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Hepatic Extraction Ratio of 5-Fluorouracil in Rats

DOSE DEPENDENCE AND EFFECT OF URACIL AND INTERLEUKIN-2

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ABSTRACT. The hepatic extraction ratios (E_H) of 5-fluorouracil (FUra) in rats were studied to clarify the disposition of FUra in the liver. The E_H of FUra in rats at infusion rates ranging from 0.375 to 3 mg/kg/min decreased from 0.750 to 0.225. The E_H of values of tegafur, a pro-drug of FUra, were 0.076 to 0.103 over the range of infusion rates, 0.577 to 4.616 mg/kg/min, and were much lower than those of FUra. The E_H of FUra at an infusion rate of 0.375 mg/kg/min combined with uracil (0.323 mg/kg/min) was 0.646, which was significantly lower than that of FUra alone, 0.750 ($P < 0.001$). The E_H of FUra combined with interleukin-2 (IL-2) at an infusion rate of 7500 U/kg/min was significantly higher than that of FUra alone ($P < 0.01$). The dose dependence of the E_H of FUra and the effects of uracil and IL-2 on the E_H of FUra corresponded with clinical findings. These results suggest that this experimental model in rats may be useful for predicting the clinical pharmacokinetics and efficacy of FUra. We also studied the effect of IL-2 on the E_H of mitomycin C (MMC). The E_H of MMC combined with IL-2 was higher than that of MMC alone, but the difference was not significant. BIOCHEM PHARMACOL 52;4: 561–568, 1996.

KEY WORDS. 5-fluorouracil; interleukin-2; immunochemotherapy; pharmacokinetics; hepatic extraction; uracil

FUra§ is one of the most widely used cancer chemotherapeutic agents [1–4]. It is employed in the treatment of various tumors, particularly cancer of the gastrointestinal tract, alone or in combination with other drugs [1, 2, 4]. FUra is also used in locoregional chemotherapy, which has the dual aim of increasing, relatively, the exposure of the tumor to drugs while reducing the systemic exposure. For example, high clinical response rates to chemotherapy administered by an intra-arterial hepatic route have been reported [1, 5]. E_H is the ratio of the removal of drugs by the liver, involving hepatic cellular uptake, metabolism, and biliary excretion. Variations in the E_H during hepatic arterial infusion affect both the exposure of the target, hepatic tumor and the systemic exposure connected with systemic toxicity [1, 4–6]. An E_H of FUra of 0.11 to 0.93 has been reported [1, 4–8]. These results show that the E_H of FUra decreases with increasing dose or infusion rate, that is, the pharmacokinetics of FUra are non-linear during hepatic arterial infusion. Therefore, the efficacy and toxicity should be dependent on the dosing protocols. It is important to clarify the non-linearity of the E_H of FUra to establish the most suitable hepatic arterial infusion protocol. In this paper, the infusion rate dependence of the E_H of FUra during constant infusion of FUra into rats was studied and found to be non-linear, as in humans. We also studied the E_H of tegafur, a pro-drug of FUra.

Recently, to improve the efficacy of FUra, a combination regimen of FUra with IL-2 [9–11] or FUra + IL-2 + other cytotoxic agents or biological modulators, for example MMC [12–14], cisplatin [2, 15], dipyridamole [3, 7, 16], and leucovorin [2, 3, 7, 17–20], was attempted, and several studies have demonstrated improved efficacy [2, 7, 11, 12, 14, 15, 17]. Since these agents have different mechanisms of action, the efficacy may be synergistic and the toxicity may be non-overlapping [9, 15]. In our previous report [12], a high response rate (76%) was found for the treatment of hepatic metastases by hepatic arterial infusion of a combination chemotherapy of IL-2, FUra, and MMC [IL · MF therapy]. There have been many reports of the pharmacological effects of IL-2 [21–26] and the effects of FUra and MMC on host immunological status [24–29], but few reports of the pharmacokinetics of FUra and/or MMC combined with IL-2; none of them involved the interaction of these agents in the liver during hepatic arterial infusion. As described above, the variation of E_H during hepatic arterial infusion directly affects both the exposure of the target, hepatic tumor, and the systemic exposure associated with systemic toxicity. Therefore, hepatic arterial infusion com-

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§ *Abbreviations:* FUra, 5-fluorouracil; E_H, hepatic extraction ratio; IL-2, interleukin-2; MMC, mitomycin C; and CL_{tot}, systemic clearance.

