Cancer Chemother Pharmacol (2003) 52: 79–85
DOI 10.1007/s00280-003-0613-0

ORIGINAL ARTICLE

**Xiao-Du Guo · Nancy Harold · M. Wasif Saif
Barbara Schuler · Eva Szabo · J. Michael Hamilton
Brian P. Monahan · Mary G. Quinn · Janet Cliatt
Diana Nguyen · Frank Grollman · Rebecca R. Thomas
Elizabeth A. McQuigan · Richard Wilson
Chis H. Takimoto · Jean L. Grem**

Pharmacokinetic and pharmacodynamic effects of oral eniluracil, fluorouracil and leucovorin given on a weekly schedule

Received: 23 October 2002 / Accepted: 6 March 2003 / Published online: 18 April 2003

Abstract *Purpose*: To determine the toxicities and pharmacokinetic effects of eniluracil (EU) given on two weekly dosing schedules with 5-fluorouracil (5-FU) and leucovorin (LV). *Methods*: A group of 26 patients received a single 24-h i.v. infusion of 5-FU 2300 mg/m^2 to provide a pharmacokinetic reference. After 2 weeks, patients received oral EU 20 mg plus LV 30 mg on days 1–3 with a single dose of 5-FU 15–29 mg/m^2 on day 2, or LV 30 mg on days 1–2 with a single dose of EU at least 1 h prior to 5-FU 29 mg/m^2 on day 2 weekly for 3 of 4 weeks. *Results*: Diarrhea was the most common dose-limiting toxicity. The recommended dose of 5-FU is 29 mg/m^2 per day. EU on either schedule decreased 5-FU plasma clearance by 48 to 52-fold, prolonged the half-life to >5 h, and increased the percentage of 5-FU excreted in the urine from 2% to 64–66%. With EU, plasma fluoro-β-alanine was not detected while urinary excretion was reduced to $<1\%$ of that seen with i.v. 5-FU alone. Marked increases in both plasma and urinary uracil were seen. Thymidylate synthase ternary complex formation was demonstrated in bone marrow mononuclear cells isolated 24 h after the first oral 5-FU dose; the average was 66.5% bound. *Conclusions*: Either a single 20-mg dose of EU given prior to or for 3 days around the oral 5-FU dose led to comparable effects on 5-FU pharmacokinetic parameters, and inhibition of dihydropyrimidine dehydrogenase and thymidylate synthase.

Keywords Fluorouracil · Thymidylate synthase · Dihydropyrimidine dehydrogenase · Eniluracil · Pharmacokinetics

X.-D. Guo · N. Harold · M. W. Saif · B. Schuler · E. Szabo
J. M. Hamilton · M. G. Quinn · J. Cliatt · D. Nguyen
F. Grollman · R. R. Thomas · R. Wilson · C. H. Takimoto
J. L. Grem (✉)
National Cancer Institute-Navy Medical Oncology,
Cancer Therapeutics Branch, Center for Cancer Research,
National Naval Medical Center, 8901 Wisconsin Ave.,
Bethesda, MD 20889-5105, USA
E-mail: gremj@mail.nih.gov
Tel.: +1-301-4355382
Fax: +1-301-4801683

B. P. Monahan
Hematology/Oncology Section, Department of Medicine,
National Naval Medical Center, Bethesda, Maryland, USA

E. A. McQuigan
Department of Radiology,
National Naval Medical Center, Bethesda, Maryland, USA

Introduction

Eniluracil (EU) is a potent inactivator of dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme in the catabolism of 5-fluorouracil (5-FU) [18]. Clinical studies have shown that EU allows essentially complete bioavailability of orally administered 5-FU, and prolongs the half-life to 5 h [1, 2, 17, 18, 22]. Two schedules were initially developed: single daily dosing of EU for 7 days with oral 5-FU and leucovorin (LV) on days 2–5 every 4 weeks, and twice-daily dosing of EU/5-FU for 28 of 35 days [13]. We have previously reported the results of a phase I trial of oral EU/5-FU/LV designed to mimic a weekly high-dose 24-h infusion of 5-FU [7]. A group of 12 patients received 20 mg EU and 15 mg LV orally twice daily on days 1–3, with a starting dose of either 10 or 15 mg/m^2 5-FU given twice on day 2; treatment was repeated weekly for 3 of 4 weeks. In the previous trial, the duration of DPD inactivation as measured directly in peripheral blood mononuclear cells in these patients was much longer (average activity 24% of baseline 19 days after the last EU dose) than reported by other investigators in subjects receiving EU alone (complete recovery within 7–14 days) [8, 22]. There is clinical relevance and real toxicity concerns of DPD

inhibition beyond simply modulating 5-FU, since administration of full doses of fluoropyrimidines to patients with abnormally low DPD activity can lead to life-threatening or fatal toxicities.

