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ORIGINAL ARTICLE

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Phase I study of phosphonacetyl-L-aspartate, 5-fluorouracil, and leucovorin in patients with advanced cancer

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Abstract Low-dose phosphonacetyl-L-aspartate (PALA) may potentiate both 5-fluorouracil (5-FU) incorporation into RNA and thymidylate synthase inhibition by 5-fluorodeoxyuridylate (5-FdUMP). The gastrointestinal toxicity of 5-FU is not increased by PALA administration. Exogenous leucovorin, on the other hand, which enhances thymidylate synthase inhibition, appears to increase the clinical toxicity of 5-FU in a dose-dependent manner. As a result, the clinical use of high-dose leucovorin requires a marked dose reduction of 5-FU. Extracellular leucovorin levels of 1 μ*M* suffice to maximize the enhancement of thymidylate synthase inhibition in several models. We conducted a trial to add leucovorin to the PALA/5-FU regimen. We chose a leucovorin dose that was predicted to yield end-infusion total reduced folate concentrations of 1 μ*M*. The major endpoint was to determine the maximum tolerated dose of 5-FU in this combination. The regimen consisted of 250 mg/m^2 PALA given on day 1 and, 24 h later, escalating 5-FU doses ranging from 1,850 to 2,600 mg/m^2 admixed with 50 mg/m^2 leucovorin and given by 24-h infusion. Courses were repeated weekly. A total of 24 patients with a median performance status of 1 were entered at three dose levels. Diarrhea was dose-limiting; 6/13 patients had grade II or worse diarrhea at 2,600 mg/m^2. Dose modification resulted in a mean dose intensity of 2,300 mg/m^2 at both the 2,600- and 2,300-mg/m^2 dose levels. The 2,300-mg/m^2 dose is suitable for phase II testing of this regimen. Three patients (two with breast cancer and 1 with sarcoma) had a partial remission. We measured steady-state concentrations (C_{ss}) of 5-FU in 23 patients. The mean C_{ss} increased with dose from 0.738 to 1.03 μg/ml. Total body clearance did not vary with dose in this range. Patients with grade II or worse diarrhea had a higher mean C_{ss} (1.10 ± 0.19) than those with grade O or I toxicity (0.835 ± 0.25, $P < 0.02$). Total bioactive folates (bound and free) were measured using a biological assay. Pretreatment values ranged from 2 to 52 n*M* and were not predictive of toxicity. End-infusion (23-h) values were somewhat lower than predicted and ranged from 400 to 950 n*M*. The risk of diarrhea was positively correlated with end-infusion total folate values. In a logistic regression analysis, total folate values obtained at 23 h were a more powerful predictor of diarrhea than were 5-FU C_{ss} values. These results confirm the contribution of leucovorin to the toxicity of the 5-FU/leucovorin combination and suggest that interpatient differences in folate pharmacology may contribute to the therapeutic index of the 5-FU/leucovorin combination.

Key words 5-FU · Biochemical modulation

Introduction

The results of clinical trials in colorectal cancer support the hypothesis that biochemical modulation may enhance the antitumor activity of 5-fluorouracil (5-FU) [14, 17]. Inhibition of pyrimidine and purine synthesis and/or modulation of thymidylate synthase activity have been demonstrated to potentiate 5-FU in preclinical models and have yielded promising response rates in clinical trials [16, 33]. A number of studies suggest that the use of more than one mechanism of modulation may yield a further increment in response [15]. The purpose of the current trial was to derive a regimen that combines both inhibition of pyrimidine synthesis and modulation of thymidylate synthase.

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The inhibition of thymidylate synthase by the 5-FU anabolite 5-fluorodeoxyuridylate (5-FdUMP) is increased by exogenous reduced folate [2]. Folate is a source of 5,10-methylenetetrahydrofolate, which stabilizes the complex of 5-FdUMP and thymidylate synthase and results in the inactivation of the enzyme. The addition of leucovorin as a source of reduced folate decreases the 50% inhibitory concentration (IC_{50}) of 5-FU in vitro [31, 35], and the coadministration of leucovorin and 5-FU decreases the maximum tolerated dose of 5-FU in murine models [18]. Clinical trials of the combination of 5-FU and leucovorin show higher response rates for the combination in patients with colorectal cancer [7, 8, 23, 27, 28]. The use of low doses of leucovorin (in a five-daily-dose schedule) in one trial was at least as effective as that of a higher dose and allowed the administration of higher doses of 5-FU [30]. Thus, the dose intensity of 5-FU may be compromised by the use of higher doses.

