RELATIONSHIPS AMONG PLASMA [2-13C]URACIL CONCENTRATIONS, BREATH 13CO2 EXPIRATION, AND DIHYDROPYRIMIDINE DEHYDROGENASE (DPD) ACTIVITY IN THE LIVER IN NORMAL AND DPD-DEFICIENT DOGS

Makoto Inada, Yukihiro Hirao, Toshihisa Koga, Minoru Itose, Jun-ichi Kunizaki, Takefumi Shimizu, and Hitoshi Sato

Research Section, Diagnostics Division, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan (M.In., M.It., J.K., T.S.); Department of Drug Metabolism, Drug Safety Research Center, Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan (Y.H., T.K., M.In., M.It.); and Department of Clinical and Molecular Pharmacokinetics/Pharmacodynamics, School of Pharmaceutical Sciences, Showa University, Tokyo, Japan (H.S.)

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ABSTRACT:
Dihydropyrimidine dehydrogenase (DPD), the first enzyme in the sequential metabolism of pyrimidine, regulates blood concentrations of 5-fluorouracil and is deeply involved in its toxicity. This study was designed to examine the effects of a DPD inhibitor on blood concentrations of [2-13C]uracil ([13C]uracil) and 13CO2 concentration (Δ13C) expired in breath after oral or intravenous administration of [13C]uracil to DPD-suppressed dogs prepared by pretreatment with 5-(trans-2-bromovinyl)uracil (BVU), a DPD inhibitor. Area under the curve (AUC) of [13C]uracil after oral administration at 20 μmol/kg to dogs pretreated with BVU at 2, 5, and 40 μmol/kg were 37-, 88- and 120-fold higher than those of the control dogs, respectively. In contrast, breath AUC values of Δ13C were reduced to 0.88-, 0.47- and 0.13-fold the control values, respectively. Upon intravenous administration of [13C]uracil at 20 μmol/kg to dogs pretreated with BVU at 0.5, 2, and 40 μmol/kg, blood AUC values of [13C]uracil were 1.4-, 4.2-, and 13-fold higher than those of the control group, respectively, whereas breath AUC values were reduced to 1.0-, 0.83-, and 0.07-fold the respective control values. DPD activities in the liver cytosol of dogs pretreated with BVU at 0.5, 2, 5, and 40 μmol/kg were decreased to 0.71-, 0.12-, 0.06-, and 0.04-fold those of the control dogs, respectively. These findings demonstrate that breath output (Δ13C) is a good marker of hepatic DPD activity in vivo.

5-Fluorouracil (5-FU) is a metabolic antagonist of pyrimidine that was first synthesized by Duschinsky et al. (1957). 5-FU and its prodrugs (tegafur, carmofur, doxifluridine, capecitabine, etc.) are anticancer agents that are widely used in the management of several common malignancies, including cancer of the colon, breast, and skin. Due to the structural similarity of 5-FU to the pyrimidine base uracil, its metabolism is identical to that of uracil. Thus, 5-FU is first converted by dihydropyrimidine dehydrogenase (DPD) to 5-fluorodihydrouracil, then by dihydropyrimidinase to α-fluoro-β-ureidopropionic acid, and finally by β-ureidopropionase to α-fluoro-β-alanine, ammonia, and carbon dioxide (Fig. 1). These final metabolites are excreted in urine and breath.

Administration of 5-FU or a prodrug to patients with abnormal pyrimidine metabolism such as DPD deficiency or concomitant use with a DPD inhibitor probably causes abnormally high blood concentrations of 5-FU, which result in serious adverse drug reactions (Lyss et al., 1993; Morrison et al., 1997). In a particularly notable example, the combined use of sorivudine (an antiviral drug) and 5-FU resulted in a large number of deaths in 1993 in Japan (Okada et al., 1997, 1998). The mechanism of this severe toxicity was attributed to the generation of 5-(trans-2-bromovinyl)uracil (BVU) from sorivudine by gut flora (Nakayama et al., 1997). BVU has strong DPD inhibitory activity. BVU, a structural analog of 5-FU, has been conclusively determined to inhibit DPD via an irreversible (i.e., covalent-binding) and mechanism-based inhibition (Desgranges et al., 1986; Nishiyama et al., 2000). Given this background, diagnostic methods able to predict and prevent adverse drug reactions to 5-FU in patients with pyrimidine metabolism disorders have been actively sought. Although several methods are now available, including quantification of urinary pyrimidine (Sumi et al., 1995, 1998), measurement of DPD activity in

ABBREVIATIONS: 5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; BVU, 5-(trans-2-bromovinyl)uracil; LC-MS/MS, liquid chromatography-tandem mass spectrometry; IRMS, isotope ratio-mass spectrometry; CV, coefficient of variation; AUC∞, area under the plasma concentration-time curve from time 0 to infinity.
enzymes and finally expired as 13CO2 (Fig. 1). In the present study, to examine relationships among the degree of DPD deficiency and uracil concentrations following oral or intravenous administration of [13C]uracil on i.v. administration. Values are mean ± S.D. (n = 3).

