DFUR (100 mg/kg).

In this investigation, the effect of coadministered UR on the intestinal first-pass metabolism of 5′-DFUR in conscious rats was directly evaluated by PS method based on local moment analysis.

## MATERIALS AND METHODS

### Chemicals

5′-DFUR was generously provided by Nippon Roche Co., Ltd., Kamakura, Japan. No contamination of 5-FU in 5′-DFUR was confirmed by HPLC. 5-FU, UR, U and 5-bromouracil (internal standard) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium heparin solution was obtained from Shimizu Pharmaceutical 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 "Principle of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

Experiments were performed according to the method reported (14). Male Wistar rats, weighting 200–250 g, fasted overnight, were lightly anesthetized with ether. After a midline incision to open the abdomen, a PE10 catheter was inserted into the portal vein from the junction of portal vein and inferior pancreaticoduodenal vein, and the tip of catheter was placed just close to the liver. The catheter was secured to the mesentery

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with an adhesive. The free end of the catheter was exteriorized to the right side of abdominal wall. The right femoral artery of each rat was also cannulated. Each rat was held in a Bollman cage and was allowed to recover from the anesthesia for more than 2 h. Rats were divided to control group (Group A) and coadministration group (Group B). 5′-DFUR dissolved in saline (50 mg/ml) was administered orally as a gastric gavage at a dose of 100 mg/kg in Group A. UR (100 mg/kg) and 5′-DFUR (100 mg/kg) in saline (50 mg/ml) were orally administered at the same time. Portal and arterial blood samples (ca. 60 μl each) were simultaneously collected from two canulas at scheduled time intervals.

### Analytical Procedure

The concentrations of 5′-DFUR, 5-FU, UR and U in blood were simultaneously determined as previously reported (14), according to assay methods (18,19) with slight modifications. 50 μl of blood was added to 0.25 ml of 50 mM phosphate buffer (pH 2.5) containing internal standard (1 μg/ml). The mixture was extracted three times with 0.75 ml of ethyl acetate and the combined organic layers were evaporated under nitrogen stream. The residue was reconstituted with 250 μl of phosphate buffer, and a 90 μl portion was injected into an HPLC system equipped with an ODS reversed-phase column. The detector wavelength, flow rate, and column temperature were at 260 nm, 1.0 ml/min, and 40°C, respectively. 5′-DFUR, 5-FU, UR, and U were eluted according to a gradient program with a mobile phases consisted of 10 mM sodium acetate buffer (pH 4.0) and methanol. Calibration lines were used to determine unknown analyte concentrations in blood samples, ranging 0.25 to 50 μg/ml for 5′-DFUR, 0.05 to 10 μg/ml for 5-FU, 2.5 to 50 μg/ml for UR, and 0.5 to 10 μg/ml for U, respectively. Using peak area ratios to internal standard, calibration parameters were determined by the variance-stabilizing transformation method (20). Accuracy and precision were within 10% at all concentrations.

### Data Analysis

The absorption rate of administered drug, $dA(t)/dt$, from the intestinal tract into the portal system was calculated with Eq. 1 (16,17).

$$\frac{dA(t)}{dt} = Q_b \left(C_b^{por}(t) - C_b^{art}(t)\right) \quad (1)$$

where $Q_b$ is the blood flow rate in the portal system, and $C_b(t)$ is the time course of 5′-DFUR concentrations in blood. The superscripts, *por* and *art*, specify portal and arterial concentrations, respectively. $Q_b$ was estimated to be 15.3 ± 2.2 ml/min per body weight (250 g) by the measurement with an electromagnetic flowmeter (14).

