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ORIGINAL ARTICLE

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Pharmacokinetics and pharmacodynamic effects of 5-fluorouracil given as a one-hour intravenous infusion

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Abstract *Purpose*: Clinical toxicity associated with 5-fluorouracil (5-FU) is related to the area under the plasma concentration-time curve (AUC). Recently, short-term infusions of 5-FU given over 30 or 60 min have been substituted for conventional "bolus" 5-FU given over 3–5 min in randomized clinical trials, but there are only limited pharmacokinetic data for these altered infusion durations. We therefore wished to determine the pharmacokinetics and toxicity associated with 5-FU given as a 1-h intravenous (i.v.) infusion. *Methods*: A group of 22 adults with advanced gastrointestinal tract cancers and no prior systemic chemotherapy for advanced disease received interferon α-2a (5 MU/m^2 s.c., days 1–7), leucovorin (500 mg/m^2 i.v. over 30 min, days 2–6) and 5-FU (370 mg/m^2 i.v. over 1 h, days 2–6). The doses of 5-FU and interferon-α were adjusted according to individual tolerance. The pharmacokinetics and clinical toxicity were retrospectively compared with patients receiving the same regimen under the same treatment guidelines except that 5-FU was given over 5 min. *Results*: The regimen was well tolerated, and 41% of the patients tolerated 5-FU dose escalations to 425–560 mg/m^2 per day. Grade 3 or worse diarrhea and fatigue ultimately occurred in 14% of the patients each. Granulocytopenia, mucositis, and diarrhea appeared to be appreciably milder in the present trial compared with our prior phase II experience in colorectal cancer. The peak 5-FU plasma levels and AUC with 370 mg/m^2 5-FU given over 1 h were 7.3-fold and 2.4-fold lower than previously measured in 31 patients who received 5-FU over 5 min. *Conclusion*: Increasing the length of 5-FU infusion to 1 h seemed to substantially reduce the clinical toxicity with this modulated 5-FU regimen, likely due to markedly lower peak 5-FU plasma levels and AUC. Changes in the duration of a short infusion of 5-FU clearly affects the clinical toxicity, but raises the concern of a potentially adverse impact on its antitumor activity. These results suggest the importance of including precise guidelines concerning the time over which 5-FU is given in clinical trials. Having a specified duration of 5-FU infusion is also important if 5-FU dose escalation is considered.

Key words 5-Fluorouracil · Infusion duration · Pharmacology · Biochemical modulation

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Introduction

The toxicity associated with 5-fluorouracil (5-FU) given by intravenous (i.v.) bolus is related to total systemic exposure as reflected by the area under the plasma concentration-time curve (AUC) [1–5]. Injection of

5-FU over a 3–5-min period typically produces peak plasma concentrations of several hundred micromolar, which profoundly influences the AUC. Increasing the time over which 5-FU is given is a strategy to lower the peak plasma levels of 5-FU and thereby reduce clinical toxicity. With administration of 5-FU by continuous infusion for periods of 24 h or longer, the tolerated daily dose is greater with 5-FU infusion than bolus injection [1].

5-FU given as either a 30- or 60-min infusion has been compared in several randomized trials with an investigational agent given over the same infusion time. 5'-Deoxy-5-fluorouridine (doxifluridine) 4000 mg/m^2 has been compared with 5-FU in two randomized trials with both drugs given by 1-h infusion daily for 5 days every 4 weeks [6, 7]. Administration of doxifluridine over 1 h rather than by bolus injection seemed to be better tolerated in phase II studies, and presumably the investigators wished to have comparable durations of infusion in both arms. Very low incidences of grade 3 mucositis and diarrhea (4% each) and grade 3–4 leukopenia (2%) were found in one trial that employed 5-FU 500 mg/m^2 over 1 h [6]. However, only one patient had a partial response (0.9%). Doxifluridine was compared with 5-FU 450 mg/m^2 over 1 h in a second trial. None of the patients receiving 5-FU had grade 3–4 hematologic toxicity, and grade 3–4 gastrointestinal toxicity of all types occurred in only 10% of patients [7]. The clinical response rate was 6.7%. These response rates are arguably lower than the 11% typically expected for single-agent 5-FU given by injection given over several minutes [8]. In another phase III trial, gemcitabine was compared with 5-FU 600 mg/m^2 over 30 min weekly as first-line therapy for pancreatic cancer [9]. The clinical toxicity with 5-FU was minimal: no patient had grade 4 toxicity of any type, and grade 3 toxicities included diarrhea (5%), WBC (1.6%), and platelets (1.6%). In each of these three trials, the clinical toxicity was greater with the investigational drug. Since 5-FU dose escalation was not permitted in these trials, this raises a concern that patients may have received suboptimal doses of 5-FU.

This concern is supported by findings from a randomized trial that compared 5-FU 500 mg/m^2 given either over 2–4 min or over 10–20 min with leucovorin (LV) 60 mg/m^2 i.v. given 40 min after the start of 5-FU, daily for 2 days every 2 weeks [10]. Dose escalation was not planned, and only one instance of grade 3 toxicity (diarrhea) occurred among 103 patients treated with the 10–20-min infusion, whereas 6 of 100 patients who received 5-FU over 2–4 min had grade 3 diarrhea. The response rate was lower in patients receiving the 10–20-min infusion (13% versus 27%). Despite these results, administration of 5-FU over 1 h is being used more commonly. Further, precise guidelines concerning how quickly 5-FU should be administered in clinical trials involving "bolus" 5-FU are often not specified, thus allowing the potential for variation in toxicity and perhaps efficacy.

