Phase I and Pharmacokinetic Trial of Weekly Oral Fluorouracil Given With Eniluracil and Low-Dose Leucovorin to Patients With Solid Tumors

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Purpose: **Fluorouracil (5-FU) given as a weekly, high-dose 24-hour infusion is active and tolerable. We evaluated an oral regimen of eniluracil (which inactivates dihydropyrimidine dehydrogenase [DPD]), 5-FU, and leucovorin to simulate this schedule.**

Patients and Methods: **Patients received a single 24-hour infusion of 5-FU (2,300 mg/m² on day 2) with leucovorin (15 mg orally [PO] bid on days 1 through 3) to provide reference pharmacokinetic data. Two weeks later, patients began treatment with eniluracil (20 mg) and leucovorin (15 mg) (PO bid on days 1 through 3) and 5-FU (10 to 15 mg/m² PO bid on day 2).**

Results: **Dose-limiting toxicity (diarrhea, neutropenia, and fatigue) was seen with 5-FU 15 mg/m² PO bid on day 2 given weekly for either 6 of 8 weeks or 3 of 4 weeks, whereas five of seven patients tolerated 5-FU 10 mg/m² PO bid given weekly for 3 of 4 weeks. Eniluracil led to a 35-fold reduction in 5-FU clearance. Fluoro-beta-alanine, a 5-FU catabolite, was not detected in plasma during oral 5-FU–eniluracil therapy. DPD activity was markedly suppressed in all patients during eniluracil therapy; the inactivation persisted after the last eniluracil dose; percentages of baseline values were 1.8% on day 5, 4.5% on day 12, and 23.6% on day 19.**

Conclusion: **The recommended oral dosage of 5-FU (10 mg/m² PO bid) given with eniluracil and leucovorin is approximately 115-fold lower than the reference dosage for 24-hour infusional 5-FU. This difference is greater than expected given the reduction in 5-FU clearance. DPD inactivation persisted for several weeks after completion of eniluracil therapy.**

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PRECLINICAL AND clinical studies have shown that both fluorouracil (5-FU) concentration and duration of exposure are critical determinants of cytotoxicity.[1] Several randomized trials have demonstrated that administration of single-agent 5-FU by protracted continuous intravenous (IV) infusion has an advantage over bolus injection in terms of response rate and toxicity profile.[2-5] A meta-analysis of six randomized trials confirmed the superior response rate associated with infusional 5-FU and revealed a small but statistically significant survival advantage.[6] A variety of infusion schedules have been developed, but there is no consensus regarding the optimal regimen. Ardalan et al[7] first evaluated the administration of high-dose 5-FU by weekly infusion over 24 hours. They and others documented that this was an active schedule that provided a high 5-FU dose-intensity but with a favorable toxicity profile.[7-10] Gastrointestinal toxicity and myelosuppression are manageable, although neurotoxicity characterized by symptoms of cerebellar ataxia may be dose limiting. Randomized studies have shown that the weekly 24-hour infusion schedule with or without leucovorin modulation is less toxic than modulated bolus 5-FU regimens.[3,11,12]

Oral administration of 5-FU was previously ruled out because of low and erratic bioavailability, due to first-pass catabolism of 5-FU by dihydropyrimidine dehydrogenase (DPD) in both the intestinal mucosa and the liver. A number of strategies have been developed to permit oral administration of 5-FU to mimic various continuous infusion schedules without the need for indwelling IV catheters and use of an infusion pump. Eniluracil is a potent mechanism-based inactivator of DPD.[13] Both preclinical and clinical studies have shown that eniluracil administration results in complete inactivation of DPD, as evidenced directly by enzyme assays and indirectly by markedly increased plasma uracil levels.[14-19] Oral administration of 5-FU with eniluracil is associated with essentially 100% bioavailability, and renal excretion is the predominant form of elimination of 5-FU in this case. An attractive feature of eniluracil therapy is the potential to prevent the formation of catabolites of 5-FU, which may contribute to host toxicity and interfere with 5-FU cytotoxicity.[20-23] Further, eniluracil prevents the neurotoxicity that has been associated with a 72-hour infusion of 5-FU in dogs.[24] Laboratory and clinical evi-

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dence suggests that increased expression of DPD in tumor tissue may be associated with insensitivity to 5-FU.[25-28] A 3-day course of twice-daily eniluracil has been shown to inactivate DPD completely in primary tumor tissue of colorectal cancer patients,[29] which suggests that eniluracil may circumvent a potential resistance mechanism.

We wished to develop a weekly oral regimen of eniluracil, 5-FU, and leucovorin that would simulate a weekly, high-dose, 24-hour infusion schedule. Fixed doses of eniluracil and leucovorin were to be given twice daily on days 1 through 3, with oral 5-FU given twice on day 2. The goals of this study were to define a tolerable dose of 5-FU, to compare the pharmacokinetic profile of a 24-hour infusion of 5-FU (with oral leucovorin on days 1 through 3) and oral 5-FU (given with eniluracil and leucovorin on days 1 through 3), and to monitor DPD activity during and after eniluracil therapy.

PATIENTS AND METHODS

Eligibility

This study was activated in January 1998, accrual was completed in October 1998, and the last remaining patient was removed from protocol therapy in June 1999. Patients with solid tumors for whom a 5-FU–leucovorin–based regimen represented a reasonable therapeutic approach or for whom no effective standard therapy was available were eligible. There were no restrictions with regard to prior chemotherapy. Patients were required to have an Eastern Cooperative Oncology Group performance status of 2 or better and adequate organ function (granulocyte count $>$ 2,000/μL, platelet count $>$ 100,000/μL, bilirubin level $<$ 2.0 mg/dL, AST level $<$ four times normal, creatinine level $<$ 1.6 mg/dL, and creatinine clearance $>$ 55 mL/min). This study (FUMA5008) had the approval of the local institutional review boards and the Cancer Therapy Evaluation Program (CTEP), National Cancer Institute; all patients gave written informed consent.

Treatment Plan

Oral tablets of eniluracil (10 mg) and 5-FU (5 and 25 mg) were formulated by Glaxo Wellcome (Research Triangle Park, NC) and supplied by CTEP. Commercial sources were used for the IV 5-FU and oral leucovorin. During the initial period, leucovorin 15 mg orally (PO) was given twice daily on days 1 through 3. On day 2, 5-FU 2,300 mg/m^2 was given by continuous infusion over a 24-hour period. The patient returned 2 weeks later to commence treatment with eniluracil (20 mg) and leucovorin (15 mg) (PO bid on days 1 through 3). 5-FU was given PO 12 hours apart on day 2; the starting dose was 15 mg/m^2. Dose escalation was planned in cohorts of three to six patients, with 50% increments until grade 1 clinical toxicity (excluding nausea and vomiting) was seen in at least two patients during their first cycle at a given level. Thereafter, 25% dose increments were planned, until dose-limiting toxicity occurred in at least two patients at a given level. Dose-limiting toxicity was defined as a nadir granulocyte count of less than 500/μL at any time, a nadir platelet count of less than 50,000/μL at any time, $\geq$ grade 2 nonhematologic toxicity (excluding nausea, vomiting, and alopecia) occurring before completion of the planned weekly treatments, $\geq$ grade 2 neurotoxicity (excluding grade 2 headache) at any time, $\geq$ grade 3 nonhematologic toxicity occurring after completion of the three weekly treatments, or the need for a treatment delay of more than 2 weeks to permit resolution of toxicity.

The treatment cycle was halted if the platelet count decreased to less than 50,000/μL, the granulocyte count decreased to less than 500/μL, or $\geq$ grade 2 nonhematologic toxicity (excluding nausea, vomiting, and alopecia) occurred. In this case, the cycle was considered complete; treatment could resume 2 weeks later provided the granulocyte count was more than 1,500/μL, the platelet count was more than 75,000/μL, and the patient had recovered from nonhematologic toxicities.

