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Journal of Chromatography B, 738 (2000) 249–258

JOURNAL OF CHROMATOGRAPHY B

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Measurement of plasma uracil using gas chromatography–mass spectrometry in normal individuals and in patients receiving inhibitors of dihydropyrimidine dehydrogenase

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Received 17 August 1999; received in revised form 5 November 1999; accepted 11 November 1999

Abstract

A sensitive gas chromatographic–mass spectrometric method is described for reliably measuring endogenous uracil in 100 μl of human plasma. Validation of this assay over a wide concentration range, 0.025 μ*M* to 250 μ*M* (0.0028 μg/ml to 28 μg/ml), allowed for the determination of plasma uracil in patients treated with agents such as eniluracil, an inhibitor of the pyrimidine catabolic enzyme, dihydropyrimidine dehydrogenase. Calibration standards were prepared in human plasma using the stable isotope, [$^{15}N_2$]uracil, to avoid interference from endogenous uracil and 10 μ*M* 5-chlorouracil was added as the internal standard. Published by Elsevier Science B.V.

Keywords: Uracil; Eniluracil; Dihydropyrimidine dehydrogenase

1. Introduction

Dihydropyrimidine dehydrogenase (DPD) is the key rate-limiting enzyme responsible for the catabolism of endogenous pyrimidine bases, such as uracil and thymine. DPD is also the major determinant of the rate of clearance of the cancer chemotherapeutic agent, 5-fluorouracil (5-FU) [1]. Recently, powerful inhibitors of this enzyme have been developed that block the catabolism of 5-FU both systemically and in tumor tissues. This allows for fluoropyrimidines to be administered orally with a potentially improved therapeutic index [2–5]. Inhibitors of DPD currently in clinical testing as cancer chemotherapeutic agents include eniluracil [2,3], and S-1 [6]. Furthermore, the antiviral agents, sorivudine [7] and netivudine [8], also have DPD inhibitory activity. The experimental anticancer agent, eniluracil, is an especially potent suicide inhibitor of DPD that binds covalently to the enzyme causing complete and essentially irreversible

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0378-4347/00/$ – see front matter Published by Elsevier Science B.V.
PII: S0378-4347(99)00528-9

inhibition [9]. Recovery of DPD-mediated pyrimidine catabolic activity following eniluracil treatment is presumed to require the synthesis of new enzyme. Inhibition of DPD can cause potentially lethal drug interactions when patients are co-administered agents that are normally metabolized by this enzyme. Fatal toxicities have been reported patients who inadvertently received standard doses of 5-FU after being treated with DPD inhibitors [10–12].

The exact duration of systemic DPD inhibition following the administration of eniluracil to patients is not known, but may last for up to several weeks [13]. In addition, the time to recover DPD activity following eniluracil therapy inhibition can vary in individual patients, and may depend on the dose of eniluracil administered [14]. Assays for determining DPD enzyme activity in peripheral blood mononuclear (PBM) cells have been developed to screen patients for genetic deficiencies in this enzyme [15], but these measurements may be less useful in monitoring dynamic drug-induced changes in systemic enzyme activity. For example, DPD recovery in PBM cells may not reflect the full systemic recovery of DPD activity in other important tissues, such as the liver or gastrointestinal tract.

A theoretically more sensitive marker for total body DPD activity is the plasma uracil concentration. Uracil, the natural substrate for DPD, accumulates in plasma when systemic DPD is inhibited [16]. For example, normal individuals have a basal plasma uracil concentration range from 100 n*M* to 300 n*M* [17,18], which is frequently below the lower limit of quantitation achievable by traditional high-performance liquid chromatography (HPLC) assays using ultraviolet detection [18]. However, after the administration of a DPD inhibitor such as eniluracil, plasma uracil concentrations can increase by 100-fold and remain elevated during the period of enzyme inhibition [14]. Thus, the plasma uracil concentration may be a clinically useful assay to monitor the dynamic status of systemic DPD activity. Therefore, we have developed a gas chromatography–mass spectrometry (GC–MS)-based method that easily measures basal endogenous human plasma uracil concentrations, yet is linear in response through the high micromolar range. This broad response allows for the measurement of plasma uracil both in normal individuals and in patients treated with DPD enzyme inhibitors. We are currently using this method to assess the duration and extent of enzyme inhibition in a clinical trial of the DPD inhibitor, eniluracil, administered in combination with oral 5-FU to adult cancer patients.

