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,[1] Jeanne Fourie,[1] Yukihiro Hirao,[3] Toshihisa Koga,[3] Renee A. Desmond,[2] Jennifer R. King,[1] Takefumi Shimizu,[3] and Robert B. Diasio[1]

Abstract

Purpose: Dihydropyrimidine dehydrogenase (DPD) deficiency is critical in the predisposition to 5-fluorouracil dose-related toxicity. We recently characterized the phenotypic [2-^{13}C]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-^{13}C]dihydrouracil formation, [2-^{13}C]uracil clearance, and DPD activity.

Experimental Design: An aqueous solution of [2-^{13}C]uracil (6 mg/kg) was orally administered to 23 healthy volunteers and 8 cancer patients. Subsequently, breath $^{13}CO_2$ concentrations and plasma [2-^{13}C]dihydrouracil and [2-^{13}C]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 $^{13}CO_2$ DOB_{50} significantly correlated with PBMC DPD activity (r_p = 0.78), plasma [2-^{13}C]uracil area under the curve (r_p = −0.73), [2-^{13}C]dihydrouracil appearance rate (r_p = 0.76), and proportion of [2-^{13}C]uracil metabolized to [2-^{13}C]dihydrouracil (r_p = 0.77; all Ps < 0.05).

Conclusions: UraBT breath $^{13}CO_2$ pharmacokinetics parallel plasma [2-^{13}C]uracil and [2-^{13}C]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.

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 fluoro-β-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 dihydrouracil area under the curve (AUC) to uracil AUC (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-^{13}C]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

Authors' Affiliations: Divisions of [1]Clinical Pharmacology and Toxicology and [2]Biostatistics, Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, Alabama and [3]Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

Received 9/15/05; revised 10/18/05; accepted 11/7/05.

Grant support: NIH grant CA62164 (R.B. Diasio) and National Center for Research Resources grant M01 RR-00032 (General Clinical Research Center, University of Alabama at Birmingham).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Robert B. Diasio, Division of Clinical Pharmacology and Toxicology, Comprehensive Cancer Center, University of Alabama at Birmingham, 1824 6th Avenue South, Wallace Tumor Institute, Room 620, Birmingham, AL 35294-3300. Fax: 205-975-5650; E-mail: robert.diasio@ccc.uab.edu.

doi:10.1158/1078-0432.CCR-05-2020

99% specificity (with 239 of 241 subjects with DPD activity in the reference range identified as normal; ref. 10). To date, however, the clinical relationship between pharmacokinetics of the [2-^{13}C]uracil probe substrate and its metabolites in plasma and breath remains to be elucidated.

Based on our initial characterization of the UraBT, we hypothesize that (*a*) [2-^{13}C]uracil metabolite pharmacokinetic variables in the breath and plasma are reflective of DPD activity and (*b*) breath $^{13}CO_2$ concentrations as measured through the UraBT correlate with plasma [2-^{13}C]uracil metabolite pharmacokinetics. In the present study, we provide a detailed characterization of the UraBT showing the relationship among breath $^{13}CO_2$ metabolite formation, plasma [2-^{13}C]dihydrouracil formation, [2-^{13}C]uracil clearance, and DPD activity.

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 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 Ficoll 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 [6-^{14}C]-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-^{14}C]-5-FU and [6-^{14}C]-5-FUH$_2$ were separated and quantified using a previously described reverse-phase high-performance liquid chromatography method (11, 12). The amount of [6-^{14}C]-5-FUH$_2$ 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-^{14}C]-5-FUH$_2$. DPD enzyme activity was calculated by dividing the formation rate of [6-^{14}C]-5-FUH$_2$ 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.

