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Phase I study of 5-fluorouracil and leucovorin by a 14-day circadian infusion in metastatic adenocarcinoma patients

Georg A. Bjarnason, Ian G. Kerr, Nancy Doyle, Moira Macdonald, Marcia Sone

Division of Medical Oncology, Toronto Bayview Regional Cancer Center, and Division of Clinical Pharmacology, Sunnybrook Health Science Center, Department of Medicine, University of Toronto, 2075 Bayview Avenue, North York, Ontario, Canada M4N 3M5

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Abstract. Initial experimental and clinical studies have indicated that 5-fluorouracil (5-FU) toxicity can be reduced by delivering 5-FU at around 4 a.m. More recent data have suggested that the toxicity might be reduced even more with delivery at around 9–10 p.m. The current study determined the maximum tolerated dose (MTD) for 5-FU and leucovorin (LV) delivered as a continuous circadian infusion over 14 days every 28 days, with the peak of the infusion occurring at around 3–4 a.m. The peak drug delivery was shifted to 9–10 p.m. in all patients developing toxicity of ≥ grade II (Eastern Cooperative Oncology Group) to determine if this timing further reduced toxicity and enabled increased dose intensity. A total of 14 patients with metastatic adenocarcinoma received an admixture of 5-FU and LV via a programmable portable infusion pump, with 62.5% of the 24-h dose being given over 7 h around the infusion peak. The starting dose level of 5-FU (200 mg/m^2 daily) and LV (5 mg/m^2 daily) was that established as the highest tolerable dose rate in a previously reported phase I study using a 14-day flat infusion of 5-FU and LV. The LV dose was first escalated to 20 mg/m^2 daily, followed by escalations of the 5-FU dose. A total of 51 courses were evaluable for toxicity. The dose-limiting toxicity was oral mucositis and hand-foot syndrome. More dose intensity could be delivered using a circadian infusion peaking at around 3–4 a.m. than was possible with a flat infusion of these drugs. Toxicity was reduced even further with peak drug delivery at around 9–10 p.m. The recommended dose for phase II studies using this schedule is 250 mg/m^2 5-FU daily and 20 mg/m^2 LV daily with the peak of the infusion occurring at 9–10 p.m. This is a 300% and 25% higher dose for LV and 5-FU, respectively, than was found to be safe for a flat infusion.

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Correspondence to: Georg A. Bjarnason

Introduction

Prospective randomized trials in patients with metastatic colorectal cancer have shown a significantly improved response rate with the combination of 5-FU and LV as compared with 5-FU alone [38]. Two prospective randomized studies have demonstrated that a long-term continuous infusion of 5-FU gives a better response rate and less toxicity than a bolus treatment in metastatic colorectal cancer [25, 37]. Experimental data suggest that the combination of 5-FU and LV may also be more active when both agents are given on an infusion schedule [29]. Anderson et al. [1] have completed a phase I study to identify the highest tolerable dose rate of delivery for an admixture of 5-FU and LV given as a 14-day continuous flat infusion every 28 days. The highest tolerable dose for 5-FU and LV was found to be 200 and 5 mg/m^2 daily, respectively. At this dose no toxicity greater than grade I (Eastern Cooperative Oncology Group, ECOG) was seen. At higher doses of either 5-FU or LV, stomatitis became the dose-limiting toxicity.

In murine studies, the toxicity of at least 20 chemotherapeutic agents has been shown to be dependent on the time of day of delivery [30]. Clinical trials have confirmed this finding for 5-fluoro-2′-deoxyuridine (FUDR) and for some anthracyclines and platinum analogs [2, 18]. Murine data on 5-FU-induced *lethal* toxicity [4, 34] were the basis for subsequent clinical trials with 5-FU, which demonstrated less toxicity when a circadian 5-FU infusion peaked at 4 a.m. [23, 24]. Recent clinical studies of 5-FU pharmacokinetics [15], 5-FU metabolism [7, 15, 44], human gut cytokinetics [3] and murine studies using *nonlethal* 5-FU doses [11, 27, 32] suggest that a 5-FU infusion peaking at 9–10 p.m. may be even less toxic than a schedule with maximal dose delivery at around 3–4 a.m.

The objective of the current study was to determine the maximum tolerated dose (MTD) for 5-FU and LV given as a continuous circadian infusion over 14 days, with the infusion peaking at 3–4 a.m. and subsequently at 9–10 p.m. The starting dose for 5-FU and LV was the highest tolerable dose for a flat infusion as defined by Anderson et al.

