pound nLc_4 These results indicate that G16-1 and G16-2 contain two side chains, NeuAcα2-3Galβ1-4HexNAc and Galβ1-4HexNAc, which are linked together to the terminal galactose of nLc_4. When intact G16-2 was trimmed on a nonsialylated branch with β1,4-galactosidase and β-*N*-acetylhexosaminidase, the product was identical to G12. These

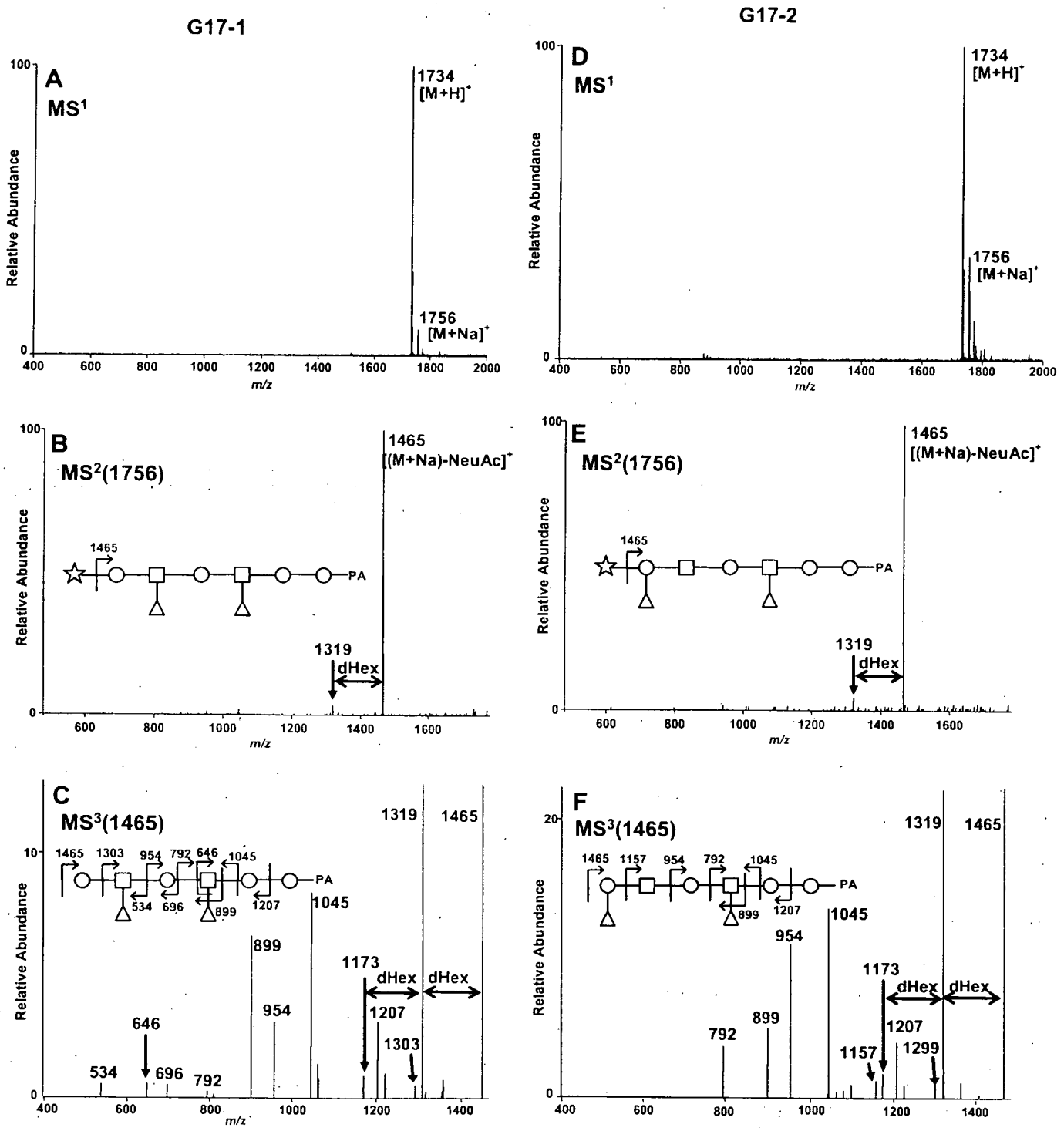

Fig. 6. MS^{1-3} spectra of G17-1 and G17-2. (A,D) MS^1 spectra of G17-1 (A) and G17-2 (D). (B,E) MS^2 spectra of $[M+Na]^+$ precursor ion at *m/z* 1756 detected in MS^1 of panel A (B) and panel D (E). (C,F) MS^3 spectra of $[(M+Na)\text{-NeuAc}]^+$ precursor ion at *m/z* 1465 detected in MS^2 of panel B (C) and panel E (F). Mass spectra in panels C and F are magnified to demonstrate clearly the minor product ions such as at *m/z* 534, 1157, and 1303. The *y* axes in panels C and F are labeled with the most abundant ions at *m/z* 1319 as 100%. Relative abundances of product ions at *m/z* 1465 in panels C and F are 38 and 45%, respectively, in these scans. Fragment ions numbered mass values in panels B, C, E, and F are sodium adduct ions. The MS/MS fragment ions were assigned as shown in Fig. 4.

results suggest the position of sialylation in G16-2 to be at the terminal galactose of the Galβ1-4GlcNAc chain on the side of the β1-3 linkage. In G16-1, the position of sialylation is at the terminal galactose of another Galβ1-4HexNAc chain (Table 3). We speculate another HexNAc to be GlcNAc and β1-6 linked to galactose; however, from the results of this study, we are unable to demonstrate this unambiguously. The structures of G16-1, G16-2, and G16-3 are consistent with MS/MS analysis data (data not shown).

