Table 2.
Postoperative Recurrence in Patients With Dukes A+B Disease

	Dukes A+B Disease						
	LR	LR+DM	DM	Others	Unknown	LR Rate (%)[a]	DM Rate (%)[a]
Period 1975–1984							
TME-P(–) (n = 103)	3	2	11	1	2	4.9	12.6
Period 1985–1999							
TME-P(+) (n = 102)	3	1	8	4	0	3.9	8.8
TME-P(–) (n = 22)	2	0	3	2	0	9.1	13.6

LR = local (pelvic) recurrence; DM = distant metastasis including liver, lung, cerebrum, or bone metastasis; Others = peritoneal seeding and inguinal nodes metastasis; TME-P(–) = total mesorectal excision without autonomic nerve preservation; TME-P(+) = total mesorectal excision with autonomic nerve preservation.
[a]No significant difference was noted among any groups.

Registry

Both clinical and histologic detailed information were registered into our computer, which was set up in 1982 using the database (dBASE III software, Ashton-Tate, Torrance, CA). Since 1982, both clinical information and histologic findings of resected specimens have been prospectively registered in detail into the database.[6–9] Before 1982, those data were retrospectively registered by reviewing the medical records and available histologic findings. Since 1995, we have been using the Visual dBASE software (Borland dBASE version 5.6J, CA) in the Windows Operating System (Microsoft).

Follow-Up

Follow-up investigation was performed by outpatient visits, letter, and telephone. We also obtained information about the condition of patients from doctors of our affiliated public or private hospitals. The most recent date of contact was regarded as the final date of confirmation in each case. The overall final follow-up date was the last day of May 2003. For the purposes of this study, patients received postoperative protocol surveillance including tumor marker measurement, chest radiography, and ultrasonography every month for the first year, every three to six months for the next two, three, and four years, and then annually. Since 1985, CT or magnetic resonance imaging (MRI) was performed to identify the recurrence site when it was suspected.

A total of 6 of 403 patients (1.4 percent) were lost to follow-up by the final follow-up date, but 4 of 6 patients had been followed for at least ten years. The median follow-up of survival patients was 218 (range, 129–328) months in the Period 1975 to 1984 and 115 (range, 27–217) months in the Period 1985 to 1999. The presence or absence of recurrence was determined by digital examination, barium enema, measurement of serum tumor marker level, and findings on chest radiography, ultrasound, CT, and MRI. The site of recurrence was recorded for each patient and defined as local (or pelvic) recurrence, distant metastasis (liver, lung, bone, or cerebrum), or peritoneal dissemination.

Statistics

The chi-squared and Fisher's exact tests were used to determine the statistical significance of any difference, and the Kaplan-Meier method[10] was used to calculate survival rates. Any significant difference in the survival rate was assessed using the log-rank test according to Peto *et al.*[11] The level of significance was defined at $P < 0.05$.

RESULTS

Dukes A+B Disease

Local Recurrence. In the TME-P(–) group of Period 1975 to 1984, LR rate was 4.9 percent (Table 2). In the TME-P(–) and TME-P(+) groups of Period 1985 to 1999, LR rates were 9.1 and 3.9 percent, respectively. No significant difference was noted among the three groups.

Distant Metastasis. In the TME-P(–) group of Period 1975 to 1984, distant metastasis (DM) rate was 12.6 percent (Table 2). In the TME-P(–) and TME-P(+) groups of Period 1985 to 1999, DM rates were 13.6 and 8.8 percent, respectively. No significant difference was noted among the three groups.

Survival. Ten-year, disease-free survival (10YDFS) rate was 77 percent in the TME-P(–) group of Period 1975 to 1984 (Fig. 1). In the Period 1985 to 1999, it

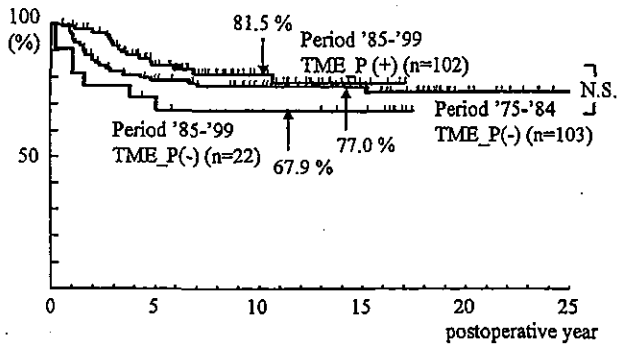

Figure 1. Ten-year, disease-free survival (10YDFS) in patients with Dukes A+B disease. The 10YDFS was 77 percent in the TME-P(−) group of Period 1975 to 1984, 81.5 percent in the TME-P(+) group of Period 1985 to 1999, and 67.9 percent in the TME-P(−) group of Period 1985 to 1999. No significant difference was noted among the three groups.

was 81.5 percent in the TME-P(+) group and 67.9 percent in the TME-P(−) group. No significant difference was noted among the three groups. More than ten years after the operation, one patient had local recurrence (127 months later) and another had liver metastasis (181 months later).

Dukes C Disease

Local recurrence. In the TME-P(−) group of Period 1975 to 1984, LR rate was 25.3 percent (Table 3). In the TME-P(−) and TME-P(+) groups of in Period 1985 to 1999, LR rates were 16.7 and 16.4 percent, respectively. No significant difference was noted among the three groups.

Distant metastasis. In the TME-P(−) group of Period 1975 to 1984, DM rate was 33 percent (Table 3). In the TME-P(−) and TME-P(+) groups of Period 1985 to 1999, DM rates were 66.7 and 34.3 percent, respectively. Significant differences were noted between the TME-P(−) group of Period 1975 to 1984 and the TME-P(−) group of Period 1985 to 1999 ($P = 0.016$), and between the TME-P(+) group and TME-P(−) group of Period 1985 to 1999 ($P = 0.027$).

Survival. The 10YDFS rate was 47.7 percent in the TME-P(−) group of Period 1975 to 1984 and 49.7 percent in the TME-P(+) group of Period 1985 to 1999 (Fig. 2). No significant difference was noted between the two groups. In the TME-P(−) group of Period 1985 to 1999, the 10YDFS was 16.7 percent and was significantly lowest than any other groups ($P < 0.001$). More than ten years after operation, one patient had liver metastasis (188 months later) and another had local recurrence (189 months later).

Urinary and Sexual Function

In the TME-P(−) group, the urinary disorder was found at a high rate of > 90 percent, and sexual function in males younger than aged 60 years did not recover (Table 4). In the TME-P(+) group, the urinary function was preserved at > 80 percent, and erection was preserved at 79 percent and ejaculation at 65 percent.

DISCUSSION

Sterns and Deddish[12] tried to establish lateral lymphadenectomy during the 1950s, but could not find any clinical significance. Therefore, many doctors in Europe renounced LLA and moved to the concept of TME. In Japan, LLA was widely performed between the mid 1970s and the early 1980s.[1–3] The term, TME, was not used during the period, because it was not known well in Japan. However, not only LLA but also rectal resection including total mesorectum was performed for lower rectal cancer even more than 20 years ago. Therefore, local recurrence reduced and survival improved in Japan. Oppositely, functional disorder such as urinary or sexual problems occurred at a high rate. The functional disorder after LLA is caused by sacrificing the autonomic nerve plexus, which occurred at an extremely high rate during Period 1975 to 1984, as reported by some authors,[1,5] because, either hypogastric or pelvic plexus was killed without paying any attention to them during this period. That is why the operation before 1985 was retrospectively defined as TME-P(−). Since 1985 in our department, autonomic nerve preservation came into wide application for selected patients to avoid functional disorder. In addition, the term, TME, came to be known well and commonly used in Japan. Therefore, the operation since 1985 was defined as TME-P(+). However, nothing except for ANP has basically changed concerning both TME and LLA during the last two decades.

As for function, urinary or sexual disorder was found at a high rate in the Period 1975 to 1984, but these disorders extremely reduced to a low rate in the Period 1985 to 1999. However, sexual function was not completely preserved, although autonomic nerves were preserved, because unilateral preservation was included or mental factor may have influenced the sexual function. Nevertheless, ANP was an excellent procedure to avoid functional disorder.

On the other hand, there are oncologically some

Table 3.
Postoperative Recurrence in Patients With Dukes C Disease

	Dukes C Disease						
	LR	LR+DM	DM	Others	Unknown	LR Rate (%)	DM Rate (%)
Period 1975–1984							
TME-P(–) (n = 91)	13	10	20	3	1	25.3[a]	33[a,b]
Period 1985–1999							
TME-P(+) (n = 67)	7	4	19	1	0	16.4[a]	34.3[a,b]
TME-P(–) (n = 18)	1	2	10	0	1	16.7[a]	66.7[b]

LR = local (pelvic) recurrence; DM = distant metastasis including liver, lung, cerebrum, or bone metastasis; Others = peritoneal seeding and inguinal nodes metastasis; TME-P(–) = total mesorectal excision without autonomic nerve preservation; TME-P(+) = total mesorectal excision with autonomic nerve preservation.

[a]No significant difference was noted among any groups.

[b]Significant difference was noted between the two groups.

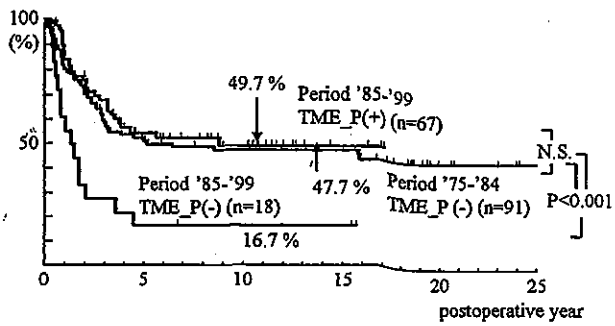

Figure 2. Ten-year, disease-free survival (10YDFS) in patients with Dukes C disease. The 10YDFS rate was 47.7 percent in the TME-P(–) group of Period 1975 to 1984, and 49.7 percent in the TME-P(+) group of period 1985 to 1999. No significant difference was noted between the two groups. In the TME-P(–) group of Period 1985 to 1999, the 10YDFS was 16.7 percent, which was significantly lower than any other groups ($P < 0.001$).

Table 4.
Urinary and Sexual Function

Urinary Function During Hospital Stay			
	Good	Fair	Poor
TME-P(–) (n = 172)	2	56	42
TME-P(+) (n = 120)	84	14	2
Sexual Function in Males Younger Than Aged 60 Years			
	Erection	Ejaculation	
TME-P(–) (n = 80)	0	0	
TME-P(+) (n = 49)	79	65	

Good = possible self-urination; Fair = frequent or prolonged urination, sense of residual urine; Poor = necessity of urethral catheter, severe incontinence.

Data are percentages.

important problems concerning ANP. When the nerve plexus is preserved, there may be a risk in which cancer cells remain around the nerves. Especially, in Dukes C disease, it is said that local recurrence will occur at an extremely high rate. These problems have been discussed for a long time in Japan. However, in our department, local recurrence, distant metastasis, or disease-free survival in patients with ANP was not inferior to patients without ANP. Our results clearly showed good prognosis in patients with ANP. Therefore, ANP is not only reasonable to avoid functional disorder but also is an excellent and successful curative surgery, as reported by other authors.[13–18] Of course, the nerve plexus should be killed when cancer cells are suspected to have directly invaded themselves. ANP is selected depending on the presence or absence of tumor invasion, which means patients selection bias. In the Period 1985 to 1999, autonomic nerves were killed only in patients having suspicious invasion to the nerves, and perineural invasion was histologically demonstrated at a high rate. That is why disease-free survival in the TME-P(–) group of Period 1985 to 1999 was the lowest among the three groups. Also, in the group of Dukes C disease, distant metastasis was the highest than any other groups. Oral administration of anticancer drug was given to patients whose autonomic nerves were killed, but we could not prevent distant metastasis. These patients of this group need intensive chemotherapy after operation.

