The Uracil Breath Test in the Assessment of Dihydropyrimidine Dehydrogenase Activity: Pharmacokinetic Relationship between Expired $^{13}$CO$_2$ and Plasma [2-13C]Dihydouracil

Lori K. Mattison, Jeanne Foure, Yukihiro Hirao, Toshihisa Koga, Renee A. Desmond, Jennifer R. King, Takefumi Shimizu, and Robert B. Diasio

Abstract

Purpose: Dihydropyrimidine dehydrogenase (DPD) deficiency is critical in the predisposition to 5-fluorouracil dose-related toxicity. We recently characterized the phenotypic [2-13C]uracil breath test (UraBT) with 96% specificity and 100% sensitivity for identification of DPD deficiency. In the present study, we characterize the relationships among UraBT-associated breath $^{13}$CO$_2$ metabolite formation, plasma [2-13C]dihydouracil formation, [2-13C]uracil clearance, and DPD activity.

Experimental Design: An aqueous solution of [2-13C]uracil (6 mg/kg) was orally administered to 23 healthy volunteers and 8 cancer patients. Subsequently, breath $^{13}$CO$_2$ concentrations and plasma [2-13C]dihydouracil and [2-13C]uracil concentrations were determined over 180 minutes using IR spectroscopy and liquid chromatography-tandem mass spectrometry, respectively. Pharmacokinetic variables were determined using noncompartmental methods. Peripheral blood mononuclear cell (PBMC) DPD activity was measured using the DPD radioassay.

Results: The UraBT identified 19 subjects with normal activity, 11 subjects with partial DPD deficiency, and 1 subject with profound DPD deficiency within the corresponding previously established ranges. UraBT breath $^{13}$CO$_2$ DOB$_{50}$ significantly correlated with PBMC DPD activity ($r_p = 0.78$), plasma [2-13C]uracil area under the curve ($r_p = -0.73$), [2-13C]dihydouracil appearance rate ($r_p = 0.76$), and proportion of [2-13C]uracil metabolized to [2-13C]dihydouracil ($r_p = 0.77$; all $P_s < 0.05$).

Conclusions: UraBT breath $^{13}$CO$_2$ pharmacokinetics parallel plasma [2-13C]uracil and [2-13C]dihydouracil pharmacokinetics and are an accurate measure of interindividual variation in DPD activity. These pharmacokinetic data further support the future use of the UraBT as a screening test to identify DPD deficiency before 5-fluorouracil-based therapy.

Dihydropyrimidine dehydrogenase (EC 1.3.1.2, DPD) is the rate-limiting enzyme in uracil and 5-fluorouracil (5-FU) catabolism, converting >80% of an administered dose of 5-FU to inactive metabolites (1, 2). The initial step of catabolism is mediated by DPD converting 5-FU to 5-dihydrofluorouracil, with subsequent catabolism by dihydropyrimidinase and β-ureidopropionase enzymes to ultimately produce fluorobeta-alanine, ammonia, and CO$_2$. The latter final metabolic end-products are excreted in the urine and breath (3).

The pharmacogenetic syndrome of complete and partial DPD deficiency is prevalent in ~0.1% and 3% to 5% of the general population, respectively (4). DPD deficiency is a significant pharmacogenetic factor in the predisposition of cancer patients to increased risk of altered 5-FU pharmacokinetics and associated toxicity. Specifically, 60% of patients presenting with severe 5-FU-related hematologic toxicity showed reduced DPD activity (5).

Recent studies have investigated the predictive value of the ratio of plasma dihydouracil area under the curve (AUUC) to uracil AUUC (DUUR) for the assessment of DPD activity and potential individualization of 5-FU therapy. Specifically, 5-FU dose optimization may be based on the plasma DUUR observed before 5-FU administration (6). Jiang et al. have also showed that the pre-5-FU treatment DUUR may be a good index of DPD activity (7, 8).

