Dose-Dependent Gastrointestinal Absorption of 5-Fluorouracil in Rats
in Vivo

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Dose-dependent gastrointestinal absorption of 5-fluorouracil (5-FU) was kinetically evaluated in rats in vivo
by analyzing gastrointestinal disposition after oral administration, where a linear model assuming first-order gastric
emptying followed by first-order intestinal absorption was fitted to remaining fraction versus time profiles for the
stomach and small intestine to estimate the rate constants of gastric emptying ($k_e$) and intestinal absorption ($k_i$).
With an increase in dose from 1.5 mmol/rat (low dose) to 15 mmol/rat (high dose), the $k_e$ decreased from 5.95 to
0.55 min$^{-1}$, suggesting the involvement of carrier-mediated transport. This study is the first to demonstrate the
dose-dependent gastrointestinal absorption of 5-FU in vivo, though it has long been suggested in situ and in vitro.
Meanwhile, at both the low and high doses, the $k_i$ values, which were unaffected by dose (0.069 and 0.082 min$^{-1}$,
respectively, for the low and high doses), were smaller than the $k_e$ values by an order of magnitude or more and
the recovery of 5-FU was negligible, compared with that of insulin (a nonabsorbable marker), in the most distal
segment of ileum. These results suggest that, regardless of dose, 5-FU is highly absorbable in a gastric emptying-
limited manner. Thus, well-publicized bioavailability problems (low and erratic) of 5-FU may be attributable to
extensive and variable first-pass metabolism rather than poor and variable gastrointestinal absorption.

Key words  intestinal absorption; 5-fluorouracil; dose dependency; rat; carrier-mediated transport; gastrointestinal
disposition analysis

5-Fluorouracil (5-FU) has been widely used in the treatment of solid tumors, such as breast and gastro-
testinal cancers, and is clinically available in oral dosage forms.$^{1}$ However, the bioavailability of orally admin-
istered 5-FU in humans is reportedly low and erratic (0—74%).$^{2}$ Carrier-mediated transport, which can cause
dose-dependent variability in gastrointestinal absorption (absorption rate constant and fraction absorbed), has long
been suggested to be involved in the intestinal absorption of 5-FU and suspected to be at least in part a source of
the problems.$^{3,12}$ However, this remains unconfirmed with little information about 5-FU absorption in vivo.

In an effort to determine the sources of the bioavailability problems of 5-FU, we kinetically evaluated the
dose-dependent gastrointestinal absorption of the drug in rats in vivo by gastrointestinal disposition analysis.$^{13}$

MATERIALS AND METHODS

Chemicals  $[^3]$H]-5-FU (555.0 GBq/mmol), $[^14]$C]-linulin (96.0 MBq/g) and Scintisol, a scintillation cocktail, were
purchased from Dupont-NEN Co. (Boston, MA, U.S.A.). Soluene-350, a tissue solubilizer, was purchased from
Packard Instrument Co. Inc. (Meriden, CT, U.S.A.). Unlabeled 5-FU (Wako Pure Chemical Industries, Ltd.,
Osaka, Japan) was commercially obtained. All other reagents were of analytical grade and commercially
obtained.

Dosing Solutions  The dosing solutions, containing 0.01 (low dose) or 10 (high dose) mM 5-FU with a trace
amount (404 kBq/0.728 nmol/ml) of $[^3]$H]-5-FU and a trace amount (33.7 kBq/0.4 mg/ml) of $[^14]$-linulin, a
nonabsorbable marker, were prepared in saline (0.9% NaCl solution).

Gastrointestinal Disposition Experiments  Male Wistar

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intestine \( (FR_{si}) \) as follows:

\[
FR_{si} = e^{-k_{si}t} \quad (1)
\]

\[
FR_{si} = (e^{-k_{si}t} - e^{-k_{pi}t})/(1-k_{si}/k_{pi}) \quad (2)
\]

Equations 1 and 2 were simultaneously fitted to \( FR_{si} \) and \( FR_{pi} \) data, which were corrected for (normalized by) the total fraction of inulin recovered from the gastrointestinal tract, for 5-FU to estimate \( k_{si} \) and \( k_{pi} \), using a nonlinear regression program, PCNONLIN (Scientific Consulting Inc., Apex, NC), and weighted according to the reciprocal of the variance.

