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Modification of 5-Fluorouracil Activity by High-dose Methotrexate or Leucovorin in Advanced Colorectal Carcinoma

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21 patients with advanced colorectal carcinoma were entered into a phase II study to evaluate efficacy and toxicity of methotrexate (MTX), 1500 mg/m² rapid infusion on day 1, combined with continuous infusion of 5-fluorouracil (5-FU), 600 mg/m² per 24 h on days 1–4. 12 patients who had progressive disease during this regimen subsequently received high-dose leucovorin, 200 mg/m² bolus injection on days 1–4, combined with 4 days' continuous infusion of 5-FU. In the MTX/5-FU group 1 pathologically proven complete remission and 3 partial remissions were seen (response rate 20%). The median progression-free interval was 30 weeks. In 12 patients with progressive disease leucovorin/5-FU stabilized disease in 2 (17%). Toxicity in both regimens was tolerable, gastro-intestinal side-effects being most frequent. There were no treatment-related deaths. Median survival time was 10 months. Serum levels of carcinoembryonic antigen before treatment or doubling-time during progression did not correlate with survival.
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INTRODUCTION

FOR advanced colorectal carcinoma there are few therapeutic options. Only 5-fluorouracil (5-FU) has a constant but low activity, inducing remission in 8–25% of patients. These remissions are usually partial and not lasting (average duration 7–8 months). Effects on survival, if any, are marginal [1–3]. Several modifications enhance the effectiveness of 5-FU [3]. Among these is continuous infusion, which augments response and decreases toxicity but does not improve survival [4, 5].

Biochemical modulation of 5-FU activity involves the pharmacological manipulation of the intracellular metabolic pathway.

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One way is to increase intracellular phosphoribosylpyrophosphate, the main enzyme for 5-FU conversion to fluorouridine triphosphate, which disrupts RNA function. Increasing phosphoribosylpyrophosphate can be brought about by methotrexate (MTX) [6], itself marginally active against colon cancer. The synergistic effect of MTX/5-FU is only observed when MTX is given before 5-FU [7–12]. However, the optimal time between both drugs has not been elucidated [13, 14].

Another way to enhance 5-FU involves inhibition of DNA synthesis. The 5-FU metabolite 5-fluorodeoxyuridylate (FdUMP) binds thymidylate synthetase (TS), thereby inhibiting DNA synthesis. The FdUMP–TS complex is stabilized by reduced folates provided by leucovorin [15, 16]. This synergistic effect has been observed in several clinical studies [9, 17–20].

We have combined 5-FU continuous infusion with high-dose MTX. With continuous infusion we hoped to improve response rates and diminish toxicity [4], and circumvent the problem of the optimal time between MTX and 5-FU—the protracted infusion of 5-FU always covered this interval. Since leucovorin modulates 5-FU in a different way from MTX, responses might still be obtained after progression on MTX/5-FU. Therefore, in patients with clearly progressive disease on MTX/5-FU, MTX was replaced by leucovorin while 5-FU dosage was unaltered. We also studied the prognostic value of carcinoembryonic antigen (CEA).

PATIENTS AND METHODS

Patients

21 patients with histologically documented metastatic colorectal cancer were entered, 13 males and 8 females (Table 1). All patients had measurable or evaluable disease consisting of parenchymal nodules that could be assessed by imaging procedures (plain X-ray for pulmonary metastases, ultrasonography or computed tomography for abdominal lesions). Laboratory data, including CEA levels, were assessed monthly. CEA was measured by ELISA (Abbott). These laboratory results were not considered for evaluation of tumour response.

Table 1. *Patients' characteristics*

	MTX/5-FU (n = 21)	Leucovorin/5-FU (n = 12)
Median age (range)	54 (34–69)	54.5 (35–70)
Prior chemotherapy	3	12
Prior radiotherapy	2	3
Site of metastases		
Liver	8	1
Peritoneum	1	
Liver + peritoneum	2	1
Lung	3	
Lung + others	7	10
Site of indicator lesion		
Liver	9	1
Peritoneum	2	1
Lung	6	7
Lung + liver	4	3
Histology of primary tumour		
Differentiated	6	5
Intermediate	6	2
Undifferentiated	7	4
Unknown differentiation	2	1

All patients had an initial Karnofsky performance score above 80. All had normal blood count and renal parameters. Median time from operation for the primary tumour to start of chemotherapy because of metastases was 5.75 months (range 0.5–61, mean 13.6).