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bined with a modulator to increase E_H can make efficient targeting of the liver possible. In this study, the effects of IL-2 on the E_H of FUra and MMC, as well as the effect of uracil, which has been used thus far in combination with FUra [7, 15, 30], on the E_H of FUra, were also studied.

MATERIALS AND METHODS

Chemicals

FUra, MMC for injection, and porfiromycin (internal standard for MMC analysis) were supplied by the Kyowa Hakko Kogyo Co. Ltd. Uracil was purchased from Wako Pure Chemical Industrial Ltd. (Tokyo, Japan), and IL-2 was a gift from the Shionogi Pharmaceutical Co. Ltd. (Osaka, Japan). 5-Chlorouracil was purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Heparin for injection was purchased from the Shimizu Pharmaceutical Co. Ltd. (Shizuoka, Japan). All other chemicals were commercially available and of analytical grade.

Animals

Adult male Wistar rats, 6-weeks-old, were obtained from Charles River Japan Inc. (Tokyo, Japan). The animals were housed for about 1 week under controlled conditions of temperature and lighting (12 hr) with free access to food and water at all times. The rats weighed between 225 and 324 g. Each group consisted of 3 or 4 rats.

Hepatic Extraction Experiments

Hepatic extraction was measured by the method of Yokota and coworkers [31, 32]. Under light ether anesthesia, the abdomen was opened through a midline incision downward, extending about 4 cm from the diaphragm. The central lobe of the liver was moved to the upper left with gauze soaked in saline. The hepatic vein junction of the left (or right) and central lobe was identified easily. As a cannula, a venous injection needle (0.65 × 25 mm; Terumo, Tokyo, Japan), bent at a right angle 3.5 mm from the tip, was connected to a PE-50 polyethylene tube. This cannula was connected to a 1-mL syringe, and then the bent needle tip of the cannula was inserted downward into the junction of the hepatic veins, and a small volume of hepatic venous blood was drained by withdrawing the syringe plunger very gently. After confirmation of blood drainage, the syringe was replaced with one filled with injectable heparin solution, and the blood in the cannula was pushed back up to the cannulated point by the heparin solution in the syringe. The cannulated point was fixed by a surgical binding agent, Aron Alpha (Sankyo Co. Ltd., Tokyo, Japan), and the central lobe was returned to its normal position. The correct cannulation was confirmed once more by the same procedure as above, and then the abdomen was sutured. Femoral arterial and venous cannulation was carried out by the usual method, with PE-50 polyethylene tubing (0.5 mm i.d.). Cannulated rats were kept in the supine position on boards and were allowed to recover from anesthesia prior to the injection of drugs. After recovery from anesthesia, drug infusion was carried out by femoral venous cannulation using a micro automatic infusion pump (model 975, Harvard Apparatus, South Natick, MA, U.S.A.). The infusion rate was 0.0652 mL/min. The infusion was started after bolus administration of the infusion dose as a loading dose. The infusion dose rate of FUra alone was 0.375, 0.75, 1.5, and 3 mg/kg/min. The infusion dose rate of tegafur alone was 0.577, 1.154, 2.308, and 4.616 mg/kg/min. The infusion rates of FUra and uracil during co-administration were 0.375 and 0.323 mg/kg/min, respectively. The infusion rates during co-administration were 0.375 mg/kg/min for FUra, and 3000 and 7500 U/kg/min for IL-2. The infusion dose rate of MMC alone was 2.5, 7.5, and 25 μg/kg/min. The infusion rates of MMC and IL-2 during co-administration were 7.5 μg/kg/min and 7500 U/kg/min, respectively. Plateaus of the plasma concentration of FUra, tegafur, and MMC were observed between 20 and 40 min. Thus, femoral arterial and hepatic venous blood samples were collected at 20, 30, and 40 min after infusion in heparinized capillary tubes (Drummond Scientific Co., Broomall, PA, U.S.A.). Plasma was separated by the usual method and stored at −20° until the day of assay.