Our laboratory recently reported the rapid noninvasive phenotypic [2-13C]uracil breath test (UraBT) for assessment of DPD activity with 96% specificity and 100% sensitivity (9). Application of the UraBT to a large population of cancer-free subjects ($n = 255$) showed an observed 86% sensitivity (with 12 of 14 DPD-deficient subjects identified as DPD deficient) and
Materials and Methods

Subjects. Thirty-one subjects (16 men and 15 women; ages 19-70 years) participated in this institutional review board–approved pharmacokinetic examination that was conducted at the General Clinical Research Center at the University of Alabama at Birmingham. Eight subjects were cancer patients who were referred by their oncologist due to known or suspected DPD deficiency. Twenty-three subjects were participants from the University of Alabama at Birmingham campus who volunteered for examination after reading an institutional review board–approved advertisement placed in the campus newspaper. Due to the rarity of DPD deficiency in the general population, we included six DPD-deficient individuals previously phenotyped (UraBT and DPD peripheral blood mononuclear cell [PBMC] radioassay) and genotyped (denaturing high-performance liquid chromatography-tandem mass spectrometry) during the 180-minute period immediately following [2-13C]uracil administration. Blood was immediately processed after collection and plasma was isolated as follows: 3 mL whole blood was centrifuged at 4°C for 10 minutes at 2200 g; plasma was immediately pipetted into polypropylene tubes and then stored at −80°C until analysis by liquid chromatography-tandem mass spectrometry.

Detection and quantification of plasma [2-13C]uracil and [2-13C]dihydrouracil concentrations. While each subject performed the UraBT, whole blood was simultaneously collected via a heparin lock placed in the participant’s arm. A baseline blood sample was collected immediately before oral administration of the [2-13C]uracil solution. Post-dose blood samples were collected into heparinized (green-top) vacutainers at 5, 10, 15, 20, 25, 30, 50, 60, 90, 120, and 180 minutes following [2-13C]uracil administration. Blood was immediately processed after collection and plasma was isolated as follows: 3 mL whole blood was centrifuged at 4°C for 10 minutes at 2200 g; plasma was immediately pipetted into polypropylene tubes and then stored at −80°C until analysis by liquid chromatography-tandem mass spectrometry.

Pharmacokinetic analysis. Concentration-time profiles of plasma [2-13C]uracil and [2-13C]dihydrouracil were constructed. Noncompartmental methods (WinNonlin version 4.1, Pharsight Corp., Mountain View, CA) were used to determine the pharmacokinetic variables of [2-13C]uracil in plasma, [2-13C]dihydrouracil in plasma, and 13CO2 in breath. Calculated pharmacokinetic variables were \( C_{\text{max}} \), \( T_{\text{max}} \), apparent clearance (CL/F), terminal apparent distribution volume (V/F), and elimination half-life (\( t_{1/2} \)). AUC was determined using the trapezoidal rule (16). \( C_{\text{max}} \) and \( T_{\text{max}} \) were taken directly from the observed concentration-time data. CL/F was calculated as dose/AUC, and V/F was calculated as dose divided by the product of terminal elimination rate constant, \( k_{\text{e}} \), and AUC, elimination rate constant was determined by linear regression of the terminal elimination phase concentration-time points; \( t_{1/2} \) was calculated as ln(2)/\( k_{\text{e}} \). [2-13C]Dihydrouracil may only be produced in appreciable quantities in vivo by the DPD-mediated catabolism of [2-13C]uracil. To assess formation of [2-13C]dihydrouracil (metabolite) from [2-13C]uracil (probe substrate) by DPD, the [2-13C]dihydrouracil appearance rate,

Statistical analysis. Summary data stratified by DPD activity are presented as mean ± SD. Comparisons of plasma [2-13C]uracil and [2-13C]dihydrouracil concentrations and pharmacokinetic variables between subjects with normal DPD activity and subjects with partial DPD deficiency were assessed by bootstrap t tests of hypotheses using PROC MULTTEST in SAS version 9.1. The bootstrap Ps were compared with the raw Ps to assess nonnormality of inferences. If the bootstrap P was close to the normality-assuming P, we concluded that nonnormality was not a concern for the particular variable. For comparisons between the subjects with normal DPD activity and the one subject with profound DPD deficiency, we used the t test to perform a single mean comparison to test the mean of subjects with normal DPD activity for each variable against the value for the profoundly DPD-deficient individual. Correlations among UraBT DOB_{50}, PBMC DPD activity, and plasma [2-13C]uracil and [2-13C]dihydrouracil pharmacokinetic variables were evaluated using Pearson’s correlation coefficient. For all analyses, P < 0.05 was deemed statistically significant.

