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# Combination Chemotherapy with Methotrexate, Leucovorin, and 5-Fluorouracil for Advanced Colorectal Cancer

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*ABSTRACT*

Purpose : To evaluate the efficacy and toxicity of combination therapy with methotrexate, leucovorin, and 5-fluorouracil (5-FU) in patients with metastatic colorectal cancer.

Methods : Methotrexate was administered intravenously at a dose of 60 mg/m². Twenty-four hours later, 60 mg/m² of leucovorin was given intravenously, after which 600 mg/m² 5-FU was administered as an intravenous bolus. This treatment was repeated every 2 weeks.

Results : Fifty patients were enrolled. Nine partial responses (PRs) occurred in 38 measurable patients, for a response rate of 24% (95% confidence interval, 13% to 37%). Of the 17 patients who had not previously received chemotherapy, 6 had PRs, for a response rate of 35% (95% confidence interval, 15% to 60%). Median response duration of the 9 PRs was 6.5 months (range, 1.2 to 34.9 months). Overall median survival time was 12.0 months (range, 0.8 to 52.7 months)

Conclusion : This combination therapy with methotrexate, leucovorin, and 5-FU given every 2 weeks may enhance cytotoxicity, without significant adverse effects, over that of two-drug regimens using either leucovorin or methotrexate combined with 5-FU for the treatment of colorectal cancer. (Jikeikai Med J 2005 ; 52 : 63-9)

Key words : chemotherapy, colorectal cancer, methotrexate, leucovorin, 5-fluorouracil, phase II study

## INTRODUCTION

5-Fluorouracil remains one of the most active agents for colorectal cancer, although its response rate is only 10% to 20%. Many attempts have been made to enhance the cytotoxicity of 5-FU through biomodulation. 5-FU inhibits protein synthesis by incorporating fluorouridine triphosphate into RNA, thereby disrupting RNA processing and function. The ribosylphosphorylation of 5-FU to its active metabolite, fluorouridine triphosphate, is limited by the availability of phosphoribosylpyrophosphate. Methotrexate potentiates 5-FU cytotoxicity by the accumulation of phosphoribosylpyrophosphate through the inhibition of purine metabolism.

The interval between the adminstration of methotrexate and that of 5-FU is the most important factor for maximizing cytotoxicity. Marsh et al.[1] have observed higher response rates and longer survival when the administration interval between methotrexate and 5-FU was 24 hours than when it was 1 hour. However, the most effective dose of methotrexate for the treatment of colorectal cancer has not been determined. Kemeny et al.[2] used methotrex-

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ate at a dose of 40 mg/m² and obtained a response rate of 32%, which was comparable to that with high-dose methotrexate.

The other mechanism of action of 5-FU is its conversion into fluorodeoxyuridylate, which inhibits thymidylate synthase. In the presence of L-5, 10-methylene tetrahydrofolate, fluorodeoxyuridylate binds tightly to thymidylate synthase and leads to the formation of a covalent ternary complex. Leucovorin potentiates 5-FU cytotoxicity through stabilization of the ternary complex.

We conducted a combination phase II study with a 2-week double biomodulation schedule using methotrexate before administering leucovorin and 5-FU for the treatment of metastatic colorectal cancer.

## Patients and Methods

*Patient selection*

Patients were eligible for this study if they had inoperable metastatic colorectal cancer that had been was histologically confirmed to be adenocarcinoma, an Eastern Cooperative Oncology Group performance status of at least 3, adequate function of other organs, and an estimated survival time of at least 2 months. Patients who had had chemotherapy were not excluded. Informed consent was obtained from all patients before the start of treatment.

*Treatment schedule*

Methotrexate was administered intravenously at a dose of 60 mg/m². Twenty-four hours later, leucovorin (60 mg/m²) was given intravenously, followed by intravenous bolus adminstration of 5-FU (600 mg/m²). These treatments were repeated every 2 weeks. Patients were usually treated on an outpatient basis. The treatment regimen was designed for a minimum of 4 courses or until progressive disease was documented. Follow-up studies, which were performed every 2 weeks, included a physical examination, complete blood counts, liver function tests, and creatinine clearance tests. Carcinoembryonic antigen was measured at least every 4 weeks, and radiography or computed tomography was performed every 2 months.

*Dose modification criteria*

The dose of methotrexate was not reduced even if patients had small amounts of pleural effusion or ascites. When grade 2 or greater mucositis or myelosuppression was observed, the treatment was withheld until recovery to pretreatment levels.

