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Phase I trial of a 5-day infusion of L-leucovorin plus daily bolus 5-fluorouracil in patients with advanced gastrointestinal malignancies

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Abstract. The combination of leucovorin [(6*d,l*)-5-formyltetrahydrofolate] and 5-fluorouracil (5-FU) has increased efficacy compared to 5-FU alone as treatment of advanced colorectal cancer. Leucovorin is metabolized to methylene tetrahydrofolate, which potentiates the antitumor actions of 5-FU by forming a ternary complex of thymidylate synthase, fluorodeoxyuridine and methylene tetrahydrofolate. Only *l*-leucovorin is metabolized to methylene tetrahydrofolate and forms this ternary complex. However, *d*-leucovorin may not be inert. *d*-Leucovorin may impair cellular uptake and metabolism of *l*-leucovorin, thereby inhibiting the actions of *l*-leucovorin. Because of this possible limitation to the effectiveness of racemic leucovorin, we have begun to explore the effects of the pure, biologically active isomer, *l*-leucovorin. In this phase I trial, patients with advanced gastrointestinal malignancies were treated with a 5-day continuous infusion of *l*-leucovorin and daily intravenous boluses of 5-FU at 370 mg/m^2. The dose of *l*-leucovorin was escalated in groups of three patients at four doses, 200 mg/m^2 per day, 400 mg/m^2 per day, 700 mg/m^2 per day and 1000 mg/m^2 per day. Treatment was repeated every 28 days. Seventeen patients with advanced gastrointestinal cancers entered the trial. Sixteen patients were evaluable for toxicity. Toxicity was similar to that expected for leucovorin plus 5-FU. The most common severe toxicities (and the number of patents affected) were: diarrhea (2), mucositis (2), nausea/vomiting (1), and abdominal/rectal pain (2). The maximum tolerated dose of *l*-leucovorin was 700 mg/m^2 per day. Twelve patients were evaluable for response. One complete, one partial and one minor response were observed. All responses occurred among the nine patients with colorectal carcinomas. The combination of *l*-leucovorin and 5-FU is well tolerated by patients and appears active for treatment of advanced colorectal carcinomas. Additional clinical trials are necessary to determine if *l*-leucovorin is more effective than *d,l*-leucovorin for modulating the effectiveness of 5-FU.

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Introduction

5-fluorouracil (5-FU) is the most active single agent for treating advanced colorectal cancer with a response rate of 10–20% [13]. Despite 30 years of effort to discover more active drugs, no single agent has a higher response rate than 5-FU. Because of the lack of success in developing new drugs, increasing effort has been made to improve the effectiveness of 5-FU. These efforts include testing alternative doses and schedules of 5-FU, which has led to some promising results [9]. Biochemical modulation of the actions of 5-FU with diverse agents such as leucovorin [3, 5, 10, 16, 18, 26] *N*-(phosphonacetyl)-L-aspartic acid (PALA) [1] or interferon [28, 29] also appears promising in preclinical and clinical trials. The combination of leucovorin and 5-FU has received the most attention. Several randomized trials have shown the therapeutic superiority of leucovorin plus 5-FU compared to 5-FU alone for treatment of advanced colorectal cancer [3, 5, 16, 18, 26]. A recent meta-analysis of nine clinical trials which compared leucovorin plus 5-FU to 5-FU alone as treatment for advanced colorectal cancer demonstrated that the combination is superior in terms of response rates [17]. One trial demonstrated an advantage in overall survival for treatment with leucovorin plus 5-FU [18].

Leucovorin (5-formyltetrahydrofolate) is metabolized to methylene tetrahydrofolate. This compound potentiates the antitumor actions of 5-FU by forming a ternary complex of methylene tetrahydrofolate, fluorodeoxyuridine, and thymidylate synthase, thereby resulting in prolonged inhibition of this critical enzyme [8, 25]. The formulation of leucovorin used in preclinical and clinical studies is the racemic mixture *d,l*-leucovorin. However, only *l*-leucovorin is metabolized to methylene tetrahydrofolate and is biologically active. Although *d*-leucovorin lacks intrinsic biological activity, it can, under some circumstances, antagonize the effects of *l*-leucovorin in vitro [21, 23, 30]. *d*-Leucovorin competes with *l*-leucovorin for uptake by cells [23, 30]. Furthermore, d-leucovorin is a substrate and inhibitor for folylpolyglutamate synthetase [21], an enzyme which is important to the effectiveness of *l*-leu-

