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Phase II Study of Epirubicin Sequential Methotrexate and 5-Fluorouracil for Advanced Colorectal Cancer

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91 patients with measurable metastatic colorectal carcinoma entered a phase II study. A three-drug schedule; epirubicin (20 mg/m²), sequential methotrexate (150 mg/m²), 5-fluorouracil (600 mg/m²) with 1-h interval (EMF) with folinic acid rescue was given weekly three times followed by 2–3 weeks rest. 85 patients were evaluable for response. 5 patients (6%) experienced a complete response (CR), 20 (23%) a partial response (PR), 31 (37%) had disease stabilisation (SD) and 29 (34%) progressive disease (PD). The median survival time was 13.7 months in all patients ($n = 91$) and 14.0 months in those evaluable for response ($n = 85$). In patients with CR, PR, SD and PD the median survival time was 46.7, 19.8, 14.7 and 8.7 months, respectively. The response rate was significantly ($P < 0.05$) higher in tumours originating from the colon (41%) than in those originating from the rectum (18%) and also significantly ($P < 0.001$) higher in non-symptomatic than in symptomatic patients, 40 vs. 4%, respectively. The treatment was fairly well tolerated as an outpatient regimen, the main dose-limiting side-effect being diarrhoea. Deaths for septic fever, which may be attributable to the treatment were encountered in 3 patients, all with progressive cancer. Further studies to disclose differences in response rate in subsets of patients are warranted.
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INTRODUCTION

ALMOST 50% of colorectal carcinoma patients may be cured by surgery. However, patients with advanced unresectable colorectal carcinoma still have a uniformly poor prognosis. Historically, in patients with metastatic colorectal carcinoma, 5-fluorouracil (5-FU) as a single agent has provided an overall response rate of 15–20% without prolonging patients' survival. However, in many recent studies using more stringent response criteria, only 3–10% of patients have responded to 5-FU treatment [1–5].

In an attempt to improve its efficacy many agents have been combined with 5-FU with variable success. Methotrexate has been shown to be capable of modulating 5-FU cytotoxicity. The mechanism of this effect is thought to involve the accumulation of phosphoribosylpyrophosphate due to methotrexate inhibition of purine metabolism, resulting in increased formation of 5-FU ribonucleotide compounds [6]. Observations on synergistic action of methotrexate and 5-fluorouracil (MF), with proper timing, have raised much interest in it as effective chemotherapy for colorectal carcinoma. In studies with various MF schedules, a considerable proportion of patients have responded [7–10] while some authors have reported less favourable results [11–14]. One report has even suggested an overall survival benefit, when compared with 5-FU treatment, for patients treated instead with MF combinations [3]. In the present study, sequential methotrexate and 5-FU at a 1-h interval was combined with low-dose weekly epirubicin. The treatment was administered on an outpatient basis.

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The idea of combining epirubicin with MF resulted from an observation that adding this drug to the MF regimen in patients whose disease progressed during MF treatment, caused some of the patients to achieve prolonged disease stabilisation or tumour marker levels; elevated liver enzymes associated with liver metastases transiently decreased. Furthermore, in the early 1980s, during the initiation of this study a few reports also appeared on some degree of efficacy of epirubicin, particularly in rectal cancers [15, 16]. Since no remarkable additive toxicity was observed due to addition of such a small dose of epirubicin, this three-drug combination was selected for this phase II study. The results of EMF treatment in patients with measurable metastatic colorectal carcinoma are herein reported. We also analysed different clinical and tumour characteristics predictive of tumour response as well as patient survival.

PATIENTS AND METHODS

Patients

91 patients with advanced colorectal cancer were entered on the study between October 1984 and July 1990. Eligibility requirements included histologically proven metastatic colorectal adenocarcinoma not amenable to surgical resection. For response evaluation, bidimensional measurable disease was required. Other prerequisites included performance status of $\geq 60\%$ (Karnofsky), age ≤ 70 years, white blood cell count $\geq 3.5 \times 10^3$ μl, platelet count $\geq 100 \times 10^3$ μl, serum creatinine ≥ 1.5 mg/dl, adequate hepatic function (defined as serum bilirubin less than three times normal), no evidence of major cardiovascular or mental disease. Patients with a previous history of cancer (except basal cell skin cancer or carcinoma *in situ* of the uterine cervix) were excluded. Before admission to this study, informed consent was obtained.