Analytical Procedures

FUra, tegafur, and MMC concentrations in plasma were determined by HPLC. For the termination of FUra in plasma, 200 μL of plasma was mixed with 50 μL of 0.5 M phosphate buffer (pH 8.0), 100 μL of an aqueous solution containing 5-chlorouracil (0.5 μg/mL) as an internal standard, and 8 mL of ethyl acetate. The mixture was shaken for 30 min and centrifuged at 2200 *g* for 10 min. The ethyl acetate layer was removed and evaporated. The residue was reconstituted in 500 μL of HPLC mobile phase (*n*-hexane:ethyl acetate:formic acid:water = 50:50:0.5:0.3) by sonicating for 10 min. An aliquot (150 μL) of the solution was injected into an HPLC chromatograph. The HPLC chromatograph (model LC-6A, Shimadzu, Kyoto, Japan) was equipped with a UV detector (SPD-6A UV-VIS detector, Shimadzu, Kyoto, Japan) and a 4.6 × 100 mm Develosil 60-3 column (Nomura Chemical Co., Aichi, Japan). The flow rate was 0.9 mL/min for 20 min, followed by 0.4 mL/min for 27 min and 0.9 mL/min for 8 min. Absorbance of FUra at 264 nm was measured, and the detection limit was 3 ng/mL plasma. For the determination of tegafur in plasma, 20 μL of plasma was mixed with 50 μL of 0.5 M phosphate buffer (pH 8.0), 100 μL of an aqueous solution containing 5-chlorouracil (0.5 μg/mL) as an internal standard and 7 mL of ethyl acetate. The mixture was shaken for 30 min and centrifuged at 2200 *g* for 10 min. The ethyl acetate layer was removed and evaporated. The residue was reconstituted with 500 μL of a solvent mixture (HPLC mobile phase of FUra:*n*-hexane = 3:2) by sonicating for 10 min. The entire volume of this solution was injected into an HPLC chromatograph. The HPLC chromatograph was

the same as that for FUra. The flow rate was constant, 0.9 mL/min. The absorbance of FUra at 264 nm was measured. The detection limit was 50 ng/mL plasma. For the determination of MMC in plasma, 50 μL plasma was added to 50 μL acetonitrile containing porfiromycin (2 μg/mL) as an internal standard. The mixture was vortexed for 0.5 min and centrifuged at 2200 *g* for 10 min at 4°. The supernatant, 50 μL, was mixed with 50 μL water. An aliquot (50 μL) of the mixture was injected into an HPLC chromatograph. The HPLC chromatograph (models L-6000 and L-6200, Hitachi, Tokyo, Japan) was equipped with a UV detector (SPD-10A, Shimadzu) and a 6 × 150 mm YMC packed column AM-312 ODS (YMC Co. Ltd., Kyoto, Japan). The column was developed using two buffers as the mobile phase: buffer A consisted of 50 mM phosphate buffer (pH 7.0):acetonitrile (90:10) containing 5 mM sodium 1-octanesulfonate, and buffer B consisted of 50 mM phosphate buffer (pH 7.0):acetonitrile (50:50) containing 5 mM sodium 1-octanesulfonate. A linear gradient of 100% buffer A to 70% buffer A/30% buffer B was run for 20 min, followed by a linear gradient to 100% buffer B over 1 min. The following linear gradients were then run in series (% buffer A/% buffer B): to 0%/100% over 5 min; 100%/0% over 1 min; 100%/0% over 10 min. The flow rate was 1 mL/min. The absorbance of MMC at 360 nm was measured, and the detection limit was 10 ng/mL plasma.