*Response evaluation*

A complete response (CR) was defined as the complete disappearance of all clinically detectable disease for at least 1 month, and a partial response (PR) was defined as reduction in the volume of detectable lesions of greater than 50%. No change (NC) was defined as a reduction in lesion volume of less than 50% or an increase in previous lesions of less than 25% but without the appearance of new lesions. Progressive disease (PD) was defined as an increase in measurable disease of greater than 25% or the appearance of new lesions. The median duration of response and survival time were estimated with the Kaplan-Meier method.

*Toxicity evaluation*

Toxicity was scored according to World Health Organization criteria[3].

## Results

*Patient characteristics*

Fifty patients were enrolled from April 1991 through September 1998. Although all patients had distant metastases, 10 patients who did not have any measurable lesions and 2 patients who refused further treatment after 1 or 2 cycles of treatment were excluded from the response analysis. The remaining 38 patients were evaluable for treatment response, and all 50 patients were evaluated for toxicity. The pretreatment characteristics of the 50 patients are shown in Table 1. Twenty-nine patients had received chemotherapy. Of these patients, 11 had received postoperative adjuvant chemotherapy, usually including of oral 5-FU. Others had chemotherapy as a first-line treatment for advanced diseases. Eleven patients received 5-FU, 5 patients received combination chemotherapy with interferon-$\alpha$ and 5-

Table 1. Patient characteristics

| | |
|---|---|
| No. of Patients | 50 |
| No. of Measurable Patients | 38 |
| Sex (M/F) | 29/21 |
| Median Age (years) | 55 (range ; 27-73) |
| Median Performance Status | 0 (0-3) |
| Primary site | |
| Colon | 29 |
| Rectum | 21 |
| Prior chemotherapy | |
| Yes | 29 |
| No | 21 |
| Metastatic site | |
| Liver | 33 |
| Lung | 14 |
| Lymph nodes | 12 |

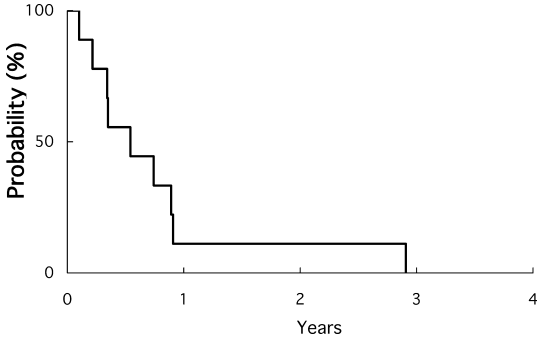


Fig. 1. Response duration for patients with advanced colon cancer treated with methotrexate, leucovorin, and 5-FU

Table 2. Response to methotrexate, leucovorin, and 5-fluorouracil for evaluable patients with colorectal cancer

| | No. of patients | PR | NC | PD | Response rate (%) |
|---|---|---|---|---|---|
| Prior chemotherapy | 17 | 6 | 8 | 3 | 35 |
| No prior chemotherapy | 21 | 3 | 8 | 10 | 14 |
| Total | 38 | 9 | 16 | 13 | 24 |

FU, and 2 patients received irinotecan and 5-FU. The median number of times of previous chemotherapy was 1 (1 to 5 regimens). The median duration of follow-up was 50 months (range, 5 to 64 months), and the median number of treatment courses was 6 (range, 2 to 34 courses).

*Response*

The evaluable patients had 9 PRs, for a response rate of 24% (95% confidence interval [CI], 13%-37%). The subgroup of 17 evaluable patients who had not had previous chemotherapy had 6 PRs, for a response rate of 35% (95% CI : 15% to 60% ; Table 2). In contrast, the subgroup of 21 patients who had had prior chemotherapy had 3 PRs (14%). The median duration of response in the 9 PR cases was 6.5 months (range, 1.2 to 34.9 months ; Fig. 1). One patient with a PR refused further chemotherapy and was transferred to another hospital after the response was evaluated 1.6 months after the start of chemotherapy. The PR duration in this patient was 1.2 months. The location of the primary tumor (colon vs rectum), major metastatic site, sex, performance status (0 or 1 versus 2), and histological subtype had no effect on response (data not shown).

*Survival*

Overall median survival time (MST) was 12 months (range, 0.8 to 52.7 months), and the MST in patients with PRs was 24.6 months (range, 1.6 to 52.7 months). The MST was 20.5 months in patients who had not received previous chemotherapy and 10.1 months in patients who had received previous therapy. The Kaplan-Meier survival curves are shown in Fig. 2.