Uracil 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-^{13}C]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-^{13}C]uracil solution. Post-dose breath samples were collected into 100 mL breath bags (Otsuka Pharmaceutical) during the 180-minute period immediately following [2-^{13}C]uracil administration. IR spectrophotometry (UBiT-IR$_{300}$, Meretek, Lafayette, CO) was used to measure breath $^{13}CO_2$ concentrations, which were reported in delta over baseline (DOB) notation as described previously (9). Breath profiles were constructed by plotting the concentration of $^{13}CO_2$ in breath at each time point (*Y* axis) against time (*X* axis). The percent dose of [2-^{13}C]uracil recovered in the breath as $^{13}CO_2$ (PDR) was calculated as described elsewhere (14). Breath $^{13}CO_2$ maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), and DOB$_{50}$ ($^{13}CO_2$ concentration in breath 50 minutes after [2-^{13}C]uracil administration) were determined by inspection of breath profiles (9). Subjects showing a DOB$_{50}$ < 128.9 DOB were classified as DPD deficient (9). Subjects showing a DOB$_{50}$ ≥ 128.9 DOB were classified as having normal DPD activity (9).

Liquid chromatography-tandem mass spectrometry analysis of plasma [2-^{13}C]uracil and [2-^{13}C]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-^{13}C]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-^{13}C]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-^{13}C]uracil and [2-^{13}C]dihydrouracil was done following minor modification of a previously described liquid chromatography-tandem mass spectrometry method (15). Briefly, isotope-labeled [$^{13}C_4$,$^{15}N_2$]uracil and [$^{13}C_4$,$^{15}N_2$]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 acetronitrile 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-^{13}C]Uracil and [2-^{13}C]dihydrouracil were separated on a Develosil RAPQUEOUS 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-^{13}C]uracil and [2-^{13}C]dihydrouracil.

Pharmacokinetic analysis. Concentration-time profiles of plasma [2-^{13}C]uracil and [2-^{13}C]dihydrouracil were constructed. Noncompartmental methods (WinNonlin version 4.1, Pharsight Corp., Mountain View, CA) were used to determine the pharmacokinetic variables of [2-^{13}C]uracil in plasma, [2-^{13}C]dihydrouracil in plasma, and $^{13}CO_2$ in breath. Calculated pharmacokinetic variables were AUC$_\tau$, C_{max}, T_{max}, apparent clearance (CL/F), terminal apparent distribution volume (V_z/F), and elimination half-life ($t_{1/2}$). AUC$_\tau$ was determined using the trapezoidal rule (16). C_{max} and T_{max} were taken directly from the observed concentration-time data. CL/F was calculated as dose/AUC$_\tau$. V_z/F was calculated as dose divided by the product of terminal elimination rate constant, λ_z, and AUC$_\tau$. The elimination rate constant was determined by linear regression of the terminal elimination phase concentration-time points; $t_{1/2}$ was calculated as $\ln(2)/\lambda_z$.

[2-^{13}C]Dihydrouracil may only be produced in appreciable quantities *in vivo* by the DPD-mediated catabolism of [2-^{13}C]uracil. To assess formation of [2-^{13}C]dihydrouracil (metabolite) from [2-^{13}C]uracil (probe substrate) by DPD, the [2-^{13}C]dihydrouracil appearance rate,

amount of [2-^{13}C]dihydrouracil formed, and proportion of [2-^{13}C]uracil metabolized to [2-^{13}C]dihydrouracil were estimated. The [2-^{13}C]dihydrouracil appearance rate in plasma was determined from the slope of the line following regression analysis of the plasma [2-^{13}C]dihydrouracil concentration-time plot from baseline (t = 0 minute) to C_{max}. The amount of [2-^{13}C]dihydrouracil formed was calculated by multiplying [2-^{13}C]dihydrouracil AUC_{τ} and clearance (17). The proportion of [2-^{13}C]uracil metabolized to [2-^{13}C]dihydrouracil was calculated by dividing the amount of [2-^{13}C]dihydrouracil formed over 180 minutes by the amount of orally administered [2-^{13}C]uracil.