2. Experimental

2.1. Chemicals and solvents

Uracil and 5-chlorouracil (5-ClU) were obtained from Sigma (St. Louis, MO, USA). [$^{15}N_2$]Uracil was obtained from Isotec (Miamisburg, OH, USA). Pentafluorobenzylbromide (PFBzBr) was purchased from Pierce (Rockford, IL, USA). All solvents were HPLC-reagent grade and were purchased from Fisher (Fair Lawn, NJ, USA).

2.2. Sample preparation

Human plasma (100 μl) was placed into 15-ml polypropylene centrifuge tubes. Calibration standards were spiked with [$^{15}N_2$]uracil to generate final concentrations of 0.025, 0.25, 2.5, 25 and 250 μ*M* and with 10 μ*M* 5-ClU added as an internal standard. Protein was precipitated by the addition of 1.0 ml of acetonitrile followed by vigorous vortex mixing for 30 s and centrifugation at 3000 *g* for 15 min at 4°C. The supernatant was removed and transferred into 4-ml screw-top glass vials. Next, 100 μl of 1 *M* K_2HPO_4, pH 11.0 and 20 μl of PFBzBr were added and the tubes were sealed, vigorously shaken, and then heated at 100°C for 1 h in a heating block. After cooling to room temperature, samples were centrifuged at 3000 *g* for 15 min at 4°C and the supernatant (about 2 ml) was applied to a Bond-Elute 1 mg C_{18} solid-phase extraction column (Varian, Harbor City, CA, USA) which had been preconditioned by sequential washing with 1 ml of methanol and three times with 1 ml of water. After loading the sample on to the extraction column, it was washed three times with water (3×1 ml) and the retained sample components were eluted with 1 ml of ethanol into a 1.5-ml microfuge tube. Samples were evaporated to dryness using a Zymark TurboVap (Hopkinton, MA, USA) with filtered compressed air and a waterbath temperature of 50°C. The

residue was resuspended in 150 μl of hexane–acetone (2:1, v/v), centrifuged at 12 000 *g* for 10 min at 25°C to remove undissolved solids and analyzed by GC–MS.

2.3. GC–MS analysis

Quantitation of the derivatized bases was performed on a HP6890 GC system with a mass-selective detector and an HP7673 autosampler. Instrument operation was monitored with daily autotunes, and samples were analyzed with a dwell time of 40 ms per ion, using an electron multiplier offset of 300 V above the autotune value. Compounds were separated on a Supelco SPB-20 capillary column, 15 m×0.25 mm, with a 0.25 μm stationary phase thickness (Bellefonte, PA, USA). Helium at a constant column flow of 1.0 ml/min was used as the carrier gas. Aliquots of the sample (1 μl) were introduced into the GC column by automatic injection via a splitless injection with the purge valve turned on 0.3 min after injection. The injection port temperature was 294°C and the mass spectrometer transfer line was maintained at 290°C. The column temperature was initially set at 100°C and the oven temperature was programmed 1 min after injection as follows: 25°C/min to 190°C, then 10°C/min to 230°C followed by 25°C/min to 290°C and maintained for 2 min for a total run time of 13.0 min.

Data were obtained from the GC–MS system by selected-ion monitoring of the pyrimidine base derivatives. At 8 min after the start of the analysis, uracil was monitored using the following ions with *m/z* values of: 472 (uracil target ion), 248 (qualifier ion 1), and 96 (qualifier ion 2). For [$^{15}N_2$]uracil in the calibration standards, the following ions were monitored: 474 ([$^{15}N_2$]uracil target ion), 249 (qualifier ion 1), and 97 (qualifier ion 2). The retention time for both uracil and [$^{15}N_2$]uracil was 9.80±0.02 min (n=10). At 9.9 min, the mass spectrometer was switched to monitor the internal standard 5-ClU ions at *m/z* 506 (target ion), with 280 (5-ClU qualifier ion 1) and 130 (5-ClU qualifier ion 2). The retention time for 5-ClU was 10.28±0.01 min (n=10). Data analysis was performed using the peak area ratio of [$^{15}N_2$]uracil (*m/z* 474) to 5-ClU (*m/z* 506) to generate a calibration curve using weighted ($1/y^2$) linear regression. Unknown samples were quantitated by calculating the uracil (*m/z* 472) to the 5-ClU (*m/z* 506) peak area ratio with extrapolation using the [$^{15}N_2$]uracil calibration curves generated during the same analytic run. The GC–MS analysis was fully automated and left unattended during analytic runs.

