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Research Paper

Effect of acute hepatic failure on the hepatic first-pass effect of 5-fluorouracil in rats

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Abstract

Objectives In cancer chemotherapy for hepatocellular carcinoma, 5-fluorouracil is widely used and has typically been given by intrahepatic arterial (i.a.) infusion to increase treatment efficacy and reduce systemic toxicity. 5-Fluorouracil is eliminated primarily by the liver and so the hepatic first-pass effect after intrahepatic arterial administration of 5-fluorouracil may be lower in patients with hepatic failure, and systemic toxicity may not be reduced. In this study, we have investigated the effect of acute hepatic failure on the first-pass effect of 5-fluorouracil in rats.

Methods Experimental acute hepatic failure was induced by treatment with carbon tetrachloride (CCl_4). 5-Fluorouracil was infused for 15 min into the hepatic artery or the saphenous vein of rats at a dose of 1.25 mg/kg.

Key findings Hepatic availability in 50% CCl_4-treated (severe hepatic failure) rats was higher than in controls.

Conclusions The hepatic first-pass effect after intrahepatic arterial administration of 5-fluorouracil was lower in severe hepatic failure. Therefore, the reducing effect of the systemic toxicity after intrahepatic arterial administration may be lower in severe hepatic failure.

Keywords first-pass effect; 5-fluorouracil; hepatic failure; intrahepatic arterial injection; pharmacokinetics

Introduction

Hepatocellular carcinoma is one of the most common malignancies worldwide.[1] In chemotherapy for hepatocellular carcinoma, anticancer agents, such as 5-fluorouracil have typically been given by intrahepatic arterial (i.a.) infusion to increase treatment efficacy and to reduce systemic toxicity by the hepatic first-pass effect.[2,3]

Hepatocellular carcinoma is strongly linked to chronic infection with hepatitis B and/or C, and so it usually presents as an advanced disease and is complicated by cirrhosis in 80% of cases.[1] Therefore, the hepatic first-pass effect after intrahepatic arterial administration of 5-fluorouracil may be lower in patients with hepatocellular carcinoma and systemic toxicity may not be reduced because 5-fluorouracil is eliminated primarily by the liver.[4]

Some studies have reported the effect of liver dysfunction on the pharmacokinetics of 5-fluorouracil; however, the results were discordant and there have been few reports about the hepatic first-pass effect with hepatic failure.[5,6] To assure safe and effective chemotherapy, we should evaluate the extent to which hepatic failure influences the pharmacokinetics of 5-fluorouracil after intrahepatic arterial injection.

In this study, we have investigated the effect of acute hepatic failure on the hepatic first-pass effect of 5-fluorouracil in rats.

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Materials and Methods

Materials

5-Fluorouracil was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in saline (0.5 mg/ml, as 5-fluorouracil). 5-Fluoro-5,6-dihydrouracil (DHFU) was obtained

from Kyowa Hakko Kirin Co., Ltd (Tokyo, Japan). Carbon tetrachloride (CCl_4) was purchased from Wako Pure Chemicals Co., Ltd (Osaka, Japan). All other chemicals and solvents were of the highest commercially available grade.

Animals

Male Wistar rats (250–310 g, 9-weeks old; Kyudo Co. Ltd, Kumamoto, Japan) were used and were maintained in the Department of Bio-resources, Division of Biotechnology, Frontier Science Research Center, University of Miyazaki, Japan. The Committee for the Ethics on Animal Experiments in University of Miyazaki approved the experimental protocol. The animal experiments were performed in accordance with The Guidelines for Animal Experiments of the University of Miyazaki.

Preparation of model rat for acute hepatic failure

Acute hepatic failure was induced according to a previously described method with minor modifications.[7] Briefly, rats were fasted for 24 h and then 10% (mild hepatic failure) or 50% CCl_4 (severe hepatic failure) in corn oil was administered 1.0 ml/kg orally. Untreated control rats received the same volume of corn oil alone. Pharmacokinetic studies were performed 24 h later.

