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Circulating endothelial progenitor cells in metronomic chemotherapy using irinotecan and/or bevacizumab for colon carcinoma: Study of their clinical significance

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Abstract. The aim of the present study was to clarify the anti-tumor efficacy of metronomic chemotherapy using irinotecan (CPT-11) combined with or without bevacizumab against colon cancer, and the significance of circulating endothelial cell (CECs) and endothelial progenitor cells (CEPs) as a surrogate marker for metronomic chemotherapy. KM12SM cells were implanted into the subcutis of nude mouse. After confirming that the implanted tumors had grown 5 mm in size, group A received an intraperitoneal injection of 40 mg/kg CPT-11 every two weeks for 4 weeks [conventional maximum-tolerated dose (MTD)], group B received 10 mg/kg twice weekly (metronomic), group C received 10 mg/kg twice weekly combined with 5 mg/kg bevacizumab twice weekly (metronomic + anti-angiogenic), and the control group received 0.2 ml of PBS every week. Serial changes of CECs and CEPs in peripheral blood and microvessel density (MVD) in the tumor tissues were evaluated. The results showed that the antitumor activity in group B and in group C was significantly higher than that in group A. A significant inhibition in CEPs on day 15 in the metronomic therapy groups B and C was noted when compared to that in the control group, while there was no significant difference in CECs and CEPs between the groups on days 4 and 8. The MVD on day 15 in metronomic groups was significantly lower than that in group A. In conclusion, metronomic chemotherapy of CPT-11 with or without bevacizumab for colon cancer was more effective than the MTD therapy via anti-angiogenic effects. Sequential measurement of CEPs may be a predictive factor for the efficacy and a decisive factor for the optimal dose of metronomic therapy in colon cancer.

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Key words: circulating endothelial cell, irinotecan, anti-angiogenesis, maximum-tolerated dose, surrogate marker for angiogenesis

Introduction

Angiogenesis plays a pivotal role in tumorigenesis and metastasis (1). Tumor angiogenesis is a complex process and is based on the concept that a tumor requires a vascular blood supply to grow beyond 1 or 2 mm (2,3). Tumors that do not establish a neovascular supply may remain dormant for a long time (4). Neovascularization has been thought to result exclusively through proliferation, migration and remodeling of fully differentiated endothelial cells derived from pre-existing blood vessels. In addition, vascular endothelial growth factor (VEGF) has been found to induce mobilization of bone marrow-derived endothelial progenitor cells resulting in increased numbers of differentiated endothelial progenitor cells and augmented neovascularization (5,6).

Conventional cytotoxic chemotherapeutic drugs are sensitive to endothelial cells in addition to directly sacrificing or inhibiting the proliferation of rapidly dividing tumor cells (7). However, conventional chemotherapy, which is administered at the more toxic maximum-tolerated dosage (MTD), requires 2- to 3-week rest periods between successive cycles of therapy. The anti-angiogenic efficacy of chemotherapy appears 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. This concept is sometimes referred to as 'metronomic' chemotherapy (8). In such a situation, mature circulating endothelial cells (CECs) and endothelial progenitor cells (CEPs) have been used as a potentially useful surrogate marker for anti-angiogenic activity (9).

Recently, various drugs have been shown to have significant anti-angiogenic activity when administered at a low dosage using a metronomic schedule (10,11). Irinotecan (CPT-11), which has resulted in improved prognosis of patients with metastatic colorectal cancer (12,13), is always administered using a therapeutic MTD approach; thus, the antitumor and anti-angiogenic efficacy of metronomic CPT-11 administration is unknown.

Humanized monoclonal antibody bevacizumab against VEGF demonstrated an antitumor effect through its administration combined with chemotherapy using CPT-11 and

Table I. Administration schedules of CPT-11 and bevacizumab.

Treatment groups	Dose of CPT-11 (mg/kg)	Dose of bevacizumab (mg/kg)	Application	Total dose of CPT-11 over 4 weeks (mg/kg)
Group A (Conv-40)	40		Day 1, 15 i.p.	80
Group B (Metro-10)	10		Twice weekly i.p.	80
Group C (Metro-10 + Beva)	10	5	Twice weekly i.p.	80

Conv, conventional; Metro, metronomic; Beva, bevacizumab; i.p., intraperitoneal.