TABLE 1

Pharmacokinetic parameters of plasma-unchanged [13C]uracil following oral or intravenous administration of [13C]uracil to fasted male dogs

\[
\begin{array}{cccccccccc}
\text{Dose} & C_{\text{max}} & \text{AUC} & t_{\text{max}} & t_{1/2} & \text{CL/F} & \text{V/F} & F^* \\
\mu g/ml & \mu g \cdot h/ml & h & h & l/h/kg & l/kg & \% \\
10 \mu g/kg & 0.0301 \pm 0.0130 & 0.0617 \pm 0.00229 & \text{N.D.} & 0.278 \pm 0.192 & \text{N.D.} & \text{N.D.} & \text{N.D.} & - \\
20 \mu g/kg & 0.137 \pm 0.059 & 0.0309 \pm 0.0160 & 0.0518 & 0.223 \pm 0.096 & 0.145^* & 43.7^* & 9.11^* & 10.4 \\
40 \mu g/kg & 0.419 \pm 0.293 & 0.100 \pm 0.064 & 0.142 & 0.167 \pm 0.000 & 0.174^* & 33.7^* & 8.48^* & 14.3 \\
80 \mu g/kg & 4.45 \pm 1.47 & 1.39 \pm 0.52 & 1.39 \pm 0.52 & 0.222 \pm 0.096 & 0.144 \pm 0.044 & 7.25 \pm 3.14 & 1.41 \pm 0.33 & 69.9 \\
\end{array}
\]

N.D., not determined; —, not calculated.

* n = 1; \( b \) n = 2 (\( \lambda \) not estimated by linear regression in some cases).

TABLE 2

Pharmacokinetic parameters of plasma-unchanged [13C]uracil concentrations following oral or intravenous administration of [13C]uracil to fasted male dogs at 20 \( \mu g/kg \)

\[
\begin{array}{cccccccccc}
\text{Route} & C_{\text{max}} & \text{AUC} & t_{\text{max}} & t_{1/2} & \text{CL/F} & \text{V/F} & F \\
\mu g/ml & \mu g \cdot h/ml & h & h & l/h/kg & l/kg & \% \\
\text{Intravenous} & 5.47 \pm 1.56 & \text{N.D.} & 0.484 \pm 0.111 & 0.497 \pm 0.114 & \text{N.D.} & 0.101 \pm 0.010 & 4.78 \pm 1.19 & 0.696 \pm 0.190 & - \\
\text{Oral} & 0.228 \pm 0.078 & 0.0511 \pm 0.0218 & 0.0504 \pm 0.0126^* & 0.333 \pm 0.258 & 0.0857 \pm 0.0176^* & - & - & 10.8 \pm 3.7^* \\
\end{array}
\]

N.D., not determined; —, not calculated.

* n = 5 (\( \lambda \) not estimated by linear regression in one case).

peripheral monocytes (Fleming et al., 1992; Johnson et al., 1997), and evaluation of DPD genotype (Ridge et al., 1998; Collie-Duguid et al., 2000), none are considered highly reliable.

[2-13C]Uracil ([13C]uracil), whose C-2 position can be labeled with a stable isotope, 13C, is metabolized by pyrimidine-metabolizing enzymes and finally expired as 13CO2 (Fig. 1). In the present study, to examine relationships among the degree of DPD deficiency and uracil concentrations in blood and breath output, we administered [13C]uracil to DPD-suppressed beagle dogs prepared by preadministration of BVU (nominated as DPD-deficient model) and measured DPD activities in the liver cytosol, 13CO2 concentrations (\( \Delta^{13}C \)) in the breath, and blood concentrations of [13C]uracil.

Materials and Methods

[2-13C]Uracil and [2,4,5,6,13C]Uracil were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA), and BVU was purchased from Sigma-Aldrich (St. Louis, MO). [6-14C]5-Fluorouracil was obtained from Moravek Biochemicals (Brea, CA). All other reagents were of analytical grade.

Study Animals. Male beagle dogs purchased from Nosan Corporation (Yokohama, Japan) were 7 to 16 months old and weighed 9.0 to 13.2 kg at the time of administration. The animals were fasted overnight before use.

Administration of [13C]Uracil. [13C]Uracil was administered by oral gavage to three dogs each at the doses of 10, 20, 40, and 80 \( \mu g/kg \). An additional six dogs were intravenously or orally administered [13C]uracil at 20 \( \mu g/kg \) in a crossover design.