Calculation of E_H and CL_{tot}

CL_{tot} and E_H were calculated from equations 1 and 2:

$$CL_{tot} = I_o/C_{p,a} \quad (1)$$

$$E_H = (C_{p,a} - C_{H,V})/C_{p,a} \quad (2)$$

where I_o is the infusion dose rate of each drug, and $C_{p,a}$ and $C_{H,V}$ are the femoral arterial and hepatic venous plasma concentrations, respectively.

Statistical Analysis

All data are presented as means ± SD. Significant differences between E_H and CL_{tot} of FUra, tegafur, or MMC at each infusion rate were estimated by Scheffe's test with equal variances, a significant difference being recognized by Bartlett's test and ANOVA, respectively. A non-parametric Scheffe's test was used when an unequal variance was recognized by Bartlett's test. A significant difference between the E_H of FUra alone and the E_H of FUra in combination with uracil was estimated by Student's *t*-test. A significant difference between the E_H of FUra alone and the E_H of FUra in combination with IL-2 was estimated by Dunnett's test with equal variances, a significant difference being recognized by Bartlett's test and ANOVA, respectively. A significant difference between the E_H of MMC alone and the E_H of MMC in combination with IL-2 was estimated by Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

Dose Dependence of Hepatic Extraction of FUra

The E_H in rats was determined by using $C_{p,a}$ and $C_{H,V}$ at steady state after femoral intravenous infusion of FUra at 0.375, 0.75, 1.5, and 3 mg/kg/min. $C_{p,a}$, $C_{H,V}$, E_H, and CL_{tot} at steady state are shown in Table 1. The E_H of FUra at each infusion rate (0.375, 0.75, 1.5, and 3 mg/kg/min) was 0.750 ± 0.010 (mean ± SD, N = 3 or 4), 0.687 ± 0.116, 0.402 ± 0.084, and 0.225 ± 0.055, respectively. The E_H at an infusion rate of 3 mg/kg/min decreased significantly, compared with that at 0.375 mg/kg/min ($P < 0.05$). The CL_{tot} at each infusion rate was 65.4 ± 3.9, 48.5 ± 3.5, 34.6 ± 3.8, and 24.3 ± 5.1 mL/min/kg, decreasing, like the E_H, as the infusion rate increased.

Dose Dependence of Hepatic Extraction of Tegafur

The E_H of tegafur in rats at infusion rates of 0.577, 1.154, 2.308, and 4.616 mg/kg/min was determined by the same

TABLE 1. Hepatic extraction ratios (E_H) of FUra following intravenous infusion at various rates into rats

Infusion rate (mg/kg/min)	C_{ss}* (μg/mL)		E_H†	CL_{tot}‡ (mL/min/kg)
	Artery	Vein		
0.375	5.75 ± 0.34	1.44 ± 0.05	0.750 ± 0.010	65.4 ± 3.9
0.75	15.5 ± 1.1	4.86 ± 1.91	0.687 ± 0.116	48.5 ± 3.5§
1.5	43.8 ± 5.3	26.4 ± 5.7	0.402 ± 0.084	34.6 ± 3.8‖¶
3	126.8 ± 24.1	97.7 ± 17.2	0.225 ± 0.055**	24.3 ± 5.1‖††

Each value is the mean ± SD of 3 or 4 rats.

* Concentration at steady state.

† Hepatic extraction ratio.

‡ Systemic clearance.

§ Significantly different from the value of infusion rate 0.375 mg/kg/min ($P < 0.01$).

‖ Significantly different from the value of infusion rate 0.375 mg/kg/min ($P < 0.001$).

¶ Significantly different from the value of infusion rate 0.75 mg/kg/min ($P < 0.01$).