covorin [20]. The two stereoisomers are metabolized at different rates in vivo. *l*-Leucovorin is metabolized with a half-life of minutes whereas *d*-leucovorin is metabolized with a much longer half-life of 4–6 h [15, 22]. This differential metabolism results in accumulation of *d*-leucovorin to concentrations which frequently exceed 30 μM in humans [15, 22]. The resulting molar ratio of *d* to *l* isomers may exceed the ratio at which *d*-leucovorin inhibits cellular uptake and polyglutamylation of *l*-leucovorin. Not all studies, however, demonstrate a deleterious effect of *d*-leucovorin [2, 32]. Zheng-Gang and Rustum [32] demonstrated that *d*-leucovorin did not impair the modulation of 5-FU cytotoxicity by *l*-leucovorin in HCT-8 cells. Bertrand and Jolivet [2] reported similar findings using CCRF-CEM cells.

One strategy for circumventing this possible inhibitory effect of *d*-leucovorin is to take advantage of the stereoselective absorption of *l*-leucovorin from the gastrointestinal tract by administering *d,l*-leucovorin orally [6, 24]. Absorption of the *l* isomer is saturable, however, and this strategy achieves only low levels of reduced folates in vivo [12]. In addition, accumulation of *d*-leucovorin still occurs after oral administration of the racemic mixture. Nevertheless, the administration of oral *d,l*-leucovorin appears to increase the effectiveness of 5-FU for treating colorectal cancer substantially [6]. This observation that low concentrations of *l*-leucovorin without the potentially opposing effect of *d*-leucovorin potentiates the effectiveness of 5-FU suggests that achievement of high concentrations of *l*-leucovorin without the opposing effects of the *d* isomer could increase the effectiveness of 5-FU to an even greater extent. Recently, *l*-leucovorin became available in a purified form, thereby permitting study of the effectiveness of high concentrations of *l*-leucovorin without the confounding effects of *d*-leucovorin. This report describes a phase I trial of a 5-day continuous infusion of *l*-leucovorin plus daily intravenous boluses of 5-FU in patients with advanced gastrointestinal malignancies. The goal of the study was to determine the safety of high doses of *l*-leucovorin when given with 5-FU on this schedule. The incidence, severity and duration of toxic effects were also assessed.

Patients and methods

Patients with metastatic or locally recurrent adenocarcinomas of the gastrointestinal tract (colon, rectum, stomach or pancreas) which could not be treated effectively by surgery or radiotherapy were eligible for the trial. Histological documentation of the primary cancer was required and histological documentation of recurrence or metastasis was encouraged. Study entry required ECOG performance status equal to or less then 2 and adequate hematological (leukocytes >4000/mm^3; platelets >130,000/mm^3), hepatic (bilirubin <1.5 mg/dl; SGOT <2 × upper limits of normal) and renal (creatinine <1.5 mg/dl) function. Full recovery from any surgery and a 4-week interval between completion of any radiotherapy and initiation of chemotherapy were required. Patients who received prior chemotherapy were excluded from the study. The trial was approved by the Institutional Review Board of each participating institution. All patients gave written informed consent before receiving treatment.

Treatment. This is a phase I trial of increasing doses of *l*-leucovorin given as a continuous 120-h infusion in combination with a fixed dose of 5-FU (370 mg/m^2) given as a daily intravenous bolus. In this regimen, one fourth of the assigned daily dose of *l*-leucovorin was given as a rapid intravenous bolus on day 1. The patients then began a continuous intravenous infusion of *l*-leucovorin for 120 h. Six hours after beginning treatment with *l*-leucovorin the patients received 5-FU (370 mg/m^2) administered as a rapid intravenous bolus. Treatment with 5-FU was repeated daily at approximately the same time of day for a total of five doses of 5-FU. With this schedule, the infusion of *l*-leucovorin continued for 18 h after the last dose of 5-FU. Patients received additional courses of treatment at 28 day intervals if they did not have grade 4 toxicity. Patients were removed from the study if they had grade 4 toxicity or disease progression.