2.4. Standard solutions

Standard solutions of uracil, [$^{15}N_2$]uracil and 5-ClU were prepared in water and stored at −20°C. Concentrations of uracil and [$^{15}N_2$]uracil were confirmed using UV spectrophotometry with a molar extinction coefficient of ϵ=8200 at a wavelength of 259.5 nm. Standards were stable under these storage conditions for at least 1 month (data not shown).

2.5. Relative recovery and solid-phase extraction efficiency

Water and plasma (100 μl) standards were prepared in triplicate containing 100 μ*M* and 1 μ*M* of uracil and 10 μ*M* 5-ClU. Samples were derivatized and processed identically as described above and the concentrations of PFBz–uracil and PFBz–5-ClU were determined using the GC–MS assay. Comparison of the resulting plasma standards relative to the water standards were used to calculate the relative recovery of uracil and 5-ClU, which were expressed as mean±standard deviation (SD). Efficiency of the solid-phase extraction was assessed by taking PFBBr-derivatized uracil and 5-ClU which corresponded to original concentrations of 100 μ*M* and 1 μ*M* uracil and 10 μ*M* of 5-ClU in 100 μl of donor plasma and subjecting them to either solid-phase extraction or no extraction in triplicate. Absolute recovery was calculated by comparing the extracted samples to the mean unextracted concentration for both uracil and 5-ClU.

2.6. Validation

Analytical methods evaluation was performed using the criteria suggested by Shah et al. [19]. Ten different calibration curves using [$^{15}N_2$]uracil in plasma were analyzed over a 3-month time period and the accuracy and precision of each of the five

calibration standards were determined by calculating the mean value and the relative standard deviation (RSD) at each concentration. The within run and the between-run precision and accuracy were determined by preparing three quality control samples of uracil at 250 μ*M*, 2.5 μ*M* and approximately 0.270 μ*M* (endogenous uracil from a single plasma donor) in human plasma analyzed in quintuplicate and determined on five separate occasions using five different sample calibration curves. The similarity of the mass selective detector response for uracil and [$^{15}N_2$]uracil was compared by preparing quintuplicate samples of each at 0.25, 2.5 and 250 μ*M* and determining the ratio of the respective molecular ion response to that of the 5-ClU internal standard at each concentration. Uracil and [$^{15}N_2$]uracil ratios at equivalent concentrations were then compared using a Student's *t*-test.

2.7. Conversion of deoxyuridine, uridine and cytosine to uracil

The potential for any pre-existing endogenous deoxyuridine, uridine, or cytosine in plasma to chemically convert into uracil during the sample preparation and derivatization steps was assessed by spiking separate aliquots of plasma to a final concentration of 100 μ*M* of either deoxyuridine, uridine, or cytosine in triplicate. Samples were then derivatized according to the preparation procedures outlined above. The relative amount of uracil formed during the sample preparation process was determined by subtracting the endogenous control plasma uracil concentration from the measured uracil concentration in each of the pyrimidine-spiked samples.

2.8. Stability of stored plasma samples

The stability of uracil was tested in plasma containing 100 μ*M* and 0.124 μ*M* (endogenous) uracil that was stored at −80°C for up to 2 months. To examine the effect of blood processing delays, fresh blood and plasma samples were spiked with 100 μ*M* uracil and incubated at on ice and at room temperature (25°C). Uracil measurements were made at 0, 30 min, and at 1, 2 and 4 h.

2.9. Analysis of patient samples

Plasma samples were drawn from 11 cancer patients enrolled in a phase I clinical trial of eniluracil and 5-FU chemotherapy. Uracil concentrations were measured at baseline and 24 h after receiving two oral doses of 20 mg eniluracil 12 h apart. Blood was collected into lithium–heparin tubes and immediately centrifuged at 4°C at 2000 *g* for 10 min to isolate plasma. Plasma samples were stored frozen at −80°C until analysis.

3. Results

3.1. Gas chromatography–mass spectrometry

Total ion electron-impact (EI) mass spectra of PFBz–uracil (Fig. 1A) and PFBz–[$^{15}N_2$]uracil (Fig. 1B) each showed prominent molecular ions at *m*/*z* 472 and 474, respectively. Loss of a PFBz from the molecular ion gave rise to the most intense ion in each spectrum at *m*/*z* 181. The derivatized internal standard, PFBz–5-ClU, was quantitated using its molecular ion with an *m*/*z* ratio of 506.