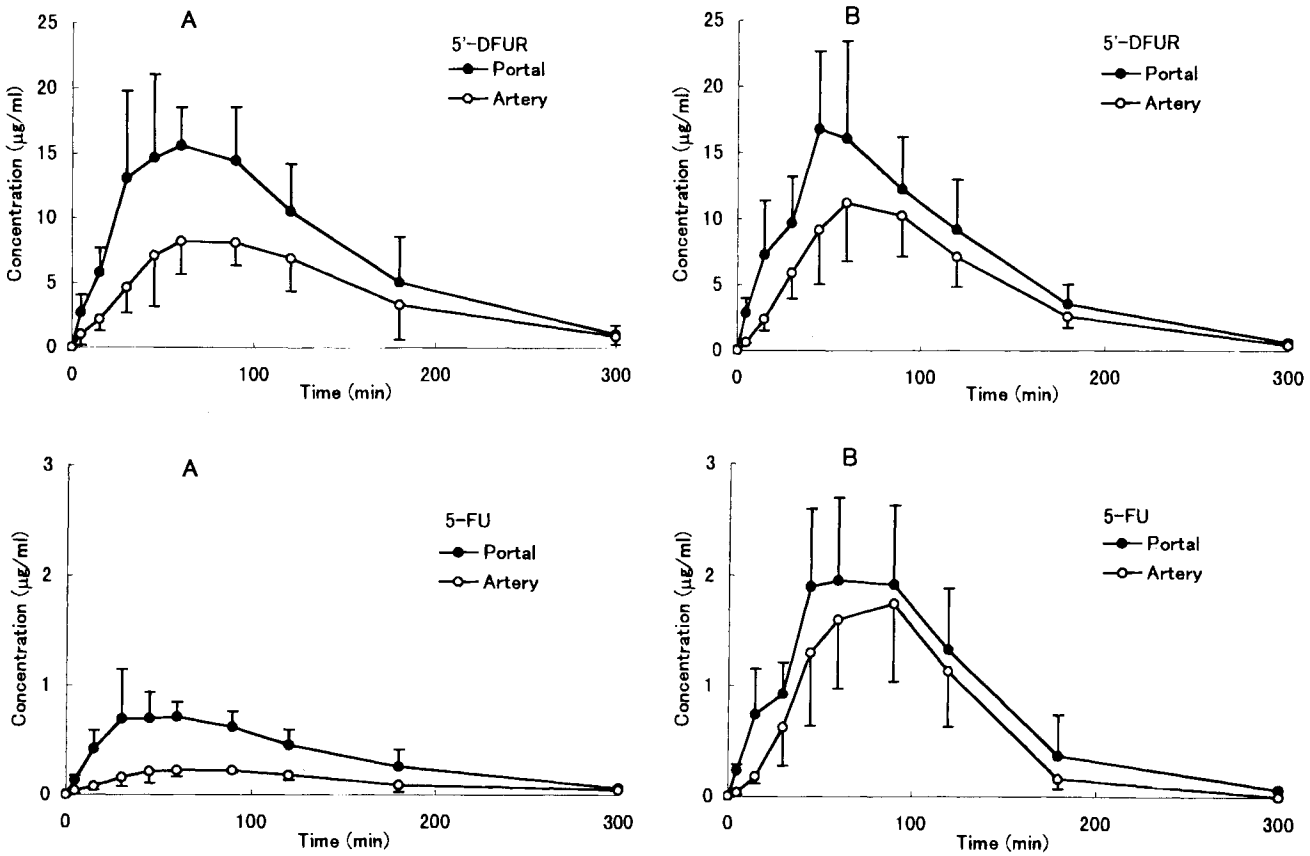


**Fig. 1.** Mean blood concentration-time course of 5′-DFUR and its metabolite 5-FU after oral administration of 100 mg/kg of 5′-DFUR alone (A, left figures) and after concomitant oral administration of 100 mg/kg of 5′-DFUR with UR (B, right figures). ● and ○ represent portal vein and artery blood, respectively. Each point represents mean and SD of five rats.