The dose of *l*-leucovorin was scheduled to be tested at four levels: 200 mg/m^2 per day, 400 mg/m^2 per day, 700 mg/m^2 per day and 1000 mg/m^2 per day. Three patients were to be treated at each level until dose-limiting toxicity occurred during the first cycle of therapy or the maximum dose of 1000 mg/m^2 per day was tested. If 2 or 3 patients at one dose level experienced toxicity of grade 3 or above, then an additional 3 patients were treated at that dose to determine more precisely the rate of severe toxicity. Dose-limiting toxicity was defined as toxicity of grade 4 or above in 2 patients or toxicity of grade 3 or above in 4 or more patients if 6 patients were treated at that level. The maximum tolerated dose of *l*-leucovorin was defined as the dose which produced toxicity of grade 3 or above in 50% or fewer patients. Toxicity was scored according to the NCI Common Clinical Trials Criteria. After the first cycle of therapy, the dose of 5-FU was decreased 20% for patients who experienced toxicity of grade 2 or above. For patients who did not experience any toxicity, the dose of 5-FU was increased by 10% each cycle until at least grade 1 toxicity was achieved. The dose of *l*-leucovorin was not adjusted.

Assessment of response and survival. Objectively measurable disease was not required for entry into this phase I trial. Nevertheless, patients who had objectively measurable disease were assessed for response after every two cycles of therapy. Previously described standard criteria for assessing complete and partial responses, stable disease and progressive disease were used to assess response [26]. A minor response was defined as a more than 25% but less than 50% decrease in the size of the measurable lesions. Time to disease progression and overall survival were calculated starting from the day written informed consent was obtained and were calculated for all evaluable patients.

Results

Patients

The trial was open to accrual from 1 September 1989 to 1 April 1991. The patient data were updated to 10 September 1991. Seventeen patients were entered into the trial. Their clinical characteristics are summarized in Table 1. One patient was not evaluable for toxicity or response because of a major protocol violation which resulted in a substantial error in the doses of *l*-leucovorin and 5-FU given. Only the remaining 16 patients are described in this report. All 16 are evaluable for toxicity and 12 patients were evaluable for response to treatment. The patients' median age was 58 years with 5 patients older than 70 years. The patients' performance status were excellent with 9 patients having ECOG performance status 0 and only 1 patient having performance status 2. Twelve patients had colorectal carcinomas and the majority had liver or lung metastases. No patients had received prior chemotherapy and only 3 had received prior radiotherapy.

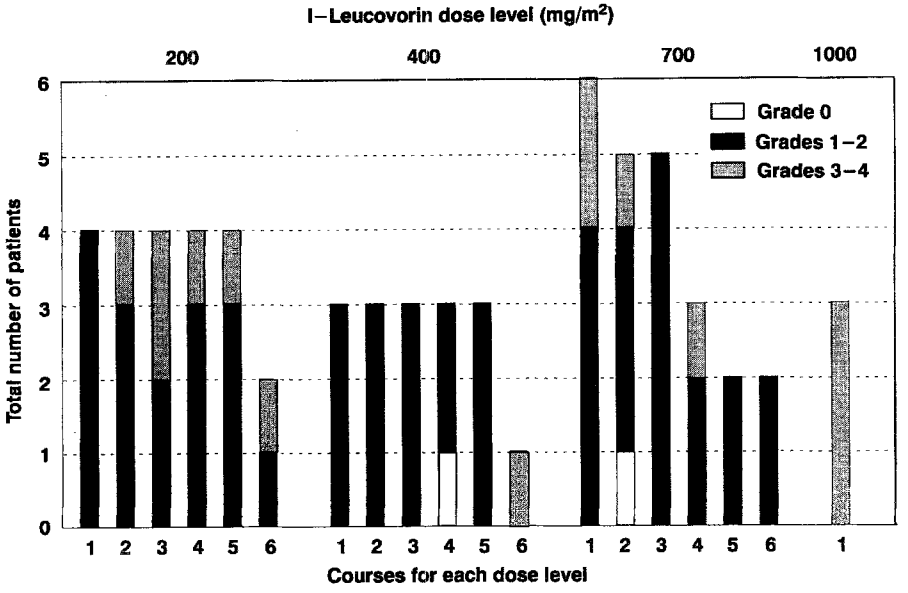

Fig. 1. Non-hematological toxicity experience of all evaluable patients for the first six courses of treatment

Table 1. Patient characteristics

Number enrolled	17
Number evaluable	
Toxicity	16
Response	12
Age, median	58
(range)	(36–76)
Performance status, median	0
(range)	(0–2)
Sex (M/F)	14/2
Disease site:	
Colorectal	12
Pancreas	3
Stomach	1
Prior therapy	
Surgery	16
Radiotherapy	3
Chemotherapy	0
Disease sites:	
Liver	8
Lung	3
Nodes	2
Other	3

Treatment delivered

The number of patients treated at each *l*-leucovorin dose level was: 200 mg/m^2, 4 patients; 400 mg/m^2, 3 patients; 700 mg/m^2, 6 patients; and 1000 mg/m^2, 3 patients. Dose-limiting toxicity (2 patients with grade 4 toxicity and 1 patient with grade 3 toxicity) was encountered at 1000 mg/m^2, so a total of 6 patients were treated at the maximum tolerated dose of 700 mg/m^2. Treatment was in general well tolerated with most patients receiving multiple courses of therapy. The median number of courses delivered was 5.5. The number of courses delivered at each dose level is shown in Fig. 1. Eleven patients stopped treatment because of disease progression; 3 stopped because of severe toxicity; and 2 continued therapy for over 1 year and then stopped with ongoing tumor responses.