Selected-ion monitoring (SIM) was used for measuring uracil, [$^{15}N_2$]uracil, and the 5-ClU internal standard. Fig. 2A shows a typical SIM chromatogram of the lowest quantitated standard of 0.025 μ*M* of [$^{15}N_2$]uracil with a signal-to-noise ratio of 6:1. The peak corresponding to a plasma uracil concentration of 48.9 μ*M* uracil measured in a patient receiving oral eniluracil therapy is shown in Fig. 2B.

3.2. Relative recovery and solid-phase extraction efficiency

The impact of the biological matrix on the assay was modest as evidenced by the relative recovery of uracil from water compared to spiked plasma samples ($n=3$) of 95.8±2.0% and 95.5±10.9%, for 1 μ*M* and 100 μ*M* uracil, respectively. The relative recovery of 10 μ*M* 5-ClU internal standard from plasma compared to water was 95.5±4.1% ($n=6$). The efficiency of the solid-phase extraction step was also excellent with absolute recoveries of PFBz–uracil and PFBz–5-ClU of 95.5±6.8% and 97.0±1.9%, respectively.

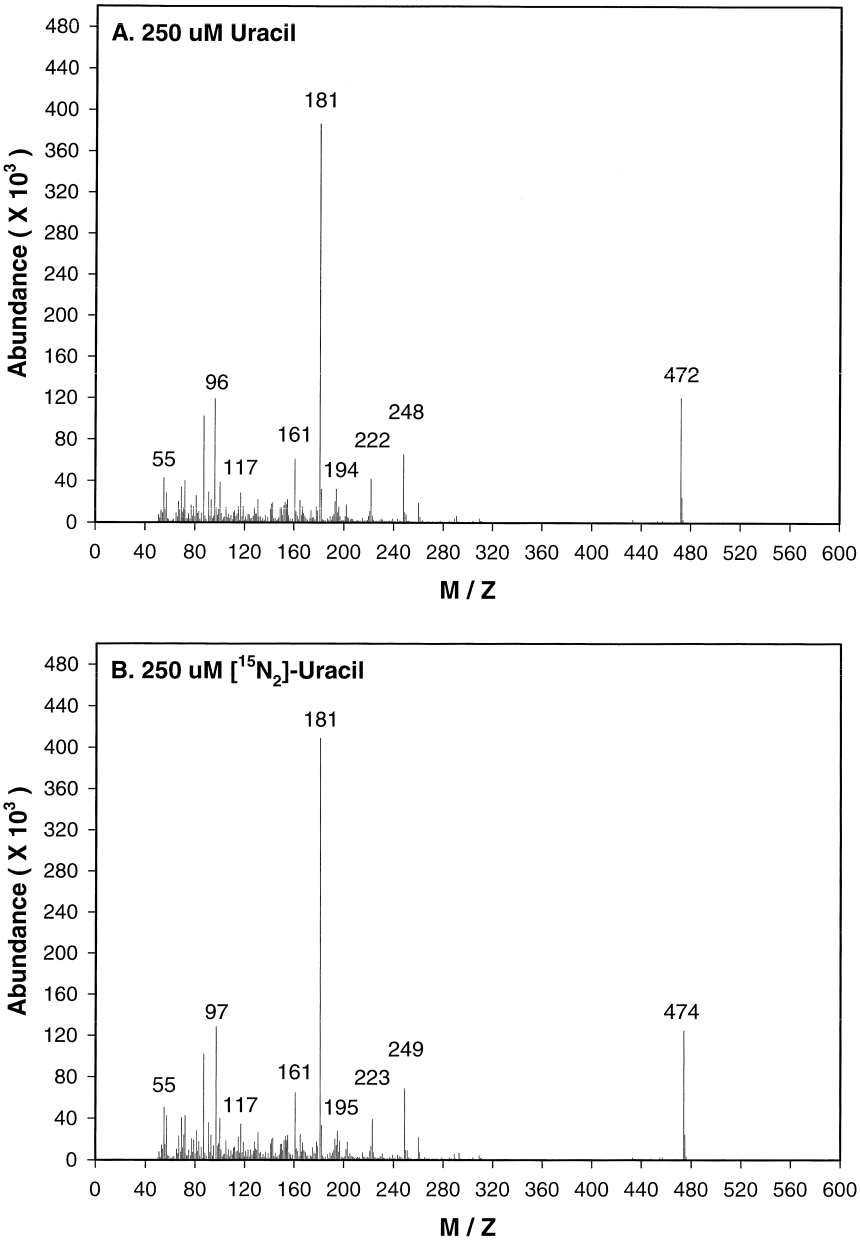

