Effect of Age on the Disposition and Tissue Clearances of Fluorinated Pyrimidines in Rats

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Abstract: The age dependency of the elimination and tissue clearances of 5-fluorouracil (FU) and its nucleoside analog, 5'-deoxy-5-fluorouridine (dFUR), was investigated in 2 to 12 months old female Fischer rats. In all age groups, the blood clearances of dFUR at infusion rates of 500 and 750 mg kg^{-1} day^{-1} and of FU at 25 and 35 mg kg^{-1} day^{-1} were independent of the dose; however, the clearance of FU at a higher infusion rate of 50 mg kg^{-1} day^{-1} was significantly lower than at 25 mg kg^{-1} day^{-1}. An inverse relationship between animal age and clearance was observed for dFUR at both 500 and 750 mg kg^{-1} day^{-1} doses, and for FU at the 50 mg kg^{-1} day^{-1} dose. By contrast, the FU clearance at the 25 and 35 mg kg^{-1} day^{-1} doses was independent of age. To examine the age effect on the metabolic activities of major eliminating organs, the metabolism of dFUR by liver and small intestine in young and old rats was compared using 13,000 × g supernatant fractions of the tissue homogenates. Data were computer-fitted to the Michaelis-Menten equation. The Km for both tissues of both age groups was approximately 120 μg ml^{-1}. The intrinsic clearance (Vmax/Km) of dFUR was 5 ml kg^{-1} min^{-1} in the liver and 8 ml kg^{-1} min^{-1} in the intestine. The intestinal intrinsic clearance was independent of animal age, but the hepatic intrinsic clearance was significantly decreased in the older rats. The blood concentrations of FU derived as a metabolite from dFUR were also dependent on the animal age; an elevated FU concentration was associated with a lower dFUR metabolic clearance in the old rats. These data indicate that the elimination of FU and dFUR in rats is age-dependent, and that the systemic concentration of FU, a determinant of dFUR selectivity, is elevated in older animals.

5-Fluorouracil (FU) is used in the treatment of human malignancies including breast cancer, ovarian and colorectal carcinomas (1). The clinical toxicity of FU includes myelosuppression and gastrointestinal disturbances (1). One of its analogs, 5'-deoxy-5-fluorouridine (dFUR), is currently under clinical investigations in Europe and Japan. dFUR was introduced as a metabolic prodrug of FU (2); it is expected to have a greater selective toxicity to some tumors based on *in vitro* (3, 4) and animal (5, 6) metabolism studies. We have examined the mechanism of action and the biological activities of dFUR, and the target site specificity in the dFUR activation to FU (4, 7, 8). In these previous studies (8), we observed an age-related variability of the disposition of dFUR and of the blood concentrations of its cytotoxic metabolite FU. To define the cause of this variability, the effects of animal age on the disposition of FU and dFUR at therapeutic doses, and on the hepatic and intestinal intrinsic clearances of dFUR were studied.

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Materials and Methods

Chemicals

dFUR (MW 246.2) was a gift from the Hoffmann LaRoche Laboratory (Nutley, NJ). FU (MW 130.1) was purchased from Sigma Chemical Co. (St. Louis, MO). dFUR (lot number 7445-114) contained 0.01% of FU and 0.16% of 5-fluorouridine as impurities. All other chemicals or solvents were of analytical grade or spectroquality.

Animal Protocol

Female Fischer rats were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA and Kengston, NJ). A female rat reaches puberty at 1 to 1.5 month of age (9). The age of the animals used in this study ranged from 2 to 12 months, and their body weight ranged from 103 to 205 g. Animals were housed in metabolic cages and maintained at 12 h dark-light cycle. Details of the pharmacokinetic study were as described previously (8). Drug solutions were administered by continuous infusion into a tail vein catheter, and venous blood samples were withdrawn through an indwelling jugular vein catheter. In the present study, drug solutions were infused for the equivalent of at least 7 half-lives when drug concentrations are expected to be at 99% of the steady state concentration (10). Three to 4 blood samples were then collected while drug infusion was ongoing. The mean drug concentration was used as the steady state drug concentration. In our previous study where groups of animals (5–9 months old) were given various dosages, we observed a decrease in FU clearance when the dose was increased from 35 to 50 mg kg^{-1} day^{-1} (8). To examine the pharmacokinetics of FU and dFUR in the younger rats and to minimize the inter-animal variability, a group of 2–3 months old rats were infused with FU (25, 35, and 50 mg kg^{-1} day^{-1}) or dFUR (500 and 750 mg kg^{-1} day^{-1}). Drug dosages were escalated in succession, and animals were infused to a steady state at each dose level. Urine samples were collected throughout the infusion and for 24 h post-infusion periods.