Statistical analysis. Summary data stratified by DPD activity are presented as mean ± SD. Comparisons of plasma [2-^{13}C]uracil and [2-^{13}C]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-^{13}C]uracil and [2-^{13}C]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 $DOB_{50} \geq 128.9$ DOB. All partially and profoundly DPD-deficient subjects showed $DOB_{50} < 128.9$ DOB. Altered breath $^{13}CO_2$ concentration-time profiles were also observed in all DPD-deficient subjects. Specifically, profoundly and partially DPD-deficient subject(s) showed an increased UraBT T_{max} and reduced UraBT $^{13}CO_2$ C_{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-^{13}C]uracil pharmacokinetics in subjects with normal and reduced PBMC DPD activity. Plasma [2-^{13}C]uracil pharmacokinetic variables (mean ± SD) obtained from subjects with normal and reduced DPD activity are summarized in Table 2. [2-^{13}C]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-^{13}C]uracil C_{max} between subjects with normal activity and those with partial or profound DPD deficiency. No significant difference was observed in plasma [2-^{13}C]uracil T_{max} from subjects with normal DPD activity and subjects with partial DPD deficiency. However, a significant difference was observed in plasma [2-^{13}C]uracil T_{max} from the subjects with normal DPD activity and the profoundly DPD-deficient subject.

Reduced [2-^{13}C]uracil catabolism was observed in all DPD-deficient subjects (Fig. 2A). Both profoundly and partially DPD-deficient subject(s) showed increased plasma [2-^{13}C]uracil $t_{1/2}$ and AUC and reduced plasma [2-^{13}C]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-^{13}C]uracil clearance (Fig. 1B) and inversely correlated with plasma [2-^{13}C]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-^{13}C]uracil clearance and inversely correlated with plasma [2-^{13}C]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

	Normal activity (n = 19)	Partial DPD deficiency (n = 11)	Profound DPD deficiency (n = 1)
DOB_{50} (DOB)*	183.2 ± 31.2†	83.7 ± 25.3†	0.9†
C_{max} (DOB)*	193.8 ± 28.1†	121.8 ± 36.7†	3.6†
T_{max} (min)*	50.5 ± 10.8†	125.4 ± 43.2†	120.0†
AUC_{τ} (‰ min)*	21,597.1 ± 2,634.2†	15,572.9 ± 4,327.4†	348.7†
PDR*	55.7 ± 4.9†	39.8 ± 9.9†	<1.0†
DPD activity (nmol/min/mg)*	0.27 ± 0.06‡	0.11 ± 0.05‡	Undetectable

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-^{13}C]uracil (6 mg/kg dose). Data are mean ± SD.

*DOB_{50}, $^{13}CO_2$ concentration in breath (DOB) 50 minutes after [2-^{13}C]uracil administration; C_{max}, maximum concentration of $^{13}CO_2$ in breath; T_{max}, time to C_{max}; AUC_{τ}, area under the $^{13}CO_2$ breath curve; PDR, percent dose of [2-^{13}C]uracil recovered in the breath as $^{13}CO_2$; 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).