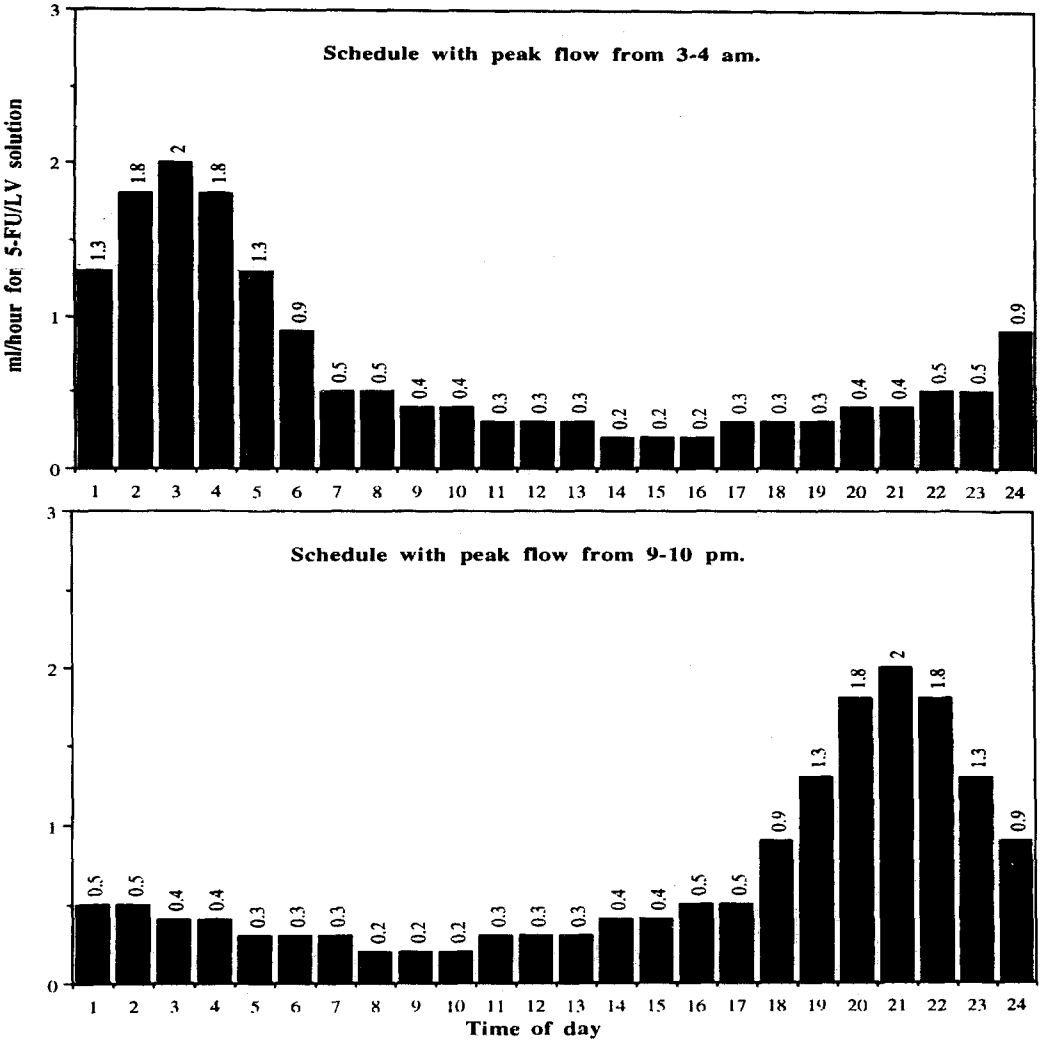

Fig. 1. Infusion rate of the 5-FU/LV solution in milliliters per hour for each hour of the day for the two schedulès tested as delivered by the Vivus 4000/2 pump

[1]. Patients who developed ≥ grade II toxicity with the infusion peak at 3–4 a.m. had the peak of the infusion shifted to 9–10 p.m. to determine if this timing reduced toxicity further and enabled increased dose intensity. Patients who did not develop ≥ grade II toxicity were continued on treatment according to the escalation scheme with the infusion peak at 3–4 a.m., since changing the peak of their infusion would not have been informative with regard to time-dependent toxicity.