Structure of G17-1 and G17-2

Sialic acid is linked α2-3 and α2-6 to the terminal residues of G17-1 and G17-2, respectively, as demonstrated by sialidase digestion as described above (Fig. 5). Desialylated G17-1 could be digested with α1,3/4-fucosidase. In contrast, desialylated G17-2 could be digested with α1,2-fucosidase but not with α1,3/4-fucosidase. The products of both digests were identical to Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-PA, which was determined as described above (Fig. 5). The digestion product from G17-1 was sequentially digested with β1,4-galactosidase, β-*N*-acetylhexosaminidase, and α1,3/4-fucosidase to yield a product identical to nLc_4 (Fig. 5). From these results, the structures of G17-1 and G17-2 are estimated to be as shown in Table 3. The structure of G17-2 has the same terminal structure as G10-2, NeuAcα2-6(Fucα1-2)Galβ1-4GlcNAcβ1, and has also not been reported previously. These structures were also confirmed by MS/MS analysis. Product ions in MS^3 spectra revealed the core structure of G17-1 and G17-2 to be Hex-HexNAc-Hex-HexNAc-Hex-Hex-PA (Fig. 6). Fucosylation of the inner HexNAc residue of G17-1 and G17-2 was suggested by the detection of a characteristic product ion at *m/z* 792, corresponding to $[dHex\text{-}HexNAc\text{-}Hex\text{-}Hex\text{-}PA+Na]^+$ in MS^3 spectra of G17-1 and G17-2. The position of attachment of the remaining fucose residue is also demonstrated by careful examination of product ions of MS^3 spectra. Product ions at *m/z* 1303, corresponding to $[dHex\text{-}HexNAc\text{-}Hex\text{-}(dHex\text{-})HexNAc\text{-}Hex\text{-}Hex\text{-}PA+Na]^+$, and at *m/z* 534, corresponding to $[Hex\text{-}HexNAc\text{-}dHex+Na]^+$, were detected in MS^3 spectra of G17-1, suggesting that the remaining fucose is linked to the subterminal HexNAc in G17-1. In contrast, in MS^3 spectra of G17-2, the product ion at *m/z* 1303 was not detected; instead, a product ion at *m/z* 1157, corresponding to [HexNAc-Hex-(dHex)HexNAc-Hex-Hex-PA], was detected, suggesting the attachment of the remaining fucose is to the outermost Hex in G17-2.

Discussion

Enzymatic release of carbohydrate moieties and fluorescent labeling, followed by 2-D mapping, MS/MS, and glycosidase digestion, revealed the structures of major acidic GSLs in colon adenocarcinoma. We were able to identify 22 kinds of acidic GSLs from approximately 20 mg of tissue. One of those identified is sulfated (G1), and the others are sialylated, including ganglio-series (G2, G3, G4, G5, G7, and G9), lacto-series (G11), and neolacto-series (G6, G8, G10-1,2, G12, G13, G14-1,2, G15, G16-1,2,3, and G17-1,2) GSLs. The structures of acidic GSLs in colon adenocarcinoma have been widely studied using a number of conventional methods, such as TLC and methylation analysis, and various kinds of acidic GSLs have been reported to be present in the tissues [8,11,13,15]. The acidic GSLs identified in this study include most of the acidic GSLs previously reported to be present in the tissue. For example, as major components, GM3 (G2), SPG (G6), $IV^6NeuAc\alpha\text{-}nLc_4$ (G8), SLe^x (G10-1), SLe^a (G11), $VI^3NeuAc\alpha\text{-}nLc_6$ (G12), and $VI^6NeuAc\alpha\text{-}nLc_6$ (G13) were identified, whereas $VI^3NeuAc\alpha III^3Fuc\alpha\text{-}nLc_6$ (G14-1), $VI^6NeuAc\alpha III^3Fuc\alpha\text{-}nLc_6$ (G15), and $VI^3NeuAc\alpha,V^3Fuc\alpha III^3Fuc\alpha\text{-}nLc_6$ (G17-1) were present as minor components. SM3 (G1) and GD1b (G9) have not been detected in the tissues in the previous studies. Furthermore, it is noteworthy that 2 novel fucogangliosides, G10-2 and G17-2, having a common carbohydrate moiety at their termini, namely NeuAcα2-6(Fucα1-2)Galβ1-4GlcNAc, were identified. To our knowledge, this unique structure has not been found in glycoproteins as well as in GSLs. In addition to G10-2 and G17-2, the structure of G16-1 has not been reported previously. Although the structures of G16-1 and G16-2 were not unambiguously determined in the current study, it is most likely that the undetermined HexNAc residue is GlcNAc linked β1-6 to terminal galactose of nLc_4 Assuming the above to be correct, G16-2 has been previously isolated from human erythrocytes [21], but G16-1 has not been isolated from any source. Several acidic GSLs, such as sulfatide and disialosyl lacto-series gangliosides (reported to be present in colon cancer tissues [11,13,15]), were not detected in this study. Sulfatide could not be detected in this study because of its high level of resistance to endoglycoceramidase II [16]. The reason for the lack of detection of the other gangliosides is not clear but may arise from sample variation.