Tissue Metabolism

Animals were fasted for 24 h prior to the studies. Rats from different age groups were used in a random fashion. In a cold room (4–6° C), a rat was decapitated and its liver (4 lobes) or small intestine (from pylorus to the distal end of ileum) were removed, rinsed and/or flushed with 1.15% KCl in 10 mM phosphate buffer, pH 7.4. The fatty tissues and/or the mesenteric attachments were carefully removed. Tissues were blot-dried and placed in a volume of 3 times the tissue weight of KCl/phosphate buffer, cut into fragments 3–5 mm in diameter or length, and homogenized using a motor- driven pestle tissue homogenizer. The homogenates were centrifuged using a Sorvall RC2B centrifuge at 4° C and 9,000 or 13,000 × g for

15 min. The supernatant fractions were centrifuged for an additional 15 min. The final supernatant preparation, obtained within 1 h after the animal was sacrificed, was free of tissue debris or fat and used as the 25 % enzyme solution. Dilutions to 12.5 and 5 % were made with the KCl/phosphate buffer. Hepatic or intestinal enzyme solution (13,000 × g supernatant fractions) was incubated with dFUR at 20 to 500 μg ml^{-1} concentrations and 2.5 mM $MgSO_4$ at 37° C under 95 % O_2 and 5 % CO_2 atmosphere. Hepatic enzyme solution (9,000 × g supernatant fractions) was incubated with FU at 20 to 200 μg ml^{-1} concentrations, 2.5 mM $MgSO_4$, 0.1 mM NADP, 10 mM nicotinamide, and 1.5 mM glucose-6-phosphate. Aliquots (200 μl) of the incubation mixture were taken at different time intervals. Proteins in the mixture were precipitated with 500 μl acetonitrile, and 5-bromouracil was added as the internal standard. The clear supernatant obtained after centrifugation at 13,000 × g for 1 min in a microcentrifuge (Fisher Scientific, St. Louis, MO) was transferred. The acetonitrile fraction was evaporated under a stream of nitrogen for 15–30 min, and the remaining aqueous fraction was analyzed by HPLC. The metabolic rates of FU and dFUR were determined from the decline in their concentrations during incubation. FU, the product of dFUR phosphorolysis, is further metabolized. We therefore monitored the disappearance rate of dFUR instead of the product formation rate.

Sample Analysis

The procedures used to quantitate FU and dFUR in blood and urine were as described previously (8). In brief, samples were extracted with ethyl acetate. 5-Bromouracil was used as the internal standard, and the concentrations of FU and dFUR in biological samples were analyzed by HPLC using an μBondapak C_{18} column (4 mm × 30 cm, Waters Assoc., Milford, MA). The HPLC mobile phase for the analysis of FU and dFUR in blood and urine samples, and of FU in liver homogenates was 2.5 mM ammonium acetate and 1.5 % methanol in water; the pH of the solution was adjusted to 5.0 with acetic acid. The flow rate was 3 ml/min, and UV detection was performed at 254 and 280 nm. The mobile phase for analysis of dFUR in the *in vitro* tissue metabolism studies was similar except that the methanol content was changed to 2.5 % for the intestine samples and to 5 % for the liver samples. Standard curves constructed with the peak height ratios of the drugs to 5-bromouracil were linear from 10 to 300 ng per sample for FU and from 0.2 to 20 μg per sample for dFUR.

Data Analysis

The blood clearances of FU and dFUR were calculated as (infusion rate) ÷ (steady state blood concentration) (10). The renal clearance was calculated as (blood clearance) × (fraction of dose excreted unchanged in urine). The Michaelis-Menten constants, Vmax and Km, of tissue metabolism of dFUR were estimated using the computer program HYPER which determines the best fit of the data to the Michaelis-Menten equation (11).

Results

Blood Clearance

The blood clearance values of FU at different dosages are presented in Table I. A group of 2–3 months old rats received infusions of FU with a step-wise increment of dosages. Groups of 5–9 months old rats were given FU at different dosages. In both age groups, the FU clearance at 50 mg kg^{-1} day^{-1} was significantly lower than that at 25 mg kg^{-1} day^{-1} dose. The age of the animals did not affect the FU clearance at the 25 and 35 mg kg^{-1} day^{-1} doses. However, at the 50 mg kg^{-1} day^{-1} dose, the old rats eliminated FU at a 15 % slower rate than the young rats.

