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First-pass Metabolism of 5-Fluorouracil in Rats

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Abstract

The first-pass metabolism of 5-fluorouracil has been investigated in rats to compare systemic bioavailability after administration by different routes, the bioavailability after intravenous bolus administration being defined as unity.

Bioavailability after oral administration (F_{po}) was compared with that after intra-intestinal administration into the closed loop (F_{loop}) in conscious rats. F_{po} was very low and variable ($0{\cdot}28 \pm 0{\cdot}30$, mean ± s.d.), in agreement with earlier studies in man, but comparable with F_{loop} ($0{\cdot}33 \pm 0{\cdot}05$), suggesting insignificant loss of 5-fluorouracil by degradation in the gastrointestinal lumen or by faecal excretion. The bioavailability after intraportal vein administration (F_{ipv}) was compared with F_{loop} in rats anaesthetized with pentobarbital, anaesthesia being used to maintain a stable portal drug infusion that mimics the sustained input of drug into the portal blood flow after intra-intestinal administration. F_{ipv} was smaller than unity ($0{\cdot}68 \pm 0{\cdot}03$), suggesting significant hepatic first-pass metabolism, but higher than F_{loop} ($0{\cdot}31 \pm 0{\cdot}10$), suggesting significant first-pass metabolism in the intestinal mucosa. The intestinal bioavailability for passage through the epithelial mucosa (F_i) was estimated, from the ratio of F_{loop} to F_{ipv}, to be 0·46.

The study revealed that both the liver and intestinal mucosa are responsible for the extensive first-pass metabolism of 5-fluorouracil after oral administration. This first-pass metabolism might be similar to that in man, in which the oral bioavailability is reportedly similar to that in the rats used in this study. The findings in this study should be of help in monitoring ways of improving oral 5-fluorouracil therapy.

5-Fluorouracil has been used as an anticancer agent for approximately 40 years, either alone or in combination with other drugs, for treatment of solid tumours such as breast and gastrointestinal cancers (Diasio & Harris 1989). To enhance the efficacy of the drug and to reduce its toxicity much attention has been devoted to the routes of administration of 5-fluorouracil—intravenous injection, intravenous infusion, intrahepatic arterial infusion, intraperitoneal administration, topical administration (Diasio & Harris 1989) and, more recently, subcutaneous administration (Borner et al 1993). Although oral administration has also been used for convenience, this route has the disadvantage that the oral bioavailability of 5-fluorouracil is low and erratic, making oral 5-fluorouracil therapy less efficient and more difficult to control (Christophidis et al 1978; Phillips et al 1980; Diasio & Harris 1989). Clarifying the mechanism behind this low and erratic bioavailability is a prerequisite for improving oral 5-fluorouracil therapy, for example by developing more efficient 5-fluorouracil delivery strategies. The intestinal transport of 5-fluorouracil is known to be partially mediated by pyrimidine carriers (Yuasa et al 1996a). Although this could be a source of dose-dependent variability in absorption, our recent study demonstrated that 5-fluorouracil is highly absorbable, in terms of its disappearance from the gastrointestinal lumen, irrespective of dose (Yuasa et al 1996b). This was attributed to good passive intestinal membrane permeability to 5-fluorouracil, which has a relatively high oil-to-water partition coefficient (0·1) for a hydrophilic drug (Buur et al 1990). Therefore, we assumed that the low and erratic bioavailability of 5-fluorouracil might be mainly a result of first-pass metabolism, which has generally been assumed to occur in the liver (Diasio & Harris 1989). Although dihydropyrimidine dehydrogenase, the major metabolizing enzyme of 5-fluorouracil, is present in the liver, significant levels

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have also been reported in the intestinal mucosa (Queener et al 1971; Naguib et al 1985). Therefore, to examine the potential involvement of the small intestine in first-pass 5-fluorouracil metabolism, we performed a comparative evaluation of the first-pass in-vivo extraction of 5-fluorouracil by the small intestine and liver in rats.

Materials and Methods

Materials

5-Fluorouracil (Wako, Osaka, Japan), 5-chlorouracil (Nacalai Tesque, Kyoto, Japan; internal standard for the HPLC determination of 5-fluorouracil) and pentobarbital sodium (Nembutal, Dainippon Pharmaceuticals, Osaka, Japan) were obtained commercially. Other reagents were of analytical or high-performance liquid chromatography (HPLC) grade and obtained commercially.