There are two possible reasons why the newly identified acidic GSLs, G10-2, G16-1, and G17-2, have been undiscovered previously. One is that the quantities of these gangliosides are quite small. However, this is unlikely given that G17-1, which is present in this tissue at nearly equivalent levels to the newly characterized acidic GSLs seen in this analysis, previously have been isolated and characterized from adenocarcinoma of colon metastases to liver, that is, the same sample used in this study. The other possibility is the potential error in identifying acidic GSLs by TLC. These acidic GSLs might not be separated and purified to homogeneity by HPLC using organic solvents and TLC, probably due to the existence of one or more other components, such as their isomers, with identical chromatographic behavior. Even in our study, G10-2, G16-1, and G17-2 coeluted on the size fractionation column with their isomers, G10-1, G16-2, and G17-1, respectively.

It was only through the use of another chromatography method (an ODS column) that the species could be separated from each other. These results show that the high-resolution techniques employed here, consisting of pyridylamination of carbohydrate moieties of acidic GSLs followed by separation on two different kinds of chromatography column (amide and ODS), enabled the isolation and characterization of novel GSLs even in a comprehensively studied tissue. Therefore, the results encourage us to look for unique structures that may be present in other types of tumor tissues using this assay. However, it should be noted that of the 22 kinds of GSLs identified in this analysis, 12 did not match to standard oligosaccharides previously prepared and analyzed on the 2-D map. To generally apply the method used in this study to a variety of cancerous tissues, the number of prepared standard oligosaccharides would seem to be insufficient, and it is essential to obtain many more standard PA-oligosaccharide libraries for 2-D mapping. Furthermore, libraries of multistage MS^n spectra of *N*-linked oligosaccharides have been constructed and have served to identify structures [22,23]. A similar approach might also be necessary for the glycomic analysis of GSLs.

Two α2-6-sialyltransferases, ST6Gal-I [24] and ST6Gal-II [25], and two α-2-fucosyltranserases, FUT I and FUT II [26], are the candidate enzymes for the biosynthesis of the unique terminal structures of G10-2 and G17-2. It is necessary to clarify the specificities of the α2-6-sialyltransferases and the α-2-fucosyltranserases to elucidate the involvement of these glycosyltransferases in forming these carbohydrate structures. Furthermore, it is also important to determine whether or not the newly characterized G10-2 and G17-2 are tumor-associated antigens. Preparation of antibodies recognizing the unique structure common to their termini, NeuAcα2-6(Fucα1-2)Galβ1-4GlcNAc, may be useful for immunohistochemical analysis of cancerous and normal tissues.

The relative quantities of each acidic GSL were revealed in detail, showing ranges from 0.1 to 58.0% of the total. However, in the previous studies, it was impossible to analyze the quantity precisely because they were analyzed by densitometry or estimated from the density of orcinol-stained bands. We believe that the accumulation of more precise quantitative data of the kind presented here from various other tumors and normal tissues will help in understanding the characteristic features of the carbohydrate structures in individual tumors.

In summary, this study has demonstrated that the techniques used are sensitive enough and have enough resolving power to find novel acidic GSL structures from small quantities of already well-studied cancerous tissues The structural analysis of acidic GSLs in malignant tissues has been performed mainly in adenocarcinoma, whereas other types of malignant tumor, such as squamous cell carcinoma and sarcoma, have not been studied in detail. Application of the methods used in this study to these tumors will be important and will lead to the discovery of novel carbohydrate structures and reveal characteristic features of individual tumors.

Acknowledgments

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Metronomic Chemotherapy using Weekly Low-dosage CPT-11 and UFT as Postoperative Adjuvant Therapy in Colorectal Cancer at High Risk to Recurrence

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This study was designed to evaluate the antitumor efficacy and feasibility of postoperative adjuvant metronomic chemotherapy using weekly low-dosage CPT-11 and UFT in colorectal cancer at high risk to recurrence. A total of 49 patients (24 stage IIIb and 25 distant metastasis) who underwent a R0 operation were enrolled in this prospective study. Forty mg/m^2 of CPT-11 were administered on day 1, day 8, and on day 15 in 28-day cycles. A dosage of 335 mg/m^2/day of UFT was given perorally on daily schedule. Cycles were repeated for 6 months, and were followed by UFT alone for further 6 months. One or more adverse effects were seen in 43 of the 49 patients. However, most of these effects were mild at grade 1 or 2: with only nausea in 3 patients, vomiting in 2, leucopenia in 2 and neutropenia in 2 at grade 3 . The overall survival rates were favorable both in the stage IIIb group (5-year: 73%) and in the distant metastases group (5-year: 62%). Postoperative adjuvant metronomic chemotherapy using weekly low-dosage CPT-11 and UFT might be safe and feasible and prolong survival time in colorectal cancer at high risk to recurrence.

