Eur J Cancer Clin Oncol, Vol. 25, No. 1, pp. 45–49, 1989.
Printed in Great Britain

0277–5379/89$3.00 + 0.00

Lack of Effectiveness of Combined 5-Fluorouracil and Leucovorin in Patients with 5-Fluorouracil-resistant Advanced Colorectal Cancer

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Abstract—*Favorable results have been reported for the treatment of advanced colorectal cancer with the combination of 5-fluorouracil (5-FU) and leucovorin (LV). In these investigations the highest response rates were obtained in non-pretreated patients. In the present study, 22 patients with primary or acquired resistance to single-agent 5-FU and documented progressive disease on 5-FU were given a bolus injection of LV at a dose of* 200 mg/m^2, 1 h *prior to a* 2 h *infusion of 5-FU at a dose of* 370–770 mg/m^2 *on* 5 *consecutive days, and this was repeated every* 3 *weeks. Whenever possible the dose of 5-FU was escalated to find the maximum tolerable dose. No objective response was observed. Five patients had short-lasting stable disease. Despite 5-FU dose escalation, toxicity was acceptable. One patient developed 5-FU-related angina pectoris with EKG changes. It is concluded that in the schedule used, combined LV/5-FU treatment is ineffective for patients with 5-FU-resistant advanced colorectal cancer.*

INTRODUCTION

THE RESULTS of treatment of advanced colorectal cancer are discouraging and have not changed essentially during the last three decades. The 'standard' treatment, systemic administration of 5-fluorouracil (5-FU), has a response rate of only approx. 20%, and 5-FU-based combination chemotherapy has not improved the results [1, 2]. No effective new antineoplastic agents have been developed for this disease. Improvement might be obtained by enhancement of the therapeutic index of 5-FU by biochemical modulation [3], for example by the addition of leucovorin (LV) to 5-FU [4]. Together, the LV metabolite, 5,10-methylenetetrahydrofolate (CH_2-THF), and the active 5-FU metabolite fluorodeoxyuridine monophosphate (FdUMP) form a covalently bound ternary complex with the target enzyme thymidylate synthase (TS), which leads to inhibition of DNA synthesis [5]. The extent and stability of the FdUMP binding is highly dependent on the availability of CH_2-THF [6]. Several *in vitro* studies have shown that in cell cultures 5-FU-induced growth inhibition can be enhanced by the addition of LV [7–9], but few *in vivo* data on the effect of the LV/5-FU combination in animals are available. No enhancement of 5-FU antitumor activity by LV was obtained in L1210 tumor-bearing mice [10]. However, experiments we performed recently in mice bearing the colon tumors Colon 26 and 38 showed that LV could potentiate the antitumor activity of 5-FU in these solid tumors [11]. The use of different tumor systems and treatment schedules might have been responsible for the observed differences in activity.

Results of LV/5-FU treatment in patients with metastatic colorectal cancer have been published since 1982. Recently, these clinical studies were reviewed by Laufman *et al.* [12]. Despite wide differences in the dosage and schedules of both 5-FU and LV, the reported response rates were in general consistently higher than would be expected from 5-FU single-agent therapy. Although it is interesting that in a number of the studies responses were observed in patients previously treated with 5-FU, few of the reports give detailed data on prior 5-FU treatment. A recently completed phase III trial in non-pretreated patients showed a statistically significantly improved response rate (48%) for LV/5-FU compared with single-agent 5-FU (response rate 11%) or methotrexate/5-FU (response rate 5%) [13]. However, it was disappointing that this higher response rate was not accompanied by improved survival of the LV/5-FU-treated patients. Because the addition of LV to

Accepted 3 August 1988.
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5-FU adds considerably to the cost of treatment of patients with advanced colorectal cancer, a phase II study on LV/5-FU in patients with prior single-agent 5-FU seemed warranted. The aim of the present study was to find out whether use of this combination is justified in the latter patient category. Only patients with measurable disease were included, and LV/5-FU was given immediately after proven resistance to single-agent 5-FU. Furthermore, the dose of 5-FU was escalated in an attempt to treat each patient with the maximum tolerable dose.

