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A clinical and pharmacological study of 5-fluorouracil, leucovorin and interferon alfa in advanced colorectal caner

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Abstract Modulation of 5-fluorouracil (FUra) using leucovorin (LV) is a standard treatment approach in patients with metastatic colorectal cancer. Modulation of FUra with interferon alfa has also shown some promise. Laboratory data have demonstrated increased cytotoxicity when FUra is combined with both LV and interferon. The current study examined the effects of double modulation of FUra using LV and interferon.Patients with measurable advanced colorectal cancer received bolus FUra 375 mg/m^2 plus LV 20 mg/m^2 daily for 5 days, repeated every 28 days. Recombinant human interferon alfa-2a, 3 million IU/m^2 subcutaneously, was given daily on the days of chemotherapy then three times weekly. There was one complete response and nine partial responses (10/41) seen for an overall response rate of 24% (95% CI 12.0–40.0%). Overall, 70% of patients experienced one or more episodes of nonhematologic toxicity of grade 3 or more. Weight loss was common, with a mean decrease of 2.9 kg over the first two months ($P < 0.0001$). Improvements in tumor-related symptoms were balanced by increased fatigue and a deterioration in body weight and performance status. There was no evidence of progressive changes in FUra metabolism from interferon usage.

Key Words 5-Fluorouracil · Interferon · Colorectal cancer

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Introduction

The most active single agent against colorectal cancer is 5-fluorouracil (FUra) which has a true response rate of less than 20% [1]. In vitro, the cytotoxicity of FUra can be enhanced by a number of agents including leucovorin (LV), levamisole, methotrexate, dipyridamole and interferon [2]. LV is a reduced folate that stabilizes the binding of the intracellular FUra metabolite 5-fluorodeoxyuridine monophosphate (FdUMP) to thymidylate synthase (TS) [8]. This leads to a more prolonged inhibition of thymidylate production and ultimately to cell death.

A number of randomized studies in colorectal cancer, and a subsequent meta-analysis have shown that FUra plus LV gives significantly higher antitumor response rates than FUra alone with little effect on survival [1]. A variety of FUra and LV dosages and schedules have been used; no one has consistently been demonstrated to be superior. A comparison of FUra plus LV 200 mg/m^2 with FUra plus LV 20 mg/m^2 with FUra alone has demonstrated equivalent improvements in response rate with the lower dose of LV [17].

While interferons can enhance the cytotoxic effects of FUra in vitro, they have no independent cytotoxic activity against colonic adenocarcinoma [21]. In vitro, they have been demonstrated to prevent the increase in TS production normally seen after FUra administration, and to increase the frequency of FUra-induced double-stranded DNA breaks [6, 11]. The initial phase II study of FUra plus interferon in patients with colorectal cancer produced a high response rate with an increase in toxicity [24]. Subsequent studies have shown lower response rates that appear comparable to those achieved with FUra/LV [12, 18, 25]. A randomized study comparing FUra and interferon to FUra/LV has demonstrated equivalent response rates and survival, but different toxicity profiles [13].

Studies in two human colon adenocarcinoma cell lines have demonstrated a 2.6–3.2-fold potentiation of FUra cytotoxicity with LV *or* interferon and a further potentiation when all three agents are combined [10]. The initial clinical results with both FUra and interferon, as well as the promising in vitro results when all three agents are combined, led to a phase I study of FUra plus LV plus interferon using the daily for 5 days schedule given every 28 days [16]. This report contains the results of a phase II study examining the effects of FUra plus LV plus interferon on tumor growth and symptoms in patients with untreated advanced colorectal cancer.

Materials and methods

Patient selection

Eligible patients were those with histologically confirmed adenocarcinoma of the colon or rectum who had measurable metastatic disease, a performance status (ECOG) ≤ 2 and normal hematologic parameters. They were also required to have a serum creatinine $< 150\ \mu M$, a serum bilirubin $< 20\ \mu M$ and an AST less than two times normal. Prior chemotherapy or immunotherapy for metastatic disease, or as adjuvant treatment was not permitted.