Pharmacokinetics of 5-fluorouracil in rats

Normal and hepatic failure rats had an indwelling cannula (PE50; Becton Dickinson, Sparks, MD, USA) implanted in the left carotid artery under pentobarbital anaesthesia (50 mg/kg normal rats; 25 mg/kg hepatic failure rats, i.p.). Heparin in saline (100 U/ml) was injected at a dose of 0.1 ml/100 g body weight through the left carotid artery to prevent blood clotting. 5-Fluorouracil (1.25 mg/kg) was infused for 15 min into the hepatic artery or the saphenous vein of rats. For intrahepatic arterial administration, rats underwent a laparotomy, and the gastroduodenal artery was exposed and dissected. Using polyethylene tubing (PE10; Becton Dickinson, Sparks, MD, USA), the gastroduodenal artery was cannulated hepatopetally. The catheter tip was positioned below the bifurcation of the gastroduodenal and proper hepatic arteries. For intravenously (i.v.) injected rats, a sham operation was performed. Blood samples (0.25 ml each) were drawn periodically through a cannula in the carotid artery at 0, 2, 5, 15, 17, 20, 25, 30, 40 and 50 min after 5-fluorouracil administration under pentobarbital anaesthesia. Blood samples were immediately centrifuged at 13 000*g* for 2 min and the plasma was separated.

Determination of hepatic blood flow

Hepatic blood flow was determined using the method of Yokota *et al.*[8] with minor modifications. Briefly, a priming dose of indocyanine green was injected into the femoral vein (5 mg/kg), followed immediately by a constant infusion of indocyanine green into the femoral vein (1.25 mg/h). Plasma was obtained from blood samples taken from the femoral artery and the hepatic vein at 50 min after start of indocyanine green infusion. After indocyanine green infusion, the livers were quickly removed, and stored at –80°C for the dihydropyrimidine dehydrogenase (DPD) activity assay. Indocyanine green in plasma was measured at a wavelength of 805 nm after 10-fold dilution.

Determination of dihydropyrimidine dehydrogenase activity

DPD activity was determined using the method of Tateishi *et al.*[9] with minor modifications. Briefly, hepatic cytosol was prepared, and the protein concentration of the cytosolic fraction was measured using the method of Bradford *et al.*[10] Cytosolic incubation was carried out in a final volume of 2.0 ml with 35 mmol/l potassium phosphate buffer (pH 7.4), 2.5 mmol/l magnesium chloride, 10 mmol/l 2-mercaptoethanol, and 200 μmol/l nicotinamide adenine dinucleotide phosphate, reduced form (NADPH). The concentration of 5-fluorouracil was 40 μmol/l. The protein content was determined as 500 μg/ml. The reaction time was determined to be 15 min because the rate of production of the 5-fluorouracil metabolite remained constant for up to 15 min under these conditions. The reaction mixture was pre-incubated at 37°C for 5 min, and the reaction was initiated by the addition of 5-fluorouracil. A sample of reaction mixture (100 μl) was removed and mixed with 2 ml ice-cold ethyl acetate. DPD activity was determined by measuring the metabolite of 5-fluorouracil formed (DHFU) by high-performance liquid chromatography (HPLC).

Assay of 5-fluorouracil or 5-fluoro-5,6-dihydrouracil concentration

The 5-fluorouracil or DHFU assay was performed according to a previously described method with minor modifications.[11] To determine 5-fluorouracil or DHFU, 100 μl plasma or the reaction mixture, 10 μl internal standard (5-chlorouracil, 20 μg/ml in distilled water), and 1 ml ethyl acetate were mixed vigorously for 30 s, and then centrifuged at 13 000*g* for 5 min. Supernatant (900 μl) was evaporated to dryness *in vacuo* at 45°C. Mobile phase (200 μl) was added to the residue, and vortexed for 30 s. A 50-μl sample of the supernatant was injected into the HPLC column.

