First-Pass Metabolism of 5-Fluorouracil in the Perfused Rat Small Intestine

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The first-pass intestinal metabolism of 5-fluorouracil (5-FU) was investigated by single-pass perfusion of the rat small intestine. At the low concentration of 0.06 mg/ml, the fraction of 5-FU absorbed into (i.e., appeared in) the mesenteric venous blood (Fₐ), was about 50% smaller than the fraction absorbed (disappeared) from the intestinal lumen (Fₛ), indicating the first-pass extraction of 5-FU in the intestinal mucosa. By addition of uracil (6 mg/ml), the Fₛ of 5-FU was reduced presumably by competition for the pyrimidine carrier at the process of intestinal uptake (entry into the mucosa). The Fₛ was also reduced, but to a lesser extent, resulting in insignificant first-pass extraction. These results suggest that the extraction of 5-FU in the absence of uracil is caused by metabolism and that uracil is a competitor for this pathway. When 5-FU concentration was raised from 0.06 to 0.6 mg/ml in the absence of uracil, the Fₛ was reduced by about 50%, consistent with the suggestion of the involvement of saturable uptake by the pyrimidine carrier, and thereafter remained unchanged at 6 mg/ml. However, since Fₐ was also reduced by a similar extent, the intestinal availability (Fₛ/Fₛ) was unchanged at about 0.5, indicating that the intestinal first-pass extraction of 5-FU is independent of concentration with the extraction ratio (difference between unity and Fₛ) of about 0.5 over the wide range of concentration from 0.06 to 6 mg/ml. Thus, the present study demonstrates that the significant first-pass metabolic extraction of 5-FU occurs in the mucosa of the small intestine, supporting our previous suggestion that 5-FU undergoes first-pass metabolism not only in the liver but also in the small intestine after oral administration. Considering that the oral bioavailability of 5-FU in the human (28%) is reportedly comparable with that in the rat (28%), it is likely that intestinal first-pass metabolism may be significant also in the human. Intestinal first-pass metabolism should be taken into account to explore more efficient and controlled oral 5-FU therapy.

Key words 5-fluorouracil; first-pass metabolism; small intestine; perfusion; intestinal absorption; rat

The clinical bioavailability of 5-fluorouracil (5-FU) following oral administration to humans has been reported to be low and erratic,1,2 imposing difficulties in controlling oral 5-FU therapy. Poor gastrointestinal absorption and extensive hepatic first-pass metabolism have been suspected as the causes of the bioavailability problem in past reports.1,2 However, our recent study in the rat revealed that the absorption of 5-FU is, regardless of dose, rapid and complete,3 suggesting that first-pass metabolism may be responsible for the bioavailability problem. Furthermore, in our preceding study, where the availabilities into the systemic circulation were compared after 5-FU administration by venous, portal, intestinal (closed loop) and oral routes, not only the liver but also the intestinal mucosa was found to be significantly involved in the first-pass extraction.4

Dihydropyrimidine dehydrogenase (DPD), the major metabolizing enzyme for 5-FU, is reportedly present not only in the liver but also in the intestinal mucosa5,6 and the degradation of 5-FU was reported by in vitro studies using the intestinal homogenates of rats7 and the isolated intestinal tissues of rabbits.8 However, the intestinal first-pass metabolism of 5-FU has not been subjected to extensive investigation; for example, there is no detailed kinetic characterization of the absorption process. We, therefore, investigated the first-pass metabolic extraction of 5-FU in the perfused rat small intestine in situ, examining the effects of uracil, a potential competitive inhibitor, and 5-FU concentration.

MATERIALS AND METHODS

Chemicals 5-Fluorouracil (5-FU) and uracil were commercially obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan), FITC-dextran (MW, 16000), a nonabsorbable marker, was from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and 5-chlorouracil, an internal standard for the HPLC determination of 5-FU, was from Nacalai Tesque Inc. (Kyoto, Japan). All other reagents were of analytical or HPLC grade, and commercially obtained.

Animals Male Wistar rats, weighing 240—280 g, were purchased from Shizuoka Laboratory Animals Center (Hamamatsu, Japan) and fed with water ad libitum prior to experiments.

