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CLINICAL TRIAL REPORT

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Biochemical modulation of fluorouracil: comparison of methotrexate, folinic acid, and fluorouracil versus folinic acid and fluorouracil in advanced colorectal cancer: a randomized trial

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Abstract Recent advances in biochemical pharmacology have revealed the basis for the biological modulation of 5-fluorouracil (5-FU) by methotrexate (MTX) and folinic acid (FA). Sequential use of MTX given 24 h prior to 5-FU has resulted in enhanced cell kill in vitro and in vivo. In addition, administration of FA prior to 5-FU has led to potentiation of 5-FU action by stabilization of the ternary complex of thymidine synthase. In the present randomized study, two groups of patients with advanced colorectal cancer were treated as follows: 43 patients (pts) in group A received 5-FU + FA, whereas 45 pts in group B received 5-FU + FA + MTX. The dosage was as follows: group A received FA i.v. at 300 mg/m^2 per day, prior to i.v. 5-FU at 500 mg/m^2 per day on days 1–4; group B was given MTX i.v. at 130 mg/m^2 per day on day 0, followed 24 h later by FA at 15 mg q6h × 6, and 5-FU + FA was started on day 1 and given at the same doses and schedule described for group A. Objective responses were achieved by 8/43 pts in group A (1 complete response and 7 partial responses) and by 18/45 pts in group B (3 complete and 15 partial responses), all occurring in the liver. There was no significant difference in the median time to progression (group A 6.1 months, group B 6.8 months) or the median survival (group A 9.2 months, group B 10.3 months). Toxicity was significantly greater in group B [grade 2–3 mucositis 20% versus only 2% in group A ($P < 0.0001$); grade 3 diarrhea in group B 15% versus 3% in group A ($P < 0.001$)]. According to our results, double biological modulation of 5-FU with MTX + FA led to an enhanced response rate with increased toxicity as compared with the 5-FU + FA regimen given at less than its maximally tolerated dose.

Key words Biochemical modulation · 5-Fluorouracil · Methotrexate · Folinic acid · Advanced colorectal cancer

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Introduction

5-Fluorouracil (5-FU) is the central cytostatic drug used in chemotherapy for colorectal cancer. The biochemical modulation of 5-FU action by folinic acid (FA) has gained major interest in the chemotherapy of advanced colorectal cancer. In this type of tumor a large number of preclinical and clinical investigations with 5-FU/FA have been conducted, clearly showing an advantage for the combination over 5-FU alone [1, 2] in terms of response rates but no effect on survival as suggested by a recent metaanalysis of published studies [3–5].

Methotrexate (MTX) has also been shown to be capable of modulating 5-FU cytotoxicity [6–9]. The mechanism of this effect is thought to involve the accumulation of phosphoribosylpyrophosphate (PRPP) due to MTX inhibition of purine metabolism, resulting in increased formation of 5-FU ribonucleotide compounds [9]. The timing of the administration of MTX followed by 5-FU is thought to be important for optimization of the biochemical interactions of these two agents, the maximal clinical effects of which are seen when 5-FU is given 4–7 h after MTX [11]. Furthermore, in a cell-culture system, concentrations of MTX (5–100 mol/l) corresponding to a delivered dose in the range of 200 mg/m^2 are required to enhance the effect of 5-FU [12].

In the present study we aimed at investigating whether the addition of an intermediate-dose MTX regimen to the combination of 5-FU and FA would increase the response rate.

Patients and methods

Patients

Between August 1990 and February 1994, 83 patients with recurrent or metastatic adenocarcinoma of the colon and rectum received 5-FU and FA with or without MTX. They were randomized into two groups (A and B). Randomization was carried out according to the method of closed envelopes. Group A (without MTX) contained 43 patients and group B (with MTX), 40 patients; there was no significant difference between the groups in terms of sex, age, performance status, primary tumor site, or metastasis. The patients' characteristics are shown in Table 1.