*Toxicity*

All 50 patients were evaluated for toxicity (Table 3). Toxicities were generally mild. Nausea and vomiting was the most common toxicity, but patients could resume treatment without interruption or dose modification. One patient discontinued treatment

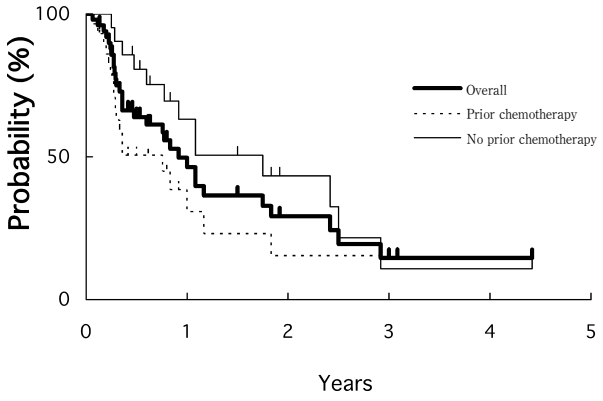


Fig. 2. Survival of patients with advanced colon cancer treated with methotrexate, leucovorin, and 5-FU.

Table 3. Toxicity percentage of methotrexate, leucovorin, and 5-fluorouracil according to World Health Organization grades

| | Grade | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| Hematological | | | | |
| Leukopenia | 31 | 9 | 8 | 2 |
| Anemia | 28 | 7 | 2 | 2 |
| Thrombocytopenia | 49 | 1 | | |
| Nonhematological | | | | |
| Nausea/Vomiting | 16 | 26 | 6 | 2 |
| Stomatitis | 30 | 17 | 2 | 1 |
| Diarrhea | 34 | 11 | 3 | 2 |

because of severe diarrhea. Leukopenia was observed in 38% patients, but no patient had leukopenic fever. No treatment-related death occurred.

## DISCUSSION

5-FU remains the primary drug for the treatment of advanced colorectal cancer. However, response rates with 5-FU alone are only 10% to 20%. Many agents, such as methotrexate, interferon, and leucovorin, have been combined with 5-FU in attempts to improve its efficacy. Biochemical modulation has been suggested to be the mechanism of the increased efficacy of these combinations.

Wadler et al.[4] have reported a response rate of 76% with the combination therapy of interferon and 5- FU, but subsequent phase II studies using the same schedule and dosage have achieved response rates of only 26% to 42%[5-7]. Meta-analysis[8,9] has shown response rates with leucovorin/5-FU and with methotrexate/5-FU of 23% and 19%, respectively. Petrelli et al.[10] have performed a randomized trial to compare 5-FU, leucovorin/5-FU, and methotrexate/5-FU. Higher response rates were achieved with leucovorin/5-FU than with the other treatments. The Corfu-A Study Group[11] has also compared leucovorin /5-FU and interferon/5-FU in a randomized trial. Response rates with both treatments were similar, but more patients withdrew from the interferon/5-FU arm because of its toxicity. These results indicate that the basic regimen for colorectal cancer in Western countries is leucovorin/5-FU. However, meta-analysis has shown that although leucovorin/5-FU has a higher response rate than does 5-FU alone, it does not confer a survival benefit. Effective drugs for colorectal cancer have recently been developed. One such drug is irinotecan, an inhibitor of topoisomerase I. Irinotecan has been combined with LV/5-FU in an attempt to response rates and survival. Saltz et al.[12] have reported that the combination of irinotecan, leucovorin, and 5-FU achieved a higher response rate (39%) and survival rate than did leucovorin/5-FU but produced severe gastrointestinal toxicities (nausea, vomiting, and diarrhea) and hematological toxicity. The dose-limiting toxicities are diarrhea and neutropenia. Because of its toxicity, irinotecan is administered only to inpatients. Furthermore, irinotecan is contraindicated if a patient has constipation or mechanical ileus. Although the combination of irinotecan and LV/5-FU remains a promising regimen, limiting its indications would be prudent.

Oxaliplatin, an analogue of cisplatin, is also an effective drug for colorectal cancer, but its effectiveness and toxicity in Japanese patients remain unclear. In a phase I/II study the response rate of oxaliplatin is 10% to 24%, and the combination of oxaliplatin and LV/5-FU, designated FOLFOX4, shows synergistic effects against colorectal cancer[13]. However, the administration of 5-FU in this combination is complicated (bolus and continuous infusion for 22 hours on days 1 and 2). The hematological toxicities are

moderate. Neurotoxicity is the dose-limiting toxicity. Hospitalization is required for patients treated with FOLFOX4. A recent phase III study by Goldberg et al.[14] has shown that FOLFOX4 is more active and less toxic, except for neurotoxicity, than is the combination of irinotecan, leucovorin, and 5-FU.