Results

Determination of PBMC DPD activity. The DPD enzyme activity was determined for all subjects (mean ± SD). Nineteen subjects showed normal DPD activity (0.27 ± 0.06 nmol/min/mg), 11 subjects showed partial DPD deficiency (0.11 ± 0.05 nmol/min/mg), and 1 subject showed profound deficiency (undetectable DPD activity).

Detection of DPD deficiency by UraBT. UraBT indices (mean ± SD) obtained in subjects with normal and reduced DPD activity are summarized in Table 1. The UraBT showed 100% agreement with the PBMC radioassay. Subjects with DPD activity in the reference range showed UraBT DOB_{50} ≥ 128.9 DOB. All partially and profoundly DPD-deficient subjects showed DOB_{50} < 128.9 DOB. Altered breath [13C]CO2 concentration-time profiles were also observed in all DPD-deficient subjects. Specifically, profoundly and partially DPD-deficient subject(s) showed an increased UraBT T_{\text{max}} and reduced UraBT 13C CO2 C_{\text{max}}, DOB_{50}, AUC, and PDR compared with subjects with normal DPD activity (all Ps < 0.05).

UraBT DOB_{50} also showed significant correlation with PBMC DPD activity (Fig. 1A).

Comparison of plasma [2-13C]uracil pharmacokinetics in subjects with normal and reduced PBMC DPD activity. Plasma [2-13C]uracil pharmacokinetic variables (mean ± SD) obtained from subjects with normal and reduced DPD activity are summarized in Table 2. [2-13C]Uracil was detectable in the plasma of most subjects 5 minutes after oral administration (Fig. 2A). No statistically significant differences were observed in plasma [2-13C]uracil C_{\text{max}} between subjects with normal activity and those with partial or profound DPD deficiency. No significant difference was observed in plasma [2-13C]uracil T_{\text{max}} from subjects with normal DPD activity and subjects with partial DPD deficiency. However, a significant difference was observed in plasma [2-13C]uracil T_{\text{max}} from the subjects with normal DPD activity and the profoundly DPD-deficient subject.

Reduced [2-13C]uracil catabolism was observed in all DPD-deficient subjects (Fig. 2A). Both profoundly and partially DPD-deficient subject(s) showed increased plasma [2-13C]uracil t_{1/2} and AUC and reduced plasma [2-13C]uracil clearance compared with subjects with normal DPD activity (all Ps < 0.05).

PBMC DPD activity was significantly correlated with several pharmacokinetic variables of uracil catabolism. Specifically, PBMC DPD was significantly correlated with plasma [2-13C]uracil clearance (Fig. 1B) and inversely correlated with plasma [2-13C]uracil AUC and t_{1/2} (all Ps < 0.05; Table 3). The UraBT DOB_{50} were also significantly correlated with several pharmacokinetic variables of uracil catabolism. Specifically, the UraBT DOB_{50} were significantly correlated with plasma [2-13C]uracil clearance and inversely correlated with plasma [2-13C]uracil AUC (Fig. 1C) and t_{1/2} (all Ps < 0.05; Table 3).