Table 1 Patients' characteristics (*NSS* Not statistically significant)

	FA + 5-FU	MTX + FA + 5-FU	*P*
Number of patients	43	40	NSS
Sex:			
M	26	27	NSS
F	17	13	NSS
Mean age in years (range)	63 (44–69)	65 (42–72)	NSS
Performance status (ECOG):			
1	23	28	NSS
2	20	12	0.06
Primary site of tumor:			
Sigmoid	19	14	NSS
Rectum	10	13	NSS
Rest	14	13	NSS
Local recurrence	26	22	NSS
Metastases:			
Peritoneum	8	3	NSS
Lung	8	4	NSS
Liver	29	37	NSS
Extraabdominal (e.g., bone, lymph nodes)	16	16	NSS

Eligibility criteria

Eligibility criteria included measurable disease; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; a life expectancy of >2 months; the absence of brain metastases or active ischemic cardiac disease; and normal hematological, renal, and hepatic function tests unless the abnormalities resulted from direct tumor invasion. A histological documentation of the measurable metastatic disease was obtained whenever possible. Informed consent was obtained from all patients according to our institutional policy.

Randomization

Randomization was carried out using closed envelopes and there was no distinction between ECOG 1 and ECOG 2 performance status.

Treatment

Two groups of patients were formed according to their therapeutic schedule (Table 1). Group A received FA given at 300 mg/m^2 per day by i.v. infusion prior to 5-FU given at 500 mg/m^2 per day in 500 ml 5% dextrose in water by i.v. infusion over 1 h for 4 days in the day clinic. Cycles were repeated every 21 days. Group B received MTX given at 130 mg/m^2 per day as a 30-min i.v. infusion in 500 ml 0.9% (N/S) at 20 h before the administration of FA at 300 mg/m^2 per day by i.v. infusion prior to i.v. infusion of 5-FU at 500 mg/m^2 in 500 ml 5% dextrose in water over 1 h for 4 days at the day clinic. Cycles were repeated every 21 days. At 24 h after the MTX infusion, patients in this group also received FA p.o. at 15 mg q6h for a total of six doses. Cycles were repeated every 21 days. In both groups the dose of 5-FU was escalated by 5–10% if no sign of toxicity was evident.

Treatment was continued for six cycles or until tumor progression. In the event of grade 2–3 myelosuppression, grade 3 mucositis, and diarrhea of grade 3 or more (WHO classification), treatment was delayed until the patient's recovery. For each degree of toxicity involving mucositis, diarrhea, and myelosuppression we decreased the dose of 5-FU by 10% (I 10%, II 20%, III 30%). In the case of neurotoxicity and cardiotoxicity we decreased the dose of 5-FU by 25%. In the event of severe toxicity the treatment was discontinued. The dose of FA was not changed at any level of toxicity. After the completion of chemotherapy, selected patients who showed a single metastasis, especially in the pelvis, received consolidation radiotherapy at that site.

Criteria for response

Before each treatment cycle, every patient underwent a complete blood count (CBC), SMA-12, EKG, chest roentgenography, and abdominal computerized tomography (CT) scan. Between the treatment cycles, CBCs were performed weekly. Patients were evaluated for response between treatment cycles during the the 2-week rest period.

A complete response (CR) was defined as complete disappearance of all clinically evident disease that lasted for at least 8 weeks as documented on two separate scans. A partial response (PR) was defined as a decrease of more than 50% in the sum of the products of the largest perpendicular diameters of the measurable lesions. A 25–50% decrease that did not satisfy the criteria of a PR was defined as stable disease (SD). Progressive disease was defined as an increase in the abovementioned measurements or the appearance of a new lesions.

Toxicity

Toxicity was estimated according to WHO criteria [13], except for nausea and vomiting [14].

Statistical evaluation

Statistical evaluation was based on the chi-square test and Student's *t*-test. Survival was estimated from the initiation of chemotherapy until the patient's death. Survival and time to progression were calculated using the Kaplan-Meier method [15]. Comparisons of the survival curves were made using log-rank tests [16].