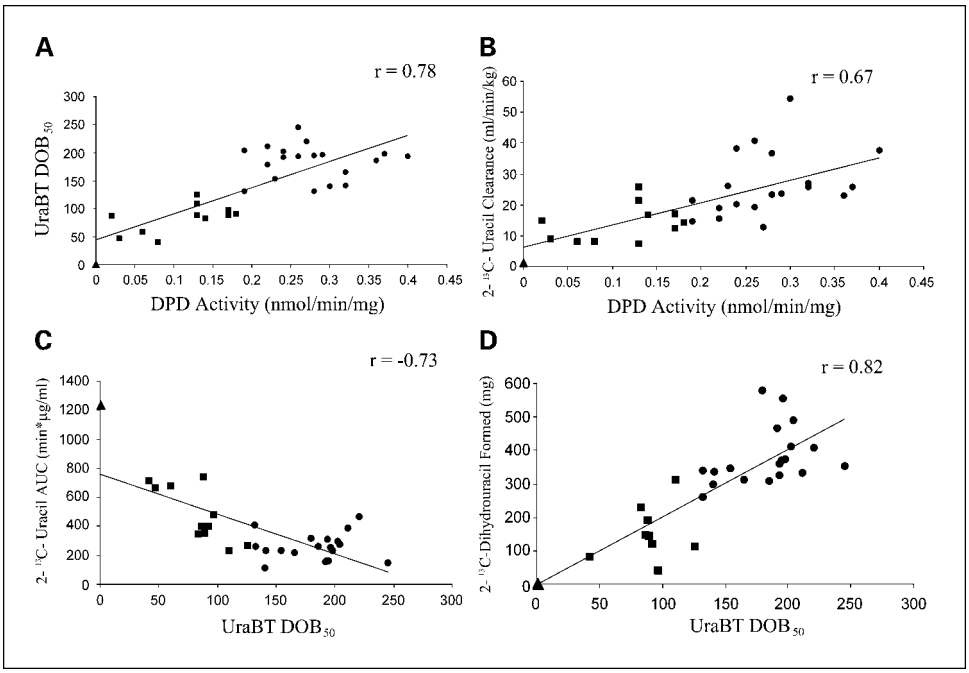

Fig. 1. Correlation of PBMC DPD activity, the UraBT, [2-^{13}C]uracil clearance, and [2-^{13}C]dihydrouracil formation. Nineteen subjects with normal DPD activity (●), 11 subjects with partial DPD deficiency (■), and 1 subject with profound DPD deficiency (▲) ingested a 6 mg/kg oral solution of [2-^{13}C]uracil and performed the UraBT. DPD activity was determined as described. Plasma samples were collected for 180 minutes after [2-^{13}C]uracil ingestion and the concentration of [2-^{13}C]uracil and [2-^{13}C]dihydrouracil was determined for each plasma sample. [2-^{13}C]Uracil clearance, [2-^{13}C]uracil AUC, and amount of [2-^{13}C]dihydrouracil formed was determined as described. Significant correlation was shown between PBMC DPD activity and UraBT DOB_{50} (*A*; $P < 0.05$), PBMC DPD activity and [2-^{13}C]uracil clearance (*B*; $P < 0.05$), UraBT DOB_{50} and [2-^{13}C]uracil AUC (*C*; $P < 0.05$), and UraBT DOB_{50} and amount of [2-^{13}C]dihydrouracil formed (*D*; $P < 0.05$).

Comparison of [2-^{13}C]dihydrouracil plasma pharmacokinetics in subjects with normal and reduced PBMC DPD activity. Plasma [2-^{13}C]dihydrouracil pharmacokinetic variables (mean ± SD) obtained in subjects with normal and reduced DPD activity are summarized in Table 4. Altered plasma [2-^{13}C]dihydrouracil concentrations were observed in DPD-deficient subjects (Fig. 2B). The profoundly deficient subject showed plasma [2-^{13}C]dihydrouracil concentrations beneath the limit of detection; thus, pharmacokinetic variables could not be determined. Partially deficient subjects showed significantly decreased plasma [2-^{13}C]dihydrouracil C_{max} and increased plasma [2-^{13}C]dihydrouracil T_{max} and $t_{1/2}$ compared with subjects with normal DPD activity (all *P*s < 0.05). Partially deficient subjects also showed a significant reduction in the proportion of [2-^{13}C]uracil metabolized to [2-^{13}C]dihydrouracil, [2-^{13}C]dihydrouracil appearance rate, amount of [2-^{13}C]dihydrouracil formed, and plasma DUUR (all *P*s < 0.05).

[2-^{13}C]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-^{13}C]uracil metabolized to [2-^{13}C]dihydrouracil, [2-^{13}C]dihydrouracil appearance rate, amount of [2-^{13}C]dihydrouracil formed, plasma C_{max}, and plasma DUUR (all *P*s < 0.05). PBMC DPD activity was also inversely correlated with plasma [2-^{13}C]dihydrouracil T_{max} ($P < 0.05$).

[2-^{13}C]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-^{13}C]uracil metabolized to [2-^{13}C]dihydrouracil, [2-^{13}C]dihydrouracil appearance rate, amount of [2-^{13}C]dihydrouracil formed (Fig. 1D), plasma DUUR, and plasma [2-^{13}C]dihydrouracil C_{max} (all *P*s < 0.05). UraBT DOB_{50} were inversely correlated with plasma [2-^{13}C]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-^{13}C]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 $^{13}CO_2$ 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 $^{13}CO_2$ kinetic profiles to plasma [2-^{13}C]uracil and [2-^{13}C]dihydrouracil kinetics.

Examination of plasma [2-^{13}C]uracil concentration-time profiles showed that orally administered [2-^{13}C]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-^{13}C]uracil (15).