Animals

Male Wistar rats, 250–280 g, 8–9 weeks, were purchased from Nihon SLC (Hamamatsu, Japan) and fasted with free access to water for 18 h before the experiments.

Oral bioavailability in conscious rats

Under light ether anaesthesia, the rats were fitted with a cannula inserted into the right jugular vein for intravenous dosing and blood sampling. For bolus intravenous and oral administration, after regaining consciousness the rats were given 5-fluorouracil (25 mg) dissolved in saline (2·5 mL kg^{-1}). Intravenous dosing was completed by filling the cannula with saline containing 100 units mL^{-1} heparin. This procedure of filling the cannula with heparinized saline, which is also required for avoidance of contamination with residual 5-fluorouracil during subsequent blood sampling, was conducted after collection of every sample. For intra-intestinal administration the rats were given 5-fluorouracil (25 mg) dissolved in phosphate buffer (20·1 mM $Na_2HPO_4 \cdot 12H_2O$, 47·0 mM KH_2PO_4, 101·0 mM NaCl, pH 6·4; 4·2 mL kg^{-1}) into a closed loop of the mid-gut (approx. 10 cm) at a volume-to-length ratio of 0·1 mL cm^{-1} shortly (within 5 min) before regaining consciousness, as described elsewhere (Yuasa et al 1997). After administration of 5-fluorouracil each rat was maintained unrestrained in a metabolic cage and 0·25 mL venous blood was obtained periodically and transferred to a tube containing 5 units heparin and centrifuged (3000 rev min^{-1} for 10 min) to separate the plasma. Plasma samples were stored at −40°C until analysis by HPLC.

Intestinal and hepatic bioavailability in anaesthetized rats

The rats were anaesthetized with pentobarbital sodium (50 mg mL^{-1} kg^{-1}, i.p.), and the right jugular vein was cannulated for intravenous dosing and blood sampling. The abdomen was opened by midline incision and an injection needle (25 gauge × 0·5 in) connected to PE-50 polyethylene tubing was inserted into the portal vein and fixed with surgical adhesive (Aron Alpha; Sankyo, Tokyo, Japan). The intraportal infusion of 5-fluorouracil (25 mg; 2·5 mL kg^{-1}) in saline was conducted at an infusion rate of 0·42 mg; 0·042 mL min^{-1} kg^{-1} for 60 min and blood samples (0·25 mL) were obtained periodically. This infusion period mimics the approximate absorption period after intra-intestinal administration (discussed later). Intravenous and intra-intestinal administration were conducted in the same manner as in conscious rats, and saline was infused intraportally at a rate of 0·042 mL min^{-1} kg^{-1} for 60 min to maintain the rats under conditions similar to those in the intraportal administration experiments (sham operation for intraportal administration). The plasma samples were stored at −40°C until analysis by HPLC.

Analytical procedure

5-Fluorouracil concentrations in plasma were determined by HPLC with ultraviolet detection at 268 nm, with 5-chlorouracil as internal standard, as described elsewhere (Watanabe et al 1985).

Data analysis

The plasma concentration–time profiles for 5-fluorouracil were analysed by use of a one-compartment model and assuming first-order absorption for both oral and intra-intestinal administration. The plasma concentrations for intravenous bolus administration, intraportal infusion and first-order absorption are described by equations 1, 2 and 3, respectively.

$$C = (D/Vd)e^{-k_e t} \quad (1)$$

$$C = (FD/T_I)(1/[Vdk_e])(e^{-k_e t'} - e^{-k_e t}) \quad (2)$$

$$C = [(FDk_a')/Vd(k_a' - k_e)](e^{-k_e t} - e^{-k_a' t}) \quad (3)$$

where D, Vd and k_e are the dose, volume of distribution and elimination rate constant, respectively. T_I is the infusion time, where $t' = 0$ when $t < T_I$ and $t' = t - T_I$ when $t > T_I$. k_a' and F are the absorption rate constant and systemic availability, respectively. Vd and k_e were estimated by fitting equation 1 to profiles of C against t after intravenous administration, using the non-linear regression

program, Winnonlin (Scientific Consulting, Apex, NC). By use of the Vd and k_e values estimated for intravenous administration, profiles of C against t after intraportal infusion were fitted to equation 2 to estimate F (F_{ipv}), and those after oral and intra-intestinal administration were fitted to equation 3 for estimation of k_a' and F (F_{po} or F_{loop}). The elimination half-life ($t_{1/2} = \ln 2/k_e$), total clearance ($CL_{tot} = k_e Vd$) and the area under the plot of plasma concentration against time to infinite time ($AUC = (DF)/CL_{tot}$) were calculated from those parameters estimated by model fitting.