Blood Sampling and Plasma Separation. Approximately 2.5 ml of blood was collected from each animal using a heparinized vacuum blood-sampling tube before dosing and at 10, 20, 30, 40, 50, 60, 90, 120, 180, and 240 min after oral administration and before dosing and at 2, 5, 10, 20, 30, 60, 90, 120, 180, and 240 min after intravenous administration. Immediately after collection, plasma was separated by centrifugation at 3500 rpm at 4°C for 15 min and kept frozen at −80°C until measurement of [13C]uracil by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Breath Sampling. Approximately 300 ml of breath was collected in a vinyl bag before and at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 210, and 240 min after administration using a valve-equipped breath collection device that allows one-way breath inflow only. Approximately 200 ml of the collected breath was withdrawn from the bag with a syringe and dispensed into breath-collecting bags, which were kept at room temperature until the measurement of 13CO2 by gas chromatography/isotope ratio-mass spectrometry (IRMS).

Effects of DPD Inhibitor on the Pharmacokinetics of [13C]Uracil. [13C]Uracil at 20 \( \mu g/kg \) was orally or intravenously administered to three beagle dogs, each at 1 h after oral administration of BVU at 2, 5, and 40 \( \mu g/kg \) or 0.5, 2, and 40 \( \mu g/kg \), respectively. Plasma and breath were then collected at the sampling times above. In addition, at 1 h after oral administration of BVU at 0.5, 2, 5, and 40 \( \mu g/kg \), the livers were excised, and cytosol was prepared (\( n = 3 \)) by the method described below.

Preparation of Liver Cytosol. The animals were killed by bleeding under ketamin/xylazine anesthesia 1 h after BVU administration, and the liver was perfused with physiological saline, removed, and homogenized in 3 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA and 0.5 mM dithiothreitol with a Polytron homogenizer followed by stirring. A portion (10 ml) of the homogenate was further homogenized with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 105,000g for 60 min at 4°C to obtain supernatant (cytosol). The cytosol was applied to a MicroSpin G-25 column (Amersham Biosciences Inc., Piscataway, NJ) to remove endogenous substances. The eluate was collected as the enzyme source, rapidly frozen in liquid nitrogen, and stored at −80°C until activity measurement.
Sample Analysis. Determination of $^{13}$C-uracil in plasma by LC-MS/MS. Plasma concentrations of $^{13}$C-uracil were measured by LC-MS/MS (TSQ7000; Thermo Finnigan, San Jose, CA) using isotope-labeled uracil ($^{13}$C$_4$, 15N$_2$) as internal standard. After 0.25 ml of a saturated aqueous ammonium sulfate solution was added to 0.25 ml of plasma, $^{13}$C-uracil was extracted with 3 ml of acetonitrile followed by centrifugation at 1800g for 10 min. After the organic layer was evaporated to dryness and the residue was reconstituted in 200 µl of purified water, 50 µl of the resulting solution was injected into the LC-MS/MS, and the analytes were separated by methanol/water (1:99 v/v) using a Develosil RPAQUEOUS column (5 µm, 2.0 mm × 150 mm; Nomura Chemical Co., Ltd., Seto, Japan). The protonated molecular ions [M + 1]$^+$ of the analytes and the internal standard, formed by atmospheric pressure chemical ionization, were fragmented, and the selected product ions were monitored (selected reaction monitoring) at a linear concentration range of 10 to 500 ng/ml. The limit of quantification, defined as the lowest concentration determined with a coefficient of variation (CV) of <20% and accuracy within 20%, was 10 ng/ml. Precision (expressed as CV%) and accuracy (expressed as bias%) were <15% and within 15%, respectively, for all analyte concentrations except the limit of quantification.

Analysis of $^{13}$CO$_2$ in breath by gas chromatography IRMS. Concentration of $^{13}$CO$_2$ in the breath was determined with a gas chromatograph IRMS (ABCA-G; PDZ Europa Ltd., Cheshire, UK). $^{13}$CO$_2$/$^{12}$CO$_2$ ratios were expressed as the $^{13}$C value (permil, ‰) relative to the Pee Dee Belemnite limestone standard, and changes in $^{13}$C value as $\Delta^{13}$C (‰) were compared with the baseline.