Following absorption of [2-^{13}C]uracil in subjects with normal DPD activity, the [2-^{13}C]uracil was observed to peak

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 (n = 19)	Partial DPD deficiency (n = 11)	Profound DPD deficiency (n = 1)
[2-^{13}C]uracil $t_{1/2}$ (min)*	15.9 ± 1.7†	39.3 ± 31.2†	306.6†
[2-^{13}C]uracil AUC_τ (min μg/mL)*	257.5 ± 93.4†	480.3 ± 187.6†	1236.1†
[2-^{13}C]uracil CL/F (mL/min/kg)*	26.6 ± 10.5†	14.1 ± 6.0†	1.3†
[2-^{13}C]uracil C_{max} (μg/mL)*	8.45 ± 3.09‡	8.87 ± 3.88‡	10.19‡
[2-^{13}C]uracil T_{max} (min)	28.9 ± 9.5§‖	33.2 ± 13.8§	60.0‖

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.

*$t_{1/2}$, elimination half-life; AUC_τ, area under the plasma [2-^{13}C]uracil concentration-time curve; CL/F, apparent clearance; C_{max}, maximum concentration of [2-^{13}C]uracil in plasma.

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

‡$P > 0.05$ 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).

‖$P < 0.05$ (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.

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 both 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_{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

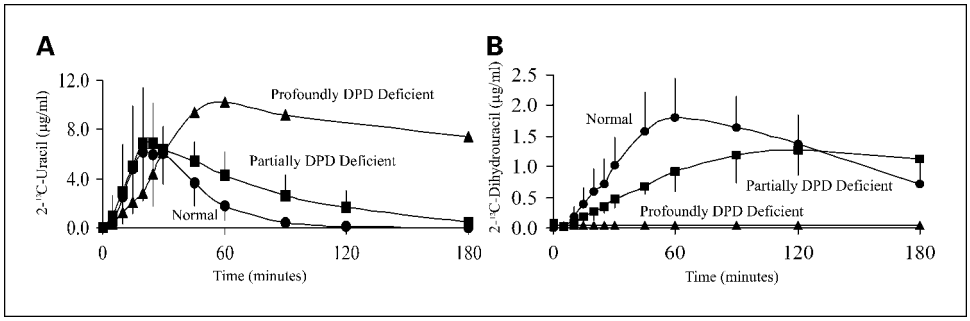

Fig. 2. Plasma [2-^{13}C]uracil (*A*) and [2-^{13}C]dihydrouracil (*B*) concentrations from subjects with normal DPD activity and partial and profound DPD deficiency. The plasma concentration-time profiles (mean ± SD) of [2-^{13}C]uracil (*A*) and [2-^{13}C]dihydrouracil (*B*) from 19 subjects with normal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

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

	DPD activity (nmol/min/mg)*	UraBT DOB_{50} (DOB)*
[2-^{13}C]Uracil CL/F (mL/min/kg)	0.67	0.59
[2-^{13}C]Uracil AUC_{τ} (min μg/mL)	−0.72	−0.73
[2-^{13}C]Uracil $t_{1/2}$ (min)	−0.54	−0.59
[2-^{13}C]Uracil metabolized (%)	0.67	0.77
[2-^{13}C]Dihydrouracil appearance rate (μg/mL/min)	0.59	0.76
[2-^{13}C]Dihydrouracil formed (mg)	0.61	0.82
DUUR†	0.67	0.65
[2-^{13}C]Dihydrouracil C_{max} (μg/mL)†	0.43	0.70
[2-^{13}C]Dihydrouracil T_{max} (min)	−0.64	−0.68

*All Pearson correlation coefficients (r_p) are significant (all Ps < 0.05).
†DUUR, ratio of plasma dihydrouracil AUC/uracil AUC; C_{max}, maximum concentration of [2-^{13}C]dihydrouracil in plasma.

$^{13}CO_2$ is produced for every 1 mol [2-^{13}C]uracil reduced to [2-^{13}C]dihydrouracil by DPD.