5-FU/LV in a phase III trial for advanced colorectal cancer (14,15). Angiogenesis inhibitors also have effects on CECs and CEPs, and these changes have emerged as a potentially useful surrogate marker (16). However, the serial change in the number of CECs/CEPs in chemotherapy, in particular in metronomic chemotherapy is still unknown. In the present study, we investigated the serial change of CECs/CEPs, and the relationship between antitumor efficacy and CECs/CEPs, in metronomic chemotherapy using CPT-11 combined with or without bevacizumab for colon cancer.

Materials and methods

Drugs. Bevacizumab was a kind gift from Genentech (South San Francisco, CA). CPT-11 was a gift from Yakult Honsha (Tokyo, Japan). CPT-11 solution was freshly prepared in 0.9% saline at a concentration of 1 mg/ml.

Cell culture. The human colon carcinoma cell line KM12SM, which produces a high level of VEGF in monolayer culture (supernatant: 2822 pg/ml/10^6/48 h, unpublished data), was kindly provided by Dr M. Nakajima (Johnson & Johnson KK, Tokyo, Japan). The tumor cells were harvested from subconfluent cultures by a 5-min treatment with trypsin-EDTA (Invitrogen, Tokyo, Japan). The dislodged cells were first washed in RPMI-1640 (Invitrogen) supplemented with 10% fetal bovine serum and re-suspended in phosphate-buffered saline (PBS) for injection. Only single cells in suspension with >90% viability were used for the injections.

Animals. Male BALB/c/nu/nu mice, aged 4 weeks, were purchased from Clea Japan, Inc. (Tokyo, Japan). The mice were maintained in a laminar-flow cabinet under specific pathogen-free conditions and were used for experiments at the age of 5 weeks. The mice were maintained in facilities according to the regulations and standards of the Kurume University School of Medicine.

Tumor xenografts and assessment of antitumor effects. A total of $1x10^6$ KM12SM cells/PBS was transplanted into the subcutis of the dorsal skin in each nude mouse. The maximum tumor diameter was set at 5 mm and then CPT-11 combined with or without bevacizumab was administered intraperitoneally at a dosage of 10-40 mg/kg of CPT-11 [up to one-fourth and one-sixteenth the dosage of the LD_{50} of 177.5 mg/kg (17)] and 5 mg/kg of bevacizumab for 28 days. After confirming that the implanted tumor had grown 5 mm in size, mice were divided into 4 groups. Group A received 40 mg/kg of CPT-11 every two weeks (Conv-40), and group B received 10 mg/kg of CPT-11 twice weekly (Metro-10). Group C received 10 mg/kg of CPT-11 twice weekly combined with 5 mg/kg of bevacizumab twice weekly (Metro-10 + Beva). The control group received 0.2 ml of PBS every week (Table I). We calculated the body weight of each mouse from day 0 to 29, and these data were used as an indicator of side effects. The tumor size was measured twice weekly using calipers, and the tumor volume was calculated by the formula: [(Maximum tumor diameter)^2 x Minimum tumor diameter/2]. We then resected the tumors 29 days after the start of the drug administration, and the tumors were fixed with 10% formalin for histological examination.

Measurement of CECs and CEPs by flow cytometry. Mice were euthanized with diethyl ether on days 0, 4, 8 and 15, in each group, and heparinized blood was obtained from the heart for CEC and CEP evaluation. CECs and CEPs were counted using a FACSVantage SE flow cytometer (BD Biosciences, San Jose, CA), and the acquired data were analyzed with FlowJo version 6.3.2 flow cytometry analysis software (Tree Star, Inc., Ashland, OR). Heparinized whole blood was hemolyzed and stained with anti-mouse CD45 monoclonal antibody, anti-mouse Flk-1 antibody, anti-mouse CD31 monoclonal antibody and anti-mouse CD117 monoclonal antibody (all from BD Bioscience, San Diego, CA). After red cell lysis, cell suspensions were evaluated by a FACSVantage SE using analysis gates designed to exclude dead cells, platelets and debris. $CD45^+$ cells were excluded by gating, and then $CD31^+$ and $Flk\text{-}1^+$ cells were separated from the $CD45^-$ cells. Among these cells, $CD117^-$ cells were regarded as CECs, and $CD117^+$ cells were regarded as CEPs (Fig. 1). After acquisition of at least 100,000 cells/sample, analyses were considered as informative when adequate numbers of events (i.e., >50, typically 100-200) were collected in the CEC and CEP enumeration gates (18,19).