Materials and methods

Patient selection. Between June 1990 and September 1991, 14 metastatic adenocarcinoma patients on a normal activity/sleep routine were entered on study. Inclusion criteria included biopsy-proven advanced or metastatic adenocarcinoma, an age of between 18 and 75 years, an ECOG performance status of ≤2, a life expectancy of >8 weeks, no radiation therapy in the preceding 4 weeks, a serum creatinine level of <150 μM, a serum bilirubin value of ≤1.5 times normal, an aspartate transferase value of ≤3 times normal, a granulocyte count of $\geq 1.5 \times 10^9$/l, and a platelet count of $\geq 100 \times 10^9$/l. The presence of measurable disease was not required. Pregnant women, nursing mothers, and patients with cerebral metastases were excluded. The study was approved by the Sunnybrook Health Science Center Ethics Review Board. Before inclusion into the study, patients were required to sign a written informed consent. Patients were withdrawn from the study if there was evidence of disease progression, if intolerable toxicity developed despite dose reduction, or at the patient's request.

Drugs and schedule. Central venous access was obtained by means of a subcutaneous P. A. S. Port (Pharmacia Canada Inc., Baie D'Urfé, Québec) in the antecubital fossa or by a Port-a-Cath (Pharmacia Canada Inc., Baie D'Urfé, Québec) in the subclavian area.

The total dose of 5-FU (Hoffmann-La Roche Ltd., Mississauga, Ontario) and LV (Lederle Laboratories, Cyanamid Canada Inc., Montreal, Québec) sufficient for 8 days of treatment was dissolved in 128 ml of normal saline in a plastic reservoir attached to the infusion pump. The daily infusion volume was 16 ml. A new freshly prepared reservoir was fitted to the pump on day 8 of each treatment cycle, thus leaving 16 ml in the reservoir to account for pump-purging volumes and possible delays in changing the reservoir on day 8. On day 15 the infusion pump was disconnected and a heparin solution (80 units, 10 units/ml), injected into the central catheter. The infusion volumes remained constant with dose escalation, but the concentration of drugs was increased appropriately. Anderson et al. [1] looked at the stability of a 5-FU/LV solution and found it to be stable for at least 15 days. In the current study a fresh solution of 5-FU/LV was prepared for each 7-day treatment period.

Each course of treatment consisted of a 14-day, time-modified, continuous infusion of 5-FU and LV given every 28 days on an outpatient basis. The time-modified shape of the infusion (Fig. 1) was such that 62.5% of the 24-h dose was given in 7 h at around 3–4 a.m. This shape

was designed to be as close to a sinusoidal curve as was possible within the limits imposed by the infusion-pump hardware and software. In patients who experienced ≥ grade II toxicity on this schedule, the peak of the infusion was shifted from 3–4 a.m. to 9–10 p.m. and treatment was repeated at the same dose level. The shape of the infusion curve was not changed. Patients who did not develop ≥ grade II toxicity continued on the initial schedule (peak, 3–4 a.m.) with appropriate dose escalations.

The Strato Micropump (Strato Medical Corporation, Beverly, Mass.) and, subsequently, the VIVUS-4000/2 multichannel infusion pump (I-Flow Corporation, Irvine, Calif.) were used for drug delivery. Both of these pumps have the ability to change the flow rate of the continuous infusion for every hour over 24 h. The accuracy of drug delivery was ascertained by measuring the residual volume in the drug reservoir at the end of each 7-day treatment period. If the residual volume was more or less than 10% of the expected value (the stated accuracy of the pumps), that course of treatment was not evaluable for toxicity, and the same dose level was given again to that patient.

The starting dose level of 5-FU and LV was the dose rate established as safe by Anderson et al. [1]. This consisted of 200 mg/m^2 5-FU daily and 5 mg/m^2 LV daily. Dose escalation was then carried out as outlined in Table 2, first escalating the LV dose by 5 mg/m^2 daily to 20 mg/m^2 daily and then escalating the 5-FU dose by 50 mg/m^2 daily per dose escalation. At least three patients were treated at each dose level. Individual patients were escalated to the next higher dose level provided that a total of three patients had completed treatment and follow-up at the current dose level with < grade II toxicity and that the individual patient had not experienced toxicity of ≥ grade II. The MTD was defined as that dose of the combination of 5-FU and LV that produced dose-limiting toxicity in at least half of the treated patients. Dose-limiting toxicity consisted of any of the following toxicities: ≥ grade II oral mucositis and/or ≥ grade II diarrhea and/or ≥ grade II hand/foot syndrome. Once the MTD was defined, the next lower dose would be considered the recommended phase II dose.