Blood to Plasma Partition of 5-FU After injecting 0.2 ml of heparin solution (1000 U/ml in saline) through the femoral vein to rats under ether anesthesia, blood was collected through carotid artery. To 1 ml of blood was added 5-FU (1 to 200 μg) with or without uracil (10 or 25 μg). The blood was then incubated at 37°C and 100 cycles/min for 30 min, and centrifuged at 3000 rpm for 15 min to separate plasma. 5-FU concentrations in plasma were determined by a HPLC method with UV detection at 268 nm as previously described,9 using 5-chlorouracil as an internal standard.

In Situ Perfusion Experiments In situ single-pass perfusion was conducted in rats anesthetized with urethane (4.5 ml/kg of 25% solution, intraperitoneally) as described previously,10 using a 10-cm midgut segment and a perfusion rate of 0.15 ml/min and collecting mesenteric venous blood with complementing blood by infusion (0.35 ml/min) through the femoral vein.

Perfusion solutions consisted of 20.1 mM Na₂HPO₄·12H₂O, 47.0 mM KH₂PO₄, and 101.0 mM NaCl (pH 6.4), and contained 5-FU at specified concentrations and FITC-dextran.

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(0.07 mg/ml) as a nonabsorbable marker. The outflow solution and the total mesenteric venous blood draining the perfused segment were collected for 30 min at 5-min intervals, starting at 15 min after the initiation of perfusion, by which time steady state was achieved.

The fraction of 5-FU absorbed (disappeared) from the intestinal lumen \( F_s \) was estimated by correcting for minor volume changes based on changes in FITC-dextran (nonabsorbable marker) concentrations. The fraction of 5-FU absorbed into (appeared in) mesenteric venous blood \( F_{ab} \) was estimated by the following equation:

\[
F_{ab} = \frac{R_b \cdot C_p \cdot Q_h}{C_n \cdot Q}
\]

where \( R_b \) is the blood to plasma concentration ratio; \( C_p \) and \( C_n \) are the concentrations of 5-FU in plasma and infusor solution, respectively; \( Q_h \) and \( Q \) are the mesenteric venous blood flow rate (0.35 ml/min as an average) and the perfusion rate (0.15 ml/min), respectively. The \( Q_h \) was estimated by dividing the volume of total mesenteric venous blood collected by the time of blood collection, where the blood volume was estimated from the blood weight, using unity as the approximate specific gravity of blood. The \( F_s \) and \( F_{ab} \) values were determined as the average of six sampling periods in each rat and then averaged for 3 or 6 animals.

**Statistical Analysis** Levels of statistical significance were assessed by analysis of variance.

**RESULTS AND DISCUSSION**

**Blood to Plasma Partition of 5-FU** To estimate the fraction absorbed into (i.e., appeared in) mesenteric venous blood \( F_{ab} \) from plasma concentrations using Eq. 1, it is necessary to determine the blood to plasma concentration ratio \( R_b \). As shown in Table 1, for the concentrations of 5-FU in blood ranging from 1 to 200 μg/ml, the concentrations of 5-FU in plasma were almost equal to those in blood, giving \( R_b \) values close to unity independent of concentration. This result was consistent with that by Au et al.,[1] who reported the \( R_b \) of 0.93 ± 0.13 in the concentration range of 0.1 to 100 μg/ml after the intravenous administration of 5-FU to rats.

The 5-FU concentration range of 1 to 200 μg/ml covers the following absorption experiments. At lower concentrations, where the effect of uracil on 5-FU absorption was examined, the \( R_b \) was not affected by uracil that was added at concentrations 10 times higher than those of 5-FU. Therefore, the \( R_b \) was assumed to be unity in data analysis in the following absorption experiments.

<table>
<thead>
<tr>
<th>Concentration in blood (μg/ml)</th>
<th>( R_b ) of 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU</td>
<td>Uracil</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2.5</td>
<td>25</td>
</tr>
</tbody>
</table>

Data are represented as the mean±S.D. (n=3).