Statistical analysis

Levels of statistical significance were assessed by use of Student's *t*-test.

Results

Characterization of profiles of plasma concentration against time

Profiles of plasma concentration against time were successfully characterized by the one-compartment model, assuming first-order absorption for intra-intestinal and oral administration in each series of experiments in conscious (Figure 1 and Table 1) and anaesthetized (Figure 2 and Table 2) rats.

In conscious rats given 5-fluorouracil-free buffer in the intestinal loop (sham operation for intra-intestinal administration), the k_e and Vd for intravenous 5-fluorouracil were $0{\cdot}062 \pm 0{\cdot}014\ min^{-1}$ and $580 \pm 118\ mL\ kg^{-1}$, respectively (mean ± s.d., n = 3) and were not significantly different from those in rats without an intestinal loop (Table 1). In anaesthetized rats given 5-fluorouracil-free buffer in the intestinal loop (sham operation for intra-intestinal administration) and intraportal infusion of saline (sham operation for intraportal administration), the k_e and Vd for intravenous 5-fluorouracil were $0{\cdot}058 \pm 0{\cdot}030\ min^{-1}$ and $582 \pm 165\ mL\ kg^{-1}$, respectively (mean ± s.d., n = 3) and were not significantly different from those in rats without an intestinal loop (Table 2). This suggests that the operation for intra-intestinal administration does not affect the distribution and elimination kinetics of 5-fluorouracil in conscious and anaesthetized rats, and so k_e and Vd determined in rats without an intestinal loop were used in the model analyses of data not only for oral and intraportal dosing but also for intra-intestinal administration.

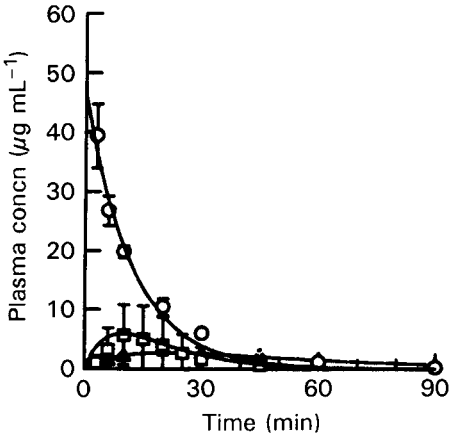

Figure 1. Plasma concentrations of 5-fluorouracil in conscious rats. Data are means ± s.d. 5-Fluorouracil ($25\ mg\ kg^{-1}$) was administered intravenously (○; n = 7), orally (□; n = 6) or into the intestinal loop (△; n = 3). The solid lines denote computer-fitted curves.

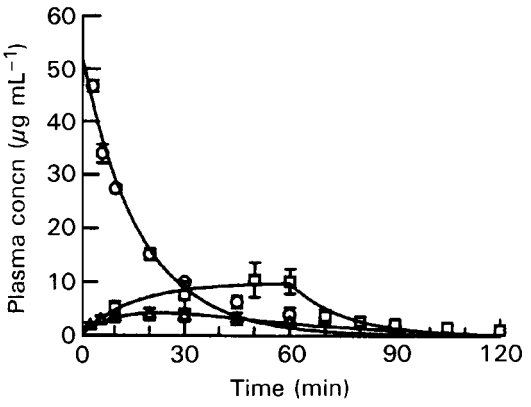

Figure 2. Plasma concentrations of 5-fluorouracil in anaesthetized rats. Data are means ± s.d. 5-Fluorouracil ($25\ mg\ kg^{-1}$) was administered intravenously (○; n = 3), intraportally (□; n = 4) or into the intestinal loop (△; n = 3). The solid lines denote computer-fitted curves.