We therefore wished to evaluate the clinical toxicity and pharmacokinetics of 5-FU given as a 1-h infusion. We have a pharmacokinetic and clinical toxicity database for patients treated with a regimen of interferon-α (IFN α-2a) given s.c. days 1–7, and high-dose LV and 5-FU 370 mg/m^2 over 5 min days 2–6 [5, 11, 12]. In order to permit comparison of the pharmacokinetics and the incidence and severity of clinical toxicities in our database, we purposely employed the same regimen except that 5-FU was given as a 1-h infusion immediately after LV. The dose modifications, treatment guidelines, and supportive care measures were identical to those employed in the prior trial [11].

Patients and methods

Eligibility

This study was activated in March 1994, and accrual was completed in October 1995. The final patient was removed from the study in February 1999 for disease progression. The study was an amendment to a protocol entitled "A pilot study of interferon γ-1b in combination with fluorouracil, LV, and interferon α-2a". The amendment shifted the focus to assessing the toxicity and pharmacokinetics associated with 5-FU given over 1 h, while the addition of interferon-γ was delayed for several cycles, as explained below [12]. Patients with solid tumors for whom a 5-FU/LV-based regimen represented a reasonable option were eligible. Patients were required to have an ECOG performance status of 2 or better and adequate organ function. Patients must have received no prior systemic chemotherapy for advanced disease. Prior regional therapy and prior adjuvant chemotherapy were permitted provided that 12 or more months had elapsed since their discontinuation. This study had the approval of the local Institutional Review Boards and the Cancer Therapy Evaluation Program, NCI; all patients gave written informed consent.

Treatment plan

Recombinant human IFN α-2a (Roferon, Hoffman La-Roche, Nutley, N.J.), recombinant human IFN γ-1b (Actimmune, Genentech, South San Francisco, Calif.) and calcium LV (Ben Venue Laboratories, Bedford, Ohio) were supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI, Bethesda, Md. IFN α-2a 5 MU/m^2 s.c. was given on days 1–7. On days 2–6, LV 500 mg/m^2 was given immediately after IFN α-2a as a 30-min i.v. infusion followed by 5-FU 370 mg/m^2 given as a 1-h i.v. infusion. As was our practice in our prior phase II study, we employed oral cryotherapy to diminish the severity of mucositis. Patients used ice chips by mouth starting 5 min prior to 5-FU and continuing during the 5-FU infusion, and were instructed to use an oral hygiene program, and to drink at least two to three quarts of fluids per day [11]. Patients maintained a calendar diary to record their side effects. The next cycle was repeated on day 22 if the granulocyte count was $>1500/\mu l$, the platelet count was $>80{,}000/\mu l$ and the patient had recovered from nonhematologic toxicities. Otherwise, treatment was delayed until resolution of toxicity.

If the prior cycle was accompanied by minimal clinical toxicity (granulocyte nadir $\geq 1200/\mu l$, platelet nadir $\geq 60{,}000/\mu l$, hematologic recovery by day 28, and not more than grade 1 mucositis and diarrhea), the 5-FU dose was increased by 15% the subsequent cycle, while the doses of IFN α-2a and LV remained the same. If dose-limiting toxicity occurred, a reduced dose of either 5-FU or IFN α-2a was given cycle 2, depending on the nature of the clinical toxicity. The dose of 5-FU was reduced if the granulocyte nadir was $<500/\mu l$ on day 10 or beyond, the platelet nadir was $<25{,}000/\mu l$, or for grade 3 or 4 nonhematologic toxicity attributed to 5-FU/LV (NCI Common Toxicity Criteria version 1). The dose of IFN α-2a was decreased by 25% for severe fatigue, malaise or anorexia.

Once tolerable doses of 5-FU/LV and IFN α-2a were established in each patient, IFN-γ was added the following cycle at 0.3 MU/m^2 s.c. days 1–7 given prior to IFN α-2a (10 μg/m^2 per day), and was then continued with each subsequent cycle. This IFN-γ dose was the lowest evaluated in the initial part of this study, and was shown to be well tolerated when given in combination with 5-FU/LV/IFN α-2a [12]. Blood counts were obtained weekly, and chemistry values were obtained on day 1 of each cycle. Radiographic studies were repeated every three cycles. Treatment was continued indefinitely until there was evidence of disease progression, provided it was tolerated.

Response criteria

Measurable disease was not required for this study. Patients with bidimensionally measurable disease on radiographic studies who had received at least three cycles of therapy with adequate pretreatment and follow-up radiographic studies were considered assessable for response. However, patients who experienced rapid disease progression prior to reassessment were included in the analysis of response as treatment failures. Standard criteria for complete and partial response were used [13], except that our definition of stable disease required a duration of at least 3 months. Disease progression was defined as an increase in tumor size by 25% or more over either the baseline or best response, or the appearance of new sites of disease. Early disease progression refers to progression within the initial 3 months of therapy.

Pharmacokinetic analysis of 5-FU

In the current trial, venous samples for pharmacokinetic analysis were drawn pretreatment, and at 50 and 55 min during the first and fourth infusions of 5-FU. Blood sampling was repeated if the dose of 5-FU was adjusted. Samples drawn into heparinized tubes were placed on ice immediately, spun at 800 *g* at 4°C for 10 min, and the plasma was separated and frozen at –70°C until analysis. Duplicate aliquots of 0.5 ml plasma were spiked with a fixed amount of the internal standard (5-chlorouracil) and 50 μl glacial acetic acid, then were extracted with 8 ml ethyl acetate/methanol (95%/5%, v/v). The supernatant was concentrated to dryness, resuspended in 500 μl water, and analyzed by reversed-phase high-performance liquid chromatography. A Waters (Milford, Mass.) Resolve Radial-pak 5 μm C18 cartridge (8 × 100 mm) and a Resolve C18 precolumn were used, and the mobile phase was 0.06% glacial acetic acid in HPLC-grade water at 1 ml/min. UV absorbance at 266 nm and 290 nm was monitored using a photodiode array detector. The retention times were as follows (means ± SD): 5-FU 8.2 ± 0.4 min, 5-chlorouracil 13.3 ± 0.2 min. The overall variation in these duplicate samples was 2.1%. The variability between the values of the two samples drawn 5 min apart at the end of infusion was estimated by the equation: $[100 \times (a - b)] \div [(a + b)]$. The average intraassay and interassay variabilities in the area of the internal standard were 6.8% and 3.8%.