If the preceding cycle was accompanied by minimal clinical toxicity (nadir granulocyte count $\geq$ 1,000/μL, nadir platelet count $\geq$ 75,000/μL, hematologic recovery by day 29, and $\leq$ grade 1 mucositis and diarrhea), the 5-FU dose was increased by 25% in the next cycle, whereas the doses of eniluracil and leucovorin remained the same. The dose of 5-FU was reduced by 33% in the case of a nadir granulocyte count of less than 500/μL, a nadir platelet count of less than 50,000/μL, or grade 3 or 4 nonhematologic toxicity at any time or grade 2 toxicity before completion of the cycle. National Cancer Institute common toxicity criteria, version 1, were used. Fatigue and malaise were considered grade 1 if the symptoms were mild and did not interfere with the patient's activities; moderate symptoms that affected some specialized activities were scored as grade 2; symptoms were deemed grade 3 if they greatly interfered with the patient's routine activities and/or were accompanied by a decrease in performance status.

The first three patients were treated weekly for 6 of 8 weeks, but dose-limiting toxicity occurred during the first cycle after 4 or 5 weeks in two patients. The schedule was therefore amended to weekly for 3 of 4 weeks, and a second cohort of patients was treated. Because dose-limiting toxicity occurred in three of six patients in cycle 1, the starting dose was decreased to 10 mg/m^2. The toxicity associated with the lower dose is described in the Results.

Patient Evaluation and Follow-Up

A blood cell count with WBC differential was obtained weekly. On day 1 of each cycle, liver function tests were performed and blood urea nitrogen, creatinine, and electrolyte levels were determined. Radiographic studies were repeated every third cycle. Treatment was continued until there was evidence of disease progression, provided treatment was tolerated and the patient agreed to continuation of therapy.

Pharmacokinetic Studies

Patients were hospitalized for the 24-hour infusion of 5-FU and after the initial oral dose of 5-FU, to permit extended blood sampling for pharmacokinetic analysis. Blood samples were obtained before treatment, at hours 0.5, 1, 4, 7, 10, 14, 18, 22, and 23 during the infusion, immediately before the end of the infusion, and 60 minutes after the infusion. On the first day of oral 5-FU therapy, blood samples were obtained at the following time points relative to administration of the 5-FU dose: 0.5, 1, 1.5, 2, 3, 6, 9, 12, 15, 18, 22, 23, 24, and 26 hours. The second dose of 5-FU was to be given immediately after the 12-hour blood sample was obtained. The blood was collected into 10-mL heparinized tubes, immediately placed on ice, and centrifuged at 800 $\times$ *g* for 15 minutes; the plasma was then frozen at $-$70°C and kept at that temperature until the time of analysis. Samples obtained at night were stored on ice and the plasma was isolated the following morning. A 24-hour urine collection was performed on both occasions. The volume of urine was measured at intervals of approximately 8 hours, and an

aliquot of urine was transferred to a 50-mL tube and stored on ice. After being transported to the laboratory, the urine was stored at −70°C.

Unless otherwise stated, chemicals were obtained from Sigma Chemical Co (St Louis, MO). 5-FU, uracil, and fluoro-beta-alanine (FBAL) (Fluorochem USA, West Columbia, SC) were measured using validated gas chromatography–mass spectroscopy methods.[30-32] Plasma (100 μL) or urine (10 μL) was spiked with the internal standard, 5-chlorouracil. Pentafluorobenzylbromide was the derivatizing agent. A Supelco (Bellefonte, PA) SPB-20 capillary column was used (15 m × 0.25 mm–inside diameter fused silica, 0.250-μm phase film). Ultrapure helium at a concentration of 1 mL/min was used as the carrier gas. Standard curves were constructed using donor plasma, with 5-FU concentrations ranging from 0.1 to 100 μmol/L, FBAL concentrations from 0.25 μmol/L to 100 μmol/L, and [$^{15}N_2$]uracil concentrations from 0.025 to 250 μmol/L.

Noncompartmental pharmacokinetic analytic methods were used to estimate the area under the concentration curve (AUC) using the WinNonLin 2.1 software package (Pharsight, Mountain View, CA). The terminal elimination half-life was estimated from the terminal portion of the concentration-versus-time curve. Oral 5-FU clearance divided by the bioavailability (CL/F) was determined by dividing the administered dose by the AUC extrapolated to infinity. For the IV administration of 5-FU, clearance was determined in the same manner. Peak and trough plasma concentrations were determined by visual inspection of the data.

Measurement of DPD Activity

Venous blood samples were collected in heparinized tubes before treatment, on day 3 of eniluracil therapy (before the morning dose of eniluracil and leucovorin), and on day 8 before eniluracil therapy. The protocol was later amended to permit collection of additional blood samples 12 and 19 days after the last dose of eniluracil whenever possible. Peripheral-blood mononuclear cells were isolated by Ficoll-Hypaque density centrifugation and then subjected to a brief hypotonic lysis (0.2% saline for 30 seconds followed by "rescue" with an equal volume of 1.6% saline) to remove erythrocytes. Intact cell pellets were stored at −70°C until analysis. The frozen cell pellet was suspended in 300 μL of DPD assay buffer (potassium phosphate 35 mmol/L [pH 7.4], magnesium chloride 2.5 mmol/L, and 2-beta-mercaptoethanol 14.3 mmol/L) and placed on ice. The cellular membrane was ruptured by sonication, and the supernatant containing a cellular lysate was collected after centrifugation at 12,000 × *g* for 30 minutes at 4°C. Protein concentration was determined using the BioRad (Hercules, CA) protein assay kit; bovine serum albumin was used to generate the standard curve. Lysate containing 200 to 300 μg of protein was incubated in a final volume of 1 mL of DPD assay buffer containing [^{3}H]5-FU 20 μmol/L (25 Ci/mmol; Moravek Biochemicals, Brea, CA) and 250 μmol/L nicotinamide adenine dinucleotide phosphate (reduced form) in a 37°C shaking water bath. At 15-minute intervals, a 250-μL aliquot was removed and transferred to a 1.5-mL microfuge tube containing an equal volume of 100% ice-cold methanol. After incubation on ice for at least 15 minutes, the sample was centrifuged, and the methanol-soluble supernatant was filtered through a 0.2-μm Acrodisc filter (Gelman Sciences, Ann Arbor, MI). The samples were then concentrated to dryness in a TurboVap (Zymark Corp, Hopkinton, MA). The samples were resuspended in 200-μL high-performance liquid chromatography (HPLC) mobile phase before analysis.

5-FU was separated from its catabolites by an ion-pairing HPLC method using a Waters (Milford, MA) HPLC system with an in-line Radiomatic 500TR series flow scintillation analyzer (Packard Instruments, Meriden, CT). A 5-μm C18 Columbus Column (4.9 × 250 mm; Phenomenex, Torrey Pines, CA) was used. The mobile phase was tetrabutylammonium hydrogen sulfate 5 mmol/L and $KH_2P0_4$1.5 mmol/L, pH 9.0, at a flow rate of 1.4 mL/min. Ultima-Flow M (Packard Instruments) at a 3:1 ratio was used for postcolumn scintillation detection. Dihydrofluorouracil and 5-FU eluted at 3 and 13 minutes, respectively. A control sample contained all assay ingredients without a source of protein, to permit correction for any possible degradation of 5-FU or exchange of tritium with water. DPD activity was defined as the amount of catabolites (in picomoles) formed per minute per milligram of protein using the linear portion of the curve.

Statistical and Graphical Analysis

Graphical analysis was performed using SigmaPlot 5.0 for Windows (SPSS, Inc, Chicago, IL), and statistical analysis was performed using SigmaStat for Windows 2.03 (SPSS, Inc). The median time to progression was calculated by a Kaplan-Meier survival curve. The Cockcroft and Gault formula was used as follows to calculate the estimated creatinine clearance: men, (140 − *age in years*) · (*body weight in kg*) / (72 · *serum creatinine level in mg/dL*); women, (0.85) · (140 − *age in years*) · (*body weight in kg*) / (72 · *serum creatinine level in mg/dL*). The strength of linear association between pairs of variables was determined using Pearson's correlation coefficient: *r* values ≥ .70 reflect a strong correlation, *r* values between .50 and .70 represent a moderate correlation, and *r* values of .3 to .5 suggest a weak to moderate correlation. The percent change in nadir granulocyte counts was determined by the following equation: 100 × (*baseline value* − *nadir value*) / (*baseline value*). The relationship between dose and percent change in blood counts was analyzed using a sigmoidal maximum effect model. Coefficients of determination (r^2) values greater than .50 indicate a strong fit between the model and the data, whereas values between .25 and .50 signify a moderately strong fit.