Results

Response to chemotherapy

There was a difference in response between the two groups (Table 2). In group A, 1 (2%) patient achieved a CR, 7 (16%) patients showed a PR, 13 (30%) displayed SD, and 22 (51%) demonstrated PD during the chemotherapy period. In group B, 3 (7.5%) patients achieved a CR, 15 (38%) showed a PR, 8 (20%) displayed SD, and 14 (35%) developed PD. We noticed an increased number of patients achieving a PR in group B as compared with group A (A 7 versus B 15, $P < 0.029$; Table 2).

Table 2 Response of patients with advanced colorectal cancer to chemotherapy with 5-FU + FA versus 5-FU + FA + MTX

	Group A FA + 5-FU Number (%) (n = 43)	Group B MTX + FA + 5-FU Number (%) (n = 40)	P	95% CI
Courses of chemotherapy	123	118	0.904	
Response:				
CR	1 (2)	3 (8)	0.272	0.13−−0.01
PR	7 (16)	15 (38)	0.029	0.13− 0.30
SD	13 (30)	8 (20)	0.284	0.36−−0.16
PD	22 (51)	14 (35)	0.287	0.94−−0.62
Response of liver metastases:				
Complete	1 (4)	3 (8)	0.272	–0.01− 0.097
Partial	6 (24)	15 (41)	0.014	0.25− 0.09
Stable disease	3 (12)	4 (11)	0.621	0.01−−0.01
Progression	15 (60)	15 (41)	0.804	0.08−−0.002
Mean survival in months	10.3	14	0.154	
Range	(3−36)	(8−26)		
Median	10	12		
Mean duration of response in months	2.8	4.2	0.163	
Range	(2−12)	(2−12)		
Median	3	4		
Mean time to progression in months	6.5	7.1	0.850	
Range	(2−11)	(6−8)		
Median	6	7		
Mean survival in months for patients with liver metastases	7.4	12	0.04	
Range	(2−18)	(8−26)		
Median	6	7		
Living patients	6	11	0.127	

The response rate with regard to liver metastases was as follows: CR 1 (3%) patient in group A and 3 (10%) patients in group B, PR 6 (20%) patients in group A and 15 (41%) in group B ($P < 0.014$), SD 3 (10%) patients in group A and 4 (10%) in group B, and PD 15 (51%) patients in group A and 15 (41%) in group B.

Response/survival

No statistically significant difference was noted in the median duration of response, which was 2.2 (range 2−12) months for group A and 2.8 (range 2−12) months for group B (mean A 2.8 months versus B 3.2 months). The median survival was 9.2 (3−36) months for group A and 10.3 (8−26) months for group B (mean A 11.3 months versus B 14 months). The time to progression was 6.1 (2−11) months for group A and 6.8 (6−8) months for group B (mean A 6.5 months versus B 7.1 months; Table 2). A difference in survival was noted for patients with liver metastases, median survival being 5.5 (mean 5.5, range 2−18) months for group A and 13 (mean 12, range 8−26) months for group B ($P < 0.044$; Table 2).

In group A, 36 patients died due to disease progression and there was 1 toxic death; 6 patients remain alive. In group B, 29 patients died due to disease progression and 11 patients remain alive.

Toxicity

Myelosuppression was more profound in group B as mirrored by the occurrence of neutropenia (grade 0: A 59% versus B 15%, $P < 0.0001$) and thrombocytopenia (grade 0: A 94% versus B 75%, $P < 0.0001$). Myelosuppression due to MTX addition was responsible for four episodes of febrile neutropenia that could be managed well with antibiotics. These four patients developed myelosuppression after they had completed four courses of chemotherapy. Mild renal failure combined with low fluid intake was responsible for the toxicity.

The following toxicities were more severe in group B: mucositis of grades 1 (A 21% versus B 35%, $P < 0.013$) and 3 (A 2% versus B 12%, $P < 0.006$), nausea and vomiting of grades 1 (A 21% versus B 35%, $P < 0.028$) and 3 (A 0 versus B 8%, $P < 0.004$), and diarrhea of grade 2 (A 15% versus B 35%, $P < 0.001$; Table 3). With respect to other parameters of toxicity, fatigue (A 22 patients versus B 35 patients, $P < 0.0001$), anorexia (A 21 patients versus B 37 patients, $P < 0.0001$), and dermatolologic features (rash,