In the current clinical trial, a single daily dose of 20 mg given with LV on days 1–3 and 5-FU on day 2 was evaluated, and was later amended to study a single dose of EU given 1–12 h prior to 5-FU. The primary objectives were to evaluate the pharmacokinetics of 5-FU and fluoro-β-alanine (FBAL, a catabolite implicated in certain host toxicities) in plasma and urine, to monitor changes in uracil levels as an indirect reflection of DPD inactivation, and to determine whether inhibition of thymidylate synthase (TS) occurred in a surrogate host tissue. The clinical results, and pharmacokinetic and pharmacodynamic effects of these two different schedules of EU/5-FU/LV are reported here. We have previously reported that DPD activity in peripheral blood mononuclear cells during oral therapy is profoundly depressed with both schedules, while DPD activity appears to recover to baseline values by 2 and 3 weeks after the single dose and daily-for-3-days schedules, respectively [10].

Patients and methods

Eligibility

Patients were accrued into this amended protocol between October 1998 and July 2000. Patients with solid tumors for whom a 5-FU/LV-based regimen represented a reasonable therapeutic approach or for whom no effective standard therapy was available were eligible provided they had adequate hematologic, hepatic and renal function as previously reported for the twice-daily schedule of EU on days 1–3 [7]. 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 EU (10 mg) and 5-FU (5 mg and 25 mg) were formulated by Glaxo Wellcome (Research Triangle, N.C.) and supplied by CTEP. Commercial sources were used for the intravenous (i.v.) 5-FU and oral LV. To provide a pharmacologic reference, it was planned that all patients should receive a single i.v. infusion of 5-FU 2300 mg/m^2 over 24 h on day 2, with oral LV 30 mg given once daily on days 1–3. Oral therapy was begun 2 weeks later. During the dose escalation portion of the study, EU 20 mg and LV 30 mg were given orally daily on days 1–3 with 5-FU on day 2 at a starting dose of 15 mg/m^2; this was repeated weekly for 3 of 4 weeks. Dose escalation was planned in cohorts of three to six patients with 25% increments until dose-limiting toxicity (defined below) was seen in at least two patients at a given level during the initial cycle. In this portion of the trial, 16 patients were enrolled (patients 13 to 28) at one of four 5-FU dose levels between 15 and 29 mg/m^2. Ten patients were subsequently enrolled and received LV 30 mg orally on days 1 and 2, with a single dose of EU 20 mg given either at bedtime the night before (patients 29 to 33) or at least 1 h prior to oral 5-FU 29 mg/m^2 orally on day 2 (patients 34 to 38).

Dose-limiting toxicity was defined as a granulocyte nadir $< 500/\mu l$ at any time, a platelet nadir $< 50{,}000/\mu l$ at any time, grade 2 nonhematologic toxicity (excluding nausea, vomiting, and alopecia) occurring prior to completion of the planned weekly treatments, grade 2 or worse neurotoxicity (excluding grade 2 headache) at any time, grade 3 nonhematologic toxicities 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 NCI Common Toxicity Criteria version 1 were used. The dose of 5-FU was reduced one dose level for a granulocyte nadir $< 500/\mu l$, a platelet nadir $< 50{,}000/\mu l$, or for grade 3 or 4 nonhematologic toxicity at any time or grade 2 toxicity prior to completing the therapy for that cycle. Individual dose escalation was permitted provided that nonhematologic toxicity was $\leq$ grade 1, hematologic toxicity was $\leq$ grade 2, and no treatment interruptions or delays were required. If no toxicity was reported, the dose was increased by 50%; a 25% increment was used if grade 1 toxicity was observed. The doses of EU and LV remained the same.

Patient evaluation and follow-up

A blood count with WBC differential was obtained weekly. Liver function tests, blood urea nitrogen, creatinine and electrolytes were obtained on day 1 of each cycle. Radiographic studies were repeated every third cycle. Treatment was continued indefinitely until there was evidence of disease progression provided it was tolerated and the patient agreed.

Pharmacokinetic studies

Patients were hospitalized for the 24-h infusion of 5-FU and following the initial oral dose of 5-FU to permit extended blood sampling for pharmacokinetic analysis. Blood samples were obtained before treatment, at 0.5, 1, 4, 7, 10, 13, 18, 22 and 23 h during the infusion, immediately prior to the end of infusion, and at 15, 30 and 60 min after infusion. On the first day of oral 5-FU, blood samples were obtained prior to and at 0.5, 1, 1.5, 2, 3, 6, 9, 12, 15, 18, 22, 24 and 26 h after 5-FU dosing. The blood was collected into heparinized tubes and immediately placed on ice. The plasma was centrifuged at 800 *g* for 15 min at 4°C, then 1.8-ml aliquots were frozen at −70°C until the time of analysis. A 24-h urine collection was obtained on both occasions. The volume of urine was measured and recorded at 8-h intervals, and an aliquot of urine was transferred to a 50-ml tube placed on ice; the urine was stored at −70°C.

Unless otherwise stated, chemicals were obtained from Sigma Chemical Company (St. Louis, Mo.). 5-FU, uracil, and FBAL (Fluorochem USA, W. Columbia, S.C.) were measured using validated gas chromatography/mass spectroscopy methods as previously described [3, 10]. Plasma (100 µl) or urine (10 µl) was spiked with the internal standard, 5-chlorouracil. Standard curves were constructed using donor plasma with 5-FU concentrations ranging from 0.01 to 100 µ*M*, FBAL concentrations ranging from 0.25 to 100 µ*M*, and [$^{15}N_2$]uracil from 0.025 to 250 µ*M*.