Phosphonacetyl-L-aspartate (PALA) is a transition-state analog inhibitor of the enzyme aspartate transcarbamylase, which catalyzes an early step in de novo pyrimidine synthesis [10]. Inhibition of this enzyme results in depletion of uridine nucleotide pools and increased incorporation of 5-fluorouridine triphosphate (5-FUTP) into RNA. Preclinical and clinical studies support the use of a low dose of PALA in modulating 5-FU. Martin et al. [17] showed that the use of low doses of PALA maximized the biochemical effects in tumorous over normal tissue, thus favoring a selective antitumor action. In addition, the use of higher doses of PALA required a reduction in 5-FU dose because of overlapping toxicity to normal tissues, thereby diminishing the overall efficacy of the combination. In a phase I clinical trial, Casper et al. [5] showed that a dose of 250 mg/m^2 resulted in inhibition of whole-body pyrimidine synthesis comparably with a dose of 2 g/m^2. Thus, the lower dose was chosen for further study [5, 26].

The maximum tolerated dose of 5-FU given by 24-h infusion was 2,600 mg/m^2 following PALA administration at 250 mg/m^2 [13]. In a phase II trial in colorectal cancer, we reported a 43% response rate using this regimen [25]. The role of PALA in modulating 5-FU in this regimen is currently being evaluated both in a randomized phase II trial [3] and in a phase III study by cooperative groups.

The purpose of the current study was to add leucovorin to PALA/5-FU so as to derive a novel regimen of modulation incorporating more than one locus of effect. To maintain maximal 5-FU dose intensity, we selected to aim for a plasma total reduced folate concentration of 1 μM. Maximal potentiation of 5-FU in vitro is observed at 1 μM leucovorin in some models and at 10 μM in others [37]. Pharmacokinetics studies have demonstrated that total reduced folate levels of nearly 10 μM are achieved with leucovorin given at 500 mg/m^2 per 24 h by continuous i.v. infusion [1]. Therefore, given the linear pharmacokinetics of leucovorin in this range, a leucovorin dose of 50 mg/m^2 was chosen for evaluation in the present study. The objectives were to establish the maximum tolerated dose of 5-FU in combination with PALA and low-dose leucovorin, to describe the toxicity of the regimen, and to verify that the desired plasma concentration of active reduced folate was achieved with this dose of leucovorin. We also wished to investigate the relationship between the pharmacokinetics of both leucovorin and 5-FU and toxicity.

Patients and methods

Patients eligible for this study had a histologic diagnosis of a malignant solid tumor and had exhausted the conventional therapeutic options for their disease or had a disease for which no established treatment exists. They were required to have recovered from all toxic effects of prior treatment, to be older than 18 years of age, and to have an Eastern Cooperative Oncology Group performance status of 0–2. Patients had adequate bone marrow function (white blood cells, ≥4,000/mm^3; platelets, ≥100,000/mm^3), adequate liver function (serum bilirubin, ≤1.5 mg/dl), and adequate renal function (creatinine, ≤1.5 mg/dl). All patients gave written informed consent in accordance with federal, state, and institutional guidelines.

Prior to therapy, a medical history, physical examination, complete blood count, biochemical profile, urinalysis, chest X-ray, and appropriate scans were performed. Patients were monitored weekly with complete blood counts and monthly with clinical examinations and biochemical profiles. Doses were reduced if necessary on the basis of the level of toxicity. If grade I gastrointestinal (GI) toxicity or grade II toxicity of any other type was observed on the day of treatment, the week's dose was held. Following resolution of the symptoms, therapy was reinstituted at a lower dose. The dose modifications for toxicity were based on the worst level experienced. For grade I or II toxicity, a 25% dose reduction was used. For grade III or IV toxicity, a 50% dose reduction was used. If the reduced dose was well tolerated for a minimum of 4 weeks, an intermediate dose escalation could be taken. Doses were not escalated within patients. Only the dose of 5-FU was modified; those of PALA and leucovorin were unchanged.

Results are reported using the Common Toxicity Criteria (Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Md., 1988). Patients with measurable disease were evaluated every 8 weeks; those whose disease had stabilized or reduced in size were continued on therapy. Response criteria were standard [20]. The maximum tolerated dose was defined as the dose of drug that would produce predictable and reversible toxicity but would not be incapacitating or interfere with the patient's well-being and general activity.