The HPLC apparatus consisted of LC-2010C_{HT} (Shimadzu Co., Kyoto, Japan). The column was a Develosil ODS-UG-5 (5 μm, 4.6 mm i.d. × 150 mm; Nomura Chemical Co. Ltd, Aichi, Japan). The mobile phase was 10 mmol/l acetate buffer (pH 4.0) for 5-fluorouracil or 50 mmol/l KH_2PO_4 (pH 4.5) for DHFU. The flow rate was 0.5 ml/min. The column effluent was monitored by a UV detector set at 266 (5-fluorouracil) or 215 nm (DHFU).

Biochemical assay

Plasma concentrations of alanine transaminase (ALT) and asparatate transaminase (AST) were measured by the pyruvate oxydase/*N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-*m*-toluidine/4-aminoantipyrine coupling method using Transaminase CII-Test Wako (Wako Pure Chemical Industries, Ltd, Osaka, Japan).

Pharmacokinetic analysis

The plasma concentration–time profiles for 5-fluorouracil were analysed using a two-compartment model with zero-order

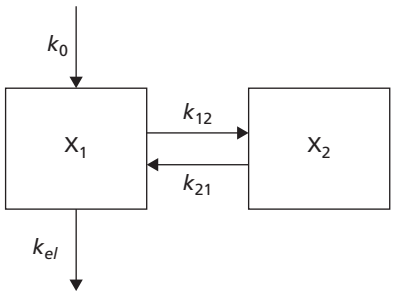

Figure 1 Schematic representation of a two-compartment model with zero-order absorption. X_1 is the amount of drug in the central compartment, X_2 is the amount of drug in the peripheral compartment. Rate constant k_{12} represents the movement from the central to peripheral compartment, and k_{21} represents the reverse. Rate constant k_{el} represents elimination from the central compartment, and k_0 represents the zero-order infusion rate of 5-fluorouracil (1.25 mg/15 min per kg), respectively

infusion (Figure 1). The differential equations describing the model in Figure 1 are:

$$dX_1/dt = k_{21}X_2 - (k_{12} + k_{el})X_1 + k_0 \quad (1)$$

$$dX_2/dt = k_{12}X_1 - k_{21}X_2 \quad (2)$$

where X, k_{12}/k_{21}, k_{el} and k_0 represent the amount of drug, the first-order transfer rate constants between the central and peripheral compartments, the first-order elimination rate constant from the central compartment, and the zero-order infusion rate of 5-fluorouracil (1.25 mg/15 min per kg), respectively. Solution by the Laplace transform method yielded the following equation:

$$C = k_0(1-e^{\alpha T})(k_{21}-\alpha)e^{-\alpha t}/[Vd_1\alpha(\alpha-\beta)] + k_0(1-e^{\beta T})(k_{21}-\beta)e^{-\beta t}/[Vd_1\beta(\beta-\alpha)] \quad (3)$$

where α and β are bi-exponential elimination rate constants from plasma and Vd_1 is the volume of distribution of the central compartment. T is the infusion time, where $T = t$ when $0 \leqq t \leqq T$ and $T = T$ when $T \leqq t$. Pharmacokinetic parameters were estimated by fitting equation 3 to profiles of C against t after 5-fluorouracil administration, using a nonlinear least-square program MULTI.[12] Hepatic availability (F) of 5-fluorouracil was calculated as the ratio of the area under the concentration–time curve (AUC) after intrahepatic arterial to intravenous administration. Total clearance of 5-fluorouracil after intravenous administration ($CL_{i.v.}$) was determined by multiplying k_{el} by Vd_1. Distribution volume at steady state (Vd_{ss}) was calculated using the following equation: $Vd_{ss} = Vd_1(1+k_{12}/k_{21})$. Half-life of the β-phase ($t_{1/2\beta}$) was determined by dividing the natural logarithm of 2 by β.

Statistical analysis

All results are expressed as the mean ± SD. Analysis of variance followed by Dunnett's test was used for multiple comparisons. Differences were considered significant at $P < 0.05$.