The local moments for the absorption rate-time curve for parent compounds are defined by the following Eqs. 2 and 3 (16):

$$F_a = \int_0^\infty \frac{dA(t)}{dt} dt \,/\, Dose = Q_b(AUC^{por} - AUC^{art}) \,/\, Dose \quad (2)$$

$$\bar{t}_a = \int_0^\infty t \cdot \frac{dA}{dt} \cdot dt \,/\, \int_0^\infty \frac{dA}{dt} \cdot dt = \frac{MRT^{por} \cdot AUC^{por} - MRT^{art} \cdot AUC^{art}}{AUC^{por} - AUC^{art}} \quad (3)$$

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 for 5′-DFUR.

The same equation of Eq. 1 can be applied to 5-FU (metabolite) generated from 5′-DFUR (parent compound), and to U generated from UR through the intestine into the portal system. The local absorption ratio ($F_a^m$) and the mean local absorption time ($\bar{t}_a^m$) of metabolites (5-FU and U) can also be defined by the same way shown in Eqs. 2 and 3 (14). $F_a^m$ is defined to be the fraction of parent drug that reaches as its metabolite to the portal system after the metabolic conversion by the intestinal flora and/or in the intestinal mucosa. Accordingly, $F_a^m$ is related to not only the metabolite generation, but also metabolite elimination in the intestine, and it is noted that Dose in Eq. 2 is that of 5′-DFUR (or UR). $\bar{t}_a^m$ is the mean time which includes mean times of the parent drug and its metabolite, that spends in the intestinal lumen and intestinal wall. $F_a^m$ was calculated by taking into consideration the molecular weight of 5′-DFUR (246.1) and 5-FU (130.1), or UR (244.2) and U (112.1).

AUC and MRT of compounds in arterial and portal bloods were computed by the trapezoidal integration with extrapolation to infinite. The statistical analysis was performed by one or two-way analysis of variance (ANOVA) at a 5% significant level.

## RESULTS

Figure 1 presents the time courses of portal and arterial blood concentrations of 5′-DFUR and 5-FU, following oral administration of 5′-DFUR alone (Group A) and coadministration of 5′-DFUR with UR (Group B) into rats. Each point in the figure represents the mean of five rats.

The blood concentration of 5′-DFUR was always higher in portal vein than in the artery in Group A. This difference reflected the absorption of 5′-DFUR from the intestinal tract into the portal system (16,17). The portal-systemic difference in 5-FU concentrations indicated the generation of 5-FU from 5′-DFUR in the intestine (14). In Group B, the blood concentration of 5′-DFUR in the portal vein was also higher than that in the artery, and the extent of concentration difference between portal and artery bloods was smaller than that in Group A. The concentrations of 5-FU in both portal and artery bloods were considerably greater in Group B than in Group A, though the portal-systemic differences in 5-FU concentration were almost the same between groups A and B.

Table I presents AUC and MRT of time courses of 5′-DFUR and 5-FU concentrations in the portal and arterial bloods in Groups A and B. In Group A, AUC values of both 5′-DFUR and 5-FU in portal blood were significantly higher than those in arterial blood. MRT values of 5′-DFUR and 5-FU in portal vein were significantly smaller than those in the artery. MRT values of 5′-DFUR in portal vein and artery were very close to corresponding MRT values of 5-FU in portal vein and artery, respectively. $F_a$ and $\bar{t}_a$ of 5′-DFUR were estimated to be 60.1 ± 10.5% of dose and 90.6±15.1 min, respectively, according to Eqs.2 and 3. $F_a^m$ of 5-FU, generated by intestinal metabolism from 5′-DFUR and reached the portal system, was 8.3 ± 1.9% of 5′-DFUR administered. $\bar{t}_a^m$ of 5-FU was estimated to be 96.8 ± 14.8 min, which was close to $\bar{t}_a$ of 5′-DFUR. The local moment parameters in Group A were almost the same as those in previous reports (14).