Assay of DPD activities in liver cytosol. Enzyme reaction was performed according to the procedure of Etienne et al. (1995). Briefly, the reaction mixture consisting of 70 mM phosphate buffer (pH 7.5), 5 mM MgCl$_2$, 0.5 mM NADPH, and 40 µM [6-$^{14}$C]-5-fluouracil was incubated at 37°C for 10 min. The reaction was then stopped by heating at 90°C for 2 min. After 25 µl of

TABLE 3
Pharmacokinetic parameters of $^{13}$C-uracil in male dogs obtained on the basis of time-related $^{13}$CO$_2$ concentration ($\Delta^{13}$C) in breath following oral administration of $^{13}$C-uracil

<table>
<thead>
<tr>
<th>Dose (µmol/kg)</th>
<th>Route</th>
<th>Breath $t_{max}$</th>
<th>Breath $C_{max}$</th>
<th>Breath AUC$_{0-t}$</th>
<th>Breath $t_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Oral</td>
<td>20 ± 8.7</td>
<td>28.9 ± 2.4</td>
<td>1.40 ± 0.52</td>
<td>18.3 ± 8.0</td>
</tr>
<tr>
<td>20</td>
<td>Oral</td>
<td>20 ± 8.7</td>
<td>59.2 ± 7.9</td>
<td>3.07 ± 0.68</td>
<td>23.8 ± 19.6</td>
</tr>
<tr>
<td>40</td>
<td>Oral</td>
<td>45.0 ± 30.0</td>
<td>98.7 ± 1.6</td>
<td>7.98 ± 1.89</td>
<td>28.1 ± 5.6</td>
</tr>
<tr>
<td>80</td>
<td>Oral</td>
<td>30.0 ± 0.0</td>
<td>193 ± 21</td>
<td>14.3 ± 1.5</td>
<td>27.1 ± 8.7</td>
</tr>
</tbody>
</table>

FIG. 2. A, plasma concentration-time profiles of $^{13}$C-uracil after oral administration of $^{13}$C-uracil to male dogs at 10, 20, 40, and 80 µmol/kg (mean ± S.D., n = 3). B, plasma concentration-time profiles of $^{13}$C-uracil after oral or intravenous administration of $^{13}$C-uracil to male dogs at 20 µmol/kg (mean ± S.D., n = 6).

TABLE 4
Pharmocokinetic parameters of $^{13}$C-uracil obtained on the basis of time-related $^{13}$CO$_2$ concentration ($\Delta^{13}$C) in breath following intravenous or oral administration of $^{13}$C-uracil to male dogs at 20 µmol/kg

<table>
<thead>
<tr>
<th>Route</th>
<th>Breath $t_{max}$</th>
<th>Breath $C_{max}$</th>
<th>Breath AUC$_{0-t}$</th>
<th>Breath $t_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>15.0 ± 0.0</td>
<td>55.2 ± 5.1</td>
<td>2.54 ± 0.62</td>
<td>20.1 ± 6.2</td>
</tr>
<tr>
<td>Oral</td>
<td>25.0 ± 12.3</td>
<td>57.3 ± 6.8</td>
<td>2.95 ± 0.87</td>
<td>17.3 ± 7.2</td>
</tr>
</tbody>
</table>

FIG. 3. A, respiratory concentrations of $^{13}$CO$_2$ ($\Delta^{13}$C)-time profiles after oral administration of $^{13}$C-uracil to male dogs at 10, 20, 40, and 80 µmol/kg (mean ± S.D., n = 3). B, respiratory concentrations of $^{13}$CO$_2$ ($\Delta^{13}$C)-time profiles after oral or intravenous administration of $^{13}$C-uracil to male dogs at 20 µmol/kg (mean ± S.D., n = 6).
Pharmacokinetic parameters of $[^{13}C]$uracil obtained on the basis of blood concentrations of $[^{13}C]$uracil in DPD-deficient male dogs treated with BVU and normal male dogs after oral administration at 20 μmol/kg

$F^*$ was calculated using the average AUC in Table 2 for control and in Table 7 for 2 μmol/kg BVU and 40 μmol/kg BVU. Numbers in parentheses indicate the ratio relative to the control value. Values are mean ± S.D.