Pharmacokinetic data were available for 39 data sets obtained from 20 patients. Seven of the data sets were from a single infusion only (day 1), and the remaining samples were from two separate infusions during the same cycle (days 1 and 4). Since 78% of the day-4 end-of-infusion plasma values were higher than the paired day-1 sample, the data were modeled with ADAPT II software [14] using a one-compartment model with a variable that allowed alteration of the clearance of 5-FU with repeated daily administration. The actual times of sample collection and the length of infusion were used for this analysis.

The pharmacokinetic data for the 1-h infusion samples were compared with data from 31 prior patients who had received 5-FU by injection over 5 min 1 h after a 30-min i.v. infusion of 500 mg/m^2 LV days 2–6, with 5 MU IFN α-2a s.c. days 1–7 in two of our previously reported trials [5, 12]. The bolus 5-FU data comprise information from 14 patients included in two prior reports [5, 12], while banked plasma samples from 17 additional patients that had not been analyzed at the time the latter trial was published were measured and the data included in the present report.

Statistical analysis

The report describing our prior phase II study included data collected through November 1992, and was published in 1993 [11]. The final patient was taken off study in Spring 1996 for disease progression. The database for the 46 patients enrolled in this prior trial was updated to include the toxicities for all cycles administered. Wilcoxon's rank sum test was used to compare continuous parameters (i.e. WBC and AGC nadirs, 5-FU pharmacokinetic values) between the two trials. Comparison of the worst grades of toxicity experienced by patients on the present and prior study was performed by Lehmann's test for differences in trends of toxicity grades [15, 16]. Comparisons of trends in toxicity grades between the two studies for all cycles, stratified for 5-FU dose, were performed using the stratified version of the Cochran-Armitage trend test [17]. Time to treatment failure was measured from the initiation of therapy until the patient was removed from study for any reason, and then analyzed by the Kaplan-Meier life-table method [18]. The paired pharmacokinetic parameters from blood samples obtained on days 1 and 4 of the same cycle were compared by a Wilcoxon's signed ranks test. All *P*-values are two-sided.

Results

Patient characteristics

A group of 22 adult patients with good performance status participated in this study (Table 1). One patient each had received either adjuvant therapy or prior regional therapy to the liver. The majority had adenocarcinomas arising in the upper gastrointestinal tract. The characteristics of 46 patients with colorectal cancer treated on our prior phase II trial of IFN α-2a, LV and

Table 1 Patient characteristics

Age (years)	
Median	52
Range	21–74
Gender	
Male	16 (72.7%)
Female	6 (27.3%)
ECOG performance status	
0	6 (27.3%)
1	16 (72.7%)
Prior therapy	
Adjuvant chemotherapy	1 (4.5%)
Chemo-embolization	1 (4.5%)
Radiation therapy	2 (9.1%)
Histology	
Pancreas/bile duct	12 (54.5%)
Gastric/gastroesophageal junction	6 (27.3%)
Colorectal	2 (9.1%)
Hepatoma/cholangiocarcinoma	2 (9.1%)
Number of cycles	
Median	3.5
Range	1–52
Laboratory values	
Albumin (g/dl)	4.0 (3.2–4.6)
LDH (U/l)	510 (335–2445)
Alkaline phosphatase (U/l)	126 (68–781)
Creatinine (mg/dl)	0.8 (0.3–1.3)
Hemoglobin (g/dl)	13.1 (9.1–15.3)

5-FU (bolus injection) have been previously reported [11]. In the prior trial, the proportion of asymptomatic patients (59%) was about twice that in this trial, and female patients comprised a slightly higher percentage (37%).

Clinical toxicity

The toxicities experienced during the first cycle for the current and prior study are shown in Fig. 1. The doses of 5-FU/LV/IFN α-2a were identical, and only the duration of 5-FU infusion differed. In the current study, diarrhea was the most frequent toxicity (91% of patients), but was of grade 3 severity in only 13.6% of patients. Prophylactic antiemetics were not used. Nausea/vomiting occurred in 54.5% of patients, and was severe in 23% of patients. Fatigue was also a prominent symptom (68% of patients), but was severe in only two patients. In contrast, mucositis was uncommon (13.6% of patients). Clinical toxicity had completely resolved by day 21 in 86.4% of patients, while the other patients needed a 1-week delay. In comparison, the incidence of mucositis, granulocytopenia and leukopenia appeared to be much higher in our prior phase II trial with injection of 5-FU over 5 min, while fatigue seemed more common in the current study (Fig. 1).

A total of 180 cycles were given. The 5-FU dose was adjusted based on individual tolerance. Nine patients (41%) tolerated a 5-FU dose escalation to 425 mg/m^2 or above, while seven patients required a 5-FU dose reduction (32%). IFN-γ was not added until a tolerable dose of 5-FU and IFN α-2a had been identified for each patient. Thus, only ten patients (45%) received one or more cycles with IFN γ-1b 0.3 MU/m^2 s.c. days 1–7 in conjunction with IFN α-2a, 5-FU and LV. No appreciable differences in the incidence or severity of toxicities were evident in these ten matched cycles in which the addition of IFN γ-1b was the only variable (data not shown).