Table 3 Toxicity encountered in patients undergoing chemotherapy with 5-FU + FA versus 5-FU + FA + MTX for advanced colorectal cancer

Parameter	WHO grade	Group A *n* (%)	Group B *n* (%)	*P*	95% CI
Neutropenia[a]	0	79 (59)	21 (15)	0.0001	0.336– 0.541
	1	28 (21)	47 (34)	0.025	–0.234––0.0247
	2	20 (15)	36 (26)	0.003	–0.204––0.0152
	3	7 (5)	24 (17)	0.003	–0.194––0.0472
	4	0	11 (8)	0.003	–0.124––0.0343
Thrombocytopenia[a]	0	126 (94)	104 (75)	0.019	0.110– 0.275
	1	8 (6)	25 (18)	0.017	–0.196––0.0447
	2	0	10 (7)	0.007	–0.115––0.0290
Mucositis[a]	0	93 (69)	50 (36)	0.0001	0.223– 0.446
	1	21 (16)	43 (31)	0.013	–0.251––0.0542
	2	17 (13)	29 (21)	0.133	–0.170– 0.0062
	3	3 (2)	17 (12)	0.006	–0.160––0.0400
Nausea and vomiting[a]	0	88 (66)	50 (36)	0.0001	0.184– 0.410
	1	28 (21)	43 (35)	0.028	–0.249––0.0385
	2	18 (13)	29 (21)	0.133	–0.143––0.0146
	3	0	11 (8)	0.004	–0.124––0.0343
Diarrhea[a]	0	79 (59)	42 (30)	0.0001	0.174– 0.400
	1	27 (20)	36 (26)	0.314	–0.157––0.0421
	2	20 (15)	49 (35)	0.001	–0.303––0.104
	3	8 (6)	12 (9)	0.421	–0.088––0.0349
Neurotoxicity[b]		8 (19)	12 (30)	0.226	–0.298– 0.0696
Alopecia[b]	0	34 (81)	28 (71)	0.062	–0.096– 0.278
	1	5 (10)	8 (21)	0.336	–0.240– 0.0730
	2	4 (9)	4 (8)	0.111	–0.134– 0.120
Dermatologic toxicity[b]		13 (30)	27 (67)	0.001	–0.308– 0.0374
Fatigue[b]		22 (51)	35 (88)	0.0001	–0.545––0.182
Anorexia[b]		21 (49)	37 (93)	0.0001	–0.607––0.266

[a] Data are expressed according to the number of chemotherapy cycles received
[b] Data are expressed according to the number of patients affected

hyperpigmentation of the skin: A 13 patients versus B 27 patients, $P < 0.001$) were more frequently encountered in group B (Table 3).

Discussion

This study clearly demonstrates that the addition of MTX to the combination of FA and 5-FU plus FA rescue may enhance the activity of the regimen, leading to a higher response rate along with a minor improvement in survival. As expected, the toxicity was greater in the MTX group but, fortunately, was not associated with toxic deaths.

The two substances examined in the present study (MTX and 5-FU) have been extensively studied by other investigators either as combinations of FA + 5-FU or as combinations of MTX + 5-FU as compared with 5-FU alone. In several studies, all three substances, were investigated by either simultaneous or sequential administration [1].

In vivo biochemical modulation of 5-FU can be achieved by a variety of substances, but clinical studies have largely been restricted to FA, MTX, *N*-phosphonacetyl-L-aspartate (PALA), and interferons. FA is the most extensively studied 5-FU modulator. Although the superiority of the combination of 5-FU + FA over 5-FU alone in terms of response has been confirmed by several studies [1, 2], its superiority with regard to survival has not been clearly demonstrated. Recently, a metaanalysis conducted by the Advanced Colorectal Cancer Metaanalysis Project [3] showed a clear advantage for the combination with regard to response rate and palliation but not survival. Although not all oncologists are convinced, the combination of FA + 5-FU is currently expected to become the new standard treatment for colorectal cancer.