The age dependency of dFUR clearance is shown in Table II. At both the 500 and 750 mg kg^{-1} day^{-1} doses, dFUR was eliminated at a significantly faster rate in 2–3 months old rats than in 5–9 months old rats. The correlations between dFUR clearance, animal body weight, and animal age are shown in Fig. 1. The average body weight of female Fischer rats increased 30 % between the ages of 2 and 3 months and then linearly from age 3 to 12 months (r^2 = 0.99). The dFUR clearance was inversely correlated with animal age and body weight, the r^2 of both regressed lines was 0.55 ($p < 0.001$).

Renal Excretion

The fraction of an FU dose excreted in the urine of 5–9 months old rats (n=5) at the 25 mg kg^{-1} day^{-1} dose was 6.5 ± 1.3 % which was not different from the 8.4 ± 4.0 % by 2–3 months old rats at the 35 mg kg^{-1} day^{-1} dose ($p > 0.5$, unpaired two-tailed t test). The fraction of a 500 mg kg^{-1} day^{-1} dose of dFUR excreted unchanged in urine and the renal clearance of dFUR was significantly higher in young rats than in old rats (Table II).

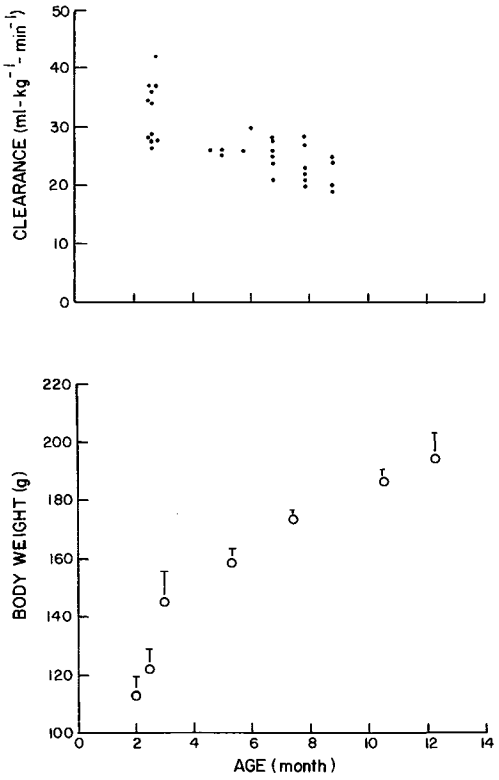

Fig. 1 Correlation of dFUR clearance with animal age and body weight. The coefficients of determination (r^2) of the least square regressed lines were 0.56 for clearance *vs* age and 0.55 for clearance *vs* body weight.

Steady State FU Concentration

The steady state FU concentrations attained during FU infusions at 25 and 35 mg kg^{-1} day^{-1} doses were independent of the animal age. By contrast, the concentration of FU derived as a metabolite of dFUR (500 mg kg^{-1} day^{-1}) in old rats was significantly higher than that in young rats (Table III).

Tissue Intrinsic Clearance

The nonenzymatic degradation rate of dFUR at an initial concentration of 50 μg ml^{-1} using boiled tissue homogenates was 0.026 μg min^{-1} ml^{-1}. An initial estimate of Km of approximately 100 μg ml^{-1} was obtained in a preliminary study using liver homogenates. The concentration of dFUR used in the subsequent experiments ranged from one-fifth to five-fold the estimated Km. Incubations were carried out using different substrate and enzyme concentrations, and for various time intervals up to 30 min. The rate of dFUR metabolism in liver enzyme preparations was linear with enzyme concentration of 5, 12.5, and 25 % (r^2 = 0.997). Decline in drug concentrations over time was linear ($r^2 > 0.9$), either as zero order or first order rate process (Fig. 2). The disappearance rates of dFUR and its corresponding concentrations at different time intervals were used to calculate the Vmax and Km of the metabolism of individual rats. For the small intestine, the dFUR disappearance rate was linear with the enzyme concentration of 5, 12.5, and 25 % (r^2 = 0.995). Unlike the metabolism by hepatic enzymes which was linear with time up to 30 min, the metabolism of dFUR by intestinal enzymes during the first 10 min of incubation was more rapid than that during the next 20 min. After 10 min, the rate of metabolism was linear with time