<table>
<thead>
<tr>
<th>Group</th>
<th>$C_{max}$ (μmol/ml)</th>
<th>AUC (μg · h/ml)</th>
<th>AUC∞ (μg · h/ml)</th>
<th>$t_{max}$ (h)</th>
<th>$t_{1/2}$ (h)</th>
<th>CL/F (l/kg)</th>
<th>V/F (l/kg)</th>
<th>$F^*$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>0.228 ± 0.078</td>
<td>0.051 ± 0.0128</td>
<td>0.0504 ± 0.0126</td>
<td>0.333 ± 0.258</td>
<td>0.0857 ± 0.0176</td>
<td>46.9 ± 10.9</td>
<td>6.01 ± 2.52</td>
<td>10.8 ± 3.7</td>
</tr>
<tr>
<td>2 μmol/kg BVU</td>
<td>1.71 ± 0.48 (7.5)</td>
<td>1.91 ± 0.09 (37)</td>
<td>1.92 ± 0.09 (38)</td>
<td>0.778 ± 0.631</td>
<td>0.628 ± 0.184 (7.3)</td>
<td>1.18 ± 0.05</td>
<td>1.08 ± 0.36</td>
<td>81.0</td>
</tr>
<tr>
<td>5 μmol/kg BVU</td>
<td>2.29 ± 0.26 (10)</td>
<td>4.51 ± 0.95 (88)</td>
<td>4.53 ± 0.95 (90)</td>
<td>0.500 ± 0.333</td>
<td>1.38 ± 0.27 (16)</td>
<td>0.515 ± 0.19</td>
<td>0.996 ± 0.117</td>
<td>—</td>
</tr>
<tr>
<td>BVU 40 μmol/kg</td>
<td>2.77 ± 0.49 (12)</td>
<td>6.11 ± 0.30 (120)</td>
<td>10.7 ± 1.8 (210)</td>
<td>0.889 ± 0.673</td>
<td>2.91 ± 1.06 (34)</td>
<td>0.216 ± 0.038</td>
<td>0.870 ± 0.180</td>
<td>105</td>
</tr>
</tbody>
</table>

...not calculated.

$* n = 5$ ($\lambda_z$ not estimated by linear regression in one case).

![Graph](https://example.com/graph.png)

FIG. 4. Plasma concentration of $[^{13}C]$uracil after oral administration of $[^{13}C]$uracil at 20 μmol/kg in male dogs with or without BVU (mean ± S.D., n = 3 or 6).

0.36 M KOH was added to the reaction mixture and kept at room temperature for 30 min, 25 μl of 0.36 M HClO4 was added to neutralize the alkaline reaction mixture. After centrifugation, 5 μl of the supernatant was applied to a thin-layer chromatography plate (Silica gel 60F254, Merck, Darmstadt, Germany) that was developed for about 5 cm with a mixture of ethanol/1M ammonium acetate (5:1 v/v). After drying, the plate was again developed for about 15 cm with diethyl ether/acetone/chloroform/water (50:50:40:1, by volume).

Radioactivity in the [6-$^{13}$C]5-fluorouracil and radioactive products regions was analyzed with a bioimaging analyzer system (BAS2000; Fuji Photo Film Co., Ltd., Tokyo, Japan). The reaction ratio, corrected by the degradation ratio calculated using a blank reaction, was expressed as the percentage of the reaction products to the total radioactivity.

**Pharmacokinetic Analysis.** Pharmacokinetic parameters for $[^{13}C]$uracil and $^{13}$CO₂ were estimated using the noncompartmental pharmacokinetic methods of analysis (WinNonlin Standard version 3.1; Pharsight, Mountain View, CA). Maximum plasma concentration ($C_{max}$) and the time to $C_{max}$ ($t_{max}$) were expressed as the mean and S.D. of the values of $C_{max}$ and $t_{max}$ assigned from observed data. Area under the plasma concentration-time curve (AUC) was calculated up to the last measurable point $t$ by the linear trapezoidal method. The apparent terminal-phase disposition rate constant ($\lambda_z$) was estimated by linear regression from the semilogarithmic curve of plasma concentration versus time using the last three or four points. Terminal elimination half-life ($t_{1/2}$) was calculated from 0.693/\lambda_z. AUC∞ was calculated by extrapolation to infinity by dividing the last measured concentration by $\lambda_z$. Apparent total clearance (CL/F) was calculated from dose/AUC, and the apparent volume of distribution (V/F) was calculated from (CL/F)/\lambda_z. Breath pharmacokinetic parameters, namely breath $t_{max}$, $C_{max}$, AUC, and $t_{1/2}$, were calculated by the same methods as plasma concentration.

**Results.**

Pharmacokinetics of $[^{13}C]$Uracil. $[^{13}C]$Uracil concentration in plasma. Pharmacokinetic parameters determined based on the plasma concentrations of $[^{13}C]$uracil after oral administration of $[^{13}C]$uracil at 10, 20, 40, and 80 μmol/kg and intravenous administration at 20 μmol/kg under fasted conditions are shown in Tables 1 and 2, respectively. Figure 2, A and B, shows plasma $[^{13}C]$uracil concentration versus time curves after oral and intravenous administration, respectively. The dose-normalized AUC values (AUC/dose) increased with the increase in the dose, showing that $[^{13}C]$uracil exhibited nonlinear pharmacokinetics. Absolute bioavailability determined from AUC∞ after oral and intravenous administration was only 10.8% (Table 2) at 20 μmol/kg.

