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 dihydropyrimidinidase and β-ureidopropionase enzymes to ultimately produce fluorobeta-alanine, ammonia, and CO₂. 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 dihydrouracil area under the curve (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

| 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 13CO₂ metabolite formation, plasma [2-13C]dihydrouracil 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 13CO₂ concentrations and plasma [2-13C]dihydrouracil 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 with PBMC DPD activity within the corresponding previously established ranges. UraBT breath 13CO₂ DOB₅₀ significantly correlated with PBMC DPD activity (rₚ = 0.78), plasma [2-13C]uracil area under the curve (rₚ = −0.73), [2-13C]dihydrouracil appearance rate (rₚ = 0.76), and proportion of [2-13C]uracil metabolized to [2-13C]dihydrouracil (rₚ = 0.77; all Ps < 0.05). Conclusions: UraBT breath 13CO₂ pharmacokinetics parallel plasma [2-13C]uracil and [2-13C]dihydrouracil 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. |
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 DPYD peripheral blood mononuclear cell (PBMC) radioassay] and genotyped (denaturing high-performance liquid chromatography analysis of the DPYD gene) in the current pharmacokinetic examination (9). Subjects with a history of gastric (i.e., dyspepsia) or respiratory (i.e., asthma) disease were excluded from the study.

DPD radioassay. PBMC DPD activity was determined for all subjects as described previously (11, 12). To minimize interassay variation in enzyme activity, 60 mL whole blood was collected into heparinized vacutainers at −12 p.m. on the day of testing and processed within 10 minutes of collection. After Ficol separation of whole blood, isolated PBMCs were washed with PBS and lysed. The cytosol was collected after cellular debris was removed by centrifugation. The concentration of cytosolic protein was quantified by the Bradford method (13). A reaction mixture containing 250 µg cytosolic protein, NADPH, buffer A, and [2-13C]5-FU was incubated for 30 minutes. Every 5 minutes, 130 µL aliquots were removed and added to an equal volume of ice-cold ethanol. This mixture was incubated overnight at −80°C, thawed, and filtered to remove protein before high-performance liquid chromatography analysis. [6-13C]-5-FU and [6-13C]-5-FUH2 were separated and quantified using a previously described reverse-phase high-performance liquid chromatography method (11, 12). The amount of [6-14C]-5-FUH2 formed at each time point (Y axis) was plotted against time (X axis). Linear regression analysis was used to calculate the equation of the line and determine the formation rate of [6-13C]-5-FUH2, 5-FUH2 and 5-FUH2K by the amount of protein used in the reaction mixture (i.e., nmol/min/mg protein). Subjects were considered to be partially DPD deficient by radioassay when their fresh PBMC DPD activity was <0.18 nmol/min/mg protein (11). Subjects were considered to be profoundly DPD deficient by radioassay when their PBMC DPD activity was undetectable.

UraBT breath test. The UraBT principle and detailed methodology has been described previously (9). At −8 a.m. on the day of testing, fasting subjects were weighed and an aqueous solution containing 6 mg/kg [2-13C]uracil (Cambridge Isotope Laboratories, Inc., Andover, MA) was prepared. Subjects donated three baseline breath samples into 1.2 L breath bags (Otsuka Pharmaceutical, Tokushima, Japan) followed by oral administration of the [2-13C]uracil solution. Post-dose breath samples were collected into 100 mL breath bags (Otsuka Pharmaceutical) during the 180-minute period immediately following [2-13C]uracil administration. IR spectrophotometry (UBIT-IR3000, Meretek, Lafayette, CO) was used to measure breath 13CO2 concentrations, which were reported in delta over baseline (DOB) notation as described previously (9). Breath profiles were constructed by plotting the concentration of 13CO2 in breath at each time point (Y axis) against time (X axis). The percent dose of [2-13C]uracil recovered in the breath as 13CO2 (PDR) was calculated as described elsewhere (14). Breath 13CO2 maximum plasma concentration (Cmax), time to Cmax (Tmax), and DOB50 ([13C]CO2 concentration in breath 50 minutes after [2-13C]uracil administration) were determined by inspection of breath profiles (9). Subjects showing a DOB50 < 128.9 DOB were classified as DPD deficient (9). Subjects showing a DOB50 ≥ 128.9 DOB were classified as having normal DPD activity (9).