Noncompartmental analysis was used to estimate the area under the concentration-time curve (AUC_{last}) with WinNonLin version 3.1 software (Pharsight, Mountain View, Calif.). The elimination half-life was estimated from the terminal portion of the plasma concentration versus time curve (AUC).

Statistical and graphical analysis

Graphical analysis was performed with SigmaPlot 2001 for Windows (SPSS, Chicago, Ill.), and statistical analysis was performed with SigmaStat for Windows version 2.03 (SPSS). The median time to progression was calculated by a Kaplan-Meier survival curve. The Cockcroft-Gault formula was used to calculate the estimated creatinine clearance. The strength of the linear association between pairs of variables was determined by Pearson correlation.

Assessment of TS inhibition

In patients who had no prior pelvic irradiation and were not receiving therapeutic anticoagulation, a bone marrow aspirate was obtained prior to any protocol therapy, and again on day 3 of period 2 (24 h after the oral 5-FU dose). The samples were first passed though a 400 μm×25 mm filter disc held in a Swinnex (Millipore) filter holder to remove bone spicules and fat globules. The mononuclear cells were isolated by Ficoll-Hypaque density centrifugation at room temperature, and erythrocytes were removed with a brief hypotonic lysis step. Aliquots of intact cell pellets were stored at −70°C until analysis.

On the day of analysis, a protease inhibitor cocktail was added to the frozen bone marrow mononuclear cell pellets, the cell membranes were disrupted by sonication, and the cellular lysate was isolated after centrifugation at 12,000 *g* for 15 min at 4°C. Free and bound TS protein was determined by a semiquantitative Western blot analysis as previously described [9, 19]. Protein samples isolated from HCT8 colon cancer cells treated with no drug or with 100 μ*M* 5-FU for 4 h served as negative and positive controls on each gel.

Table 1 Patient characteristics

Number of patients	26
Age (years)	
Median	61.5
Range	32–80
Gender	
Male	17
Female	9
ECOG performance status	
0	4
1	18
2	4
Prior therapy	
Radiationtherapy	8
Chemotherapy	24
Number prior regimens	
Median	2
Range	0–4
Histology	
Colorectal	19
Pancreas	3
Cholangiocarcinoma	2
Other	2

Results

Of 26 patients enrolled (Table 1), the majority had colorectal cancer, and all but one had received prior chemotherapy. Three patients received only the initial 24-h i.v. 5-FU infusion due to a personal decision to withdraw (one patient) or rapid disease progression (two patients). The i.v. 5-FU infusion was omitted in one patient due to poor venous access. Therefore, 23 patients were assessable for clinical toxicity with oral 5-FU/LV/EU.

Among the first 15 patients treated with three daily doses of EU/LV, dose-limiting diarrhea was seen during the initial cycle in one of six patients entered at 29.3 mg/m^2 (Table 2). Dose escalation and reduction were tailored to individual patient tolerance (Table 3). One patient escalated to 29.3 mg/m^2 had dose-limiting

Table 2 Toxicity cycle 1 for both schedules

EU schedule	5-FU (mg/m^2)	Patients	Mucositis grade[a]		Diarrhea grade[a]			AGC nadir (per μl)	
			1	2	1	2	3	Median	Range
Daily ×3	15	3	2	0	2	0	0	2103	1680–3976
Daily ×3	18.8	3	0	0	0	1	0	4658	2425–6502
Daily ×3	23.4	3	0	0	0	0	0	2852	1952–6502
Daily ×3	29.3	6	1	0	2	0	1	3790	1638–7370
Daily ×1	29.3	8	0	0	2	1	0	4678	2091–6253

[a]Common Toxicity Criteria

Table 3 Dose-limiting toxicities during any cycle for 15 patients treated with EU/LV days 1–3 and 5-FU day 2. Dose escalation in individual patients was allowed if minimal toxicity was seen the previous cycle. The dosage increment was 50% if no grade 1 toxicity was seen, and 25% if grade 1 toxicity occurred

5-FU dose		Patients			Cycles		Dose-limiting toxicities			
mg/m^2/day	Total mg/day	New	Total	With DLT	Total	With DLT (%)	Toxicity type	Grade	Cycle	No. of patients
15	25–35	3	4	0	6	0	–	–	–	–
18.8	25–45	3	6	1 (16.7%)	21	4.8	Diarrhea	3	2	1
23.4	35–60	3	6	1 (16.7%)	13	7.7	Sensorimotor neuropathy	2	7	1
29.3	45–60	6	7	2 (28.6%)	11	18.2	Diarrhea	3	1	1
								3	2	1
34–44	55–70	0	5	2 (40%)	10	20	Diarrhea/AGC	4/4	2	1
							Fatigue	3	3	1
51–55	90–105	0	2	2 (100%)	6	33	Diarrhea	3	5	1
							Sensorimotor neuropathy	2	9	1
63	135	0	1	1 (100%)	1	100	Sensorimotor neuropathy	2	7	1

diarrhea in cycle 2. Although some individuals tolerated higher doses, two of five patients escalated from lower tolerated doses to 34–44 mg/m^2 experienced dose-limiting toxicity. Based on these safety considerations, a decision was made not to pursue further dose escalation for subsequent patient cohorts beyond 29.3 mg/m^2. Two male patients unexpectedly developed delayed onset symptoms of unsteady gait and reduced sensation in the legs during the seventh cycle of therapy. Electromyogram and nerve conduction studies documented axonal sensorimotor polyneuropathy with secondary demyelinative features; details of these two cases have been previously published [21].