Treatment

Chemotherapy consisted of FUra 375 mg/m^2 immediately preceded by LV 20 mg/m^2, both given by intravenous bolus daily for 5 days. Treatment cycles were repeated every 28 days if normal tissue toxicity had resolved. In addition recombinant human interferon alfa-2a (Roferon-A) 3 million IU/m^2 was given by subcutaneous injection daily for the 5 days of chemotherapy then three times weekly during the intervening 3 weeks. For the third and subsequent cycles the interferon was only given during the 5 days of chemotherapy. All patients gave written consent to this study on a form approved by the University of Toronto institutional review board.

FUra dosage in subsequent cycles was reduced by 50 mg/m^2 in patients who experienced grade 4 neutropenia (granulocytes < 500/mm^3) or grade 3 non-hematologic toxicity, and by 100 mg/m^2 in those experiencing grade 4 nonhematologic toxicity. Interferon dosage was reduced by 25% in those experiencing persistent fatigue or fever.

Evaluation

Response and toxicity were assessed by standard WHO criteria [15]. Response assessments were made monthly in those with palpable subcutaneous nodules or lung metastases and bimonthly in those with intra-abdominal disease. In most cases assessment was based on CT scanning of the liver.

A questionnaire was completed independently by patients prior to each cycle of treatment. This asked them to describe a number of their symptoms on a numerical scale where 0 represented no symptoms and 4 was the worst possible. These included symptoms related to their disease such as pain, those attributed to treatment and some, such as fatigue or anorexia, that could relate to either. They also rated their overall quality of life and indicated which of the listed ECOG performance status criteria best described them. An independent assessment as to whether the patient was improved, stable or worse was also made by the nurse closely involved with day-to-day patient care. Patients who had improvements of 1 integer or more in any one of performance status, quality of life or symptoms (without progression in any of the other two) sustained for 2 months or more and who were assessed by the nurse as stable or improved, were deemed to have had a palliative response. Those who had a deterioration of 1 integer or more in performance status or quality of life, or 2 integers or more in fatigue, anorexia or pain and were assessed as worse by the nurse were considered a palliative progression. Those not fitting these categories were assessed as having a stable palliative response.

Changes in score of symptoms, weight, quality of life or performance status between cycles were tested using a paired t-test (two-tailed). For this analysis only those patients continuing on therapy were included to limit the influence of progressive disease on changes in symptom scores and quality of life.

Pharmacological studies

FUra concentrations were measured in 13 patients by high-performance liquid chromatography prior to injection and at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min afterwards on the 1st and 5th day of the first and second cycles of treatment [4]. On the 1st day of the first cycle the chemotherapy was given prior to the interferon. Each data set was analyzed independently using a non-linear computer fitting program. The values for elimination rate constant, area under the curve (AUC), distribution volume, and total body clearance were identified from the one compartment fits. The AUC was normalized by a direct proportional adjustment if a FUra dose other than 375 mg/m^2 was used. Comparisons between mean parameter values on days 1 and 5 and between cycles 1 and 2 were done using a paired two-tailed t-test.

Results

There were 43 patients entered on study with all being evaluable for toxicity and 41 being evaluable for response. The 2 patients inevaluable for response withdrew after the first cycle due to toxicity. The basic demographic characteristics are shown in Table 1.