Treatment plan

Patients were admitted to the Mary S. Schinagl Clinical Studies Unit at Fox Chase Cancer Center for 24–48 h surrounding the initial infusion of 5-FU. PALA was supplied by the National Cancer Institute as the disodium salt in 10-ml ampules containing 100 mg/ml water for injection. It was further diluted in 5% dextrose or 0.9% sodium chloride and given as a rapid infusion over 10 min. 5-FU was commercially available in 500-mg/10-ml ampules, reconstituted in 500 ml of 0.9% sodium chloride or 5% dextrose. [6RS]-L-Formyl tetrahydrofolate (leucovorin) was obtained commercially in 50- or 100-mg vials and was reconstituted using normal saline. Leucovorin was added to the 5-FU solutions at the appropriate doses.

PALA was given at 250 mg/m^2 by i.v. push on day 1; 24 h later, an infusion of 5-FU and leucovorin was begun. The starting dose of 5-FU was 1,850 mg/m^2. The 5-FU dose was mixed with 50 mg/m^2 leucovorin and given i.v. by continuous infusion over 24 h. Doses were repeated weekly. There were three levels of 5-FU dose escalation; three to six patients were treated at each level.

Pharmacokinetic sampling

All but one patient had blood drawn for 5-FU analyses before and at 6, 12, and 23 h into the 5-FU infusion. In addition, blood was drawn before and at 23 h after the infusion of leucovorin to measure the level

of reduced folate. Heparinized blood samples were placed on ice for 15–20 min, then centrifuged to separate plasma. Ascorbic acid was added to aliquots of plasma to give a concentration of 5 mg/ml. Samples were stored at –70° C and transported on dry ice. Aliquots from the pretreatment and the 23-h samples were removed before the addition of ascorbate and subjected to ultracentrifugation through Amicon Centrifree filters. Following the ultracentrifugation step, ascorbate was added in a manner similar to that described above.

Analytic procedure

5-Fluorouracil

Sample preparation. Plasma samples (1 ml) in 17- × 100-mm snap-top polypropylene tubes (Baxter, Scientific Products Division, McGraw Park, Ill., USA) were prepared for extraction by the addition of 125 µl of water for the plasma blank or 25 µl of the appropriate 5-FU standard and 100 µl of the internal standard, 5-chlorouracil (10 µg/ml), dissolved in water. Patients' samples were prepared by adding 25 µl of water and 100 µl of 5-chlorouracil solution. After brief vortexing of the samples, 2 ml of saturated ammonium sulfate solution and 100 µl of 1.0 M ammonium phosphate were added and the samples were again vortexed. This was followed by the addition of 8 ml of ethyl acetate and rocking of the samples for 15 min. The organic and aqueous layers were separated by centrifugation at 1,200 g for 10 min. The upper organic layer was transferred to a glass conical tube and 400 µl of 0.5 M potassium hydroxide was added. After vortexing for 15 min, the organic and aqueous layers were separated by centrifugation at 100 g for 10 min. The upper organic layer was aspirated and discarded; 20 µl of the aqueous layer was injected onto the high-performance liquid chromatography (HPLC) column.

High-performance liquid chromatography. The chromatographic system consisted of a Hewlett-Packard (Palo Alto, Calif., USA) HP-1090 Series A liquid chromatograph equipped with an autoinjector/autosampler and an HP1046A diode-array detector [34]. The column effluent was monitored at 254 nm. The chromatograph was operated with an HP-85B personal computer, and the data were processed with a DPU multichannel integrator. Chromatography was performed on a Spherisorb ODS-2 C18 reverse-phase analytical column (5 µm, 250- × 4.6-mm inside diameter; Alltech Associates, Inc., Deerfield, Ill., USA) preceded by a 15- × 3.2-mm, 7-µm Newguard C18 guard column (Applied Biosystems, Inc., San Jose, Calif., USA). The isocratic mobile phase consisted of 95% 0.05 M ammonium phosphate (pH 6.8) and 5% methanol (v/v) at a flow rate of 1 ml/min. Standard curves were plotted as the peak-height ratio of 5-FU to 5-chlorouracil versus the concentration of 5-FU. The linear regression lines were calculated by the method of least squares and were unweighted.

Biologically active folates

The procedures for deproteinization of the plasma samples and the determination of biologically active folates by the growth of bacteria in folate-deficient growth medium have been described previously [22]. Plasma ultrafiltrates were analyzed similarly to determine free bioactive folate. The concentrations of total bioactive folates were determined by the growth of *Lactobacillus casei* and the concentrations of leucovorin by *Pediococcus cerevisae*.