Immunohistochemistry for microvessels and assessment of microvessel density. The dorsal subcutaneous tumor was fixed by formalin and embedded into paraffin. Serial sections 3 μm were cut from each block. One section was stained using hematoxylin and eosin (H&E), and a second was immunostained for CD31. Immunoreactivities were determined using the avidin-biotin peroxidase complex method (Vector Laboratories, Burlingame, CA) using anti-mouse CD31 (Abcam, Cambridge, MA) at no dilution as the primary anti-

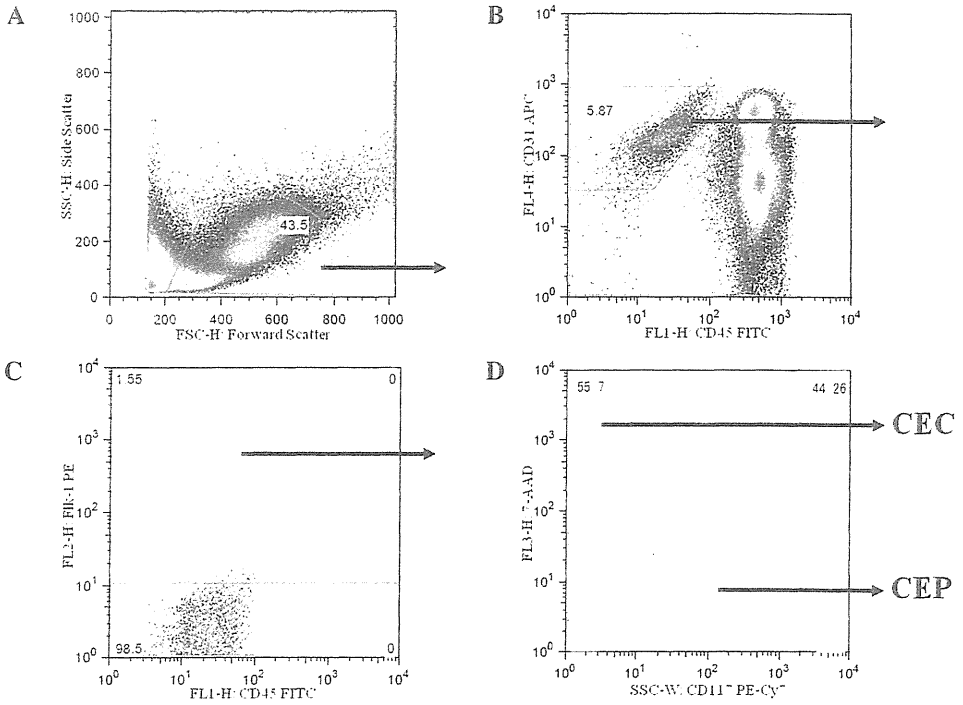

Figure 1. Representative flow cytometric evaluation of CEC and CEP enumeration. (A) Initial gate used to exclude red cells, platelets and debris. (B) Selection of $CD45^-$ and $CD31^+$ cells. (C) Gate for the enumeration of $CD45^-$, $CD31^+$ and $Flk\text{-}1^+$ cells. (D) Gate for enumeration of the $CD45^-$, $CD31^+$, $Flk\text{-}1^+$ and $CD117^+$ cells (CECs) and $CD45^-$, $CD31^+$, $Flk\text{-}1^+$ and $CD117^+$ cells (CEPs).

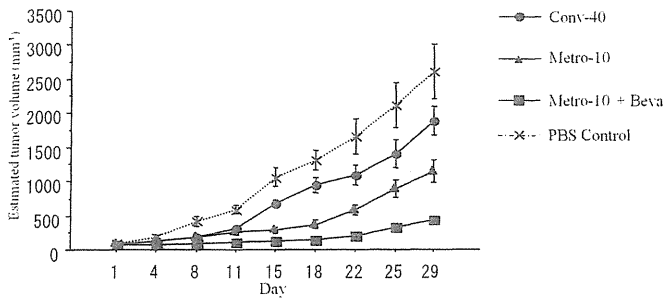

Figure 2. Growth curves of the subcutaneous (KM12SM cell) tumors implanted in nude mouse. The maximum tumor diameter was set at 5 mm, and CPT-11 combined with or without bevacizumab was administered intraperitoneally.

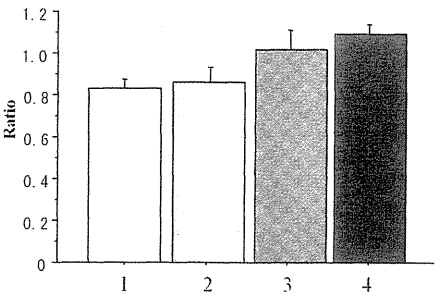

Figure 3. The weight-loss ratio (day 29/day 0): 1, PBS control; 2, Conv-40 group; 3, Metro-10 group; 4, Metro-10 + Beva group.

body. Hematoxylin was used as the counterstain. The negative controls used reagents except for the primary antibody. Positive staining of a small tubular formation for CD31 was defined as a macrovessel, and the microvessel density (MVD) was assessed as the average number of vessels/mm^2, over three areas, at x200 magnifi-cation (20).