In the present study, where FA + 5-FU was considered the standard treatment, MTX was added with the purpose of enhancing the effectiveness of the regimen. MTX has been extensively studied as a 5-FU modulator, but until recently the results of clinical studies have been conflicting [17, 18]. Obviously the timing of the administration of MTX followed by 5-FU is very important. The Piedmont Oncology Group [19] has demonstrated a significant survival advantage for patients treated with a 24-h as compared with 1-h interval between MTX and 5-FU infusions. Most recently the Advanced Colorectal Metaanalysis Project, based on individual data on 1178 patients included in 8 randomized studies, demonstrated a higher response rate of 19% for the MTX + 5-FU combination versus 10% for 5-FU alone. This improvement in tumor response also translated into a slight improvement in survival (10.7 versus 9.1 months) [5].

The rationale of the therapeutic schedule used in the present study was based on data derived from preclinical and clinical studies. The delivered MTX dose was consid-

ered sufficient to achieve the appropriate drug concentration for 5-FU modulation [10]. Also, the interval between MTX and FA + 5-FU administration was 24 h, a difference of considerable clinical relevance as demonstrated in clinical studies [9, 18, 19]. In several other studies in which 5-FU is being modulated by MTX in regimens such as the MFL [MTX + leucovorin (LV) + 5-FU] regimen, the two drugs are given every 3 weeks, whereby 5-FU is given 3 and 23 h after MTX. In the MFL studies, FA is given only in small doses for rescue [5, 20] after MTX. The response rate in MFL studies varies from 24% to 50% [5, 21].

In our study, it is possible that overrescue from the MTX dose occurred due to the administration of i.v. FA at 300 mg/m^2 at 20 h after the MTX infusion followed by six oral doses of FA at 15 mg q6h. It is possible that the efficacy of the regimen could be improved if the FA given i.v. on the 1st day were omitted and only low-dose oral FA were given for both rescue from MTX and modulation of 5-FU. Higher rates of response are usually reported in single-institution as opposed to multicenter studies, where the results are less favorable [4, 20]. To our knowledge there has been only one study where equally higher dose 5-FU (600 mg/m^2) and FA (500 mg/m^2) have been given at doses equivalently higher than those used in the present study. However, the FA doses were very low (15 mg) and were given q6h for six doses. The response rate for this regimen was 27% and included a CR rate of 10% [22]. As used in our study, the 5-FU + FA regimen is definitely a full-dose combination, being at least as potent as or more intense than the conventional 5-day 5-FU (370–425 mg/m^2)/FA (200 mg/m^2) schedule. As expressed according to body surface area, the total dose used in our regimen was 2000 mg/m^2 for 5-FU and 1200 mg/m^2 for FA; in contrast, the conventional regimen comprises 5-FU at 1850–2125 mg/m^2 and FA at 1000 mg/m^2.

With regard to the results of the present study, it is noteworthy that although the response rate in the MTX group was quite high (45%), no significant advantage in terms of survival was achieved except in patients with liver metastases. Moreover, the proportion of patients with liver metastases was higher in the MTX group (37/40 versus 29/43 in group A, $P = 0.01$), and it is known that liver involvement is the main cause of death in colorectal cancer patients. In this case, even considering the prolongation of survival achieved for patients with liver metastases by MTX, the median survival of the whole group did not improve significantly as compared with that of group A (with less critical metastatic sites such as lymph nodes and soft tissue). As for toxicity, obviously the two treatment arms were not equitoxic. The addition of MTX plus the round-the-clock FA administration for the first 36 h had an enhancing effect on 5-FU-induced toxicity. Most probably, FA administration also led to an increase in the bioavailability of FA, resulting in a higher response rate as compared with that obtained for the 5-FU + FA arm, which we have to admit, was given at less than its maximally tolerated dose.

In conclusion, MTX may improve the response rate for the "standard regimen" (FA + 5-FU), but advantages in survival cannot be demonstrated, at least for the number of patients enrolled in the present study. Since the regimen is not very toxic, it would be interesting to study further the double biological modulation of 5-FU (by MTX and LV). In the future we plan to apply the same regimen modified to a shorter duration (1 day of LV + 5-FU) every week, provided that toxicity is acceptable and permits its administration.

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