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Ⅱ．過去の研究歴

1983年8月-1990年7月　中国北京中日友好病院薬剤部薬剤師

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Ⅲ．過去の研究実績

1. 顧健：Acetylspiramycin錠剤の溶出率及び生物学的利用率に関する検討．第1回全国病院若い薬剤師優秀論文報告学術会議，1991年11月，北京．（二等賞）

2. 顧健，湯浅博昭，松田憲治，林弥生，渡辺淳：　ラットにおける5-フルオロウラシルの生物学的利用率の変動性に関する検討．第5回日本病院薬学会年会．1995年7月22日-23日，東京．

Ⅳ．本年度の研究業績

(1) 学会、研究会等においての口頭発表（学会名・内容）

顧健，湯浅博昭，渡辺淳：　ラット小腸における5-フルオロウラシルの初回通過代謝．日本薬学会第117年会，1997年3月26日-28日（予定）．

(2) 学会誌等に発表した論文　　無　・　(有)　（雑誌名・論文名）

Yuasa, H., Matsuda, K., Gu, J., Suzuki, E., Yokouchi, I. and Watanabe, J.: Dose-dependent gastrointestinal absorption of 5-fluorouracil in rats *in vivo*. *Biol. Pharm. Bull.*, 19, 1494 - 1498, 1996.

Ⅴ．今後の研究計画及び希望

5-フルオロウラシルの初回通過代謝における肝臓と小腸の相対的寄与および投与量依存性を明らかにし，生物学的利用率および薬効の変動との関係を探りたい．また，修士課程終了後，博士課程に進学し，さらに研究経験を積むと共に，先端的学問の修得に努めたい．

Ⅵ. 研　究　報　告（日本語、又は英語で書いて下さい。 2,000字程度で記載して下さい。）

5-フルオロウラシル(5-FU)は経口抗癌剤として利用されているが，生物学的利用率（消化管から血漿中への移行率）の低さと変動性に起因していると考えられる個体間および個体内での薬効の変動が問題とされている．生物学的利用率にこのような問題を生じる原因としては，(1)腸管からの吸収性（腸管膜透過性）の問題と(2)循環血に到達するまでの代謝分解（初回通過代謝）による消失の問題が考えられる．我々は，その原因を明らかにし，5-FUを用いた経口療法の改善のための基礎情報を得るために研究を進めてきている．

我々は，ラット腸管灌流法を用いた検討により，5-FUの腸管吸収は良好である可能性が高いことを既に報告している．本年度，in vivo実験法を用いてさらに詳細な検討を続けた結果，能動輸送の関与により若干の投与量依存性があるが，治療域を含む広い投与量範囲で5-FUの吸収は良好で，吸収率はほぼ100%に達することを示すことができた（*Biol. Pharm. Bull.*, 19, 1494 - 1498, 1996）．したがって，5-FUの生物学的利用率の問題は，主に，初回通過代謝の問題に起因しているものと考えられる．

一方，初回通過代謝の問題についても平行して検討を進めてきており，先の第5回病院薬学会年会（1995年7月22日-23日，東京）において，肝臓だけでなく腸管組織においても5-FUの代謝が起こっている可能性があることを指摘したが，本年度，腸管灌流法を用いた実験により腸管での代謝について検討した．実験にはWistar系雄性ラット（約260ｇ，非絶食）を用い，urethane麻酔下で，小腸中央部10 cmを0.15 ml/minの流速で一回灌流しながら腸管膜静脈血を採取した．また，採血による失血を補うため，大腿静脈より輸血した．定常状態での灌流液中及び血漿中5-FU濃度をHPLC法（UV検出）により測定し，吸収率（管腔からの消失率）と血中への出現率を求めた．その結果，腸管膜静脈血中には腸管腔から消失した5-FUの約50%しか出現しないことが明らかとなった．残りの約50%は腸管組織内で代謝により消失しているものと考えられる．現在，5-FUの腸管での代謝の濃度（投与量）依存性などについて，さらに詳細な検討を続けており，日本薬学会第117年会（1997年3月26日-28日）にて発表予定である．

以上のように，5-FUの腸管での利用率（代謝されずに通過する割合）は50%程度であることが明らかとなったが，生物学的利用率（循環血中への到達率）は25%程度であり，肝臓での初回通過代謝も無視できないと考えられる．今後，5-FUの初回通過代謝における肝臓と小腸の相対的寄与および投与量依存性等について検討し，生物学的利用率および薬効の変動との関係を明らかにしていくことが必要であると考えられる．

Ⅶ. 指導教官の意見

顧健さんは，学習，研究意欲が豊かであり，また非常に勤勉である．日本人の若い学生が研究室に現れない午前中の時間に、すでに実験を開始している姿もしばしば見られる．研究員時代から通算して3年近く当研究室で研究に従事しているが，最近は，日本語も格段に上達し，日本人学生ともよく協調，協力して，上記の研究報告にあるように，着実に成果を上げつつある．何より自分の実験のテーマをよく理解し，直接の指導している湯浅博昭助教授とよく打ち合わせを行っているのがよい結果を生んでいる原因であるとみられる．また，本年度は，貴協会からの助成が頂けたことも本人にとって大きな励みとなったものと思われる．今後も変わらず努力を重ねて研究を実らせると共に，将来，日中の友好，協力に貢献できる人材に育つことを期待する次第である．