The mean number of cycles of treatment given was 4.1 (1–9) with 5 patients receiving only one cycle (3 progression, 2 toxicity) and 15 receiving six or more cycles. Treatment was stopped in 31 patients because of progressive disease, in 9 due to toxicity. Two patients

Table 1 Patient demographic characteristics

Entered on study	43
Eligible for response	41
Pharmacokinetic study	13
Median age (range) (years)	59 (37–74)
Sex (F:M)	14:29
Major metastatic sites[a]	
Liver	31
Lung	9
Retroperitoneal nodes	5
Pelvic recurrence	2
Performance status (ECOG)	
0	11
1	24
2	8

[a] Some patients had more than site

Table 2 Worst grade of toxicity experienced by each patient ($n = 43$)

Toxicity type	≤ Grade 1	Grade 2	≥ Grade 3
Stomatitis	24	8	11
Diarrhea	16	15	12
Fatigue	8	17	18
Nausea & vomiting	25	11	7
Granulocytopenia	32	5	6

completed a planned eight cycles of therapy and one died of a colonic perforation at the site of the primary tumor.

Toxicity

The worst grades of toxicity experienced by each patient are shown in Table 2. Overall, 30/43 (70%) experienced one or more episodes of nonhematologic toxicity of grade 3 or more. Stomatitis, diarrhea and fatigue (grade 3 or more) were experienced by 26%, 28% and 42% of patients, respectively. Weight loss of more than 1 kg was seen after one cycle in 26/38 patients and after two cycles in 21/31 patients while 3 and 2 patients gained 1 kg or more over the same period. There was a mean weight loss of 2.9 kg over the first 2 months of treatment ($P < 0.0001$) in those who did not have documented progression by the end of two cycles.

Response and palliation

There was one complete response and nine partial responses (10/41) seen for an overall response rate of 24% (95% CI 12.0–40.0%). The median duration of response was 6 months (range 4–8). Three patients had a palliative response, defined previously as improvements in one or more of performance status, overall quality of life or symptoms without deterioration in any of the others. Under these palliative response criteria, 31 patients were worse and 7 were stable after 2 months of therapy. The palliative evaluations of the 19 who received less than four cycles of therapy (13 progressive disease, 6 toxicity), the 14 who were stable for three or more cycles and the 10 who achieved a partial or complete response are shown in Table 3.

Table 4 Changes in mean scores (0 = none, 4 = worst possible) for fatigue, pain, anorexia, and overall quality of life and ECOG performance status by treatment cycle

	Baseline	Cycle 1	Cycle 2	Cycle 3
Fatigue	1.73	3.11	2.30	1.70
Pain	1.38	1.10	1.00*	1.00
Anorexia	1.75	2.36*	1.70	1.17
Performance status (ECOG)	1.00	1.46**	1.30*	1.30
Quality of life	1.11	1.48*	1.54*	1.03

*$P \leq 0.05$; **$P \leq 0.001$

Changes in fatigue, pain, performance status and overall quality of life are shown Table 4. An analysis was performed on the 24 patients who received more than three cycles of therapy (and thus had not been taken off study early due to progressive disease or toxicity). This showed a significant decrease in weight ($P \leq 0.003$), an increase in fatigue ($P < 0.0001$), a decrease in pain ($P = 0.04$), a deterioration in performance status ($P = 0.007$) and no change in overall quality of life. The median survival of all patients on study was 10 months with 4/43 being alive with disease at 18 months.

Pharmacological studies

Summary pharmacokinetic data are shown in Table 5. A modest increase in FUra AUC was seen between the 1st and 5th day of therapy on cycle one. However, there was no evidence of progressive changes with the continued usage of interferon. There was notable interpatient variability in pharmacokinetics with AUC ranging from 30.0 to 142.4 μM/h, and clearance from 0.57 to 2.68 l/min. There was no apparent correlation between FUra AUC and toxicity, although with this degree of interpatient variability the sample size was much too small to adequately address this issue.