No antiemetic was given unless patients complained of nausea, in which case 10 mg prochlorperazine was given orally every 4 h as needed. Patients were instructed to use a sodium bicarbonate mouthwash solution during and after their course of chemotherapy. If serious mucositis developed, this was treated appropriately. Vitamin-B6 (50 mg orally three times per day) was given to patients who experienced ≥ grade II hand/foot syndrome [28], and treatment at the same dose level was repeated before the infusion peak was shifted based on toxicity.

Evaluation during the study included a history and physical examination prior to every treatment course, a weekly toxicity assessment, a weekly complete blood count, determinations of electrolyte and creatinine levels, liver-function studies prior to each course, and radiological investigations of measurable disease and appropriate tumor markers prior to every other course.

As in Anderson's trial [1], ECOG toxicity criteria were used to grade oral mucositis and diarrhea. The most significant toxicity was graded as follows. *Oral mucositis*: Soreness/erythema (grade I), erythema, ulcers, can eat solids (grade II), ulcers, requiring a liquid diet only (grade III), and alimentation not possible (grade IV). *Diarrhea*: Transient, lasting for <2 days (grade I); tolerable but lasting for >2 days (grade II), intolerable, requiring therapy (grade III); and hemorrhagic with dehydration (grade IV). *Hand/foot syndrome*: Hand/foot tenderness (grade I); hand/foot tenderness and erythema (grade II); and hand/foot tenderness, erythema, swelling, and desquamation (grade III).

Results

Patients' characteristics

Table 1 describes the patients' characteristics. The 14 patients with metastatic adenocarcinoma received from 1 to 10 courses of treatment (mean, 4.8 courses/patient). Most patients had a performance status of 0 or 1. Seven patients had not received prior therapy: two with pancreatic cancer, four with renal-cell cancer, and one with adenocarcinoma of the lung. Seven patients had received either chemotherapy, hormonal therapy, radiation therapy, or a combination of these modalities. Radiation therapy had been given to areas of bone metastases or postoperatively to the breast. No patient had received prior abdominal radiotherapy that might have potentiated gastrointestinal toxicity. Two heavily pretreated patients with metastatic breast cancer had received chemotherapy with 5-FU in the past.

Table 1. Patients' characteristics

Patients entered on study	14
Sex (M/F)	8/6
Age	30–64 (mean, 47.5) years
Performance status:	
0	2
1	11
2	1
Primary tumor sites:	
Colorectal	3
Pancreas	2
Kidney	5
Breast	2
Endometrium	1
Lung	1
Prior treatment:	
Untreated	7
Chemotherapy	6
Radiation therapy	4
Hormonal therapy	2

Table 2. Dose levels and number of courses given at each dose level

Dose level and drug doses[a]	Number of new patients entered at this dose level	Number of patients treated at this dose level	Number of courses given at this dose level	Number of courses evaluable for toxicity
1. 5-FU 200/LV 5	3	3	7	5
2. 5-FU 200/LV 10	2	3	7	5
3. 5-FU 200/LV 15	3	7	9	8
4. 5-FU 200/LV 20	3	9	16	11
5. 5-FU 250/LV 20	3	8	16	15
6. 5-FU 300/LV 20	0	5	10	7
Totals	14		65	51

[a] Doses are expressed in mg/m^2 per day

Dose escalation and toxicity

As described in Table 2, a total of 65 courses were given at 6 escalating dose levels. Of these 65 courses, 51 were evaluable for toxicity. The reasons for the 14 incomplete nonevaluable courses are detailed in Table 3. These were related to mechanical problems with the vascular access devices (3 courses) or the infusion pumps (11 courses). During 2 of the 51 evaluable courses the infusion was stopped early because of toxicity at dose levels 5 and 6,

Table 3. Reasons for incomplete nonevaluable courses

Problems with the vascular access devices:	
Needle dislodged from the P. A. S. Port	1
Infected P. A. S. Port	1
Blocked Port-a-Cath	1
Problems related to the infusion pump:	
Pump inadvertently turned off early by the patient	3
Residual volume on day 7 or 14 more than the accepted ±10% of the expected value	6
Leakage of the pump tubing connection	2
Total	14

respectively. These courses are included as being evaluable for toxicity.