RESULTS

Patient Characteristics and Clinical Toxicity

Twelve adult patients with good performance status who had undergone a median of two prior chemotherapy regimens participated in this study (Table 1). The majority of patients had adenocarcinomas arising in the gastrointestinal tract. All patients were assessable for toxicity.

Two treatment periods made up the first cycle of therapy. The initial period consisted of 3 days of oral leucovorin (15 mg PO bid), with 5-FU 2,300 mg/m^2 given as a 24-hour IV infusion starting on day 2. This initial treatment was well tolerated. Oral chemotherapy was started 2 weeks later. The first cohort was treated weekly for 6 of 8 weeks at a starting 5-FU dose of 15 mg/m^2, given PO bid day 2, with leucovorin 15 mg and eniluracil 20 mg PO bid given on days 1 through 3. Two of the first three patients experienced either grade 4 granulocytopenia (after four doses) or grade 3 diarrhea (after five doses) before receiving all six planned weekly doses. Therefore, the protocol was amended to feature a schedule of weekly administration for 3 of 4 weeks. One of the next three patients experienced grade 4 diarrhea during the first cycle, and two other patients had minimal toxicity. The cohort was then expanded to a total of

Table 1. Patient Characteristics

Age, years	
Median	57
Range	42-72
Sex, male/female	6/6
ECOG performance status	
0	3 (25%)
1	8 (67%)
2	1 (8%)
Prior chemotherapy	
Adjuvant	5
5-FU–based	4
Metastatic disease	9
5-FU–based	6
Irinotecan	5
No. of prior regimens	
Median	2
Range	0-4
Prior immunotherapy	3
Prior radiation therapy	2
Histology	
Colorectal cancer	5
Pancreatic or bile duct cancer	3
Appendiceal	1
Breast cancer	1
Non–small-cell lung cancer	1
No. of cycles	
Median	2.5
Range	1-10
Mean laboratory values at study entry (range)	
Albumin level, g/dL	4.0 (3.7-4.7)
LDH level, U/L	614 (295-1550)
AST level, U/L	28 (21-63)
Creatinine level, mg/dL	0.9 (0.7-1.0)
Hemoglobin level, mg/dL	12.3 (10.6-14.4)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; AST, aspartate aminotransferase.

six patients. Two other patients experienced either grade 3 diarrhea or grade 3 fatigue, and this dose level was believed to have exceeded the maximum-tolerated dose. The starting dosage was then lowered to 10 mg/m^2 PO bid. One of three new patients had grade 3 diarrhea during cycle 1. Seven patients received 10 mg/m^2 (including four patients who underwent a dose reduction from 15 mg/m^2). One patient experienced grade 3 fatigue after four cycles and underwent a dose reduction for subsequent cycles. The other five patients tolerated the 10 mg/m^2 oral dose.

A median of 2.5 cycles were given. The worst toxicity experienced by each patient across all cycles of therapy is listed in Table 2. Grade 3 or 4 diarrhea occurred in 33% of patients, and grade 3 fatigue occurred in 25%. Mucositis did not exceed grade 1. Only one patient had a nadir granulocyte count of less than 500/μL. One quarter of patients experienced a nadir hemoglobin level of less than 8.0 mg/dL, but thrombocytopenia was negligible. During the study, one patient with primary bile duct cancer died from refractory upper gastrointestinal bleeding, without thrombocytopenia or coagulation abnormalities. Endoscopy showed no evidence of esophagitis, gastritis, or duodenitis and confirmed that tumor invading the duodenum had caused the bleeding. This death was attributed to rapid progression of the underlying malignancy.

Table 2. Worst Toxicity per Patient Across All Cycles of Therapy (n = 12)

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Nausea or vomiting	6	5	1	0
Mucositis	7	0	0	0
Diarrhea	3	1	3	1
Anorexia	5	0	0	0
Constipation	1	0	0	0
Abdominal discomfort	2	0	0	0
Fatigue	6	1	3	0
Skin	3	0	0	0
Ocular toxicity	2	0	0	0
Leukocytic toxicity	4	1	1	0
Granulocytopenia	1	2	0	1
Hemoglobin level-related toxicity	7	2	3	0
Thrombocytopenia	6	1	0	0

Prophylactic antiemetics were not used, and one or more episodes of nausea or vomiting occurred in 11 of 12 patients. The nausea or vomiting occurred during cycle 1 in 10 patients. Five patients had only one episode of nausea, whereas six patients had multiple episodes. Of 48 episodes of nausea or vomiting, the onset was within a median of 1 day of 5-FU therapy, and within 3 days of 5-FU therapy in 81% of patients. The median duration of nausea or vomiting was 2 days, and nausea or vomiting lasted $\leq$ 3 days in 85% of the episodes.

A total of 14 cycles at 15 mg/m^2 PO bid were given to nine patients (Table 3). Six cycles (43%) were complicated by dose-limiting toxicity, including grade 3 or 4 diarrhea, grade 3 fatigue, and grade 4 granulocytopenia. Three patients received one cycle at 18.75 mg/m^2. Two patients tolerated this dose but were taken off study because of disease progression. The other patient experienced grade 2 fatigue and requested that the 5-FU dose be reduced to 15 mg/m^2. A total of 12 cycles at 10 mg/m^2 PO bid were given to seven patients. Two patients (16.7%) had dose-limiting toxicity (either grade 3 diarrhea or fatigue). No dose-limiting toxicities occurred during 15 cycles at 6.7 mg/m^2. Therefore, we consider 10 mg/m^2 PO bid to be a safe starting dosage. In the case of patients treated weekly for 3 of 4 weeks, treatment intervals between cycles were 4 weeks in 49% of cycles and 5 weeks in 30%.

No objective responses were seen. The median time to treatment failure was 3.8 months (range, 2.2 to 12.1

Table 3. Dose-Limiting Clinical Toxicity for All Cycles, by 5-FU Dose

Dosage (mg/m^2 bid)	Total Dose (mg/d)	No. of Patients		No. of Cycles	No. of Patients With Dose-Limiting Toxicity
		New	Total		
6.7	20-30	0	2	9	0
10	30-40	3	7	12	2 (1 with grade 3 diarrhea, 1 with grade 3 fatigue)
15	40-60	9	9	14	6 (3 with grade 3-4 diarrhea, 1 with grade 4 granulocytic toxicity, 2 with grade 3 fatigue)
18.75	60-70	0	3	3	0

months). Eight patients (75%) had stable disease for 3 months or longer.

Pharmacokinetic Studies

The plasma concentrations of 5-FU during the initial 24-hour infusion of 2,300 mg/m^2 are shown in Fig 1. The average 5-FU plasma concentrations 30 minutes and 24 hours into the infusion were 5.8 and 8.0 μmol/L, respectively. One hour after completion of the infusion, the plasma concentration had decreased to 0.16 μmol/L. 5-FU pharmacokinetic parameters are listed in Table 4. Within individual patients, the peak 5-FU plasma concentration was 2.1 $\pm$ 0.4-fold (mean $\pm$ SD) greater than the trough 5-FU plasma concentration. The average clearance was 1,953 mL/min/m^2 (median, 1,947 mL/min/m^2); for the population, the fastest clearance was 1.9-fold greater than the slowest clearance. Only 1% of the total dose was recovered as parent drug in the urine during the 24-hour infusion.

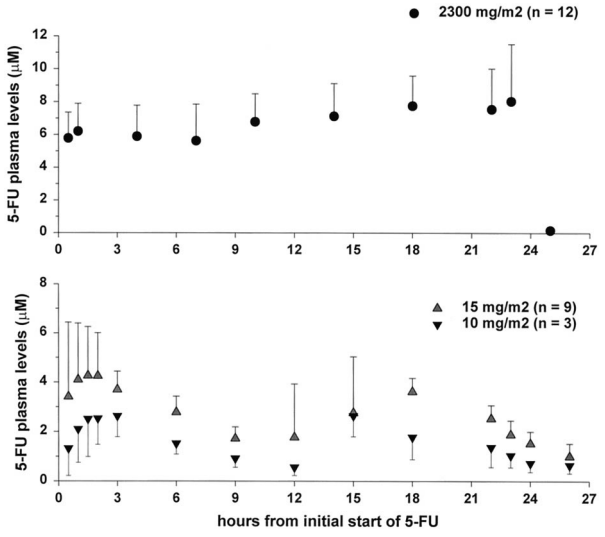

Fig 1. 5-FU plasma levels during the 24-hour infusion of 5-FU 2,300 mg/m^2 (top) and after administration of 5-FU 10 to 15 mg/m^2 PO bid on day 2 with eniluracil 20 mg PO bid on days 1 through 3 (bottom). Data are presented as mean $\pm$ SD.