Treatment

The treatment regimen was MTX 1500 mg/m^2 as a rapid infusion over 15 min, immediately followed by continuous infusion of 5-FU, 600 mg/m^2 per 24 h over 4 days. Leucovorin rescue was started 24 h after MTX administration: 30 mg orally every 6 h for seven doses (more doses were given if serum MTX levels after 48 h were above 250 μg/l). Urine was alkalized by infusion of 1.4% $NaHCO_3$ before (1 l in 6 h) and after (2 l in 48 h) MTX administration. Courses were repeated every 4 weeks, until progression of disease.

If a patient had progressive disease, therapy was changed in those who still had Karnofsky status of 70 or more. This second-line regimen was leucovorin, 200 mg/m^2 bolus injection on days 1–4 with continuous infusion of 5-FU 600 mg/m^2 per 24 h on the same days. Treatment was repeated every 4 weeks.

Evaluation

After each two courses of therapy relevant imaging procedures were done. Response criteria were complete remission (CR), the disappearance of all perceptible tumour and partial response (PR), 50% reduction in product of largest perpendicular diameter of the indicator lesion with no increase in the size of other measurable disease and no appearance of new lesions. Duration of response was calculated from the time the response began until progression. Stable disease (SD) was defined as no change in size of measurable lesion or a decrease in tumour size by less than 50% or an increase of a quarter with no appearance of new lesions; SD required a minimum of 8 weeks' duration. Progressive disease (PD) was the appearance of any new lesions and/or growth of any existing lesion by more than a quarter from the start of treatment.

Evaluation of toxicity followed WHO guidelines [21].

RESULTS

MTX/5-FU continuous infusion

20 patients were evaluable for response; 1 patient stopped treatment after one course due to grade 4 mucositis. Total number of treatment courses was 180 and the median number per patient was 7 (range 1–33).

1 patient (5%) had CR, histologically proven by second-look laparotomy. This CR lasted 48 weeks. 3 patients (15%) had PR, lasting 20 weeks, 48 weeks and 152+ weeks, respectively. The overall remission rate was 20% (95% CI 6–40%). SD was

Table 2. *Toxicity*

Toxicity	MTX/5-FU	Leucovorin/5-FU
Mucositis		
Grade 1–2	12 (57%)	6 (50%)
Grade 3–4	2 (9.5%)	0
Nausea and vomiting grade 1–2	3 (14%)	2 (17%)
Diarrhoea grade 1–2	3 (14%)	4 (33%)
Nephrotoxicity		
Grade 1	1 (4.7%)	0
Grade 2	2 (9.5%)	0

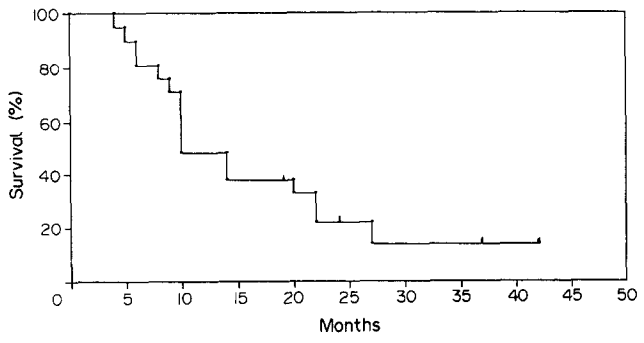

Fig. 1. Overall survival of all patients (n = 21). Lines = patients alive.

achieved in 13 patients (65%) (95% CI 41–85%), with a median duration of 24 weeks (range 8–48). 3 patients (15%) had PD within 8 weeks. The overall median progression-free interval was 30 weeks.

Toxicity is summarized in Table 2. In addition, conjunctivitis was observed in 3 patients (14%). The regimen was potentially nephrotoxic due to MTX. The nephrotoxicity grade 2 that occurred in 2 patients was rapidly and completely reversible after adequate hydration and alkalization of urine. Dose reduction of MTX was necessary once because of nephrotoxicity. 4 patients were admitted for management of toxicity: mucositis grade 4 (1 patient), nephrotoxicity grade 2 (2) and elevated MTX concentration (1). Total inpatient days due to adverse effects was 19. No leukopenia or thrombocytopenia was seen, nor signs of hand–foot syndrome. There were no treatment-related deaths.