The pretreatment characteristics of the 91 patients treated with epirubicin-sequential methotrexate-5-FU are shown in

Table 1. Characteristics of 91 patients treated with epirubicin-sequential methotrexate-5-FU

Variable	*n*	%
Sex		
Male	39	43
Female	52	57
Age (years)		
Mean	53	
Range	27–70	
Karnofsky performance score		
100	41	45
90	25	27
80	11	12
70	10	11
60	4	4
Site of primary tumour		
Right colon	26	29
Left colon	19	21
Rectum	46	51
Treatment of primary vs. recurrent disease		
Recurrent disease	43	47
Primarily unresectable	48	53
Previous treatment of metastases		
None	74	81
Chemotherapy	5	5
Radiotherapy	8	9
Chemotherapy and radiotherapy	4	4
Site of metastases		
Limited to one anatomic site or organ	56	62
Inoperable primary tumour	3	3
Pelvis or abdomen	14	15
Liver	27	30
Lung	12	13
Multiple sites	35	38
Inoperable primary tumour	3	3
Pelvis or abdomen	27	30
Liver	27	30
Lung	17	19

Table 1. All patients were evaluated for side-effects of the treatment. 6 patients of the 91 (7%) were non-evaluable for response. 2 patients refused further treatment after one and two cytostatic courses. 2 patients died of septic infections after two and three cytostatic courses. 1 patient died due to accidental subdural haematoma after two cytostatic courses. A solitary hepatic metastasis was excised in 1 patient after five cytostatic courses.

Treatment

A three-drug schedule (epirubicin 20 mg/m^2; sequential methotrexate 150 mg/m^2 plus 5-FU 600 mg/m^2 with a 1-h interval) was administered once a week for 3 successive weeks (day 1, day 8 and day 15) followed by a 2–3-week rest period. Folinic acid rescue (15 mg every 6 h given eight times) was initiated 24 h after methotrexate administration. The treatment was continued until progression, or discontinued with any appearance of intolerable side-effects.

Evaluation

Pretreatment evaluation included a complete history and physical examination, determination of blood counts (haemoglobin, leucocytes, platelets), blood alkaline phosphatase, serum glutamic oxaloacetic transaminase, bilirubin, and serum tumour markers such as CEA or CA19-9. Baseline ECG was also obtained. Blood counts were checked prior to every drug administration, and other laboratory values listed were obtained every 4 weeks.

The response was evaluated by clinical examination, lung X-rays every 4 weeks, and computed tomography scans or ultrasound every 2–3 months. The relief of symptoms was also registered. UICC response criteria were used [17], complete response (CR) being defined as disappearance of all measurable metastases, partial response (PR) defined as 50% reduction of areas of all measurable metastases for at least a 1-month period, and progressive disease (PD) was defined as an increase of 25% in area of all measurable metastases; stable disease (SD) was that stage between PR and PD. The side-effects of the treatment were classified using the WHO criteria [18].

Statistical description and analysis

Differences between mean values were analysed using the Student's *t*-test, and differences between frequencies with contingency tables. A stepwise logistic regression analysis was used to reveal factors predicting tumour response [19].

For calculation of progression-free survival and overall survival from the beginning of the treatment, product-limit survival analysis was performed using the BMDP 1L computer program [19]. Calculations of the significance of observed differences were performed using the log rank test (Mantel-Cox).

The relative prognostic importance of all parameters was investigated using Cox's regression model and the BMDP 2L computer program. A prognostic variable with two or more categories of outcome is represented by a number of variables and parameters equal to the number of its categories minus one. The reference category was not included as a variable.

RESULTS

Response

A median of 14 chemotherapy courses were administered (range 1–71). Overall response rate was 29%; 5 patients (6%) achieved CR and 20 patients (23%) PR (Table 2). The median time to partial response after the initiation of therapy was 2.2 months (range 0.7–5.8) and to complete response 5.7 months (range 2.5–8.4), respectively. The response rate was significantly higher in tumours originating from the colon than in those originating from the rectum: 41 vs. 18% ($P < 0.05$). The response rate was even higher in tumours originating from the left colon than from the right colon: 50 vs. 35% ($P > 0.05$), respectively. Patients having metastases or unresectable primary tumour at the time of diagnosis (stage D) tended to have a higher response rate (39%) than patients with recurrent disease (20%, $P = 0.053$). Patients with abdominal or pelvic metastases showed a lower response rate than those without abdominal or pelvic metastases, 18 vs. 38% ($P < 0.05$), respectively. In stepwise logistic regression analysis of the aforementioned variables, however, only the site of the primary tumour was a significant factor in predicting tumour response.