CONCLUSIONS

To establish the significance of ANP, a randomized controlled trial may be needed. In Japan, randomized controlled trials are not ethically accepted. Therefore, clinicopathologic data based on reliable long-term follow-up is extremely important. A few patients had recurrence at more than ten years after operation. This means the necessity of long-term follow-up. We

concluded that ANP was oncologically and functionally excellent and suitable for almost patients with advanced lower rectal cancer. Intensive chemotherapy is needed for patients whose autonomic nerves were killed in suspicion of nerve invasion.

REFERENCES

1. Hojo K, Sawada T, Moriya Y. An analysis of survival and voiding, sexual function after wide iliopelvic lymphadenectomy in patients with carcinoma of the rectum, compared with conventional lymphadenectomy. Dis Colon Rectum 1989;32:128–33.
2. Koyama Y, Moriya Y, Hojo K. Effect of extended systemic lymphadenectomy for adenocarcinoma of the rectum – significant improvement of survival rate and decrease of LR. Jpn J Clin Oncol 1984;14:623–32.
3. Moriya Y, Sugihara K, Akasu T, Fujita S. Importance of extended LN dissection for advanced lower rectal cancer. World J Surg 1997;21:728–32.
4. Heald RJ, Ryall RD. Recurrence and survival after total mesorectal excision for rectal cancer. Lancet 1986;1: 1479–82.
5. Enker WE. Total mesorectal excision – the new golden standard of surgery for rectal cancer. Ann Med 1997;29: 127–33.
6. Shirouzu K, Isomoto H, Kakegawa T. A prospective clinicopathologic study of venous invasion in colorectal cancer. Am J Surg 1991;162:216–22.
7. Shirouzu K, Isomoto H, Kakegawa T. Prognostic evaluation of perineural invasion in rectal cancer. Am J Surg 1993;165:233–37.
8. Shirouzu K, Isomoto H, Morodomi T, Kakegawa T. Carcinomatous lymphatic permeation. Prognostic significance in patients with rectal carcinoma: a long-term prospective study. Cancer 1995;75:4–10.
9. Shirouzu K, Isomoto H, Kakegawa T. Distal spread of rectal cancer and optimal distal margin of resection for sphincter-preserving surgery. Cancer 1995;76:388–92.
10. Kaplan EL, Meier P. Non-parametric estimation from incomplete observation. J Am Stat Assoc 1958;53:457–81.
11. Peto R, Pike MC, Armitage NE, *et al.* Design and analysis of randomized clinical trials requiring prolonged observation of each patients. II. Analysis and examples. Br J Cancer 1977;35:1–39.
12. Sterns MW, Deddish MR. Five-year results of abdominopelvic lymph node dissection for carcinoma of the rectum. Dis Colon Rectum 1959;2:169–72.
13. Di Matteo G, Mascagni D, Peparini N, Di Matteo FM. Lymphadenectomy and nerve sparing technique in radical surgery of rectal cancer. Ann Ital Chir 1996;67: 593–602.
14. Mori T, Takahashi K, Yasuno M. Radical resection with autonomic nerve preservation and LN dissection techniques in lower rectal cancer surgery and its results: the impact of lateral LN dissection. Langenbecks Arch Surg 1999;384:405–8.
15. Havenga K, Enker WE, McDermott K, Cohen AM, Minsky BD, Guillem J. Male and female sexual and urinary function after total mesorectal excision with autonomic nerve preservation for carcinoma of the rectum. J Am Coll Surg 1996;182:495–502.
16. Masui H, Ike H, Yamaguchi S, Oki S, Shimada H. Male sexual function after autonomic nerve preserving operation for rectal cancer. Dis Colon Rectum 1996;39: 1140–5.
17. Sugihara K, Moriya Y, Fujita S. Pelvic autonomic nerve preservation for patients with rectal carcinoma: oncologic and functional outcome. Cancer 1996;78:1871–80.
18. Enker WE, Havenga K, Polyak T, Thaler H, Cranor M. Abdominoperineal resection via total mesorectal excision and autonomic nerve preservation for low rectal cancer. World J Surg 1997;21:715–20.

Int J Clin Oncol (2004) 9:98–106
DOI 10.1007/s10147-003-0374-0

ORIGINAL ARTICLE

**Masahiko Watanabe · Osamu Nishida · Yasuo Kunii
Susumu Kodaira · Takashi Takahashi · Takeshi Tominaga
Keiichi Hojyo · Tomoyuki Kato · Minoru Niimoto
Kazufumi Kunitomo · Hiroyasu Isomoto · Yasuo Ohashi
Masayuki Yasutomi**

Randomized controlled trial of the efficacy of adjuvant immunochemotherapy and adjuvant chemotherapy for colorectal cancer, using different combinations of the intracutaneous streptococcal preparation OK-432 and the oral pyrimidines 1-hexylcarbamoyl-5-fluorouracil and uracil/tegafur

Received: June 10, 2003 / Accepted: December 11, 2003

Abstract

Background. We investigated the efficacy and safety of adjuvant immunochemotherapy and adjuvant chemotherapy for colorectal cancer, using different combinations of the intracutaneous streptococcal preparation OK-432 and the oral pyrimidines 1-hexylcarbamoyl-5-fluorouracil (carmofur, HCFU) and uracil/tegafur (UFT).

Methods. Patients with stage II, III, or IV (Dukes' B, C) colorectal cancer were enrolled and randomly assigned to one of three groups: an immunochemotherapy group (mitomycin C [MMC] + 5-fluorouracil [5-FU] + HCFU + OK-432), a chemotherapy group (MMC + 5-FU + HCFU), and a control group (surgery alone) for those with colon cancer (study 1); and an immunochemotherapy group (MMC + 5-FU + UFT + OK-432), a chemotherapy group (MMC + 5-FU + UFT), and a control group (surgery alone) for those with rectal cancer (study 2).

Results. A total of 760 patients with colon cancer and 669 patients with rectal cancer were entered into this randomized clinical trial (RCT). The incidence of side-effects was in the order of: immunochemotherapy group > chemotherapy group > control group in both the cohort of patients with colon cancer and the cohort with rectal cancer. In particular, the frequency of leucopenia and skin disorders was significantly higher than control groups. There were no severe adverse events such as death related to the adjuvant therapy. In both the colon cancer and rectal cancer cohorts, no significant difference in the 5-year survival rate and disease-free survival rate was noted among the three groups.

Conclusion. The results of an RCT demonstrated that the combination of MMC + 5-FU + HCFU + OK-432 for colon cancer and that of MMC + 5-FU + UFT + OK-432 for rectal cancer could not prolong the survival of patients with surgically resected colorectal cancer, but that both combinations were well tolerated as adjuvant therapy.

Key words Colorectal cancer · Adjuvant immunochemotherapy · OK-432 · HCFU · UFT

M. Watanabe (✉)
Department of Surgery, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara 228-8555, Japan
Tel./Fax 81-42-778-8735
e-mail: gekaw@med.kitasato-u.ac.jp

M. Watanabe · O. Nishida · Y. Kunii · S. Kodaira · T. Takahashi · T. Tominaga · K. Hojyo · T. Kato · M. Niimoto · K. Kunitomo · H. Isomoto · Y. Ohashi · M. Yasutomi
The Japanese Foundation for Multidisciplinary Treatment of Cancer, JFMC15-8901, Tokyo, Japan

Introduction

Recurrence of colon cancer is often found as hepatic metastasis, and the proportions of hepatic metastases and local recurrences are still high in rectal cancer. The prevention and treatment of recurrence pose major problems in the treatment of colorectal cancer. Clinical trials of various adjuvant chemotherapies have been conducted vigorously worldwide with the aim of improving the clinical results of curative resection of colorectal cancer.[1–3]

The JFMC07-8601 trial conducted by the Japanese Foundation for Multidisciplinary Treatment of Cancer (JFMC) is one of the largest scale clinical trials in Japan.[4,5] The results showed a significant improvement in the survival rate in the mitomycin C (MMC) + 1-hexylcarbamoyl-5-fluorouracil (carmofur, HCFU) group for stage IV or V

colon cancer compared with the group treated by surgery alone.[1] In rectal cancer, the disease-free survival rate of the MMC + uracil/tegaur (UFT) group was significantly higher than that of the group having surgery alone.[5] Based on the results of the JFMC07-8601, in the current study, we investigated a new adjuvant chemotherapy using two kinds of oral anticancer drugs, the pyrimidine fluoride HCFU for colon cancer and UFT for rectal cancer, with the aim of improving the clinical results. HCFU, an oral derivative of 5-fluorouracil (5-FU), is reported to have improved the survival rate and disease-free survival rate as adjuvant chemotherapy for colorectal cancer.[1,6,7] UFT, a compound prepared by mixing tegafur, a 5-FU derivative, and uracil (which inhibits the degradation of 5-FU) at the molecular weight ratio of 1:4 is reported to have improved the disease-free survival rate in rectal cancer.[5,8]

Immediately before this study was planned, promising results in the treatment of colon cancer using levamisole (LEV) as an immunoactivator and 5-FU were reported.[9] In the current study, a streptococcal agent (OK-432), a nonspecific immunoactivator, instead of LEV, was added to the regimen of JFMC07-8601 in order to reinforce the efficacy of chemotherapy. Accordingly, OK-432 was added to MMC + 5-FU + HCFU in patients with colon cancer and it was added to MMC + 5-FU + UFT in patients with rectal cancer in order to clarify the efficacy of adjuvant immunochemotherapy.

Patients and methods

Patients

This study, conducted as JFMC15-8901, consisted of two studies; namely, study 1 for colon cancer and study 2 for rectal cancer. The protocol was prepared by the JFMC15-8901 Committee and then approved by the Scientific Screening Committee of the JFMC. Hospitals participating in the study were recruited through medical journals, and 262 hospitals were selected by the Scientific Screening Committee.

This study adhered to the guidelines set out in *The general rules for clinical and pathological studies on cancer of colon, rectum and anus (4th edition)*,[10] which were applicable in Japan at the time. These rules for the classification of staging differ from the TNM classification of the International Union Against Cancer (UICC) as follows: stage II refers to cases in which lymph node metastasis is negative and the tumor spreads beyond the proper muscle, but does not invade other organs. Stage III refers to cases in which metastasis to paracolic lymph nodes is positive or the tumor directly invades other organs. Stage IV refers to cases in which metastasis to intermediate lymph nodes or main lymph nodes is positive. Therefore, stage II corresponds to Dukes' B, and stages III and IV to Dukes' C.

Cases meeting the following criteria: (1) stage II, III, or IV (Dukes' B, C) colorectal cancer and (2) patient age less than 75 years were registered from January 1989 to the end of December 1989. Consent to participate in this study was obtained from patients in advance. A central telephone registration system was adopted. The registered cases were randomly assigned to the treatment groups according to the assignment table.

Methods

In both study 1 and study 2, there were three treatment groups; namely, an immunochemotherapy group (group A and group D), a chemotherapy group (group B and group E), and a control group (group C and group F).

In study 1, for colon cancer, MMC (Kyowa Hakko Kogyo, Tokyo, Japan), 6 mg/m^2, was administered intravenously on the day of surgery, 1 week after surgery, and 1, 2, 3, 4, 5, and 6 months after surgery; 5-FU, 250 mg/day, was administered intravenously for 1 week from postoperative day 1, HCFU (Nihon Schering, Osaka, Japan) 300 mg/day was administered orally for 1 year from 2 weeks after surgery; and OK-432, 1 KE, 3 KE, and 5 KE, was administered intracutaneously on the day of surgery and on postoperative days 3 and 7, and 5 KE was administered every 2 weeks after the postoperative day 14 up to 6 months after surgery in group A. In group B, OK-432 was not administered, but MMC + 5-FU + HCFU were administered as in group A. In group C, surgery alone was performed.

In study 2, on rectal cancer, UFT (Taiho Pharmaceutical, Tokyo, Japan) 400 mg/day (in place of HCFU in group A) was administered orally for 1 year from the second postoperative week in group D, and the other drugs were administered by the same administration method as in group A. In group E, no OK-432 was administered, and MMC + 5-FU + UFT were administered as in group D. In group F, surgery alone was performed (Fig. 1).