Table 1. Comparison of UraBT indices from subjects with normal DPD activity and partial and profound DPD deficiency

<table>
<thead>
<tr>
<th></th>
<th>Normal activity (n = 19)</th>
<th>Partial DPD deficiency (n = 11)</th>
<th>Profound DPD deficiency (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOB_{50} (DOB)*</td>
<td>183.2 ± 31.2</td>
<td>83.7 ± 25.3</td>
<td>0.9</td>
</tr>
<tr>
<td>C_{\text{max}} (DOB)*</td>
<td>193.8 ± 28.1</td>
<td>121.8 ± 36.7</td>
<td>3.6</td>
</tr>
<tr>
<td>T_{\text{max}} (min)*</td>
<td>50.5 ± 10.8</td>
<td>125.4 ± 43.2</td>
<td>120.0</td>
</tr>
<tr>
<td>AUC (mg/mL)*</td>
<td>215.567 ± 2.634.2</td>
<td>15,572.9 ± 4,327.4</td>
<td>348.7</td>
</tr>
<tr>
<td>PDR*</td>
<td>55.7 ± 4.9</td>
<td>39.8 ± 9.9</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>DPD activity (nmol/min/mg)*</td>
<td>0.27 ± 0.06</td>
<td>0.11 ± 0.05</td>
<td>Undetectable</td>
</tr>
</tbody>
</table>

NOTE: Data were obtained from 19 subjects with normal DPD activity, 11 subjects with partial DPD deficiency, and 1 subject with profound DPD deficiency following oral administration of [2-13C]uracil (6 mg/kg dose). Data are mean ± SD.

*DOB_{50}, [13C]CO2 concentration in breath (DOB) 50 minutes after [2-13C]uracil administration; C_{\text{max}}, maximum concentration of [13C]CO2 in breath; T_{\text{max}}, time to C_{\text{max}}; AUC, area under the [13C]CO2 breath curve; PDR, percent dose of [2-13C]uracil recovered in the breath as [13C]CO2; DPD activity, fresh PBMC DPD enzyme activity.

* P < 0.05 for both pairwise comparisons (normal DPD activity versus partial DPD deficiency and normal DPD activity versus profound DPD deficiency).

* P < 0.05 (normal DPD activity versus partial DPD deficiency).

Plasma [2-13C]dihydrouracil pharmacokinetic variables (mean ± SD) obtained in subjects with normal and reduced DPD activity are summarized in Table 4. Altered plasma [2-13C]dihydrouracil concentrations were observed in DPD-deficient subjects (Fig. 2B). The profoundly deficient subject showed plasma [2-13C]dihydrouracil concentrations below the limit of detection; thus, pharmacokinetic variables could not be determined. Partially deficient subjects showed significantly decreased plasma [2-13C]dihydrouracil C_max and increased plasma [2-13C]dihydrouracil T_max and t_1/2 compared with subjects with normal DPD activity (all P < 0.05). Partially deficient subjects also showed a significant reduction in the proportion of [2-13C]uracil metabolized to [2-13C]dihydrouracil, [2-13C]dihydrouracil appearance rate, amount of [2-13C]dihydrouracil formed, and plasma DUUR (all P < 0.05).

[2-13C]Dihydrouracil formation and concentrations were significantly correlated with PBMC DPD activity (Table 3). Specifically, PBMC DPD activity was significantly correlated with the proportion of [2-13C]uracil metabolized to [2-13C]dihydrouracil, [2-13C]dihydrouracil appearance rate, amount of [2-13C]dihydrouracil formed, plasma C_max and plasma DUUR (all P < 0.05). PBMC DPD activity was also inversely correlated with plasma [2-13C]dihydrouracil T_max (P < 0.05).

[2-13C]Dihydrouracil formation and concentrations were significantly correlated with UraBT DOB 50 (Table 3). In particular, UraBT DOB 50 were significantly correlated with the proportion of [2-13C]uracil metabolized to [2-13C]dihydrouracil, [2-13C]dihydrouracil appearance rate, amount of [2-13C]dihydrouracil formed (Fig. 1D), plasma DUUR, and plasma [2-13C]dihydrouracil C_max (all P < 0.05). UraBT DOB 50 were inversely correlated with plasma [2-13C]dihydrouracil T_max (P < 0.05).