Pharmacokinetic analysis

Steady-state 5-FU concentrations (C_{ss}) were estimated from the mean of three values obtained during the 24-h infusion. The individual area under the curve was estimated from the C_{ss} times the hours of infusion. Total body clearance (CL_{tot}) was calculated from *Dose rate*/C_{ss} and expressed in milliliters per minute per square meter.

Statistical analysis

Two endpoints were considered in the statistical analysis: the development of severe diarrhea and the time from the beginning of treatment to the development of severe diarrhea. Univariate comparisons between patients with and without diarrhea were performed by the nonparametric Mann-Whitney test for continuous response variables. Contingency tables were constructed and chi-square or exact tests were applied for categorical variables. Logistic regression and likelihood ratio tests were used to construct a predictive model. To assess the dependence of the time to diarrhea on the variables analyzed, proportional hazard regression was used. Statistical analysis was performed using a BMDP computer software package. The critical significance level of 0.05 was chosen.

Results

A total of 24 patients were evaluated; their demographic characteristics are summarized in Table 1. Pharmacokinetic data were obtained from all but one patient for 5-FU and all but two patients for total bioactive folates. The most common diagnoses were colorectal and breast cancers. The patients were minimally symptomatic from their tumors (median performance status, 1) and had not been heavily pretreated. Four patients had received no prior chemotherapy; two with pancreatic cancer, one with colon cancer, and one with unknown-primary cancer.

Gastrointestinal toxicity

The major and dose-limiting toxicity of this trial was diarrhea. Grade II or worse diarrhea occurred in 9 of the 24 patients (Table 2). At the 2,600-mg/m^2 5-FU dose level,

Table 1 Patients' characteristics

Total number of patients	24
Sex:	
F	15
M	9
Age (years):	
Range	24–77
Median	53
Primary site of disease:	
Colorectal	8
Breast	7
Pancreas	2
Sarcoma	2
Head/neck	1
Ovarian	1
Lung	1
Unknown	2
Performance status:	
Range	0–2
Median	1
Prior therapy:	
Chemotherapy	10
Radiation	2
Chemotherapy/radiation	8
None	4

6 of 13 patients were affected, requiring dose modification that resulted in a mean tolerable dose of 2,300 mg/m^2 5-FU. On the basis of these findings, 2,300 mg/m^2 5-FU given by continuous infusion over 24 h was established as the appropriate phase II dose in combination in 250 mg/m^2 PALA and 50 mg/m^2 leucovorin. The episodes of diarrhea occurred within the first 4 weeks of the initiation of chemotherapy in 5 of 9 affected patients. This pattern of early dose-limiting toxicity was also observed in our previous phase II study of 5-FU and PALA [25].

An additional manifestation of GI toxicity was mucositis. Grade III or worse toxicity was observed in only two patients, both of whom were also affected by grade II or worse diarrhea. Eight patients reported mild nausea, and half of them also had an episode of vomiting. This toxicity was easily controlled with standard antiemetic drugs and was not dose-limiting.

Hematologic toxicity

Grade II neutropenia occurred in 2 of 13 patients treated at the 2,600-mg/m^2 dose level of 5-FU and in a total of 4 patients altogether, but this toxicity was not dose-limiting. A 76-year-old patient with metastatic rectal cancer experienced an episode of grade III hand-and-foot syndrome at the 2,600-mg/m^2 dose level in association with grade II diarrhea and mucositis as well as grade III ataxia. His 5-FU steady-state concentration was high (1.312 μg/ml), suggesting that these effects might be predictable.

Pharmacokinetic/pharmacodynamic relationships

We measured steady-state concentrations (C_{ss}) of 5-FU in 23 of 24 patients. The mean C_{ss} increased with the dose of 5-FU from 0.738 to 1.03 μg/ml (Table 3). The CL_{tot} did not vary with the dose. There was, however, significant interindividual variation.

Mean C_{ss} values were significantly lower ($P = 0.0332$) in the group of patients without severe diarrhea (mean, 0.838 μg/ml; SEM, 0.062 μg/ml; $n = 15$) than in the group of patients with severe diarrhea (mean, 1.071 μg/ml; SEM, 0.082 μg/ml; $n = 8$). Correspondingly, mean CL_{tot} values were significantly higher ($P = 0.0282$) in the group of patients without severe diarrhea (mean, 2,094 ml min^{-1} m^{-2}; SEM, 191 ml min^{-1} m^{-2}; $n = 15$) than in the group of patients with severe diarrhea (mean, 1,575 ml min^{-1} m^{-2}; SEM, 137 ml min^{-1} m^{-2}; $n = 8$). As expected, C_{ss} and CL_{tot} were highly correlated ($r = -0.827$, $P < 0.001$). This relationship between CL_{tot} and toxicity was observed by Danhauser et al. [6] in a phase I trial of 5-FU and α-interferon 2B, who found that pretreatment with the interferon reduced the CL_{tot} of 5-FU. They reported that a reduction in 5-FU CL_{tot} by more than 20% was associated with a 4-fold increase in the incidence of diarrhea as compared with patients with less than a 20% reduction in CL_{tot}.