$[^{13}C]$CO₂ concentration ($\Delta^{13}$C) in breath. The pharmacokinetic parameters of breath output calculated from the $[^{13}$CO₂ concentration ($\Delta^{13}$C) in the breath versus time curve after oral administration of $[^{13}C]$uracil at doses of 10, 20, 40, and 80 μmol/kg or intravenous administration at 20 μmol/kg under fasted conditions are shown in Tables 3 and 4, respectively. Figure 3, A and B, shows the $[^{13}$CO₂ concentration ($\Delta^{13}$C) in breath versus time curves. Linear dose relationships were obtained for both breath AUC and breath $C_{max}$ (Table 3).

TABLE 6

Pharmacokinetic parameters of $[^{13}C]$uracil obtained on the basis of $[^{13}$CO₂ concentration ($\Delta^{13}$C) in breath in DPD-deficient male dogs treated with BVU and normal male dogs after oral administration at 20 μmol/kg

<table>
<thead>
<tr>
<th>Group</th>
<th>Breath $t_{max}$ (min)</th>
<th>Breath $C_{max}$ (μg/ml)</th>
<th>Breath AUC (μg · h/ml)</th>
<th>Breath $t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>25.0 ± 12.3</td>
<td>57.3 ± 6.8</td>
<td>2.95 ± 0.87</td>
<td>17.3 ± 7.2</td>
</tr>
<tr>
<td>2 μmol/kg BVU</td>
<td>80.0 ± 22.9</td>
<td>22.6 ± 1.4 (0.39)</td>
<td>2.59 ± 0.20 (0.88)</td>
<td>19.9 ± 13.9 (1.2)</td>
</tr>
<tr>
<td>5 μmol/kg BVU</td>
<td>85.0 ± 31.2</td>
<td>10.8 ± 3.2 (0.19)</td>
<td>1.38 ± 0.63 (0.47)</td>
<td>25.7 ± 17.4 (1.5)</td>
</tr>
<tr>
<td>BVU 40 μmol/kg</td>
<td>80.0 ± 34.6</td>
<td>3.0 ± 1.1 (0.05)</td>
<td>0.39 ± 0.227 (0.13)</td>
<td>69.2 ± 91.6 (4.0)</td>
</tr>
</tbody>
</table>
Pharmacokinetics of $[^{13}C]$Uracil in DPD-Suppressed Dogs after Oral Administration. Pharmacokinetic parameters calculated from plasma concentrations of $[^{13}C]$uracil together with the relative ratios of $C_{\text{max}}$, AUC, and $t_{1/2}$, after oral administration at 20 $\mu$mol/kg to DPD-suppressed dogs obtained by pretreatment with BVU at 2, 5, and 40 $\mu$mol/kg are shown in Table 5, and the plasma concentration of $[^{13}C]$uracil versus time curve is shown in Fig. 4. Pharmacokinetics in the breath calculated from $^{13}$CO$_2$ concentration ($\Delta^{13}$C) in breath versus time curve together with the relative ratios of breath $C_{\text{max}}$, breath AUC, and breath $t_{1/2}$ are shown in Table 6, and $\Delta^{13}$C in the breath versus time curve is shown in Fig. 5.

Pharmacokinetics of $[^{13}C]$Uracil in DPD-Suppressed Dogs after Intravenous Administration. Pharmacokinetic parameters calculated from the plasma concentrations of $[^{13}C]$uracil together with the relative ratio of AUC, $t_{1/2}$, and AUC$\infty$ after intravenous administration at 20 $\mu$mol/kg to DPD-suppressed dogs obtained by pretreatment with BVU at 0.5, 2, and 40 $\mu$mol/kg are listed in Table 7, and the plasma concentration of $[^{13}C]$uracil versus time curve is shown in Fig. 6. Pharmacokinetics in the breath calculated from $^{13}$CO$_2$ concentration ($\Delta^{13}$C) in breath versus time curve together with the relative ratio of breath $C_{\text{max}}$, breath AUC, and breath $t_{1/2}$ are shown in Table 8, and $\Delta^{13}$C in the breath versus time curve is shown in Fig. 7.

DPD Activities in Liver Cytosol. DPD activities in the untreated group and the groups pretreated with BVU at 0.5, 2, 5, and 40 $\mu$mol/kg are shown in Fig. 8. Relative ratios with respect to the untreated group were 0.71, 0.12, 0.06, and 0.04, respectively.

Discussion
As part of our studies into the prediction of adverse reactions to treatment with the anticancer agent 5-FU, we investigated the effects of inhibition of DPD on blood concentrations of $[^{13}C]$uracil and breath concentrations of $^{13}$CO$_2$ ($\Delta^{13}$C) after oral or intravenous administration of $[^{13}C]$uracil to dogs prepared by pretreatment with BVU, a DPD inhibitor. Results showed that the breath output ($\Delta^{13}$C) is a good marker of hepatic DPD activity in vivo.

