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Postsurgical Sequential Methotrexate, Fluorouracil, and Leucovorin for Advanced Colorectal Carcinoma: A Preliminary Study

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Background and Objectives: The present study compared the effects of sequential methotrexate and fluorouracil followed by leucovorin rescue (MFL), as an adjuvant chemotherapy versus a combination of tegafur (UFT) and mitomycin C (MMC), on patient survival and recurrence after surgery for colorectal carcinoma.

Methods: Between January 1990 and December 1995, a total of 46 patients with advanced colorectal cancer were treated postsurgically by adjuvant chemotherapy using MFL or UFT-MMC. Surgical treatment was performed according to standardized procedures for radical resection of colorectal cancer. The patients were stratified into two groups after surgery. The MFL regimen consisted of MTX (100 mg/m^2) and 5-FU (600 mg/m^2) at hour 24, followed by leucovorin rescue. The UFT-MMC regimen consisted of MMC (12 mg/m^2) intraoperatively and MMC (6 mg/m^2) ever other week after surgery for 2 months and oral UFT (375 mg/m^2/day), a combination of tegafur and uracil in a molar ratio of 1:4, was continued for 3 years or longer depending on the patients tolerance.

Results: The overall survival rates after surgery was significantly ($P < 0.05$) higher in the MFL than the UFT-MMC group. Recurrence rates were significantly lower in the MFL than the UFT-MMC Group, especially for liver recurrence. Disease-free survival was significantly ($P < 0.05$) higher in the MFL than the UFT-MMC group.

Conclusions: The present results demonstrated the superiority of MFL therapy for improving postsurgical survival in patients with advanced colorectal cancer, in particular for those patients with a high risk of recurrence following potential curative resection.

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KEY WORDS: surgery; MTX-5-FU; colorectal cancer

INTRODUCTION

Carcinoma recurrence and metachronous metastasis are occasionally experienced in patients with advanced colorectal carcinoma even after macroscopical curative resection of the original lesion due to the presence of residual occult disease and distant micrometastasis [1,2]. Thus adjuvant treatment has been employed to improve the prognosis of patients. Although a variety of adjuvant chemotherapies have been tried, most have failed to show any significant advantage in preventing recurrence, since colorectal carcinoma is resistant to conventional chemotherapy. Even though 5-fluorouracil (5-FU) is still considered one of the most active drugs, a recent meta-analysis showed that overall response rates to 5-FU were ~11%, with a median survival of only 11 months [3,4].

At present, tegafur (1-(2-tetrahydrofuryl)-5-fluorouracil), or UFT, a combination of uracil and tegafur in a

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molar ratio of 1:4, are often used for adjuvant chemotherapy after surgery for colorectal carcinoma [5,6]. A combination of the intravenous administration of mitomycin C (MMC) and these oral fluorouracil derivatives also is widely used postsurgically [7,8]. However, the combination of MMC and these oral agents has yet to be proved more effective than the oral agents alone [9].

Recently, sequential methotrexate (MTX) and 5-FU followed by leucovorin (LV) rescue (MFL) have been used to overcome the resistance of cancer cells to 5-FU alone. Synergistic antitumor activity of MFL has been observed, first in experimental tumors [10], then in human cancer of the breast, stomach, and colon [11]. Further, MFL has proved to be superior to 5-FU alone or a combination of 5-FU and LV in treatment of gastrointestinal cancer in clinical trials [12,13]. Few reports, however, have been published on the effects of MFL for adjuvant therapy after surgery for advanced colorectal cancer. In the present study, the clinical appraisal of MFL was superior to that of UFT, as evaluated by comparing disease-free interval, patient survival, and objective responses.