MATERIALS AND METHODS

The study was done in 22 patients, eight males and 14 females with a median age of 62 years (range 29–77 years). The primary tumor was colon cancer in 16 patients and rectal carcinoma in six. All patients were in moderate to good general condition with a median WHO performance status of 1 (range 0–2). Sites of metastases were the liver (in 20 patients), the lungs (8), lymph nodes (6), bone (4), peritoneum (4) and the skin (2). Sixteen patients had metastatic disease at multiple sites. Besides distant metastases, two patients had locally recurrent disease. In 20 patients the primary tumor had been removed surgically, and the other two had received local cryosurgery.

Two patients had been treated with adjuvant radiotherapy for a primary rectal carcinoma. With respect to prior chemotherapy, all of the patients had had previous treatment with 5-FU given as weekly bolus injections. In three cases this had led to a partial response, in 10 the disease had stabilized, and in nine the disease had progressed during this therapy. Before entering the present study all patients had progressive disease on treatment with single agent 5-FU which was given at the maximum tolerated dose. Thus, all patients were considered resistant to the fluoropyrimidine. The interval between 5-FU single agent and LV/5-FU did not exceed 4 weeks. Furthermore, three patients had had prior hepatic intra-arterial 5-FU, one had had i.v. 5-aza-2′-deoxycytidine, and one patient i.v. cisplatin and hepatic intra-arterial 5-FU. All patients had normal values for hematological, renal and hepatic parameters except those in whom abnormal liver enzyme levels were due to metastatic liver disease. All patients had objectively measurable metastatic disease diagnosed by physical examination and/or radiologic procedures such as chest film or CT scanning. For lesions measured by CT scanning the lower size limit was put at a diameter of 3 cm. Bone metastases, elevated carcinoembryonic antigen levels and abnormal liver enzyme levels were not considered measurable disease. Oral informed consent was obtained from all patients.

Treatment regimen

Leucovorin (200 mg/m^2) was administered as an i.v. bolus injection daily for 5 days; 1 h later, 5-FU was given at an initial dose of 370 mg/m^2 as a 2 h i.v. infusion daily for 5 days. This treatment was repeated at 21-day intervals. If toxicity was grade ≤ 1 (WHO), the dose of 5-FU was escalated by 20% in each successive course. When toxicity grade > 2 was observed, the 5-FU dose was decreased to the preceding dose level. Leucovorin was always given at a fixed dose of 200 mg/m^2. Response was evaluated every two to three courses.

Study parameters

Before each course of therapy, a complete blood count was performed, serum liver enzyme and serum creatinine levels were determined and a chest film was made. Radiological studies to evaluate the response to therapy were repeated every two to three cycles. All patients were seen between days 10 and 12 of each course to evaluate toxicity of the treatment on the basis of the history, physical examination and complete blood count.

Response criteria were defined as follows: complete response = disappearance of all known tumor; partial response = a $\geq$ 50% reduction of the tumor parameters and no appearance of new lesions; stable disease = no change in size of the measurable lesions or a decrease of $<$ 50% or an increase of $<$ 25% with no appearance of new lesions, lasting for a minimum of six weeks; progression = increase of $\geq$ 25% in the size of the measurable lesions and/or appearance of new lesions after the start of treatment.

RESULTS

Response to therapy

The 22 patients received a total of 81 courses of LV/5-FU. The median number of courses per patient was 3 (range 1–9). In 41 courses it was possible to increase the 5-FU dose by 20% of the dose in the preceding course. This led to five 5-FU dose levels, i.e. 370, 440, 530, 640 and 770 mg/m^2. Table 1 shows the number of courses for each dose level. In four courses the 5-FU dose had to be decreased to the preceding level because of toxicity. In the remaining courses the dose was kept at the level giving acceptable toxicity. One patient, in whom toxicity did not develop in the first course, accidentally did not receive an increased dose in her second course.