The dosage of 5-FU was adjusted in response to each patient's toxicity experience as described in Table 2. It is noteworthy that 4 of 6 patients treated with *l*-leucovorin at 700 mg/m^2 never required adjustment of their dose of 5-FU despite treatment for 6 or more months in some cases.

Non-hematological toxicity

The total non-hematological toxicity experience for the first six courses of therapy is summarized in Fig. 1. The first course of treatment was used to determine dose escalation and to identify the maximum tolerated dose. All patients experienced at least grade 1 toxicity during the first course. Grade 3–4 toxicity during the first course was observed in none of the patients at 200 mg/m^2 or 400 mg/m^2, in 2 of 6 patients at 700 mg/m^2, and in 3 of 3 patients at 1000 mg/m^2. Most patients experienced grade 3–4 toxicity during at least one subsequent course of therapy. However, 2 patients at 200 mg/m^2 and 1 patient at 700 mg/m^2 never experienced grade 3–4 toxicity despite treatment for five, five and four courses respectively. Dose-limiting toxicity occurring during the first course at 1000 mg/m^2 was: grade 4, nausea/vomiting, grade 3 diarrhea, and myocardial infarct in 1 patient; grade 3 stomatitis in 1 patient; and grade 4 diarrhea in 1 patient.

The maximum tolerated dose of *l*-leucovorin was 700 mg/m^2. The types of toxicity encountered at that dosage were similar to that encountered at the other dosages. The overall grade 3–4 toxicity experience for the 16 patients was similar to that expected for the combination of leucovorin and 5-FU. The most common grade 3–4 toxicities (and the number of patients affected) were: diarrhea (2), mucositis (2), nausea/vomiting (1), and abdominal/rectal pain (2). All patients recovered fully from these toxicities with standard supportive care.

Table 2. Adjustment of dosage of 5-fluorouracil (5-FU) based on toxicity experience

l-Leucovorin dose	No. of evaluable patients	5-FU dose adjustment			
		None	10% increase	20% decrease	One cycle only
200 mg/m^2	4	0	3	1	0
400 mg/m^2	3	1	1	1	0
700 mg/m^2	6	4	0	1	1
1000 mg/m^2	3	0	0	0	3

Grade 1–2 non-hematological toxicity was more frequent. Nine patients had a total of 19 episodes of grade 1–2 nausea and/or vomiting. The median duration of this toxicity was 1 day. Eight patients had a total of 15 occurrences of mild/moderate mucositis with a median duration of 12 days. Ten patients had a total of 19 occurrences of grade 1–2 diarrhea which had a median duration of 2 days but which persisted up to 21 days.

Hematological toxicity

Hematological toxicity was mild in this study with most patients having only grade 1 or 2 toxicity. Analysis of the lowest hematological parameters observed during any course of therapy for each patient revealed the following nadir values: leukocytes, 3600/mm^3 (median), 1600–8200/mm^3 (range); platelets, 182000/mm^3 (median), 90000–428000/mm^3 (range); hemoglobin, 10.05 g/dl (median), 7.5–12.5 g/dl (range).

Response and survival analysis

Twelve patients, including 9 with colorectal carcinomas, were evaluable for response to treatment. One patient had a complete response of liver metastases lasting over 52 weeks; 1 patient had a partial response of lung metastases lasting 39 weeks; and 1 patient had a minor response in lung metastases lasting 10 weeks. All responses occurred in patients with colorectal carcinomas. The median overall survival was 30 weeks with 3 patients alive more than 79, 87 and 97 weeks after study entry.