The worst toxicities experienced by patients across all cycles of therapy are shown in Fig. 2. Diarrhea was the most common toxicity, but was of grade 3 severity in only 13.6% of patients in the present study. Mucositis, ultimately noted by 45% of patients, was generally mild. Fatigue was also common, but was severe in only 13.6% of patients. Skin rash occurred infrequently. No patient experienced a WBC or an AGC nadir below 1000/μl or 500/μl, respectively, and less than 10% experienced grade 3 toxicity. Mild to moderate anemia was recorded in most patients, but 59% of patients were anemic when they enrolled on this trial (at baseline: grade 1, 11 patients; grade 2, 2 patients). Anemia reached grade 3–4 severity in two patients (9.1%). In comparison to the present trial, the incidence and severity of granulocytopenia, leukopenia, diarrhea, and mucositis appeared to be significantly higher in the prior trial involving 5-FU given over 5 min.

Toxicities complicating each cycle of therapy were also evaluated. Figure 3 shows the distribution of leukocyte and granulocyte nadirs for all cycles at 315, 370 and 425 mg/m^2 5-FU in both trials. The WBC and AGC nadir values were significantly higher in the present trial at each dose level. Grade 2 mucositis complicated only 2.4% of cycles at 370 mg/m^2 and one of four cycles at 490 mg/m^2. Diarrhea of grade 3 severity complicated only 3.6% of cycles at 370 mg/m^2, and was not observed at any other dose. Grade 2 diarrhea complicated 21%, 28%, 25% and 50% of cycles at 370, 425, 490 and 560 mg/m^2, respectively. Nausea/vomiting

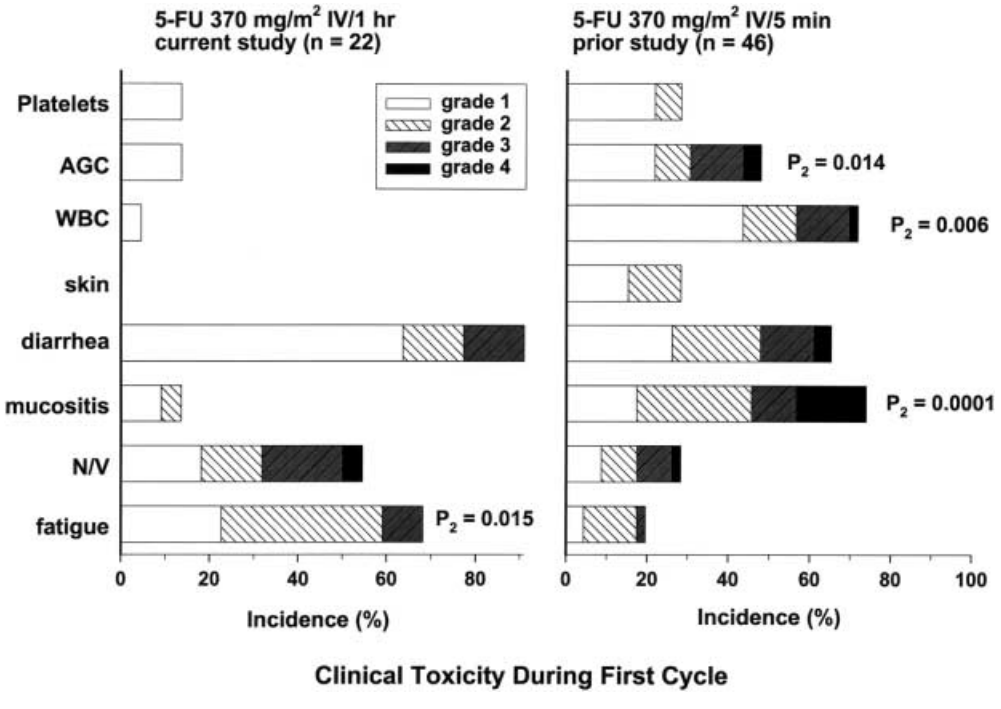

Fig. 1 Clinical toxicity during the initial cycle of therapy. The incidence of toxicities of various types during the first cycle of therapy are presented in a stacked bar graph format for both the present trial and the prior phase II study. Comparison of grades 0–1 vs 2–4 hematologic toxicity between the two studies indicates that grade 2 or worse leukopenia and granulocytopenia occurred significantly more often with 5-FU given over 5 min. Mucositis (grades 0–1 vs 2 vs 3–4) was a more significant problem with bolus 5-FU, while fatigue (grades 0–1 vs 2–3) was more common in the present trial. Lehmann's *P*-values are shown (two-sided)