Key Words: Postoperative adjuvant chemotherapy, UFT, Irinotecan, Colorectal carcinoma, Metronomic chemotherapy

Colorectal cancer remains one of the leading killers in the world. The golden standard for treatment of colorectal cancer with curative intent is surgical resection even in the presence of distant metastasis. Worldwide, infusion of 5-fluorouracil and leucovorin (5-FU/LV) combination chemotherapy has been considered to be standard as postoperative chemotherapy for stage III colon cancer over the past decade (1,2). Recently, FOLFOX which is oxaliplatin added to bolus plus infusion 5-FU/LV has been reported to be more effective than 5-FU/LV for stage III colon cancer (3). On the other hand, no effective postoperative adjuvant chemotherapy has been established for stage IV or recurrent colorectal cancer which has a higher risk to recurrence than stage III disease after surgery.

Recently, oral fluoropyrimidines such as capacitabine and UFT (tegafur + uracil, in molar ratio 1: 4) with oral LV have been reported to be not inferior to intravenous 5-FU/LV as postoperative adjuvant chemotherapy for stage II or stage III colon cancer (4, 5). In Japan, since the 1980s, oral fluoropyrimidines such as UFT and 5'-deoxy-5-fluorouridine (5'-DFUR), an intermediate of capecitabine, have been used as postoperative adjuvant chemotherapy for colorectal cancer. A recent meta-analysis has reported that surgery combined with oral fluoropyrimidines was more effective in preventing recurrence in patients with a colorectal cancer at Dukes' B or C rather than surgery alone (6). Our previous retrospective study (7) assumed the existence of a subgroup having a high risk to recurrence and being less-responsive to oral fluoropyrimidines alone among stage III tumors. This subgroup has been termed stage IIIb according to Japanese criteria (8). This stage IIIb tumor is associated with metastasis to the mesenteric lymph nodes (intermediate or principal lymph node), and oral fluoropyrimidenes have not demonstrated any clear increase in disease-free survival rate or in overall survival rate in

stage IIIb colorectal cancer. Accordingly, potentially more effective chemotherapy such as FOLFOX should be applied for those patients at high risk to recurrence at stage IIIb, as well as for those at stage IV, and recurrent colorectal cancer, after R0 resection.

Irinotecan (CPT-11) also has been reported to be effective for advanced colorectal cancer. It has been shown that the response rate to CPT-11 was 11 to 25% in patients with advanced colorectal cancer, that was refractory to 5-FU based chemotherapy (9,10). Data have suggested a lack in tumor cross-resistance between these two agents. Recently, favorable results from combination chemotherapies using CPT-11 and 5-FU/LV for advanced colorectal cancer have been reported (11,12), and a CPT-11 and bolus plus infusion 5-FU/LV regimen FOLFIRI has been recommended as a first-line therapy for advanced colorectal cancer. However, the standard dosages in CPT-11 and 5-FU based regimens have not shown any clear benefit in an adjuvant setting in randomized trials recently reported in the USA and in Europe (13,14). Thus, the administration of CPT-11 and 5-FU could be modified to improve efficacy in an adjuvant setting. Moreover, in these FOLFIRI and FOLFOX regimens that consist of the maximum tolerated dosages (MTDs), several adverse effects at grade 3 or worse have been noted resulting in a subsequent decrease in the patient's quality of life (QOL).

Besides, conventional cytotoxic chemotherapeutics have been shown to affect the endothelium of the growing tumor vasculature (15). The antiangiogenic efficacy of chemotherapy seems to be optimized by administering comparatively low-dosages of the drug on a frequent (daily, several times a week, or weekly) or continuous schedule, with no extended interruptions - sometimes referred to as 'metronomic' chemotherapy (16). Such metronomic administration has the advantage of being less acutely toxic, therefore making more prolonged treatments possible. Of interest, since an angiogenic switch in micrometastasis is thought to be the key event for the development of recurrence after surgery (17), metronomic chemotherapy should be worthwhile investigating as postoperative adjuvant chemotherapy. Oral fluoropyrimidine is the common agent for metronomic chemotherapy, and then weekly low-dosage CPT-11 could be inserted in this pattern. Here, we report the results of a prospective pilot phase II study conducted to assess the safety and antitumor effect of weekly low-dosage CPT-11 and daily oral UFT combination metronomic chemotherapy for stage IIIb, stage IV, and for recurrent colorectal cancer, as postoperative adjuvant therapy.

Patients and Methods

Patients selection

A total of 49 patients were enrolled in this prospective pilot study, between January 2001 and November 2004. The criteria for eligibility were as follows: pathological stage IIIb, or stage IV, or recurrent colorectal carcinoma according to the Japanese criteria (8), curatively resection (R0), no preoperative radiation therapy or chemotherapy, an ECOG performance status of 0-1, adequate bone marrow function (leucocytes $\geq$ 4,000 per mm^3, platelets $\geq$100,000 per mm3), adequate liver function (bilirubin $\leq$1.5 mg/dl), adequate renal function (creatinin $\leq$1.5 mg/dl), no serious or uncontrolled concurrent medical illness, and no other active malignancy. Patients were required to be $\geq$ 20 years and <80 years of age, and not pregnant. All patients were fully informed of the investigational nature of this treatment and gave their written informed consent.