Results

Laboratory data in rats after CCl_4 treatment

The biochemical parameters in hepatic failure rats are shown in Table 1. In CCl_4-treated rats, serum AST and ALT levels were significantly elevated compared with control rats, and serum AST/ALT levels in 50% CCl_4-treated rats (severe hepatic failure) were higher than in 10% CCl_4-treated rats (mild hepatic failure).

Hepatic availability in rats

Figure 2 shows the plasma 5-fluorouracil concentration–time profiles in control and hepatic failure rats after intravenous and intrahepatic arterial administration. In control and hepatic failure rats, the plasma concentration and AUC of 5-fluorouracil after intrahepatic arterial injection were significantly lower than following intravenous injection (Figure 2, Table 1).

Hepatic availability in control rats (31.1%) was similar to the values in mild hepatic failure rats (35.5%). In contrast,

Table 1 Pharmacokinetics of 5-fluorouracil in control and hepatic failure rats

Parameter	Intravenous			Intrahepatic arterial		
	Control	Mild hepatic failure	Severe hepatic failure	Control	Mild hepatic failure	Severe hepatic failure
Number of animals	8	8	8	8	8	8
Body weight (g)	282 ± 4	277 ± 17	281 ± 12	279 ± 12	274 ± 14	276 ± 19
AST (IU/l)	34 ± 9	225 ± 141	970 ± 617**	39 ± 22	207 ± 80	1065 ± 537**
ALT (IU/l)	8 ± 3	66 ± 50	342 ± 261**	7 ± 3	55 ± 34	267 ± 115**
Vd_{ss} (l/kg)	0.431 ± 0.055	0.504 ± 0.111	0.422 ± 0.075	–	–	–
$CL_{i.v.}$ (l/min per kg)	0.0454 ± 0.0143	0.0391 ± 0.0075	0.0309 ± 0.0059*	–	–	–
$t_{1/2\beta}$ (min)	16.0 ± 3.5	19.5 ± 4.5	20.4 ± 2.8*	16.3 ± 3.9	18.5 ± 4.7	25.9 ± 6.1**
AUC (mg.min/l)	29.7 ± 8.1	33.2 ± 7.4	41.9 ± 8.6*	9.2 ± 2.7	11.8 ± 1.9	24.9 ± 9.7**
F (%)				31.1	35.5	59.5

Administration of 5-fluorouracil was intrahepatic arterial or intravenous. Data were measured 24 h after oral administration of corn oil (control) or CCl_4 (0.1 ml/kg, mild hepatic failure; 0.5 ml/kg, severe hepatic failure) to rats. ALT, alanine transaminase; AST, asparatate transaminase; AUC, area under the concentration–time curve; Vd_{ss}, distribution volume at steady state; $CL_{i.v.}$, total clearance of 5-fluorouracil after intravenous administration; F, hepatic availability of 5-fluorouracil; $t_{1/2\beta}$, half-life of the β-phase, $t_{1/2\beta}$. Each value represents the mean ± SD. $^*P < 0.05$; $^{**}P < 0.01$ compared with control values.

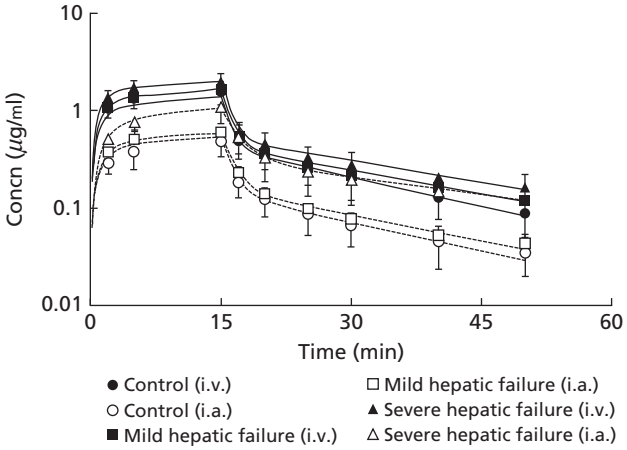

Figure 2 Plasma concentration–time profiles of 5-fluorouracil in control and hepatic failure rats. 5-Fluorouracil was infused for 15 min into the hepatic artery or saphenous vein of rats at a dose of 1.25 mg/kg 24 h after the administration of corn oil (control) or CCl_4 (0.1 ml/kg, mild hepatic failure; 0.5 ml/kg, severe hepatic failure). Each value represents the mean ± SD of eight rats. Lines denote computer-fitted curves