Since analysis of the duration of DPD inhibition in the patients treated with the three daily doses of 20 mg EU indicated that enzyme activity remained markedly depressed up to 12 days after EU dosing [6], we subsequently evaluated a single dose of EU given with 29.3 mg/m^2 oral 5-FU, and LV 30 mg on days 1 and 2 only. Four patients received the EU at bedtime the night before the first oral 5-FU dose (no more than 12 h prior to the 5-FU dose), while the final four patients received EU the morning of 5-FU dosing (at least 1 h prior to 5-FU). None of these eight patients experienced dose-limiting toxicity during their initial cycle with 29 mg/m^2 5-FU.

When the worst toxicity experienced by each patient across all cycles of therapy was considered (Table 4), grade 3 or worse nonhematologic toxicities of the following types were seen (percent of patients): diarrhea (17.4%), and abdominal discomfort and fatigue (4.3% each). One patient developed confusion of grade 3 severity, which was considered possibly related to therapy. Hematologic toxicity was generally of mild to moderate severity.

For all 26 patients (intention to treat), the median time to treatment failure (TTF) was 2.8 months. Two confirmed partial responses were seen among 17 assessable patients (11.8%) with colorectal cancer who had progressed on prior 5-FU/LV therapy (median TTF 11.8 and 12.8 months). The median 5-FU dose these two patients received was 33.8 and 18.8 mg/m^2.

Table 4 Worst toxicity per patient across all cycles of therapy. Of 26 patients enrolled, 23 were assessable for toxicity with EU/5-FU (three patients received only the initial 24-h i.v. infusion of 5-FU)

Toxicity	Number of patients experiencing toxicity			
	Grade 1	Grade 2	Grade 3	Grade 4
Nausea/vomiting	6	5	0	0
Mucositis	8	1	0	0
Diarrhea	4	7	3	1
Anorexia	7	1	0	0
Constipation	5	0	0	0
Abdominal discomfort	3	2	1	0
Fatigue	12	2	1	0
Skin	6	0	0	0
Ocular	3	0	0	0
WBC	2	2	2	0
AGC	3	3	0	1
Hemoglobin	12	7	4	0
Platelet	8	1	0	0
Peripheral neuropathy	0	2	0	0
Confusion	0	0	1	0

Pharmacokinetic results

In 22 patients who received the initial 24-h i.v. infusion of 5-FU 2300 mg/m^2, near steady-state plasma levels of 5-FU were observed 30 min after the start of the infusion, and were in the 5–6 μ*M* range (data not shown). Upon completion of the 24-h infusion, the 5-FU levels dropped quickly with a half-life of 11 min. FBAL levels in plasma approached steady-state by 7 h into the infusion, and were on average about 9–14 times higher than 5-FU through the end of the infusion. Urinary excretion of parent drug over the 24-h period represented about 2% of the total drug administered.

The 5-FU plasma concentration-time curve (AUC) during oral EU therapy is shown in Fig. 1. There was no appreciable difference in the pharmacokinetic results for the patients who received a single dose of EU either the night before or the morning of initial oral 5-FU dosing, and the data are combined for these eight patients. The 5-FU pharmacokinetic parameters observed in patients receiving either the daily for 3 days or single EU dose schedule were similar (Table 5). The 5-FU elimination half-life was increased almost 30-fold to 5.1–5.6 h. Plasma clearance was reduced by about 50-fold. The proportion of 5-FU excreted as parent drug in the urine over a 24-h period was dramatically increased to about 65% of the administered dose. In contrast to the high plasma levels of FBAL during the i.v. 5-FU infusion without EU, FBAL was not detected in plasma in any patient during oral EU/5-FU therapy. FBAL was detected in the urine in all patients, but the amount was