MATERIALS AND METHODS

Between January 1990 and December 1995, surgical resection of primary lesions was performed in a total of 85 patients with advanced colorectal carcinoma classified as stage 2 or greater. Forty-six of the 85 patients were treated with MFL or UFT-MMC and were eligible for this study. The patients were basically allocated to the two treatment groups every two to one cases alternatively to make the patient characteristics as homogeneous as possible. A rare patient with impression of more severe macroscopic disease at the time of surgery was allocated to Group B, since this study was performed in open label, unrandomized fashion. However, the two treatment groups were homogeneous and well balanced in clinical and pathological characteristics (see Table II). The other 39 patients were mostly stage 2 without lymph node metastasis and treated with other protocols using tegafur, carmofur (1-hexylcarbamoyl-5-fluorouracil), or oral 5-FU.

Surgery

Surgical treatment was performed by the same surgeons according to standardized procedures of radical resection for colorectal cancer. The pathologic diagnosis and classification were evaluated according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum, and Anus in Japan (Table I) [14]. For the diagnosis of S2, definite serosal invasion was required, whereas for S3, invasion to contiguous structures was evident. Lymph nodes in groups 1, 2, and 3 are referred to as n1, n2, and n3, respectively, and distant lymph nodes located beyond group 3 (n3) are referred to as n4. Colorectal resection based on lymph node dissection was classified as follows: R0, colorectal resection including the incomplete removal of the group 1 lymph nodes; R1, colorectal resection including the complete resection of only the group 1 lymph nodes; R2, colorectal resection including the complete resection of the group 1 and 2 lymph nodes; R3, colorectal resection including the complete resection of the group 1, 2, and 3 lymph nodes.

TABLE I. Histologic Staging According to General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum, and Anus in Japan

	Factor			
Stage	Depth of invasion[a]	Lymph node metastasis	Peritoneal dissemination	Liver or distant metastasis
I	m, sm, pm	n(−)	(−)	(−)
II	ss, se, a1*, a2*	n(−)	(−)	(−)
IIIa	si, ai	n1(+)	(−)	(−)
IIIb	si, ai	n2(+), n3(+)	(−)	(−)
IV	si, ai	n4(+)	(+)	(+)

[a]m: mucosa; sm: submucosa; pm: muscularis propria; ss: subserosa; s: serosa; se: serosa exposed; si: serosa (infiltrating adjacent organs); a1 (site without serosa): beyond muscularis propria, but does not penetrate deeper; a2 (site without serosa): beyond muscularis propria infiltrating deeper but does not infiltrate adjacent organs; ai: infiltrating adjacent organs.

Chemotherapy

The MFL regimen consisted of MTX (100 mg/m^2) as an intravenous (IV) injection, followed by 5-FU (600 mg/m^2) IV infusion at hour 24. Leucovorin rescue was given at hour 30, with a 15 mg IV injection followed by seven oral doses of leucovorin, 15 mg every 6 hours. The dosage adjustments were made according to hematological toxicity. The treatment was started 14–21 days after surgery and repeated every 14 days for 8 courses and subsequently every 3–4 weeks. The treatment was not to be terminated before the fourth course unless severe adverse effects occurred. After eight courses of treatment, therapy was continued for as long as it was considered to be of palliative value. The treatment was maintained even when the disease recurred, unless otherwise indicated. The UFT-MMC regimen consisted of MMC (12 mg/m^2) IV intraoperatively, followed by MMC (6 mg/m^2) IV every other week after surgery for 2 months. Oral UFT (375 mg/m^2/day) was continued for 3 years or as long as the patients could tolerate.

Evaluation

All patients were re-evaluated every 2 weeks with a physical examination, and their complete blood chemistry was checked every 4 weeks. Tumor markers such as carcinoembryonic antigen (CEA) and carcinogenic anti-

gen (CA)-19-9 were measured before and after surgery, and every 2–3 months. Image diagnostic assessment, including computed tomography, ultrasonography, and/or magnetic resonance imaging, were carried out every 3–6 months to detect the recurrence of lesions.