Total bioactive folates were determined prior to treatment and at 23 h. The range of pretreatment total bioactive folate ranged from 2 to 52 n*M*. The mean 23-h value was 648 n*M* and the range was 400–950 n*M*. [6S]-Leucovorin (1-folinic acid), which was not detectable in any of the pretreatment samples, ranged from 86 to 170 n*M* at 23 h. Pretreatment total bioactive folate levels were not correlated with posttreatment values ($r = 0.281$; Table 4).

Total bioactive folate values obtained at 23 h were significantly lower ($P = 0.041$) in the group of patients without severe diarrhea (mean, 625 nmol; SEM, 35 nmol; $n = 13$) than in the group with severe diarrhea (mean, 743 nmol; SEM, 36 nmol; $n = 8$). The free bioactive folate values were also lower ($P = 0.0707$) in those without severe diarrhea (mean, 377 nmol; SEM, 22 nmol; $n = 12$) than in those with severe diarrhea (mean, 448 nmol; SEM, 32 nmol; $n = 8$). Total folate and free folate levels were highly correlated ($r = 0.835$, $P < 0.001$). An analysis of interaction between reduced folate levels and 5-FU pharmacokinetics was performed. Among the four pairs of folate and free folate values combined pairwise with 5-FU, C_{ss}, and CL_{tot}, the only significantly correlated pair was CL_{tot} and total folate ($r = 0.453$, $P = 0.039$).

Additional demographic characteristics were analyzed as risk factors for diarrhea; age, creatinine level, body surface area, sex, and the 23-h plasma concentration of

Table 2 Clinical toxic effects . of grade 2 or greater

5-FU dose	Total number of patients	Number of patiens with grade 2 or greater toxicity				
		Diarrhea	Mucositis	Leukopenia	Neurologic	Hand/foot
1,850 mg/m^2	6	2	–	1	–	–
2,300 mg/m^2	5	1	–	1	–	–
2,600 mg/m^2	13	6	2	2	1	1

Table 3 5-FU pharmacokinetic parameters

Dose (mg)	*n*	CL_{tot} (ml min^{-1} m^{-2})[a]		C_{ss} (μg/ml)[b]	
		Mean ± SD	Range	Mean ± SD	Range
1,850	6	1.928±757	1,521–3323	0.737±0.22	0.595–0.971
2,300	5	1,917±464	1,349–2,388	0.875±0.22	0.695–1.167
2,600	12	1,905±766	1,315–4,172	1.027±0.24	0.437–1.330

