Phase I-II Trial of High-Dose Calcium Leucovorin and 5-Fluorouracil in Advanced Colorectal Cancer

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ABSTRACT

Twenty-six patients with metastatic colorectal adenocarcinoma were entered into a Phase I-II study of 5-fluorouracil (5-FUra)-high-dose leucovorin (CF). The starting dose of 5-FUra was 300 mg/sq m with escalation to 750 mg/sq m/week in 6 doses given by rapid i.v. injection midway during a 2-hr infusion of CF, 500 mg/sq m. Partial responses were seen in 9 of 23 patients (6 of 12 who had had previous 5-FUra). Complete normalization of liver enzymes was seen in two of these patients. Side effects were seen sporadically with 5-FUra doses up to 600 mg/sq m. At a 600-mg/sq m 5-FUra dose, 8 of 18 patients had diarrhea, and 2 of 18 had white blood cell counts <3000/µl. At a 750-mg/sq m dose of 5-FUra, 6 of 11 patients had severe diarrhea and 6 of 11 had white blood cell counts <3000/µl. Other toxicities were mild conjunctivitis and lacrimation, thinning of the nails, and alopecia. In bioavailability studies of CF p.o., no plasma CF could be detected. After CF i.v., mean plasma peak was 111.3 ± 40.3 (S.D.) µM. 5-FUra-CF appears to be effective in patients clinically resistant to 5-FUra. This study is being extended to randomized trial of 5-FUra-CF versus 5-FUra alone.

INTRODUCTION

5-FUra used alone produces an objective response in 15 to 20% of patients with advanced colorectal cancer (8). Various early combination studies have yielded results that are not superior to those with 5-FUra alone (6). Recently, attempts have been made to enhance the effectiveness of 5-FUra based on considerations of the biochemical pathways of 5-FUra activation and site of action. Two mechanisms of the antitumor effect of 5-FUra have been proposed: (a) activation to 5-fluorouridine triphosphate and incorporation into RNA; and (b) activation to 5-FdUMP with competitive inhibition of TS by formation of a ternary complex with the active site of TS and 5,10-CH2FH4. Modulation of the first mechanism should be achieved by coadministration of thymidine which blocks catabolism of 5-FUra, thus increasing its intracellular availability, but provides an end product of the blocked TS reaction, thus obviating the effect of this block. However, in clinical trials, thymidine increased the toxicity of 5-FUra but did not affect its therapeutic ratio (12). The use of concomitant CF should modulate the second mechanism. Evans et al. (5) have shown that the stability of the ternary complex is maximal when the extracellular CF concentration is 10 µM in cell culture.

Since studies in tumor-bearing mice have also shown that the recovery of TS is a critical factor in the activity of 5-FUra in vivo, the possibility that coadministration of large doses of CF might retard the enzyme recovery and thereby increase 5-FUra activity was considered worthy of investigation in colorectal carcinoma (7).

The Phase I study was conducted to evaluate: (a) the toxicity of 5-FUra in combination with CF; and (b) the therapeutic efficacy of this combination against advanced human colorectal carcinoma.

MATERIALS AND METHODS

Twenty-six patients were entered into the study. All had histological proof of metastatic colorectal carcinoma, and all gave written informed consent for entry into the study. All patients had normal hematological, renal, hepatic, and cardiac parameters unless the abnormalities resulted for direct tumor invasion. All of the patients had objectively measurable disease. There were 13 males and 13 females; the median age was 60 years with a range of 19 to 71 years. Twelve patients had had prior chemotherapy with 5-FUra. Two patients had only liver metastases, one had only lung metastases, 7 had both liver and lung metastases, 11 had abdominal wall metastases, and 5 patients had bone metastases.

Treatment Plan. CF was supplied by Lederte in 50-mg vials. The bioavailability studies of CF p.o. were conducted in 5 patients prior to the treatment. The i.v. dose of CF was dissolved in 250 ml of lactated Ringer's solution and given over a period of 2 hr. 5-FUra was given by rapid i.v. injection in the middle of CF infusion. Treatment was given every week for 6 weeks (one course) followed by a 2-week rest period.

Dose. Doses of 500 and 1000 mg/sq m CF p.o. were selected for the bioavailability studies. The i.v. CF dose was 500 mg/sq m. The starting dose of 5-FUra was 300 mg/sq m in the absence of dose-limiting toxicity, escalations were made every second course. Doses of 300, 600, and 750 mg/sq m were evaluated. A minimum of 3 new patients were entered at each dose level, and then doses were escalated for individual patients.

Study Parameters. Prior to each course of therapy, every patient had a complete blood count, serum electrolyte determination, liver function tests, serum creatinine, blood urea nitrogen, carcinoembryonic antigen, chest X-ray, liver scan, abdominal and pelvic computer-assisted tomography scan, or ultrasound where appropriate. During each course, the complete blood count, liver function tests, creatinine, and blood urea nitrogen were repeated weekly.