**Intestinal First-Pass Metabolism of 5-FU** The highest inflow 5-FU concentration of 6 mg/ml in the present perfusion experiments was the concentration used in our preceding study to evaluate the intestinal availability in vivo.[4] In the preceding study, a pharmacological dose (25 mg/kg) orally used in the human was administered in the closed loop of the rat small intestine, and the intestinal availability \( F_s \) was estimated to be 0.46 by dividing the systemic availability after the intraintestinal administration (hepato-intestinal availability) by that after intraportal administration (hepatic availability). Because the luminal concentration decreases as absorption proceeds in the intestinal loop, 5-FU absorption was examined in the present study in a concentration range below 6 mg/ml down to 0.06 mg/ml.

As shown in Table 2, the fraction absorbed into the mesenteric venous blood \( F_{ab} \) of 0.131 was significantly lower than that which disappeared from the intestinal tract \( F_s \) of 0.294 when 5-FU concentration was 0.06 mg/ml. Because both ends of the perfused intestinal segment were cannulated for perfusion and isolated from the rest of the intestine and the total blood draining the perfused segment was collected, 5-FU could not be lost by leaking out. Because no metabolite was detected in the outflow solution, 5-FU was least likely degraded in the intestinal lumen. Therefore, the difference between \( F_s \) and \( F_{ab} \) was most likely caused by first-pass extraction in the intestinal mucosa. The \( F_{i} \) was calculated to be 0.451 as the ratio of \( F_{ab} \) to \( F_s \), and the extraction ratio (difference between unity and \( F_{i} \)) was 0.549.

If 5-FU is extracted in the intestinal mucosa by metabolic degradation, it may be competitively inhibited by uracil, an analog of 5-FU, as reported for the homogenates of the rat liver.[12] Therefore, we examined the effect of uracil on the intestinal extraction of 5-FU by adding uracil at the concentration of 0.6 mg/ml, which was 10 times higher than that of 5-FU (Table 2). By addition of uracil, the \( F_s \) of 5-FU was reduced, presumably, by competition for the pyrimidine carrier at the process of intestinal uptake (entry into the mucosa).[13] The \( F_{ab} \) was also reduced, but by a lesser extent, resulting in an insignificant extraction. This result suggests that the extraction of 5-FU in the absence of uracil was caused by metabolism in which uracil is a competitor. It should also be noted that the \( F_s \) value in the presence of uracil was not statistically different from those for higher 5-FU concentrations, where passive transport was presumed to be predominant as described later. Therefore, it seemed that uracil inhibited almost completely not only the metabolic degradation of 5-FU.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>( F_s )</th>
<th>( F_{ab} )</th>
<th>( F_{i} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>0.294±0.018</td>
<td>0.131±0.036</td>
<td>0.451±0.122</td>
</tr>
<tr>
<td>0.60</td>
<td>0.120±0.019a</td>
<td>0.047±0.011a</td>
<td>0.408±0.125</td>
</tr>
<tr>
<td>6.00</td>
<td>0.124±0.054a</td>
<td>0.060±0.031a</td>
<td>0.497±0.152</td>
</tr>
<tr>
<td>0.06</td>
<td>0.073±0.011a</td>
<td>0.065±0.012a</td>
<td>0.586±0.054b</td>
</tr>
</tbody>
</table>

Data are represented as the mean±S.D. (n=3 or 6). A 10-cm midgut segment was perfused at the perfusion rate of 0.15 ml/min. \( F_s \) fraction absorbed (disappeared) from the intestinal lumen; \( F_{ab} \) fraction absorbed into (i.e., appeared in) the mesenteric venous blood; \( F_{i} \), intestinal availability (\( F_{ab} / F_{s} \)).

a) \( p<0.05 \) compared with the value for 0.06 mg/ml 5-FU in the absence of uracil;

b) \( p<0.05 \) compared with the value for any concentration of 5-FU in the absence of uracil.
but also the carrier-mediated transport.