Oral bioavailability in conscious rats

In conscious rats (Table 1) the oral bioavailability (F_{po}) was low and erratic (average 0·28; range 0·03–0·84), similar to that measured in previous studies with man (average 0·28; range 0–0·80) as reviewed by Diasio & Harris (1989). Therefore, in terms of apparent kinetic characteristics, the rat seems to be a good animal model for assessing the bioavailability of 5-fluorouracil.

In the closed intestinal loop where the luminal contents were replaced with buffer 5-fluorouracil cannot be excreted in faeces and is least likely to be degraded in the intestinal lumen. Therefore, complete absorption (entry into the intestinal mucosa) can be assumed in this model analysis extrapolated to infinite time, and the low systemic availability after intra-intestinal administration into the closed loop ($F_{loop} = 0{\cdot}33$) was most likely a result of first-pass metabolism after absorption.

Table 1. Pharmacokinetic parameters for 5-fluorouracil in conscious rats.

Parameter	Intravenous	Oral	Loop
Elimination rate constant (min^{-1})	0·083 ± 0·014	0·083*	0·083*
Absorption rate constant (min^{-1})	–	0·128 ± 0·036	0·023 ± 0·002†
Half-life (min)	8·4 ± 1·4	–	–
Volume of distribution ($mL\,kg^{-1}$)	532 ± 86	532*	532*
Total clearance ($mL\,min^{-1}\,kg^{-1}$)	43 ± 4	–	–
Bioavailability	1	0·28 ± 0·30‡	0·33 ± 0·05‡
Area under the plasma concentration–time plot ($\mu g\,min\,mL^{-1}$)	584 ± 54	164 ± 70‡	194 ± 32‡

Data are means ± s.d. 5-Fluorouracil ($25\,mg\,kg^{-1}$) was administered intravenously (n = 7), orally (n = 6) or into the intestinal loop (n = 3). *Parameters were fixed at those determined for intravenous data. † $P < 0·01$, significantly different from result for oral dosing. ‡ $P < 0·01$, significantly smaller than unity or the result for intravenous dosing.

Table 2. Pharmacokinetic parameters for 5-fluorouracil in anaesthetized rats.

Parameter	Intravenous	Intraportal	Loop
Elimination rate constant (min^{-1})	0·059 ± 0·004	0·059*	0·059*
Absorption rate constant (min^{-1})	–	–	0·022 ± 0·003
Half-life (min)	11·9 ± 0·8	–	–
Volume of distribution ($mL\,kg^{-1}$)	481 ± 14	481*	481*
Total clearance ($mL\,min^{-1}\,kg^{-1}$)	28 ± 1	–	–
Bioavailability	1	0·68 ± 0·03†	0·31 ± 0·10†‡
Area under the plasma concentration–time plot ($\mu g\,min\,mL^{-1}$)	891 ± 45	606 ± 106†	280 ± 86†‡

Data are means ± s.d. 5-Fluorouracil ($25\,mg\,kg^{-1}$) was administered intravenously (n = 3), intraportally (n = 4) or into the intestinal loop (n = 3). *Parameters were fixed at those determined for intravenous data. † $P < 0·01$, significantly smaller than unity or the result for intravenous dosing. ‡ $P < 0·01$, significantly different from the result for intraportal dosing.

That F_{po} was comparable with F_{loop} suggests that the loss of 5-fluorouracil by faecal excretion and luminal degradation is negligible after oral administration also, and F_{po} is mainly defined by its first-pass metabolic extraction after absorption. This is in agreement with our earlier suggestion after analysis of the disposition of 5-fluorouracil in the gastrointestinal tract (Yuasa et al 1996b).

Intestinal and hepatic availability in anaesthetized rats

To examine whether the small intestine is involved in the first-pass metabolism of 5-fluorouracil, it is necessary to evaluate the systemic availability after intraportal vein administration (F_{ipv}) to enable comparison with F_{loop}. In this study intraportal vein administration was achieved by constant-rate infusion to mimic the sustained input of the drug into the portal blood flow after intra-intestinal administration. To maintain stable portal infusion this series of experiments was performed in rats anaesthetized with pentobarbital and, to furnish similar conditions, saline was infused intraportally in the intravenous and intra-intestinal administration experiments. The infusion period was set at 60 min, twice the absorption half-life of 30 min (from a k_a' of $0·022\,min^{-1}$ in Table 2) or the time required for a major fraction (three quarters) of the dose to be absorbed.