Patients were considered evaluable for toxicity if they received at least one course. Patients were evaluated for response after 2 courses (12 doses). Standard response criteria were used to evaluate antitumor effect: (a) complete response, complete disappearance of all recognizable tumor masses; (b) partial response, a 50% reduction in the product of the largest perpendicular diameters of the most clearly measurable area of known malignant disease. There should be no increase in the size of other measurable disease and no appearance of new lesions; (c) stable, a decrease in tumor size less than partial response with no appearance of new lesions; (d) progressive disease, an increase in the size of measurable disease; (e) unknown response, definition to be adopted as necessary to meet the requirements of the International Union against Cancer for the system of staging and therapy response criteria.

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of new lesions; and (d) study termination, for which individual patients were removed from the study when objective tumor progression occurred.

Pharmacokinetic studies of CF and its 5,10-CH2FH4 form were carried out using a reverse-phase ion-pair high-performance liquid chromatographic system consisting of a C18-Bondapak reverse-phase column and using 5 μmol tetraethylammonium phosphate in 30% methanol as the mobile phase. The detection limit of the assay was 0.98 μM, and the reproducibility was within 10% range.

RESULTS

Response. Three patients were not evaluable for response or toxicity. One patient died after the third dose of CF and 5-FUra from unrelated causes, and 2 others were lost to follow-up after 1 to 3 doses.

Twenty-three of the 26 patients had objectively measurable disease and received at least 2 cycles of 5-FUra and CF at 600 and 500 mg/sq m i.v., respectively. Partial responses were seen in 9 patients, 6 of whom had had 5-FUra previously (Table 1). Two of these patients had complete normalization of their liver enzymes. The range of the response has been 6 to 18 months with a median at the time of this report of 10 months (Table 2).

One of the patients who was classified as a partial responder had the diagnosis of a Dukes C2 (26 of 66 lymph nodes positive for tumor) adenocarcinoma of the sigmoid colon at the age of 18 years. Following surgery, the patient was placed on 5-FUra for 1 year as adjuvant treatment. Two years following the sigmoid resection and a left oophorectomy, the patient was found to have an unresectable pelvic tumor as well as multiple implants scattered throughout the abdominal cavity including the surface of the liver documented at laparotomy. Following the latter surgery, the patient developed massive ascites and was subsequently placed on the present protocol. Within 6 months of treatment, the pelvic tumor had dramatically decreased in size and the ascites resolved completely. The patient underwent exploratory laparotomy. The previously gross carcinomatosis had disappeared, and the pelvic mass was now resectable. This patient subsequently succumbed to her disease 6 months later.

Toxicity. The dose-limiting toxicities were leukopenia and gastrointestinal toxicity, mainly diarrhea (Table 3). The side effects were seen sporadically at 5-FUra doses up to 600 mg/sq m. Of 18 patients who received 5-FUra, 600 mg/sq m and CF, 500 mg/sq m i.v., 6 developed diarrhea which subsequently required intensive i.v. rehydration in 3 patients. Three of 18 patients also experienced leukopenia of 600, 2700, and 3100/μl. Full recovery was established in all patients within 1 week with no further problems when the dose of 5-FUra was decreased to 500 mg/sq m. Six of 11 patients who received 5-FUra, 750 mg/sq m, and CF, 500 mg/sq m i.v., experienced severe diarrhea lasting 5 to 7 days, and 8 patients had WBC nadir at <3000/μl. Full recovery was established in all but one of these patients within 7 to 10 days, and subsequent treatment was continued at 5-FUra doses of 500 to 600 mg/sq m without any further problems. One patient with a WBC nadir of 500/μl died subsequently of infection in a presumed drug-related death.

Maximum toxicity was usually seen at the end of the first course at the 600-mg/sq m dose of 5-FUra or after the third dose of the first course at a 750-mg/sq m dose of 5-FUra.

### Table 1

**Response to chemotherapy with weekly CF-5-FUra**

<table>
<thead>
<tr>
<th>No prior 5-FUra</th>
<th>Prior 5-FUra</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Partial</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Stable</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Progression</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

### Table 2

**Characteristics of responders**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Diagnosis</th>
<th>Present treatment</th>
<th>Previous chemotherapy</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>66</td>
<td>5/80</td>
<td>3/83</td>
<td>5-FUra-Interferon</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>61</td>
<td>8/81</td>
<td>2/83</td>
<td>5-FUra-CGP15720A</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>64</td>
<td>6/77</td>
<td>2/83</td>
<td>5-FUra-MeCCNU-straptozotocin-CGP15720A-DAMP</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>19</td>
<td>8/81</td>
<td>11/82</td>
<td>5-FUra</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>71</td>
<td>8/82</td>
<td>2/83</td>
<td>5-FUra</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>60</td>
<td>8/82</td>
<td>12/82</td>
<td>DAMP-CGP15720A</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>60</td>
<td>8/82</td>
<td>11/82</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>60</td>
<td>2/80</td>
<td>11/82</td>
<td>5-FUra</td>
</tr>
<tr>
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<td>F</td>
<td>61</td>
<td>9/82</td>
<td>3/83</td>
<td>CPG15720A</td>
</tr>
</tbody>
</table>

### Table 3

**Toxicity of weekly CF with 5-FUra**

<table>
<thead>
<tr>
<th>5-FUra (mg/sq m)</th>
<th>WBC nadir&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Gastrointestinal toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;4000 cells/μl</td>
</tr>
<tr>
<td>300</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>600</td>
<td>18</td>
<td>15</td>
</tr>
</tbody>
</table>

<sup>a</sup> Total number of patients was 23; some of these were treated on 2 or 3 dose levels.