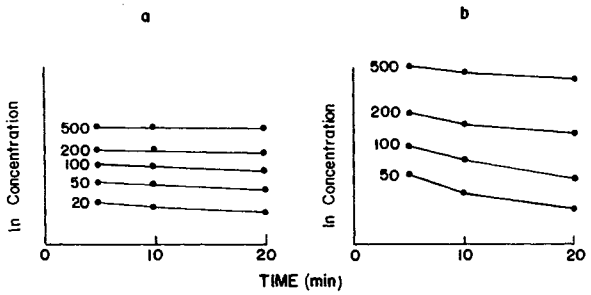

Fig. 2 Metabolism of dFUR by 25 % (w/v) of 13,000 × g supernatant of a rat (a) liver and (b) intestinal homogenates. The initial drug concentrations per ml of incubation (unit is μg ml^{-1}) are indicated on the corresponding lines.

Table I. Effect of age on the blood clearance (CL) of FU in rats. FU was administered by continuous infusion to reach and maintain a steady state concentration. CL was calculated as (infusion rate) ÷ (steady state blood concentration). Data are presented as mean ± S.D. with range and number of observations in parentheses.

Dose	CL (ml kg^{-1} min^{-1})		p^a
mg $kg^{-1}day^{-1}$	2.5–2.7 months	5–9 months	
25	142.0±13.1[b] (127.0–159.3, n=5)	128.9±15.7[c] (111.2–152.5, n=8)	>0.1
35	132.8±11.9 (118.1–145.6, n=5)	122.4±11.9 (110.3–141.8, n=5)	>0.2
50	126.5±8.7[b] (114.3–132.9, n=5)	107.9±16.5[c] (72.8–127.6, n=14)	= 0.05

[a] Unpaired two-tailed t-test.
[b] $p < 0.01$, paired two-tailed t-test.
[c] $p < 0.05$, unpaired two-tailed t-test.

Table II. Effect of age on the disposition of dFUR in rats. dFUR was administered by continuous infusion to reach and maintain a steady state concentration. The blood clearance (CL) was calculated as (infusion rate) ÷ (steady state blood concentration). Data are presented as mean ± S.D. with range of observations and number of animals in parentheses. Fe is the fraction of the dose (500 mg kg^{-1} day^{-1}) excreted unchanged in urine. Renal clearance (CL_R) is the product of (CL) and (Fe).

	Age (months) 2.5–2.7	5–9	p^a
Body weight, g	144.5±8.0 (128.5–158.0, n=11)	189.7±12.1 (106.0–203.0, n=10)	<0.001
CL, ml kg^{-1} min^{-1}			
500 mg kg^{-1} day^{-1}	33.0±5.5 (26.2–42.0, n=11)	22.8±3.1 (19.0–28.3, n=10)	<0.001
750 mg kg^{-1} day^{-1}	32.9±3.6 (27.1–35.8, n=5)	23.4±8.0 (20.7–29.7, n=11)	<0.05
Fe	0.33±0.11 (0.19–0.46, n=5)	0.22±0.07 (0.14–0.33, n=7)	<0.02
CL_R, ml kg^{-1} min^{-1}	10.89±3.33 (5.81–14.4, n=5)	5.83±3.82 (3.6–12.3, n=7)	<0.02

[a] Unpaired two-tailed t-test.

either as zero or first order rate processes ($r^2 > 0.94$) (Fig. 2). The cause of the nonlinearity between the first 10 min and the following 20 min was not apparent. The Vmax and Km of dFUR metabolism by intestinal homogenates were established using only the rates during the first 10 min at 50 to 500 μg ml^{-1} initial concentrations.

The Vmax, Km and intrinsic clearances of dFUR by the hepatic and intestinal tissues are presented in Table IV. the tissue intrinsic clearance, defined as Vmax/Km, gives a measure of the maximal drug clearance by the tissue (12). The standard error of the mean values (S.E.M.) of Km and Vmax, determined from the best-fit of the data to the Michaelis-Menten equation, ranged between 5 to 40 % but was less than 20 % in 51 out of 60 cases. The average S.E.M. of Km and Vmax were 25.0 ± 9.3 % (mean ± S.D.) and 9.5 ± 4.6 % for the hepatic tissue; and 16.9 ± 8.1 % and 12.5 ± 8.5 % for the intestinal tissue. The Km in both tissues was about 120 μg ml^{-1}, but the Vmax of the intestine was about twice that of the liver. Accordingly, the intestinal intrinsic clearance of dFUR was about twice that of the hepatic clearance. The hepatic intrinsic clearance in young rats was significantly higher than those in old rats if the data were normalized on a body weight basis, but there was no difference if the data were expressed per g tissue weight or per whole tissue. The intrinsic clearance of small intestines was not altered by age.