$F_a$ of 5′-DFUR (38.0 ± 18.6% of dose) in Group B was about one third less than that in Group A (60.1%). AUC values of 5-FU both in portal vein and artery were increased intensively

**Table I.** Moment Characteristics of 5′-DFUR, 5-FU and Uracil After Oral Administration of 5′-DFUR or Oral Coadministration of 5′-DFUR and Uridine

| Administration | 5′-DFUR alone | 5′-DFUR+Uridine |
|---|---|---|
| Body weight (g) | 222 ± 19 | 226 ± 16 |
| Qb[a] (ml/min) | 13.6 ± 1.2 | 13.8 ± 1.0 |
| (A) 5′-DFUR | | |
| AUC (μg*min/ml) | | |
| Portal | 2340 ± 290[c] | 2040 ± 540[c] |
| Artery | 1350 ± 330 | 1420 ± 350 |
| MRT (min) | | |
| Portal | 110 ± 21.1[c] | 102 ± 11.0[c] |
| Artery | 123 ± 26.7 | 107 ± 11.5 |
| Fa (%) | 60.1 ± 10.5 | 38.0 ± 18.6[b] |
| $\bar{t}_a$ (min) | 90.6 ± 15.1 | 89.6 ± 16.3 |
| (B) 5-FU | | |
| AUC (μg*min/ml) | | |
| Portal | 109 ± 23.0[c] | 262 ± 66.7[b,c] |
| Artery | 38 ± 9.2 | 187 ± 66.7[b] |
| MRT (min) | | |
| Portal | 108 ± 16.7 | 102 ± 11.6 |
| Artery | 125 ± 34.7 | 94.2 ± 14.3 |
| $F_a^m$ (% of 5′-DFUR) | 8.3 ± 1.9 | 8.7 ± 4.0 |
| $\bar{t}_a^m$ (min) | 96.8 ± 14.8 | 133 ± 39.8 |
| (C) Uracil | | |
| AUC (μg*min/ml) | | |
| Portal | | 840 ± 340[c] |
| Artery | | 530 ± 210 |
| MRT (min) | | |
| Portal | | 94.9 ± 7.5 |
| Artery | | 89.4 ± 3.5 |
| $F_a^m$ (% of Uridine) | | 41.9 ± 26.8 |
| $\bar{t}_a^m$ (min) | | 102 ± 13.0 |

*Note*: (A) and (B) correspond to the moment parameters obtained by 5′-DFUR and 5-FU blood concentrations, respectively, following oral administration of 100mg/kg of 5′-DFUR or oral coadminstration of 100mg/kg each of 5′-DFUR and uridine in rats (mean ± S.D., n = 5 each). (C) represents the moment parameters of uracil generated from uridine after oral coadminstration of 5′-DFUR and uridine. Uridine concentrations were less than the quantitation limit (2.5μg/ml) throughout the study.

[a] Portal blood flow rate was calculated as Qb = 15.3 × body weight/250.

[b] Significantly different ($p < 0.05$) from the group administered 5′-DFUR alone.

[c] Significantly different ($p < 0.05$) from that in artery.

in Group B, and AUC in the artery of Group B was about five-fold of that in the artery of Group A. However, $F_a^m$ of 5-FU in Group B was estimated to be 8.7 ± 4.0% of administered 5'-DFUR, which was very close to that in Group A. $F_a^m$ of 5-FU in Group B was slightly greater than that in Group A, although the difference was statistically insignificant.

Figure 2 shows the time courses of portal and arterial blood concentrations of U in Group A (triangle) and Group B (circle). No remarkable fluctuation in endogenous U concentrations was observed both in portal and arterial bloods in Group A. UR was not detected in portal and arterial bloods (at quantification limit of 2.5 μg/ml) in Group B. The concentration of U were considerably increased by UR coadministration and U concentration in portal vein was always higher than that in artery, indicating the intestinal metabolic conversion from UR to U. The time profile of U concentration in the artery was comparable to that of 5-FU (Fig. 1). Table I also presents AUC and MRT of U concentrations in portal and arterial bloods and local moment parameters in Group B. $F_a^m$ of U was estimated to be 41.9 ± 26.8% of coadministered UR, which demonstrates that UR is eliminated 100% through the intestinal wall and arrives 40% at portal system as its metabolite (U).