The accumulation of cases began in January 1989. Because the indication of OK-432 for colorectal cancer was deleted from the list of drugs covered by the Japanese Medical insurance system in December 1989, due to a lack of evidence of an effect on colorectal cancer, the accumulation in groups A and D, using OK-432, was terminated on December 31, 1989. Clinical trials conducted during the period from January to December 1989 were designated as the first-term trial of JFMC15-8901. Registration of cases continued up to the end of December 1990 in groups B and C of study 1 and in groups E and F of study 2. This was designated as the second-term trial of JFMC15-8901. This article reports the results of the first-term trial of JFMC15-8901.

All the registered cases were followed-up for 5 years after surgery. The contents of reports were verified by a data manager. When the accuracy of the information entered was in doubt, an inquiry was made with the physician in charge.

Statistical analysis

The required sample size was calculated on the basis of the following assumptions: a 5-year survival rate of 70% for the

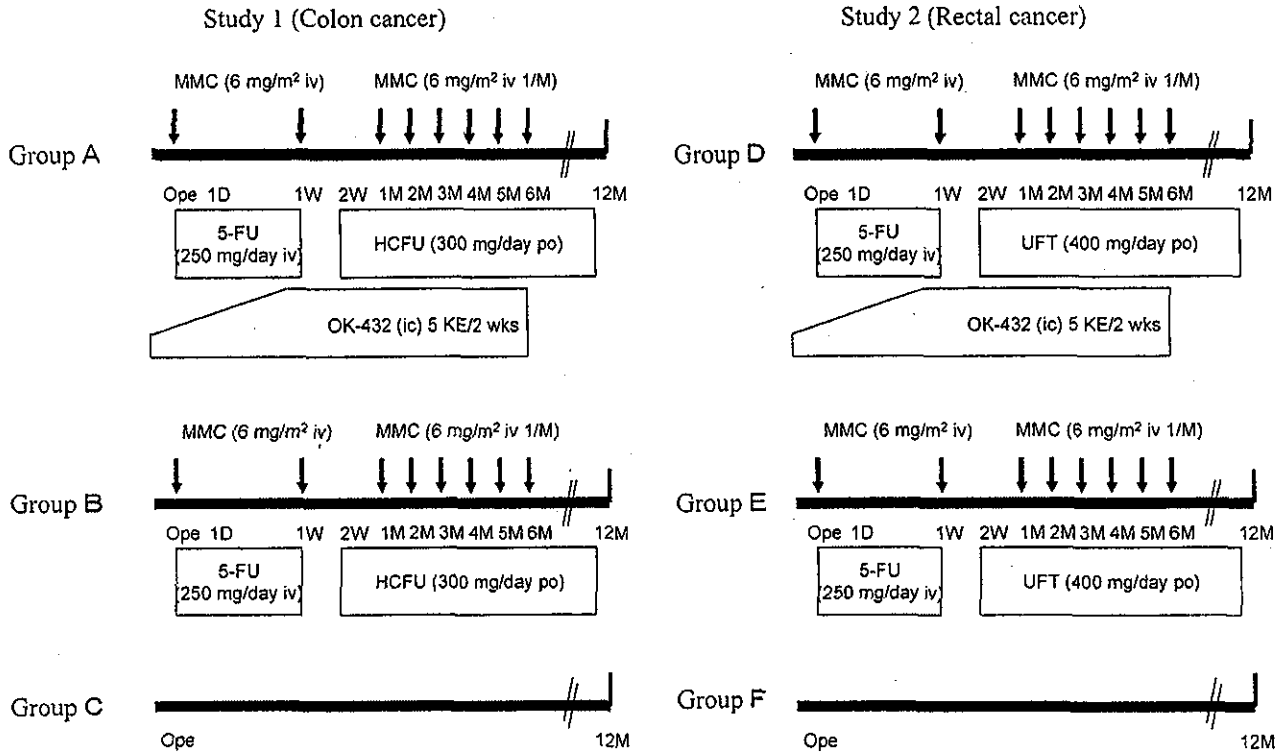

Fig. 1. Medication schedule. *MMC*, mitomycin C; *5-FU*, 5-fluorouracil; *HCFU*, 1-hexyl-carbamoyl-5-fluorouracil; *UFT*, uracil/tegafur; *D*, day; *W*, week(s); *M*, month(s); *ope*, operation

control group, and 75% for the drug treatment groups with colon cancer, and a 5-year survival rate of 60% for the control group, and 65% for the drug treatment groups with rectal cancer. The number of patients required for a group was estimated to be 600 for colon cancer and 700 for rectal cancer.

Statistical analysis was performed with Statistical Analysis System (SAS version 6.12, SAS Institute, Cary, NC, USA) software at the data center, and the procedure for the analysis and the results of this study were approved by the Clinical Trial Committee of the JFMC. The χ^2 test and Kruskal-Wallis test were used to evaluate the clinical characteristics. The survival rate and disease-free survival rate were estimated by the Kaplan-Meier method, and statistical significance was evaluated by the log-rank test and generalized Wilcoxon test (g-Wilcoxon test). The final report of this study was approved by the Clinical Trial Committee of the JFMC.

Results

Study 1 (colon cancer)

A total of 760 registered patients with colon cancer were randomly assigned to treatment in group A (5-FU + MMC + HCFU + OK-432; 254 patients); group B (5-FU + MMC + HCFU; 259 patients); or group C (control; 247 patients). In this study, 12 cases were ineligible (Table 1). No significant differences in the main clinicopathological background factors were found among the three groups (Table 2).

The compliance rate, based on the prescribed administration of 100 ± 20%, was 57.1% for MMC (group A, 56.3%; group B, 57.9%), 92.8% for 5-FU (group A, 92.9%; group B, 92.7%); 59.6% for HCFU (group A, 59.4%; group B, 59.8%) and 47.6% for OK-432 (Table 3). No significant difference in compliance was found between the groups with respect to MMC, 5-FU, and HCFU.

The toxicity profiles of these treatments are presented in Table 4. The incidence was in the order of group A > group B > group C. The most common significant toxicities were hematological disorders. Parameters showing significant differences in groups A and B compared with group C were stomatitis, anorexia, nausea, vomiting, increased blood urea nitrogen (BUN) and creatinine, skin disorders, dizziness, and feeling hot. The parameter showing a significantly high incidence in group A compared with groups B and C was fever. There were no severe adverse events such as death related to the adjuvant therapy.

The 5-year follow-up rate was 98.4%. No statistically significant difference in the 5-year survival rate was found among the three groups, with the rate being 75.4% in group A, 81.9% in group B, and 76.9% in group C (log-rank test, P 0.203; g-Wilcoxon test, P 0.220; Fig. 2). The 5-year

Table 1. Distribution of patients among treatment groups

	Study 1 (colon cancer)			Study 2 (rectal cancer)		
	Group A	Group B	Group C	Group D	Group E	Group F
Entered cases	254	259	247	222	218	229
Eligible cases	251 (98.8)	255 (98.5)	242 (98.0)	218 (98.2)	216 (99.1)	223 (97.4)
Ineligible cases	3 (1.2)	4 (1.5)	5 (2.0)	4 (1.8)	2 (0.9)	6 (2.6)
Treated case before surgery				1		
Benign tumor			1			
Nonepithelial tumor				1	1	
Double cancer	1	1	1			2
Multicentric cancer	1		1			
Location violation		1	1	1		1
Stage violation		1				1
Macroscopic noncurative resection	1	2	1	1	1	2

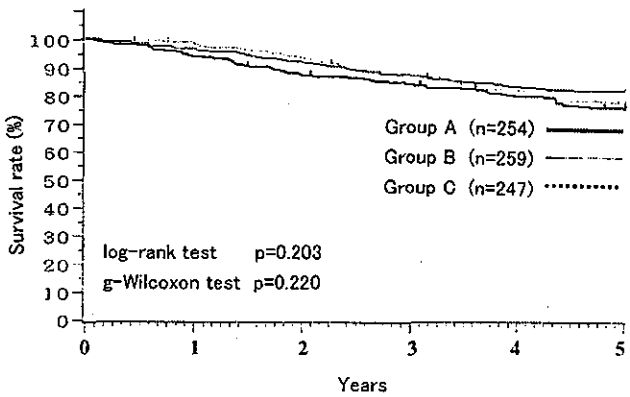

Fig. 2. 5-Year survival curves in study 1 (colon cancer). *g-Wilcoxon*, generalized Wilcoxon

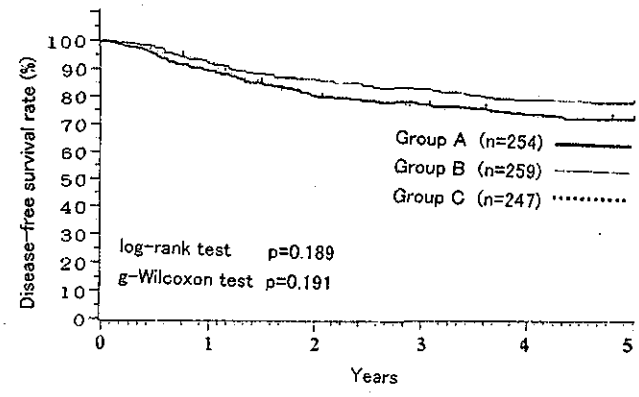

Fig. 3. 5-Year disease-free survival curves in study 1 (colon cancer)

disease-free survival rate was 71.5% in group A, 77.6% in group B, and 71.3% in group C. There were no significant differences between the groups (log-rank test, $P = 0.189$; g-Wilcoxon test, $P = 0.191$; Fig. 3). When compared by staging, the results for Dukes' B were poor in group A compared with groups B and C (log-rank test, $P = 0.038$; g-Wilcoxon test, $P = 0.039$). The 5-year disease-free rate for Dukes' B was 78.8% in group A, 81.8% in group B, and 74.3% in group C. However, no significant difference was found (log-rank test, $P = 0.129$; g-Wilcoxon test, $P = 0.132$).

Study 2 (rectal cancer)

A total of 669 registered patients with rectal cancer were randomly assigned to treatment in group D (5-FU + MMC + UFT + OK-432; 222 patients); group E (5-FU + MMC + UFT; 218 patients); or group F (control; 229 patients). In this study, 12 cases were ineligible (Table 1). No significant differences in the main clinicopathological background factors were found among the three groups (Table 2).

The compliance rate, based on the prescribed administration of 100 ± 20%, was 50.5% for MMC (group D, 49.1%; group E, 51.8%); 91.4% for 5-FU (group D, 91.0%; group E, 91.7%); 52.5% for UFT (group D, 49.5%; group E, 55.5%); and 45.0% for OK-432 (Table 3). As regards MMC, 5-FU, and UFT, no difference in compliance was found between the groups.

The toxicity profiles of these treatments are presented in Table 4. The incidence of toxicities was in the following order: group D > group E > group F. The most common toxicity was skin disorders. Parameters showing significant differences in groups D and E compared with group F were hematologic disorders, anorexia, nausea, vomiting, diarrhea, and respiratory disorders. The parameter showing a significantly high incidence in group D compared with groups E and F was fever. There was no severe toxicity such as death related to the adjuvant therapy.

The 5-year follow-up rate was 98.4%. No statistically significant difference in the 5-year survival rate was found among the three groups, with the rate being 73.5% in group D, 71.8% in group E, and 72.6% in group F (log-rank test, $P = 0.933$; g-Wilcoxon test, $P = 0.934$; Fig. 4). The 5-year disease-free survival rate was 67.8% in group D, 65.4% in group E, and 64.8% in group F, but no significant difference was found (log-rank test, $P = 0.785$; g-Wilcoxon test, $P = 0.745$; Fig. 5).