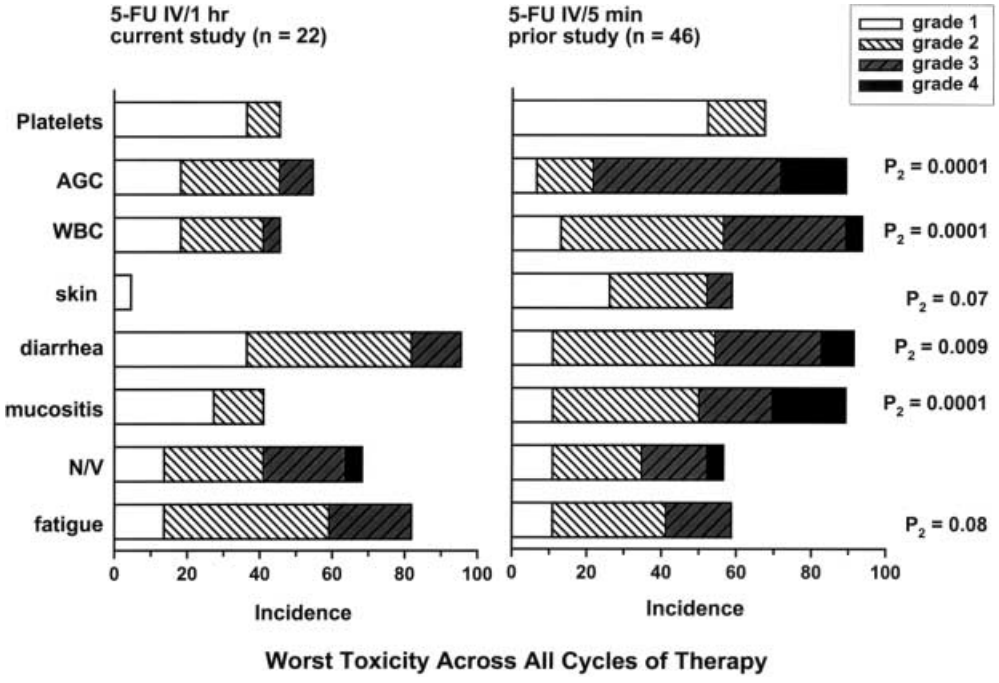

Fig. 2 Worst toxicity across all cycles of therapy. The worst grade of toxicity experienced by each patient at any time during protocol therapy are shown in a stacked bar graph format. In general, the incidence of grades 0–1 vs 2 vs 3–4 toxicities were compared between the two studies, using Lehmann's procedure. For fatigue and skin rash, the incidence of grade 0–1 vs grade 2–3 toxicities were compared between the two studies. A significantly higher proportion of patients experienced grade 2 or worse leukopenia, granulocytopenia, mucositis and diarrhea with 5-FU given over 5 min

responded well to antiemetic therapy, and grade 2 or worse nausea/vomiting complicated only 12%, 17%, and 0% of cycles at 315, 370 and 425 mg/m^2. Fatigue occurred across all doses of 5-FU, but generally was grade 2 or less. When the toxicities complicating all cycles of therapy at the three major dosage levels were compared with the prior phase II database (Fig. 4), a significant trend for greater incidence and severity of mucositis, diarrhea and skin rash was noted with 5-FU given over 5 min, while nausea/vomiting tended to be worse in the present trial.

Clinical pharmacology studies

The half-life of 5-FU given by bolus administration in this dose range is about 10 min, and we expected to reach steady-state by the end of infusion. To avoid any possible effect of circadian variation in 5-FU plasma levels, the blood samples for all patients were collected during the first and fourth 5-FU infusion at approximately the same time of day. Blood sampling was repeated if the dose of 5-FU was changed in subsequent cycles. A total of 39 data sets were available. When measured from the start of infusion, the two samples were drawn at 50.2 ± 3.4 min (mean $\pm$ SD, $n = 70$) and 55.3 ± 3.2 min ($n = 71$) into the infusion. The first sample was drawn at 11:18 a.m. ± 9.3 min (mean $\pm$ SD, $n = 70$), while the second sample was drawn at 11:23 a.m. ± 8.0 min ($n = 71$).

The maximum plasma concentrations (Cmax) of 5-FU for all samples are presented in Table 2. The variability between the values for the two time-points at the end of each infusion was $9.5 \pm 7.3\%$ (mean $\pm$ SD, $n = 68$), suggesting that steady-state had been achieved. The clearance and AUC values were estimated with a one-compartment model using each 5-FU plasma level and the actual times at which samples were drawn relative to the length of infusion. The clearance appeared to be independent of dose for both the day-1 and day-4 samples (Table 2). Among the 32 paired samples (combining all 5-FU doses), the day-1 clearance was significantly higher ($P_2 = 0.003$) than on day 4 (median, range): 1764 (871–5655) ml/min per m^2 vs 1297 (262–4190) ml/min per m^2. Consequently, the AUC was significantly higher ($P_2 < 0.001$) on day 4: 1721 (503–3755) $\mu M \cdot$ min vs 2018 (679–10,852) $\mu M \cdot$min. These data are consistent with a time-dependent decrease in 5-FU clearance.

The mean ($\pm$SD) duration of infusion was 61.0 ± 4.9 min ($n = 66$). Variations in the infusion duration are expected to influence the Cmax. To permit comparison of the Cmax values obtained on either day 1 or day 4 for the 32 paired patient cycles the Cmax values were therefore normalized to a 60-min infusion. Cmax levels during the fourth 5-FU infusion were higher than those measured during the first 5-FU infusion in 25 of 32 paired samples. The average increase was 1.46-fold, and was ≥1.25-fold higher in two-thirds of the samples. When the paired data for all 5-FU doses were combined, the distribution of values for day 1 was significantly different ($P_2 < 0.001$) than for day 4 (median, range): 27.2 (8.2–67.7) μM vs 36.9 (12.4–91.6) μM. Similar results were obtained if the analysis was restricted to 18 paired cycles at 5-FU 370 mg/m^2 (median, range): 22.1 (8.2–50.0) μM vs. 32.2 (12.4–72.7) μM; $P_2 = 0.006$. The Cmax values were also significantly higher on day 4 than on day 1 when the data were not adjusted for differences in length of infusion (data not shown). Since the times of day of the paired 5-FU infusions were very similar, the differences cannot be attributed to circadian variation.