<sup>b</sup> No thrombocytopenia was noted (all platelet counts >100,000/μl).

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All patients receiving 5-FUra, 500 to 750 mg/sq m, experienced watering eyes with mild conjunctivitis. Some relief was achieved with dexamethasone eye drops.

Three patients receiving 5-FUra, 600 mg/sq m, developed a transient short-lived macular rash on the lower extremities during the second and third courses of treatment. Biopsies of the skin lesions were inconclusive, and the rash disappeared spontaneously without interruption of the treatment. Three patients noticed thinning of their nails, and 2 others had hair loss at the 600-mg/sq m dose of 5-FUra.

**Pharmacokinetic Studies.** In 10 patients who received CF, 500 mg/sq m, as a 2-hr i.v. infusion, the peak plasma level of CF ranged between 33.1 and 194.0 μM (mean, 111.3 μM) had occurred within minutes from the onset of the infusion. The peak plasma concentration of 5,10-CH₂FH₄ ranged from 1.8 to 22.8 μM (mean, 11.0 μM) and was attained within minutes from the start of the infusion.

In 5 patients who received CF, 500 or 1000 mg/sq m p.o., the peak plasma level of 5,10-CH₂FH₄ achieved in their plasma was 2.4 to 3.6 μM, and this level was attained within 40 to 180 min following drug administration. No CF was detected in the plasma during this period.

**DISCUSSION**

Thymidine rescue and protection studies have revealed that the inhibition of TS by 5-FdUMP is the primary site of the growth inhibitory action of 5-FUra in many although not all cells (4). In the presence of cofactor, 5,10-CH₂FH₄, 5-FdUMP is a powerful inhibitor of TS, having a Kᵢ of 1.9 mM for the enzyme of human chronic myelocytic leukemia cells (3) and 0.06 mM for the enzyme from Lactobacillus casei (10). According to the work of Ullman et al., the levels of folates which were sufficient for the normal growth of L1210 cells did not provide for maximal binding of 5-FdUMP to TS (11). These authors demonstrated a 3-fold increase in sensitivity of L1210 cells to 5-fluorouridine when the CF content of the medium rose from 0.01 to 1 μM or that of folate rose from 28 to 23 μM. It appears that the rate of recovery of TS activity plays a more significant role in the potency and site of action of 5-FUra than does the amount of 5-FdUMP which is formed and that this recovery can be greatly retarded with an excess of folate or CF. We have examined a potentiating effect of excess CF in cell types such as HeP-2, which do not easily respond to increases in 5-FdUMP levels. This effect is due to stabilization of the 5-FdUMP-TS complex which leads to a marked slowdown in the recovery of the enzyme activity and potentiation of growth inhibition. It has been shown further that CF increases the effectiveness of 5-FUra as an antitumor agent without increasing its toxicity in mice.

A Phase I study of this combination was carried out by Bruckner et al. (2). Low-dose CF (25 mg every 8 hr) was combined with 5-FUra (1100 mg/sq m/day by continuous infusion for up to 4 days) with moderate toxicity. In a more recent study by Bruckner et al., (1) there was an indication of better response to this combination than to 5-FUra alone. The combination of 5-FUra with high-dose CF has been published by Machover et al. (9). 5-FUra (370 to 400 mg/sq m daily for 5 days) was combined with CF (200 mg/sq m daily for 5 consecutive days). Only 7 episodes of marked granulocytopenia (WBC < 1000/μl) were observed in 210 courses, and a response rate of >50% was seen in previously untreated colorectal carcinoma, which was considerably higher than that seen with 5-FUra alone.

In the present study, i.v. CF infusion, 500 mg/sq m, over 2 hr, combined with 5-FUra, 600 to 750 mg/sq m, resulted in benefit in 15 of 23 patients with colorectal carcinoma (stable disease plus partial response). Six of 12 previously 5-FUra-resistant patients responded to the 5-FUra-CF combination. Hematological toxicities, mainly leukopenia, were rarely seen; diarrhea and emesis were easily manageable at the 600-mg/sq m dose of 5-FUra.

These data are very suggestive that simultaneous administration of 5-FUra and high-dose CF may enhance the antitumor activity of 5-FUra even in colorectal tumors previously resistant to 5-FUra alone. This study is therefore being extended to a randomized trial of 5-FUra-CF versus 5-FUra alone, to other gastrointestinal cancers, and to exploration of other doses and schedules of 5-FUra and CF. The recommended dose of 5-FUra for the Phase II study is 600 mg/sq m. The dose of 5-FUra alone for the Phase III study is 400 mg/sq m daily for 5 days followed by 200 mg/sq m every other day for 11 days.

**REFERENCES**

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