hepatic availability in severe hepatic failure rats (59.5%) was higher than in their controls.

Effects of acute hepatic failure on the blood disposition of 5-fluorouracil in rats

After intravenous or intrahepatic arterial injection, there was no significant difference in the plasma concentration and AUC of 5-fluorouracil between mild hepatic failure rats and their controls (Figure 2, Table 1). In contrast, the plasma concentration and AUC of 5-fluorouracil in rats with severe hepatic failure were significantly higher than in control rats. The AUC ratio of severe hepatic failure rats to control rats with intrahepatic arterial administration (271%) was higher than the ratio of severe hepatic failure rats to control rats with intravenous administration (141%) (Table 1). These results indicated that severe hepatic failure influenced the pharmacokinetics of 5-fluorouracil, and the influence of hepatic failure on plasma 5-fluorouracil concentration was greater via the intrahepatic arterial route than the intravenous route.

Effects of acute hepatic failure on dihydropyrimidine dehydrogenase activity and hepatic blood flow in rats

There was no significant difference in hepatic blood flow between rats with mild hepatic failure and their controls (Table 2). In contrast, hepatic blood flow in rats with severe hepatic failure was significantly lower than in control rats. DPD activity in mild and severe hepatic failure rats was 23 and 54% lower, respectively, than in control rats.

Discussion

5-Fluorouracil is eliminated primarily by the liver; therefore, the drug may undergo a hepatic first-pass effect after intrahepatic arterial administration.[4] The results of this study showed that the plasma concentration and AUC of 5-fluorouracil after intrahepatic arterial injection were significantly lower than following intravenous injection (Figure 2, Table 1). Yuasa *et al.*[13] showed that the AUC of 5-fluorouracil after oral and intraportal administration was significantly lower than after intravenous administration. These results suggested that the hepatic first-pass effect for 5-fluorouracil through the liver was appreciable.

Hepatic dysfunction can influence the hepatic first-pass effect of 5-fluorouracil. In this study, we showed that hepatic availability in rats with severe hepatic failure (59.5%) was higher than in controls (31.1%). This result suggested that the first-pass effect after intrahepatic arterial administration of 5-fluorouracil was lower in severe hepatic failure. Therefore, in severe hepatic failure, the reducing effect of the systemic toxicity after intrahepatic arterial administration may be lower.

The hepatic availability (F), namely avoidance ratio of the hepatic first-pass effect, is expressed by equation 4 as follows:

$$F = Q/(Q + f \cdot CL_{int}) \quad (4)$$

where Q *is* the hepatic blood flow, f is the unbound ratio to protein binding, and CL_{int} is the hepatic intrinsic clearance. Since the protein binding of 5-fluorouracil to rat plasma was approximately 0% (i.e. f = 1),[14] the increase of F was induced by an increase of Q or a decrease of CL_{int}. The results of this study showed Q and DPD activity in rats with severe hepatic failure were significantly lower than in control rats. This suggested that the increase of the hepatic

Table 2 Hepatic blood flow and dihydropyrimidine dehydrogenase activity in control and hepatic failure rats

Parameter	Control	Mild hepatic failure	Severe hepatic failure
Number of animals	7	6	6
Body weight (g)	279 ± 18	289 ± 6	273 ± 7
AST (IU/l)	40 ± 14	284 ± 69	1604 ± 1793*
ALT (IU/l)	9 ± 6	82 ± 16	407 ± 482**
Hepatic blood flow (ml/min per kg)	50.7 ± 10.8	50.6 ± 21.2	31.8 ± 3.8*
DPD activity (nmol/min per mg protein)	0.683 ± 0.124	0.527 ± 0.051**	0.314 ± 0.035**