As shown in Table 2, the F_{ipv} was smaller than unity, suggesting significant hepatic first-pass metabolism, but larger than F_{loop}, suggesting significant first-pass metabolism in the intestinal mucosa also. As discussed in the preceding section, complete absorption (entry into the intestinal mucosa) can be assumed after intra-intestinal administration and, hence, F_{loop} can be represented as the product of the hepatic availability (F_h), which can be assumed to be equivalent to F_{ipv}, and the intestinal availability for passage through the epithelial mucosa (F_i). Because F_{loop} is 0·31 and F_h (or F_{ipv}) is 0·68 for the availability after intraportal administration, $F_i(=F_{loop}/F_h)$ is 0·46. The values of extraction ratio (or the difference between unity and the respective bioavailability) are 0·32 and 0·54, respectively, for liver and intestine. Thus, in terms of the extraction ratio, intestinal extraction is comparable with, or more extensive than, hepatic extraction.

The intestinal extraction ratio of 0·54 is equivalent to the fraction extracted in the intestine after intra-intestinal administration, whereas the total

fraction extracted during the first passage through the intestine and liver after intra-intestinal administration is 0·69, the difference between unity and an F_{loop} of 0·31. This analysis suggests that intestinal extraction accounts for most (approximately 80%) of the total extraction.

Discussion

Although it has been reported that dihydropyrimidine dehydrogenase, the major metabolizing enzyme of 5-fluorouracil, is present in the liver, there are also significant levels in the intestinal mucosa (Queener et al 1971; Naguib et al 1985) and metabolic degradation of 5-fluorouracil occurs in the intestinal tissue (Smith et al 1988) and homogenates (Hamada et al 1996) in-vitro. Therefore, the finding that 5-fluorouracil undergoes first-pass extraction, probably by metabolic degradation, in the intestine is not very surprising. What is important is that in this study we evaluated the in-vivo intestinal first-pass extraction of 5-fluorouracil quantitatively and found that intestinal extraction might be more extensive than hepatic extraction. It is also important to note that first-pass metabolic extraction is suggested as being responsible for the low oral bioavailability of 5-fluorouracil.

According to a recent study in rats by Jarugula et al (1997) the disposition of 5-fluorouracil after intravenous administration remained linear when the dose was increased from 10 to 50 mg kg^{-1}, although there was a reduction in the total clearance when the dose was further increased to 100 mg kg^{-1}. In another recent study in rats, Fuse et al (1996) evaluated the hepato-splanchnic extraction (E_{hs}) of 5-fluorouracil by comparing the steady-state plasma concentrations in the systemic artery and hepatic vein achieved by constant-rate intravenous infusion. They found that the hepato-splanchnic extraction was almost constant (E_{hs} approximately 0·7) at systemic plasma concentrations below 15 μg mL^{-1} and saturable at concentrations above 40 μg mL^{-1}. Therefore, the dose of 25 mg kg^{-1} and systemic plasma concentrations below 50 μg mL^{-1} (mostly below 20 μg mL^{-1}) for the different dosage routes in the present study (Figures 1 and 2) fall within the range of doses and concentrations in which the distribution and elimination of 5-fluorouracil can be assumed to be linear, although the observed concentrations were spread over a relatively wide range. This supports the implicit and basic assumption of linearity in the current model analysis (equations 1–3), in which the elimination and distribution parameters (k_e and Vd) estimated for intravenous administration were used as fixed parameters in the data analyses for other administration pathways. Also from the study by Fuse et al (1996), assuming a blood-to-plasma concentration ratio (R_b) of unity and using the hepatic blood flow rate (Q_h) of approximately 55 mL kg^{-1} (Davies & Morris 1993), the hepato-splanchnic clearance ($CL_{hs} = Q_h E_{hs} R_b$) for the lower concentrations for which extraction is linear and E_{hs} is approximately 0·7 is calculated to be 38·5 mL min^{-1} kg^{-1}. This estimate of CL_{hs} is comparable with the non-renal clearance (approximately 30 mL min^{-1} kg^{-1}), which accounts for approximately 80% of total clearance in the study by Jarugula et al (1997). Therefore, we can also assume that 5-fluorouracil is mainly eliminated by metabolic degradation in the hepato-splanchnic region, and approximating the R_b to unity is reasonable.