Bevacizumab, a monoclonal antibody against endothelial growth factor, has shown clinical activity against colorectal cancer. A randomized, controlled phase III study by Hurwitz et al.[15] has shown that the addition of bevacizumab to irinotecan, leucovorin, and 5-FU results in a clinically meaningful improvement in survival in patients with metastatic colorectal cancer. The combination of irinotecan, leucovorin, and 5-FU with oxaliplatin or bevacizumab or both will be evaluated in the next several years.

We investigated the efficacy and toxicity of the sequential combination of methotrexate, leucovorin, and 5-FU in patients with metastatic colorectal cancer. The response rate was 35% in patients who had not received previous chemotherapy and 14% in previously treated patients. The response duration and overall survival were 6.6 and 8+months, respectively. This result suggests that the addition of methotrexate enhances the cytotoxicty of LV/5-FU.

The toxicities in the present study were usually mild, and the incidence of grade 3 leukopenia, nausea and vomiting, diarrhea, or stomatitis ranged from 2% to 4%. Conti et al.[16] have reported that diarrhea of grade 3 or 4 occurred in 17% of patients receiving sequential trimethotrexate, fluorouracil, and high-dose leucovorin. Petrelli et al.[10] have reported that high-dose leucovorin (50 mg/m²) shows a greater therapeutic effect against metastatic colorectal cancer than does low-dose leucovorin (25 mg/m²). However, severe diarrhea occurred in 26% of patients receiving high-dose leucovorin. The optimal dose and schedule of leucovorin have not been determined. In the present study, in which leucovorin at a dose of 60 mg/m² was administered every 2 weeks, the incidence of grade 3 diarrhea was 4%. Therefore, we do not consider diarrhea to be a dose-limiting toxicity. The combination of methotrexate, leucovorin, and 5-FU might have a lower response rate than does FOLFOX4 or irinotecan, leucovorin, and 5-FU but it is less toxic. However, combination chemotherapy with methotrexate, leucovorin, and 5-FU has advantages over other regimens in that it can be administered safely, especially to elderly patients, and does not require hospitalization.

Several reports have been published about double modulation of 5-FU with methotrexate and leucovorin in the treatment of advanced colorectal carcinoma. The results of these studies are summarized in Table 4[17-22]. Response rates ranged from 20% to 30% and were higher than those with leucovorin /5-FU or 5-FU alone. However, Valone et al.[17] have reported that combination therapy with methotrexate, leucovorin, and 5-FU prolongs the time to failure but does not improve overall survival compared with 5-FU alone. Abad et al.[20] also failed to show a significant difference in overall survival between the combination of methotrexate, leucovorin, and 5-FU and that of leucovorin /5-FU. We achieved better

Table 4. Summary of methotrexate, leucovorin, and 5-fluorouracil in previously untreated patients with colorectal cancer

| Author | methotrexate (mg/m²) | leucovorin (mg/m²) | 5-fluorouracil (mg/m²) | Interval between MTX and FU (hours) | Treatment Interval (wks) | Response Rate (%) | Median Survival Time (mo) |
|---|---|---|---|---|---|---|---|
| Valone[17] | 250 | 50 | 500 | 24 | 2 | 19.8 | 12 |
| NGTATG[18] | 250 | 120 | 1,000 | 2 | 2 | 24 | 8.5 |
| Balaban[19] | 30-70 | 100 | 600 | 17-24 | 2 | 29 | 9.3 |
| Abad[20] | 500 | 200 | 600 | 12 | 2 | 25.7 | 14.3 |
| Glimelius[21] | 250 | 120 | 1,000 | 2 | 2 | 17 | 7.5 |
| Comella[22] | 500 | 250 | 600 | 24 | 2 | 26 | 14.3 |
| Present study | 60 | 60 | 600 | 24 | 2 | 35 | 20.5 |

NGTATG: Nordic Gastrointestinal Tumor Adjuvant Therapy Group

response rates and less toxicity in our present study. The double modulation of 5-FU for treating patients with metastatic colorectal cancer should be re-evaluated.