[a] Total body clearance of 5-FU
[b] Concentration at steady state

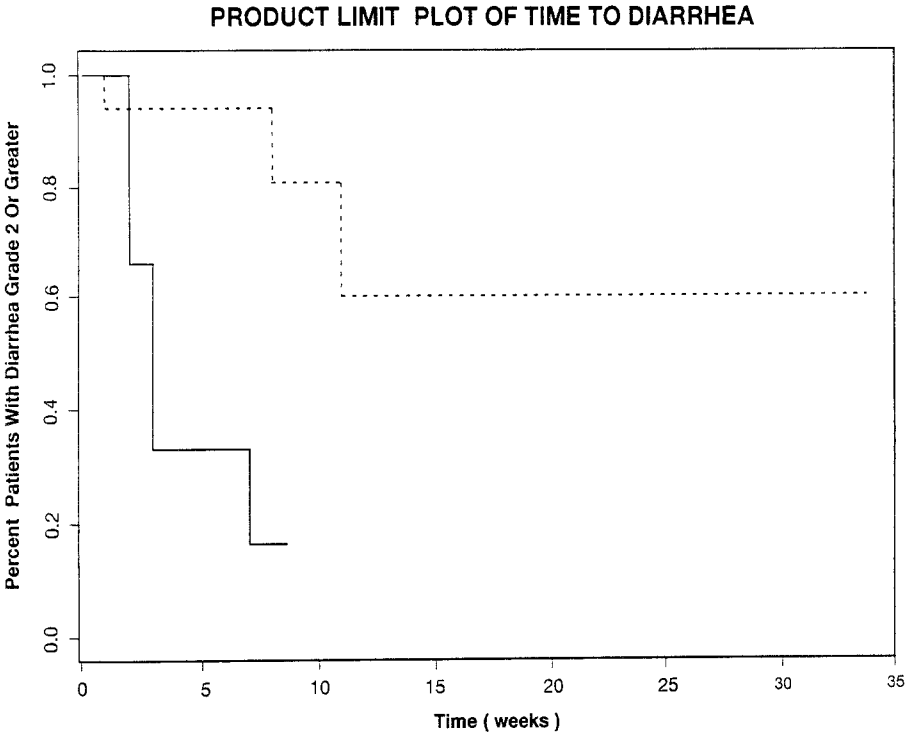

Fig. 1 Product-limit plots of time to diarrhea for patients with a CL_{tot} of 5-FU of ≥1,400 ml min^{-1} m^{-2} (*broken line*; 1,400 ml min^{-1} m^{-2} is the first quartile from the total sample) and patients with a CL_{tot} of 5-FU of <1,400 ml min^{-1} m^{-2} (*solid line*; $P = 0.0059$ for this binary variable)

leucovorin did not differ significantly between the two groups. When all variables were considered simultaneously in a logistic model, the 23-h total bioactive folate value was selected as the best predictor for severe diarrhea ($P = 0.049$).

In addition to the incidence of diarrhea, we also considered the rapidity with which it developed on weekly dosing, with the mean period being 6 weeks (range, 1–11 weeks). Univariate proportional hazard regressions showed that 5-FU CL_{tot} is a significant predictor of the time to severe diarrhea ($P = 0.0477$), whereas C_{ss} is not ($P = 0.0928$). Univariate proportional hazard regressions also showed that total bioactive folate and free folate are significant predictors of the time to severe diarrhea ($P = 0.0214$ and $P = 0.0274$, respectively). The coefficients of the other variables were not significantly different from zero. Again, when all variables were considered simultaneously, total folate alone was selected as the best predictor of the time to severe diarrhea. Figure 1 depicts the product-limit plots of the time to diarrhea for patients with a CL_{tot} of 5-FU of ≥1,400 ml min^{-1} m^{-2} (broken line; 1,400 is the first quartile from the total sample) and patients with a CL_{tot} of 5-FU of <1,400 ml min^{-1} m^{-2} (solid line; $P = 0.0059$ for this binary variable). Similarly, Fig. 2 depicts the product-limit plots of the time to diarrhea for patients with total folate levels of ≥648 nmol (solid line; 648 is the median value for the total sample) and patients with total folate levels of <648 nmol (broken line; $P = 0.0090$ for this binary variable).

C_{ss}, CL_{tot}, age, and sex

In this sample, the C_{ss} of 5-FU was not correlated with age ($P = 0.104$) or sex ($P = 0.462$). Similarly, CL_{tot} was not correlated with age ($P = 0.274$) or sex ($P = 0.266$). Sex-related differences in regard to the pharmacologic response to a drug or the incidence of adverse effects have been reported for several drugs. In a study of 378 patients (301 men and 79 women) with squamous-cell carcinoma of the head and neck treated with cisplatin and 5-FU by continuous infusion, Milano et al. [19] reported that the capacity to clear 5-FU was lower in women than in men. In the present study with 23 patients assessable for 5-FU pharmacokinetics (15 women and 8 men), we observed no significant difference between the mean CL_{tot} of women (1,940 ml min^{-1} m^{-2}; range, 1,349–4,172 ml min^{-1} m^{-2}) compared with men (1,862 ml min^{-1} m^{-2}; range, 1,315–2,388 ml min^{-1} m^{-2}).

Table 4 Folate pharmacokinetic parameters

5-FU dose (mg/m^2)	*n*	Total bioactive folate (nmol)		Leucovorin (nmol) Mean ± SD
		Mean ± SD	Range	
1,850	5	722±149.7	500–850	125±38
2,300	5	656±136	510–880	172±23
2,600	11	652±123	400–810	135±29