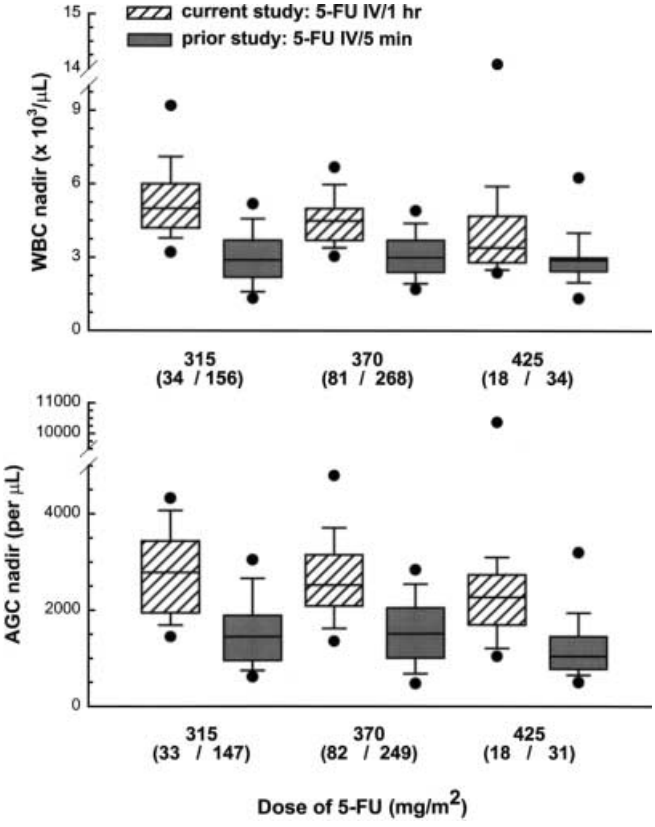

Fig. 3 Leukocyte and granulocyte nadirs for all cycles of therapy. The lowest leukocyte (WBC) and granulocyte (AGC) nadirs for each patient cycle for the present study ($n = 22$ patients) and the prior phase II study ($n = 46$ patients) were evaluated. The data are presented in box and whisker plot format, in which the median values are represented by the horizontal lines, the 25th and 75th percentiles by the bars, the 10th and 90th percentiles by the whiskers, and the 5th and 95th percentiles by the solid circles. The number of assessable cycles are shown in parentheses. The distribution of values for each category was compared by the Wilcoxon's rank sum test. The WBC and AGC nadirs were significantly lower for each dose level with 5-FU given over 5 min (WBC 425 mg/m^2, $P_2 = 0.015$; all other comparisons $P_2 = 0.0001$)

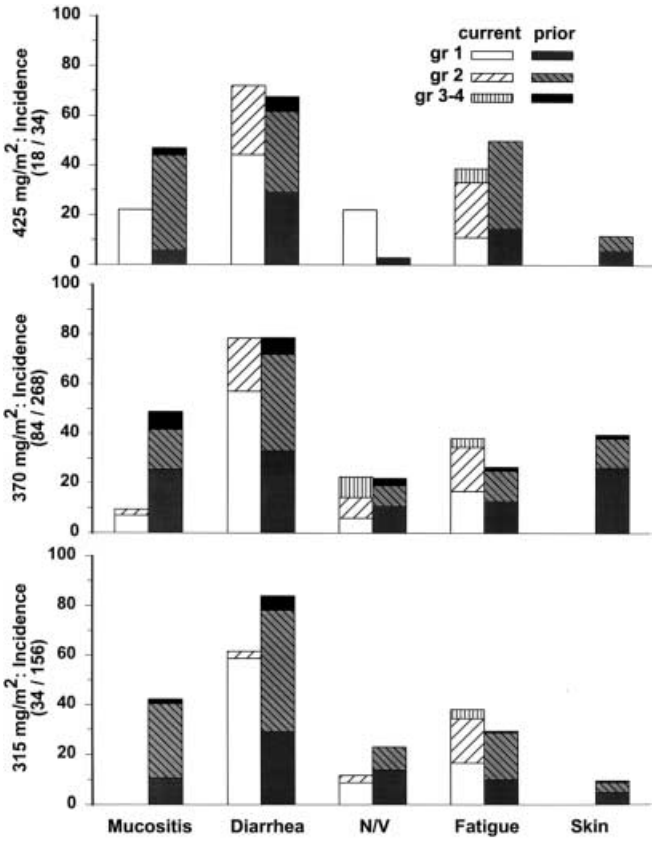

Fig. 4 Percent of cycles complicated by nonhematologic toxicities. The incidence of various types of toxicity is shown for all cycles at 315, 370 and 425 mg/m^2 5-FU for both the present and prior phase II study. The numbers of assessable cycles per dose for each trial are shown in parentheses. The data are presented as a stacked bar graph with three groupings: grade 1, grade 2, and grade 3–4. The stratified version of the Cochran-Armitage trend test was used to compare trends in toxicity grades between the present study and the prior phase II study, stratified by dose, using grades 0–1 vs 2 vs 3–4. The trends in toxicity grades were significantly different for mucositis ($P_2 = 0.0001$), diarrhea ($P_2 = 0.0001$), nausea/vomiting ($P_2 = 0.036$), and skin rash (grade 0–1 vs 2–3, $P_2 = 0.002$)

We next compared the pharmacokinetic parameters in patients receiving 5-FU at 370 mg/m^2 given over either 5 min or 1 h in combination with 500 mg/m^2 LV days 2–6 and 5 MU/m^2 IFN α-2a s.c. days 1–7, using the values obtained with the fourth dose of 5-FU. The blood samples in all three trials were obtained between 10 a.m. and 1 p.m. The median 5-FU plasma concentration immediately following the 5-min injection was 7.3-fold higher than the median Cmax measured in the present trial, and the median AUC was 2.4-fold higher (Fig. 5). The lower systemic exposure to 5-FU with the 1-h infusion are consistent with the lower apparent clinical toxicity.