Liquid chromatography-tandem mass spectrometry analysis 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: 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 was done following minor modification of a previously described liquid chromatography-tandem mass spectrometry method (15). Briefly, isotope-labeled [13C4,15N2]uracil and [13C5,15N2]dihydrouracil (Cambridge Isotope Laboratories) were used as internal standards. Plasma protein was precipitated by adding 500 µL of a saturated ammonium sulfate solution and 4 mL acetonitrile to 500 µL plasma. Following centrifugation, the organic layer was collected, evaporated, and reconstituted in 200 µL purified water. The mixture was injected into the liquid chromatography-tandem mass spectrometry system (TSQ7000, Thermo Finnigan, San Jose, CA). [2-13C]Uracil and [2-13C]dihydrouracil were separated on a Developosil RAQQUEOUS reverse-phase column (5 µm, 2.0 × 150 mm; Normura Chemical Co., Ltd., Seto, Japan) in a mobile phase of 1:99 (v/v) methanol/water. Atmospheric pressure chemical ionization was used to form protonated analytes and fragment them. Selected reaction monitoring was used to detect the fragmentation pattern of parent and daughter ions and quantify the concentrations of [2-13C]uracil and [2-13C]dihydrouracil.

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 AUCt, Cmax, Tmax, apparent clearance (CL/F), terminal apparent distribution volume (Vd/F), and elimination half-life (t1/2). AUCt was determined using the trapezoidal rule (16). Cmax and Tmax were taken directly from the observed concentration-time data. CL/F was calculated as dose/AUCt. Vd/F was calculated as dose divided by the product of terminal elimination rate constant, k2, and AUCt. The elimination rate constant was determined by linear regression of the terminal elimination phase concentration-time points. t1/2 was calculated as ln(2)/k2.


**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 DOB50, 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 DOB50 ≥ 128.9 DOB. All partially and profoundly DPD-deficient subjects showed DOB50 < 128.9 DOB. Altered breath 13CO2 concentration-time profiles were also observed in all DPD-deficient subjects. Specifically, profoundly and partially DPD-deficient subject(s) showed an increased UraBT Tmax and reduced UraBT 13CO2 Cmax, DOB50, AUC, and PDR compared with subjects with normal DPD activity (all Ps < 0.05).

UraBT DOB50 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 Cmax between subjects with normal activity and those with partial or profound DPD deficiency. No significant difference was observed in plasma [2-13C]uracil Tmax from subjects with normal DPD activity and subjects with partial DPD deficiency. However, a significant difference was observed in plasma [2-13C]uracil Tmax from 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 t1/2 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 t1/2 (all Ps < 0.05; Table 3).

The UraBT DOB50 were also significantly correlated with several pharmacokinetic variables of uracil catabolism. Specifically, the UraBT DOB50 were significantly correlated with plasma [2-13C]uracil clearance and inversely correlated with plasma [2-13C]uracil AUC (Fig. 1C) and t1/2 (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>DOB50 (DOB)*</td>
<td>183.2 ± 31.2</td>
<td>83.7 ± 25.3</td>
<td>0.9*</td>
</tr>
<tr>
<td>Cmax (DOB)*</td>
<td>193.8 ± 28.1</td>
<td>121.8 ± 36.7</td>
<td>3.6*</td>
</tr>
<tr>
<td>Tmax (min)*</td>
<td>50.5 ± 10.8</td>
<td>125.4 ± 43.2</td>
<td>120.0*</td>
</tr>
<tr>
<td>AUC, (%min)*</td>
<td>12,507.9 ± 4,327.4</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.

*DOB50, 13CO2 concentration in breath (DOB) 50 minutes after [2-13C]uracil administration; Cmax, maximum concentration of 13CO2 in breath; Tmax, time to Cmax; AUC, area under the 13CO2 breath curve; PDR, percent dose of [2-13C]uracil recovered in the breath as 13CO2; DPD activity, fresh PBMC DPD enzyme activity.