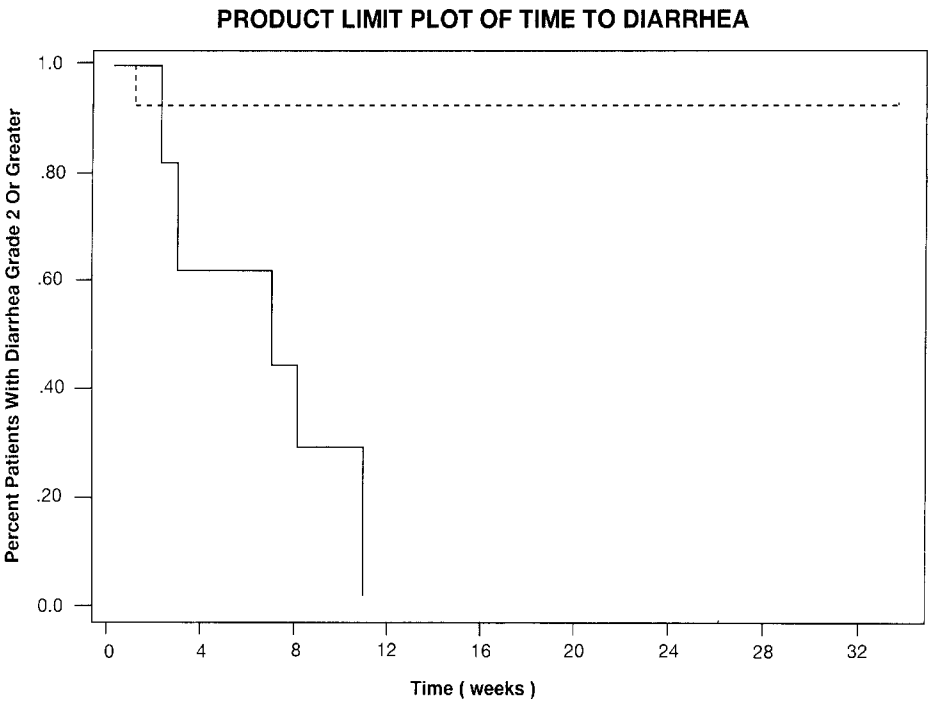

Fig. 2 Product-limit plots of time to diarrhea for patients with total folate levels of ≥648 nmol (*solid line*; 648 nmol is the median value for the total sample) and patients with total folate levels of <648 nmol (*broken line*; $P = 0.0090$ for this binary variable)

Clinical response

Among 24 patients assessable for response, there were 3 partial remissions (2 in breast cancer and 1 in sarcoma) and 4 additional minor responses. In all, 4 responses [2 partial responses (PR) and 2 minor responses (MR)] were observed among 7 patients with metastatic breast carcinoma, with the duration of response ranging from 4 to 34 weeks. Among 8 patients with colorectal cancer, 1 patient with rectal cancer showed an MR. The other responses were observed in a patient with angiosarcoma of the scalp (PR) and a patient with carcinoma of unknown primary origin that was metastatic to the liver and bone (MR). All responders had previously been treated with chemotherapy and/or radiotherapy. Only 1 of the 7 responders had any significant toxicity (grade II diarrhea).

Discussion

In preclinical models [18] and in clinical trials in colorectal cancer [12] the dose intensity of 5-FU is an important determinant of its efficacy. Thus, the weekly 24-h infusion provides one of the most dose-intense regimens available (2,600 mg/m^2 per week), comparable with protracted infusion (2,100 mg/m^2 per week) and much more potent than the intermittent or weekly schedules (450–600 mg/m^2 per week) [24]. Low-dose PALA appears to potentiate the activity of 5-FU without compromising the dose intensity. The addition of leucovorin (LV), however, potentiates the toxicity of 5-FU and requires a dose reduction, more for high than for low LV doses. In a randomized trial, the addition of low-dose LV required a 15% reduction and that of high-dose LV, a 25% reduction in the 5-FU dose [29]. We hypothesized that a therapeutic advantage may result from maintaining the dose intensity of 5-FU. In light of preclinical data that demonstrated close to maximal potentiation of 5-FU by 1 μ*M* total reduced folate, we sought to add LV at this level to the PALA/5-FU regimen.

The recommended phase II dose of 5-FU given by continuous infusion over 24 h in combination with low-dose PALA (250 mg/m^2) and 50 mg/m^2 LV was 2,300 mg/m^2. The 5-FU, PALA, and LV regimen studied in this trial was well tolerated. Over half of the patients required no dose modification. The patients who experienced toxicity had a gradual appearance of the side effects on this weekly schedule, allowing timely dose modification. This was especially important in the management of diarrhea, for which the treatment must be withheld until the diarrhea resolves. The dose-limiting toxicity was this regimen is diarrhea.