For routes other than intravenous, portal plasma concentrations might be topically and temporarily higher than systemic concentrations because of additional input of the drug by portal infusion or intestinal absorption, potentially affecting availability estimates. However, assuming an R_b of unity, again, and using a portal blood flow rate (Q_p) of approximately 39 mL min^{-1} kg^{-1} (Davies & Morris 1993), the intraportal infusion rate (v_{ipv}) of 0·42 mg min^{-1} kg^{-1} gives an additional concentration (v_{ipv}/Q_p) of only approximately 11 μg mL^{-1}. The k_a' of approximately 0·02 min^{-1} (Tables 1 and 2) after intra-intestinal administration (25 mg kg^{-1}) gives a maximum absorption rate ($k_a'D$) of approximately 0·5 mg min^{-1} kg^{-1} and would give at most, even if there were no first-pass intestinal extraction, an additional concentration ($k_a'D/Q_p$) of only approximately 13 μg mL^{-1}. Although portal infusion lasted for 60 min and intestinal absorption from the loop needed to be sustained for approximately 60 min for approximately three quarters of the dose to be absorbed because of the flip-flop kinetics ($k_a < k_e$), adding approximately 10 μg mL^{-1} to the systemic concentrations below 10 μg mL^{-1} observed for portal and intestinal doses would not raise portal concentrations high enough (above 40 μg mL^{-1}) to saturate hepatic extraction. Thus, it still seems acceptable to assume linearity in hepatic extraction for portal and intestinal dosing and to assume that the bioavailability estimates are not biased by saturable extraction.

For oral dosing only a k_a' of approximately 0·128 min^{-1} (Table 1) initially gives the maximum absorption rate ($k_a'D$) of 3·2 mg min^{-1} and, if the intestinal availability (F_i) is approximately 50% according to results from anaesthetized rats, the additional concentration ($k_a'DF_i/Q_p$) could be as high as 41 μg mL^{-1}. Because k_a' was highly variable for oral dosing, in some rats with faster

absorption the hepatic extraction could be saturated to some extent, causing extensive variation in oral bioavailability (F_{po}). In an attempt to demonstrate saturability in hepatic extraction we were unable to detect hepatic first-pass extraction for intraportal bolus dosing (data not shown), where 5-fluorouracil concentrations in portal plasma could have been initially as high as $640\,\mu g\,mL^{-1}$. Although, theoretically, an increase in the average F_{po} might also be expected, the large amount of variation observed might have masked this. Because the disposition of 5-fluorouracil after oral administration conformed to ordinary kinetics with $k_a > k_e$, the problem possibly caused by increased and variable portal concentrations would only be associated with the initial absorption phase, without affecting the kinetics during the elimination phase. However, because intestinal extraction might play a greater role in total first-pass extraction than in hepatic extraction, variation in intestinal extraction might also be involved in the extensive variability in F_{po}. The potential saturability and variability of intestinal extraction might need to be investigated further to clarify the underlying mechanism.

Considering that the hepatic extraction ratio (E_h) of 0·32 in this study is probably not biased by saturation, the approximately 2-fold greater hepato-splanchnic extraction ratio (E_{hs}) of approximately 0·7 reported by Fuse et al (1996) suggests significant prehepatic splanchnic extraction of 5-fluorouracil, as found in dogs (Gustavsson et al 1979).

The CL_{tot} was approximately 30% smaller in rats anaesthetized with pentobarbital than in conscious rats and, accordingly, the AUC values were greater in anaesthetized rats for both intravenous and intra-intestinal dosing. Because plasma concentrations in anaesthetized rats, for which there was no portal saline infusion were almost identical with those in conscious rats (data not shown), the reduction in CL_{tot} was probably caused by intraportal saline infusion or associated procedure. It might have been specifically caused by a reduction in hepatic blood flow as a result of hydrodynamic interference caused by the infusion needle. As discussed above, it is suggested that the total clearance is mainly (80%) defined by hepato-splanchnic clearance, which would be mainly limited by blood flow, as implied by the relatively high extraction ratio of 0·7 (Fuse et al 1996). Therefore, the moderate reduction in CL_{tot} by approximately 30% might reflect a reduction in the hepato-splanchnic clearance brought about by reduction of the blood flow to a similar extent. If that is the mechanism, although hepatic extraction might be moderately altered, intestinal first-pass extraction in the absorption process would be least likely to be affected. Because it accounts for most (80%) of the total first-pass extraction after intra-intestinal administration, as suggested in anaesthetized rats, the presumed moderate change in hepatic extraction would have only a very small effect on systemic bioavailability. Thus, it seems reasonable that the bioavailabilities were comparable for intra-intestinal dosing in conscious and anaesthetized rats.