Statistical analysis. The deta were analyzed using the Student's t-test. The tumor volume was analyzed using two-way repeated ANOVA. A P-value <0.05 was considered statistically significant. Analyses were computed using the StatView v.5.0 software (SAS Institute Inc., Cary, NC).

Results

Growth inhibition of the tumors implanted into the mouse subcutis. Conventional treatment of CPT-11 (Conv-40) showed significantly higher antitumor activity compared with the PBS control group (P=0.019). In addition, metronomic treatment using CPT-11 (Metro-10) showed more effective antitumor activity compared to the conventional (Conv-40) group (P<0.01). An additive antitumor effect was found when bevacizumab was combined with metronomic chemotherapy using CPT-11 (Metro-10 + Beva) (n=10 in each group) (P<0.01) (Fig. 2).

The weight-loss ratio (day 29/day 0) was statistically lower in the conventional group (Conv-40) than that in the metronomic-treated (Metro-10 ± Beva) groups (P=0.004), although there was no significant difference in the weight loss ratio between the conventional (Conv-40) group and that in the PBS control group (n=7 in each group) (P=0.909) (Fig. 3).

Serial changes of CECs and CEPs. CEC and CEP enumeration by flow cytometry is depicted in Fig. 1. The numbers of CECs in the control group and the conventional (Conv-40) group on day 4 and 8 showed no difference compared to the numbers on day 0, while the numbers of CECs on day 4 and 8 tended to

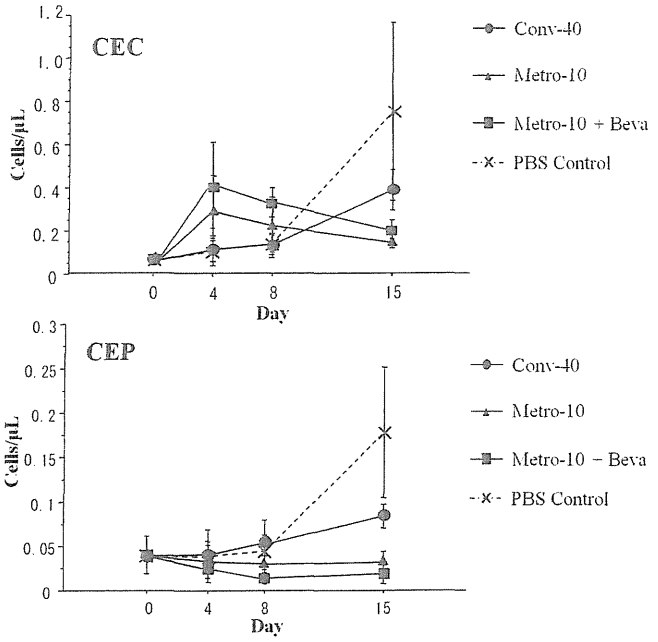

Figure 4. CEC and CEP kinetics before and after treatment in BALB/c/nu/nu mice implanted with KM12SM cell tumors.

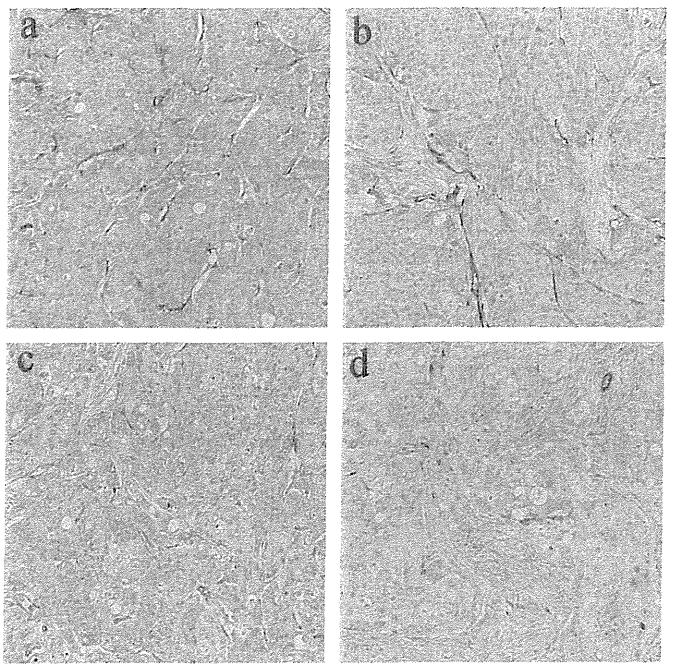

Figure 5. Immunohistochemical staining for CD31 of subcutaneously implanted tumors: (a) PBS control, (b) Conv-40, (c) Metro-10, (d) Metro-10 + Beva.