Table 2. Clinicopathological background factors

	Study 1 (colon cancer)				Study 2 (rectal cancer)			
	Group A	Group B	Group C	*P* value	Group D	Group E	Group F	*P* value
	254	259	247		222	218	229	
Age (years)								
≦49	34	40	45		26	38	38	
50–59	79	65	75	$P = 0.277$ (K–W test)	62	71	67	$P = 0.128$ (K–W test)
60–69	91	120	90	$P = 0.063$ (χ^2 test)	109	86	86	$P = 0.074$ (χ^2 test)
70–74	50	34	37		25	23	38	
Sex								
Male	136	129	125	$P = 0.674$ (χ^2 test)	143	127	133	$P = 0.298$ (χ^2 test)
Female	118	130	122		79	91	96	
Location of tumor								
I	0	0	0		0	0	0	
V	0	0	0		0	0	0	
C	24	22	26		0	0	0	
A	54	60	41		0	0	0	
T	34	42	34		0	0	0	
D	16	22	22	$P = 0.532$ (χ^2 test)	0	0	0	$P = 0.796$ (χ^2 test)
S	126	112	123		0	0	0	
Rs	0	0	1		61	50	62	
Ra	0	1	0		71	69	67	
Rb	0	0	0		89	99	99	
P	0	0	0		1	0	1	
E	0	0	0		0	0	0	
Depth of tumor invasion								
m	0	0	0		0	1	1	
sm	1	2	2		6	3	6	
pm	24	24	23	$P = 0.623$ (K–W test)	34	47	41	$P = 0.522$ (K–W test)
ss.a1	145	151	132	$P = 0.886$ (χ^2 test)	89	83	81	$P = 0.682$ (χ^2 test)
s.a2	77	72	77		87	78	91	
si.ai	6	10	12		4	6	9	
Unknown	1	0	1		2	0	0	
Lymph node metastasis								
n0	147	157	138		119	122	121	
n1	62	62	60		65	63	58	
n2	34	31	35	$P = 0.470$ (K–W test)	33	32	44	$P = 0.479$ (K–W test)
n3	9	9	12	$P = 0.872$ (χ^2 test)	3	1	5	$P = 0.517$ (χ^2 test)
n4	0	0	1		0	0	1	
Unknown	2	0	1		2	0	0	
Histological stage								
Stage I	18	21	19		29	31	36	
Stage II	124	130	113		88	89	79	
Stage III	66	68	65	$P = 0.490$ (K–W test)	66	65	61	$P = 0.456$ (K–W test)
Stage IV	43	38	46	$P = 0.972$ (χ^2 test)	36	33	49	$P = 0.252$ (χ^2 test)
Stage V	2	2	3		1	0	4	
Unknown	1	0	1		2	0	0	

Duke's classification								
A	18	21	19		29	31	36	
B	128	136	119	$P = 0.595$ (K–W test)	89	91	84	$P = 0.804$ (K–W test)
C	105	100	105	$P = 0.960$ (χ^2 test)	101	96	105	$P = 0.387$ (χ^2 test)
D	2	2	3		1	0	4	
Unknown	1	0	1		2	0	0	
Lymphatic invasion								
ly0	74	73	73		65	55	70	
ly1	107	113	87		82	100	92	
ly2	51	55	57	$P = 0.753$ (K–W test)	53	43	55	$P = 0.819$ (K–W test)
ly3	15	12	19	$P = 0.521$ (χ^2 test)	13	13	9	$P = 0.465$ (χ^2 test)
ly(−)	0	0	1		0	1	0	
Unknown	7	6	10		9	6	3	
Venous invasion								
v0	143	151	136		113	114	110	
v1	73	71	70		64	64	77	
v2	24	21	22	$P = 0.872$ (K–W test)	27	25	26	$P = 0.819$ (K–W test)
v3	5	6	3	$P = 0.903$ (χ^2 test)	8	9	8	$P = 0.952$ (χ^2 test)
v(−)	0	0	1		0	0	0	
Unknown	9	10	15		10	6	8	
Histological type								
Benign tumor	0	0	1		0	0	0	
Well-differentiated adenocarcinoma	134	140	119		115	112	116	
Moderately differentiated adenocarcinoma	98	100	107		97	92	101	
Poorly differentiated adenocarcinoma	13	10	11		1	7	3	
Mucinous adenocarcinoma	7	8	8		7	5	7	
Signet-ring cell carcinoma	0	0	1	$P = 0.822$ (χ^2 test)	0	0	0	$P = 0.554$ (χ^2 test)
Squamous cell carcinoma	0	0	0		0	1	2	
Adenosquamous carcinoma	0	0	0		0	0	0	
Undifferentiated carcinoma	1	1	0		0	0	0	
Unclassified carcinoma	0	0	0		0	0	0	
Others	0	0	0		1	1	0	
Unknown	1	0	0		1	0	0	
Lymph node dissection								
D0	0	0	0		0	0	0	
D1	11	8	2	$P = 0.217$ (K–W test)	9	7	11	$P = 0.413$ (K–W test)
D2	79	98	109	$P = 0.010$ (χ^2 test)	104	103	91	$P = 0.446$ (χ^2 test)
D3	163	153	136		108	108	127	
Unknown	1	0	0		1	0	0	
Microscopic curability								
Absolute curative resection	229	239	215		196	193	192	
Relative curative resection	22	16	24	$P = 0.171$ (K–W test)	21	20	27	$P = 0.171$ (K–W test)
Relative noncurative resection	1	3	4	$P = 0.423$ (χ^2 test)	0	0	6	$P = 0.038$ (χ^2 test)
Absolute noncurative resection	1	1	3		3	5	4	
Unknown	1	0	1		2	0	0	

K–W, Kruskal–Wallis

Table 3. Compliance with prescribed doses

Percentage compliant	Study 1 (colon cancer)		Study 2 (rectal cancer)	
	Group A	Group B	Group D	Group E
	254	259	222	218
MMC (i.v.)				
0%	3 (1.2)	1 (0.4)	4 (1.8)	4 (1.8)
<80%	97 (38.2)	99 (38.2)	101 (45.5)	96 (44.0)
80%–120%	143 (56.3)	150 (57.9)	109 (49.1)	113 (51.8)
>120%	10 (3.9)	7 (2.7)	6 (2.7)	5 (2.3)
Unknown	1 (0.4)	2 (0.8)	2 (0.9)	0 (0.0)
5-FU (i.v.)				
0%	9 (3.5)	8 (3.1)	9 (4.1)	8 (3.7)
<80%	8 (3.1)	8 (3.1)	8 (3.6)	9 (4.1)
80%–120%	236 (92.9)	240 (92.7)	202 (91.0)	200 (91.7)
>120%	0 (0.0)	1 (0.4)	1 (0.5)	1 (0.5)
Unknown	1 (0.4)	2 (0.8)	2 (0.9)	0 (0.0)
HCFU (p.o.)				
0%	15 (5.9)	8 (3.1)		
<80%	68 (26.8)	74 (28.6)		
80%–120%	151 (59.4)	155 (59.8)		
>120%	19 (7.5)	20 (7.7)		
Unknown	1 (0.4)	2 (0.8)		
UFT (p.o.)				
0%			20 (9.0)	6 (2.8)
<80%			73 (32.9)	76 (34.9)
80%–120%			110 (49.5)	121 (55.5)
>120%			17 (7.7)	15 (6.9)
Unknown			2 (0.9)	0 (0.0)
OK-432 (i.c.)				
0%	14 (5.5)		11 (5.0)	
<80%	104 (40.9)		104 (46.8)	
80%–120%	121 (47.6)		100 (45.0)	
>120%	14 (5.5)		5 (2.3)	
Unknown	1 (0.4)		2 (0.9)	

Figures in parentheses are percentages
MMC, mitomycin C; 5-FU, 5-fluorouracil; HCFU, 1-hexylcarmaboyl-5-fluorouracil; UFT, uracil/tegafur

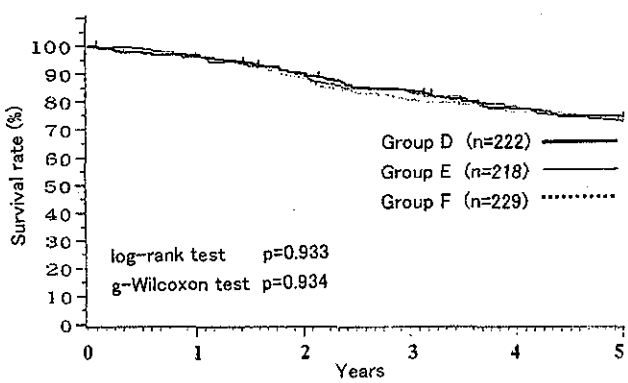

Fig. 4. 5-Year survival curves in study 2 (rectal cancer)

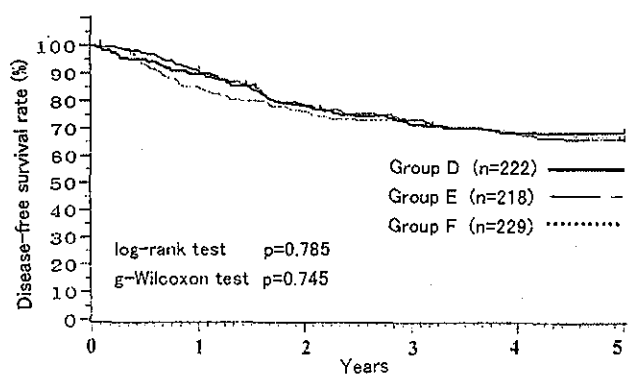

Fig. 5. 5-Year disease-free survival curves in study 2 (rectal cancer)

Discussion

The rates of ineligible cases and of those lost to follow-up were low, with the former being 12/760 cases (1.6%) of colon cancer and 12/669 cases (1.8%) of rectal cancer and the latter being 1.4% overall. Therefore, the present study is considered to be a multi-institutional joint control study in which the accuracy is very high. The compliance rate, based on the prescribed administration of 100 ± 20%, was high for 5-FU, at about 90%, versus about 50% for MMC, HCFU, UFT, and OK-432. Presumably, this was due mainly to the relatively simple administration method for 5-FU versus the intermittent administration method for MMC

Table 4. Incidence of toxic effects (%)