We assumed that the DPD reaction is the rate-limiting step in uracil or 5-FU elimination. Because this reaction is the first step in uracil metabolism, whereas CO$_2$ is the end product of the final step, we reasoned that if an intermediate step became rate-limiting, breath analysis would reflect that limitation. In contrast, in DPD-deficient subjects, BVU would be rate-limiting for overall pyrimidine metabolism.

Results showed that the pharmacokinetic parameters (breath AUC, and breath $C_{\text{max}}$) obtained from the $^{13}$CO$_2$ concentrations ($\Delta^{13}$C) in the breath exhibited a linear relationship within the dose range of 10 to 80 $\mu$mol/kg. In contrast, pharmacokinetic parameters ($C_{\text{max}}$ and AUC) determined from $[^{13}C]$uracil concentrations in plasma were nonlinear. The time profile of $\Delta^{13}$C in breath after intravenous administration was closely similar to that after oral administration (Fig. 3B), and similar values were observed with respect to those pharmacokinetic parameters (Table 4). This finding indicates that the absorption of $[^{13}C]$uracil was extremely rapid and the extent of absorption was very high. This is further supported by the finding that radioactivity recoveries in the breath, urine, and feces after oral [2-14C]uracil administration at 20 $\mu$mol/kg to dogs were 90.6, 5.6, and 0.7%, respectively, of the dose given by 168 h (data not shown). This nonlinearity in blood concentration is likely due to the saturation of first-pass metabolism in the liver. In contrast, $[^{13}C]$uracil that escaped the first-pass effect and entered the systemic circulation should be finally metabolized in the liver and excreted as $^{13}$CO$_2$. In other words, breath $C_{\text{max}}$ and breath AUC, represent the maximal metabolic activity ($V_{\text{max}}$) but not its affinity ($K_{m}$). This would explain why $\Delta^{13}$C in the breath output remained linear regardless of dosage route and increased with the increase in dose.

The relative ratios of $C_{\text{max}}$, AUC, $t_{1/2}$, and AUC$\infty$ were signifi-

![Fig. 5. Expired $^{13}$CO$_2$ profiles after oral administration of $[^{13}C]$uracil in male dogs at a dose of 20 $\mu$mol/kg with or without BVU (mean ± S.D., n = 3 or 6).](image)

![Fig. 6. Plasma concentration of $[^{13}C]$uracil after intravenous administration of $[^{13}C]$uracil at 20 $\mu$mol/kg in male dogs with or without BVU (mean ± S.D., n = 3).](image)

**TABLE 7**

Pharmacokinetic parameters of $[^{13}C]$uracil obtained on the basis of blood concentrations of $[^{13}C]$uracil in DPD-deficient male dogs treated with BVU and normal male dogs after intravenous administration at 20 $\mu$mol/kg

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC</th>
<th>AUC$\infty$</th>
<th>$t_{1/2}$</th>
<th>CL</th>
<th>$V_z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>0.564 ± 0.070</td>
<td>0.580 ± 0.075</td>
<td>0.0917 ± 0.0183</td>
<td>3.94 ± 0.51</td>
<td>0.520 ± 0.087</td>
</tr>
<tr>
<td>0.5 $\mu$mol/kg BVU</td>
<td>0.766 ± 0.124 (1.4)</td>
<td>0.776 ± 0.114 (1.3)</td>
<td>0.122 ± 0.018 (1.3)</td>
<td>2.96 ± 0.45</td>
<td>0.511 ± 0.046</td>
</tr>
<tr>
<td>2 $\mu$mol/kg BVU</td>
<td>2.35 ± 0.30 (4.2)</td>
<td>2.37 ± 0.29 (4.1)</td>
<td>0.433 ± 0.083 (4.7)</td>
<td>0.964 ± 0.120</td>
<td>0.619 ± 0.146</td>
</tr>
<tr>
<td>40 $\mu$mol/kg BVU</td>
<td>7.25 ± 0.41 (13)</td>
<td>10.2 ± 0.8 (18)</td>
<td>2.10 ± 0.20 (23)</td>
<td>0.224 ± 0.017</td>
<td>0.661 ± 0.040</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the ratio relative to the control value. Values are mean ± S.D. (n = 3).
cantly increased as BVU dosage increased (Table 5). Moreover, the bioavailability (\(F\)) of \([13C]\)uracil increased from 10.8 (control) to 105% (BVU, 40 \(\mu\)mol/kg), indicating that the saturation of DPD by BVU is stronger and complete at higher doses. The \(V_z/F\) value therefore decreased with the increase in BVU dose, whereas the \(V_z\) value calculated using \(F\) ranged from 0.7 to 0.9 l/kg (Table 5), close to the 0.7 l/kg (Table 2) obtained after intravenous administration. Thus, it was indicated that BVU has no substantial effect on the drug distribution process. On the contrary, the relative ratios of breath C\(_{\text{max}}\) and breath AUC\(_t\) decreased as the BVU dose increased (Table 6), indicating that the breath output of \(^{13}\)CO\(_2\) reflects DPD activity in vivo. The delay of \(t_{\text{max}}\) (Fig. 5) may be explained by the decrease in \(^{13}\)CO\(_2\) production due to the DPD suppression. The direct correlation between breath C\(_{\text{max}}\) and liver DPD activity (Fig. 9A) and breath C\(_{\text{max}}\) and plasma clearance of \([13C]\)uracil (Fig. 9B) demonstrates that the change in breath C\(_{\text{max}}\) is a sensitive indicator of a decrease in DPD activity.