Treatment protocol

Chemotherapy was started within 8 weeks after the operation. CPT-11 was administered once in each of 3 consecutive weeks followed by one week of rest, in 4-week-treatment cycles. Forty mg/m^2 of CPT-11 was given intravenously in a 2 hrs infusion. UFT was given perorally in three divided doses for a total dosage of 335 mg/m^2/day (400 mg, 450 mg, 500 mg, or 600 mg/day) on days 3-7, days 10-14, days 17-21, and on days 24-28 (Fig. 1). With regard to the schedule of drug administration, *in vitro* studies have shown that CPT-11 down-regulates thymidylate synthase expression in tumor cells, leading to synergic effects between CPT-11 and 5-FU that are maximized when CPT-11 is given 24 hrs prior to 5-FU (18). In addition, 2 days of resting per week of UFT administration is thought not to decrease the antitumor effect of UFT and can decrease the incidence and severity of any adverse effects, compared to 7 days of consecutive administration, according to Sadahiro *et al.* (19). It thus seemed reasonable to administer of CPT-11 followed by UFT with a 2-day interval in our regimen.

Therapy was temporarily suspended for grade 2 or higher stomatitis or any diarrhea, or for total bilirubin >2.5mg/dl, creatinin >1.5mg/dl, GOT/ GPT >150U/l, leucocytes <3000/ mm^3, granulocytes <1500/ mm^3, or platelets <75000/ mm^3, or on the patient's request. When leucocytes <2000/mm^3, granulocytes <1000/ mm^3, platelets <50000/ mm^3, or grade 3 non-hematological toxicity excluding nausea/vomiting and general fatigue was observed, or if the treatment was sus-

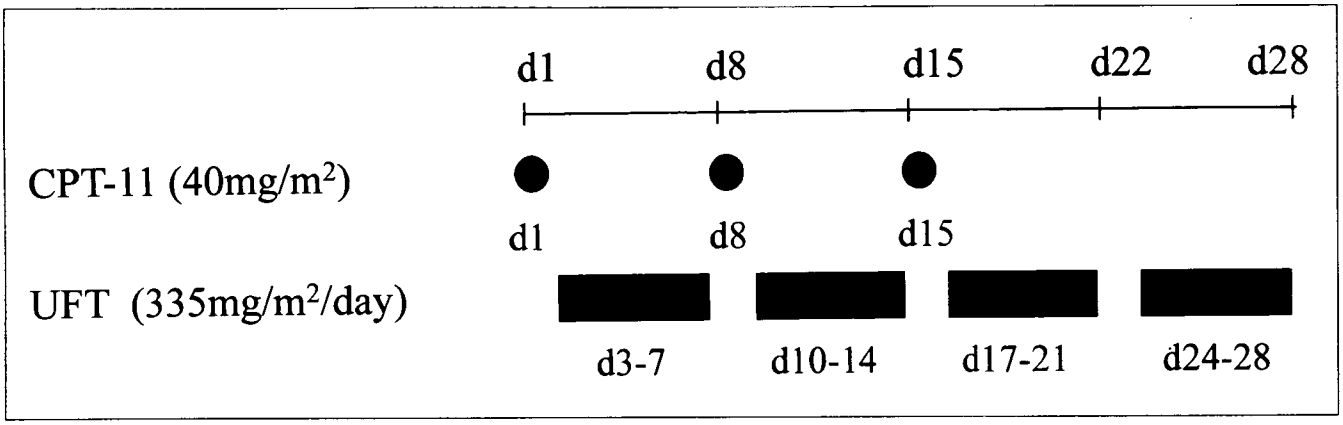

Fig. 1 - Schedule of CPT-11 plus UFT combination metronomic chemotherapy.

pended for longer than 14 days from the schedule, therapy was re-instituted using a reduced dosage of CPT-11 (30 mg/m^2) and UFT (250 mg/m^2: 300 mg, 400 mg, 450 mg, or 500 mg/day) after recovery in all toxicity levels to the administration criteria mentioned above. The therapy was permanently discontinued if a further dosage reduction was required, or if grade 4 non-hematological toxicity was found, or if the treatment was suspended for more than 21 days from the schedule, or if any recurrence occurred. The cycle was repeated for up to 6 cycles within 6 months, meaning no re-introduction since 5 months and 1 week after the initiation of therapy. The CPT-11/UFT therapy was followed by oral administration of UFT alone for a further 6 months, for a total chemotherapy period of 1 year. Treatment was performed on an outpatient basis.

Assessment of toxicity

Adverse reactions were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0.

Follow-up evaluation

Within the 2 weeks prior to initiating chemotherapy, all patients were assessed by physical examination, routine hematology and biochemistry analyses, ECG, and chest X-ray. Complete blood cell counts with platelet and differential counts were determined weekly during chemotherapy, and serum chemistries were repeated once or twice during each 28-day course. Subjective symptoms, body weight, physical examination, performance status, and all adverse effects were recorded and evaluated before each 28-day treatment cycle.