	Study 1 (colon cancer)				Study 2 (rectal cancer)			
	Group A	Group B	Group C	*P* Value	Group D	Group E	Group F	*P* Value
Anorexia	15.0 (2.0)	13.5 (1.5)	1.6 (0.4)	$P < 0.001$	18.0 (0.5)	21.1 (2.8)	3.9 (0)	$P < 0.001$
Nausea, vomiting	13.0 (2.4)	10.0 (0.8)	2.4 (0.4)	$P < 0.001$	11.3 (0.9)	17.4 (2.8)	2.2 (0)	$P < 0.001$
Diarrhea	7.1 (1.2)	5.4 (1.2)	2.8 (0)	$P = 0.089$	9.0 (2.7)	11.0 (2.8)	3.9 (0)	$P = 0.013$
Stomatitis	3.1 (0)	1.9 (0)	0 (0)	$P = 0.022$	2.3 (0)	2.3 (0)	0 (0)	$P = 0.066$
Respiratory system disorders	3.9 (2.0)	1.9 (0.8)	0 (0)	$P = 0.006$	5.4 (0.5)	5.0 (1.4)	1.3 (0)	$P = 0.042$
Fever	10.6 (0.4)	3.4 (0)	1.6 (0)	$P < 0.001$	9.5 (0)	3.7 (0)	2.2 (0)	$P < 0.001$
Sensory abnormality	3.5 (1.6)	0.4 (0)	0 (0)	$P < 0.001$	2.7 (1.4)	0.9 (0.5)	0 (0)	$P = 0.026$
Skin disorders	6.3 (0.8)	6.6 (0.8)	0 (0)	$P < 0.001$	7.2 (1.4)	3.2 (0)	0.4 (0)	$P < 0.001$
Alopecia	1.6 (0)	1.2 (0)	0 (0)	$P = 0.165$	3.2 (0)	1.4 (0)	0.4 (0)	$P = 0.063$
Consciousness disturbance	1.2 (0.4)	0.4 (0)	0 (0)	$P = 0.165$	1.4 (0)	0 (0)	0 (0)	$P = 0.045$
Depression	0.8 (0.4)	0.4 (0)	0 (0)	$P = 0.369$	1.8 (0)	0.5 (0)	0 (0)	$P = 0.066$
Phlebitis	1.2 (-)	1.9 (-)	0 (-)	$P = 0.107$	1.4 (-)	0 (-)	0 (-)	$P = 0.046$
Dizziness	5.1 (-)	5.8 (-)	0.4 (-)	$P = 0.004$	3.6 (-)	4.1 (-)	1.7 (-)	$P = 0.265$
Frequent urination	2.0 (-)	2.7 (-)	0.4 (-)	$P = 0.153$	5.9 (-)	2.8 (-)	3.9 (-)	$P = 0.266$
Feeling hot	5.9 (-)	4.2 (-)	0 (-)	$P = 0.001$	3.6 (-)	2.3 (-)	0.9 (-)	$P = 0.127$
Hemoglobin decreased	29.5 (3.5)	22.0 (1.2)	13.4 (0.8)	$P < 0.001$	25.7 (4.1)	24.3 (2.8)	12.2 (0)	$P < 0.001$
Leucopenia	39.4 (3.9)	28.6 (1.9)	7.7 (0)	$P < 0.001$	33.8 (2.7)	33.9 (4.1)	8.7 (0)	$P < 0.001$
Neutropenia	21.7 (3.5)	12.7 (2.3)	3.6 (0.8)	$P < 0.001$	15.8 (2.3)	16.5 (5.5)	5.7 (0)	$P < 0.001$
Thrombopenia	12.6 (4.3)	7.3 (1.9)	1.6 (1.2)	$P < 0.001$	18.9 (5.4)	16.5 (2.3)	2.6 (0.9)	$P < 0.001$
T. Bilirubin increased	2.0 (0)	2.7 (0)	1.2 (0)	$P = 0.507$	3.2 (0)	5.5 (0.5)	1.3 (0)	$P = 0.048$
BUN increased	7.9 (0.8)	5.8 (1.2)	0 (0)	$P < 0.001$	3.2 (0)	3.2 (0)	0.9 (0)	$P = 0.181$
Creatinine increased	5.1 (0.4)	2.7 (1.2)	0 (0)	$P = 0.002$	2.7 (0)	1.8 (0)	0 (0)	$P = 0.054$
Hematuria	5.9 (0)	5.8 (0.4)	2.4 (0)	$P = 0.109$	7.2 (0)	6.4 (0)	3.5 (0)	$P = 0.191$
Hypotension	1.6 (0.4)	0 (0)	0 (0)	$P = 0.018$	0 (0)	1.4 (0)	0 (0)	$P = 0.046$

Figures in parentheses are percentages of toxic effects of grade 3 or more

and OK-432. The long administration period of HCFU and UFT, 1 year after surgery, may be a factor in the reduced compliance rate. However, no difference in the total dose was found among the groups.

With all the registered groups, the incidence of side-effects was significantly higher in the chemotherapy group than in the surgery-alone group. Furthermore, the incidence of side-effects was higher in the immunochemotherapy groups (groups A and D) than in the chemotherapy groups (groups B and E) for both colon cancer and rectal cancer. The addition of OK-432 tended to cause the incidence of side-effects to increase. Whether this was due to the side-effects of OK-432 alone or whether it was the result of the side-effects of chemotherapy being amplified by OK-432 remains to be seen. In both study 1 and study 2, the proportion of side-effects accounting for the discontinuation of medication was lower in the immunochemotherapy group than in the chemotherapy group, and the side-effects themselves in the immunochemotherapy group were considered to be mild. In both study 1 and study 2, the incidence of toxicity was higher in the chemotherapy group and the immunochemotherapy group than in the control group, but there was no severe toxicity such as death related to treatment. The adjuvant therapy was considered to be highly tolerable and safe.

It is well known that there is a limit to the improvement in prognosis that can be gained by surgical treatment alone for advanced colorectal cancer. There is no objection to the necessity for some adjuvant therapies. The present study, following JFMC07-8601, is of significance as the basis of argument for the propriety of adjuvant therapy centered around 5-FU. However, MMC + 5-FU + OK-432 + oral fluoropyrimidine could not prolong the survival of patients with surgically resected colorectal cancer, and it led to no conclusion on the significance of the combined use of MMC and the optimal administration method of 5-FU. In both study 1 and study 2, the compliance rate for MMC, HCFU, UFT, and OK-432 in the treatment groups was only about 50%. Whether the low compliance rate for the drug has an influence on the lack of effectiveness found in the chemotherapy and immunochemotherapy groups remains unknown. In this study, registration of the immunotherapy groups had to be stopped in the middle of the study because the insurance coverage on OK-432 for colorectal cancer was no longer available. Therefore, the number of cases fell short of the required sample size, resulting in a decline of the statistical power. This may be mentioned as one of the reasons that no significant difference in survival rate or disease-free survival rate was found between the chemotherapy group and immunotherapy group, on the one hand, and the drug-treatment groups and the control group on the other.

As adjuvant therapy for Dukes' C colon cancer, 5-FU/LEV therapy was recommended by the American NIH in 1990, based on various clinical trials.[9] Subsequently, 5-FU/leucovorin (LV) therapy following 5-FU/LEV therapy started to draw attention, and a large-scale clinical trial of 5-FU/LV therapy was conducted.[11,12] As a result, 5-FU/LV therapy was found to be the most effective adjuvant therapy for Dukes' C colon cancer, in terms of the duration of administration, side-effects, recurrence rate, and survival rate, compared with 5-FU/LEV therapy.[13] At present,

therefore, 5-FU/LV therapy is regarded as the standard adjuvant therapy. On the other hand, group comparison studies of 5-FU/LV and UFT/LV have been conducted in Europe and the United States, and preliminary analysis of the toxicity findings has indicated that both regimens were well tolerated and had similar toxicity profiles.[11] This shows that the safety and convenience of oral fluoropyrimidine as adjuvant chemotherapy are being recognized in Europe and the United States. The current study is significant in that it demonstrated the safety of oral fluoropyrimidine as adjuvant therapy prior to those studies.

The combined use of irinotecan (CPT-11)[15] or oxaliplatin[16] has been reported to exert an effect on metastatic colorectal cancer that is superior to that of 5-FU/LV alone. Studies may focus on determining which treatments are valid as adjuvant chemotherapy for colorectal cancer – combined therapy with CPT-11 or oxaliplatin or combined therapy with an oral fluoropyrimidine. In the future, clinical trials similar to JFMC 15-8901, including the question of how to select subjects responsive to adjuvant therapy, should be conducted as soon as possible.

References

1. Macdonald JS, Astrow AB (2001) Adjuvant therapy of colon cancer. Semin Oncol 28:30–40
2. Wils J, O'Dwyer P, Labianca R (2001) Adjuvant treatment of colorectal cancer at the turn of the century: European and US perspectives. Ann Oncol 12:13–22
3. Ragnhammar P, Hafstrom L, Nygren P (2001) A systematic overview of chemotherapy effects in colorectal cancer. Acta Oncol 40:282–308
4. Yasutomi M, Takahashi T, Kodaira S, et al. (1997) Prospective controlled study on the usefulness of carmofur as a postoperative adjuvant chemotherapy for colorectal cancer. Gan To Kagaku Ryoho 24:1953–1960
5. Kodaira S, Kikuchi K, Yasutomi M, et al. (1998) *Postoperative* adjuvant chemotherapy with mitomycin C and UFT for curatively resected rectal cancer. Results from the Cooperative Project No. 7 Group of the Japanese Foundation for Multidisciplinary Treatment of Cancer. Int J Clin Oncol 3:357–364
6. Sugimachi K, Maehara Y, Ogawa M, et al. (1996) Postoperative *chemotherapy for* colorectal cancer by combining 5-fluorouracil infusion and 1-hexylcarbamoyl-5-fluorouracil administration after curative resection. Cancer 77:36–43
7. Ito K, Yamaguchi A, Miura K, et al. (1996) Prospective adjuvant therapy with mitomycin C and carmofur (HCFU) for colorectal cancer, 10-year follow-up: Tokai HCFU Study Group, the first study for colorectal cancer. J Surg Oncol 62:4–9
8. Kato T, Ohashi Y, Nakazato H, et al. (2002) Efficacy of oral *UFT* as adjuvant chemotherapy to curative resection of colorectal cancer: multicenter prospective randomized trial. Langenbeck's Arch Chir 386:575–581
9. Windle R, Bell PR, Shaw D (1987) Five-year results of a randomized trial of adjuvant 5-fluorouracil and levamisole in colorectal cancer. Br J Surg 74:569–572
10. Japanese Research Society for Cancer of Colon and Rectum (1985) General rules for clinical and pathological studies on cancer of colon, rectum and anus. 4th edn. Kanehara Shuppan, Tokyo
11. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators (1995) Efficacy of adjuvant fluorouracil and folinic acid in colorectal cancer. Lancet 345:939–944
12. Zaniboni A (1997) Adjuvant chemotherapy in colorectal cancer with high-dose leucovorin and fluorouracil: impact on disease-free survival and overall survival. J Clin Oncol 15:2432–2441
13. Wolmark N, Rockette H, Mamounas E, et al. (1999) Clinical trial to assess the relative efficacy of fluorouracil and leucovorin, fluorouracil and levamisole, and fluorouracil, leucovorin, and *levamisole in patients with* Dukes' B and C carcinoma of colon: results from National Surgical Adjuvant Breast and Bowel Project C-04. J Clin Oncol 17:3553–3559
14. Smith RE, Lembersky BC (2000) UFT/leucovorin vs 5-FU/leucovorin in colon cancer. Oncology(Huntingt) 14:24–27
15. Douillard J, Cunningham D, Roth A, et al. (2000) Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a *multicenter* randomized trial. Lancet 355:1041–1047
16. Levi F, Zidani R, Misset JL (1997) Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. International Organization for Cancer Chemotherapy. Lancet 350:681–686

Surg Endosc (2004) 18: 1113–1117
DOI: 10.1007/s00464-003-9150-5

SURGICAL ENDOSCOPY
and Other Interventional Techniques

Port-site metastasis after CO_2 pneumoperitoneum

Role of adhesion molecules and prevention with antiadhesion molecules

Y. Hirabayashi,[1] K. Yamaguchi,[2] N. Shiraishi,[1] Y. Adachi,[1] I. Saiki,[2] S. Kitano[1]

[1] Department of Surgery I, Oita Medical University, 1-1 Idaigaoka, Hasama-machi, Oita 879-5593, Japan
[2] Department of Pathogenic Biochemistry, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan

Received: 25 June 2003/Accepted: 9 December 2003/Online publication: 12 May 2004

Abstract
Background: Port-site metastasis is a continuing problem in laparoscopic cancer surgery. To clarify the role of adhesion molecules in the development of port-site metastasis, particularly with regard to prevention, we performed experiments in which port-site metastasis was inhibited using antibodies against extracellular matrix proteins or the active Arg–Gly–Asp (RGD) peptide after CO_2 pneumoperitoneum in a murine model.
Methods: We examined the development of port-site metastasis under the following conditions: (1) CO_2 pneumoperitoneum with or without hyaluronic acid and anti-integrin or anti-CD44 antibody and (2) CO_2 pneumoperitoneum and a RGD peptide or pseudo-RGD sequence peptide (FC-336). BALB/c mice ($n = 130$) were injected with 5×10^5 human gastric cancer cells (MKN45) and either antibody or peptide, treated with CO_2 pneumoperitoneum, and injected intraperitoneally with antibody or peptide for 5 days. Three weeks after CO_2 pneumoperitoneum, the frequency and weight of port-site metastatic tumors were determined.
Results: Anti-integrin antibody significantly decreased the weight of port-site metastatic tumors without hyaluronic acid (control vs anti-integrin: 8.2 ± 7.1 vs 3.6 ± 4.5 mg; $p < 0.05$) but not the frequency of port-site metastases. With hyaluronic acid, the frequency of port-site metastasis and the weight of port-site metastatic tumors were significantly decreased both by anti-integrin and by anti-CD44 antibody (control vs anti-integrin and anti-CD44; 95% and 8.5 ± 7.2 mg vs 50% and 3.1 ± 4.3 mg and 55% and 3.3 ± 5.1 mg, respectively; $p < 0.05$). RGD peptide and FC-336 also inhibited port-site metastasis in a dose-dependent manner.
Conclusion: Cell adhesion molecules integrin and CD44 play an important role in the development of port-site metastasis after laparoscopic cancer surgery. Intraperitoneal injection of RGD peptide or pseudo-RGD sequence peptide (FC-336) can prevent port-site metastasis.