The relative ratios of AUC\(_t\) and \(t_{1/2}\) after intravenous administration of \([13C]\)uracil increased markedly as BVU dosage increased (Table 7), although to a lesser degree than after oral administration. The relative ratios of breath C\(_{\text{max}}\) and breath AUC\(_t\), remarkably decreased as BVU dosage increased (Table 8). These decreases in breath parameters were approximately the same as those after oral administration at the same BVU doses (2 and 40 \(\mu\)mol/kg).
The rate-limiting factor of clearance for drugs with a high first-pass effect is hepatic blood flow after intravenous administration but hepatic intrinsic clearance after oral administration (Shand et al., 1975). The limiting factor of clearance is therefore different between intravenous and oral routes of administration. Approximately 90% of [13C]uracil is subject to first-pass metabolism, a similar ratio to that for 5-FU (Almersjo et al., 1980; Kuan et al., 1996; Sawai et al., 1997). DPD activity is localized predominately in the liver (Ho et al., 1986). DPD activity in the liver of the group pretreated with BUV at 0.5 μmol/kg as a ratio of that of the untreated group was 0.71, or in other words decreased by approximately 30%. Thus, given that blood flow is the rate-limiting factor in intravenous administration, even though DPD activity was decreased by about 30%, the decrease had little influence on blood concentration (Fig. 6), and the change in breath reaction was small (Fig. 7). On the contrary, blood concentration after oral administration directly reflects a change in DPD activity (capacity of enzyme reaction). The differences observed in the relative blood concentration ratios between the intravenous and oral administration routes with respect to the untreated controls can therefore be explained by the difference in the susceptibility of clearance to the decrease in intrinsic clearance.

Because approximately 90% of the dose was excreted as [13C]CO2 in breath in the DPD-normal model, it is assumed that 10% remained as parent drug in the blood. If 70% of the dose were excreted in breath in the DPD-deficient model, the remaining parent drug would be noticeably elevated 3-fold above the normal model (10–30%), whereas changes in the breath response would not be large, namely only a 20% reduction (90–70%). This interpretation may explain the pharmacokinetic discrepancy seen between breath excretion (ΔΔ13C) and plasma [13C]uracil concentrations following the moderate suppression (i.e., 30%) of DPD activity. On the other hand, when DPD activity was markedly decreased (i.e., to 10% of the control), the breath output of [13C]CO2 was remarkable and easily detected.

The most important finding of the present study is that the breath response (ΔΔ13C) reflected the degree of DPD activity in the liver (Fig. 9, A and B). Sludden et al. (1998) showed that the species order of DPD activity was mouse > rat > human > dog ≈ cynomolgus monkey ≈ rhesus monkey, whereas Collins (1985) showed that the clearance values of 5-FU were similar in dogs and humans. Khor et al. (1997) stated that the pharmacokinetics of 5-FU in the DPD-deficient state in humans could be predicted from animal data, including dogs. We therefore speculate that the DPD-deficient dog model is a good surrogate model for humans. Our DPD-deficient model may be useful for the preliminary assessment of interindividual variability in 5-FU pharmacokinetics and toxicity caused by genotypic-dependent DPD deficiency in humans. Further study is required to evaluate the clinical use of [13C]uracil breath testing in the identification of DPD-deficient patients before 5-FU cancer chemotherapy.

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Address correspondence to: Dr. Makoto Inada, Otsuka Pharmaceutical Co., Ltd., 224-18 Bisenimo, Hiraiishi, Kawaucho-cho, Tokushima 771-0182, Japan. E-mail: inadam@otsuka.jp