Fig. 1. Electron-impact mass spectra of the pentafluorobenzyl derivatives of (A) 250 μ*M* uracil and (B) 250 μ*M* [$^{15}N_2$]uracil. The large response at 181 *m*/*z* is the pentafluorobenzyl ion.

3.3. Quantitation of uracil by GC–MS: standard curves

Assay reproducibility was examined by analyzing standard curves containing five standard concentrations of [$^{15}N_2$]uracil at 0.025, 0.25, 2.5, 25 and 250 μ*M* and 10 μ*M* 5-ClU. The similarity of the mass-selective detector response for uracil or [$^{15}N_2$]uracil over the range of concentrations measured in our assay is demonstrated in Table 1. Analysis of 10

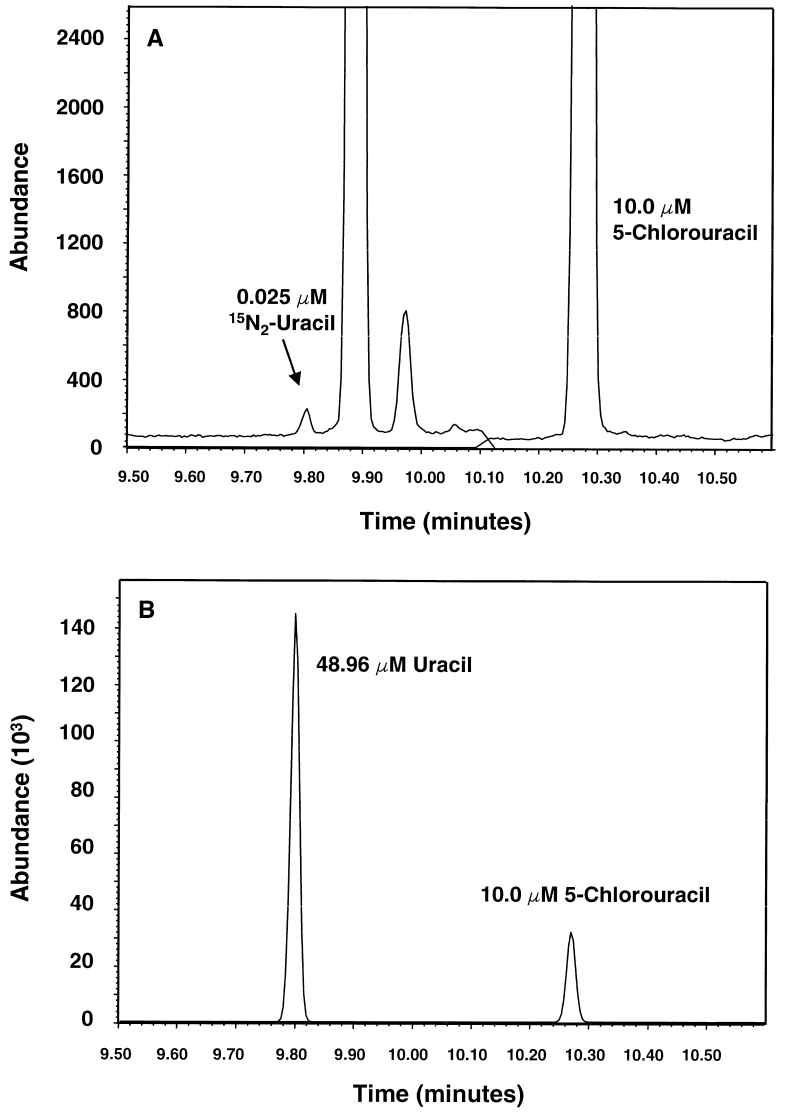

Fig. 2. Selected-ion monitoring tracing of plasma extracts obtained from the analysis of (A) the lowest quantitated [$^{15}N_2$]uracil standard of 0.025 μ*M* and (B) uracil at a measured concentration of 48.96 μ*M* observed in a patient undergoing treatment with 5-fluorouracil and with the dihydropyrimidine dehydrogenase inhibitor, eniluracil.