Key words: Laparoscopic surgery — Port-site metastasis — Adhesion molecule — RGD site — Murine model

Introduction

Laparoscopic surgery has been performed worldwide for gastrointestinal cancer since 1991 because it is minimally invasive, allowing early recovery and early discharge [1, 10]. To our knowledge, more than 160 cases of port-site metastasis after laparoscopic cancer surgery have been reported [15], but the clinical problem of port-site metastases is considerably less than initial studies suggested. Indeed, there are now many large-scale studies of laparoscopic oncologic surgery demonstrating that the incidence of port-site metastases is no different from that of open surgery [6]. Port-site metastasis occurs mainly in the subperitoneal tissue at the port sites, grows rapidly, and is diagnosed generally within 6 months after laparoscopic surgery [18]. Whether port-site metastasis influences patient prognosis is not clear, but clarifying its pathogenesis and means of prevention is considered important. Therefore, many animal studies have been performed to clarity the pathogenesis and prevention of port-site metastasis after laparoscopic cancer surgery [7].

Cell surface adhesion molecules, including integrin and CD44, participate in implantation of cancer cells into the peritoneum during peritoneal dissemination [13]. Inhibition of peritoneal dissemination by adminis-

Correspondence to: S. Kitano

tration of antibodies and inhibitors against integrin and CD44 has been attempted in animal models [17]. We have shown that the secretion of hyaluronic acid increases in the peritoneal cavity after CO_2 pneumoperitoneum [20] and that this hyaluronic acid increases the frequency of port-site metastases [19]. A light microscopy and scanning electron microscopy study showed that free cancer cells attach to the matrix at the injured port sites immediately after CO_2 pneumoperitoneum [8]. Thus, the adhesion molecules integrin and CD44 may play an important role in the development of port-site metastasis.

To clarify the role of adhesion molecules in the development of port-site metastasis with a focus on prevention, we investigated the possibility of inhibiting port-site metastasis using antibodies against integrin and CD44, the active Arg–Gly–Asp (RGD) sequence peptide derived from integrin, and a pseudopeptide analogue (FC-336) of the RGD sequence in an animal model.

Materials and methods

Animals

A total of 130 female BALB/c nude mice aged 6–8 weeks and weighting 20–25 g were used. All animals were kept under standard laboratory conditions with free access to water and food. All procedures were performed under the guidelines for animal experimentation of Oita Medical University.

Cell line

Human gastric carcinoma cell line (MKN45) was obtained from the Japanese Cancer Resources Bank Tokyo. The cells were grown in RPMI 1640 medium (Gibco BRL, Life Technologies, Rockville, MD, USA) supplemented with 10% fetal bovine serum (Gibco BRL) and an antibiotic–antimycotic agent containing 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 2.5×10^{-4} mg amphotericin B (Gibco BRL). The cells were cultured in dishes in a 5% CO_2 atmosphere at 37°C.

Hyaluronic acid

Sodium hyaluronate, which is used clinically to treat patients with chronic arthritis and to prevent adhesions after abdominal surgery, was donated by Kaken Pharmaceutical Company (Tokyo).

Antibodies

Murine monoclonal antibodies used in the characterization of the gastric cancer cell line and for intraperitoneal treatment were anti-CD44 (reactive mouse IgG2b, clone G44-26, PharMingen, USA), anti-integrin (reactive mouse IgG1 clone MAR4, PharMingen), and non-reactive mouse IgG (clone G18-145, PharMingen).

Chemicals

Arg–Gly–Asp–Ser (RGDS) tetrapeptide and control tetrapeptide Arg–Gly–Glu–Ser (RGBS) were purchased from the Peptide Institute (Osaka, Japan). A pseudopeptide RGD sequence FC-336, Ph (CH2NH-DR-COCH2-D)2, described by Fujii et al. [4], was dissolved in phosphate-buffered saline (PBS) before use.

Preparation of tumor cells

For each mouse, tumor cells were initially incubated with 2 μg of antibody or with RGDS, RGBS, or FC-336 in 0.5 ml of PBS for 30 min at 4°C to ensure adequate antibody or peptide coating. After resuspension, the cells were injected intraperitoneally along with antibody or peptide.

Port-site metastasis

The animal model used for port-site metastasis in this study was described by Yamaguchi et al. [19]. Seventy mice were used to examine the effect of anti-CD44 antibody and anti-integrin antibody on port-site metastasis. After the mice were anesthetized with diethyl ether, two 20-gauge intravenous cannulas were inserted in the left and right lower quadrant: one was used for gas insufflation and the other for pressure measurements. Two 20-gauge intravenous cannulas were inserted in the left and right upper quadrants as the port sites. Mice underwent intraperitoneal injection of 5×10^5 MKN45 cancer cells in the presence of either anti-CD44 antibody ($n = 10$) or anti-integrin antibody ($n = 10$) without hyaluronic acid and in the presence of anti-CD44 antibody ($n = 10$), anti-integrin antibody ($n = 10$), or both ($n = 10$) with hyaluronic acid (2 mg) in 0.5 ml of PBS. Hyaluronic acid was added to increase the frequency of port-site metastasis [20]. Control groups (two groups, $n = 10$ each) were given normal mouse IgG at concentrations matching those of the other two antibodies. Pneumoperitoneum was established and maintained with CO_2 insufflation at 4–6 mmHg for 20 min with a syringe pump [16]. This was followed by injection of 2 μg of antibody at various doses for 5 days. Three weeks after the procedure, the mice were killed, and the frequency and weight of port-site metastatic tumors were determined. Tumor nodules at the port sites measuring more than 1 mm were considered port-site metastases. Nodules in the peritoneal cavity were excluded. Nodules at the port site were easily recognized, were harvested for weighing under a scanning stereoscopic microscope to remove as much normal tissue adjacent to the tumor as possible, and were confirmed microscopically as metastatic foci of adenocarcinoma.

Sixty mice were used to clarify the effects of RGDS and FC-336 in lieu of antibiotics on port-site metastasis without hyaluronic acid. RGDS was given at 0.1, 0.2, or 1.0 mg/mouse ($n = 10$ in each group) in 0.5 ml PBS, FC-336 was given at 1.0 or 10 mg/mouse ($n = 10$ in each group) in 0.5 ml PBS, and control RGBS was given at 1.0 mg/mouse ($n = 10$).

Statistical analysis

Data are shown as mean ± standard deviation. Fisher's PLSD test was used to analyze the frequency and weight of port-site metastases, and a p value of less than 0.05 was considered significant.

Results

When no hyaluronic acid was injected, anti-integrin antibody did not significantly decrease the frequency of port-site metastases (Fig. 1a), but the antibody significantly decreased the weight of port-site metastatic tumors (control vs anti-integrin: 8.2 ± 7.1 vs 3.6 ± 4.5 mg; $p < 0.05$) (Fig. 1b). Anti-CD44 antibody decreased the frequency and weight of port-site metastatic tumors, but not significantly (Fig. 1).

When hyaluronic acid was injected into the abdominal cavity, the frequency and weight of port-site metastases were significantly inhibited in the group treated with anti-integrin antibody, anti-CD44 antibody, or a combination of anti-integrin and anti-CD44 with hyaluronic acid compared to the control group (control vs anti-integrin; 95% and 8.5 ± 7.2 mg vs 50% and

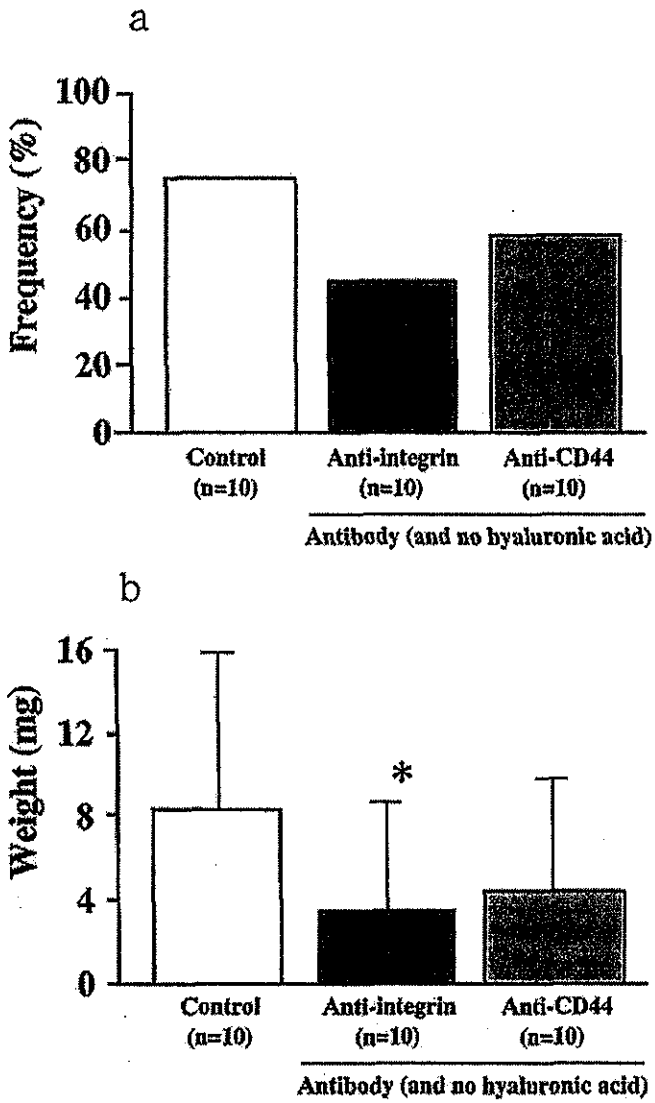

Fig. 1. The effect of antibodies against adhesion molecules on the frequency of port-site metastasis (a) and the weight of port-site metastatic tumors without hyaluronic acid (b). Each group included 20 port sites in 10 mice. Control; injection of normal mouse IgG, anti-integrin; injection of anti-integrin antibody, anti-CD44; injection of anti-CD44 antibody. $*p < 0.05$ vs control; Fisher's PLSD test.

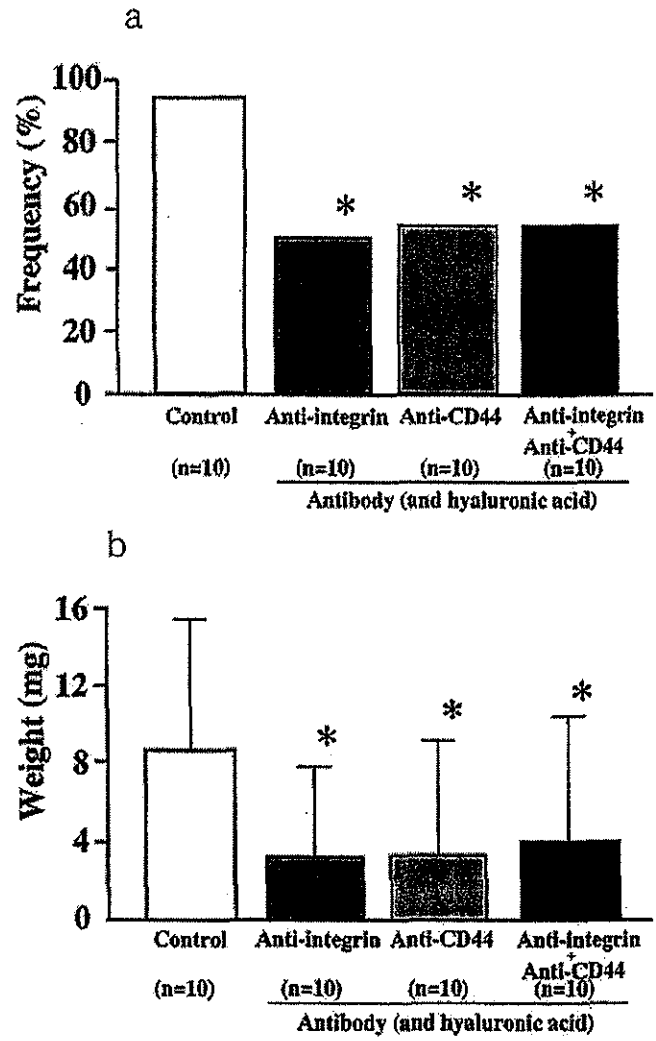

Fig. 2. The effect of antibodies against adhesion molecules on the frequency of port-site metastasis (a) and the weight of port-site metastatic tumors with hyaluronic acid (b). Each group included 20 port sites in 10 mice. Control; injection of normal mouse IgG; anti-integrin; injection of anti-integrin antibody; anti-CD44; injection of anti-CD44 antibody; anti-integrin + anti-CD44; injection of anti-integrin antibody and anti-CD44 antibody. $*p < 0.05$ vs control; Fisher's PLSD test.