Data were measured 24 h after oral administration of corn oil (control) or CCl_4 (0.1 ml/kg, mild hepatic failure; 0.5 ml/kg, severe hepatic failure) to rats. ALT, alanine transaminase; AST, asparatate transaminase; DPD, dihydropyrimidine dehydrogenase. Each value represents the mean ± SD. *$P < 0.05$; **$P < 0.01$ compared with control values.

availability in severe hepatic failure rats could not be explained by the alterations of Q and would be brought about by the decrease in CL_{int}, which was suggested by the reduction in DPD activity because DPD is an initial and rate-limiting enzyme for the catabolism of 5-fluorouracil.[4,15]

The hepatic extraction ratio of 5-fluorouracil was approximately 70% because the hepatic availability of 5-fluorouracil in control rats was 31.1% (Table 1); therefore, $CL_{i.v.}$ would be mainly limited by hepatic blood flow. In this study, we showed no significant difference in the plasma concentration and AUC of 5-fluorouracil between mild hepatic failure rats and their controls after intravenous administration (Figure 2, Table 1). This result could be attributed to no difference in hepatic blood flow between mild hepatic failure rats and their controls (Table 2). On the other hand, the decreased hepatic blood flow in rats with severe hepatic failure resulted in a high plasma concentration of 5-fluorouracil in severe hepatic failure rats after intravenous administration. These results suggested that the pharmacokinetics of 5-fluorouracil with intravenous administration was influenced by hepatic blood flow.

In hepatically metabolized drugs such as 5-fluorouracil, the apparent clearance after intrahepatic arterial administration ($CL_{i.a}$) is expressed by equation 5:

$$CL_{i.a.} = CL_{i.v.}/F \tag{5}$$

Since the contribution of renal clearance to total clearance of 5-fluorouracil was not considerable, the $CL_{i.v}$ was expressed by equation 6:

$$CL_{i.v} = CL_H = Q \cdot f \cdot CL_{int}/(Q + f \cdot CL_{int}) \tag{6}$$

where CL_H is the hepatic clearance. Equations 4, 5 and 6 yielded the following equation:

$$CL_{i.a.} = f \cdot CL_{int} \tag{7}$$

Therefore, $CL_{i.a}$ of 5-fluorouracil as expressed would be mainly limited by CL_{int} because the protein binding of 5-fluorouracil to rat plasma was approximately 0%.[14] We showed the plasma concentration of 5-fluorouracil in rats with severe hepatic failure was significantly higher than the values in control rats. In addition, the DPD activity in severe hepatic failure rats was significantly lower than in control rats. These results suggested that the pharmacokinetics of 5-fluorouracil with intrahepatic arterial administration was mainly influenced by DPD activity.

The hepatic intrinsic clearance values calculated by equation 4 in control and severe hepatic failure rats were 112.4 and 21.7 ml/min per kg, respectively. Therefore, the hepatic intrinsic clearance in severe hepatic failure rats was approximately one-fifth of their controls. Meanwhile, DPD activity in severe hepatic failure rats was approximately one-half of their controls. These results suggested that the increase of AUC with intrahepatic arterial administration in severe hepatic failure rats could not be explained only by the alterations of DPD activity. Choi *et al.*[16] reported that 5-fluorouracil was metabolized via hepatic cytochrome P450 (CYP)1A in rats. In addition, the enzyme activity and the expression of CYP1A2 decreased significantly in CCl_4-induced hepatic failure rats.[7] Therefore, the pharmacokinetics of 5-fluorouracil with intrahepatic arterial administration would be influenced not only by DPD activity but also by other 5-fluorouracil metabolic enzymes (such as CYP1A).