increase in the metronomic therapy (Metro-10 ± Beva) groups. While the numbers of CECs increased in the control group and the conventional (Conv-40) group on day 15, the numbers of CECs on day 15 in the metronomic therapy groups tended to decrease compared to the numbers on day 4 and 8, but did not reach significance.

There was no significant difference in the number of CEPs between each group on day 4 and 8. However, the numbers of CEPs tended to decrease in the metronomic therapy groups on day 8 compared to those on day 0. Although a statistically

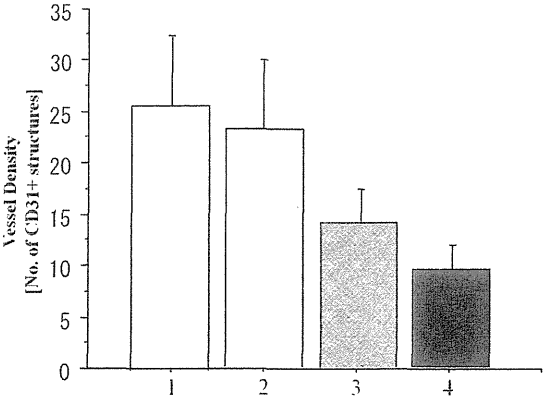

Figure 6. Quantification of the microvessel density in the implanted KM12SM cell tumors on day 15: 1, PBS control; 2, Conv-40; 3, Metro-10; 4, Metro-10 + Beva.

significant increase in the numbers of CEPs in the control and conventional (Conv-40) group on day 15 was noted compared to those on day 0, 4 and 8 (P=0.028), there was no increase in numbers of CEPs in the metronomic therapy groups (n=5 in each group) even on day 15 (Fig. 4).

Analysis of MVD. To investigate the antitumor mechanism of the metronomic CPT-11 treatment combined with or without bevacizumab, we evaluated the MVD in the implanted colon cancer tumors in the subcutis on day 15 after the beginning of drug administration (Fig. 5). The MVD values on day 15 in the metronomic treatment (Metro-10 ± Beva) groups were significantly lower than that in the conventional (Conv-40) group (P<0.001), although there was no significant difference between the conventional group and the PBS control group (P=0.173). Additive effects of the inhibition of vascularization were found when bevacizumab was combined with metronomic treatment of CPT-11 (Metro-10 + Beva vs. the Metro-10; P<0.001) (n=7 in each group) (Fig. 6).

Discussion

The purpose of the present study was to clarify the efficacy of metronomic chemotherapy using CPT-11 and its combination with bevacizumab, a specific anti-angiogenic agent, and the significance of CECs and CEPs as a surrogate marker for efficacy in metronomic chemotherapy/anti-angiogenic therapy for colon cancer. The concept of metronomic chemotherapy was summarized by Kerbel and Kamen (8) and Klement *et al* (21) as follows. (i) Conventional cytotoxic anticancer drugs have anti-angiogenic effects which could contribute to their efficacy. (ii) The anti-angiogenic effects of chemotherapy appear to be optimized by administering such drugs 'metronomically', in other words in small dosages using a frequent schedule (daily, several times a week or weekly) in an uninterrupted manner, over a relatively long period. (iii) Conventional chemotherapy, which is administered at a more toxic MTD, requires 2- to 3-week rest periods between successive cycles of therapy (which counteracts the potential for sustained therapeutically effective anti-angiogenic effects). (iv) In preclinical models, metronomic chemotherapy can be effective in treating tumors in which cancer cells have developed resistance to the same chemo-

therapeutics in an MTD administration (which also has the advantage of being less acutely toxic, therefore making a more extended treatment possible). (v) The efficacy of metronomic chemotherapy can be significantly increased when administered in combination with anti-angiogenic drugs, such as antibodies against VEGF or VEGF receptor 2. Finally, (vi) some metronomic chemotherapy regimens induce sustained suppression in CEPs and increase the levels of the endogenous angiogenesis inhibitor thrombospondin-1, both of which can suppress neovascularization.