The dose-limiting toxicity in this study was oral mucositis and hand/foot syndrome. No hematological toxicity was noted. Table 4 describes the number of courses giving toxicity at each dose level and the type of toxicity experienced. In all, 54% of courses (28/51) were associated with ≤ grade I toxicity. Oral stomatitis was the most common toxicity and was noted to some extent in ten patients. Hand/foot syndrome was noted in four patients. Darkening of nails and pigmentation of soles and palms was noted in three dark-skinned individuals. One patient had a local recall reaction over a previously radiated breast, with itching and erythema. Two patients noted a metallic taste, both at dose level 1. Grade I nausea was reported for two courses at dose levels 3 and 4, respectively. One patient had minimal hair loss at dose level 1. Diarrhea was noted by four patients at dose levels 5 and 6. Toxicity was reproducible over time in patients receiving several treatments at the same dose level. There was no evidence of cumulative toxicity in patients receiving up to ten courses of treatment.

The MTD for an infusion peaking at 3–4 a.m. was reached at dose level 5 (5-FU, 250 mg/m^2 daily; LV, 20 mg/m^2 daily), with four of eight patients developing dose-limiting toxicity. Since toxicity was reduced in two patients at dose level 5 by shifting the peak of the infusion to 9–10 p.m., further dose escalation was allowed to dose level 6. The MTD for an infusion peaking at 9–10 p.m. was reached at dose level 6 (5-FU, 300 mg/m^2 daily; LV, 20 mg/m^2 daily), with three of five patients developing dose-limiting toxicity. The recommended dose for phase II studies using this schedule is therefore 250 mg/m^2 5-FU daily and 20 mg/m^2 LV daily, with the peak of the infusion occurring at 9–10 p.m.

Six patients who experienced ≥ grade II toxicity on the schedule peaking at 3–4 a.m. had the peak of their circadian infusion changed from 3–4 a.m. to 9–10 p.m. on subsequent treatments. This resulted in decreased toxicity for all six patients. In three of these patients, further dose excalation was possible. Table 5 compares the toxicity of these six patients when the infusion peaked at 3–4 a.m. and at 9–10 p.m. Patients who did not develop ≥ grade II toxicity were continued on escalating doses with the infusion peak at 3–4 a.m., since changing their schedule would not have been informative with regard to time-dependent toxicity.

Although the response rate was not an endpoint in this study, patients with measurable disease were followed radiologically. Two heavily pretreated patients with metastatic breast cancer had received 5-FU in the past. One of these patients progressed on treatment, whereas the other came off study with stable disease after ten cycles of treatment. One chemotherapy-naive patient with inoperable rectal cancer, who had not responded to radiation therapy, had a partial response. His cancer was subsequently successfully resected. Minor short-term responses were seen in three patients with metastatic renal-cell cancer, but one of these patients continued on treatment with stable disease

Table 4. Number of courses giving toxicity at each dose level

Dose level	Evaluable courses	Toxicity grade[a]	Oral stomatitis	Hand-foot syndrome	Diarrhea	Nausea	Skin rash or pigmentation
1	5	1	2	1			1
2	5	1	2				1
		2	1				
		3	1				
3	8	1	1	1		1	
		2	2				
		3		2			
4	11	1		4		1	1
		2	1				
		3		1			
5	15	1	5		3		2
		2	5	1	2		
6	7	1	1	2			
		2	2		3	1	
		3	1	1			

[a] Eastern Cooperative Oncology Group toxicity criteria

Table 5. Six patients with reduced toxicity with the infusion peak at 9–10 p.m.

Infusion peak at 3–4 a.m.			Infusion peak at 9–10 p.m.		
Dose level	Type of toxicity	Toxicity grade[a]	Dose level	Type of toxicity	Toxicity grade[a]
2	Stomatitis	3	3[b]	Stomatitis	2
3	Hand/foot syndrome	3	4[b]	Hand/foot syndrome	1
4	Stomatitis	2	6[b]	No toxicity	
5	Stomatitis	2	5	Stomatitis	1
5	Diarrhea Stomatitis	2 2	5	Diarrhea	1
6	Stomatitis Diarrhea Nausea Hand/foot syndrome	2 2 2 1	6	Stomatitis	2

[a] Eastern Cooperative Oncology Group toxicity criteria. Each line in the table represents the data for a single patient
[b] Dose escalation was possible after the peak of the infusion had been shifted from 3–4 a.m. to 9–10 p.m.