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

2 *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 beneath the limit of detection; thus, pharmacokinetic variables could not be determined. Partially deficient subjects showed significantly decreased plasma [2-13C]dihydrouracil clearance, [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, 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 was 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 [2-13C]uracil in subjects with a profound deficiency, mean, bars, SD. Points, mean; bars, SD. Reduced 5-FU clearance with an increased 5-FU dihydouracil in both metabolism and elimination were noted as indicated by the appearance of [2-13C]dihydrouracil in plasma (within 10 minutes) and 13CO2 in the breath. The plasma concentration-time profiles of [2-13C]uracil, with 1 mol [2-13C]dihydrouracil being converted to 1 mol [2-13C]dihydrouracil is the exclusive and direct indicator of DPD activity. This conclusion is supported by the biochemical pathway of uracil catabolism where 1 mol [2-13C]uracil being converted to 1 mol [2-13C]dihydrouracil is the exclusive and direct indicator of DPD activity. This conclusion is based on the rationale that DPD-mediated metabolism of [2-13C]uracil to [2-13C]dihydrouracil is the exclusive and singular source of plasma [2-13C]dihydrouracil, with 1 mol [2-13C]uracil being converted to 1 mol [2-13C]dihydrouracil by DPD.

Several previous studies of DPD-deficient cancer patients have reported reduced 5-FU clearance with an increased 5-FU elimination half-life, Cmax, and area under the plasma [2-13C]uracil concentration-time curve; CL/F, apparent clearance; Cmax, maximum concentration of [2-13C]uracil in plasma. Our observations with orally administered [2-13C]uracil parallel these findings. Specifically, we observed significantly reduced plasma [2-13C]uracil clearance in partially and profoundly DPD-deficient subjects, which resulted in increased plasma [2-13C]uracil t1/2 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 t1/2 and DPD activity as well as positive correlations between 5-FU clearance and DPD activity (25, 26). Our observations with orally administered [2-13C]uracil also parallel these studies. We reported inverse correlations between PBMC DPD activity and plasma [2-13C]uracil AUC and t1/2 as well as a positive correlation between PBMC DPD activity and plasma [2-13C]uracil clearance. Using [2-13C]uracil, we noted significant correlations between PBMC DPD activity and several [2-13C]dihydrouracil pharmacokinetic variables. In particular, PBMC DPD activity was significantly correlated with plasma [2-13C]dihydrouracil appearance rate, amount of [2-13C]dihydrouracil formed, and [2-13C]dihydrouracil Cmax. In turn, a significant correlation between DPD-mediated plasma [2-13C]dihydrouracil formation and breath 13CO2 formation was observed, suggesting that the UraBT 13CO2 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

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**Table 2. Comparison of plasma [2-13C]uracil 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>[2-13C]uracil t1/2 (min)*</td>
<td>15.9 ± 1.7</td>
<td>39.3 ± 31.2*</td>
<td>306.6*</td>
</tr>
<tr>
<td>[2-13C]uracil AUC, (min μg/mL)*</td>
<td>257.5 ± 93.4</td>
<td>480.3 ± 187.6*</td>
<td>1236.1*</td>
</tr>
<tr>
<td>[2-13C]uracil CL/F (mL/min/kg)*</td>
<td>28.6 ± 10.5</td>
<td>14.1 ± 6.0*</td>
<td>1.3*</td>
</tr>
<tr>
<td>[2-13C]uracil Cmax (μg/mL)*</td>
<td>8.45 ± 3.09</td>
<td>8.87 ± 3.88*</td>
<td>10.19*</td>
</tr>
<tr>
<td>[2-13C]uracil Tmax (min)</td>
<td>28.9 ± 9.5</td>
<td>32.2 ± 13.8</td>
<td>60.0*</td>
</tr>
</tbody>
</table>

**NOTE:** [2-13C]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 [2-13C]uracil concentrations, [2-13C]uracil pharmacokinetic variables were determined. Data are mean ± SD. *t1/2, elimination half-life; AUC, area under the plasma [2-13C]uracil concentration-time curve; CL/F, apparent clearance; Cmax, maximum concentration of [2-13C]uracil in plasma. 1 P < 0.05 for both pairwise comparisons (normal DPD activity versus partial DPD deficiency and normal DPD activity versus profound DPD deficiency). 1P > 0.05 for both pairwise comparisons (normal DPD activity versus partial DPD deficiency and normal DPD activity versus profound DPD deficiency). 1P < 0.05 (normal DPD activity versus partial DPD deficiency). 1P > 0.05 (normal DPD activity versus profound DPD deficiency).