The accumulation of FBAL in plasma during the IV infusion of 5-FU in the absence of eniluracil is shown in Fig 2. FBAL levels rose exponentially and seemed to approach maximum level by 14 hours into the infusion. The steady-state plasma concentration of FBAL (average of hours 22 and 23) was 68.3 $\pm$ 20.1 μmol/L (mean $\pm$ SD), and the AUC of FBAL was 1,316.7 $\pm$ 384.1 μmol/L·hr (mean $\pm$ SD). The ratio of the AUC of FBAL over the AUC of 5-FU was 8.5 $\pm$ 2.2 (mean $\pm$ SD). The apparent terminal elimination half-life of FBAL was 91.2 $\pm$ 28.8 minutes, 6.6-fold greater than the observed 5-FU plasma half-life of 13.8 $\pm$ 6.0 minutes in the absence of eniluracil after the IV infusion of 5-FU. Compared with the FBAL value at end of the infusion, the plasma concentration decreased by one third 1 hour after the end of the infusion. The mean molar recovery of FBAL in the urine over the 24-hour infusion period was 24% of the administered 5-FU dose.

Oral therapy began during period 2. After the first oral dose of 5-FU on day 2 (with eniluracil and leucovorin PO bid on days 1 through 3), absorption occurred quickly; the mean plasma level at 30 minutes was 76% of the peak level observed at 1.5 hours for the group of 12 patients (Fig 1). The time at which the initial peak occurred varied among patients, but the peak occurred by 1.5 hours in 75% and by 2 hours in 83% of patients. We had intended that the 12-hour blood sample be obtained before the second oral dose of 5-FU. In 11 of the 12 patients, the plasma level at 12 hours was 62% $\pm$ 11% of the 9-hour value. One patient, however, took the second dose before the 12-hour blood sample was obtained, and the plasma level was 3.5-fold higher than that of the 9-hour sample. This outlier explains the similarity between the mean 5-FU concentrations at 9 and 12 hours for the cohort that received 5-FU 15 mg/m^2. The CL/F of 5-FU was 34.6 $\pm$ 11.3-fold lower in the presence of eniluracil, and the half-life was 5.4 $\pm$ 1.4 hours. The average recovery of parent drug in the urine collected over the 24-hour period after the first oral dose of 5-FU was 26%. FBAL was not detected in the plasma of any patient during oral eniluracil–5-FU therapy. In contrast, FBAL was

Table 4. Pharmacokinetic Data for 5-FU and FBAL

	5-FU					FBAL		
5-FU (mg/m^2)*	Average Cp Hours 0.5-23 (μmol/L)	AUC (μmol/L · h)	Clearance (mL/min/m^2)	Excreted in Urine (hour 0-24) (μmol)	% Recovered	Mean Cp (μmol/L)	Excreted in Urine (hour 0-24) (μmol)	% Recovered
2,300 as a 24-hour CI, no eniluracil (n = 12)	6.60 ± 1.68 Max: 9.00 ± 2.25 Min: 4.34 ± 1.18	159.3 ± 38.7	1,953.3 ± 453.1	326.7 ± 167.0	1.0 ± 0.6	48.6 ± 10.2	7,583 ± 3,682	23.7 ± 12.2
10 PO bid day 2 + eniluracil (n = 3)	1.74 ± 0.76 Max: 3.16 ± 0.98 Min: 0.26 ± 0.31	32.6 ± 11.5	64.0 ± 13.6	69.2 ± 21.2	26.8 ± 5.1	Not detected	38.2 ± 40.6	13.5 ± 12.4
15 PO day 2 + eniluracil (n = 9)	3.05 ± 0.77 Max: 6.30 ± 1.69 Min: 0.72 ± 0.51	55.9 ± 15.4	57.2 ± 12.7	112.3 ± 37.8	28.03 ± 8.08	Not detected	13.1 ± 8.6	3.6 ± 2.4
10-15 PO day 2 + eniluracil (n = 12)	2.73 ± 0.94 Max: 5.51 ± 2.07 Min: 0.65 ± 0.11	50.1 ± 17.6	58.9 ± 12.7	101.5 ± 38.8	27.7 ± 7.2	Not detected	20.0 ± 22.8	6.6 ± 7.8

NOTE. Data are presented as mean ± SD. The 5-FU pharmacokinetic parameters were modeled using the actual times the blood samples were drawn relative to the start of the 5-FU infusion or the initial 5-FU dose. The percentage recovered in the urine was calculated by dividing the amount of 5-FU (in micromoles) given to the patient divided by the amount (in micromoles) of either 5-FU or FBAL excreted in the urine over the 24-hour period.

Abbreviations: Cp, plasma concentration; CI, continuous infusion; max, maximum; min, minimum.

*Average, highest, and lowest 5-FU plasma concentrations during the first 23 hours of the sampling period were determined for each patient; the mean values for all patients are shown. During the 24-hour continuous infusion, the average number of venous blood samples was 9 (range, 8-10). During oral 5-FU dosing with eniluracil, an average of 11.5 venous blood samples were obtained during the initial 23 hours (range, 11-12), and two additional samples were obtained at hours 24 and 26.

detected in the urine, but recovery of 5-FU as FBAL accounted for only 7% of the two doses.

Because the dose of oral 5-FU was rounded down to the nearest 5 mg, the correlation between plasma AUC and 5-FU dose was examined. Over the relatively narrow dose range, the AUC tended to increase with increasing 5-FU dose (Fig 3, top), whereas CL/F was independent of dose (Fig 3, middle). CL/F of 5-FU in the presence of eniluracil correlated closely with creatinine clearance estimated using the serum creatinine level obtained on day 1 of period 2 (Fig 3, bottom). There was no correlation between plasma AUC of 5-FU and severity of diarrhea or absolute nadir granulocyte count (Fig 4).

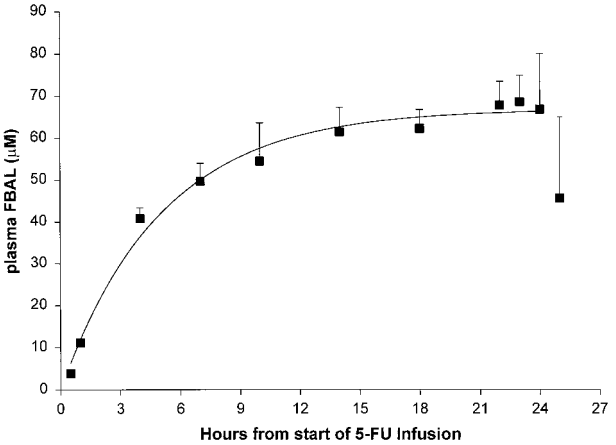

Fig 2. Mean ± SD plasma concentrations of FBAL during the initial 24-hour infusion of 5-FU and 1 hour afterward.