Pharmacokinetic Analysis of Plasma Concentration Data

Male Wistar rats, weighing about 300 g and fasted overnight, were cannulated in the right jugular vein under light ether anesthesia. After regaining consciousness and allowing a recovery period of 1 h, each rat was orally (through a gastric tube) or intravenously (through the cannula) given a low dose (1.5 nmol/0.15 ml/rat) of \( ^{3}H \)-5-FU, and left free in a metabolic cage at an ambient temperature of 25°C; 100 μl of blood was taken periodically through the cannula and placed in a centrifuge tube containing 5 units of heparin and centrifuged for 3 min with a Microfuge E (Beckman Instruments, Inc., Palo Alto, CA, U.S.A.) to obtain plasma. The plasma (20 μl) was placed in a counting vial to which was added 3 ml Scintisol, to determine the radioactivity with a liquid scintillation counter (LSC-1000, Aloka Co., Tokyo, Japan).

Plasma concentration \( (C) \) versus time \( (t) \) profiles of 5-FU were analyzed by a one-compartment model with first-order absorption, where the plasma concentrations after oral and intravenous administration are described by Eqs. 3 and 4, respectively,

\[
C = A \cdot e^{-k_{a}t} \quad (3)
\]

\[
C = A \cdot F_{a} \cdot k_{a} \cdot (e^{-k_{a}t} - e^{-k_{a}t})/(k_{a} - k_{a}) \quad (4)
\]

where \( k_{a} \), \( k_{a} \) and \( F_{a} \) are the elimination rate constant, the apparent absorption rate constant and the fraction absorbed, respectively, and \( A \) is a constant. The values of \( A \) and \( k_{a} \) were estimated by fitting Eq. 3 to the concentration versus time profiles after intravenous administration using a nonlinear regression program, PCNONLIN. With the values of \( A \) and \( k_{a} \) fixed, the values of \( k_{a} \) and \( F_{a} \) were estimated by fitting Eq. 4 to the concentration versus time profiles after oral administration.

Gastric Absorption

Male Wistar rats, weighing about 300 g and fasted overnight, were anesthetized with urethane (1.25 g/kg, i.p.), and a low dose of 5-FU was administered to the stomach, which was ligated at the cardia and the pylorus. 5-FU remaining in the gastric contents was determined 60 min after administration as described previously.

Biliary Excretion

Male Wistar rats, weighing about 300 g and without fasting, were anesthetized with urethane (1.25 g/kg, i.p.). The common bile duct was cannulated with PE-10 tubing, and bile was collected for 60 min after injection (0.5 ml) of \( ^{3}H \)-5-FU solution (0.01 mm), which was prepared in phosphate buffer (20.1 mm Na₂HPO₄·12H₂O, 47.0 mm KH₂PO₄, 101.0 mm NaCl, pH 6.4) and added with \( ^{14}C \)-inulin as a nonabsorbable marker, into a 5-cm intestinal (midgut) loop. Fifty microliters of the bile sample was placed in a counting vial, to which 5 ml of Scintisol was added for radioactivity determination. At the end of experiments, 5-FU remaining in the intestinal lumen was also determined to evaluate 5-FU absorption (disappearance) from the loop.

Stability in the Gastrointestinal Contents

Male Wistar rats, weighing about 300 g and fasted overnight, were sacrificed by puncturing the heart under ether anesthesia. Gastric and midgut contents were collected and added with citrate buffer (30.0 mm HCl, 32.8 mm citric acid, 60.0 mm NaOH, 71.8 mm NaCl, pH 2.0) and phosphate buffer (pH 6.4), respectively, to make 20% homogenates. \( ^{3}H \)-5-FU solutions (0.001 mm or 555 kBq/ml) were also prepared in the buffers of pH 2.0 and 6.4. The experiments were initiated by adding 0.65 ml of a 5-FU solution to 0.65 ml of a homogenate in a centrifuge tube (0.5 μm \( ^{3}H \)-5-FU in 10% homogenate). After 60 min of incubation at 37°C, the mixture was centrifuged at 4°C and 15000 g for 10 min with a MRX-150 centrifuge (Tomy Seiko Co., Tokyo, Japan), and the supernatant was filtrated with a disposable filter (DISMIC-25CS 0.45 μm, ADVANTEC Co., Tokyo, Japan). The filtrate was analyzed with a HPLC system (LC-10A, Shimazu Co., Kyoto, Japan) equipped with a radio analyzer (RLC-700, Aloka Co., Tokyo, Japan) under previously reported conditions with slight modifications for the column (Wakopak, WakoSil 10C18-200 4.0 mm i.d. x 250 mm, Wako Pure Chemical Industries, Ltd., Tokyo, Japan) and injection volume (200 μl).