Discussion

Identification of DPD-deficient cancer patients is important in optimizing 5-FU chemotherapy and minimizing life-threatening dose-related toxicity. We developed the UraBT, which may be used to screen cancer patients for DPD deficiency before administration of 5-FU (9). The principle of the UraBT was based on earlier metabolic studies that showed uracil and 5-FU are both degraded by the enzymes of the pyrimidine catabolic pathway, with the DPD enzyme having similar affinities for 5-FU and uracil (18–20). These studies provided a basis for use of the nontoxic [2-13C]uracil probe substrate in the UraBT to assess in vivo pyrimidine catabolism. Our initial examination of 50 volunteers and 8 DPD-deficient subjects suggested that the UraBT may be a good indicator of DPD activity. In this study, significantly reduced breath 13CO2 concentrations (DOB 50, C_max, AUC, and PDR) were observed from enrolled subjects with DPD deficiency versus those with normal DPD activity. Furthermore, the UraBT detected DPD deficiency with 96% specificity and 100% sensitivity (9). A more recent study of 255 subjects has corroborated our initial findings, with the UraBT showing 99.2% specificity and 85.7% sensitivity for detecting DPD deficiency (10). In the present study, we further validate the UraBT in a population of subjects with normal and reduced DPD activity by comparing breath 13CO2 kinetic profiles to plasma [2-13C]uracil and [2-13C]dihydrouracil kinetics.

Examination of plasma [2-13C]uracil concentration-time profiles showed that orally administered [2-13C]uracil was rapidly absorbed and detected in the plasma of most subjects within 5 minutes of administration. This observation is in agreement with an earlier animal study, which also reported rapid absorption following oral administration of [2-13C] uracil (15).

Following absorption of [2-13C]uracil in subjects with normal DPD activity, the [2-13C]uracil was observed to peak...
Following absorption of $[2-^{13}C]$uracil in subjects with a reverse points, mean; bars, SD.

have reported reduced 5-FU clearance with an increased 5-FU by DPD.

as indicated by the appearance of $[2-^{13}C]$dihydrouracil in the formations decreased reflecting both metabolism and elimination by decreased plasma $[2-^{13}C]$uracil clearance, decreased in both metabolism and elimination were noted as indicated partial and profound DPD deficiency, significant differences plasma (within 10 minutes) and $^{13}$CO$_2$ in the breath. These results suggest profoundly DPD-deficient subject showed no detectablejects and subjects with normal DPD activity, whereas the $[2-^{13}C]$uracil being converted to 1 mol $[2-^{13}C]$dihydrouracil is the exclusive and direct indicator of DPD activity. This conclusion is based on the rationale that DPD-mediated metabolism of $[2-^{13}C]$uracil to $[2-^{13}C]$dihydrouracil is the exclusive and singular source of plasma $[2-^{13}C]$dihydrouracil, with 1 mol $[2-^{13}C]$uracil being converted to 1 mol $[2-^{13}C]$dihydrouracil by DPD.

Several previous studies of DPD-deficient cancer patients have reported reduced 5-FU clearance with an increased 5-FU $t_{1/2}$ and AUC after oral and i.v. 5-FU administration (21–24).

Our observations with orally administered $[2-^{13}C]$uracil parallel these findings. Specifically, we observed significantly reduced plasma $[2-^{13}C]$uracil clearance in partially and profoundly DPD-deficient subjects, which resulted in increased plasma $[2-^{13}C]$uracil $t_{1/2}$ and AUC compared with subjects with normal DPD activity. Several clinical studies of plasma 5-FU concentrations in cancer patients have also observed inverse correlations between plasma 5-FU concentrations or $t_{1/2}$ and DPD activity as well as positive correlations between 5-FU clearance and DPD activity (25, 26). Our observations with orally administered $[2-^{13}C]$uracil also parallel these studies. We reported inverse correlations between PBMC DPD activity and plasma $[2-^{13}C]$uracil AUC and $t_{1/2}$ as well as a positive correlation between PBMC DPD activity and plasma $[2-^{13}C]$uracil clearance.