Although we observed significant correlations between PBMC DPD activity and [2-^{13}C]uracil clearance as well as between PBMC DPD activity and [2-^{13}C]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 $^{13}CO_2$ 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 $^{13}CO_2$ concentrations than PBMC DPD activity (15). Hepatic DPD activity was significantly correlated with systemic DPD-mediated reduction of [2-^{13}C]uracil as measured in breath $^{13}CO_2$ concentrations (r = 0.9999; ref. 15). This animal study suggests that hepatic DPD activity should strongly correlate with breath $^{13}CO_2$ 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}C]uracil and [2-^{13}C]dihydrouracil concentrations in subjects with normal and reduced DPD activity. In the present study, we showed significant differences

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

	Normal DPD activity (n = 19)	Partial DPD deficiency (n = 11)	Profound DPD deficiency (n = 1)*
[2-^{13}C]Dihydrouracil C_{max} (μg/mL)	2.00 ± 0.53†	1.36 ± 0.43†	—
[2-^{13}C]Dihydrouracil T_{max} (min)	66.3 ± 22.5†	125.4 ± 29.4†	—
[2-^{13}C]Dihydrouracil $t_{1/2}$ (min)	70.4 ± 24.2†	367.9 ± 354.0†	—
[2-^{13}C]Dihydrouracil formed (mg)	380.0 ± 86.2†	153.7 ± 80.9†	—
[2-^{13}C]Dihydrouracil appearance rate (μg/mL/min)	0.03 ± 0.01†	0.01 ± 0.00†	—
DUUR	0.9 ± 0.4†	0.4 ± 0.3†	—
[2-^{13}C]Uracil metabolized (%)	75.4 ± 9.8†	31.6 ± 19.4†	—

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]dihydrouracil concentrations, [2-^{13}C]dihydrouracil pharmacokinetic variables were determined. Data are mean ± SD.
*No [2-^{13}C]dihydrouracil was detected in the plasma from the profoundly DPD-deficient subject.
†P < 0.05 (normal DPD activity versus partial DPD deficiency).

in [2-^{13}C]uracil and [2-^{13}C]dihydrouracil kinetics and UraBT $^{13}CO_2$ concentrations (e.g., DOB_{50}) in subjects with decreased DPD activity versus those with normal DPD activity. The significant correlations between DPD activity and either plasma [2-^{13}C]uracil clearance, [2-^{13}C]dihydrouracil formation, or $^{13}CO_2$ breath concentrations provide further support that the UraBT may be useful for assessment of DPD deficiency before administration of 5-FU.