In comparing response rate with cancer-related symptoms, a significantly higher ($P < 0.001$) response rate was observed in symptom-free patients. Only 1 out of 25 patients (4%) with symptoms experienced PR, while 24 out of 60 symptom-free

Table 2. Response to epirubicin-sequential methotrexate-5-fluorouracil treatment related to characteristics of 85 evaluable patients

	n	CR	PR	CR+PR (%)	SD	PD	SD+PD (%)
All	85	5	20	29	31	29	71
Sex							
Male	37	2	7	24	18	10	76
Female	48	3	13	33	13	19	67
Age (years)							
< 55	43	2	11	30	14	16	70
≥ 55	42	3	9	29	17	13	71
Site of primary tumour							
Colon	41	4	13	41	12	12	58
Rectum	44	1	7	18	19	17	82
Treatment of primary vs. recurrent disease							
Recurrent disease	41	0	8	20	19	14	81
Primarily unresectable disease	44	5	12	39	12	15	61
Site of metastases							
Limited to one site or organ	54	5	12	31	19	18	69
Inoperable primary tumour	3	2	0	67	0	1	33
Pelvis or abdomen	13	1	2	23	6	4	77
Liver	26	2	7	35	6	11	65
Lung	12	0	3	25	7	2	75
Multiple sites	31	0	8	26	12	11	74
Inoperable primary tumour	2	0	1	50	1	0	50
Pelvis or abdomen	25	0	4	16	11	10	84
Liver	23	0	6	26	9	8	74
Lung	16	0	5	31	3	8	69
CEA (n = 83)							
< 5 μg/l	23	2	4	26	9	8	74
≥ 5 μg/l	60	3	16	32	21	20	68
CA 19-9 (n = 75)							
< 37	40	5	9	35	16	10	65
≥ 37	35	0	9	26	11	15	74

patients (40%) responded. However, rapid subjective relief of symptoms was experienced by almost half of the patients (12 out of 25) without objective tumour response. Among these 12 patients, 8 had SD and 4 had PD. Analysis of further response by site of the original tumour in the non-symptomatic patients revealed 9 out of 15 patients (60%) with tumours originating from the left colon responding and 8 of 17 (47%) with tumours from the right colon, while only 7 of 28 (25%) originating from the rectum responded in this subset of patients.

Progression-free and overall survival

In all patients evaluable for response, the median time to progression was 4.6 months. In patients with CR, PR, SD and PD the median time to progression was 17.8, 7.9, 5.7 and 1.9 months, respectively ($P < 0.0001$, log rank test).

Thus far 76 of the 91 patients have died. The median survival time was 13.7 months in all patients (n = 91) and 14.0 months in those evaluable for response (n = 85). In patients with CR, PR, SD, and PD the median survival time (95% confidence interval) was 46.7 months (lower 95% confidence limit 20 months, upper limit not calculable), 19.8 months (11.5–28.5 months), 14.7 months (12.6–21.4 months), and 8.7 months (6.0–11.8 months), respectively (Fig. 1, $P < 0.0005$, log rank test).

In univariate analysis, a lack of response (vs. response), a poor Karnofsky score (vs. Karnofsky ≥ 80), multiple sites of metastases (vs. one site), the presence of liver metastases (vs. none), the presence of abdominal or pelvic metastases(vs. none), and an elevated serum CA 19-9-level (vs. CA 19-9 level < 37 U/ml) correlated significantly with a poor survival. Age, sex, site of the primary tumour, or serum CEA level (≥ 5 vs. < 5 ng/ml) showed no correlation with survival.

In stepwise regression analysis, elevated serum CA 19-9 level, lack of tumour response and a poor Karnofsky score were independent adverse prognostic factors. To exclude a possible bias caused by early deaths of patients not responding to therapy, a separate analysis was made excluding all patients not surviving for 3 months [20]. In this analysis, elevated serum CA 19-9 level, a poor Karnofsky score, lack of response at 3 months after the onset of therapy and the extent of metastases correlated significantly with a poorer survival rate. Determining whether the improved prognosis of patients responding to therapy is related to the therapy or to some other confounding factors, would require a randomised study. The observation concerning serum CA 19-9 level as the most significant prognostic marker has been analysed and discussed more thoroughly elsewhere [21].

The treatment was discontinued in 66 out of 85 patients (78%) due to progression of the disease. In 1 case, the treatment was interrupted 2.5 months after detection of CR and in 3 cases due to long-lasting stable disease. In addition, 2 patients underwent surgery (an excision of pulmonary metastases after SD and excision of pelvic metastasis after PR).