Table 6. Comparison of toxicity per patient treated by dose level and study[a]

5-FU dose (mg/m^2 daily)	LV dose (mg/m^2 daily)			
	5	10	15	20
200	**3/3/0** *7/7/0*	**3/3/1 (0)** *5/3/4*[b]	**7/7/3 (2)** *1/1/1*	**9/9/2 (1)**
250	 *7/4/5*	 *2/0/2*		**8/7/4 (2)**[c]
300	 *4/0/4*			**5/4/ (3)**[d]

[a] Toxicity observed in the current study as compared with that reported by Anderson et al. [1]. The data format for each dose level is as follows: number of patients/number of patients completing all courses/number of patients with ≥ grade II toxicity. Data in parentheses represent the number of patients with ≥ grade II toxicity after a change in the peak of infusion from 3–4 a.m. to 9–10 p.m. Bold numbers represent data from the present study and italic numbers represent data from Anderson et al. [1]
[b] MTD for a flat infusion
[c] MTD for an infusion peaking at 3–4 a.m.
[d] MTD for an infusion peaking at 9–10 p.m.

for eight courses. All other patients were withdrawn from study with progressive disease.

Discussion

In this phase I study we defined the MTD for a 14-day circadian infusion of 5-FU/LV, with the peak of the infusion occurring at either 3–4 a.m. or 9–10 p.m. An infusion peaking at 9–10 p.m. was less toxic than an infusion peaking at 3–4 a.m., allowing more dose intensity. The ability to compare our toxicity results with those from the study by Anderson et al. [1] lends some credence to our results with regard to time-dependent toxicity. This comparison is of course flawed by the many potential biases associated with any historical comparison of data between different clinical trials and institutions. The patients in Anderson et al.'s study were comparable with our patients with regard to age, diagnosis, and previous therapy. On the other hand, a larger proportion of Anderson et al.'s patients had a poorer performance status. Since there were substantial differences in the attainable dose intensity between these two studies, we present a comparison of the toxicity and MTD in Table 6. This format was used by Anderson et al. to report the toxicity observed in their study, and we used the same format to compare the toxicities noted in these two studies. For each dose level, the number of patients treated, the number of patients capable of completing all courses, and the number of patients developing ≥ grade II toxicity are indicated. The sequence of dose escalation was different in the two studies, but the results can be compared with regard to the MTD (≥ grade II toxicity in at least half of the treated patients). Some of the patients on our study had more than one course of treatment at each dose level to satisfy the escalation criteria, but only the number of patients treated at each dose level are indicated in Table 6.

In Anderson et al.'s study the MTD was reached at the first dose escalation step (5-FU, 200 mg/m^2 daily; LV, 10 mg/m^2 daily), with four of five patients experiencing dose-limiting toxicity and only three patients completing the full course of treatment. Four other combinations of doses of 5-FU and LV led to dose-limiting toxicity in the majority of patients, with only 5 of 14 patients being able to complete a full treatment course. It was concluded that the optimal dose rate for this combination on this flat-infusion schedule was 200 mg/m^2 5-FU daily and 5 mg/m^2 LV daily [1]. In the current study the MTD for an infusion peaking at 3–4 a.m. was reached at dose level 5 (5-FU, 250 mg/m^2 daily; LV, 20 mg/m^2 daily), with four of eight patients developing dose-limiting toxicity. Since toxicity was reduced for two patients at dose level 5 by shifting the peak of the infusion to 9–10 p.m., further dose escalation was allowed to dose level 6. The MTD for an infusion peaking at 9–10 p.m. was reached at dose level 6 (5-FU, 300 mg/m^2 daily; LV, 20 mg/m^2 daily), with three of five

patients developing dose-limiting toxicity. The recommended dose for phase II studies using this schedule is therefore 250 mg/m^2 5-FU daily and 20 mg/m^2 LV daily, with the peak of the infusion occurring at 9–10 p.m. This is a 300% and 25% higher dose for LV and 5-FU, respectively, than was suggested to be safe for a flat infusion by Anderson et al. [1]. If this comparison is valid, a flat infusion of 5-FU/LV is more toxic than a circadian infusion peaking at either 3–4 a.m. or 9–10 p.m. A prospective randomized study is required to prove a significant difference in toxicity and achievable dose intensity between these three schedules of delivery. It could also be argued that several more circadian schedules should be tested before a firm conclusion can be drawn about the best time to deliver 5-FU/LV on this schedule. For practical purposes this was not done in the current study. On the basis of these results, we have started a phase II study in patients with previously untreated colorectal cancer to evaluate the activity of this schedule. We are also studying this schedule in conjunction with radiotherapy [20]. Since no hematological toxicity was noted, there will be ample opportunity to add myelosuppressive agents to this regimen in patients with 5-FU-responsive malignancies.