3.1 ± 4.3 mg; anti-CD44, 55% and 3.3 ± 5.1 mg; or anti-integrin and anti-CD44, 55% and 3.8 ± 5.9 mg; $p < 0.05$) as shown in Fig. 2. No synergism occurred when anti-integrin antibody and anti-CD44 antibody were combined (combination antibodies at 55% vs anti-integrin at 50% and anti-CD44 at 55%).

The frequency and weight of port-site metastasis were also inhibited by RGDS peptide in a dose-dependent manner, ranging from 0.5 to 1 mg/mouse without hyaluronic acid. As with RGDS, treatment with pseudopeptide FC-336 inhibited the frequency and weight of port-site metastatic tumors in a dose-dependent manner, ranging from 1.0 to 10 mg/mouse (Fig. 3). The frequency and weight of port-site metastases were inhibited completely by FC-336 at a dose of 10 mg/mouse. The control RGBS peptide at a high concentration (1.0 mg/mouse) did not inhibit port-site metastasis (Fig. 3). There were no differences between the RGES group and the pneumoperitoneum-only group with regard to the frequency and weight of port-site metastatic tumors (data not shown).

Discussion

In this animal study, the port-site metastatic tumor of human gastric cancer after CO_2 pneumoperitoneum weighed significantly less in the anti-integrin antibody without hyaluronic acid group than in the control group. When hyaluronic acid was injected into the peritoneal cavity, the frequency and weight of port-site metastatic tumors were significantly decreased in the anti-integrin and anti-CD44 antibody groups compared to the control group, but the effect of the antibodies was not synergistic when given together. The RGD sequence

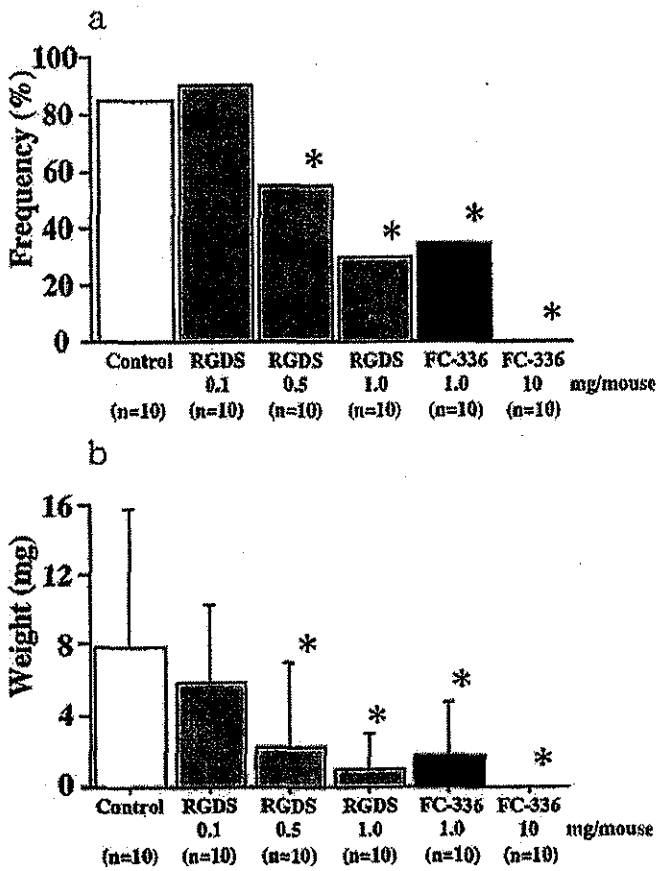

Fig. 3. The effect of RGDS peptide and FC-336 pseudopeptide on the frequency of port-site metastasis (**a**) and the weight of port-site metastatic tumors (**b**). *$p < 0.05$ vs control; *Fisher's PLSD test.*

is a common integrin-binding and CD44-binding motif in many kinds of cells. Injection of excess RGD peptide or the pseudo-peptide FC-336 into the peritoneal cavity inhibited port-site metastasis in a dose-dependent manner. Our previous light microscopy and scanning electron microscopy study showed that free cancer cells attach to the naked extracellular matrix proteins at the port sites in the early process of port-site metastasis [8]. The free cancer cells may attach to the extracellular matrix proteins at the port sites via RGD domains of integrin or CD44.

For antibody inhibition, we used 2 μg of anti-integrin antibody or anti-CD44 antibody. Strobel et al. [17] successfully used 45 μg of anti-CD44 antibody for 10×10^6 36 M2 ovarian cancer cells/mouse to determine the effects of anti-CD44 antibody on peritoneal dissemination. In the present study, 5×10^5 MKN45 cells (i.e., 1/20 the number of cells used in the prior study) were injected to the peritoneal cavity. The injection of 2 μg of each antibody seemed to be excessive and it did inhibit port-site metastasis. However, port-site metastasis was inhibited only 50% by antibodies. The reason is not clear. It is possible that port-site metastasis developed via other adhesion molecules with the RGD sequence. It is also unclear why the affinity of antibody to the adhesion molecules was weaker than the affinity of active RGD domains of adhesion molecules to the extracellular matrix proteins.

The inhibition of port-site metastasis by anti-integrin and by CD44 antibody with hyaluronic acid was marked in comparison to that without hyaluronic acid. Yamaguchi et al. reported that more hyaluronic acid was secreted in the intraperitoneal cavity of mice after CO_2 pneumoperitoneum than after laparotomy [20], and additional injection of hyaluronic acid into the intraperitoneal cavity under CO_2 pneumoperitoneum increased the frequency of port-site metastasis in a murine model [19]. Hyaluronic acid is known to bind extracellular matrix proteins such as collagen and to bind cells via RGD domains of integrin or CD44. The addition of hyaluronic acid may increase binding of free cancer cells to the extracellular matrix proteins at the port sites and enhance the inhibition of port-site metastasis effected by antibodies to integrin or CD44.

The RGD sequence is reported to play an important role in the progression of cancer, particularly in the development of peritoneal dissemination [17] and hematogenous metastasis [21]. More than 160 clinical cases of port-site metastasis have been reported for laparoscopic cancer surgery since 1991 [15]. There are now many large-scale studies of laparoscopic oncologic surgery demonstrating that the incidence of port-site metastases is no different from that of open surgery [6]. However, there is considerable uncertainty regarding the etiology and pathgenesis of port-site metastases. Several animal studies have investigated the cause of port-site metastasis, which is now thought to be multifactorial. Causes include intraoperative manipulation of cancer cells [7], aerosolization, metabolic changes in the abdominal cavity [11], and immunosuppression by pneumoperitoneum [3]. In the current study, we showed that the RGD sequence of the integrin and CD44 on the surface of cancer cells plays an important role in the early development of port-site metastasis.

There have been several animal studies investigating prevention of port-site metastasis. Intraperitoneal administration of heparin and taurolidine decreased the frequency of port-site metastasis [9]. Intraperitoneal administration of cytotoxic agents, such as povidone–iodine and methotrexate, reduced the development of port-site metastasis [12]. Excision and repair of the abdominal wall at the port sites prevented port-site metastasis [2], In the current study, RGD peptides in excess and pseudopeptides of the RGD sequence (FC-336) inhibited port-site metastasis dose dependently. At 10 mg, these peptides completely inhibited port-site metastasis. The FC-336 pseudopeptide inhibits the degradation activity of the matrix metalloproteinases and exhibits an inhibitory effect on lung metastasis in animals with B16-BL6 melanoma [5]. It also inhibits liver metastasis of colon 26-L5 carcinoma cells [14]. The FC-336 peptide may also be useful for the prevention of port-site metastasis in cases of laparoscopic cancer surgery.

We note the importance of binding between free cancer cells and naked extracellular matrix proteins at the port sites in the development of port-site metastasis after laparoscopic cancer surgery. The RGD sequence in integrin and CD44 plays an important role in the initial port-site metastasis process, and excess RGD peptides or pseudo-peptides of the RGD sequence (FC-336) may

be useful clinically for the prevention of port-site metastasis.

References

1. Adachi Y, Shiraishi N, Shiromizu A, Bandoh T, Aramaki M, Kitano S (2000) Laparoscopy-assisted Billroth I gastrectomy compared with conventional open gastrectomy. Arch Surg 135: 806–810
2. Aoki Y, Shimura H, Li H, Mizumoto K, Date K, Tanaka M (1999) A model of port-site metastases of gallbladder cancer: the influence of peritoneal injury and its repair on abdominal wall metastases. Surgery 125: 553–559
3. Chekan EG, Nataraj C, Clary EM, Hayward TZ, Brody FJ, Stamat JC, Fina MC, Eubanks WS, Westcott CJ (1999) Intraperitoneal immunity and pneumoperitoneum. Surg Endosc 13: 1135–1138
4. Fujii H, Inobe M, Hayakawa Y, Kimura F, Murakami M, Onishi Y, Azuma I, Uede T, Saiki I (1998) Vaccination with B7-1$^+$ tumor and anti-adhesion therapy with RGD pseudo-peptide (FC-336) efficiently induce anti-metastatic effect. Clin Exp Metastasis 16: 141–148
5. Fujii H, Nishikawa N, Komazawa H, Suzuki M, Kojima M, Itoh I, Obata A, Ayukawa K, Azuma I, Saiki I (1998) A new pseudopeptide of Arg–Gly–Asp (RGD) with inhibitory effect on tumor metastasis and enzymatic degradation of extracellular matrix. Clin Exp Metastasis 16: 94–104
6. Hartley JE, Mehigan BJ, MacDonald AW, Lee PW, Monson JR (2000) Patterns of recurrence and survival after laparoscopic and conventional resections for colorectal carcinoma. Ann Surg 232: 181–186
7. Hewett PJ, Thomas WM, King G, Eaton M (1996) Intraperitoneal cell movement during abdominal carbon dioxide insufflation and laparoscopy. An in vivo model. Dis Colon Rectum 39: 62–66
8. Hirabayashi Y, Yamaguchi K, Shiraishi N, Adachi Y, Kitamura K, Kitano S (2002) Development of port-site metastasis after pneumoperitoneum: a scanning electron microscopy study. Surg Endosc 16: 864–868
9. Jacobi CA, Ordemann J, Bohm B, Zieren HU, Sabat R, Muller JM (1997) Inhibition of peritoneal tumor cell growth and implantation in laparoscopic surgery in a rat model. Am J Surg 174: 359–363
10. Kitano S, Shimoda K, Miyahara M, Shiraishi N, Bandoh T, Yoshida T, Shuto K, Kobayashi M (1995) Laparoscopic approaches in the management of patients with early gastric carcinomas. Surg Laparosc Endosc 5: 359–362
11. Neuhaus SJ, Texler M, Hewett PJ, Watson DI (1998) Port-site metastases following laparoscopic surgery. Br J Surg 85: 735–741
12. Neuhaus SJ, Watson DI, Ellis T, Rofe AM, Jamieson GG (1999) Influence of cytotoxic agents on intraperitoneal tumor implantation after laparoscopy. Dis Colon Rectum 42: 10–15
13. Nishimura S, Chung YS, Yashiro M, Inoue T, Sowa M (1996) CD44H plays an important role in peritoneal dissemination of scirrhous gastric cancer cell. Jpn J Cancer Res 87: 1235–1244
14. Ohnishi Y, Fujii H, Murakami K, Sakamoto T, Tsukada K, Fujimaki M, Kojima M, Saiki I (1998) A new pseudo-peptide analogue of Arg–Gly–Asp (RGD) sequence inhibits liver metastasis of colon 26-L5 carcinoma cells. Cancer Lett 124: 157–163
15. Schaeff B, Paolucci V, Thomopoulos J (1998) Port site recurrences after laparoscopic surgery. Dig Surg 15: 124–134
16. Suematsu T, Shiromizu A, Yamaguchi K, Shiraishi N, Adachi Y, Kitano S (1999) Convenient murine pneumoperitoneal model for study of laparoscopic cancer surgery. Surg Laparosc Endosc 9: 279–281
17. Strobel T, Swanson L, Cannistra SA (1997) In vivo inhibition of CD44 limits intra-abdominal spread of a human ovarian cancer xenograft in nude mice: a novel role for CD44 in the process of peritoneal implantation. Cancer Res 57: 1228–1232
18. Wexner SD, Cohen SM (1995) Port site metastases after laparoscopic colorectal surgery for cure of malignancy. Br J Surg 82: 295–298
19. Yamaguchi K, Hirabayashi Y, Shiromizu A, Shiraishi N, Adachi Y, Kitano S (2001) Enhancement of port site metastasis by hyaluronic acid under CO_2 pneumoperitoneum in a murine model. Surg Endosc 15: 504–507
20. Yamaguchi K, Hirabayashi Y, Suematsu T, Shiraishi N, Adachi Y, Kitano S (2001) Hyaluronic acid secretion during carbon dioxide pneumoperitoneum and its association with port-site metastasis in a murine model. Surg Endosc 15: 59–62
21. Yun Z, Menter DG, Nicolson GL (1996) Involvement of integrin α_v β_3 in cell adhesion, motility, and liver metastasis of murine RAW117 large cell lymphoma. Cancer Res 56: 3103–3111