** Significantly different from the value of infusion rate 0.375 mg/kg/min ($P < 0.05$).

†† Significantly different from the value of infusion rate 0.75 mg/kg/min ($P < 0.001$).

method as that used for FUra. $C_{p,a}$, $C_{H,V}$, E_H, and CL_{tot} at steady state are shown in Table 2. The E_H of tegafur at each infusion rate, 0.577, 1.154, 2.308, and 4.616 mg/kg/min, was 0.076 ± 0.040 (N = 3), 0.042 ± 0.034, 0.103 ± 0.054, and 0.098 ± 0.041, respectively; these values were much lower than those for FUra. There was no significant difference between E_H values at each infusion rate ($P > 0.05$). CL_{tot} at each infusion rate was 13.0 ± 0.8, 13.1 ± 0.5, 12.2 ± 0.2, and 11.7 ± 1.6 mL/min/kg, and there was no significant difference between these values ($P > 0.05$).

Effect of Uracil on CL_{tot} and Hepatic Extraction of FUra and Tegafur

As shown in Table 3, the E_H of FUra at an infusion rate of 0.375 mg/kg/min combined with uracil, at an equal molar equivalent of FUra, 0.323 mg/kg/min, was 0.646 ± 0.022 (N = 3) and was significantly lower than that of FUra alone, 0.750 ± 0.010 ($P < 0.001$). The CL_{tot} of FUra combined with uracil was 45.3 ± 9.7 mL/min/kg and was significantly lower than that of FUra alone, 65.4 ± 3.9 mL/min/kg ($P < 0.05$). As shown in Table 3, no significant effects of uracil on the E_H and CL_{tot} of tegafur were found ($P > 0.05$).

Effect of IL-2 on CL_{tot} and Hepatic Extraction of FUra

The E_H of FUra alone was 0.750 ± 0.010 at an infusion rate of 0.375 mg/kg/min, while the E_H values of FUra combined with IL-2 (3000 and 7500 U/kg/min) were 0.761 ± 0.015 (N = 4) and 0.794 ± 0.022, respectively (Table 4). The E_H of FUra combined with IL-2, 7500 U/kg/min, increased significantly compared with the E_H of FUra alone ($P < 0.01$). As shown in Table 4, no significant effects of IL-2 on CL_{tot} were found at the tested infusion rates ($P > 0.05$).

Dose Dependence of Hepatic Extraction of MMC

The E_H of MMC in rats at infusion rates of 2.5, 7.5, and 25 μg/kg/min was determined by the same method as for FUra and tegafur. $C_{p,a}$, $C_{H,V}$, E_H, and CL_{tot} values at steady state are shown in Table 5. The E_H values of MMC at each infusion rate, 2.5, 7.5, and 25 μg/kg/min, were 0.332 ± 0.069 (mean ± SD, N = 3), 0.358 ± 0.018, and 0.360 ± 0.041, respectively. There was no significant difference between E_H at each infusion rate ($P > 0.05$). CL_{tot} at each infusion rate was 38.3 ± 5.3, 36.1 ± 1.3, and 35.3 ± 0.7 mL/min/kg, and there was no significant difference between them ($P > 0.05$).

Effect of IL-2 on CL_{tot} and Hepatic Extraction of MMC

We also studied the effect of IL-2 on the E_H of MMC (Table 6). The E_H of MMC alone was 0.358 ± 0.018 at an infusion rate of 7.5 μg/kg/min, while the E_H of MMC combined with IL-2, 7500 U/kg/min, was 0.405 ± 0.062. The E_H of MMC combined with IL-2 was higher than that of MMC alone, but the difference was not significant ($P > 0.05$). No significant effects of IL-2 on CL_{tot} were found ($P > 0.05$).