We were unable to accurately measure the *in vitro* metabolic rate of FU by tissue homogenates at an initial concentration of below 20 μg ml^{-1} due to its rapid and complete disappearance within 5 min. The metabolic rate of FU at higher concentration of 20 to 200 μg ml^{-1} after 1–5 min incubation was estimated

Table III. Effect of age on the steady state blood concentrations of FU during an FU infusion or an dFUR infusion. FU or dFUR was administered by continuous infusion to reach and maintain steady state concentrations. Data are presented as mean ± S.D. with range and number of observations in parentheses.

Dose mg $kg^{-1}day^{-1}$	Steady State FU Concentration (ng ml^{-1}) 2.5–2.7 months	6–9 months	p[a]
dFUR 500	153.0±24.0 (117.2–193.7, n=11)	201.0±34.7 (163.0–252,5, n=10)	<0.001
FU 25	123.7±9.5 (113.4–136.7, n=5)	136.4±15.9 (113.9–155.6, n=8)	>0.1
FU 35	197.2±25.4 (165.6–251.3, n=11)	200.0±18.2 (171.4–220.5, n=5)	>0.2

[a] Unpaired two-tailed t-test.

Table IV. Metabolism of dFUR by 13,000 × g supernatants of rat hepatic and intestinal tissue homogenates (25 % W/V). Vmax and Km were determined by computer fitting of the experimental data using the HYPER program. Vmax is the maximum rate of dFUR metabolism by 25 % tissue homogenates. Vmax/Km, the tissue intrinsic clearance, was standardized per g wet weight of tissue, or per kg of animal body weight. Data are presented as mean ± S.D. with ranges of observation in parentheses. The difference between different age groups was not significant unless noted otherwise.

	Liver		Intestine	
	2.4 months[a] (n=12)	8.5 months[b] (n=12)	2.5 months (n=5)	12 months (n=5)
Tissue Weight, g	3.4±0.2 (3.0–3.9)	4.4±0.6 (3.5–5.4)	3.4±0.2 (3.2–3.6)	3.9±0.6 (3.5–4.9)
Body Weight, g	123±16 (103–157)	177±17 (153–201)	141±4 (136–146)	192±10 (181–205)
Vmax μg min^{-1}	4.68±1.81 (2.37–8.44)	5.42±1.88 (2.76–9.72)	10.51±3.40 (9.60–14.30)	9.59±1.83 (7.80–12.54)
Km μg ml^{-1}	110.4±50.5 (38.4–213.5)	136.5±46.8 (71.3–224.1)	124.3±32.3 (94.7–164.0)	115.3±43.9 (70.3–172.4)
Vmax/Km, ml-min^{-1}				
per g tissue	0.18±0.04 (0.15–0.25)	0.16±0.03 (0.11–0.20)	0.33±0.06 (0.24–0.40)	0.36±0.09 (0.26–0.48)
per tissue	(0.63±0.17 (0.38–0.87)	0.71±0.13 (0.43–0.96)	1.13±0.24 (0.79–1.41)	1.36±0.33 (0.91–1.97)
per kg body weight	5.11±1.34[c] (3.83–7.27)	3.96±0.70[c] (2.70–5.05)	8.02±1.50 (5.81–9.89)	7.05±1.61 (5.06–10.21)

[a] Mean, the range is 2.0 to 3.0 months and the S.D. is 0.4 months.
[b] Mean, the range is 5.3 to 12.5 months and the S.D. is 2.9 months.
[c] $p < 0.05$, unpaired two-tailed t-test.