The decision to escalate the LV dose only to 20 mg/m^2 daily before escalating the 5-FU dose was partly empirical but partly based on clinical trials. Poon et al. [33] found that 5-FU (425 mg/m^2 daily) plus low-dose LV (20 mg/m^2 daily) as compared with 5-FU (370 mg/m^2 daily) plus high-dose LV (200 mg/m^2 daily) were equivalent with regard to response rate and survival in metastatic colorectal cancer when these drugs were given as a bolus daily for 5 days every 4 weeks. The dose of LV (20 mg/m^2 daily) per course (4 weeks) as given in our study is 2.8 times higher than that delivered on the low-dose LV plus 5-FU bolus schedule described by Poon et al. [33] and 3.7 times higher than that achievable by Leichman et al. [21, 22] in their study of continuous-infusion 5-FU with weekly bolus LV. Even though LV is itself a nontoxic drug, it is clear from the studies of Anderson et al. [1] and Leichman et al. [21, 22] that the ability to escalate the dose of this 5-FU modulator in conjunction with infusional 5-FU is a significant finding.

Does a focused infusion at "any time" decrease toxicity as compared with a flat infusion? This question has been addressed by Von Roemeling et al. [36] in murine studies. FUDR, 1000 mg/kg was given by continuous intravenous infusion over 48 h to female 344 Fisher rats. The drug was delivered either by constant-rate infusion or by variable-rate infusion with peak drug delivery during one of six different times of day. FUDR lethal toxicity, which was secondary to gut damage, was lowest when the peak of the infusion was delivered in the late activity span. This was also the best time with regard to the tumor response in animals with a transplanted 13762-adenocarcinoma given 700 mg/kg FUDR as a 48-h intravenous infusion. Since some of the circadian-shaped infusions studied were toxicologically and therapeutically inferior to the constant-rate infusion, the circadian timing and not the quasi-intermittency of circadian FUDR administration appears primarily responsible for these circadian pharmacodynamic differences.

The time dependence of 5-FU toxicity has been documented in several experimental studies. In these studies, the optimal time to reduce intestinal toxicity [11] and bone marrow toxicity [27, 32] with *nonlethal* doses of 5-FU seemed to be several hours later in the day than the optimal time for reducing whole-animal toxicity from *lethal doses* of the drug [4, 12, 34]. Subsequent clinical trials have confirmed that toxicity can be reduced by delivering the highest proportion of a 5-FU continuous infusion at around 4 a.m. [23, 24]. Experimental studies have also demonstrated the time-dependent toxicity of an FUDR infusion [36, 45]. This effect has been confirmed in clinical trials [35] in which less toxicity was demonstrated with an evening administration of FUDR.

The mechanisms for the time-dependent toxicity of fluoropyrimidines are only partially understood. More than 80% of a delivered dose of 5-FU is rapidly catabolized in the liver and extrahepatic sites. Thus, catabolism largely determines the availability of 5-FU for anabolism to its active nucleotide analogs [17]. The activity of dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme for fluoropyrimidine catabolism, has been shown to be highly circadian-time-dependent in the liver and bone marrow of rats [7, 13, 45] and in the mouse liver [31]. Harris et al. [14] used an isolated perfused rat-liver model to study whether the hepatic elimination rate of 5-FU and total 5-FU metabolites exhibited a circadian pattern similar to that observed for DPD activity in rat-liver homogenates. A circadian variation was observed in the elimination rate of 5-FU and its catabolites. There was a reciprocal relationship between the elimination rate of 5-FU and 5-FU catabolites. The circadian variation in the plasma levels of 5-FU in rats during continuous infusion was prevented with cyano-2,6-dihydroxypyrimidine, a strong inhibitor of DPD [10]. Harris et al. [15] and Tuchman et al. [44] have independently demonstrated a circadian variation of DPD activity in human mononuclear cells, with peak values occurring between 10 p.m. and 2 a.m. and at 1 a.m., respectively. An inverse relationship between DPD activity in peripheral blood mononuclear cells and plasma 5-FU concentration was demonstrated by Harris et al. [15] in their study of cancer patients receiving a protracted continuous infusion of 5-FU. The importance of DPD activity in determining clinical toxicity from fluoropyrimidines is further suggested by the extreme toxicity seen in DPD-deficient patients [8, 16, 43].