Discussion

The treatment of advanced colorectal cancer is palliative. Under these circumstances the beneficial effects of treatment on the cancer have to be balanced against

Table 3 Palliative response in patients who had progressive disease, or who were stable or should a partial or complete response

"Objective" criteria	n	"Palliative" criteria Response	Stable	Worse
Complete or partial response	10	3	1	6
Stable for > 2 courses	14	0	6	8
Withdrew due to toxicity	6	0	0	6
Progressive disease by 2 courses	13	0	2	11
	43	3	9	31

Table 5 FUra pharmacokinetics. Values are means (± SD)

	Course 1 Day 1	Day 5	Course 2 Day 1	Day 5
AUC (μM/h)	64.9 (13.3)	82.8 (28.3)*	66.6 (14.6)	72.8 (18.8)
Volume of distribution (l)	16.5 (11.1)	15.9 (6.4)	15.7 (5.7)	21.0 (8.4)
Clearance (l/min)	1.35 (0.46)	1.11 (0.37)	1.24 (0.37)	1.19 (0.36)

*$P = 0.03$ (vs course 1 day 1)

the normal tissue toxicity. Modulation of FUra with LV improves the antitumor effects with some increase in toxicity; overall the therapeutic index appears to be enhanced. While no detailed analyses of the effects of FUra plus LV on quality of life have been performed, one study has shown that 33% of patients had improvement in performance status, 28% of patients had weight gains of 5% or more and 54% had improvements in tumor-related symptoms [17].

Modulation of FUra with LV and interferon is a more intensive treatment. In an initial phase I study the dose of FUra that could be given in combination with both LV and interferon was less than could be ginen with LV alone [16]. Other studies that have combined these three agents in a variety of schedules have recently been reported (Table 6). The response rates [14–54%] are comparable to those seen in the original phase II studies of FUra and LV. Those studies with the higher interferon dosages also have a high incidence of serious toxicity.

Several studies, including this one, examined the effects of interferon on FUra pharmacokinetics and have produced somewhat contradictory results. In patients receiving FUra by a 5-day infusion there was no difference in FUra steady-state concentrations (Css) when interferon 9 million units three times per week was added [19]. However, using the same FUra schedule with interferon given daily, a 20–35% decrease in FUra Css associated with increased toxicity has beeen noted [7]. When FUra is given by a 28-day infusion, the concurrent administration of interferon 8–10 million units does not alter FUra pharmacokinetics but does lead to an increase in gut toxicity [23]. The most detailed studies by Grem et al. [9] have demonstrated an inhibition of FUra clearance at interferon doses of ≥ 5 million U/m^2. This was associated with an increase in FUra AUC that may have accounted for the increased toxicity seen. At a dosage of 3 million U/m^2 we did not see any consistent effect of interferon on FUra metabolism that would have accounted for the increased toxicity seen.

Despite the intensive nature of this treatment the response rate was similar to that achieved with FUra plus LV [1]. The 95% confidence limits for response (12–40%) indicates that this regimen is unlikely to be a major advance on FUra plus LV. Assessment of the palliative effects of treatment demonstrates an unfavorable balance between the antitumor effects and normal tissue toxicity. In contrast to studies with FUra and LV, our study showed improvements in performance status in just three patients, weight loss was common, and reductions in pain were balanced by increases in fatigue and anorexia.

Further attempts at FUra modulation using interferon are still warranted. As these treatments are palliative, the effects on quality of life also need to be considered. If interferon does modulate FUra cytotoxicity then it will only be clinically useful if this can be achieved using a less toxic combination schedule than that examined in this current study.

Table 6 Other studies of FUra, leucovorin and interferon in colorectal cancer

FUra dose (mg/m2)	Interferon dose (units)	# patients	Response rate (%)	≥ grade 3 toxicity (%)	Author [Ref]
2400 by 48 h infusion, weekly	10 million days 1, 3, 5	45	25	47	Punt [20]
370 IV bolus day 2 to 6	5 million/m2. day 1 to 7	46	54	> 50	Grem [9]
500 IV bolus weekly	3 million every 2nd day	44	14	10	Sobrero [22]
370 IV bolus day 2 to 6	3 million day 1 to 7	45	51	20	Cascinu [5]
430 IV bolus day 1 to 5	4 million/m2. day 1 to 5	55	27		Bukowski [3]
400 IV bolus day 1 to 5	10 million q2 days	16	25	63	LaBianca [14]

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