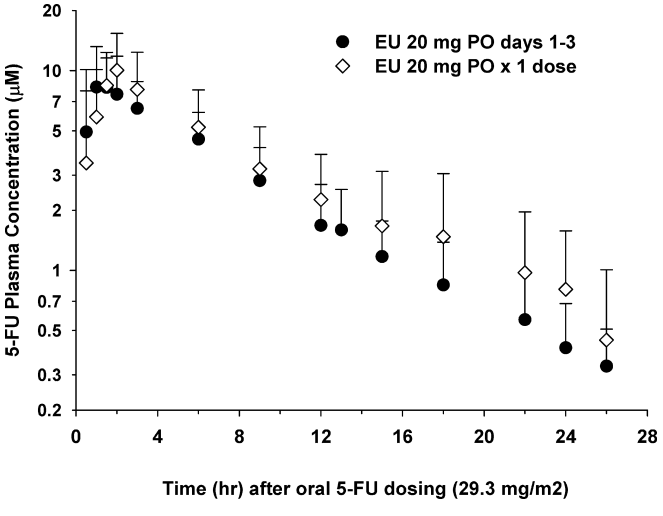

Fig. 1 Plasma concentration-time curve for 5-FU during oral therapy with EU. 5-FU plasma levels (means ± SD) following the initial oral dose of 29.3 mg/m^2 are shown (20 mg EU daily for 3 days schedule, $n = 6$; single EU dose schedule, $n = 8$)

Table 5 Summary of pharmacokinetic parameters. The data were analyzed by non-compartmental analysis using the actual times the samples were drawn and are presented as the means ± SD (*ND* not detected)

Parameter	5-FU 2300 mg/m^2 i.v. ($n=22$)	Oral 5-FUdose (mg/m^2) (5-FU day 2 + EU 20 mg orally days 1–3 or for one dose)				
		15 ($n=3$) (30–35 mg)	18.8 ($n=3$) (25–35 mg)	23.4 ($n=3$) (35–45 mg)	29.3 ($n=6$) (45–60 mg)	29.3[a] ($n=8$) (55–66 mg)
C_{max} 5-FU (μ*M*)	9.6 ± 3.8	8.0 ± 3.2	8.0 ± 2.0	8.9 ± 3.2	9.7 ± 3.6	11.1 ± 5.1
AUC_{last} 5-FU (μ*M*·h)	140.4 ± 45.8	39.1 ± 5.8	73.7 ± 32.4	50.5 ± 1.7	66.3 ± 24.5	80.1 ± 42.5
Terminal half-life 5-FU(h)	0.19 ± 0.1	4.6 ± 1.4	15.0 ± 16.6[b]	6.5 ± 0.9	5.7 ± 0.9	6.4 ± 2.6
$Vz_{observed}$ (l/m^2)	45.9 ± 24.1	19.6 ± 4.8	21.7 ± 7.1	31.6 ± 4.4	29.0 ± 9.6	28.6 ± 11.6
Cl or Cl/F (ml/min/m^2)	2680.3 ± 1087.9	50.3 ± 10.6	28.8 ± 16.5	56.8 ± 5.2	58.4 ± 17.2	62.6 ± 48.8
Urinary excretion of5-FU, 0–24 h (% of dose)	2.0 ± 0.5	88.9 ± 4.9	55.0[c]	62.3 ± 20.2[d]	60.6 ± 10.4	63.9 ± 19.2
C_{max} FBAL (μ*M*)	90.4 ± 34.9	ND	ND	ND	ND	ND
Urinary excretion ofFBAL over 24 h (μmol)	8165 ± 5599	80.3 ± 16.3	156.9[c]	22.9 ± 4.6[d]	17.7 ± 7.5	66.7 ± 23.8
C_{max} uracil (μ*M*)	1.1 ± 0.4	49.6 ± 11.1	51.1 ± 10.5	79.3 ± 52.1	56.5 ± 10.6	65.4 ± 32.1
Urinary excretion ofuracil over 24 h (μmol)	79.6 ± 44.8	4017 ± 412	2080[c]	2878 ± 1401[d]	2170 ± 499	2787 ± 883

[a]Cohort receiving a single dose of eniluracil
[b]The median (range) is 6.6 h (4.3–34.1 h)
[c]Only one patient had a complete 24-h urine collection
[d]Only two patients had a complete 24-h urine collection, and the mean ± one-half range is shown

reduced by over two orders of magnitude with oral EU/5-FU.

Pretreatment plasma uracil levels averaged 0.28 ± 0.13 μ*M*. During the 24-h i.v. infusion of 5-FU without EU, the plasma uracil levels increased throughout the infusion, and the maximum levels averaged 1.11 ± 0.35 μ*M*. This finding may reflect competition between 5-FU and uracil for catabolism by DPD. During the 24-h sampling period after oral dosing with EU-modulated 5-FU, uracil levels in plasma were dramatically elevated 49- to 62-fold above those seen at the end of the 5-FU i.v. infusion, and were 193- to 248-fold higher than the baseline uracil levels. Further, urinary excretion of uracil increased about 34-fold. The effects of both the single and three daily dose schedules on plasma and urine uracil levels were comparable.

The AUC of 5-FU during oral dosing did not correlate with either the degree of granulocytopenia or the severity of diarrhea, presumably because the incidence of grade 3–4 toxicities during the initial cycle was very low. A strong correlation was seen between the estimated creatinine clearance and 5-FU clearance during oral dosing that included EU ($r=0.728$, $P=0.00012$).