Follow-up investigations were performed through outpatient visits, by letter, and by telephone, and the most recent date of contact for each patient was regarded as the final date of confirmation. The median follow-up period was 39 months. The presence or absence of any recurrence was determined according to our follow-up protocol consisting of physical examination including digital anorectal examination every 2 to 3 months, measurement of serum tumor marker (carcinoembryonic antigen, CEA) level every 2 to 3 months, and/or by findings on barium enema or colonoscopy every 1 to 2 years, chest radiography every 6 months, and abdominal ultrasound (US), abdominal computed tomography (CT) or abdominal magnetic resonance imaging (MRI) every 6 months up to 5 years, and according to a modified protocol case-by-case thereafter.

Assessment of overall survival and disease-free survival

All data were compiled and analyzed using Statistical Analysis Software (SAS) version 6.12, (SAS Institute, Cary, NC, USA). Overall survival (OS) and disease-free survival (DFS) rates were calculated as the time from the operation to any death or recurrence using the Kaplan-Meier product -limit method.

Results

Patient characteristics

The 49 patients enrolled in this study consisted of 24 patients at stage IIIb, 16 at stage IV, and 9 patients with recurrent colorectal cancer. Their average age was 62±10 years old, with age ranging from 29 to 76 years. Twenty-seven were male, and twenty-two were female. The number of metastatic lymph nodes in 24 patients at stage IIIb was 5.6±4.6. The resected distant

metastases in stage IV or recurrent patients involved only peritoneal metastases in 10 patients, only distant lymph node metastases in 8 patients, only lung metastases in 5 patients, and only liver metastases in 2 patients.

Adverse effects

Forty-three of the 49 patients showed single or multiple adverse effects - nausea in 28 patients (57%), vomiting in 12 (24%), fatigue in 28 (57%), leucopenia in 24 (49%), neutropenia in 22 (45%), alopecia in 16 (33%), diarrhea in 9 (18%), liver dysfunction in 4 (8%), renal dysfunction in 2 (4%), thrombocytopenia in 2 (4%), and stomatitis in 2 (4%). However, most of these effects were mild at grade 1 or 2. Only nausea in 3 (6%), vomiting in 2 (4%), leucopenia in 2 (4%) and neutropenia in 2 (4%) were at grade 3 (Table I). No febrile neutropenia or treatment-related death was noted.

Administered dosages of CPT-11 and UFT as functions of the normal full dosages

The treatment was temporarily suspended by neutropenia for more than 14 days from the schedule in 1 patient, and then the dosages of CPT-11 and UFT were reduced according to the dosage-reduction criteria. In addition, 3 patients requested a dosage-reduction in CPT-11 in response to grade 2 or 3 nausea/vomiting. The administration of CPT-11 was skipped on grade 2 or 3 neutropenia, diarrhea, or on patient's request due to nausea/vomiting, general fatigue, or personal reasons, on a few occasions. Six patients requested dropping out from this study; 1 in response to grade 2 liver dysfunction after 2 cycles, 1 to grade 3 neutropenia after 3 cycles, 1 to grade 2 diarrhea after 3 cycles, 1 to grade 2 vomiting after 4 cycles, and the other 2 for non-medical personal reasons after 2 and 4 cycles, respectively. The therapy was stopped in 6 patients due to recurrence within 6 months after initiation of the metronomic chemotherapy. The average number of cycles of the CPT-11 and UFT treatment was 5.2, over all 49 patients. Table II lists the amount of CPT-11 and UFT chemotherapy actually administered, relative to the normal full dosage, in a treatment cycle. The relative dosages of CPT-11 and UFT were high in every treatment cycle. An additional 6 months of UFT monotherapy after initial CPT-11/UFT combination was performed in 37 patients. The UFT monotherapy was stopped in 8 patients out of the 37 due to recurrence. The relative dosage of UFT during the UFT monotherapy was also high at 97%.

Disease-free survival and overall survival

The median follow-up period was 45 months in those at stage IIIb, and 30 months in those at stage IV or with recurrence. The overall survival rates were favorable in the Stage IIIb Group (5-year: 73%), and in the Stage IV or Recurrence Group (5-year: 62%). The disease-free survival rate in the Stage IIIb Group was also favorable (5-year: 54%) (Fig. 2). Eight out of the 24 patients with stage IIIb disease eventually developed recurrent disease. Among those 8 patients, 6 underwent R0 re-surgery. Among the 25 patients with stage IV or recurrence, 19 patients had recurrence with a median disease-free survival time of 11 months. Among those 19 patients, 9 underwent R0 re-surgery.