Leucovorin/5-FU continuous infusion

12 patients who had tumour progression on the MTX/5-FU regimen were entered, 8 males, 4 females (Table 1). The total number of treatment courses was 42, median per patient 2.5 (range 1–8).

No CR or PR were seen. 2 patients (17%) had stabilization of previously progressive disease at weeks 18 and 20. Both patients had a differentiated primary tumour. All remaining patients had PD within 8 weeks.

Adverse effects are summarized in Table 2. No admissions were required because of toxicity. Myelosuppression was not observed. Dose reduction was not necessary and there were no treatment-related deaths.

Overall survival

The median survival time was 10 months, range 4 to more than 42 months (Fig. 1). This time was significantly longer in responders (over 22 months) than in non-responders (8.5 months) ($P < 0.05$, log-rank test). The median CEA level at the beginning of treatment was 10.4 μg/l (range 0.5–2590). No correlation was found between initial CEA level and survival. Median doubling time of CEA after progression had been diagnosed was 19 weeks (range 4–80). No correlation between doubling time and survival from start of therapy or progression could be detected.

DISCUSSION

The rationale for continuous infusion in this study was to eliminate the problem of determining the optimum interval between administration of MTX and 5-FU. In addition, continuous infusion improves the tolerance of the bone marrow and, possibly, the response of the tumour [4]. High-dose MTX compared with low-dose, as most often used [7-14], might be useful since it improves drug distribution in large solid tumours. It might also overcome the intrinsic resistance of tumour cells and prevent emergence of drug-resistant clones [15].

In patients on MTX/5-FU we observed a 20% remission rate (1 CR and 3 PR). The disease stabilized in 65%, with a median SD time of 24 weeks. The overall median progression-free interval was 7 months. These results were reflected by the median survival of 10 months. Remission rates with MTX/5-FU are reported to be 5–50% [7–14]. Median survival in these studies were 8.3–12.5 months. These data are similar to our results. If the higher dose MTX used in our study (1500 compared with 50–800 mg/m^2) is of any benefit, it was reflected in the occurrence of 1 CR and some prolonged PRs.

Studies with 5-FU alone or in combination with other agents have found remission rates of 6–40%, and median survival of 4–14.6 months. Within this group the studies of a combination of 5-FU and leucovorin gave better results [2, 4, 17–20, 22]. Median survival in patients with advanced colorectal carcinoma without treatment is 10.5 months [23], indicating the limited impact of chemotherapy on survival.

MTX enhances the effect of 5-FU on RNA synthesis, but it depletes the pool of reduced folate cofactors. These cofactors are necessary in increasing the effect of FdUMP on TS. Leucovorin exerts its effect by optimizing the quantity of intracellular reduced folates, thereby enhancing the inhibitory effect of 5-FU on DNA synthesis. Leucovorin probably has no enhancing effects on 5-FU inhibition of RNA [15]. On the basis of this mechanism, it would be hoped that substituting leucovorin for MTX in cases of tumour progression during MTX/5-FU therapy would for some patients overcome tumour resistance. This change in treatment indeed resulted in stabilization of previously progressive disease in 2 out of our 12 patients. Although this observation may underline the difference in modulation by MTX and leucovorin, its clinical value is limited. Possibly the low-dose leucovorin given for rescue of MTX toxicity in the MTX/5-FU regimen negatively influenced the response on the subsequent leucovorin/5-FU regimen.

Toxicity in both regimens was acceptable and was lower than that reported [7–14, 17–20, 24]. The main side-effects were gastrointestinal, mucositis grade 1–2 being most frequent. Despite a median of seven courses of high-dose MTX, no persisting decrease in renal function was seen. No drug-related deaths were observed.

Thus, high-dose MTX combined with 5-FU continuously infused has an anti-tumour response in colon cancer and has limited toxicity. However, improved survival is unlikely and our results do not support starting a phase III trial of this regimen. Patients showing progression on MTX/5-FU are not expected to benefit from substituting MTX by high-dose leucovorin.

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