Toxicity

The toxicity analysis included altogether 1542 chemotherapy courses. In 12 (14%) patients treatment was discontinued due to side-effects: aversion and/or fatigue (5 patients), gastrointestinal side-effects (3 patients), psychic problems (1 patient), conjuctivitis and fatigue (1 patient), painful injection sites (1 patient) and facial swelling (1 patient). In addition, 1 patient committed

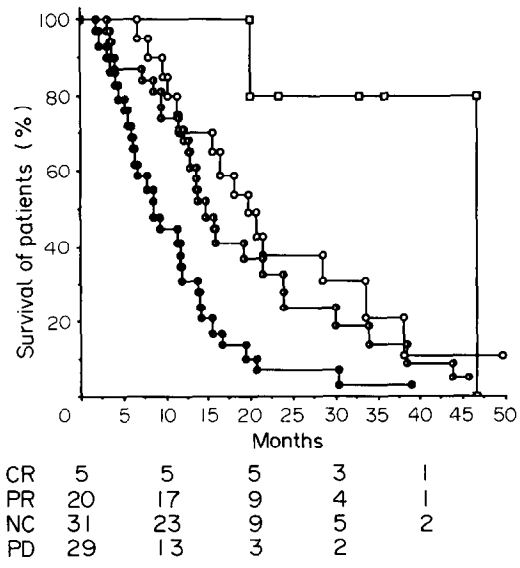

Fig. 1. Survival of patients according to response ($P < 0.0005$, log rank test). Symbols used: complete response (CR) □, partial response (PR) ○, stable disease (NC) ◑, progressive disease (PD) ●.

Table 3. Side-effects of epirubicin-sequential methotrexate-5-fluorouracil treatment in 91 patients according to WHO classification

	WHO grade (%)				
	0	1	2	3	4
Haematological					
Haemoglobin	48 (53)	31 (34)	9 (10)	2 (2)	1 (1)
Leucocytes	34 (37)	22 (24)	21 (23)	9 (10)	5 (5)
Platelets	86 (95)	3 (3)	1 (1)	1 (1)	0 (0)
Gastrointestinal					
Nausea/vomiting	27 (30)	30 (33)	26 (29)	8 (9)	0 (0)
Diarrhoea	35 (39)	27 (30)	18 (20)	9 (10)	2 (2)

suicide. In spite of interruptions, the vast majority of the patients tolerated the treatment well, as an outpatient regimen. The main toxicity requiring modifications in the treatment schedules, usually prolonging the intervals between treatment cycles, was gastrointestinal toxicity (Table 3). Of 91 patients, 2 had a severe haemorrhagic diarrhoea, and 9 patients had diarrhoea requiring fluid therapy in a hospital. Haematological toxicity was generally mild. 35 patients (38%) showed a nadir or leucocytes below $3.0 \times 10^3/\mu l$, 5 of them below $1.0 \times 10^3/\mu l$.

During the treatment, 12 patients had leukopenic fever, 10 had negative blood cultures, 1 patient suffered from a *Staphylococcus aureus*, and 1 *Pneumococcus* and *Klebsiella pneumonia* growing in the blood culture. 4 patients died from septic infection in the terminal phase of the disease. 3 of these deaths might be attributable to the treatment. The contributing factor was deteriorated general condition due to progressive metastatic disease.

No evidence of cardiac toxicity was observed although 4 patients received more than 800 mg/m^2 and 3 even more than 1000 mg/m^2 cumulative dose of epirubicin. The highest cumulative epirubicin dose was 1412 mg/m^2. A few patients experienced mild conjuctivitis or lacrimal symptoms, both of which have been almost totally alleviated during the past years by administering one drop of liquid folinic acid a few times a day into the eyes. Other possible toxicity signs such as alopecia were minimal. No renal or neurotoxicity were observed.

DISCUSSION

5-FU has been a mainstay of therapy in metastatic colorectal carcinoma. Combining either methotrexate or folinic acid with 5-FU has been a means to increase cytotoxicity of this drug. Although there has been some controversial experience regarding the efficacy of combining methotrexate with 5-FU most of the studies reveal that synergistic effect is achievable when methotrexate is administered prior to 5-FU [3, 7–10]. Two randomised trials also reveal that survival advantage can be achieved when combining prior methotrexate treatment to 5-FU [3, 10].

In the present study we have shown that with epirubicin-sequential-methotrexate-5-FU treatment of patients with metastatic colorectal carcinoma an overall objective response of 29% is achievable with acceptable toxicity. In addition, almost half of the symptomatic patients experienced relief of tumour-related symptoms, even though no objective response in tumour size could be verified.