Int J Clin Oncol (2004) 9:364–368
DOI 10.1007/s10147-004-0407-3

ORIGINAL ARTICLE

Ayumu Goto · Yasuhide Yamada · Ayumu Hosokawa
Takashi Ura · Tatsuhiro Arai · Tetsuya Hamaguchi
Kei Muro · Yasuhiro Shimada · Kuniaki Shirao

Phase I/II study of irinotecan, 5-fluorouracil, and *l*-leucovorin combination therapy (*modified Saltz regimen*) in patients with metastatic colorectal cancer

Received: January 15, 2004 / Accepted: March 23, 2004

Abstract
Background. A combination of irinotecan 125 mg/m^2, 5-fluorouracil (5-FU) 500 mg/m^2, and leucovorin (LV) 20 mg/m^2 (Saltz regimen; treatment on days 1, 8, 15, and 22 every 6 weeks) is widely used for the treatment of metastatic colorectal cancer. A modified schedule with chemotherapy on days 1 and 8 of a 21-day cycle was recommended in 2001 because of early treatment-related mortality. We conducted a phase I/II study of this modified Saltz regimen as first-line therapy in Japanese patients with metastatic colorectal cancer to assess the maximum tolerated dose (MTD) and the recommended dose of 5-FU when given with fixed doses of *l*-LV and irinotecan, and to evaluate the efficacy and the feasibility of this regimen.
Methods. Irinotecan, 5-FU, and *l*-LV were administered on days 1 and 8 of a 21-day cycle. Irinotecan 100 mg/m^2 was given intravenously over the course of 90 min on day 1, followed by *l*-LV 10 mg/m^2, and then 5-FU. The dose of 5-FU was escalated from 400 mg/m^2 (level 1) to 500 mg/m^2 (level 2). If neither level met the criteria for the MTD, the recommended dose was defined as level 2, and dose escalation was discontinued, because the maximum approved weekly dose of irinotecan alone in Japan is 100 mg/m^2 and the dose of 5-FU in the original Saltz regimen was 500 mg/m^2.
Results. One patient had grade 4 neutropenia with fever at level 1, and four patients had grade 3 neutropenia at level 2. There was no treatment-related death. Level 2 did not meet the criteria for the MTD. The relative dose intensities of the first five cycles were 91% for both 5-FU and irinotecan at level 1 and 86% for 5-FU and 93% for irinotecan at level 2. The response rates were 58% for all patients, and 69% for patients at level 2.
Conclusion. Our results confirm that the modified Saltz regimen is safe and efficacious for Japanese patients. The recommended doses for phase II studies are irinotecan 100 mg/m^2, 5-FU 500 mg/m^2, and *l*-LV 10 mg/m^2.

Key words Colorectal cancer · 5-Fluorouracil · Irinotecan · *l*-Leucovorin · Phase I/II study

Introduction

A combination of 5-fluorouracil (5-FU) and leucovorin (LV), the standard first-line therapy for advanced colorectal cancer for two decades, has a response rate of only 23% and a median survival time (MST) of 11.5 months.[1] Irinotecan is a potent inhibitor of topoisomerase I. In randomized phase III trials, irinotecan extended survival significantly as compared with best supportive care or 5-FU infusion when given as second-line therapy.[2,3] Moreover, two other randomized phase III trials showed that a combination of irinotecan, 5-FU, and LV had higher response rates, a longer time to progression (TTP), and better overall survival than did 5-FU/LV therapy.[4,5] The MST in patients who received this three-drug therapy was 14.8–17.4 months.

This three-drug regimen was designated one of the standard first-line treatments for metastatic colorectal cancer in the United States and Europe. However, patients who received a combination of irinotecan, bolus 5-FU, and LV had a three fold higher rate of early treatment-related mortality (2.5%–3.5%) from gastrointestinal toxicity or thromboembolic events compared with patients who received 5-FU/LV or oxaliplatin-based regimens (0.8%–1.1%) in subsequent phase III trials (Cancer and Leukemia Group B protocol C89803 and North Center Cancer Treatment Group protocol N9741).[6]

Irinotecan 125 mg/m^2, 5-FU 500 mg/m^2, and LV 20 mg/m^2 are given on days 1, 8, 15, and 22 every 6 weeks in

A. Goto · Y. Yamada (✉) · A. Hosokawa · T. Ura · T. Arai · T. Hamaguchi · K. Muro · Y. Shimada · K. Shirao
Gastrointestinal Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
Tel. +81-3-3542-2511; Fax +81-3-3542-3815
e-mail: yayamada@ncc.go.jp

the original Saltz regimen. In 2001, several investigators questioned how many patients received the Saltz regimen without dose reductions. The percentages of patients given irinotecan, 5-FU, and LV therapy who received the recommended doses of irinotecan and 5-FU were as follows: 89% and 88% on day 8, 64% and 64% on day 15, and 45% and 45% on day 22 of cycle 1; 47% and 48% on day 1 of cycle 2; and 42% and 41% on day 1 of cycle 3.[7] Subsequently, Elfring et al.[8] reported, in a United States study, phase III that patients received median doses of 418 mg/m^2 of irinotecan and 1602 mg/m^2 of 5-FU in the original Saltz regimen[4] during the first cycle. Knight et al.[9] recommended that the treatment schedule be modified to days 1 and 8 of a 21-day cycle, or that the initial dosage of cycle 1 be revised to irinotecan 100 mg/m^2, 5-FU 400 mg/m^2, and LV 20 mg/m^2. However, the feasibility of these modified regimens has not been studied in Japan, and many patients with colorectal cancer continue to receive 5-FU/l-LV as first-line therapy.

The present phase I/II study was designed to evaluate the safety and efficacy of a modified Saltz regimen (treatment on days 1 and 8 of a 21-day cycle) and to determine the recommended dose (RD) of irinotecan in combination with 5-FU/l-LV in Japanese patients with colorectal cancer.

Patients and methods

Eligibility

Eligible patients had histologically confirmed metastatic colorectal adenocarcinoma with measurable disease, defined as the presence of at least one index lesion able to be measured on computed tomographic (CT) scans. Other eligibility criteria included age between 20 and 75 years; Eastern Cooperative Group (ECOG) performance status of 0–2; adequate baseline bone marrow (white blood cell (WBC) count between 4000 and 12000/μl and platelets more than 100000/μl), suitable hepatic function (serum bilirubin level, 1.1 mg/dl or less, and serum aspartate aminotransferase and alanine aminotransferase 100 U/l or less), and suitable renal function (serum creatinine level, 1.2 mg/dl or less); and the ability to orally ingest food and liquids. Patients who had received prior irinotecan, bolus 5-FU therapy, or pelvic radiotherapy were excluded. Patients could have previously received adjuvant fluoropyrimidine-based chemotherapy, provided that such therapy had been terminated at least 4 weeks before study entry. Patients were also excluded if they had severe pleural effusion, ascites, diarrhea, uncontrolled infection, symptomatic brain metastases, bowel obstruction, or a high risk of a poor outcome because of concomitant uncontrollable non-malignant disease, such as diabetes, cardiac failure, or renal failure. Pregnant or breast-feeding women were also excluded. This study was approved by the institutional review board. All patients gave written informed consent before enrollment.

Treatment plan and dose escalation

Eligible patients received the following regimen: irinotecan 100 mg/m^2 by 90-min intravenous infusion; followed by *l*-LV 10 mg/m^2, administered over the course of 15 min; and 5-FU, given by bolus intravenous injection after *l*-LV. The three drugs were given on days 1 and 8 of a 21-day cycle. 5-FU was given at a dose of 400 mg/m^2 for level 1 or 500 mg/m^2 for level 2. All patients routinely received 3 mg of granisetron plus 8 mg dexamethasone before the irinotecan. Treatment continued until disease progression, unacceptable toxicity, or patient refusal.

Dose-limiting toxicity (DLT) was defined as any of the following findings during cycle 1 or 2: grade 3 non-hematologic toxicity other than nausea, vomiting, anorexia, fatigue, and hyponatremia; grade 4 leukopenia lasting for 5 days; grade 3 febrile neutropenia; grade 4 thrombocytopenia or grade 3 thrombocytopenia with hemorrhage; a WBC count of less than 3000/μl; a platelet count of less than 100000/μl, or non-hematologic toxicity of grade 2 or higher on day 22, requiring treatment to be discontinued for at least 8 days. Patient cohorts comprised a minimum of three patients for each dose level. If all three patients at level 1 completed two cycles of treatment without DLT, the next three patients were entered at level 2. If one of the three patients had DLT, three additional patients were recruited at the same dose level. If two of three or three of six patients had DLT, the maximum tolerated dose (MTD) was defined as the dose level given to this cohort. Dose reduction was not permitted during the first two cycles. If DLT occurred at level 1, the dose of irinotecan was reduced to 75 mg/m^2 from cycle 3 onward. The RD was defined as the dose one level below the MTD. If neither level 1 nor level 2 met the criteria for the MTD, the RD was defined as level 2, and dose escalation was discontinued, because the maximum approved weekly dose of irinotecan alone in Japan is 100 mg/m^2 and the dose of 5-FU in the original Saltz regimen was 500 mg/m^2. After determination of the RD, 14 patients were additionally enrolled to confirm tolerability.

Patient evaluation

Toxicity was assessed according the National Cancer Institute common toxicity criteria (NCI-CTC), version 2.0.[10] Pretreatment evaluation included a clinical examination, complete blood cell count (CBC), and chemistry profile. During treatment, toxicity was assessed weekly during cycle 1 and on days 1 and 8 of subsequent cycles.

Dose intensity was calculated by dividing the total dose received by the patient by the total duration of treatment, expressed in weeks. Relative dose intensity was calculated by dividing the delivered dose intensity by the dose intensity planned according to protocol. Dose intensity was defined within a maximum of five cycles for each patient.

The responses of assessable disease sites were evaluated according to the *New guidelines to evaluate the response to treatment in solid tumors* (RECIST).[11] Assessable lesions were reassessed every 8 weeks by CT scanning.