Using $[2-^{13}C]$uracil, we noted significant correlations between PBMC DPD activity and several $[2-^{13}C]$dihydrouracil pharmacokinetic variables. In particular, PBMC DPD activity was significantly correlated with plasma $[2-^{13}C]$dihydrouracil appearance rate, amount of $[2-^{13}C]$dihydrouracil formed, and $[2-^{13}C]$dihydrouracil $C_{\text{max}}$. In turn, a significant correlation between DPD-mediated plasma $[2-^{13}C]$dihydrouracil formation and breath $^{13}$CO$_2$ formation was observed, suggesting that the UraBT $^{13}$CO$_2$ kinetic variables are an accurate and sensitive index of systemic DPD activity. This conclusion is supported by the biochemical pathway of uracil catabolism where 1 mol

| Table 2. Comparison of plasma $[2-^{13}C]$uracil pharmacokinetic variables from subjects with normal DPD activity and partial and profound DPD deficiency |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Normal DPD activity | Partial DPD deficiency | Profound DPD deficiency |
|                                | $(n = 19)$          | $(n = 11)$         | $(n = 1)$         |
| [2-^{13}C]uracil $t_{1/2}$ (min) | 15.9 ± 1.7$^c$     | 39.3 ± 31.2$^c$   | 306.6$^c$         |
| [2-^{13}C]uracil AUC, (min µg/mL) | 257.5 ± 93.4$^c$  | 480.3 ± 187.6$^c$ | 1236.1$^c$       |
| [2-^{13}C]uracil CL/F (mL/min/kg)$^*$ | 26.6 ± 10.5$^c$ | 14.1 ± 6.0$^c$  | 1.3$^c$          |
| [2-^{13}C]uracil C$_{\text{max}}$ (µg/mL)$^*$ | 8.45 ± 3.09$^c$ | 8.87 ± 3.88$^c$  | 10.19$^c$        |
| [2-^{13}C]uracil $T_{\text{max}}$ (min) | 28.9 ± 9.5$^c$  | 33.2 ± 13.8$^c$  | 60.0$^c$         |

NOTE: [2-^{13}C]Uracil (6 mg/kg) was orally administered to 19 subjects with normal DPD activity, 11 subjects with partial DPD deficiency, and 1 subject with profound DPD deficiency. Following quantification of plasma [2-^{13}C]uracil concentrations, [2-^{13}C]uracil pharmacokinetic variables were determined. Data are mean ± SD. $^a$t$_{1/2}$, elimination half-life; AUC, area under the plasma [2-^{13}C]uracil concentration-time curve; CL/F, apparent clearance; C$_{\text{max}}$, maximum concentration of [2-^{13}C]uracil in plasma.

$^b$P < 0.05 for both pairwise comparisons (normal DPD activity versus partial DPD deficiency and normal DPD activity versus profound DPD deficiency).

$^c$P > 0.05 for both pairwise comparisons (normal DPD activity versus partial DPD deficiency and normal DPD activity versus profound DPD deficiency).

$^d$P < 0.05 (normal DPD activity versus partial DPD deficiency).

$^e$P < 0.05 (normal DPD activity versus profound DPD deficiency).

at ~28.9 ± 9.5 minutes. Subsequently, plasma concentrations decreased reflecting both metabolism and elimination as indicated by the appearance of [2-^{13}C]dihydrouracil in the plasma (within 10 minutes) and $^{13}$CO$_2$ in the breath. Following absorption of [2-^{13}C]uracil in subjects with partial and profound DPD deficiency, significant differences in both metabolism and elimination were noted as indicated by decreased plasma [2-^{13}C]uracil clearance, decreased appearance of [2-^{13}C]dihydrouracil in plasma, and decreased $^{13}$CO$_2$ concentrations in breath compared with subjects with normal DPD activity. In particular, a significant reduction in the appearance of [2-^{13}C]dihydrouracil in the plasma was observed between partially DPD-deficient subjects and subjects with normal DPD activity, whereas the profoundly DPD-deficient subject showed no detectable plasma [2-^{13}C]dihydrouracil (Fig. 2B). These results suggest that the [2-^{13}C]dihydrouracil appearance rate may be a direct indicator of DPD activity. This conclusion is based on the rationale that DPD-mediated metabolism of [2-^{13}C]uracil to [2-^{13}C]dihydrouracil is the exclusive and singular source of plasma [2-^{13}C]dihydrouracil, with 1 mol [2-^{13}C]uracil being converted to 1 mol [2-^{13}C]dihydrouracil by DPD.