In our experiment, the metronomic dispensing method of CPT-11 showed a higher tumor proliferation-controlling effect associated with reduced tumor MVD in nude mice transplanted with KM12SM colon carcinoma cells when compared with the conventional dispensing method. Moreover, the tumor proliferation-controlling effect of metronomic administration of CPT-11 was significantly increased when combined with bevacizumab, an anti-angiogenic agent. In addition, in the metronomic administration groups weight loss as an adverse effect was milder compared with that in the conventional MTD administration group. These results from our colon cancer model also support the concept of low-dosage metronomic chemotherapy suggesting the ability of long-term administration and tumor proliferation control.

It has been reported that, although the numbers of CEPs markedly increase during rest periods between MTD administrations of a chemotherapeutic agent to tumor-bearing mice, CEPs are absent during the metronomic administration and the development of tumors was not noted (18). We also investigated the serial changes in CECs/CEPs and their relationship with tumor vasculature (MVD) after treatment with CPT-11 and the vascular-targeting agent bevacizumab. Our data provide evidence that metronomic administration of CPT-11 and its combination with bevacizumab can have opposing effects in the early phase on days 4 and 8 and then similar effects in the late phase on day 15 on the number of CEPs and mature CECs just prior to the next MTD administration. Namely, the metronomic chemotherapy tended to increase the numbers of mature CECs and to decrease the numbers of CEPs in the early phase after the beginning of treatment (day 4 and 8), and tended to decrease both CECs and CEPs in the late phase after the beginning of treatment (day 15) in the KM12SM cell tumor-bearing mice. In particular, the difference in the numbers of CEPs in the late phase between the metronomic chemotherapy and conventional MTD chemotherapy was statistical significant. The small numbers of CEPs was associated with a concomitant inhibition in tumor vasculature and in tumor growth, suggesting that continuous suppression of CEPs may be a marker for anti-angiogenic activity, including metronomic chemotherapy in a clinical situation.

Our results support the conclusion that the antitumor effects of low dosage metronomic chemotherapy are attributable, at least in part, to a mechanism involving inhibition of tumor blood vessel formation. In addition to anti-angiogenic mechanisms in which fully differentiated endothelial cells are growth-inhibited and/or sacrificed by metronomic low-dosage chemotherapy (6), an anti-vasculogenic process may also be involved which is mediated by reduced CEP mobilization and viability. It is also interesting to consider whether MTD chemotherapy may sometimes accelerate tumor (re)growth and drug resistance by increased mobilization of CEPs. This may also help explain the robust reversal of the damage inflicted by MTD chemotherapy on tumor blood vessel endothelial cells as noted by Browder *et al* (22). An influx of mobilized CEPs during the rest periods between cycles of MTD therapy may replace damaged or sacrificed endothelial cells. In this regard, evaluating the mobilization, viability, and levels of CEPs detected in cancer patients treated with low-dosage metronomic chemotherapy regimens, (e.g., daily low-dosage oral chemotherapy and twice weekly oral methotrexate for breast cancer (23) or leukeran for lymphoma) (24) may be of considerable interest. Such studies and our data may provide a surrogate marker with which to monitor the anti-vasculogenic effects of metronomic chemotherapy protocols. In murine studies, the anti-angiogenic agent endostatin decreased the number of viable CEPs (25), whereas cyclophosphamide either induced or inhibited CEPs depending on whether it was administered in a conventional (every 21 days) or metronomic (every 6 days) dosing schedule (18).

With regard to the increase in the number of CECs early after the start of metronomic chemotherapy, it was found that mature CECs increased after 3 days of treatment with ZD6474 targeting the tumor vasculature in tumor-bearing mice but not in non-tumor-bearing mice (16), suggesting that the increase in mature CECs was due, at least in part, to the presence of the tumor and that ZD6474 or metronomic chemotherapy has at least some degree of selectivity for tumor endothelial cells rather than endothelial cells from normal vasculature. On the other hand, metronomic chemotherapy or anti-angiogenic therapy decreased the number of CECs on day 15 as well as the number of CEPs, while the CECs increased on day 15 in the control group and the MTD conventional chemotherapy group. The changes in number of CECs were similar to the changes of CEPs on day 15 after each treatment and in the control. These data suggest that mature CECs may originate from differentiation of CEPs in addition to the sloughing of tumor endothelial cells. Thus metronomic chemotherapy can consistently inhibit an increase in CEPs for a long time, while the number of CECs may be dependent on various factors such as anti-angiogenic efficacy, tumor volume, the status of tumor vasculature and time after chemotherapy, resulting in large individual variations in the number of CECs.