## DISCUSSION

It has been reported that 5'-DFUR blood concentrations increased proportionally with the dosing levels at 5, 20, and 50 mg/kg after oral administration of $^{14}$C-labeled 5'-DFUR in rats (21). After oral administration of 5'-DFUR (500 mg/kg) in rat, the absolute bioavailability (F) of 5'-DFUR was about 60% (22), which is very close to $F_a$ of 5'-DFUR obtained in the present study. The preceding study demonstrated that the hepatic recovery ratio was close to 100% (14). Thus, it is considered that the absorption kinetics of 5'-DFUR into the portal vein is linear at the dose (100 mg/kg).

It is known that orally administered 5'-DFUR is metabolically converted to 5-FU by PyNPase in the intestinal wall (3,6), and that the intestinal toxicity of oral 5'-DFUR is partly due to 5-FU generated by the intestinal first-pass metabolism (8). Therefore, it is expected that the inhibition of intestinal PyNPase activity and hence the block of intestinal 5-FU generation potentially decreases its intestinal toxicity. Since UR is a metabolic substrate to PyNPase, UR is predicted to inhibit competitively the conversion of 5'-DFUR to 5-FU in small intestine (9), accompanying with the formation of U as its metabolite. In this experiment, UR was not detected in portal and arterial bloods after its oral administration, which may suggest that UR was completely eliminated in the intestine and arrived 40% at the portal system as U. Since metabolites (U and 5-FU) are further degraded to corresponding dehydro-derivatives by an enzyme, dihydropyrimidine dehydrogenase (DPD) (23,24), U inhibits competitively the metabolic degradation of 5-FU in the liver (25).

After oral administration, a portion of 5'-DFUR was converted to 5-FU in the intestine and about 7% of 5'-DFUR arrived as 5-FU at the portal system (14). The experiment *in*