RESULTS

Gastrointestinal Distribution Profiles

The recovery of 5-FU was, regardless of dose and sampling time, comparable with that of inulin in the stomach, but far lower than that of inulin in the small intestine (Fig. 1). The gastric absorption of 5-FU was, as estimated in the closed stomach of rats under urethane anesthesia, 18 ± 2% (mean ± S.E.; n = 3) in 60 min, giving an absorption rate constant of 0.0034 ± 0.0004 min⁻¹, which was negligible compared with 20 to 30 times larger gastric emptying rate constants described later. 5-FU was quite stable in gastric and intestinal (midgut) contents, where the fractions recovered were 96 and 99%, respectively, after 60 min of incubation in 10% homogenate at the 5-FU concentration of 0.5 μM. These results suggest that 5-FU is, regardless of dose, rapidly absorbed in the small intestine without gastric absorption or degradation in the gastrointestinal tract. The total recovery of inulin from the stomach and small intestine was about 100% throughout the experimental period of 60 min, assuring that its distribution was restricted within the region of the gastrointestinal tract and transit from the small intestine to the large intestine can be neglected. It was also confirmed that the biliary excretion of 5-FU was negligible: only 0.37 ± 0.08% (mean ± S.E.; n = 3) of dose was excreted in 60 min after administration to the closed midgut loop, as estimated in rats under urethane anesthesia, where the absorption
from the loop was almost complete (98%). All these results meet the assumptions in the model analysis incorporated with only gastric emptying and intestinal absorption (Eqs. 1 and 2).

**Kinetic Analysis of Gastrointestinal Disposition**

The remaining fractions of 5-FU from all intestinal segments were summed for each time to obtain the total fraction of the drug remaining in the small intestine for model analysis. The profiles of remaining 5-FU versus time for stomach and small intestine were successfully described by the model (Eqs. 1 and 2) up to 20 min (Fig. 2), though the model appeared to fit the data somewhat poorly for the low dose. The parameters are summarized in Table 1. After 20 min, the remaining fractions of 5-FU in the small intestine were independent of dose and time, and could not be explained by the proposed model. Because the plasma concentrations of total radioactivity were also independent of time after 20 min as shown in Fig. 3 for the low dose, the luminal radioactivity may be equilibrated with the plasma radioactivity, representing not only 5-FU but also its metabolites (dihydro-5-fluorouracil, 5-fluorouridopropionic acid and α-fluoro-β-alanine).  

While the gastric emptying was not affected by dose, intestinal 5-FU absorption was reduced with dose, as reflected by larger fractions remaining in the small intestine and a smaller \( k_a \) value for the higher dose. Because the \( k_a \) value was associated with a large S.E. for the low dose, additional simulations were performed for \( k_a \) values of 1 and 10 min\(^{-1}\) to further confirm the dose dependency in \( k_a \). The data points for small intestine were within the range of the simulation lines for the \( k_a \) values of 1 and 10 min\(^{-1}\), suggesting that \( k_a \) for the low dose would not

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**Table 1. Kinetic Parameters of Gastrointestinal Disposition of 5-FU in Rats**

<table>
<thead>
<tr>
<th>Dose</th>
<th>( k_a ) ( (\min^{-1}) )</th>
<th>( k_i ) ( (\min^{-1}) )</th>
<th>( C_{L_{app}} ) ( (\muL/min/cm) )</th>
</tr>
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<tbody>
<tr>
<td>1.5 nmol/rat</td>
<td>0.069 ± 0.008</td>
<td>5.95 ± 2.53</td>
<td>143</td>
</tr>
<tr>
<td>15 μmol/rat</td>
<td>0.082 ± 0.010</td>
<td>0.55 ± 0.29</td>
<td>13</td>
</tr>
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</table>

Values of \( k_a \) (gastric emptying rate constant) and \( k_i \) (intestinal absorption rate constant) are represented as the computer-fitted parameter with S.E.; \( C_{L_{app}} \), apparent membrane permeability clearance as \( k_i \); \( V_{mu} \), where \( V_{mu} \) is the average intestinal lumen volume (24 μL/cm).
be smaller than 1 min⁻¹ and at least 2 times larger than that for the high dose (0.55 min⁻¹). This is the first demonstration of dose dependency in intestinal 5-FU absorption in vivo, though carrier-mediated transport has long been suggested in situ and in vitro.³⁻¹² The kinetic parameters of gastric emptying-limited absorption of 5-FU is gastric emptying-limited regardless of dose. It should also be noted that, for both the low and high doses, the recovery of 5-FU from the most distal segment of ileum was negligibly lower than that of inulin (a nonabsorbable marker), as observed at 40 and 60 min (Fig. 1). Thus 5-FU was strongly suggested to be rapidly and completely absorbed regardless of dose.