Although the intestinal absorption of 5-fluorouracil is known to be partially mediated by pyrimidine carriers, it has been suggested that carrier-mediated transport is saturated and its contribution is reduced to an insignificant level, compared with passive transport, at a dose of $6{\cdot}5\,mg\,kg^{-1}$ (Yuasa et al 1996b). Therefore, in the current study at a dose of $25\,mg\,kg^{-1}$ we can assume that 5-fluorouracil was absorbed predominantly by passive transport. Analysis of the gastrointestinal disposition (Yuasa et al 1996b) also suggested that gastrointestinal absorption of 5-fluorouracil might be limited by gastric emptying because the intestinal absorption rate constant (k_a) of $0{\cdot}55\,min^{-1}$ was much greater than the gastric emptying rate constant (k_g) of $0{\cdot}082\,min^{-1}$. The passive transport of 5-fluorouracil was assumed to be fast enough, probably because of its relatively high oil-to-water partition coefficient (0·1) for a hydrophilic drug (Buur et al 1990), for its oral absorption rate to be limited by the rate of gastric emptying. In agreement with the suggestion of gastric emptying-limited absorption, the k_a' after oral administration in this study was comparable with the k_g of $0{\cdot}082\,min^{-1}$ and, according to the suggestion, the variability in k_a', regarded as being at least partly responsible for the extensive variability of F_{po}, represents the variability in the rate of gastric emptying.

In intra-intestinal administration experiments, in which gastric emptying is not involved, k_a' is expected to represent k_a, which in turn represents the ratio of the membrane permeability clearance (CL_m) to the luminal volume (V). Consistently, the k_a' values of approximately $0{\cdot}02\,min^{-1}$ in both conscious and anaesthetized rats (Tables 1 and 2) are comparable with the k_a ($=CL_m/V$) of approximately $0{\cdot}031\,min^{-1}$ predicted from the CL_m of approximately $3\,\mu L\,min^{-1}\,cm^{-1}$ for passive transport (Yuasa et al 1996a) and the luminal volume of $100\,\mu L\,cm^{-1}$ in the current study. The result that k_a' values were comparable in conscious and anaesthetized rats is also in agreement with our earlier finding that pentobarbital does not significantly affect the intestinal transport of 5-fluorouracil (Yuasa et al 1995a). Consequently, the difference between the values of k_a, the specific

rate-constant for intestinal absorption, for oral ($0{\cdot}55\,min^{-1}$) and intra-intestinal (approximately $0{\cdot}02\,min^{-1}$) dosing would be even greater than that in the apparent values (k_a'). Although the reason for the difference in k_a values is not completely understood, the larger $k_a = (CL_m/V)$ in-vivo is at least partly attributable to smaller luminal volume in-vivo ($24\,\mu L\,cm^{-1}$; Yuasa et al 1995b) than in the loop ($100\,\mu L\,cm^{-1}$). The incomplete recovery of rats from the surgical procedure in the intra-intestinal administration experiments might also contribute to the difference in k_a values.

In summary, in rats it was found that 5-fluorouracil undergoes significant first-pass extraction, probably by metabolic degradation, not only in the liver but also in the intestinal mucosa. It is suggested that the first-pass metabolic extraction is mainly responsible for the low oral bioavailability of 5-fluorouracil. For various dosing routes most of the pharmacokinetic behaviour of 5-fluorouracil exhibited the same linear kinetics. However, for oral dosing it was suggested that faster and variable absorption, gastric emptying-limited in nature, might have initially led to higher and more variable portal plasma concentrations, causing saturation of hepatic extraction in some instances and, consequently, resulting in variable bioavailability. Considering that this study was conducted at a dose of $25\,mg\,kg^{-1}$, which is within the range of pharmacological doses ($10–25\,mg\,kg^{-1}$) used in man, and that oral bioavailability in rats was similar to that in man (Diasio & Harris 1989), the outcomes should be of some significance in providing basic information to help guide ways of improving oral 5-fluorouracil therapy.

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