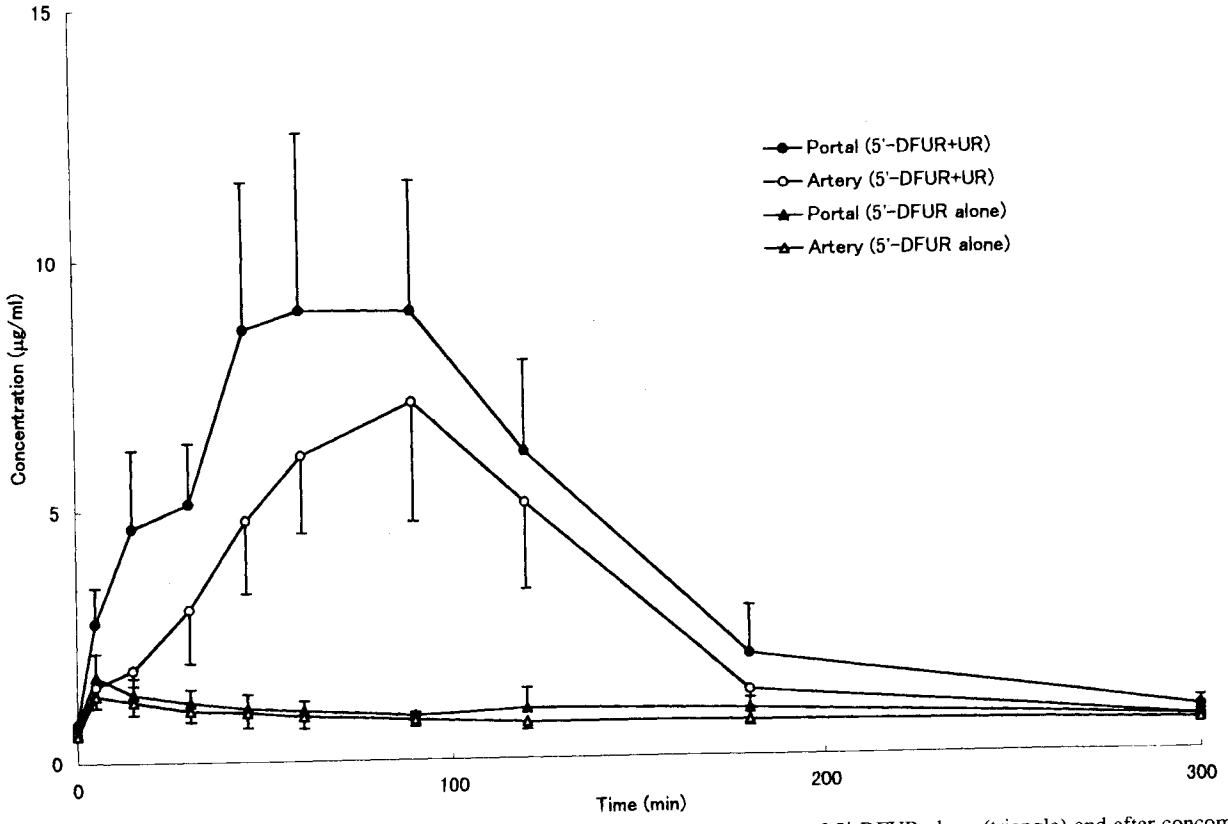


**Fig. 2.** Mean blood concentration-time course of U after oral administration of 100 mg/kg of 5'-DFUR alone (triangle) and after concomitant oral administration of 100 mg/kg of 5'-DFUR with UR (circle). The closed and open symbols represent portal vein and artery blood, respectively. Each point represents mean and SD of five rats.

the intestinal homogenate predicted about 10% of 5-FU generation *in vivo* from 5′-DFUR, which agreed with the result in the present experiment (26). The coadministration of UR was anticipated to block 5-FU generation in the intestine by the competitive inhibition of PyNPase and consequently to increase $F_a$ of 5′-DFUR and decrease $F_a^m$ of 5-FU. In contrast against this expectation, $F_a^m$ of 5-FU was unaffected and $F_a$ of 5′-DFUR was decreased 37% by coadministered UR. This contradiction may indicate that a considerably large portion of 5-FU from 5′-DFUR is further degraded through the intestinal wall in Group A and that U from UR blocks the 5-FU degradation through the intestine in Group B. It is reported that UR inhibits the transport of 5′-DFUR into the intestinal wall (13), which also explains the decrease in $F_a$ of 5′-DFUR with UR coadministration. Despite that $F_a$ of 5′-DFUR was decreased, AUC of 5′-DFUR in the arterial blood was not altered by UR administration, which may suggest that U decreases 5′-DFUR elimination in the systemic circulation (9). In Group B, 5-FU concentration in the systemic circulation was considerably increased, in spite that no remarkable change in $F_a^m$ of 5-FU. The time-profile of 5-FU and its local moment parameters ($t_a^m$ and MRT) were comparable to those of U, suggesting that the competitive inhibition of 5-FU degradation by U in the systemic circulation.

## CONCLUSIONS

The *in vitro* experiment, using an intestinal homogenate, predicts that coadministered 5′-DFUR with UR would increase 5′-DFUR level and decrease 5-FU level in the portal vein. The present study based on PS method demonstrates in a direct way that the prediction from the *in vitro* study does not always agree with the *in vivo* experiment using conscious rat. The local moment analysis by PS method provides an experimental information on the extent and rate of metabolite generation in the first-pass processes in a single conscious rat, as well as that of the parent drug disposition. Therefore, this analysis method is expected to also be effective to evaluate the drug-drug interaction of orally administered drugs.

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