**Table 3. Pharmacokinetic variables of [2-13C]uracil catabolism correlate with DPD activity and the UraBT**

<table>
<thead>
<tr>
<th>DPD activity (nmol/min/mg)</th>
<th>UraBT DOB50 (DOB)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>[2-13C]Uracil CL/F (mL/min/kg)</td>
<td>0.67</td>
</tr>
<tr>
<td>[2-13C]Uracil AUC (min µg/mL)</td>
<td>−0.72</td>
</tr>
<tr>
<td>[2-13C]Uracil t1/2 (min)</td>
<td>−0.54</td>
</tr>
<tr>
<td>[2-13C]Uracil metabolized (%)</td>
<td>0.87</td>
</tr>
<tr>
<td>[2-13C]Dihydrouracil appearance rate (µg/mL/min)</td>
<td>0.59</td>
</tr>
<tr>
<td>[2-13C]Dihydrouracil formed (mg)</td>
<td>0.61</td>
</tr>
<tr>
<td>DUUR</td>
<td>0.67</td>
</tr>
<tr>
<td>[2-13C]Dihydrouracil Cmax (µg/mL)</td>
<td>0.43</td>
</tr>
<tr>
<td>[2-13C]Dihydrouracil Tmax (min)</td>
<td>−0.64</td>
</tr>
</tbody>
</table>

*All Pearson correlation coefficients (r) are significant (all Ps ≤ 0.05).
†DUUR, ratio of plasma dihydrouracil AUC/uracil AUC; Cmax, maximum concentration of [2-13C]dihydrouracil in plasma.

13CO2 is produced for every 1 mol [2-13C]uracil reduced to [2-13C]dihydrouracil by DPD.

Although we observed significant correlations between PBMC DPD activity and [2-13C]uracil clearance as well as between PBMC DPD activity and [2-13C]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 formation being the liver. Hence, the 13CO2 detected in our assay should be primarily formed in the liver. However, ethical considerations prevented the measurement of hepatic DPD 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 13CO2 concentrations than PBMC DPD activity (15). Hepatic DPD activity was significantly correlated with systemic DPD-mediated reduction of [2-13C]uracil as measured in breath 13CO2 concentrations (r = 0.9999; ref. 15). This animal study suggests that hepatic DPD activity should strongly correlate with breath 13CO2 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 DOB50 and DUUR.

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

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**Table 4. Comparison of plasma [2-13C]dihydrouracil pharmacokinetic variables from subjects with normal DPD activity and partial and profound DPD deficiency**

<table>
<thead>
<tr>
<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>[2-13C]Dihydrouracil Cmax (µg/mL)</td>
<td>2.00 ± 0.53†</td>
<td>1.36 ± 0.43†</td>
</tr>
<tr>
<td>[2-13C]Dihydrouracil Tmax (min)</td>
<td>66.3 ± 22.5†</td>
<td>125.4 ± 29.4†</td>
</tr>
<tr>
<td>[2-13C]Dihydrouracil t1/2 (min)</td>
<td>70.4 ± 24.2†</td>
<td>367.9 ± 354.0†</td>
</tr>
<tr>
<td>[2-13C]Dihydrouracil formed (mg)</td>
<td>380.0 ± 86.2†</td>
<td>153.7 ± 80.9†</td>
</tr>
<tr>
<td>[2-13C]Dihydrouracil appearance rate (µg/mL/min)</td>
<td>0.03 ± 0.01†</td>
<td>0.01 ± 0.00†</td>
</tr>
<tr>
<td>DUUR</td>
<td>0.9 ± 0.4†</td>
<td>0.4 ± 0.3†</td>
</tr>
<tr>
<td>[2-13C]Uracil metabolized (%)</td>
<td>75.4 ± 9.8†</td>
<td>31.6 ± 19.4†</td>
</tr>
</tbody>
</table>

**NOTE:** [2-13C]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 [2-13C]dihydrouracil concentrations, [2-13C]dihydrouracil pharmacokinetic variables were determined. Data are mean ± SD.

*No [2-13C]dihydrouracil was detected in the plasma from the profoundly DPD-deficient subject.

†P < 0.05 (normal DPD activity versus partial DPD deficiency).
in [2-13C]uracil and [2-13C]dihydrouracil kinetics and UraBT 13CO2 concentrations (e.g., DObα) 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 $^{13}$CO$_2$ and Plasma [2-$^{13}$C]Dihydrouracil

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