Inhibition of TS during oral 5-FU/LV/EU therapy

A major objective of this trial was to assess whether this weekly oral regimen resulted in inhibition of TS, and the surrogate tissue employed was bone marrow mononuclear cells. Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot techniques, TS bound in a ternary complex with 5,10-methylenetetrahydrofolate and 5-fluoro-2′-deoxyuridine monophosphate has slower migration than unbound TS. The patient samples were analyzed on four separate Western blots. Only free protein was detected in the bone marrow samples taken prior to receiving any protocol therapy, while bound TS was detected in all patients in a paired bone marrow sample obtained about 24 h after the initial oral 5-FU dose (Fig. 2). Compared to the pretreatment sample, an increase in total TS content (free plus bound) was seen in 18 of 19 samples obtained after 5-FU dosing: the median increase was 4.05-fold (range 0.85- to 34.54-fold). An increase in free TS content in the post-treatment sample was less striking (median 1.29-fold, range 0.3- to 11.9-fold). When evaluated according to two ranges of oral 5-FU dose, the proportion of TS bound was significantly higher in patients receiving 50 to 60 mg (mean ± SD 70.1 ± 5.0%, $n=12$) than in patients receiving 30 to 45 mg (60.3 ± 4.2%, $n=7$, $P<0.001$, *t*-test). There was no relationship with hematologic toxicity, presumably because this regimen produced little myelosuppression.

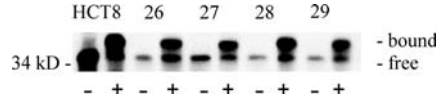

Fig. 2 Assessment of bound and free TS in paired bone marrow mononuclear cells before therapy and after oral 5-FU/LV/EU. Equal amounts of protein (50 μg) from lysates prepared from bone marrow mononuclear cells were resolved by 10% SDS-polyacrylamide gel electrophoresis (*minus sign* sample obtained prior to receiving any protocol therapy, *plus sign* sample taken the day after the initial oral 5-FU dose). Lysate (20 μg protein) from HCT8 cells treated with diluent or 100 μ*M* 5-FU for 4 h served as a reference for each blot. This represents one of four Western blots that were run to analyze the 19 patient samples

Discussion

A single oral dose of 5-FU 29 mg/m^2 on day 2 given with oral LV 30 mg on days 1–3 and either three daily doses or one dose of EU 20 mg was well tolerated on a weekly for 3 of 4 weeks schedule (only 1 of 14 patients had dose-limiting toxicity during cycle 1 at 29.3 mg/m^2 5-FU). Although some individuals tolerated at least one

cycle at higher 5-FU doses, three patients who received multiple cycles ultimately required dose reduction back to either 29.3 or 33.9 mg/m^2. Further dose escalation of 5-FU with the single oral dose of EU might have been attempted, but suspension of the clinical development of EU precluded additional protocol modifications. With our previous schedule involving twice-daily dosing of EU and LV on days 1–3 with 5-FU on day 2, the highest tolerated dose of 5-FU was 10 mg/m^2 (20 mg/m^2 total) [7]. Switching to the current schedules thus allowed a 45% 5-FU dose increase. In addition, the total 5-FU $AUC_{0-26\ h}$ at the recommended dose (10 mg/m^2 5-FU twice daily) for both doses given 12 h apart averaged 33 $\mu M \cdot$h, compared to 66 and 80 $\mu M \cdot$h with 5-FU 29.3 mg/m^2 on the current schedules. Potential explanations for the improved tolerance with the current schedules despite a higher total daily 5-FU dose and AUC include the possibility that the plasma AUC may not accurately reflect the local concentration of 5-FU metabolites in the gastrointestinal mucosa with oral 5-FU/EU therapy; further, the clinical toxicity may be influenced by the duration of DPD inhibition. Another variable with the current study was administration of LV as a single versus twice-daily dose. There were no appreciable differences between the pharmacokinetic profile of 5-FU or the urinary excretion of FBAL with either three daily doses or a single dose of EU in the current trial. The increase in uracil plasma levels and its urinary excretion were also similar with either three doses or a single dose of EU.

While TS is thought to be the primary target of 5-FU-based therapy, there is limited information to confirm whether inhibition of TS occurs in patients receiving various clinical regimens. We documented the formation of TS ternary complex in bone marrow mononuclear cells isolated during therapy with oral EU/5-FU/LV in all subjects, whereas only free TS was noted in the pretreatment samples. The extent of ternary complex formation in all patient samples was similar for both EU schedules. Total TS content was increased 24 h after oral 5-FU/EU therapy by a median of fourfold. This observation is consistent with prior reports that exposure to TS inhibitors leads to an acute increase in total TS protein content in both in vitro and in vivo models. This increase is generally attributed to translational autoregulation, although a longer half-life when TS is bound in the ternary complex may also contribute [4, 5, 11].