Table I - Adverse Effects among 43 of the 49 Patients

Adverse effect	No. of cases according to the grade				Total (%)	Grade 3-4 (%)
	1	2	3	4		
Leucopenia	17	5	2	0	24 (49)	2 (4)
Neutropenia	16	4	2	0	22 (45)	2 (4)
Thrombocytopenia	2	0	0	0	2 (4)	0
Nausea	11	14	3	0	28 (57)	3 (6)
Vomiting	7	5	2	0	16 (33)	2 (4)
Fatigue	19	9	0	0	28 (57)	0
Alopecia	16	0			16 (33)	
Diarrhea	5	4	0	0	9 (18)	0
Liver dysfunction	1	3	0	0	4 (8)	0
Renal dysfunction	2	0	0	0	2 (4)	0
Stomatitis	1	1	0	0	2 (4)	0

Table II - Administered Dosage of CPT-11 and UFT as a Function of Normal Full Dosage

Cycle No.	No. of patients	CPT-11 dosage administered/ Normal (mean;%)	Patients receiving >80% of normal CPT-11 dosage (%)	UFT dosage administered/ Normal (mean;%)	Patients receiving >80% of normal UFT dosage (%)
1	49	97	95	100	100
2	49	93	92	99	100
3	47	90	86	97	95
4	44	88	86	93	91
5	36	91	83	96	97
6	28	90	86	96	95

Numbers in these columns indicate the mean percentage of CPT-11 and UFT actually administered as a function of normal full dosage for all patients beginning a given cycle of treatment, and the percent of patients receiving more than 80% of the normal full dosage for that cycle, respectively.

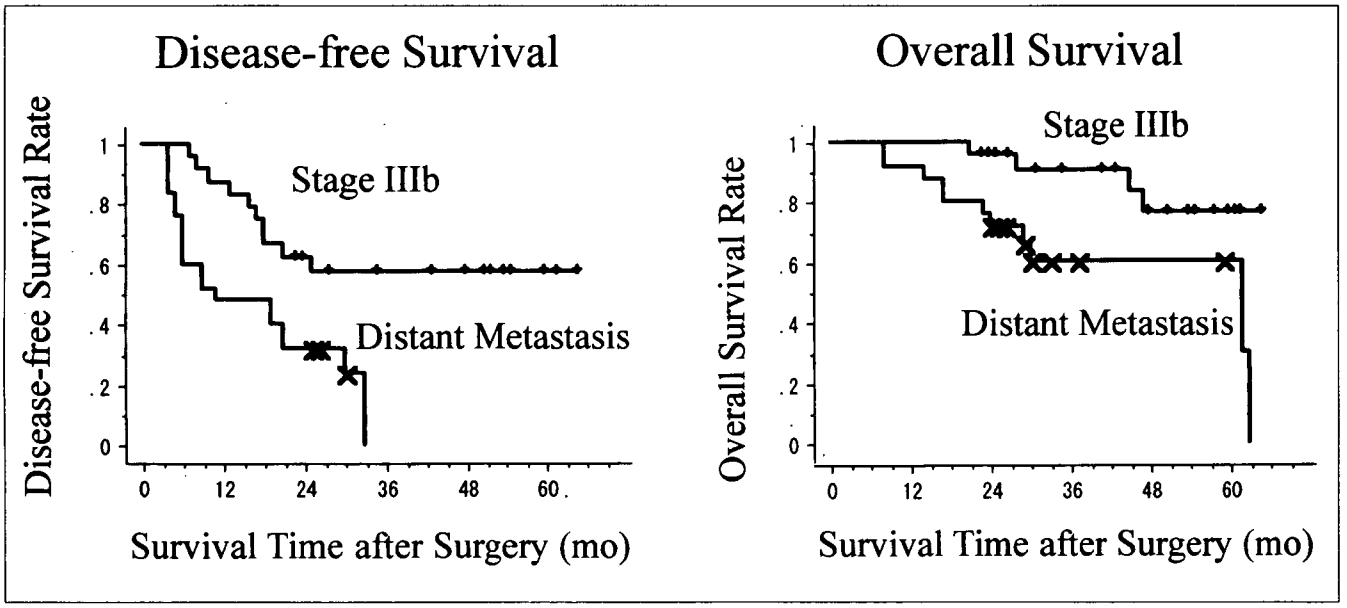

Fig. 2 - Disease-free survival (left) and overall survival (right) curves in patients with a stage IIIb colorectal cancer, and in patients with a stage IV or recurrent colorectal cancer.

Discussion

In general, chemotherapy using oral fluoropyrimidines, when compared to either intravenous 5-FU/LV or its combination with irinotecan (11,12) or oxaliplatin (20), has been characterized by a lower incidence of adverse effects, especially infrequent adverse effects at grade 3 or higher (21). As a result, chemotherapy using oral fluoropyrimidines can be continued on an outpatient basis without decreasing the patient's QOL. For patients, therapy on an outpatient basis is a critical benefit for choosing oral fluoropyrimidines.

It is now well recognized that the role of neovascularization is important for the proliferation of metastatic cells in the process of hematogenous metastasis (22). When tumor cells are able to continue proliferating and when a tumor mass is formed, neovascularization is required to deliver a supply of nourishment, oxygen, and various growth factors. Moreover, it has been suggested that the neovascularization is not only indispensable to tumor growth but also important for the occurrence of hematogenous or lymphatic metastasis through intra- or extra-vasation of the tumor cells. Thus, antiangiogenic therapy would be ideal for pre-

venting postoperative recurrence. With regard to the antiangiogenic activity of UFT, it has been shown that UFT and its metabolites, gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL), have an antiangiogenic activity in a dosage-dependent manner. And the inhibitory effects by UFT, GHB, and GBL on angiogenesis were increased with administration by continuous infusion (23), suggesting oral administration of UFT as a concept for metronomic chemotherapy, mentioned in the introduction.