When the concentration was raised from 0.06 to 0.6 mg/ml, the $F_p$ was reduced from 0.294 to 0.120, consistent with the suggestion of the involvement of the saturable uptake by the pyrimidine carrier. When the concentration was further raised from 0.6 to 6 mg/ml, the $F_p$ of 5-FU remained unchanged. These results suggest that, in agreement with our earlier report,\(^1\) the carrier-mediated transport (uptake) that is predominant at lower concentrations such as 0.06 mg/ml is saturated and reduced to an insignificant level, compared with passive transport, at concentrations of about 0.6 mg/ml. However, since $F_{s_{ab}}$ was also reduced by a similar extent as $F_s$, $F_p$ (the ratio of $F_{s_{ab}}$ to $F_p$) was unchanged at about 0.5, indicating that the intestinal first-pass extraction of 5-FU is independent of concentration with the extraction ratio of about 0.5 over the wide range of concentrations from 0.06 to 6 mg/ml. Thus, it was suggested that about 50% of 5-FU that entered the intestinal mucosa is metabolized before reaching blood flow.

The concentration of 5-FU in the mesenteric venous plasma ranged from 3 to 150 µg/ml and 5-FU concentrations would not be lower in the intestinal mucosa during absorption. Because saturability was not detected in intestinal 5-FU extraction in that concentration range, the Michaelis constant ($K_m$) of intestinal 5-FU metabolism was presumed to be much higher than 150 µg/ml. However, for hepatic 5-FU extraction in rats, saturability was reportedly detected at systemic plasma concentrations of about 40 µg/ml and the $K_m$ of 5-FU degradation by dihydropyrimidine dehydrogenase (DPD) was 5.2 µg/ml in liver homogenates.\(^2\) Thus, the $K_m$ of 5-FU metabolism seems to be much higher in the intestinal mucosa than in the liver, suggesting that the enzyme responsible for intestinal 5-FU metabolism may be different from DPD in the liver. Although DPD is reportedly present not only in the liver but also in the intestinal mucosa,\(^3\) the kinetic characteristics, for example, $K_m$ of metabolism by intestinal DPD have not been thoroughly investigated. The metabolism of 5-FU in the intestinal mucosa should be the subject of a more detailed investigation in the future, including the possibility that multiple DPD isozymes may be involved.

Intestinal 5-FU extraction was linear up to 6 mg/ml on the basis of the concentration in the inflow perfusion solution; however, it was inhibited by uracil at an inflow concentration of only 0.60 mg/ml and, hence, it seems that uracil may have higher, by as much as more than an order of magnitude, affinity for the metabolizing enzyme than 5-FU. Also, for hepatic DPD, uracil reportedly has higher affinity than 5-FU, but only moderately by a factor of 4 in terms of $K_m$ determined in the homogenates.\(^2\) This issue of substrate specificity also needs to be addressed in the course of more detailed characterization of intestinal 5-FU metabolism in the future, considering substrate concentrations in the intestinal mucosa or the site of metabolic reaction. It should be noted here that since transcellular diffusion, rather than paracellular diffusion, has been suggested to be predominant in the passive transport of 5-FU\(^1\) and carrier-mediated transport can be assumed as transcellular transport by nature, it is reasonable to assume that 5-FU is almost fully exposed to cytosolic enzymes, such as DPD,\(^1\) during absorption.

The fraction absorbed in the perfused intestine is in general smaller than that expected in vivo after oral administration, mainly due to shorter transit time in the intestinal lumen: about 7 min in the present study, where a 10-cm segment with a luminal volume of about 1 ml was perfused at the rate of 0.15 ml/min.\(^1\) Versus about 70 min in rats in vivo.\(^1\) A series of our studies in rats suggested that 5-FU is almost completely absorbed, in terms of entry into the intestinal mucosa, after oral administration even at higher doses where passive transport is predominant,\(^3\) and the low oral bioavailability of 5-FU is mainly defined by first-pass extraction not only in the liver but also in the small intestine.\(^1\) The finding that the small intestine may also be involved in first-pass 5-FU extraction was further substantiated by the finding in the present study that the extraction ratio of about 0.5 in the perfused intestine was comparable with that in vivo (0.54),\(^2\) where luminal concentrations were presumed to be in a range covered by that in this study. Almost complete absorption of 5-FU can be expected also in the human after oral administration, as discussed in another report.\(^1\) Therefore, it is likely that the oral bioavailability in the human (28%),\(^1\) which is comparable with that in the rat (28%),\(^3\) would also be mainly defined by first-pass extraction with a significant contribution by the small intestine. The intestinal first-pass metabolism should be taken into account to explore more efficient and controlled oral 5-FU therapy.

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