using five 12 months old rats. There was considerable data scattering, partly because of the rapid metabolism of FU which might have occurred during sampling. The disappearance rate of FU was plotted against incubation time of one to five min. Only the data which showed a linear disappearance rate over time ($r^2 > 0.9$) were used. Data were fitted to the Michaelis-Menten equation to solve for Km and Vmax. Estimates of Km and Vmax with standard errors of less than 30 % were included in the data analysis. Using these criteria, two different sets of Vmax and Km were found. In three rats, the Vmax, Km, and Vmax/Km of FU were 10.35 ± 2.28 μgmin^{-1}, 13.69 ± 5.53 μg ml^{-1}, and 87.82 ± 18.68 ml kg^{-1} min^{-1}, respectively. In the other two rats, they were 6.88 ± 0.33 μg min^{-1} (average ± range), 3.92 ± 0.28 μg ml^{-1}, and 191.3 ± 9.2 ml kg^{-1} min^{-1}, respectively. The determination of Km and Vmax was limited by the fact that the disappearance rate of FU at initial concentrations lower than that of Km cannot be accurately measured. The intrinsic clearance of FU by a perfused rat liver is currently under investigation.

Discussion

Effect of Age on dFUR and FU Clearances

Data comparing the total body clearance and tissue clearances in 2–3 and 5–12 months old rats consistently indicate a reduction of dFUR metabolism in older animals. However, the age effect on FU clearances is less well defined.

The total body clearance of dFUR at 500 and 750 mg kg^{-1} day^{-1} doses in the young and old rats was independent of the infusion rate, but its elimination expressed as clearance normalized for animal weight was 30–40 % more rapid in the young rats. The faster elimination of dFUR correlated with the higher renal and extrarenal clearances of dFUR in the young rats (10.9 and 22.1 ml kg^{-1} min^{-1}) as compared to those in the old rats (5.8 and 14.0 ml kg^{-1} min^{-1}). dFUR is mainly metabolized by phosphorylases which are most abundant in the liver and intestines (6). The intrinsic clearances and the effect of age were therefore studied in these two tissues *in vitro*. The hepatic intrinsic clearance (Vmax/Km) but not the intestinal clearance was affected by age. The Km of dFUR metabolism by either liver or intestinal tissues in different age groups was consistently approximately 120 μg ml^{-1}, which suggests that the substrate affinity of the phosphorylase did not vary between the two tissues nor with animal age. The higher Vmax in livers of young rats could be explained by the 10 % higher liver to body weight ratio in these rats compared to that of the old rats (Table IV).

The elimination of FU was described by dose dependent kinetics. In both age groups, the blood clearance of FU at 50 mg kg^{-1} day^{-1} was significantly less than that at 25 or 35 mg kg^{-1} day^{-1}. At the lower doses (25–35 mg kg^{-1} day^{-1}) the FU clearance tended to be decreased in old rats, but the difference between the two groups was not statistically significant. Only at the higher dose, the elimination of FU was significantly more rapid in the young rats.

Tissue Extraction

The *in vitro* rate of metabolism of FU by the liver was 10–40 fold higher than that of dFUR by liver or intestines. On the basis of the intrinsic clearances from *in vitro* experiments and tissue blood flow rates reported in the literature (14, 15), the tissue extraction ratio (ER) of these compounds, equal to the (intrinsic clearance) ÷ (the sum of intrinsic clearance and blood flow) (9, 12), can be estimated. With a hepatic and mesenteric blood flow of 50 to 100 ml kg^{-1} min^{-1}, the ER of dFUR is 0.04–0.09 for the liver and 0.07–0.14 for the intestines, and the ER of FU is 0.5–0.8 for the liver. It should be emphasized that the *in vitro* data with tissue homogenates may not accurately predict the intrinsic clearance in an intact organ. These data serve to establish the relative metabolism rates of FU and dFUR and show that the extraction ratio of FU is 10 fold that of dFUR. If a similar relationship exists *in vivo*, then FU and dFUR differ in their tissue extraction in that dFUR is a low extraction drug and FU a high extraction drug. FU has been characterized as a high extraction drug in the dog where an ER of 0.95 was found in perfused dog livers (16). The tissue clearance of a low extraction drug is governed by the tissue enzyme activity, and that of a high extraction drug by the tissue blood flow (12). The tissue enzyme activity would affect the clearance of a high extraction drug only when the concentration or amount of the drug presented to the tissue exceeds the tissue intrinsic clearance. It follows that for a low extraction drug, an age-related alteration in enzyme activity will change its total body clearance. For a high extraction drug, an age-related reduction in enzyme activity will result in a reduced total body clearance only at high drug concentrations or dosages. This may explain the more pronounced age effect on dFUR disposition compared to that of FU. We speculate that the dose-dependent age effect on FU elimination can be a result of a shift in the rate-limiting factor of drug tissue-clearance at increasing dosages. To establish the rate-limiting factors in tissue clearance of FU and dFUR, the extraction ratios and intrinsic clearances of these compounds are currently being examined in perfused rat livers.