Recently, oral daily fluoropyrimidines such as capecitabine and UFT/LV have not been proven inferior to bolus and/or infusion MTD chemotherapy using 5-FU in randomized control studies for colon cancer (26,27). Also combination therapies of oral fluoropyrimidine and oxaliplatin/CPT-11 have been developed for colorectal cancer (28,29). Oral fluoropyrimidine would be a typical agent for metronomic chemotherapy (30). We previously reported the safety and efficacy of metronomic chemotherapy using low-dosage weekly CPT-11 and daily 5'-deoxy-5-fluorouridine, an intermediate metabolite of capecitabine, for advanced colorectal cancer (31). However, one of the major problems is a definition of the optimal dosage based on the concept of metronomic chemotherapy. This is a key reason why metronomic chemotherapy has not been widely adopted in clinical trials. Our data suggests that one possible means of determining the recommended dosage for metronomic chemotherapy is to monitor the serial change of CEPs rather than that of unstable

CECs. The optimal dosage for metronomic chemotherapy can be established as the lowest level which is associated with no increase or decrease in the number of CEPs for an individual patient.

We conclude that metronomic chemotherapy using CPT-11 against colon cancer was more effective than conventional therapy, via an anti-angiogenic effect. The combination with the specific anti-angiogenic agent, bevacizumab, may realize the advantage of metronomic chemotherapy. Measurement of CEPs may be a consistent predictive factor for metronomic chemotherapy in colon cancer. The assessment of serial changes in CEP values is recommended in clinical trials of metronomic chemotherapy.

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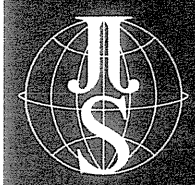

SURGERY TODAY

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切除不能大腸癌に対する2nd-lineとしてのオキサリプラチン+レボホリナート+5-フルオロウラシル併用化学療法の多施設共同第II相臨床試験：日本人における結果

A Multicenter Phase II Clinical Study of Oxaliplatin, Folinic Acid, and 5-Fluorouracil Combination Chemotherapy as Second-Line Treatment for Advanced Colorectal Cancer: A Japanese Experience

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切除不能大腸癌に対する2nd-lineとしてのオキサリプラチン+レボホリナート+5-フルオロウラシル併用化学療法の多施設共同第II相臨床試験：日本人における結果

抄　録

目　的

本試験は、日本人における切除不能大腸癌を対象とした2nd-lineとしてのオキサリプラチン+レボホリナート+5-フルオロウラシル併用化学療法（FOLFOX4法）を施行した際の有効性および忍容性を明らかにするために計画された。

方　法

1st-lineの化学療法施行後に腫瘍の増悪を認めた53例が本試験に登録された。治療は、腫瘍の増悪、忍容不能な毒性の発現または患者の中止希望のいずれかまで2週間ごとに繰り返された。

結　果

不適格が4例、プロトコール治療未施行が1例であった。したがって、奏効率、全生存期間（OS）、無増悪生存期間（PFS）の評価対象は48例、毒性の評価対象は、プロトコール治療未施行例を除く52例となった。PRは10例で、奏効率は20.8%（95%信頼区間［CI］10.5～35.0%）であった。PFS中央値は5.6カ月（95%CI 4.1～7.0カ月）であり、OS中央値は19.6カ月（95% CI 11.4～24.3カ月）であった。最も高頻度に発現したGrade 3/4の血液毒性は好中球減少であった（43.1%）。毒性プロファイルは全体的に予測可能かつ管理可能であった。

結　論

切除不能大腸癌に対する2nd-lineとして、FOLFOX4法は良好な忍容性および有効性を示した。以上より、日本人における切除不能大腸癌に対する2nd-lineとしてのFOLFOX4法は有望であることが示された。

●監修者コメント

FOLFOX法は、海外のエビデンスをもとに2005年に本邦で承認されたため、当時、本邦のエビデンスは皆無であった。そのため、海外では大腸癌治療において良好な成績が報告されていたにもかかわらず、国内での使用は困難であると考えられていた。

このような背景のもと、本試験は2nd-lineとしてのFOLFOX法のエビデンス創出を目的として九州消化器癌化学療法研究会（KSCC）により実施されたプロスペクティブな多施設共同臨床試験である。その成績は、GERCOR（V308）[1]試験における成績（奏効率15%、PFS中央値4.2カ月）と同等以上であった。また、同グループは1st-lineとしてのFOLFOX法についても検討を行っており、現在、FOLFOX法が本邦でも標準的治療として使用できるのは、これら質の高い臨床試験に寄与するところが大きい。