Direct and Indirect Monitoring of DPD Activity

Population analyses have suggested a cut point of DPD activity of ≤ 100 pmol/min/mg for distinguishing patients with significant DPD deficiency.[33, 34] Baseline blood samples for determining DPD activity were obtained at 9:00 AM ± 47 minutes (mean ± SD). The average pretreatment DPD activity in peripheral-blood mononuclear cells was 479.5 pmol/min/mg (Fig 5, top). The large SD reflects the 13-fold range of DPD activity in this patient cohort (129.3 to 1,697.6 pmol/min/mg). Samples for determining DPD activity were obtained on the third day of eniluracil therapy before the morning dose at 8:39 AM ± 33 minutes (mean ± SD). DPD activity was markedly depressed on day 3 (12 hours after the prior eniluracil dose) and remained suppressed 5 and 12 days after the last dose of eniluracil. Nineteen days after the last dose of eniluracil, DPD activity remained below 100 pmol/min/mg in four of five patients (range, 30.3 to 82.9 pmol/min/mg) and was 101 pmol/min/mg in the other patient. When relative DPD activity as a percentage of each patient's pretreatment value was considered (Fig 5, bottom), DPD was found to have recov-

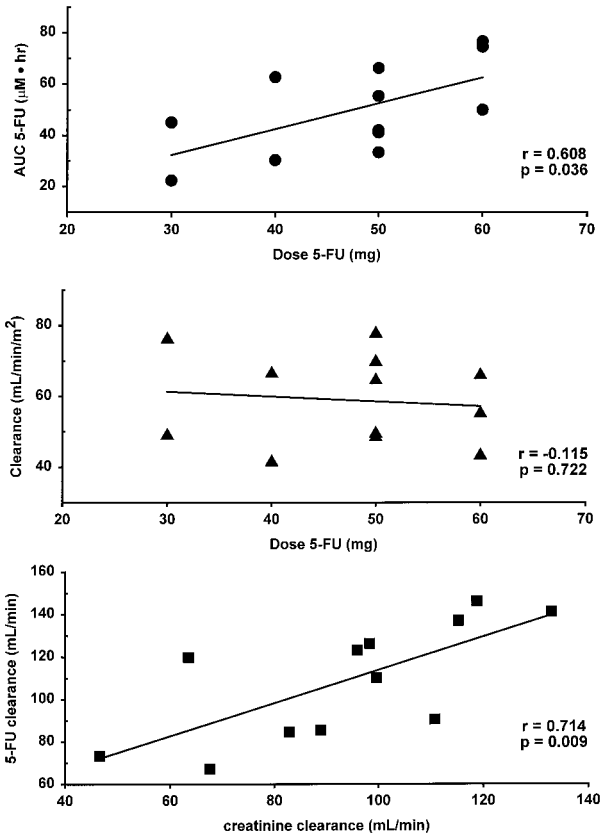

Fig 3. Correlations between 5-FU dose and either $AUC_{0\text{-}26\ hours}$ (top) or estimated creatinine clearance (middle) and between estimated creatinine clearance and 5-FU clearance (bottom). Pearson's correlation coefficients are shown.

ered to 1.8%, 4.5%, and 23.6% by 5, 12, and 19 days after the last dose of eniluracil, respectively.

Because uracil is also a substrate for DPD, plasma uracil concentration serves as an indirect measure of DPD inhibition. Pretreatment uracil levels averaged 0.17 μmol/L (Table 5). By the final 5 hours of the initial 24-hour IV infusion of 5-FU, plasma uracil levels had increased on average by 4.3-fold compared with the pretreatment value. Urinary excretion of uracil averaged 39 μmol over the 24-hour collection period.

During the second period, plasma uracil levels were measured on day 2 before the morning drug doses and throughout the next 24 hours. The uracil plasma concentration on day 2 averaged 18 μmol/L (approximately 12 hours after the prior dose of eniluracil). After oral dosing with 5-FU, eniluracil, and leucovorin on day 2, uracil levels had increased to approximately 44 μmol/L between hours 18 and 23, an average increase of 2.1-fold during the oral 5-FU dosing period. A 32-fold increase in

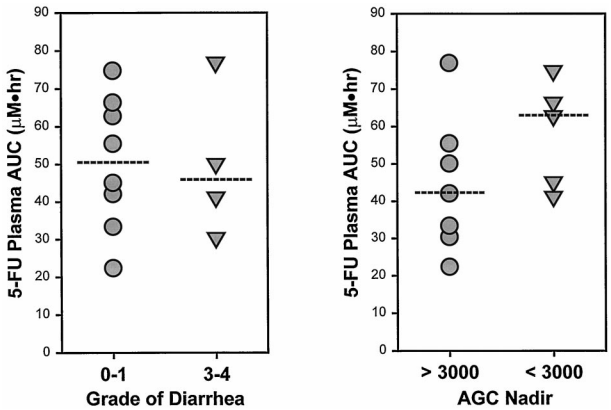

Fig 4. AUC values according to severity of diarrhea (left) or absolute granulocyte count (AGC) nadir (right). Dashed lines represent median values. There was no difference between the AUC values for each group for either diarrhea or AGC nadir.

urinary excretion of uracil over a 24-hour period was seen compared with excretion during the IV 5-FU infusion period 1.

In six patients in whom plasma uracil levels were measured 5 or 6 days after the last eniluracil dose, the uracil

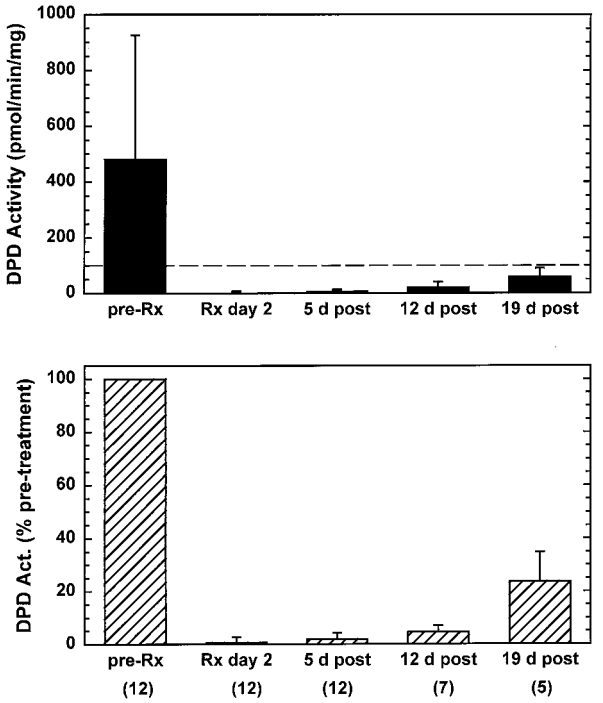

Fig 5. DPD activity in peripheral-blood mononuclear cells during and after eniluracil therapy. Activity (mean ± SD) is expressed as absolute values (top) or percentages of pretreatment values (bottom). The dashed line indicates a putative cut point for DPD deficiency. Numbers of patients are given in parentheses.

Table 5. Change in Uracil Levels in Plasma and Urine During 5-FU Treatment

5-FU (mg/m^2)	Uracil (μmol/L)				
	Hours 18, 22, and 23				
	Before 5-FU Therapy	Average	Versus Before 5-FU Therapy	Average Cp	Uracil Excreted in Urine in 24 Hours (μmol)
2,300 as a 24-hour CI, no eniluracil (n = 12)	0.17 ± 0.05	0.72 ± 0.25	4.3-fold (±1.2)	0.53 ± 0.15	39.2 ± 19.7
10 PO bid day 2 + eniluracil (n = 3)	18.11 ± 6.56	41.03 ± 4.74	2.0-fold (±0.1)	30.45 ± 4.26	1,535.9 ± 1,139.6
15 PO bid day 2 + eniluracil (n = 9)	18.20 ± 5.28	44.74 ± 10.61	2.1-fold (±0.3)	33.69 ± 8.72	1,159.6 ± 298.8
10-15 PO bid day 2 + eniluracil (n = 12)	18.2 ± 5.3	43.8 ± 9.4	2.1-fold (±0.3)	32.9 ± 7.9	1,253.7 ± 575.5

NOTE. The dosage of eniluracil was 20 mg PO bid on days 1-3. Data are presented as mean ± SD.

level had decreased from 45.8 ± 12.2 μmol/L (mean ± SD) on day 3 to 8.5 ± 4.3 μmol/L (20.7% of the peak value). More extended sampling was performed in three patients (Fig 6). The apparent "half-life" of uracil was calculated as 5.5, 3.9, and 4.2 days for the three patients. The measured uracil values had returned to the normal range by day 40 (37 days after the last dose of eniluracil) and day 30 in two of these patients and were predicted to reach the normal range by day 35 in patient no. 6.