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_g) and intestinal absorption (k_a). With an increase in dose from 1.5 nmol/rat (low dose) to 15 μmol/rat (high dose), the k_a 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_g 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_a values by an order of magnitude or more and the recovery of 5-FU was negligible, compared with that of inulin (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 gastrointestinal cancers, and is clinically available in oral dosage forms.[1] However, the bioavailability of orally administered 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]inulin (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}C]inulin, a nonabsorbable marker, were prepared in saline (0.9% NaCl solution).

Gastrointestinal Disposition Experiments Male Wistar rats, weighing about 300 g and fasted overnight, were given an oral dose of 1.5 nmol/0.15 ml/rat (low dose) or 15 μmol/1.5 ml/rat (high dose) of 5-FU using a gastric tube. The rats were then left free in a metabolic cage at the ambient temperature of 25 °C, and sacrificed at 5, 10, 20, 40, or 60 min after dosing by puncturing the heart under ether anesthesia to sample the gastrointestinal contents and tissues of stomach, duodenum and three equal lengths of small intestinal segments (jejunum, midgut and ileum) as described in our previous report.[13] After adding the appropriate amount of saline, the gastrointestinal contents and tissues were homogenized, and a portion of each homogenized sample was solubilized for the determination of radioactivity as also described previously,[13] using Soluene-350 (1 ml) as a tissue solubilizer and Scintisol EX-H (5 ml) as a scintillation cocktail.

The remaining fraction (*FR*) of 5-FU in the gastrointestinal tract of each segment was estimated to be the sum of that in the contents sample and that in the fluid adhering to the tissue. The volume of adherent fluid was estimated from the amount of inulin associated with the tissue.

As discussed earlier,[13] assuming that the apparent intestinal membrane permeability clearance (CL_{app}) and the average intestinal lumen volume for unit length (V_{av}) are constant along the small intestine, the intestinal absorption can be described as a first-order process with a rate constant of k_a. Therefore, further assuming that the gastric emptying is described by a first-order rate constant of k_g, and that the gastric absorption and the transfer from the small intestine to the large intestine are negligible, the same model equation as those for a linear compartment model consisting of the stomach and small intestine compartments can be used to describe the remaining fraction of dose in the stomach (FR_s) and small

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intestine (FR_{si}) as follows:

$$FR_s = e^{-k_g \cdot t} \quad (1)$$

$$FR_{si} = (e^{-k_a \cdot t} - e^{-k_g \cdot t})/(1 - k_a/k_g) \quad (2)$$

Equations 1 and 2 were simultaneously fitted to FR_s and FR_{si} data, which were corrected for (normalized by) the total fraction of inulin recovered from the gastrointestinal tract, for 5-FU to estimate k_a and k_g, 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,

$$C = A \cdot e^{-k_{el} \cdot t} \quad (3)$$

$$C = \frac{A \cdot F_a \cdot k'_a}{(k'_a - k_{el})} \cdot (e^{-k_{el} \cdot t} - e^{-k'_a \cdot t}) \quad (4)$$

where k_{el}, 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_{el} 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_{el} 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.[14]

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 $Na_2HPO_4 \cdot 12H_2O$, 47.0 mM KH_2PO_4, 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.[15,16]

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[17] with slight modifications for the column (Wakopak, Wakosil 10C18-200 4.0 mm i.d. × 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