Oral EU/5-FU has shown phase II activity in a number of solid tumors. In two studies, a regimen involving EU 50 mg on days 1–7, and either 5-FU 20 mg/m^2 with LV 50 mg or 5-FU 25 mg/m^2 without LV on days 2–6 has been shown to be active in patients with previously untreated advanced colorectal cancer (response rates of 13% and 21%), but there was a high incidence of neutropenia [16, 23]. The combination of EU 10 mg/m^2 and 5-FU 1 mg/m^2 twice daily for 28 of 35 days, designed to simulate a protracted i.v. infusion of 5-FU, has been more extensively studied. This regimen has an acceptable safety profile, and hematologic toxicity is unusual. In phase II studies, in patients with previously untreated advanced disease this regimen has demonstrated activity in squamous cell cancer of the head and neck, colorectal cancer, and breast cancer [12, 14, 26]. In two trials, objective responses have been shown in 10% and 18% of patients with anthracycline- and taxane-refractory breast cancer [20, 25]. The combined results of two prior studies of EU/5-FU in colorectal cancer patients whose disease had progressed on prior 5-FU therapy indicated only 1 of 96 patients achieved a partial response [15, 23]. In the present study, we observed two confirmed partial responses among 17 colorectal cancer patients who had failed prior 5-FU/LV.

Two randomized trials comparing the twice-daily 28 of 35 days schedule of oral EU/5-FU to a standard monthly schedule of bolus 5-FU modulated by LV in advanced colorectal cancer have failed to show equivalence [24, 27]. Do these disappointing results suggest that inhibition of DPD as a therapeutic strategy has failed? Perhaps not. Compliance with an oral regimen is a potential issue. Since diarrhea is dose limiting with the twice-daily 28-day EU/5-FU schedule, local exposure of the gastrointestinal mucosa and the intracellular levels of active 5-FU metabolites are likely greater than anticipated from the plasma AUC. EU by itself does not have antitumor activity, and the biochemical data suggest that far greater doses of EU were used than necessary to achieve DPD inhibition. Additional studies exploring alternate doses/schedules of EU may be reasonable.

References

1. Baker SD, Khor SP, Adjei AA, Doucette M, Spector T, Donehower RC, Grochow LB, Sartorius SE, Noe DA, Hohneker JA, Rowinsky EK (1996) Pharmacokinetic, oral bioavailability, and safety study of fluorouracil in patients treated with 776C85, an inactivator of dihydropyrimidine dehydrogenase. J Clin Oncol 14:3085
2. Baker SD, Diasio RB, O'Reilly S, Lucas VS, Khor SP, Sartorius SE, Donehower RC, Grochow LB, Spector T, Hohneker JA, Rowinsky EK (2000) 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
3. Bi D, Anderson LW, Shapiro J, Shapiro A, Grem JL, Takimoto CH (2000) 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
4. Chu E, Koeller DM, Casey JL, Drake JC, Chabner BA, Elwood PC, Zinn S, Allegra CJ (1991) Autoregulation of human thymidylate synthase messenger RNA translation by thymidylate synthase. Proc Natl Acad Sci U S A 88:8977
5. Chu E, Voeller DM, Jones KL, Takechi T, Maley GF, Maley F, Segal S, Allegra CJ (1994) Identification of a thymidylate synthase ribonucleoprotein complex in human colon cancer cells. Mol Cell Biol 14:207
6. Grem JL, Harold N, Keith B, Nguyen D, Zentko S, Takimoto CH, Shapiro J, Monahan BP, Hamilton JM, Quinn MG, Morrison G, Schuler B (2000) A phase I and pharmacodynamic trial of oral eniluracil, 5-fluorouracil and leucovorin on a weekly schedule. Proc ASCO 19:abstract 684