Clinical response

A median of three cycles were given. Two patients had metastatic disease that was surgically documented but not detectable by radiographic studies. Disease progression was documented after 19 weeks in both of these patients. Five of 20 patients with measurable disease had a partial response. The histologies included pancreas (two patients), and bile duct, cholangiocarcinoma, and adenocarcinoma of the gastroesophageal junction (one patient each). Four additional patients had stable disease lasting for at least 12 weeks (range 14.4–56 weeks). A baseline carcinoembryonic antigen (CEA) was available in all 22 patients (median 8.2 ng/ml, range 0.7–170 ng/ml), and was elevated above 5 ng/ml in 12 patients. Three of these patients experienced more than a 50% decrease while on therapy. Fourteen patients had baseline carbohydrate antigen 19-9 (CA 19-9) values (median 662 ng/ml, range < 15 to $> 40{,}000$ ng/ml), which were elevated above 15 ng/ml in 12 patients, 7 of whom had a decrease in the CA 19-9 level by more than 50% while on therapy. The median time to treatment failure was 13.2 weeks (range 4–212 weeks), and median survival was 34.1 weeks.

Table 2 Summary of pharmacokinetic parameters by 5-FU dose. A total of 39 samples were available after the first dose of 5-FU at the indicated dose levels, while 32 samples were available after the fourth 5-FU dose. The Cmax values represent the average of the two end-of-infusion plasma values, and the clearance and AUC were modeled using the actual times of blood sampling and length of infusion. The data are presented as the means ± SEM

5-FU dose (mg/m^2)	Day 1				Day 4			
	n	Cmax (μM)	Clearance ($ml/min/m^2$)	AUC ($\mu M \cdot min$)	n	Cmax (μM)	Clearance ($ml/min/m^2$)	AUC ($\mu M \cdot min$)
315	5	20.4 ± 3.7	1988 ± 682	1056 ± 315	4	29.7 ± 1.8	1092 ± 314	1495 ± 429
370	20	25.0 ± 2.4	2289 ± 260	1516 ± 154	18	34.8 ± 4.6	1800 ± 249	2390 ± 538
425	9	35.3 ± 4.7	1820 ± 273	2096 ± 282	6	45.8 ± 9.6	1563 ± 373	2713 ± 593
490	4	24.9 ± 7.1	3580 ± 1349	1487 ± 413	3	50.3 ± 3.0	1256 ± 68	3019 ± 169
560	1	48.6	1479	2913	1	76.7	1012	4256

Discussion

The principal goal of this study was to evaluate the clinical toxicities and pharmacokinetics associated with 5-FU given by 1-h infusion. The toxicity data were compared with our prior phase II study in colorectal cancer patients that used an identical schedule and dose modification guidelines [11]. Since this was not a randomized study, we recognize that any toxicity comparisons must be viewed with caution. However, 5-FU given as a 1-h infusion appeared to be associated with significantly less granulocyte and leukocyte toxicity when considering either the initial cycle, the worst grade of toxicity across any cycle while on therapy, or all cycles at 5-FU doses of 315–425 mg/m^2. Further, the incidence and severity of mucositis, diarrhea, and skin rash seemed to be substantially lower than expected. The observation that fatigue seemed to be worse in the current study may in part be due to the greater number of symptomatic patients. The National Surgical Adjuvant Breast and Bowel Project protocol C-05 evaluated the worth of IFN α-2a given with 5-FU/LV according to the NCI schedule for six cycles as adjuvant therapy for colon cancer. Serious nonhematologic toxicities occurred at a similar frequency in the 1060 patients receiving IFN α-2a to that seen in the patients treated in our prior phase II trial [19]. Grade 3–4 toxicities were (C-05 vs phase II) diarrhea 43% vs 37%,

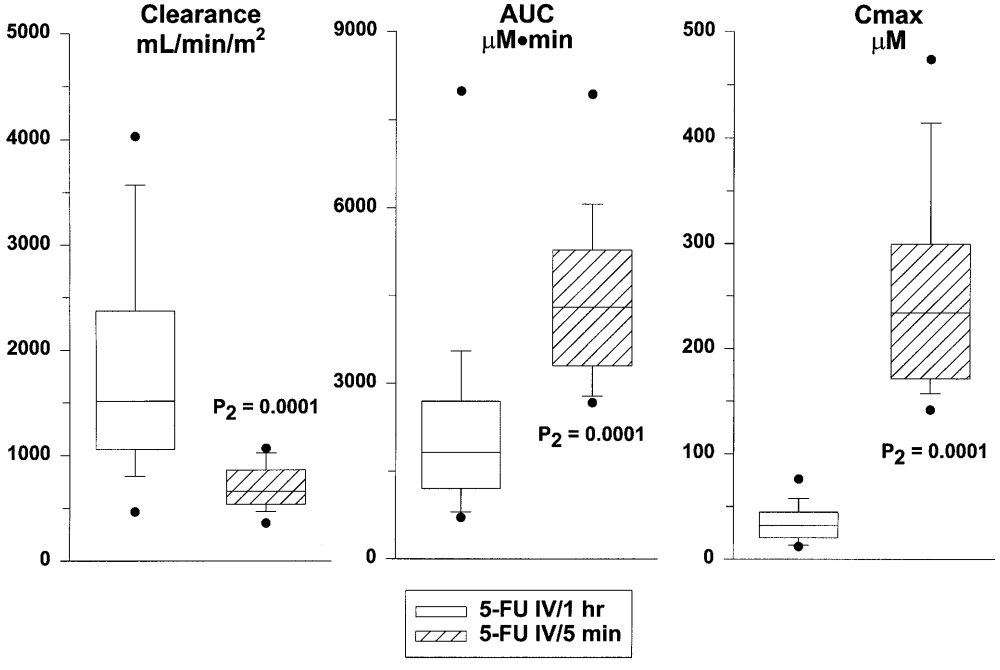

Fig. 5A–C Comparison of pharmacokinetic parameters of 5-FU 370 mg/m^2 given by bolus or 1-h i.v. infusion. 5-FU pharmacokinetic analysis was performed on patients receiving IFN α-2a 5 MU/m^2 s.c. days 1–7 with leucovorin 500 mg/m^2 over 30 min and 5-FU 370 mg/m^2 given either over 5 min 1 h after leucovorin (prior trial, $n = 31$) or over 1 h starting after the completion of the leucovorin infusion (present trial, $n = 18$) [5, 12]. The data are from samples obtained after the fourth dose of 5-FU. The clearance (left panel) is significantly lower and the AUC data (middle panel) are significantly higher with bolus administration of 5-FU. The 5-FU plasma concentration values immediately after the 5-min infusion and at the end of the 1-h infusion are shown in (right panel). The median Cmax observed with 5-FU given over 5 min was significantly higher

and mucositis 36% vs 39%, which supports our perception that the incidence and severity of nonhematologic toxicities was lower than expected with 5-FU given by 1-h infusion.