Toxicity

The evaluation of adverse effects followed the World Health Organization (WHO) guidelines [15]. The records of all patients having grades 2–4 toxicity were evaluated.

Statistics

Differences between means were tested with the Student's *t*-test, and differences between proportions were tested with the Chi-square test or Fischer's exact test. Survival curves were calculated using the Kaplan-Meier method, and differences were evaluated with the Cox-Mantel test. *P* values from one-tailed test < 0.05 were considered statistically significant.

RESULTS

Clinicopathological details of the 46 eligible cases of colon cancer or rectal cancer are summarized in Table II. Thirty patients were treated with UFT-MMC (Group A) and 16 patients were treated with MFL (Group B). Concerning the prognostic factors of colorectal cancer, no significant difference was obtained between the groups. In Group A, the patients received 186±25 (mean±SEM) g of UFT and 39±4 mg of MMC, and in Group B, all the patients received four or more treatment courses with an average of nine courses and were thus evaluable for objective response.

The patients' survival rates, calculated by the Kaplan-Meier method, 1, 3, and 5 years after surgery were 89.8%, 54.5%, and 48.5%, respectively, in Group A, and 100%, 84.6%, and 84.6%, respectively, in Group B (Fig. 1). The patients' survival rates after surgery were significantly ($P < 0.05$) higher in Group B than in Group A. The median survival duration for Group A was 28.0 months and for Group B was 31.4 months. The disease-free survival rates 1, 3, and 5 years after surgery were 63.2%, 40.5%, and 40.5%, respectively, in Group A, and 85.7%, 68.2%, and 68.2%, respectively, in Group B. Disease-free survival was significantly ($P < 0.05$) higher in Group B than in Group A (Fig. 2).

The patterns and sites of recurrence are summarized in Table III. There were a total of 21 incidences of recurrence in 17 patients of Group A and a total of 4 incidences in 4 patients of Group B. Recurrence rates were significantly decreased in Group B compared to Group A, especially liver recurrence.

Drug toxicities, including hematological and gastrointestinal toxicities, occurred in both groups. Most of the toxicities were grade II according to WHO criteria (Table IV). Leukocytopenia and gastrointestinal symptoms such as nausea, vomitting, and diarrhea were observed. Nausea was frequent in the MFL group, whereas liver dysfunction was frequent in the UFT-MMC group. There was no significant difference in the incidence of drug toxicities between the groups.

TABLE II. Clinicopathologic Characteristics of Patients With Colorectal Cancer*

Variables[a]	Group A[b] (n = 30)	Group B[c] (n = 16)	*P* values
Age	65.6 ± 1.9	66.5 ± 1.8	NS
Gender			
Male	17	12	NS
Female	13	4	
Location of tumor			
Ascending	8	5	NS
Transverse	5	2	
Descending	2	1	
Sigmoid	4	4	
Rectum	11	4	
Stage (histologic)			
II	10	6	NS
III	14	4	
IV	6	6	
Histologic findings			
Well diff	4	3	NS
Moderately diff	24	12	
Poorly diff	2	1	
Histologic depth of invasion			
sm, pm,	4	2	NS
ss, se, a1, a2	21	12	
si, ai	5	2	
Lymph node involvement			
n0	12	9	NS
n1	8	4	
n2, n3	10	3	
Operation performed			
Right hemicolectomy	13	7	NS
Left hemicolectomy	3	1	
Anterior resection	10	6	
Mile's operation	4	2	
Curability			
Curative	26	13	NS
Noncurative	4	3	

*Values are expressed as mean ± SEM.
[a]Diff: differentiated; sm: submucosa; pm: muscularis propria; s: serosa; ss: subserosa; se: serosa exposed; a1: beyond muscularis propria, but does not penetrate deeper; a2: beyond muscularis propria infiltrating deeper, but does not infiltrate adjacent organs; si: serosa (infiltrating adjacent organs); ai: infiltrating adjacent organs.
[b]UFT-MCC = tegafur plus mitomycin-C.
[c]MFL = sequential methotrexate plus fluorouracil followed by leukovorin rescue.
NS = no significant difference.