7. Grem JL, Harold N, Shapiro J, Bi DQ, Quinn MG, Zentko S, Keith B, Hamilton JM, Monahan BP, Donavan S, Grollman F, Morrison G, Takimoto CH (2000) Phase I and pharmacokinetic trial of weekly oral fluorouracil given with eniluracil and low-dose leucovorin to patients with solid tumors J Clin Oncol 18:3952
8. Heslin MJ, Yan J, Shao L, Weingarten J, Mangum SG, Ertel PJ, Lucas VS, Diasio RB (2000) Dihydropyrimidine dehydrogenase rapidly regenerates after inactivation by eniluracil (776C85) in primary and metastatic colorectal cancer. Proc ASCO 19:abstract 682
9. Johnston PG, Drake JC, Trepel J, Allegra CJ (1992) Immunological quantitation of thymidylate synthase using the monoclonal antibody TS106 in 5-fluorouracil-sensitive and -resistant human cancer cell lines. Cancer Res 52:4306
10. Keith B, Guo XD, Zentko S, Harold N, Schuler B, Quinn M, Shapiro J, Grem JL (2002) Impact of two weekly schedules of oral eniluracil given with fluorouracil and leucovorin on the duration of dihydropyrimidine dehydrogenase inhibition. Clin Cancer Res 8:1045
11. Kitchens ME, Forsthoefel AM, Rafique Z, Spencer HT, Berger FG (1999) Ligand-mediated induction of thymidylate synthase occurs by enzyme stabilization: implications for autoregulation of translation. J Biol Chem 274:12544
12. Knowling M, Browman G, Siu L, Khoo K, Cooke A, Tannock I, Klaassen D, Cripps C, Goss G, Matthews S, Clarke R, Seymour L (2001) A National Cancer Institute of Canada clinical trials group phase II study of eniluracil (776C85) and oral 5-fluorouracil in patients with advanced squamous cell head and neck cancer. Ann Oncol 12:919
13. Levin J, Hohneker J (2000) Clinical development of eniluracil/fluorouracil: an oral treatment for patients with solid tumors. Invest New Drugs 18:383
14. Mani S, Hochster H, Beck T, Chevlen EM, O'Rourke MA, Weaver CH, Bell WN, White R, McGuirt C, Levin J, Hohneker J, Schilsky RL, Lokich J (2000) Multicenter phase II study to evaluate a 28-day regimen of oral fluorouracil plus eniluracil in the treatment of patients with previously untreated metastatic colorectal cancer. J Clin Oncol 18:2894
15. Marsh J, Catalano P, Huang J, Haberman D, Cornfeld MJ, O'Dwyer P, Benson AB III (2001) Phase II trial of oral fluorouracil (5-FU) plus oral eniluracil (GW776C85) administered over 28 days for patients with advanced colorectal cancer (E4296): a trial of the Eastern Cooperative Oncology Group (ECOG). Proc ASCO 20:abstract 547
16. Meropol NJ, Niedzwiecki D, Hollis D, Schilsky RL, Mayer RJ (2001) Phase II study of oral eniluracil 5-fluorouracil and leucovorin in patients with advanced colorectal carcinoma. Cancer 91:1256
17. Ochoa L, Hurwitz HI, Wilding G, Cohen D, Thomas JP, Schwartz G, Monroe P, Petros WP, Ertel VP, Hsieh A, Hoffman C, Drengler R, Magnum S, Rowinsky EK (2000) Pharmacokinetics and bioequivalence of a combined oral formulation of eniluracil an inactivator of dihydropyrimidine dehydrogenase and 5- fluorouracil in patients with advanced solid malignancies. Ann Oncol 11:1313
18. Paff MT, Baccanari DP, Davis ST, Cao S, Tansik RL, Rustum YM, Spector T (2000) Preclinical development of eniluracil: enhancing the therapeutic index and dosing convenience of 5-fluorouracil. Invest New Drugs 18:365
19. Ren Q-F, Kao V, Grem J (1998) Cytotoxicity and DNA fragmentation associated with sequential gemcitabine and 5-fluoro-2′-deoxyuridine in HT29 colon cancer cells. Clin Cancer Res 4:2811
20. Rivera E, Sutton L, Colwell B, Graham M, Frye D, Somerville M, Conklin HS, McGuirt C, Levin J, Hortobagyi GN (2002) Multicenter phase II study of a 28-day regimen of orally administered eniluracil and fluorouracil in the treatment of patients with anthracycline- and taxane-resistant advanced breast cancer. J Clin Oncol 20:987
21. Saif MW, Wilson RH, Harold N, Keith B, Dougherty DS, Grem JL (2001) Peripheral neuropathy associated with weekly oral 5-fluorouracil leucovorin and eniluracil. Anticancer Drugs 12:525
22. Schilsky RL, Hohneker J, Ratain MJ, Janisch L, Smetzer L, Lucas VS, Khor SP, Diasio R, Von Hoff DD, Burris HA 3rd (1998) Phase I clinical and pharmacologic study of eniluracil plus fluorouracil in patients with advanced cancer. J Clin Oncol 16:1450
23. Schilsky RL, Bukowski R, Burris H 3rd, Hochster H, O'Rourke M, Wall JG, Mani S, Bonny T, Levin J, Hohneker JA (2000) A multicenter phase II study of a five-day regimen of oral 5-fluorouracil plus eniluracil with or without leucovorin in patients with metastatic colorectal cancer. Ann Oncol 11:415
24. Schilsky RL, Levin J, West WH, Wong A, Colwell B, Thirlwell MP, Ansari RH, Bell WN, White RL, Yates BB, McGuirt PV, Pazdur R (2002) Randomized open-label phase III study of a 28-day oral regimen of eniluracil plus fluorouracil versus intravenous fluorouracil plus leucovorin as first-line therapy in patients with metastatic/advanced colorectal cancer. J Clin Oncol 20:1519
25. Skovsgaard T, Davidson NG, Piccart MJ, Richel DJ, Bonneterre J, Cirkel DT, Barton CM (2001) A phase II study of oral eniluracil/fluorouracil in patients with anthracycline-refractory or anthracycline- and taxane-refractory advanced breast cancer. Ann Oncol 12:1255
26. Smith IE, Johnston SR, O'Brien ME, Hickish TF, de Boer RH, Norton A, Cirkel DT, Barton CM (2000) Low-dose oral fluorouracil with eniluracil as first-line chemotherapy against advanced breast cancer: a phase II study. J Clin Oncol 18:2378
27. Van Cutsem E, Sorensen J, Cassidy J, Daniel F, Harper P, Bailey N, Peachey M, Somerville M (2001) International phase III study of oral eniluracil plus 5-fluorouracil versus intravenous 5-FU plus leucovorin in the treatment of advanced colorectal cancer. Proc ASCO 20:abstract 522