Table 1
Ratio of uracil or [$^{15}N_2$]uracil to 5-ClU mass-selective detector response

Concentration (μ*M*)	Ratio of uracil or [$^{15}N_2$]uracil to 5-ClU mass-selective detector response		*P* value
	Uracil ratio[a] (*n*=5)	[$^{15}N_2$]Uracil ratio[a] (*n*=5)	
0.25	0.020±0.002	0.019±0.001	0.527
2.5	0.214±0.009	0.217±0.018	0.526
250	23.387±0.463	23.520±0.319	0.984

[a] Mean±SD.

Table 2
[$^{15}N_2$]Uracil standard curve

Theoretical concentration (μM)	Measured concentration (mean±SD, $n=10$) (μM)	Relative standard deviation (%)
0.025	0.025±0.001	4.03
0.25	0.242±0.013	5.51
2.5	2.52±0.19	7.48
25	26.0±1.4	5.41
250	249.0±5.4	2.18

different plasma standard curves examined over a 3-month period resulted in a correlation coefficient of (mean±SD, $n=10$) of 0.9995±0.0006 with a slope of 0.084±0.013 and a *y*-intercept of 0.00148±0.0013 which was not significantly different from zero (Student's *t*-test, $P=0.327$). The RSDs ranged from 2.18 to 7.48% over the concentration range of 0.025 to 250 μM (Table 2).

The within- and between-run accuracy and precision was determined by analyzing five separate plasma standards at concentrations of 0.27 μM (endogenous donor uracil concentration), 25 μM uracil and 250 μM uracil. The RSDs for the within-run analysis ($n=5$) were 7.04%, 5.40% and 1.24%, respectively, while the between-run RSDs were 6.73%, 6.95% and 5.96%, respectively for the three concentrations (Table 3). The deviation of the measured concentration from the theoretically ''true'' uracil concentration in the 25 μM and 250 μM standards was less than 6.0% in all cases (Table 3).

3.4. Stability of uracil in plasma and whole blood

The stability of uracil in fresh blood and plasma was determined to establish conditions for sample processing and storage. Incubation of 100 μM uracil in either whole blood or plasma at 0°C (on ice) or at room temperature (25°C) for up to 4 h prior to derivatization resulted in no loss of uracil signal intensity (Fig. 3). In these experiments, the measured plasma uracil concentration after incubation of whole blood for 4 h was not significantly different from time zero ($P=0.331$). Plasma samples containing either 100 μM or 0.124 μM (endogenous) uracil were also stable when stored at −80°C for up to at least 2 months prior to sample derivatization (*t*-test, $P=0.555$ and $P=0.327$, respectively). Nor were any discernable changes in measured concentrations observed when freshly processed samples were compared to samples subjected to three freeze–thaw cycles in triplicate ($P=0.763$).

3.5. Conversion of deoxyuridine, uridine and cytosine to uracil

Chemical conversion to uracil of any endogenously present pyrimidine-containing compounds in plasma, such as deoxyuridine, uridine or cytosine, could

Table 3
Within- and between-run accuracy and precision for the measurement of uracil in human plasma

Theoretical concentration (μM)	Measured concentration (μM)	Percentage of the theoretical concentration (%)	Standard deviation (μM)	Relative standard deviation (%)
Within run ($n=5$)				
Donor plasma[a]	0.270	–	0.019	7.04
25 μM Uracil	25.57	102.3	1.38	5.40
250 μM Uracil	237.3	94.9	3.0	1.26
Between run ($n=5$)				
Donor plasma[a]	0.274	–	0.018	6.73
25 μM Uracil	26.50	106.0	2.53	9.56
250 μM Uracil	261.4	104.6	14.1	5.38

[a] Plasma from a single patient donor with no added uracil.