DISCUSSION

The CL_{tot} and E_H of FUra in rats decreased as the dose increased in the same way as described in previous reports on humans. FUra is subjected to metabolism by various enzymes: inactivation by catabolism and activation by anabolism [1–4, 33, 34]. Probably the decrease in E_H with increasing dose is due mainly to saturation of the metabolism by these enzymes. The mechanism of active transport is known to be present in the intestine and in some cultured mammalian cells [34–36]. The same mechanism may exist for uptake into liver, and the uptake may be saturated as the dose increases. The CL_{tot} of FUra at the infusion rate of 3 mg/kg/min was 24.3 mL/min/kg and the decrease was 41.1 mL/min/kg, compared with the CL_{tot} of 65.4 mL/min/kg at an infusion rate of 0.375 mg/kg/min. CL_H, the hepatic clearance, is a product of hepatic plasma flow (Q_H, 29.8 mL/min/kg [37]) and E_H. The calculated CL_H at 0.375 mg/kg/min was 22.4 mL/min/kg, and the decrease in CL_{tot}, 41.1 mL/min/kg, could not be explained by the decrease in CL_H. Actually, urinary excretion (renal clearance) and metabolism by organs other than the liver are reported as non-hepatic clearance [1, 3, 4, 34], and the saturation of these eliminating mechanisms could have been reached. Therefore, it is important to establish a suitable dose for efficient delivery to the hepatic tumor with minimal distribution into non-hepatic organs during hepatic arterial infusion of

TABLE 2. Hepatic extraction ratios (E_H) of tegafur following intravenous infusion at various rates into rats

Infusion rate (mg/kg/min)	C_{ss}* (μg/mL)		E_H†	CL_{tot}‡ (mL/min/kg)
	Artery	Vein		
0.577	44.4 ± 2.8	41.2 ± 1.4	0.076 ± 0.040	13.0 ± 0.8
1.154	88.2 ± 3.2	86.7 ± 7.9	0.042 ± 0.034	13.1 ± 0.5
2.308	189.9 ± 2.8	170.3 ± 11.3	0.103 ± 0.054	12.2 ± 0.2
4.616	397.7 ± 54.3	352.8 ± 27.4	0.098 ± 0.041	11.7 ± 1.6

Each value is the mean ± SD of 3 rats.
* Concentration at steady state.
† Hepatic extraction ratio.
‡ Systemic clearance.

TABLE 3. Hepatic extraction ratios (E_H) of FUra and tegafur with or without uracil following intravenous infusion into rats

Drugs (mg/kg/min)	C_{ss}* (μg/mL) Artery	Vein	E_H†	CL_{tot}‡ (mL/min/kg)
FUra	5.75 ± 0.34	1.44 ± 0.05	0.750 ± 0.010	65.4 ± 3.9
FUra + uracil	8.56 ± 2.03	3.04 ± 0.75	0.646 ± 0.022§	45.3 ± 9.7‖
Tegafur	44.4 ± 2.8	41.2 ± 1.4	0.076 ± 0.040	13.0 ± 0.8
Tegafur + uracil	48.7 ± 2.0	45.5 ± 2.4	0.077 ± 0.015	11.8 ± 0.5

Each value is the mean ± SD of 3 or 4 rats. Infusion rates of FUra and tegafur were 0.375 and 0.577 mg/kg/min, respectively. Infusion rate of uracil was 0.323 mg/kg/min.

* Concentration at steady state.

† Hepatic extraction ratio.

‡ Systemic clearance.

§ Significantly different from the value of FU alone ($P < 0.001$).

‖ Significantly different from the value of FU alone ($P < 0.05$).

FUra. FUra itself is essentially harmless to mammalian host and tumor cells [1, 3, 4, 33, 34], but FUra is known to be converted into active metabolites in tumor cells [3, 4, 38–40]. Detailed studies are needed involving the saturation mechanisms in tumor cells. Saturation of non-hepatic clearance of FUra was found, and, therefore, extraction and clearance in non-hepatic organs also should be considered in establishing the proper dose for the hepatic arterial infusion of FUra.