With respect to the antitumor effect of the treatment, none of the patients experienced a complete or even a partial response. Five patients had stable disease with a duration of 2–6 months. Seventeen patients had progressive disease at the first evaluation and in 12 of them this was the reason for

Table 1. *Dose escalation of* 5-*FU*

5-FU dose level	5-FU dose (mg/m²)	No. of patients	No. of courses
I	370	22	23
II	440	17	17
III	530	14	21
IV	640	7	17
V	770	3	3
		Total	81

discontinuation of the therapy. However, in five patients with progressive disease at the first evaluation treatment was continued, because their general condition was still good and they had not reached the maximum tolerable dose of 5-FU. In spite of this dose escalation, further tumor progression was observed in these five patients. Due to rapid progession of the disease in combination with deterioration of the general condition, four patients received only one course.

Toxicity

Despite the attempt to treat the patients at the maximum tolerable dose of 5-FU, the regimen was generally tolerated very well. At the first two 5-FU dose levels, toxicity was absent or minimal. Even at the higher 5-FU dose levels a number of patients did not experience any toxicity. However, at these higher dose levels, too, moderate to sometimes severe toxicity was observed (Table 2).

Besides the side-effects listed in Table 2, one patient developed angina pectoris in the third and fourth courses at 5-FU doses of 530 and 640 mg/m², respectively. In the fifth course (5-FU dose 530 mg/m²), angina pectoris was again observed, this time accompanied by transient signs of ischemia on the EKG. Treatment was discontinued in this patient, who also had progressive disease. One of the two patients with grade 3 diarrhea had to be admitted to the hospital for i.v. rehydration.

DISCUSSION

This study failed to demonstrate any benefit of treatment with LV/5-FU in single-agent 5-FU-resistant patients with advanced colorectal cancer. No objective responses were observed. Only five of the 22 patients had disease stabilization for a short period, and all of the other seventeen had progressive disease. Several of the reported studies on LV/5-FU treatment in metastatic colorectal cancer included previously treated patients, but only a few of these studies were published as full papers [12, 14–17]; the other reports were abstracts. Only three of the former studies [15–17] were done in a sufficient number of patients considered resistant to prior fluoropyrimidines to permit meaningful conclusions as to the efficacy of LV/5-FU in these patients. The study done by Machover *et al.* [15] had 27 patients with prior 5-FU, four of whom showed an objectively evaluated response. Bertrand *et al.* [16] treated 35 previously treated patients and saw three partial responses. In a study performed by the NCOG [17], there were 43 fluoropyrimidine-resistant patients, of whom only two responded to subsequent LV/5-FU. These data combined with the results of the present study (nine responses in 127 patients) should discourage combined LV/5-FU treatment in 5-FU-resistant patients on a routine basis. However, in view of the good results obtained with LV/5-FU in non-pretreated patients, there seem to be grounds for extending its use to patients not responding to 5-FU. Much higher response rates have been reported for studies that included only non-pretreated patients. It might be that for this category of patients LV/5-FU represents an advantage over single agent 5-FU. However, until the conclusive reports of ongoing and future phase III trials, including survival data, become available, LV/5-FU should be considered experimental for all patients with advanced colorectal cancer. The present study aimed at treating the individual patient at the maximum tolerable dose by escalating the 5-FU dose by 20% in each subsequent course. Interestingly, most of the patients tolerated multiple dose increases before dose-limiting toxicity occurred. Thus, even 5-FU doses as high as 640 and 770 mg/m² could be administered to some patients. This is clearly in contrast with the studies done by Machover *et al.* [15] and Bertrand *et al.* [16], where 5-FU doses of 400 and 370 mg/m²,