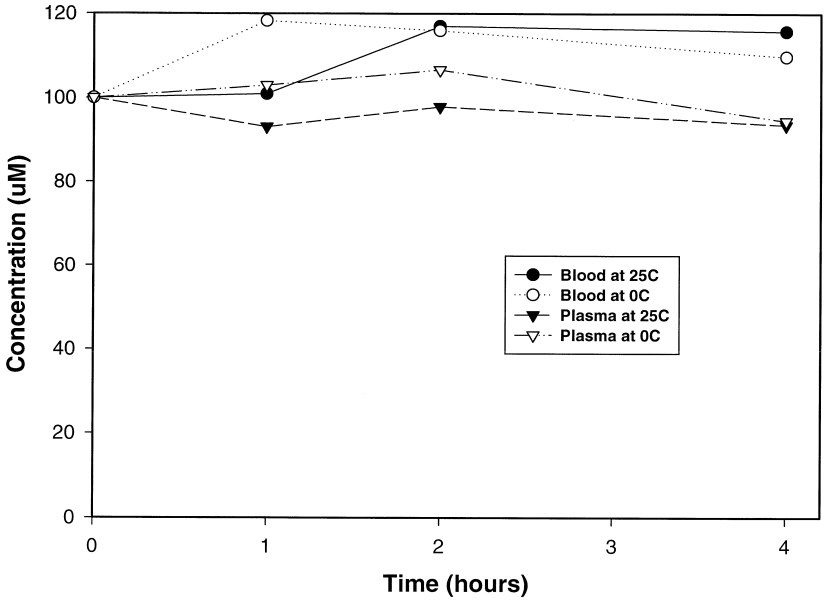

Fig. 3. Stability of uracil in fresh whole blood and plasma. Uracil at 100 μ*M* was added to fresh whole blood and fresh human plasma and incubated for up to 4 h at 0°C and 25°C. The uracil concentrations in whole blood shown were not significantly different at 4 h compared to time zero ($n=3$, $P=0.331$).

potentially confound our assay. Either the loss of a sugar moiety from any uridine or deoxyuridine nucleosides, or the deamination of any cytosine present in plasma would both cause spurious increases in plasma uracil levels. Therefore, human plasma containing 100 μ*M* deoxyuridine, uridine or cytosine was processed in triplicate and analyzed as described previously. Overall, only 0.13±0.5% of the cytosine and 0.38±0.35% of the deoxyuridine was converted to uracil during the derivatization process, and no conversion of uridine to uracil was detected. Furthermore, the presence of 5-FU also did not interfere with the measurement of plasma uracil concentrations (data not shown). Thus, conversion of other related pyrimidine compounds to uracil was not likely to interfere with the assay of uracil in human plasma.

3.6. Patient plasma samples

Plasma uracil concentrations were measured in four patients participating in a phase I clinical trial of an orally administered DPD inhibitor, eniluracil, given in combination with oral 5-fluorouracil. Plasma samples were obtained at time 0 and at hour 24 after the administration of two oral doses of 20 mg of eniluracil given 12 h apart, prior to the administration of any 5-fluorouracil. Plasma concentrations of uracil in 11 patients at baseline was 0.17±0.05 μ*M* and uracil concentrations were easily measured in all subjects analyzed. The administration of oral eniluracil increased plasma uracil concentrations by about 100-fold (Table 4). When eniluracil therapy was terminated, plasma uracil concentrations slowly returned to the baseline concentrations over the ensuing weeks (data not shown).

Table 4
Plasma uracil concentrations in patients at baseline and 24 h after eniluracil therapy

Patient No.	Baseline uracil (μ*M*)	Uracil after eniluracil treatment (μ*M*)
1	0.177	14.077
2	0.229	18.475
3	0.159	13.070
4	0.184	19.503

4. Discussion

Measurement of endogenous uracil concentrations in human plasma have been limited by the sensitivity of previously used techniques such as HPLC with UV detection [18]. However, the increased sensitivity offered by gas chromatography with electron impact mass selective detection can easily determine uracil concentrations in human plasma with a linear response from 250 μ*M* down to 0.025 μ*M*. This broad analytic range allows for the measurement of plasma uracil both in normal individuals and in patients with highly elevated uracil concentrations due to the administration of a DPD enzyme inhibitor, such as eniluracil.

Our initial assay development efforts utilized normal uracil standards with added [$^{15}N_2$]uracil as the internal standard using a more traditional stable isotope dilution technique. However, because uracil is endogenously present in human plasma, calibration curves had to be prepared using either dialyzed human plasma or another artificial matrix, such as saline, which differed from the unknown samples. Ultimately, we changed our calibration curves to utilize actual human plasma and we substituted [$^{15}N_2$]uracil standards for the normal uracil to avoid interference from the naturally occurring compound. The similarity of the mass-selective detector response to both uracil and its stable isotope, [$^{15}N_2$]uracil, is demonstrated in Table 1. Furthermore, the excellent GC–MS signal obtained from 10 μ*M* 5-ClU allowed us to substitute this compound as the internal standard for our assay. Our validation tests showing excellent accuracy and precision for both within run and between run comparisons support our use of the stable isotope [$^{15}N_2$]uracil standards for measuring endogenous plasma uracil (Table 3).