The activity of three important enzymes of fluoropyrimidine anabolism, thymidine kinase (TK), orotate phosphoribosyltransferase (OPRTase), and uridine phosphorylase (UrdPase), has recently been shown to be circadian-time-dependent in rat bone marrow, intestinal mucosa, spleen, and liver [45] as well as in mouse liver [31]. In both of these studies, there was an inverse correlation between the activity of DPD and the activity of these anabolic enzymes such that when DPD (catabolism) was at its highest activity the anabolic enzyme activity was at a nadir, and vice versa. Zhang et al. [45] found that the survival rate was inversely correlated with the TK activity (FUDR anabolism) and directly correlated with the DPD activity (FUDR catabolism) in rats given FUDR at one of six circadian times. Studies in which inhibitors of UrdPase

have been shown to increase the concentration and salvage of uridine and protect against host toxicity from both 5-FU and FUDR attest to the importance of the circadian activity of UrdPase [5, 26]. El Kouni et al. [9] have indeed shown that the plasma concentration of uridine follows a circadian rhythm that is the inverse of that observed for UrdPase activity in rodents. The peak activity of OPRTase recorded in the study by Naguib et al. [31] concurs with the time reported for maximal incorporation of orotic acid into pyrimidine components of acid-soluble extract, RNA, and DNA in rat liver [40]. No circadian variation was found in the activity of thymidine phosphorylase (dThdPase), another important fluoropyrimidine enzyme, in mouse liver [31], rat liver [7], or human mononuclear cells [6]. The above-mentioned experimental and clinical data support the contention that at the time of day when fluoropyrimidine catabolism is most active, anabolic activity is at its nadir, and vice versa. This time-dependent variation in fluoropyrimidine metabolism could at least in part explain the apparent time-dependent toxicity of 5-FU observed in our study.

In addition to the circadian pattern of activity of important biochemical pathways, circadian patterns of cytokinetic activity in normal tissues damaged by these drugs may be of equal importance in explaining the circadian pharmacodynamics. Both 5-FU and FUDR are most active against dividing cells during the process of DNA synthesis. Depending on the schedule of delivery, the gut, skin, and bone marrow are the primary targets of fluoropyrimidine toxicity. Circadian rhythms have been demonstrated with regard to the amount of ongoing DNA synthesis in human skin [39], human bone marrow [41], and human colorectal mucosa [3].

The rationale for changing the peak delivery of 5-FU/LV from 3–4 a.m. to 9–10 p.m. in the six patients experiencing $\geq$ grade II toxicity was based on some of the above findings. This timing (9–10 p.m.) corresponds to the time of least toxicity from 5-FU observed in murine studies when *nonlethal* doses of 5-FU were given [11, 27, 32]. Harris et al. [15] found the peak value for plasma 5-FU at 11 a.m. and the trough value at 11 p.m. in patients receiving 5-FU at 300 mg/m^2 daily for 14 days. Thus, 5-FU seems to be metabolized faster in the evening than early in the day. This observation is in keeping with the findings that DPD activity in human mononuclear cells is highest in the evening [15, 44]. Studies of human gut cytokinetics also suggest that fluoropyrimidines might do less damage to the gastrointestinal mucosa at 9–10 p.m. than at 3–4 a.m. [3]. Serial rectal biopsies were taken every 3 h for 24 h from 24 human volunteers in both the fed and fasting states. Much greater in vitro tritiated thymidine uptake occurred in colonic epithelial cells removed during the early morning hours (4 a.m. to 10 a.m.) than in those taken later in the day and evening.

Clinical benefit from using a circadian scheduling of chemotherapy is dependent on a certain cytokinetic and metabolic asynchrony between the circadian susceptibility patterns of the normal tissues at risk for drug toxicity and the tumor. If this asynchrony exists, then at the time when the normal tissues are less vulnerable to the toxic effects of a drug, allowing a higher dose to be given, the tumor may not be protected to the same extent. This circumstance would improve the therapeutic index. Experimental studies showing improved responses in tumor-bearing rodents given chemotherapy at the best time to reduce toxicity as compared with other times support this contention [30]. Recent human data indicate that such an asynchrony between normal tissues and tumor proliferation may indeed exist [19, 42]. Clinical trials to date have confirmed that circadian scheduling of several chemotherapy agents reduces the toxicity and allows safe escalation of the dose intensity [2]. Whether circadian scheduling of chemotherapy translates into a better response rate and/or survival is the subject of ongoing prospective randomized trials.

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