Pharmacokinetic Analysis of Plasma Concentration To further confirm the gastric emptying-limited absorption of 5-FU, the plasma concentrations of total radioactivity for the low dose were analyzed. Although these concentrations, presumably representing 5-FU and its metabolites,¹¹ were maintained at a quasi-steady state after 20 min for both intravenous and oral administration, the initial phase of concentrations up to 10 min was successfully described by the one-compartment model with first-order absorption (Eqs. 3 and 4). The kinetic parameters (mean ± S.E.; n = 3) were obtained as follows: A = 0.457 ± 0.033% of dose/ml, k₂₁ = 0.0649 ± 0.0031 min⁻¹, k₂ = 0.041 ± 0.012 min⁻¹ and Fₐ = 1.00 ± 0.0001. The k₂ₙ was comparable with the kₙ of 0.069 min⁻¹. Although k₂ₙ may be modified by the potential involvement of first-pass metabolism, the appearance rate of [³H]5-FU-derived radioactivity in plasma should not exceed the absorption (disappearance) rate of [³H]5-FU from the gastrointestinal lumen. Thus this result suggests that 5-FU absorption is rapid enough to be gastric emptying-limited.

DISCUSSION

With the estimate of the average intestinal lumen volume (Vₐᵥ), intestinal membrane permeability clearance (CL_app) can be estimated as the product of k₂ₙ and Vₐᵥ.¹³,¹⁶ Although dosing volume was larger for the high dose (1.5 ml/rat or 5 ml/kg) than the low dose (0.15 ml/rat or 0.5 ml/kg), our earlier studies showed that Vₐᵥ of 24 µl/cm in fasted rats is not affected by dosing volume up to 5 ml/kg. Using the predetermined Vₐᵥ value, CL_app values were estimated and listed in Table 1.

The intestinal carrier-mediated transport of 5-FU has been extensively characterized in situ and in vitro,³⁻¹² where, with the Michaelis constant of 20 to 100 µM, the membrane permeability clearance is maximized (30 to 70 µl/min/cm²) at concentrations below 10 µM with carrier-mediated transport predominant, and minimized (2 to 6 µl/min/cm²) at concentrations above 1 mM with passive transport predominant.¹¹,¹² On the other hand, 5-FU concentrations in the dosing solutions in this study were 0.01 and 10 mM, respectively, at the low and high doses, and can be lower in the intestinal lumen because of dilution by luminal fluid and absorption. For the low dose, luminal 5-FU concentrations should be lower than 10 µM, suggesting that carrier-mediated transport would be predominant. For the high dose, since 5 to 10% of dose (15 µmol/rat) was distributed in the duodenum to midgut region, of which luminal volume is about 1 ml,¹³ luminal 5-FU concentrations would be comparable with or higher than 1 mM, suggesting that passive transport would be predominant. Thus it seems reasonable that CL_app at the low dose is an order of magnitude larger than that at the high dose, in agreement with results in vitro and in situ. However, CL_app values in vivo are an order of magnitude larger than those for corresponding conditions in situ and in vitro (30 to 70 and 2 to 6 µl/min/cm², respectively, for low and high concentrations). This remains unexplained and is a subject for future investigation.

As suggested for D-xyllose absorption, which is intestinal absorption-limited,¹⁸,¹⁹ the rate and extent of intestinal absorption by passive transport is comparable between rats and humans. Gastric emptying rate constants reported in humans (0.02—0.2 min⁻¹)²⁰ are, although variable, also comparable with those in rats in this study (Table 1), suggesting that the two species are comparable for the rate limiting process in the gastrointestinal absorption (gastric emptying or intestinal absorption). Since 5-FU was suggested to be rapidly and completely absorbed even at the high dose, where passive transport is presumably predominant, the same is expected in humans at high doses and also at low doses, where its absorption can be more efficient. Thus, the present study successfully demonstrated dose dependency in intestinal 5-FU absorption in rats in vivo, but also suggested that the gastrointestinal 5-FU absorption can be practically independent of dose, being completely absorbed in a gastric emptying-limited manner in rats, and also in humans. The bioavailability problems (low and erratic) of 5-FU may be attributable to extensive and variable first-pass metabolism rather than to poor and variable absorption.

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