DISCUSSION

Following curative resection of colorectal carcinoma, various types of recurrence have been observed during

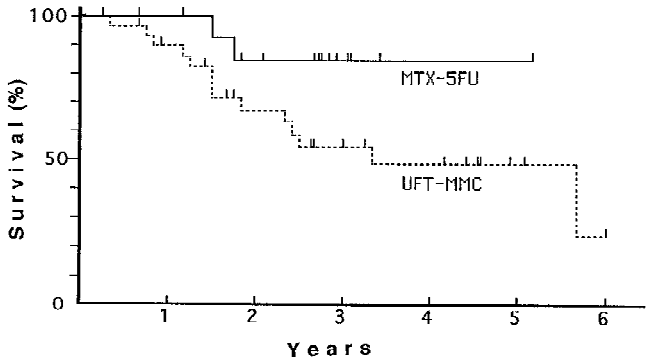

Fig. 1. Patient survival rates, calculated by the Kaplan-Meier method, after surgery were significantly ($P < 0.05$ by the Cox-Mantel test) higher in the MFL than the UFT-MMC group.

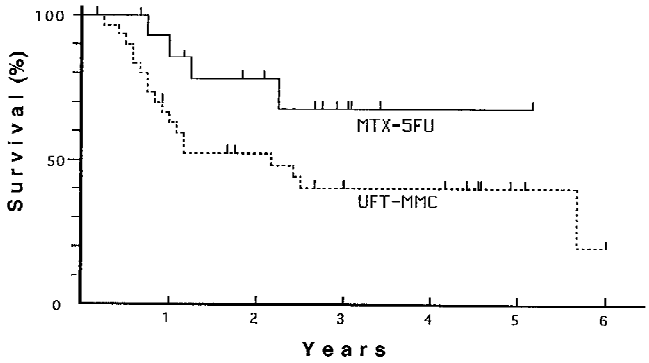

Fig. 2. Disease-free survival rates after surgery, calculated by the Kaplan-Meier method, were significantly ($P < 0.05$ by the Cox-Mantel test) higher in the MFL than the UFT-MMC group.

TABLE III. Recurrence Site and Rate in Patients With Colorectal Cancer*

Recurrence pattern	Group A[a] (n = 30)	Group B[b] (n = 16)	*P* value
Peritoneum	3	1	
Liver	7	1	
Lymph node	2	0	
Bone	3	0	
Pelvic organs	6	2	
Total (incidences/cases)	21/17	4/4	
Total (cases)	17/30	4/16	$P = 0.04$

*Several cases developed plural sites of recurrence.
[a]UFT-MCC = tegafur plus mitomycin-C.
[b]MFL = sequential methotrexate plus fluorouracil followed by leukovorin rescue.

long-term follow-up due to the presence of residual occult disease and distant micrometastasis, although the recurrence has been suppressed in some cases [1,2]. A variety of adjuvant therapies to improve the disease-free interval and survival have been developed and tried in these patients [12,16]. However, the majority of them have failed to show any significant advantage. Furthermore, for patients after noncurrative resection of advanced disease, few of the standardized protocols have successfully controlled the residual disease.

TABLE IV. Drug Toxicities in Colorectal Cancer Patients on Two Adjuvant Chemotherapeutic Regimens

Toxicity (Grade II or greater)[a]	Group A[b] (n = 30)	Group B[c] (n = 16)	*P* value
Hematologic			
Hemoglobin (<9.5g/dL)	1	0	
White blood cells (<3,000/uL)	5 (2)	4	
Platelets (<75,000/uL)	4 (1)	1	
Gastrointestinal			
Nausea, vomiting	1	3	
Liver dysfunction	3	1	
Diarrhea	0	1	
Renal (blood urea nitrogen >40 mg/dL)	1	0	
Total (incidences/cases)	15/11	10/7	
Total (cases)	11/30 cases	7/16 cases	NS

[a]Several cases had plural patterns of toxicities. Toxicity grade III is in parentheses.
[b]UFT-MCC = tegafur plus mitomycin-C.
[c]MFL = sequential methotrexate plus fluorouracil followed by leukovorin rescue.
NS = no significant difference.