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## REFERENCES

1. Marsh JC, Bertino JR, Katz KH, Davis CA, Durivage HJ, Rome LS, et al. The influence of drug interval on the effect of methotrexate and fluorouracil in the treatment of advanced colorectal cancer. J Clin Oncol 1991; 9: 371-80.
2. Kemeny NE, Ahmed T, Michaelson RA, Harper HD, Yip LC. Activity of sequential low-dose methotrexate and fluorouracil in advanced colorectal carcinoma: attempt at correlation with tissue and blood levels of phosphoribosylpyrophosphate. J Clin Oncol 1984; 2: 311-5.
3. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47: 207-14.
4. Wadler S, Schwartz EL, Goldman M, Lyver A, Rader M, Zimmerman M, et al. Fluorouracil and recombinant alfa-2a-interferon: an active regimen against advanced colorectal carcinoma [see comments]. J Clin Oncol 1989; 7: 1769-75.
5. Kemeny N, Younes A, Seiter K, Kelsen D, Sammarco P, Adams L, et al. Interferon alpha-2a and 5-fluorouracil for advanced colorectal carcinoma: assessment of activity and toxicity. Cancer 1990; 66: 2470-5.
6. Pazdur R, Ajani JA, Patt YZ, Winn R, Jackson D, Shepard B, et al. Phase II study of fluorouracil and recombinant interferon alfa-2a in previously untreated advanced colorectal carcinoma. J Clin Oncol 1990; 8: 2027-31.
7. Wadler S, Lembersky B, Atkins M, Kirkwood J, Petrelli N. Phase II trial of fluorouracil and recombinant interferon alfa-2a in patients with advanced colorectal carcinoma: an Eastern Cooperative Oncology Group study. J Clin Oncol 1991; 9: 1806-10.
8. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. Advanced Colorectal Cancer Meta-Analysis Project. J Clin Oncol 1992; 10(6): 896-903.
9. Meta-analysis of randomized trials testing the biochemical modulation of fluorouracil by methotrexate in metastatic colorectal cancer. Advanced Colorectal Cancer Meta-Analysis Project. J Clin Oncol 1994; 12: 960-9.
10. Petrelli N, Herrera L, Rustum Y, Burke P, Creaven P, Stulc J, et al. A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. J Clin Oncol 1987; 5: 1559-65.
11. Phase III randomized study of two fluorouracil combinations with either interferon alfa-2a or leucovorin for advanced colorectal cancer. Corfu: a study group. J Clin Oncol 1995; 13: 921-8.
12. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 2000; 343: 905-14.
13. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000; 18: 2938-47.
14. Goldberg RM, Sargent DJ, Morton RF, Fucks CS, et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 2004; 22: 23-30.
15. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Eng J Med 2004; 350: 2335-42.
16. Conti JA, Kemeny N, Seiter K, Goker E, Tong W, Andre M, et al. Trial of sequential trimethotrexate, fluorouracil, and high-dose leucovorin in previously treated patients with gastrointestinal carcinoma. J Clin Oncol 1994; 12: 695-700.
17. Valone FH, Friedman MA, Wittlinger PS, Drakes T, Eisenberg PD, Malec M, et al. Treatment of patients with advanced colorectal carcinomas with fluorouracil alone, high-dose leucovorin plus fluorouracil, or sequential methotrexate, fluorouracil, and leucovorin: a randomized trial of the Northern California Oncology Group. J Clin Oncol 1989; 7: 1427-36.
18. Superiority of sequential methotrexate, fluorouracil, and leucovorin to fluorouracil alone in advanced symptomatic colorectal carcinoma: a randomized trial. The Nordic Gastrointestinal Tumor Adjuvant Therapy Group. J Clin Oncol 1989; 7: 1437-46.
19. Balaban EP, Graham M, Perkins S, Sheehan RG, Frenkel EP, Ross M, et al. Double modulation of 5-fluorouracil in the treatment of advanced colorectal carcinoma: report of a trial with sequential methotrexate, intravenous (loading dose) folinic acid, 5-fluorouracil, and a literature review (review). Cancer Invest 1994; 12: 12-9.
20. Abad A, Garcia P, Gravalos C, Tusquets I, Font A, Perez G, et al. Sequential methotrexate, 5-fluorouracil (5-FU), and high dose leucovorin versus 5-FU and high dose leucovorin versus 5-FU alone for advanced colorectal cancer: a multi-institutional randomized trial. Cancer 1995; 75: 1238-44.

21. Glimelius B. Biochemical modulation of 5-fluorouracil : a randomized comparison of sequential methotrexate, 5-fluorouracil and leucovorin versus sequential 5-fluorouracil and leucovorin in patients with advanced symptomatic colorectal cancer. The Nordic Gastrointestinal Tumor Adjuvant Therapy Group. Ann Oncol 1993 ; 4 : 235-40.
22. Comella P, Palmieri G, Lorusso V, Catalano G, Nicollela D, Ianniello GP, et al. Double biochemical modulation of 5-fluorouracil by methotrexate and levo-folinic acid in the treatment of advanced digestive tract malignancies. Eur J Cancer 1996 ; 32A(10) : 1719-26.