Table 2. *Incidence and grading (WHO) of toxicity of LV/5-FU in* 81 *courses*

5-FU dose (mg/m²)	No. of patients/No. of courses	Nausea and vomiting 0 1 2 3	Stomatitis 0 1 2 3	Diarrhea 0 1 2 3	Leucopenia 0 1 2 3
370	22/23	22 1 — —	23 — — —	23 — — —	22 1 — —
440	17/17	15 2 — —	17 — — —	17 — — —	17 — — —
530	14/21	16 3 2 —	17 — 4 —	18 1 1 1	21 — — —
640	7/17	15 1 — 1	11 2 4 —	13 1 2 1	16 — 1 —
770	3/3	3 — — —	1 1 1 —	2 — 1 —	1 1 — 1

respectively, were judged to be the maximum tolerable doses. These differences might be partly explained by differences in the schedules of 5-FU and the dose/schedules of LV applied. However, when the data in these reports are analyzed carefully, minimal or no toxicity was apparently seen in a substantial number of patients, which suggests that higher 5-FU doses could have been applied in those cases. In spite of the use of 5-FU dose escalation in the present study, no benefit in terms of response was achieved.

The dose-limiting toxicity of the present regimen was gastrointestinal rather than myelosuppressive. Overall, toxicity was acceptable and transient. No life-threatening toxicity occurred and only one patient needed hospitalization, which was for diarrhea requiring i.v. hydration. It is of interest that one patient developed angina pectoris, accompanied on one occasion by EKG changes. This side-effect of 5-FU might be related to the increased use of 5-FU in prolonged infusions [18].

The findings of the present study give rise to important questions concerning the mechanisms underlying resistance to 5-FU combined with LV. As yet, little information on this topic is available from clinical investigations. Allegra *et al.* [19] showed in tumor biopsy specimens of two patients with advanced 5-FU-resistant breast cancer that 24 h after administration of single-agent 5-FU, 52% and 0% of tumor TS was bound by FdUMP. However, when 5-FU was preceded by LV in the same two patients, 98% and 100% of TS binding sites were occupied by FdUMP. Both patients responded to the combination therapy. Interestingly, when one of these patients subsequently showed progressive disease while on therapy with LV/5-FU, similar measurements showed no binding of the enzyme by the active metabolite. Alterations in folate metabolism or in the interaction of CH_2-THF/TS/FdUMP might explain this resistance to LV/5-FU. In this respect it is of interest to know whether higher doses of LV would improve the FdUMP binding. A suggestion that higher doses of LV might indeed exert a better effect was recently reported by Hines *et al.* [20]. They treated 31 patients with advanced colorectal cancer, 27 of whom were considered refractory to 5-FU. Of these 27 patients, 10 had an objective response (two CR and eight PR), for a response rate of 37%. This in clear contrast with our results in fluoropyrimidine-resistant patients. The most important differences between their study and the present and the other studies in fluoropyrimidine-resistant patients [15–17], were that they gave 5-FU as a weekly bolus injection and the dose of LV was much higher (500 mg/m^2, given as a 2-h infusion). In the studies with negative results, 5-FU was given on 5 consecutive days which was repeated every 3–4 weeks, and the dose of LV was lower (200 mg/m^2) except in the study done by Bertrand *et al.* [16], who administered LV at a dose of 500 mg/m^2/day in a continuous 6-day infusion. Although the findings in the pilot study performed by Hines *et al.* [20] are of interest, they require confirmation in order to definitively establish the significance of dose and schedule of 5-FU and LV. Obviously, these initial clinical observations with respect to resistance to LV/5-FU are very interesting and further research is certainly warranted.

In conclusion, the results of the present study show that treatment with LV/5-FU, at least in the dose/schedule applied, is not effective in patients with advanced 5-FU-resistant colorectal cancer. Participation of such patients in studies with new drugs or newly developed modes of therapy will contribute to advances in the treatment of this difficult disease.

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