Fig. 2. Plasma [2-^{13}C]uracil (A) and [2-^{13}C]dihydrouracil (B) concentrations from subjects with normal DPD activity and partial and profound DPD deficiency. The plasma concentration-time profiles (mean ± SD) of [2-^{13}C]uracil (A) and [2-^{13}C]dihydrouracil (B) from 19 subjects with normal DPD activity (●), 11 subjects with partial DPD deficiency (○), and 1 subject with profound DPD deficiency (▲) are shown. Points, mean; bars, SD.


\[^{13}\text{C}O_2\] is produced for every 1 mol \([2-^{13}\text{C}]\text{uracil}\) reduced to \([2-^{13}\text{C}]\text{dihydrouracil}\) by DPD.

Although we observed significant correlations between PBMC DPD activity and \([2-^{13}\text{C}]\text{uracil}\) clearance as well as between PBMC DPD activity and \([2-^{13}\text{C}]\text{dihydrouracil}\) formation, not all the variability in these pharmacokinetic variables could be attributed to variability in PBMC DPD activity. In fact, wide variation in DPD activity levels have been observed throughout various tissues (i.e., PBMC, kidney, colon, and liver), with the primary site of pyrimidine catabolism being the liver. Hence, the \(^{13}\text{CO}_2\) detected in colon, and liver), with the primary site of pyrimidine metabolism being the liver. Hence, the \(^{13}\text{CO}_2\) detected in our assay should be primarily formed in the liver. However, ethical considerations prevented the measurement of hepatic DPD activity in this human study. An examination of the relationship present between the UraBT and hepatic DPD activity in dogs suggested that systemic DPD activity may be more accurately reflected in breath \(^{13}\text{CO}_2\) concentrations than PBMC DPD activity (15). Hepatic DPD activity was significantly correlated with systemic DPD-mediated reduction of \([2-^{13}\text{C}]\text{uracil}\) as measured in breath \(^{13}\text{CO}_2\) concentrations \((r = 0.9999, \text{ref. 15})\). This animal study suggests that hepatic DPD activity should strongly correlate with breath \(^{13}\text{CO}_2\) formation in humans.

5-FU is characterized by a narrow therapeutic index and significant interpatient variability in its pharmacokinetics, which are both implicated in the wide interpatient variation in efficacy and toxicity (6, 26–29). These observations have led to the development of assays to measure plasma DUUR (or 5-dihydrofluorouracil/5-FU ratio) as a potential index on which 5-FU dose individualization strategies may be based (6, 28, 30). Notably, Jiang et al. suggested the importance of monitoring the formation of dihydrouracil under physiologic conditions, by examining the DUUR, to assess variability in DPD activity and 5-FU pharmacokinetics (7). Our results also parallel their observations. Specifically, we observed a significant correlation between PBMC DPD activity and DUUR. We also observed a significant correlation between UraBT DOB\(_{50}\) and DUUR.

In summary, we evaluated the UraBT with respect to PBMC DPD activity and plasma \([2-^{13}\text{C}]\text{uracil}\) and \([2-^{13}\text{C}]\text{dihydrouracil}\) concentrations in subjects with normal and reduced DPD activity. In the present study, we showed significant differences

### Table 3. Pharmacokinetic variables of \([2-^{13}\text{C}]\text{uracil}\) catabolism correlate with DPD activity and the UraBT