References

1. Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003;3:330–8.
2. Heggie GD, Sommadossi JP, Cross DS, Huster WJ, Diasio RB. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. Cancer Res 1987;47:2203–6.
3. Naguib FN, el Kouni MH, Cha S. Enzymes of uracil catabolism in normal and neoplastic human tissues. Cancer Res 1985;45:5405–12.
4. Lu Z, Zhang R, Carpenter JT, Diasio RB. Decreased dihydropyrimidine dehydrogenase activity in a population of patients with breast cancer: implication for 5-fluorouracil-based chemotherapy. Clin Cancer Res 1998;4:325–9.
5. Van Kuilenburg AB, Meinsma R, Zoetekouw L, Van Gennip AH. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. Pharmacogenetics 2002;12:555–8.
6. Gamelin E, Boisdron-Celle M, Guerin-Meyer V, et al. Correlation between uracil and dihydrouracil plasma ratio, fluorouracil (5-FU) pharmacokinetic parameters, and tolerance in patients with advanced colorectal cancer: a potential interest for predicting 5-FU toxicity and determining optimal 5-FU dosage. J Clin Oncol 1999;17:1105.
7. Jiang H, Lu J, Jiang J, Hu P. Important role of the dihydrouracil/uracil ratio in marked interpatient variations of fluoropyrimidine pharmacokinetics and pharmacodynamics. J Clin Pharmacol 2004;44:1260–72.
8. Jiang H, Lu J, Ji J. Circadian rhythm of dihydrouracil/uracil ratios in biological fluids: a potential biomarker for dihydropyrimidine dehydrogenase levels. Br J Pharmacol 2004;141:616–23.
9. Mattison LK, Ezzeldin H, Carpenter M, Modak A, Johnson MR, Diasio RB. Rapid identification of dihydropyrimidine dehydrogenase deficiency by using a novel 2-^{13}C-uracil breath test. Clin Cancer Res 2004;10:2652–8.
10. Mattison LK, Fourie J, Carpenter M, et al. Evidence for increased incidence of dihydropyrimidine dehydrogenase (DPD) deficiency in African Americans compared to Caucasians. Proc Amer Soc Clin Onc 23, 136s, Abstract #2004, 2005.
11. Lu Z, Zhang R, Diasio RB. Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients, and clinical implication in 5-fluorouracil chemotherapy. Cancer Res 1993;53:5433–8.
12. Johnson MR, Yan J, Shao L, Albin N, Diasio RB. Semi-automated radioassay for determination of dihydropyrimidine dehydrogenase (DPD) activity. Screening cancer patients for DPD deficiency, a condition associated with 5-fluorouracil toxicity. J Chromatogr B Biomed Sci Appl 1997;696:183–91.
13. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
14. Amarri S, Weaver LT. ^{13}C-Breath tests to measure fat and carbohydrate digestion in clinical practice. Clin Nutr 1995;14:149–54.
15. Inada M, Hirao Y, Koga T, et al. Relationships among plasma [2-(13)C]uracil concentrations, breath (13)CO(2) expiration, and dihydropyrimidine dehydrogenase (DPD) activity in the liver in normal and dpd-deficient dogs. Drug Metab Dispos 2005;33:381–7.
16. Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker; 1982.
17. Rowland R, Tozer TN. Metabolite kinetics. In: Balado D, editor. Clinical pharmacokinetics: concepts and applications. 3rd ed. Baltimore: Lippincott Williams and Wilkins; 1995. p.386.
18. Daher GC, Harris BE, Diasio RB. Metabolism of pyrimidine analogues and their nucleosides. Pharmacol Ther 1990;48:189–222.
19. Canellakis ES. Pyrimidine metabolism. I. Enzymatic pathways of uracil and thymine degradation. J Biol Chem 1956;221:315–22.
20. Lu ZH, Zhang R, Diasio RB. Purification and characterization of dihydropyrimidine dehydrogenase from human liver. J Biol Chem 1992;267:17102–9.
21. Christophidis N, Vajda F, Lucas I, Drummer O, Moon WJ. Fluorouracil therapy in patients with carcinoma of the large bowel: a pharmacokinetic comparison of various rates and routes of administration. Clin Pharmacokinet 1978;3:330–6.
22. Cohen JL, Irwin LE, Marshall GJ, Darvey H, Bateman JR. Clinical pharmacology of oral and intravenous 5-fluorouracil (NSC-19893). Cancer Chemother Rep 1974;58:723–31.
23. Diasio RB, Beavers TL, Carpenter JT. Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. J Clin Invest 1988;81:47–51.
24. Maring JG, van Kuilenburg AB, Haasjes J, et al. Reduced 5-FU clearance in a patient with low DPD activity due to heterozygosity for a mutant allele of the *DPYD* gene. Br J Cancer 2002;86:1028–33.
25. Fleming RA, Milano G, Thyss A, et al. Correlation between dihydropyrimidine dehydrogenase activity in peripheral mononuclear cells and systemic clearance of fluorouracil in cancer patients. Cancer Res 1992;52:2899–902.
26. Harris BE, Song R, Soong SJ, Diasio RB. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. Cancer Res 1990;50:197–201.
27. Milano G, Chamorey AL. Clinical pharmacokinetics of 5-fluorouracil with consideration of chronopharmacokinetics. Chronobiol Int 2002;19:177–89.
28. Gamelin E, Boisdron-Celle M. Dose monitoring of 5-fluorouracil in patients with colorectal or head and neck cancer-status of the art. Crit Rev Oncol Hematol 1999;30:71–9.
29. Desoize B, Bouche O, Berthiot G, Coninx P. Puzzles in the clinical pharmacokinetics of fluorouracil. Anticancer Res 1998;18:4607–10.
30. Levi F, Zidani R, Misset JL. Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. International Organization for Cancer Chronotherapy. Lancet 1997;350:681–6.