As others have shown for more prolonged schedules of 5-FU infusion, the mean C_{ss} of 5-FU was higher in patients who experienced toxicity than in those who did not [9]. Patients with grade II or worse diarrhea had a higher mean C_{ss} (1.10 μg/ml) than those with grade I or 0 diarrhea (0.835 μg/ml). Interestingly, in this study in which the delivered dose varied from 1,800 to 2,600 mg/m^2, the CL_{tot} of 5-FU was also closely correlated with toxicity. Whereas the mean C_{ss} increased with dose, the CL_{tot} of 5-FU was independent of the dose in this range, consistent with the findings of Fleming et al. [9] using a 5-day continuous infusion of 5-FU at lower doses. This indicates that the dose modification of 5-FU by continuous infusion would result in proportional changes in total body exposure, which provides the opportunity to perform adaptive dosing based on C_{ss} or CL_{tot}. Both dose escalation and dose

reduction based on the initial week's C_{ss} or CL_{tot} may individualize dosing and lead to an improved therapeutic index.

This strategy has been attempted with 96-h infusional regimens of 5-FU in patients with head and neck cancer. Santini et al. [32] observed that the 5-FU area under the concentration-time curve (AUC) was predictive of toxicity in the first group of patients that they studied retrospectively. This information was then used to adjust the 5-FU dose on the basis of its AUC in a second group of patients studied prospectively. In this group the 5-FU dose was reduced in 40% of the patients during the second half (48 h) of the treatment course. A statistically significant difference in the complete response rate, but not in the overall response rate, and a reduction in the incidence of toxicity were observed in the group in which pharmacokinetically guided dose adjustments had been made.

The optimal modulating dose and scheduling of folinic acid in combination with 5-FU remains controversial. Concentrations of 1–10 μ*M* were found to be optimal for maximal potentiation of 5-FU activity in vitro [37]. Since folinic acid appears to increase the toxicity of 5-FU in a dose-dependent manner in rodents [18] and in humans [29], the concern is that too high a dose of LV will require a reduced and potentially less effective dose of 5-FU. This concern seems to be justified by the close correlation found between the level of folate at 23 h after the initiation of therapy and the risk for grade II or greater diarrhea. Although the value obtained for total bioactive folate in these patients at 23 h was lower than predicted, the median value above which there was an increased risk for diarrhea was 0.6 μ*M*. This finding, however, is not consistent with the data of Ardalan et al. [4], who reported on a regimen of PALA, LV, and 5-FU that was identical except that the LV dose was 500 mg/m^2. The toxicity associated with the 5-FU dose of 2,600 mg/m^2 (7 of 14 patients with grade II or worse diarrhea) was similar to that encountered by us. Since previous pharmacokinetic data would suggest that the 500-mg/m^2 dose of LV would result in a total reduced folate level of between 1 and 10 μ*M* and, therefore, in more severe toxicity, it is clear that high levels of reduced folate alone are insufficient to account for the increased risk. Other factors involved in the mechanism of development of diarrhea may include interpatient variability in the cellular pharmacology of reduced folate, in the kinetics of formation of the ternary complex, or in thymidine salvage. Population pharmacokinetics and pharmacodynamics studies directed toward these endpoints are warranted.

The association of a bioactive folate level of 0.6 μ*M* with diarrhea is consistent with in vitro data that show a requirement for concentrations of 1–10 μ*M* for maximal potentiation of 5-FU. Moran and Scanlon [21] showed that the interaction of 5-FU and LV in colon cancer cells was dependent on the duration of exposure to both 5-FU and LV, with greater modulation being observed with prolonged exposure periods. In a comparison of human colon- and renal-carcinoma cell lines, optimal modulation of 5-FU cytotoxicity occurred with 1 μ*M* [6S]-LV for 5 h, whereas the renal-carcinoma cell line required 10 μ*M* [6S]-LV for 5 days [36, 37]. Houghton et al. [11] showed that human colon tumor xenografts differ in the activity of folylpolyglutamate synthetase by a factor of 4. Treatment of mice bearing these tumors with 5-FU and LV demonstrated maximal potentiation of 5-FU at [6S]-LV concentrations ranging from 2.5 to 7.2 μ*M* and given by 24-h infusion [11]. The effects of schedule variation may differ in normal colon mucosal cells, which lack detectable folylpolyglutamate synthetase and, hence, are dependent upon continuous folate exposure to maintain intracelluar reduced folate content. The further investigation of folate modulation in both tumor and bowel mucosa may identify optimal schedules for folate administration to maximize the therapeutic index of 5-FU in combination with LV.

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