We reported previously that the pharmacokinetics of epirubicin were affected in mild hepatic failure rats.[17] On the other hand, there was no significant difference in the plasma concentration of 5-fluorouracil between rats with mild hepatic failure and their controls after both intravenous and intrahepatic arterial injection. In severe hepatic failure rats, the plasma concentration of 5-fluorouracil was affected. These results suggested that the pharmacokinetics of 5-fluorouracil would not be influenced much by hepatic failure in comparison with that of epirubicin.

In this study, we have investigated the effect of acute hepatic failure in rats and showed that DPD activity in rats with severe hepatic failure was significantly lower than in control rats. On the other hand, Tateishi *et al.*[5] reported that DPD activity and protein levels in rat models of chronic hepatic failure were significantly greater than their controls; therefore, further investigations using hepatic cirrhosis model rats are necessary.

Conclusions

The hepatic first-pass effect after intrahepatic arterial administration of 5-fluorouracil was lower in rats with severe hepatic failure. Therefore, the reducing effect of the systemic toxicity after intrahepatic arterial administration may be lower in severe hepatic failure.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Llovet JM *et al*. Hepatocellular carcinoma. *Lancet* 2003; 362: 1907–1917.
2. Cagol PP *et al*. Potential advantages of loco-regional intra-arterial chemotherapy. *In Vivo* 2006; 20: 777–779.
3. Kemeny NE *et al*. Hepatic arterial infusion versus systemic therapy for hepatic metastases from colorectal cancer: a randomized trial of efficacy, quality of life, and molecular markers (CALGB 9481). *J Clin Oncol* 2006; 24: 1395–1403.
4. Pinedo HM, Peters GF. Fluorouracil: biochemistry and pharmacology. *J Clin Oncol* 1988; 6: 1653–1664.
5. Tateishi T *et al*. Dihydropyrimidine dehydrogenase activity and fluorouracil pharmacokinetics with liver damage induced by bile duct ligation in rats. *Drug Metab Dispos* 1999; 27: 651–654.
6. Innocenti F *et al*. 5-Fluorouracil catabolism to 5-fluoro-5,6-dihydrouracil is reduced by acute liver impairment in mice. *Toxicol Appl Pharmacol* 2005; 203: 106–113.

7. Yokogawa K *et al*. Serum aminotransferase activity as a predictor of clearance of drugs metabolized by CYP isoforms in rats with acute hepatic failure induced by carbon tetrachloride. *Int J Pharm* 2004; 269: 479–489.
8. Yokota M *et al*. Simple method of hepatic venous blood sampling in the rat. *J Appl Physiol* 1976; 41: 439–441.
9. Tateishi T *et al*. Preliminary examination of the influence of incubation time or cytosolic protein concentration on dihydropyrimidine dehydrogenase activity. *Clin Chim Acta* 1996; 252: 1–9.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–254.
11. Loos WJ *et al*. Determination of 5-fluorouracil in microvolumes of human plasma by solvent extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1999; 735: 293–297.
12. Yamaoka K *et al*. A pharmacokinetic analysis program (multi) for microcomputer. *J Pharmacobiodyn* 1981; 4: 879–885.
13. Yuasa H *et al*. First-pass metabolism of 5-fluorouracil in rats. *J Pharm Pharmacol* 1998; 50: 1019–1025.
14. Yamashita S *et al*. 5-Fluorouracil derivatives with serum protein binding potencies. *Chem Pharm Bull* 1989; 37: 2861–863.
15. Naguib FN *et al*. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* 1985; 45(11 Pt 1): 5405–5412.
16. Choi YH *et al*. Pharmacokinetics of 5-fluorouracil in mutant Nagase analbuminemic rats: faster metabolism of 5-fluorouracil via CYP1A. *Biopharm Drug Dispos* 2007; 28: 87–95.
17. Nagata M *et al*. Effect of acute hepatic failure on epirubicin pharmacokinetics after intrahepatic arterial injection in rats. *Biol Pharm Bull* 2008; 31: 493–496.