The marked increase in plasma uracil concentrations observed when patients are treated with inhibitors of the DPD enzyme suggest that this may be a valuable assay for monitoring the status and duration of enzyme inhibition. We are currently examining the temporal relationships between 5-FU pharmacokinetics, PBM cell DPD enzyme activity, and plasma uracil concentrations in cancer patients on eniluracil therapy [14]. Other agents and conditions may also contribute to an increase in plasma uracil concentrations in certain situations. For example, non-pyrimidine antifolate inhibitors of thymidylate synthase (TS), such as raltitrexed (Tomudex), can cause a two- to three-fold increase in plasma uracil concentrations (data not shown). This may be due to the intracellular accumulation deoxyuridine nucleotides, which are the natural substrate for TS, and the subsequent breakdown and leakage of the free deoxyuridine out of the cell. Any circulating deoxyuridine in plasma may then be converted to free uracil by the action of thymidine phosphorylase, a ubiquitous enzyme present in liver and other tissues. Normally, endogenous plasma deoxyuridine concentrations range from 0.020 μ*M* to 0.115 μ*M*, [20] but they can increase up to four-fold following inhibition of thymidylate synthase by non-pyrimidine inhibitors of this enzyme [20]. Further testing is necessary to confirm this hypothesis. However, the increased plasma uracil concentration observed under these conditions is clearly not due to the artifactual chemical conversion of deoxyuridine to uracil occurring during our sample preparation process. Only 0.38% of endogenous deoxyuridine is converted to uracil during the derivatization steps required by our assay. Other acquired conditions potentially associated with increased uracil production include metabolically-active tumors, such as medulloblastomas, and acute leukemias [21].

Another potential application of this assay is to screen for inborn errors of pyrimidine metabolism. Congenital syndromes of decreased DPD activity in adult cancer patients have not been associated with any other medical symptoms. Traditional methods for screening cancer patients for DPD deficiency, which has been identified in about 2.5% of the adult population [22], involves biochemical measurements made in PBM cell extracts, which is expensive and laborious. Urinary uracil excretion has been proposed as another method of screening for pyrimidine catabolic defects, but the absolute urine concentration can vary, and must be corrected for creatinine excretion in order to be accurately quantitated [17]. Screening based upon plasma uracil concentrations has been less well tested, largely due to limitations in the analytic assay sensitivity. More recently, the dihydrouracil-to-uracil ratio in plasma has been proposed as a predictor of DPD activity and 5-FU drug clearance [23]. However, our assay has success-

fully measured baseline uracil concentrations in all subjects and controls analyzed to date (data not shown), and, in our hands, the normal human plasma uracil concentrations of uracil appear to be quite narrowly distributed with a mean value of 0.17 ± 0.05 μM ($n=11$) [14]. Thus, a highly sensitive assay for measuring plasma uracil concentrations, either alone, or in combination with other metabolite measurements, [23] may have potential utility as a screening test for pharmacogenetic syndromes of DPD deficiency. The clinical importance of identifying this syndrome is great because patients with complete or even partial DPD deficiency are at high risk for severe, life-threatening toxicity following standard doses of 5-FU chemotherapy [18]. Further testing of our assay for measuring plasma uracil concentrations as a screening test for DPD deficiency in adult cancer patients is necessary.

In conclusion, we have developed a simple, sensitive, and validated analytic method for measuring both basal endogenous and highly elevated uracil concentrations in only 100 μl of human plasma. This test may have great clinical utility in monitoring the status of pyrimidine catabolic pathway activity under a variety of conditions, including during the administration of a pharmacological inhibitor of DPD, such as eniluracil. In addition, the PFBBr derivatization procedure utilized in our assay is similar to those used in previously validated assays for 5-fluorouracil [24] and its catabolic metabolite, fluoro-β-alanine [25], allowing for the easy measurement of these compounds with minimal modifications (data not shown). Additional studies are ongoing to better define the clinical utility of this analytical method.

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