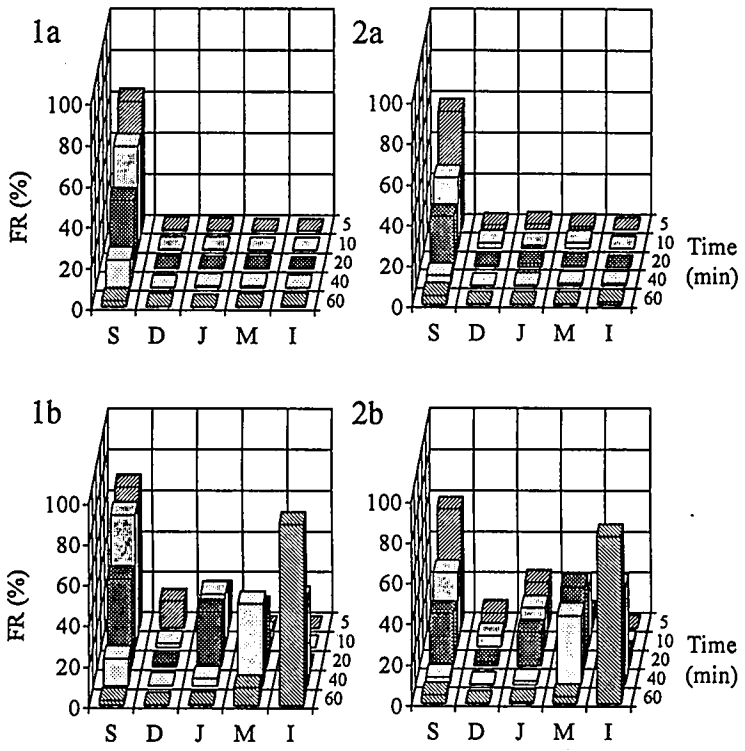

Fig. 1. Gastrointestinal Disposition of [^{3}H]5-FU and Coadministered [^{14}C]Inulin after Oral Administration of Varied Doses of 5-FU in Rats

Results are represented as the mean ($n=4$). Panels 1a (5-FU) and 1b (inulin) are for the low dose (1.5 nmol/rat), and panels 2a (5-FU) and 2b (inulin) are for the high dose (15 μmol/rat). Keys: S, stomach; D, duodenum; J, jejunum; M, midgut; I, ileum.

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-fluoroureidopropionic acid and α-fluoro-β-alanine).[1)]

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

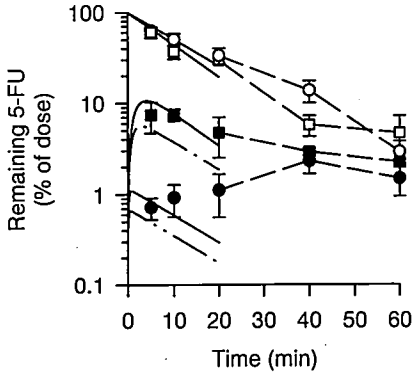

Fig. 2. Remaining [^{3}H]5-FU *versus* Time Profiles for the Stomach and Small Intestine after Oral Administration of Varied Doses of 5-FU in Rats

Results are represented as the mean±S.E. ($n=4$). The solid lines represent the computer-fitted profiles up to 20 min. For the data after 20 min, where the model was not applicable, each series of data were connected by broken lines for clarity. Keys: ○ (stomach) and ● (small intestine) for low dose (1.5 nmol/rat), and □ (stomach) and ■ (small intestine) for high dose (15 μmol/rat). The lines of —·— and —··— are simulation lines using k_a of 1 and 10 min^{-1}, respectively, for the low dose.

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

Dose	k_g (min^{-1})	k_a (min^{-1})	CL_{app} (μl/min/cm)
1.5 nmol/rat	0.069±0.008	5.95±27.53	143
15 μmol/rat	0.082±0.010	0.55± 0.29	13

Values of k_g (gastric emptying rate constant) and k_a (intestinal absorption rate constant) are represented as the computer-fitted parameter with S.E.; CL_{app}, apparent membrane permeability clearance as $k_a \cdot V_{av}$, where V_{av} is the average intestinal lumen volume (24 μl/cm).[13)]

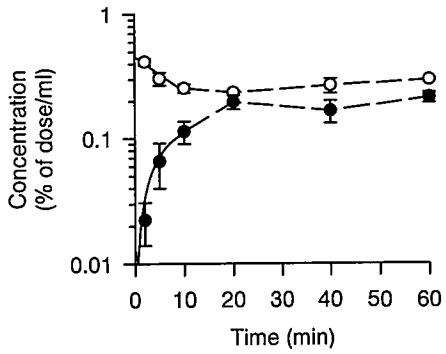

Fig. 3. Plasma Concentrations of Radioactivity after Intravenous and Oral Administration of [^{3}H]5-FU in Rats

Results are represented as the mean±S.E. (n=3). The solid lines represent the computer-fitted profiles. A low dose of 5-FU (1.5 nmol/rat) was administered intravenously (○) and orally (●).

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 k_a values were larger than k_g values by an order of magnitude or more at both the low and high doses, suggesting that the gastrointestinal 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 maker), 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,[1] 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, k_{el}=0.0649±0.0031 min^{-1}, k'_a=0.041±0.012 min^{-1} and F_a=1.00±0.0001. The k'_a was comparable with the k_g of 0.069 min^{-1}. Although k'_a may be modified by the potential involvement of first-pass metabolism, the appearance rate of [^{3}H]5-FU-derived radioactivity in plasma should not exceed the absorption (disappearance) rate of [^{3}H]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 (V_{av}), intestinal membrane permeability clearance (CL_{app}) can be estimated as the product of k_a and V_{av}.[13,18] 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 V_{av} of 24 μl/cm in fasted rats is not affected by dosing volume up to 5 ml/kg. Using the predetermined V_{av} 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/cm) at concentrations below 10 μM with carrier-mediated transport predominant, and minimized (2 to 6 μl/min/cm) 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/cm, respectively, for low and high concentrations). This remains unexplained and is a subject for future investigation.

As suggested for D-xylose 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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