The E_H of tegafur, a pro-drug of FUra, was much lower than the E_H of FUra. Hence, its efficiency of delivery will not be high in the treatment of liver tumors.

In our study, the combination of FUra with uracil decreased the E_H of FUra, as expected. FUra is converted mainly to dihydro-5-fluorouracil by dihydrouracil dehydrogenase, followed by catabolism to 2-fluoro-β-alanine (2-FA) [1, 3, 4, 33, 34]. Uracil as well as FUra is a substrate for the enzyme and is thought to inhibit the catabolic pathway of FUra [1, 3, 4, 7, 34, 41, 42]. The decreased E_H could be due mainly to metabolic inhibition. A decreased CL_{tot} of FUra in combination with uracil was also found and contributed to inhibition of the enzyme in the liver and other organs. Actually, dihydrouracil dehydrogenase is markedly distributed into organs other than the liver in humans and rats [41, 43–45].

On the other hand, no significant effect of uracil on the E_H of tegafur was found. Tegafur is usually used clinically, as UFT, with an amount of uracil four times higher than that of tegafur (uracil:tegafur = 4:1). The ratio of uracil to tegafur in our studies may not have been sufficient to find the effect of uracil on the E_H of tegafur. However, uracil mainly inhibits the degradation of FUra, and the conversion of tegafur to FUra is not affected [46]. Hence, no effect of uracil on the E_H of tegafur itself may be found even if the dose of uracil is increased.

The E_H of FUra and MMC increased in the combination with IL-2, unlike uracil. The increased E_H of these anticancer drugs may partially contribute to the improved response rate of MF therapy (MMC plus FUra) [12, 14]. The augmentation of the effects of these cytotoxic agents, such as FUra, by IL-2 is generally thought to be synergistic due to different mechanisms of action [9, 13]. Both FUra and MMC inhibit host immunological status in spleen [28], although some conflicting reports have appeared [25, 27]. IL-2 may contribute to the immunological variation by these drugs.

Many mechanisms for the effects of IL-2 on combined chemotherapy have been proposed [9, 10, 12, 14, 21–26]. Pharmacokinetic interaction in combination therapy should, however, be considered [1, 4, 7]. It is important to consider drug–drug interactions in liver during hepatic arterial infusion. Metabolism, membrane permeability (influx

TABLE 4. Hepatic extraction ratios (E_H) of FUra with or without IL-2 following intravenous infusion into rats

IL-2 (U/kg/min)	C_{ss}* (μg/mL) Artery	Vein	E_H†	CL_{tot}‡ (mL/min/kg)
Control	5.75 ± 0.34	1.44 ± 0.05	0.750 ± 0.010	65.4 ± 3.9
3000	6.63 ± 0.86	1.58 ± 0.20	0.761 ± 0.015	57.3 ± 7.5
7500	5.87 ± 0.98	1.21 ± 0.23	0.794 ± 0.022§	65.1 ± 9.8

Each value is the mean ± SD of 4 rats. Infusion rate of FUra was 0.375 mg/kg/min.

* Concentration at steady state.

† Hepatic extraction ratio.

‡ Systemic clearance.

§ Significantly different from the value of FU alone ($P < 0.01$).

TABLE 5. Hepatic extraction ratios (E_H) of MMC following intravenous infusion at various rates into rats

Infusion rate (mg/kg/min)	C_{ss}* (μg/mL)		E_H†	CL_{tot}‡ (mL/min/kg)
	Artery	Vein		
2.5	66.1 ± 9.2	44.4 ± 9.5	0.332 ± 0.069	38.3 ± 5.3
7.5	208 ± 8	134 ± 9	0.358 ± 0.018	36.1 ± 1.3
25	708 ± 14	454 ± 36	0.360 ± 0.041	35.3 ± 0.7

Each value is the mean ± SD of 3 rats.
* Concentration at steady state.
† Hepatic extraction ratio.
‡ Systemic clearance.