The proper timing of sequential methotrexate and 5-FU is still an unsolved question. It has been argued that schedules with an increasing interval between methotrexate and 5-FU will increase the response rate. This view is supported by basic research and also by comparisons made with separate clinical phase II studies [7–9]. Some studies reveal that the most pronounced synergistic effect is achieved when the time period between administering these drugs is prolonged from a few hours to 20–24 h [3, 8–10]. A recent randomised study also shows the superiority of a 24-h interval between methotrexate and 5-FU compared with a 1-h interval [10].

At the time this study was initiated, not enough data existed on toxicity of prolonged interval between these two drugs. Thus in the present study methotrexate and 5-FU were administered as a 30-min infusion with 1-h interval combined with bolus epirubicin at the beginning of the treatment to reveal whether a significant response rate could be achieved with acceptable side-effects. Also the possibility of treating patients on an outpatient basis was considered important. The response rate achieved in the present study does not differ from that reported from schedules using a longer interval between methotrexate and 5-FU. One must, however, recognise that in most of the studies using either higher doses of methotrexate and 5-FU or a longer time interval between the administration of these drugs the treatment has been given every 2 or 3 weeks. In our schedule the treatment was intended to be given three times weekly followed by a 2-week rest period, i.e. three doses within a month. Although during prolonged treatment this scheme could not always be applied, the average dose intensity of methotrexate and 5-FU was comparable with many other studies with these drugs. According to the recent favourable results by Marsh and co-workers [10] on 24-h interval of methotrexate and 5-FU without extra toxicity, one could suppose that our EMF combination might be still more effective with adapting this time interval between methotrexate and 5-FU.

The most controversial issue in the treatment scheme is the role of epirubicin. During the initiation of this trial some evidence existed that epirubicin may be active, particularly in rectal cancer [15, 16]. However, in most of the recent studies no objective responses have been achieved [22–25], although in some patients prolonged stabilisation may have been noticed [25]. The role of epirubicin in this combination would still require evaluation in a randomised study.

A large proportion of the patients in this study were 'good-risk' patients; Karnofsky performance status was 80–100 in 77 patients and 60 patients were asymptomatic. Further analysis also disclosed some differences in response rates in subsets of patients. The response rate was highest (50%) in metastases originating from the left side of the colon, reaching even as high as 60% in this group of patients without symptoms. Another response indicator was stage at primary presentation. All the complete responders were among those patients with unresectable (stage D) disease at primary presentation to clinic, and overall response rate was higher among these patients than among those with relapsed disease. These results reveal that although colorectal tumours in general are relatively insensitive to chemotherapeutic drugs, there might be subgroups of patients more liable to benefit from this kind of treatment.

Most chemotherapy studies on colorectal cancer present none or very limited information on results in different subgroups of patients. Such differences might not have been detected or more probably such analysis has been for the most part ignored. While many studies on the behaviour of early-stage colorectal carcinomas have disclosed subgroup differences [26] it seems

quite obvious that similar differences would also be observed in advanced disease and also in chemosensitivity as proposed by Graf *et al.* [27]. The present observations indicate that patient characteristics should be defined in chemotherapy studies, particularly concerning existence of symptoms, site of primary tumour or treatment initiation at primary presentation or in a recurrent phase. Otherwise, treatment results in different studies are poorly comparable, only being based upon variability of chemosensitivity owing to different patient characteristics.

The side-effects of the treatment were generally acceptable. The main dose-limiting toxicity was gastrointestinal toxicity, mainly diarrhoea lasting a few days. In 2 patients a severe haemorrhagic diarrhoea was induced by the treatment. The most probable explanation of a sensitivity to this treatment is differences in pharmacokinetics between different patients. Haematological toxicity was rather mild; however, in 5 patients a severe transient leukopenia developed, complicated in 4 by sepsis. 3 septic deaths might be at least partly attributable to the treatment in patients with deteriorated general condition.

We conclude that EMF treatment produces an objective response in one third of patients with advanced colorectal carcinoma. The treatment is generally well tolerated and most important as a palliative treatment; a significant number of patients achieve relief of tumour-related symptoms. The possible contribution of EMF chemotherapy to prolonged survival and possible improvement of results by increasing the interval between methotrexate and 5-FU needs to be explored in a prospective randomised trial. Our subset analyses reveal that more attention should be focused on this disease to disclose those subgroups of patients with the greatest probability of benefitting from chemotherapy.

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