Irinotecan is now a key drug in the management of metastatic colorectal cancer, after several randomized studies have established a survival benefit. Because 5-FU/LV based combination therapy with irinotecan such as FOLFIRI has similar efficacy to FOLFOX regimens against metastatic disease in a first-line setting, the impact of irinotecan based regimens in an adjuvant setting deserves further randomized clinical trials. The main reason for the use of CPT-11 as a metronomic partner for UFT instead of oxaliplatin in the present study is avoidance of oxaliplatin-associated neuro-toxicity which often distresses patients over a long period of time. With regard to a combination therapy of CPT-11 and UFT, only a few clinical studies for advanced colorectal cancer including a phase I study have been reported (24,25). These studies were designed to determine the maximum tolerated dosage (MTD) and the recommended dosage (RD) for further studies. As a weekly dosage of CPT-11, Alonso *et al.* (24) suggested in a phase I study that either (a) 110 mg/m^2 on day 1, 8 and 15 plus UFT 250 mg/m^2/day on days 1 through to 21, every 28 days, or (b) CPT-11 100 mg/m^2 plus UFT 300 mg/m^2/day were the recommended dosages. Also Mendez *et al.* (25) have reported the antitumor efficacy and safety of combination therapy of weekly CPT-11 and UFT/LV at dosages of CPT-11 125 mg/m^2 on day 1, 8 and 15 every 28 days. A full-dosage weekly CPT-11 might also be associated with antiangiogenic effetcs and might result in maximal tumor control if the therapy was continued without temporary suspension. However, the RD of weekly CPT-11 in those phase II studies combined with UFT resulted in the 18-28% of grade 3/4 diarrhea and the 11-13% of grade 3/4 neutropenia (25, 26). These grade 3/4 toxicities might necessitate temporary suspending the therapy, and then the high dosage of CPT-11 could not realize the optimal metronomic advantage of daily UFT. Accordingly, our dosage of weekly CPT-11 on day 1, day 8 and day 15 every 28 days was determined as 40 mg/m^2 which was a dosage lower than half the RDs in those previous studies, in order to continue the therapy for 6 months without risk to temporary suspension of CPT-11 and UFT administration. Indeed, in our study the incidence of grade 3/4 toxicity was extremely low, and the drop-out rate was also low (12%, in 6 of 49). The relative dosage intensities of CPT-11 and UFT were high in every cycle, indicating low risk to temporary suspension of CPT-11 and UFT administration. This low dosage and weekly administration of CPT-11 was based on a concept of metronomic chemotherapy having an antiangiogenic effect in addition to having an anti-proliferating effect against tumor cells. Another basis for determination of dosage of CPT-11 was a favorable objective response rate at 36% achieved by our similar metronomic chemotherapy using weekly CPT-11 of 40 mg/m^2 and daily 5'-DFUR against metastatic colorectal cancer (29). Since we have not yet performed a phase I study to determine the optimum metronomic RD, an adequate dosage should be determined using both toxicity at view point of continuation without temporary suspension and monitoring of circulating endothelial cells or endothelial progenitor cells from bone marrow which has been shown to be a biomarker for antiangiogenic therapy (27,28) rather than use only toxicity as a conventional determination of MTD.

Our regimen also showed favorable overall survival and disease-free survival rates for stage IIIb, and stage IV or recurrent colorectal cancer. In general, the 5-year-survival rate after surgery for stage IIIb colorectal cancer which involves intermediate or principle lymph nodes has been shown to be approximately 50-60 % (30). In stage IV or recurrent colorectal cancer, the survival rate after surgery has been reportedly favorable at 30 to 40 % in cases of liver metastasis or lung metastasis (31). However, in cases of distant lymph node metastasis or peritoneal dissemination, surgery has not shown any survival benefit. In comparing to these outcomes, the results from our metronomic chemotherapy are promising despite of small sample. Since angiogenic switch in micrometastasis is thought to be a key event for the development of metastasis after surgery (17), our metronomic chemotherapy using weekly low-dosage CPT-11 and UFT is attractive for its potential efficacy of antiangiogenesis in colorectal cancer as a postoperative adjuvant therapy. Thus, the efficacy of low-dosage CPT-11/UFT may be worth further investigation to clarify whether circulating endothelial cells or endothelial progenitor cells represent a valid surrogate marker for anti-angiogenesis.

In conclusion, metronomic chemotherapy using weekly low-dosage CPT-11 plus daily UFT showed promising antitumor activity and extremely low toxicity as postoperative adjuvant therapy for patients with

a stage IIIb, stage IV, or recurrent colorectal cancer. The achieved antitumor efficacy, together with the absence of febrile neutropenia, the absence of toxic deaths and good tolerance, supports the use of metronomic CPT-11/UFT as an acceptable adjuvant therapy for R0 resected colorectal cancer at high risk to recurrence. The results warrant further clinical studies of this adjuvant therapy using weekly low-dosage CPT-11 plus UFT including the search for surrogate markers in antiangiogenic therapy

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