and efflux), biliary excretion, and tissue distribution could be cited as interactions [1, 4, 7, 16, 47]. As described above, FUra is metabolized by various enzymes, and MMC is activated by various reductases [48–55]. The direct and/or indirect effects of IL-2 on metabolism by these enzymes should be considered. In this study, one reason for the increasing E_H of FUra and MMC in combination with IL-2 may be activation by these enzymes. However, IL-2 does not affect the cellular metabolism of thymidine [10]. In addition, IL-2 exerted an effect on the E_H of both FUra and MMC that was dose dependent and dose independent, respectively. It is suggested that the mechanism(s) of the increase of the E_H by IL-2 may be non-specific. Hence, the possibility of enzyme activation by IL-2 is unlikely.

One of the pharmacological effects of IL-2 is fluid retention followed by extravasation of intravascular fluid [23]. One possibility for the increase in the E_H of FUra/MMC by IL-2 may be the enhancement of membrane permeability and increased distribution volume of FUra/MMC. As shown by Sabo *et al.* [56], the majority of radioactivity 1 hr after intravenous administration of ^{14}C-labeled IL-2 to rats was located in the carcass (46%) and skin (15%). The severity of capillary or vascular leak syndrome is clearly dose dependent [23]. It is important to test the effect of IL-2 at higher doses than 7500 and 3000 U/kg/min, the doses used in our studies, to determine conclusively the effect of IL-2 on the E_H of FUra/MMC. However, as IL-2 is not on the market, we were unable to conduct studies at higher doses. Though the doses of 7500 and 3000 U/kg/min were comparable to clinical doses [12, 14], they were lower than the doses at which IL-2 induces the extravasation of ^{125}I-albumin in liver [23]. This may be the reason that the effect of IL-2 on the E_H of FUra/MMC in our studies was not clear.

Another reason may be decreased hepatic blood flow. Actually, fever and chills accompanied by a change in body temperature have been reported as side-effects of IL-2 in humans [57], and IL-2 may affect physiological factors, such as blood flow. Fluid retention, as described above, may cause a decrease in blood flow. In our study, hepatic blood flow was not determined. Hence, we do not know whether hepatic blood flow was changed. More detailed studies are needed to discover the exact mechanism for the increase in E_H by IL-2. Further studies on the effects of IL-2 on the distribution volume of a marker of extracellular space such as inulin are planned.

In our studies, the E_H of FUra and MMC in liver increased during combined administration with IL-2. It should be investigated, using tumor-bearing rats, whether the increased E_H truly contributes to the therapeutic benefits. In our studies, the extraction of these drugs in liver was focused, considering the difficulty in preparing the tumor-bearing rats. The study on the E_H of these drugs using tumor-bearing rats will be one of our future projects, if the model animals can be prepared in a more stable condition.

In conclusion, the E_H of FUra in rats decreased as the dose increased, in the same way as described in clinical reports. Uracil reduced the E_H of FUra, and IL-2 increased the E_H of FUra and MMC. The dose dependence of the E_H of FUra and the effects of uracil and IL-2 on the E_H of FUra

TABLE 6. Hepatic extraction ratios (E_H) of MMC with or without IL-2 following intravenous infusion into rats

IL-2 (U/kg/min)	C_{ss}* (μg/mL)		E_H†	CL_{tot}‡ (mL/min/kg)
	Artery	Vein		
None	208 ± 8	134 ± 9	0.358 ± 0.018	36.1 ± 1.3
7500	226 ± 21	135 ± 21	0.405 ± 0.062	33.4 ± 3.3

Each value is the mean ± SD of 3 rats. Infusion rate of MMC was 7.5 μg/kg/min.
* Concentration at steady state.
† Hepatic extraction ratio.
‡ Systemic clearance.

corresponded with clinical findings. These results suggest that this experimental model in rats may be useful for predicting the clinical pharmacokinetics and efficacy of FUra.

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