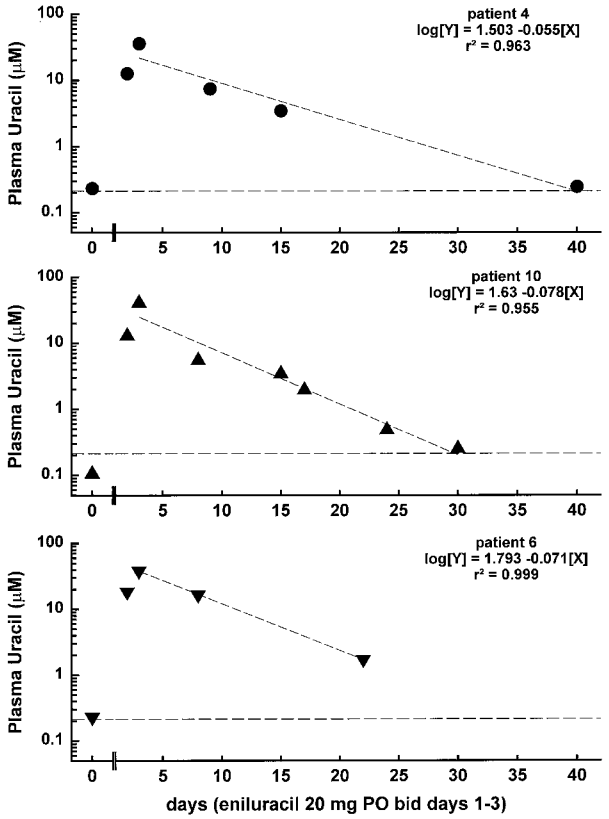

Fig 6. Uracil plasma levels in three patients during and after eniluracil therapy (20 mg PO bid days 1 to 3). The dashed line represents the upper 99% confidence interval for the pretreatment levels. The regression line is fit between the day 3 uracil value (before the morning dose) and the recovery values.

DISCUSSION

In this trial, we explored a weekly schedule of eniluracil 20 mg and leucovorin 15 mg given orally twice daily on days 1 through 3, with two doses of oral 5-FU given on day 2. The starting dose of 5-FU, 15 mg/m^2, was not tolerable when given either weekly for 6 of 8 weeks or weekly for 3 of 4 weeks. Diarrhea and fatigue were the most common dose-limiting toxicities. Mucositis was common (58% of patients) but mild. Only one patient had grade 3 neutropenia. Hand-foot syndrome was not seen, although a few patients had mild skin rash or thickening or splitting of fingernails. The recommended 5-FU dosage is 10 mg/m^2 PO bid on day 2. CNS toxicity was not seen, but the small sample size precludes drawing a definitive conclusion about the possible impact of eniluracil on neurotoxicity.

A 24-hour continuous infusion of 5-FU 2,300 mg/m^2 was given on day 2 of period 1 with leucovorin 15 mg PO bid on days 1 through 3 to provide reference pharmacokinetic and pharmacodynamic values. When the pharmacokinetic data for all patients were combined, no clear evidence of diurnal variation in plasma 5-FU levels was found. Within individual patients, the highest plasma 5-FU level was 2.1-fold greater than the lowest plasma 5-FU level. The 5-FU catabolite FBAL accumulated exponentially in the plasma and seemed to reach steady-state by the end of the infusion.

In the presence of eniluracil, FBAL was not detected in plasma during the oral 5-FU dosing period. Although FBAL was detected in the urine, the cumulative amount was approximately 379-fold lower than that measured during the IV infusion of 5-FU. 5-FU CL/F was 33-fold slower in the presence of eniluracil, and the half-life was prolonged to 5.4 hours. Because IV 5-FU was not administered with eniluracil in our study, direct determination of the oral bioavailability of 5-FU was not possible. However, our findings are in close agreement with those of an earlier study by Baker et al,[17] in which the oral bioavailability of 5-FU adminis-

tered twice daily was essentially 100%. Our estimate (59 mL/min/m^2) of CL/F of 5-FU administered with eniluracil is close to that reported in two other studies by Baker et al[17,35] (58 and 51 mL/min/m^2). The AUC of 5-FU in our trial did not correlate with the severity of diarrhea. In contrast, Baker et al found that the mean steady-state plasma 5-FU concentration was significantly higher in patients with grade 3 or 4 diarrhea than in those with milder diarrhea, when patients were given eniluracil and 5-FU twice daily for 28 days. The lack of correlation in our trial might be due to the use of a different schedule with higher 5-FU doses, the smaller sample size, or the use of leucovorin. It is also possible that local accumulation of active metabolites of 5-FU in the intestinal mucosa may contribute to gastrointestinal toxicity with this schedule. The AUC did not correlate with either the absolute nadir granulocyte count or the percent change in granulocyte count, but neutropenia was not prominent with this schedule.

Like other investigators,[18,19,29] we also found that DPD activity in peripheral-blood mononuclear cells is profoundly inhibited during eniluracil therapy. We prospectively planned to measure DPD activity in all patients on day 8 of cycle 1 (5 days after the last dose of eniluracil) and found that DPD activity remained markedly depressed in all patients. When this trial was initiated, a 3-week interval between the last dose of eniluracil and subsequent fluoropyrimidine-based therapy was recommended. The rationale behind this recommendation related to the duration of DPD inactivation in a study of oral eniluracil given once daily for 7 days at a dose of 0.74, 3.7, or 18.5 mg/m^2.[18] In that trial, DPD activity in peripheral-blood mononuclear cells was inactivated within 1 hour of eniluracil dosing and remained inhibited by 93% to 98% 24 hours after dosing.[18] Fourteen days after eniluracil therapy, mean DPD activity was approximately 60% (at the 0.74-mg/m^2 dose), 70% (3.7-mg/m^2 dose), and 125% (18.5-mg/m^2 dose) relative to baseline values. While this trial was in progress, CTEP notified investigators conducting studies of eniluracil therapy that life-threatening or fatal toxicities had been observed in a few patients treated with conventional doses of fluoropyrimidines, despite a 3-week waiting period after the last dose of eniluracil. A 4-week interval was then recommended. Because there was only limited information on the duration of DPD inactivation in clinical studies, and we observed pronounced enzyme inactivation 5 days after completing the first 3 days of eniluracil therapy, we amended our protocol to allow measurement of DPD activity after the completion of a full cycle of eniluracil therapy whenever possible. DPD activity remained substantially inhibited 12 days after eniluracil therapy; 19 days after eniluracil therapy, DPD activity had recovered to 100 pmol/min/mg in only one of five patients. Subsequently, investigators were notified by CTEP that several life-threatening and fatal toxicities had occurred in patients receiving standard-dose fluoropyrimidine therapy 4 and 5 weeks after eniluracil therapy; an 8-week wash-out period is now recommended. The most common schedule for eniluracil and 5-FU in clinical testing is 11.5 and 1.15 mg/m^2, respectively, PO twice daily for 28 of 35 days.[36,37] Additional studies geared toward characterizing the duration of DPD inactivation are being conducted by the pharmaceutical sponsor. It is critical to determine what level of DPD activity is sufficient to prevent toxicity associated with conventional-dose 5-FU therapy. Although 100 pmol/min/mg has been suggested as a cutoff for severe DPD deficiency, activity levels of 100 to 150 pmol/min/mg have been reported to reflect moderate DPD deficiency.[33,34,38,39]

Dramatic increases in plasma and urinary uracil levels are observed in patients with genetically based DPD deficiency, and a similar phenomenon is seen with pharmacologic inactivators of DPD, including eniluracil and sorivudine.[18,29,40-42] The value of monitoring "recovery" of uracil values in place of monitoring recovery of DPD activity is as yet unproven. Plasma uracil levels have generally been measured by HPLC methods. The sensitivity of this approach is hampered by the presence of endogenous uracil in plasma and the limits of ultraviolet absorption detection. Quantitation of uracil by gas chromatography–mass spectroscopy has several advantages. A stable isotope of uracil that contains ^{15}N rather than ^{14}N can be used to construct the standard curve, without interference from endogenous uracil. Further, mass spectroscopy has greater sensitivity than does ultraviolet detection. In our trial, baseline uracil levels were detectable in all patients in the submicromolar range (0.09 to 0.25 μmol/L). An unexpected finding was a consistent increase in uracil levels during the initial 5-FU infusion. Although decreased catabolism of uracil by DPD due to competition from 5-FU is a possible explanation, it does not fully account for the observation that the uracil levels continued to increase throughout the 24-hour infusion period. The plasma uracil levels were greatly increased the morning of day 2 of eniluracil therapy and were on average 121-fold greater than pretreatment values. Plasma uracil levels also increased throughout the 24-hour period of oral 5-FU dosing. The average uracil levels between hours 18 and 23 were 2.1-fold higher than the levels observed before the first oral dose of 5-FU, and they were 291-fold higher than pretreatment baseline values. Because DPD is maximally inactivated with bid dosing of eniluracil 20 mg, the additional increase in uracil levels during the oral 5-FU dosing period also suggests that a different process may be involved. Increases in plasma deoxyuridine levels have been reported in patients receiving antifolate thymidylate synthase inhibitors.[42,43] Accumulation of deoxyuridine

monophosphate occurs with thymidylate synthase inhibition, and conversion of the monophosphate to deoxyuridine intracellularly leads to efflux of the nucleoside into the extracellular compartment in preclinical models.[44-46] Deoxyuridine can be converted to uracil through the action of thymidine phosphorylase. This phenomenon should be investigated in future studies.