The pharmacokinetic data for the current study were compared with the data from patients who had received 5-FU 370 mg/m^2 given over 5 min with the same doses of LV and IFN α-2a as part of two previously reported clinical trials [5, 12]. The median Cmax values at the end of the 1-h infusion were substantially lower, and the median 5-FU AUC was 2.4-fold lower. In one prior study, the pharmacokinetics of 5-FU 500 mg/m^2 given as an injection over either 2 min or 20 min were evaluated in 14 patients [19]. In that study, peak 5-FU plasma levels and AUC were 2.1-fold (341 vs 161 μ*M*) and 1.8-fold higher (6158 vs 3355 μ*M*·min) with the 2-min injection.

Among 32 paired plasma samples obtained following the first and fourth dose of 5-FU, the median Cmax and AUC values were significantly higher (36% and 17%, respectively), and clearance was 0.74-fold lower. In our pilot trial using bolus 5-FU/LV/IFN α-2a, paired blood samples were obtained following the fourth dose of 5-FU during two cycles in which the presence or absence of IFN α-2a was the only variable [11]. A dose-dependent decrease in 5-FU clearance was noted with increasing IFN α-2a dose. With 5 MU/m^2, the median 5-FU AUC was 19.4% higher compared to the previous cycle without IFN α-2a, which is remarkably similar to the increase on day 4 vs day 1 in the present trial [11]. We later demonstrated a time-dependent decrease in 5-FU catabolism in intact peripheral blood mononuclear cells isolated from patients on days 1, 2, and 4 prior to the daily treatment with 5-FU/LV and IFN α-2a [20]. The difference between day-1 and day-4 5-FU pharmacokinetic parameters in the present trial supports such a time-dependent effect of IFN α-2a, and may explain the apparent lack of an IFN α-2a effect on 5-FU pharmacokinetics in other trials in which the 5-FU sampling was done following the initial IFN α-2a dose.

Our clinical interest in 5-FU/IFN combinations followed the demonstration that IFN potentiates 5-FU cytotoxicity in cancer cell lines [21, 22]. The results of several phase II studies, including our own, appeared promising, and numerous randomized studies were conducted to evaluate the potential benefit of adding IFN to different 5-FU schedules. While waiting for the completion of these studies, we sought to improve upon our results by various strategies [12, 23, 24]. The accrual to this trial was completed prior to the publication of most phase III trials in advanced disease. The NSABP has recently reported that IFN α-2a confers no benefit when added to 5-FU/LV in the adjuvant treatment of colon cancer [18]. Similarly, the meta-analysis group in advanced colorectal cancer recently reported that IFN-α does not improve the results when added to 5-FU ± LV [19].

Prior studies have shown a correlation between 5-FU AUC or steady-state levels and clinical toxicity [4, 5, 25, 26, 27, 28, 29]. The relationship between antitumor activity and 5-FU pharmacokinetics is less clear. Two nonrandomized trials have suggested an association between clinical response and higher 5-FU plasma exposure during 96- or 120-h infusions given with cisplatin on day 1 in head and neck cancer patients [30, 31]. A randomized trial in head and neck cancer in which standard dosing was compared with pharmacokinetically guided dose adaptation of 5-FU, however, showed that while the AUC and certain clinical toxicities were appreciably greater in the standard arm, the objective response rates were comparable [32]. In contrast, administration of 5-FU over 10–20 min was associated with a lower response rate in colorectal cancer patients compared to patients receiving 5-FU over 2–4 min [10]. Although higher-dose regimens have not generally proved to be superior to standard doses in ''chemosensitive'' solid tumors including breast, ovarian and testicular cancer, several randomized clinical trials have shown that substandard therapy is worse than standard dose therapy.

In conclusion, we found that the clinical toxicities and the 5-FU AUC seemed to be substantially lower than expected in the present trial in which 5-FU was given by 1-h infusion compared with those observed in a prior trial using the same 5-FU/LV/IFN α-2a regimen except that 5-FU was given over 5 min. Dose escalation according to patient tolerance was permitted in both trials. Over the dose range used, 5-FU clearance seemed to be independent of dose. A potential concern is that the lower 5-FU plasma exposure and reduced clinical toxicity might adversely impact the antitumor effect. This regimen had clinical activity, but further conclusions are precluded by the nature of the trial.

An important issue for protocol design is that arbitrary changes in the length of 5-FU infusion may potentially lead to subtherapeutic dosing of patients. Further, differences in the method of injection, such as use of a short-term infusion with an i.v. minibag, may result in unintentional changes in the duration of 5-FU infusion. Due to the potential impact on clinical toxicity and response, greater attention should be paid to standardizing the precise method of 5-FU administration, especially in multicenter clinical trials. If a change in the duration of 5-FU infusion is contemplated, preliminary dose-finding studies may be warranted to define a recommended dose. If dose escalation within patients is considered according to individual tolerance, our findings suggest it would be important to ensure that the time over which 5-FU is given is fixed, since alterations in the duration of infusion might lead to alterations in clinical toxicity, and perhaps, clinical efficacy.

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