Steady State FU Concentration

Compared to the old rats, the young rats had a higher extrarenal and liver clearance for dFUR; however, the concentration of the dFUR metabolite FU was lower in the young rats. The metabolism of dFUR to FU and to FU-derived metabolites can be viewed as a catenary reaction, i. e. dFUR → FU → FU metabolites. The steady state concentration of FU is equal to (the sum of the fraction of the dFUR dose that is converted to FU in a given organ) × (the availability of FU as it exists from the organ to the general circulation) ÷ (the blood clearance of FU) (17). dFUR is not known to have metabolites other than FU and FU-derived compounds, and is not expected to have a significant biliary excretion because its molecular weight is below that of compounds excreted in the bile (18). Thus its extrarenal clearance represents the metabolism to FU, which equals 67 % of the total clearance in the young rats and 78 % in the old rats. FU clearance in the concentration range of 130 to 200 ng ml^{-1} was slightly slower in old rats, but the difference is not statistically significant (Tables I and III). If we calculate from the above equation the FU availability using the two clearance values at 35 mg kg^{-1} day^{-1}, i. e. 132.8 ml kg^{-1} min^{-1} for young rats and 122.4 ml kg^{-1} min^{-1} for old rats, the FU availability is identical in both groups. However, if the clearance for both groups were equal, the FU availability in old rats is 16 % higher than that in young rats. To clarify this, experiments using perfused livers are underway to define the relationship between FU availability and the tissue clearance of FU prodrugs.

In conclusion, the age of an animal is a source of variability of the disposition of FU and dFUR, and the reductions in tissue and total body clearances of dFUR in older rats were associated with an increase of FU concentration. The cytotoxicity of

FU is not selective for the tumor tissues, and its host toxicity follows a very steep dose-toxicity relationship (1). The tissue selectivity of dFUR depends on the site of its activation and the subsequent fate of FU. When the FU concentration in blood is increased, the host tissue toxicity will be increased and the target specificity of dFUR will be diminished. The molar dosage of dFUR used clinically (19) is 20–30 fold higher than the maximally tolerated dose of FU (1). Thus, even a minor variability in the dFUR disposition may alter the systemic FU concentrations and therefore its selectivity and therapeutic efficacy. There is a growing interest in developing FU prodrugs with an improved selectivity (1). The kinetic relationship between the tissue clearances of a prodrug and the systemic availability of FU may serve as guidelines for future prodrug development.

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In Vitro Drug Absorption Models. I. Brush Border Membrane Vesicles, Isolated Mucosal Cells and Everted Intestinal Rings: Characterization and Salicylate Accumulation

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Abstract: Brush border membrane vesicles, isolated mucosal cells and everted rings from rat intestine were compared for their suitability for drug uptake studies. Vesicles from brush border membranes were judged to be metabolically and morphologically functional on the basis of biochemical and microscopic criteria. With the use of a collagenase-vascular-perfusion method, populations of villus, mid villus and crypt cells were separated. An alternative approach that is based on an EDTA-dissociation procedure afforded fractions enriched in villus and crypt cells. Although several enzymatic and metabolic activities of these two cell preparations were comparable, cell viability based on the Trypan Blue dye exclusion test, ultrastructural appearance and glucose uptake more closely conformed to *in vivo* values for cells isolated according to the EDTA-dissociation method. These cells were chosen as a model for drug transport investigation. The morphological and functional integrity of everted rings was verified by histological examination, extracellular space estimation and assessment of glucose transport ability. Sodium salicylate uptake studies using brush border membrane vesicles and isolated mucosal cells were highly variable, whereas everted segments exhibited good reproducibility in uptake experiments. Time dependence of salicylate uptake was demonstrated with membrane vesicles and everted rings. Time dependence was not observed in mucosal cell uptake studies, probably because of the time required to separate the cells from the incubation solution. Based on ease of preparation, technical aspects of *in vitro* incubation and reproducibility of results, everted intestinal rings were considered to be a good potential model for *in vivo* drug absorption. Brush border membrane vesicles were generally regarded as unacceptable because of variations after storage and between experiments. Isolated cells offered certain advantages, but the utility of cells as an *in vitro* model remains equivocal.

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