<table>
<thead>
<tr>
<th></th>
<th>DPD activity (nmol/min/mg)</th>
<th>UraBT DOB(_{50}) (DOB)</th>
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</thead>
<tbody>
<tr>
<td>([2-^{13}\text{C}]\text{Uracil CL/F (mL/min/kg)})</td>
<td>0.67</td>
<td>0.59</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Uracil AUC} (min µg/mL))</td>
<td>-0.72</td>
<td>-0.73</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Uracil t_{1/2} (min)})</td>
<td>-0.54</td>
<td>-0.59</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Uracil metabolized (%)})</td>
<td>0.67</td>
<td>0.77</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil appearance rate (µg/mL/min)})</td>
<td>0.59</td>
<td>0.76</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil formed (mg)})</td>
<td>0.61</td>
<td>0.82</td>
</tr>
<tr>
<td>DUUR</td>
<td>0.67</td>
<td>0.65</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil C}_\text{max (µg/mL)}) (^{†})</td>
<td>0.43</td>
<td>0.70</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil T}_\text{max (min)})</td>
<td>-0.64</td>
<td>-0.68</td>
</tr>
</tbody>
</table>

*All Pearson correlation coefficients \((r_p)\) are significant (all \(P < 0.05\)).

\(^{†}\)DUUR, ratio of plasma dihydrouracil AUC/uracil AUC; \(C_{\text{max}}\), maximum concentration of \([2-^{13}\text{C}]\text{dihydrouracil}\) in plasma.

### Table 4. Comparison of plasma \([2-^{13}\text{C}]\text{dihydrouracil}\) pharmacokinetic variables from subjects with normal DPD activity and partial and profound DPD deficiency

<table>
<thead>
<tr>
<th></th>
<th>Normal DPD activity ((n = 19))</th>
<th>Partial DPD deficiency ((n = 11))</th>
<th>Profound DPD deficiency ((n = 1))*</th>
</tr>
</thead>
<tbody>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil C}_{\text{max}} (µg/mL))</td>
<td>2.00 ± 0.53</td>
<td>1.36 ± 0.43</td>
<td>—</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil T}_{\text{max}} (min))</td>
<td>66.3 ± 22.5</td>
<td>125.4 ± 29.4</td>
<td>—</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil t}_{1/2} (min))</td>
<td>70.4 ± 24.2</td>
<td>367.9 ± 354.0</td>
<td>—</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil formed (mg)})</td>
<td>380.0 ± 86.2</td>
<td>153.7 ± 80.9</td>
<td>—</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Dihydrouracil appearance rate (µg/mL/min)})</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0.00</td>
<td>—</td>
</tr>
<tr>
<td>DUUR</td>
<td>0.9 ± 0.4</td>
<td>0.4 ± 0.3</td>
<td>—</td>
</tr>
<tr>
<td>([2-^{13}\text{C}]\text{Uracil metabolized (%)})</td>
<td>75.4 ± 9.8</td>
<td>31.6 ± 19.4</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE: \([2-^{13}\text{C}]\text{Uracil}\) (6 mg/kg) was orally administered to 19 subjects with normal DPD activity, 11 subjects with partial DPD deficiency, and 1 subject with profound DPD deficiency. Following quantification of plasma \([2-^{13}\text{C}]\text{dihydrouracil}\) concentrations, \([2-^{13}\text{C}]\text{dihydrouracil}\) pharmacokinetic variables were determined. Data are mean ± SD.

*No \([2-^{13}\text{C}]\text{dihydrouracil}\) was detected in the plasma from the profoundly DPD-deficient subject.

\(^{\dagger}\)\(^{\dagger}\) \(< 0.05\) (normal DPD activity versus partial DPD deficiency).
13CO2 concentrations (e.g., DOB50) in subjects with decreased 
DPD activity versus those with normal DPD activity. The significant correlations between DPD activity and either plasma 
[2-13C]uracil clearance, [2-13C]dihydrouracil formation, or 
13CO2 breath concentrations provide further support that the 
UraBT may be useful for assessment of DPD deficiency before 
administration of 5-FU.

References

The Uracil Breath Test in the Assessment of Dihydropyrimidine Dehydrogenase Activity: Pharmacokinetic Relationship between Expired $^{13}\text{CO}_2$ and Plasma [2-$^{13}\text{C}$]Dihydrouracil

Lori K. Mattison, Jeanne Fourie, Yukihiro Hirao, et al.


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