At least two mechanisms may account for the decrease in plasma uracil levels after eniluracil therapy: urinary excretion and recovery of DPD activity. Reversal of the acute 5-FU–associated increase in plasma uracil concentrations may also be a factor. In six patients in whom plasma uracil levels were measured on day 3 of eniluracil therapy and 5 days later, the values had decreased 4.8-fold, consistent with a half-life of approximately 2 days. More extended uracil sampling, performed in three patients, suggested an average half-life of 4.5 days. The projected time to reach the upper 99% confidence limits for pretreatment uracil values averaged 35 days, and the plasma uracil levels were projected to decrease to ≤ 1 μmol/L by 24 days. Additional studies are necessary to determine whether patients with normalized plasma uracil concentrations after eniluracil therapy can be safely treated with standard doses of 5-FU. These calculations serve to highlight the potential limitations associated with the use of surrogate markers to assess the pharmacodynamic effect of a drug: depending on the sensitivity of the assay, different conclusions might be drawn concerning when the effect of eniluracil had worn off.

Two schedules of 5-FU–plus–eniluracil therapy have been previously evaluated: one intended to simulate the monthly bolus schedule of 5-FU, and the other intended to simulate a protracted infusion for 4 of 5 weeks. With eniluracil 3.7 mg/m^2 PO on days 1 through 7, the highest tolerated dose of 5-FU given on days 2 through 6 was 25 mg/m^2; the recommended dose of 5-FU in single-agent therapy is 500 mg/m^2.[19] Thus, eniluracil therapy reduces the tolerated dose of 5-FU 20-fold. When given with 50 mg each of eniluracil and leucovorin on the same schedule, the recommended dose of oral 5-FU was 15 mg/m^2, which is 28-fold lower than the customary 425/20-mg/m^2 dose of 5-FU/leucovorin on a monthly schedule.[19] The 28-day schedule involves twice-daily administration of eniluracil and 5-FU, with recommended total daily doses of 11.5 mg/m^2 (eniluracil) and 1.15 mg/m^2 (5-FU), given PO for 28 of 35 days. The total daily 5-FU dose is thus 150-fold lower than the customary dose of 300 mg/m^2 when 5-FU is given as a single agent by protracted infusion. The required 5-FU dose reduction for the schedules involving eniluracil plus 5-FU intended to mimic infusional regimens is greater than the observed reduction in 5-FU clearance. Given that the activity of DPD indirectly influences the amount of 5-FU available for anabolism to its active nucleotide metabolites, a possible explanation is that twice-daily dosing of both 5-FU and eniluracil may result in more sustained exposure of normal tissues to 5-FU, with enhanced intracellular accumulation of cytotoxic 5-FU metabolites. A recent study of the effects of eniluracil on the metabolism of 5-FU in mice bearing colon 38 tumors using ^{19}F nuclear magnetic resonance spectroscopy confirmed a higher accumulation of 5-FU nucleotides and increased retention of 5-FU in both normal and tumor tissues.[47]

DPD expression may be a predictive factor for response to 5-FU–based therapy.[25-28,48] A recent clinical study has suggested that eniluracil therapy is capable of completely inhibiting DPD activity in tumor tissue.[29] It is hoped that pharmacologic inactivation of DPD will provide an opportunity to circumvent a specific mechanism of 5-FU resistance. Preclinical studies have indeed shown an improved therapeutic index with treatment with oral eniluracil plus 5-FU compared with continuous infusion of 5-FU alone.[49] Ongoing phase III trials comparing treatment with oral 5-FU plus eniluracil and commonly used IV 5-FU regimens will determine whether a similar improvement in clinical outcome can be achieved in cancer patients.

REFERENCES

1. Grem JL: 5-Fluorinated pyrimidines, in Chabner BA, Longo DL (eds): Cancer Chemotherapy and Biotherapy: Principles and Practice. Philadelphia, PA, Lippincott-Raven, 1996, pp 149-210

2. Lokich JJ, Ahlgren JD, Gullo JJ, et al: A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: A Mid-Atlantic Oncology Program study. J Clin Oncol 7:425-432, 1989

3. Leichman CG, Fleming TR, Muggia FM, et al: Phase II study of fluorouracil and its modulation in advanced colorectal cancer: A Southwest Oncology Group study. J Clin Oncol 13:1303-1311, 1995

4. Hansen RM, Ryan L, Anderson T, et al: Phase III study of bolus versus infusion fluorouracil with or without cisplatin in advanced colorectal cancer. J Natl Cancer Inst 88:668-674, 1996

5. Rougier P, Paillot B, LaPlanche A, et al: 5-Fluorouracil (5-FU) continuous intravenous infusion compared with bolus administration: Final results of a randomised trial in metastatic colorectal cancer. Eur J Cancer 33:1789-1793, 1997

6. Meta-Analysis Group in Cancer: Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. J Clin Oncol 16:301-308, 1998

7. Ardalan B, Singh G, Silberman H: A randomized phase I and phase II study of short-term infusion of high-dose fluorouracil with or without *N*-(phosphonacetyl)-L-aspartic acid in patients with advanced pancreatic and colorectal cancers. J Clin Oncol 6:1053-1058, 1988

8. Atkins JN, Muss HB, Case D, et al: High-dose 24-hour infusion of 5-fluorouracil in metastatic prostate cancer: A phase II trial of the

Piedmont Oncology Association. Am J Clin Oncol 14:526-529, 1991

9. Haas NB, Hines JB, Hudes GR, et al: Phase I trial of 5-fluorouracil by 24-hour infusion weekly. Invest New Drugs 11:181-185, 1993

10. Hsu CH, Yeh KH, Chen LT, et al: Weekly 24-hour infusion of high-dose 5-fluorouracil and leucovorin in the treatment of advanced gastric cancers: An effective and low-toxic regimen for patients with poor general condition. Oncology 54:275-280, 1997

11. O'Dwyer PJ, Ryan LM, Valone FH, et al: Phase III trial of biochemical modulation of 5-fluorouracil by IV or oral leucovorin or by interferon in advanced colorectal cancer: An ECOG/CALGB phase III trial. Proc Am Soc Clin Oncol 15:207, 1996 (abstr)

12. Kohne CH, Schoffski P, Wilke H, et al: Effective biomodulation by leucovorin of high-dose infusion fluorouracil given as a weekly 24-hour infusion: Results of a randomized trial in patients with advanced colorectal cancer. J Clin Oncol 16:418-426, 1998

13. Porter DJ, Chestnut WG, Merrill BM, et al: Mechanism-based inactivation of dihydropyrimidine dehydrogenase by 5-ethynyluracil. J Biol Chem 267:5236-5242, 1992

14. Baccanari DP, Davis ST, Knick VC, et al: 5-Ethynyluracil (776C85): A potent modulator of the pharmacokinetics and antitumor efficacy of 5-fluorouracil. Proc Natl Acad Sci U S A 90:11064-11068, 1993

15. Spector T, Harrington JA, Porter DJ: 5-Ethynyluracil (776C85): Inactivation of dihydropyrimidine dehydrogenase in vivo. Biochem Pharmacol 46:2243-2248, 1993