For decades, the agent most widely used in the treatment of colorectal cancer has been the antimetabolite 5-FU. This fluoridated pyrimidine has been available for >30 years; yet to-date, no other single agent has proven to be more effective [17]. In patients after radical colorectal resection, tegafur-MMC or UFT-MMC often has been used for adjuvant chemotherapy [18]. Also in our institute, many patients have been managed with UFT-MMC after surgery for advanced colorectal carcinoma. Many clinical studies have been conducted to evaluate their efficacy [6–8]. Significant improvement in the disease-free interval and/or patient survival after surgery was sometimes observed with these oral fluoridated pyrimidine derivatives [6]. However, most studies did not succeed in proving significant differences in these values for different regimens [7,8]. Moreover, carcinoma recurrence and metachronous metastasis are occasionally experienced even during adjuvant chemotherapy, in particular, liver metastasis and pelvic recurrence were not prevented [9,18]. This may have resulted from the drug delivery route since they were administered orally. In a study measuring the serum level of 5-FU in patients with oral tegafur or UFT, minimal serum levels were maintained in 75% of the patients, even with regular administration according to the prescription [19].

Recently, in patients with advanced primary colorectal carcinoma without surgical intervention, MFL chemotherapy has proved to be effective [13]. Meta-analysis of randomized trial testing of sequential MFL versus 5-FU alone showed a statistically significant benefit in contrast to the lack of benefit reported in a meta-analysis comparing 5-FU alone with 5FU/LV [20,21]. Consequently

in surgically treating patients, MFL may be expected to kill the residual carcinoma cells and prevent the recurrence of carcinoma more effectively than previous adjuvant chemotherapy. Although the optimal combination of MTX, 5-FU, and LV still remains to be elucidated, recent studies indicated that a long-time interval between MTX and 5-FU, as used in our regimen, results in higher response rates for colorectal carcinoma [22–24]. Using short-time intervals, no benefit of sequential treatment has been found [25–27]. A clinical review indicated an interval of 24 hours was most effective for colorectal carcinoma [12]. In this study, therefore, 5-FU was administered 24 hours after MTX. Furthermore, a 24-hour interval proved convenient for patients when MFL therapy was continued on an outpatient basis.

This study clearly demonstrates that a combination of cytostatics based on MFL is superior to UFT-MMC for adjuvant chemotherapy in postsurgical colorectal carcinoma. This superiority was shown by the decreased recurrence rates and prolonged patient survival as well as the increased disease-free interval. MFL definitely prevented hepatic recurrence in this study. The treatment groups contained relatively small numbers of patients who varied in clinical stage, since this is a first preliminary study. However, the two groups were well balanced concerning all clinical characteristics, which indicates that the outcome was probably not influenced by the differences between the two groups.

The toxicity of the two groups was comparable. The incidence of liver dysfunction was frequent in the UFT-MMC group, whereas nausea occurred frequently in the MFL group. Renal toxicity, which affected the MFL group, was not experienced in this clinical trial, perhaps because the number of treatment courses was relatively small. To date, the toxicity of MFL has been limited to less than grade 2. From the viewpoint of toxicity, therefore, MFL can be continued on an outpatient basis.

The present results demonstrate the superiority of sequential MTX-5-FU treatment for improving the postsurgical survival of patients with advanced colorectal cancer, in particular those patients with a high risk of recurrence following a potential curative resection. A prospective study based on the stage of the disease is now in progress.

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