Discussion

This phase I study of a 5-day continuous infusion of *l*-leucovorin plus five daily boluses of 5-FU revealed that the maximum tolerated dose of *l*-leucovorin is 700 mg/m^2 per day. The most frequently encountered toxicities were those one would expect for this combination, namely diarrhea, mucositis, nausea, and vomiting. The treatment regimen employed in this trial was based on a previously described regimen of a 5.5-day continuous infusion of *d,l*-leucovorin at 500 mg/m^2 per day plus a daily bolus of 5-FU at 370 mg/m^2 [3]. The starting dose of *l*-leucovorin in the current study (200 mg/m^2 per day) was slightly lower than the quantity of *l*-leucovorin delivered in the racemic mixture used for the previous study. The lower dose was selected because of concerns over excess toxicity with administration of only the biologically active isomer. Nevertheless, no severe toxicity was encountered. Dose-limiting toxicity was encountered at a dose of *l*-leucovorin which was 4 times that used previously. A dose of *l*-leucovorin which was nearly 3 times the previous dose was well tolerated. The toxicity in the current trial and the earlier one, which used *d,l*-leucovorin, is similar. Comparison of hematological toxicity between the regimens employing *l*-leucovorin and *d,l*-leucovorin revealed: nadir leukocyte counts of less than 3000/mm^3 in 0% and 22% of patients and nadir platelet counts lower than 90000/mm^3 in 0% and 6% of patients respectively. Grade 3–4 non-hematological toxicity for the *l*-leucovorin and *d,l*-leucovorin regimens was: diarrhea, 13% and 6%; nausea/vomiting, 6% and 6%; and, mucositis, 13% and 13%, respectively.

This regimen of continuous infusion *l*-leucovorin is more cumbersome than regimens which employ short infusions or boluses of leucovorin. However, the efficacy of leucovorin in enhancing the cytotoxicity of 5-FU is schedule-dependent with the duration of cell exposure to high concentrations of reduced folates being an important determinant of efficacy [7, 14, 31]. Thus, infusions of leucovorin may deliver this agent in a more pharmacodynamically optimal manner than that of other treatment schedules. A direct comparison of continuous infusion versus bolus leucovorin would be required to determine which schedule is superior.

The pharmacokinetics of *l*-leucovorin were examined in another phase I trial employing a 5.5-day continuous infusion of *l*-leucovorin and five daily boluses of 5-FU [4]. Non-compartmental pharmacokinetic parameters at 250 mg/m^2 per day and 1000 mg/m^2 per day included $C_{ss} = 2.7 \pm 0.2$ μM and $16.0 \pm$ μM respectively. Although the plasma half lives were similar (55 ± 6 min and 76 ± 9 min, respectively) non-renal clearance may be saturated at the 1000 mg/m^2 per day dose. The plasma levels of reduced folates are similar to those achieved during infusion of *d,l*-leucovorin at 500 mg/m^2 per day [15] and are over 10-fold higher than the levels achieved with oral loading of *d,l*-leucovorin [6]. A second study examined the pharmacokinetics of *l*-leucovorin after administration of 350–550 mg/m^2 of *l*-leucovorin as a 2-h intravenous infusion [11]. *l*-Leucovorin was cleared from the plasma with $t_{1/2}$ alpha and $t_{1/2}$ beta of 7.2+1.8 min and 126+6.5 min, respectively. These pharmacokinetic parameters for *l*-leucovorin are similar to those observed after bolus administration of *d,l*-leucovorin [10].

This combination of *l*-leucovorin and 5-FU appears to be active for treating advanced colorectal carcinomas. This phase I trial revealed one complete response, one partial response and one minor response among 9 evaluable patients with colorectal cancer. Similarly, a recent phase I/II trial demonstrated a 52% response rate among 25 patients with colorectal cancer [11]. Although this response rate is high, it is consistent with previous studies of *d,l*-leucovorin plus 5-FU [3, 5, 10, 16, 18, 19, 26]. Randomized trials which compare combinations of 5-FU and *d,l*-leucovorin are required to determine if the efficacy of *l*-leucovorin is superior to that of *d,l*-leucovorin.

Dose-response studies which compared low and high doses of *l*-leucovorin plus 5-FU for treatment of colorectal cancer produced conflicting results [16, 19]. Combinations of 5-FU with high doses of leucovorin were superior to combinations with low doses of leucovorin when administered on a weekly schedule [16]. In contrast, there was no difference in efficacy between low and high doses when 5-FU and leucovorin were administered on days 1–5 of a 28-day treatment cycle [19]. These comparisons of doses of leucovorin are confounded by the accumulation of *d*-leucovorin, which may limit the effectiveness of high levels of *l*-leucovorin. Our study and the other phase I trials demonstrate that *l*-leucovorin achieves high serum levels and can be combined safely with 5-FU. Thus, it is now possible to test properly the dose-response relationship for combinations of *l*-leucovorin and 5-FU.

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