16. Khor SP, Amyx H, Davis ST, et al: Dihydropyrimidine dehydrogenase inactivation and 5-fluorouracil pharmacokinetics: Allometric scaling of animal data, pharmacokinetics and toxicodynamics of 5-fluorouracil in humans. Cancer Chemother Pharmacol 39:233-238, 1997

17. Baker SD, Khor SP, Adjei AA, et al: Pharmacokinetic, oral bioavailability, and safety study of fluorouracil in patients treated with 776C85, an inactivator of dihydropyrimidine dehydrogenase. J Clin Oncol 14:3085-3096, 1996

18. Schilsky RL, Hohneker J, Ratain MJ, et al: Phase I clinical and pharmacologic study of eniluracil plus fluorouracil in patients with advanced cancer. J Clin Oncol 16:1450-1457, 1998

19. Humerickhouse RA, Dolan ME, Haraf DJ, et al: Phase I study of eniluracil, a dihydropyrimidine dehydrogenase inactivator, and oral 5-fluorouracil with radiation therapy in patients with recurrent or advanced head and neck cancer. Clin Cancer Res 5:291-298, 1999

20. Zhang R, Soong SJ, Liu TP, et al: Pharmacokinetics and tissue distribution of 2-fluoro-beta-alanine in rats: Potential relevance to toxicity pattern of 5-fluorouracil. Drug Metab Dispos 20:113-119, 1992

21. Okada R, Shibutani M, Matsuo T, et al: Experimental neurotoxicity of 5-fluorouracil and its derivatives is due to poisoning by the monofluorinated organic metabolites, monofluoroacetic acid and alpha-fluoro-beta-alanine. Acta Neuropathol (Berl) 81:66-73, 1990

22. Akiba T, Okeda R, Tajima T: Metabolites of 5-fluorouracil, alpha-fluoro-beta-alanine and fluoroacetic acid, directly injure myelinated fibers in tissue culture. Acta Neuropathol (Berl) 92:8-13, 1996

23. Spector T, Cao S, Rustum YM, et al: Attenuation of the antitumor activity of 5-fluorouracil by (R)-5-fluoro-5,6-dihydrouracil. Cancer Res 55:1239-1241, 1995

24. Davis ST, Joyner SS, Baccanari DP, et al: 5-Ethynyluracil (776C85): Protection from 5-fluorouracil-induced neurotoxicity in dogs. Biochem Pharmacol 48:233-236, 1994

25. Ishikawa Y, Kubota T, Otani Y, et al: Dihydropyrimidine dehydrogenase activity and messenger RNA level may be related to the antitumor effect of 5-fluorouracil on human tumor xenografts in nude mice. Clin Cancer Res 5:883-889, 1999

26. Fischel JL, Etienne MC, Spector T, et al: Dihydropyrimidine dehydrogenase: A tumoral target for fluorouracil modulation. Clin Cancer Res 1:991-996, 1995

27. Etienne MC, Cheradame S, Fischel JL, et al: Response to fluorouracil therapy in cancer patients: The role of tumoral dihydropyrimidine dehydrogenase activity. J Clin Oncol 13:1663-1670, 1995

28. Diasio RB, Danenberg K, Johnson M, et al: Quantitation of dihydropyrimidine dehydrogenase expression in tumor specimens of patients treated with 5-fluorouracil using a quantitative polymerase chain reaction assay. Proc Am Assoc Cancer Res 39:188, 1998 (abstr 1286)

29. Ahmed FY, Johnston SJ, Cassidy J, et al: Eniluracil treatment completely inactivates dihydropyrimidine dehydrogenase in colorectal tumors. J Clin Oncol 17:2439-2445, 1999

30. Anderson LW, Parker RJ, Collins JM, et al: Gas chromatographic-mass spectrometric method for routine monitoring of 5-fluorouracil in plasma of patients receiving low-level protracted infusions. J Chromatogr 581:195-201, 1992

31. Bi D, Anderson LW, Shapiro J, et al: Measurement of plasma uracil using gas chromatography-mass spectrometry in normal individuals and in patients receiving inhibitors of dihydropyrimidine dehydrogenase. J Chromatogr B Biomed Sci Appl 738:249-258, 2000

32. Anderson D, Kerr DJ, Blesing C, et al: Simultaneous gas chromatographic-mass spectrophotometric determination of alpha-fluoro-beta-alanine and 5-fluorouracil in plasma. J Chromatogr B Biomed Sci Appl 688:87-93, 1997

33. Lu A, 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 53: 5433-5438, 1993

34. Lu Z, Zhang R, Carpenter JT, et al: Decreased dihydropyrimidine dehydrogenase activity in a population of patients with breast cancer: Implication for 5-fluorouracil-based chemotherapy. Clin Cancer Res 4:325-329, 1998

35. Baker SD, Diasio RB, O'Reilly S, et al: Phase I and pharmacologic study of oral fluorouracil on a chronic daily schedule in combination with the dihydropyrimidine dehydrogenase inactivator eniluracil. J Clin Oncol 18:915-926, 2000

36. Smith I, Johnston S, O'Brien M, et al: High activity with eniluracil (776C85) and continuous low dose oral 5-fluorouracil (1 $mg/m^2 \times 2$ daily) as first-line chemotherapy in patients with advanced breast cancer: A phase II study. Proc Am Soc Clin Oncol 18:106a, 1999 (abstr 402)

37. Mani S, Beck T, Chevlen E, et al: A phase II open-label study to evaluate a 28-day regimen of oral 5-fluorouracil plus 776C85 for the treatment of patients with previously untreated metastatic colorectal cancer. Proc Am Soc Clin Oncol 17:281a, 1998 (abstr 1083)

38. Fleming RA, Milano GA, Gaspard MH, et al: Dihydropyrimidine dehydrogenase activity in cancer patients. Eur J Cancer 29A:740-744, 1993

39. Milano G, Etienne MC, Pierrefite V, et al: Dihydropyrimidine dehydrogenase deficiency and fluorouracil-related toxicity. Br J Cancer 79:627-630, 1999

40. Okuda H, Watabe T, Kawaguchi Y, et al: Lethal drug interactions of sorivudine, a new antiviral drug, with oral 5-fluorouracil prodrugs. Drug Metab Dispos 25:270-273, 1997

41. Yan J, Tyring SK, McCrary MM, et al: The effect of sorivudine on dihydropyrimidine dehydrogenase activity in patients with acute herpes zoster. Clin Pharmacol Ther 61:563-573, 1997

42. Rafi I, Boddy AV, Calvete JA, et al: Preclinical and phase I clinical studies with the nonclassical antifolate thymidylate synthase inhibitor nolatrexed dihydrochloride given by prolonged administration in patients with solid tumors. J Clin Oncol 16:1131-1141, 1998

43. O'Dwyer PJ, Laub PB, DeMaria D, et al: Phase I trial of the thymidylate synthase inhibitor AG331 as a 5-day continuous infusion. Clin Cancer Res 2:1685-1692, 1996

44. Grem JL, Mulcahy RT, Miller EM, et al: Interaction of deoxyuridine with fluorouracil and dipyridamole in a human colon cancer cell line. Biochem Pharmacol 38:51-59, 1989

45. Aherne GW, Hardcastle A, Raynaud F, et al: Immunoreactive dUMP and TTP pools as an index of thymidylate synthase inhibition: Effect of tomudex (ZD1694) and a nonpolyglutamated quinazoline antifolate (CB30900) in L1210 mouse leukaemia cells. Biochem Pharmacol 51:1293-1301, 1996

46. Ismail A-SA, Van Groeningen CJ, Hardcastle A, et al: Modulation of fluorouracil cytotoxicity by interferons alpha and gamma. Mol Pharmacol 53:252-261, 1998

47. Adams ER, Leffert JJ, Craig DJ, et al: In vivo effect of 5-ethynyluracil on 5-fluorouracil metabolism determined by ^{19}F nuclear magnetic resonance spectroscopy. Cancer Res 59:122-127, 1999

48. McLeod HL, Sludden J, Murray GI, et al: Characterization of dihydropyrimidine dehydrogenase in human colorectal tumours. Br J Cancer 77:461-465, 1998

49. Cao S, Rustum YM, Spector T: 5-Ethynyluracil (776C85): Modulation of 5-fluorouracil efficacy and therapeutic index in rats bearing advanced colorectal carcinoma. Cancer Res 54:1507-1510, 1994