KSCCは大腸癌臨床研究の中心的な役割を担ってきた研究グループの一つである。これらの研究の他にも、分子標的治療薬を取り入れた治療戦略の開発、術前・術後補助化学療法の検討、さらには治療期間の延長を図るべくCa/Mgを用いたオキサリプラチン特有の神経毒性の緩和などにも取り組んでいる。癌治療の進歩のためにも本邦のエビデンスを発信していくことは非常に有意義であり、日本を代表する臨床研究グループとしてKSCCのさらなる発展を期待する。

1) Tournigand C, et al. J Clin Oncol 22:229-37, 2004

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SURGERY TODAY

Original Article

A Multicenter Phase II Clinical Study of Oxaliplatin, Folinic Acid, and 5-Fluorouracil Combination Chemotherapy as Second-Line Treatment for Advanced Colorectal Cancer: A Japanese Experience

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Abstract

Purpose. This multicenter phase II study was designed to determine the efficacy and tolerability of oxaliplatin, levoforinate, and infusional 5-fluorouracil (FOLFOX4) as a second-line therapy for Japanese patients with unresectable advanced or metastatic colorectal cancer.

Methods. A total of 53 patients with progressive disease after first-line chemotherapy were enrolled in the study. The treatment was repeated every 2 weeks until disease progression or unacceptable toxicity occurred, or the patient chose to discontinue the treatment.

Results. Four patients were ineligible and one did not receive the protocol therapy. Therefore, the response rate, overall survival (OS), and progression-free survival (PFS) were evaluated in 48 patients; toxicity was evaluated in 52 patients, excluding the patient who had not received the protocol therapy. A partial response was observed in 10 patients. The overall response rate was 20.8% (95% confidence interval [CI], 10.5%–35.0%). The median PFS was 5.6 months (95% CI, 4.1–7.0 months) and the median OS was 19.6 months (95% CI, 11.4–24.3 months). The most frequently encountered grade 3/4 hematological symptom was neutropenia (43.1%). The toxicity profile was generally predictable and manageable.

Conclusion. The results showed good tolerability and efficacy for second-line FOLFOX4 in patients with advanced colorectal cancer, thus indicating the promise of this regimen as an effective second-line therapy for advanced colorectal cancer in the Japanese population.

Key words FOLFOX4 · Multicenter phase II clinical trial · Advanced colorectal cancer · Second-line chemotherapy

Introduction

In Japan, colorectal cancer is one of the most rapidly increasing malignancies. More than 90000 people develop colorectal cancer every year, and patients with colorectal cancer are expected to outnumber those with gastric cancer early in the 21st century.[1] Advanced colorectal cancer often has a poor prognosis, even if the primary tumor can be resected, and metastasis, usually to the lungs, liver, lymph nodes, and peritoneum, is the usual cause of death in these patients. Because removal of these metastases is usually difficult, chemotherapy is the treatment of choice.[2,3]

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Based on the results of controlled studies,[4,5] irinotecan (camptothecin-11; CPT-11)+5-fluorouracil (5-FU)/leucovorin (LV) therapies (IFL, Douillard, and AIO regimens) were approved as first-line treatment for advanced colorectal cancer between 1999 and 2000 in Europe and the United States, and then went on to become the standard chemotherapy. Meanwhile, de Gramont et al.[6] have conducted a controlled study comparing infusional 5-FU/LV therapy (LV5FU2 regimen) with oxaliplatin+infusional 5-FU/LV therapy (FOLFOX4 regimen) in patients with previously untreated advanced colorectal cancer (study number: EFC2962). Although no difference was observed in survival between the two groups in their study, the response rate was significantly higher (50.7% vs 22.3%, $P = 0.0001$) and progression-free survival (PFS) (the primary end point) was significantly longer (median: 9.0 months vs 6.2 months, $P = 0.0003$) in the FOLFOX4 group. Moreover, Giacchetti et al.[7] reported similar results with a regimen that differed from FOLFOX4 (oxaliplatin+chronomodulated 5-FU/LV). Based on these two reports, oxaliplatin+infusional 5-FU/LV therapy was approved in France in 1998 and throughout the European Union (EU) in 1999 as first-line treatment for unresectable recurrent advanced colorectal cancer. Rothenberg et al.[8] conducted a controlled study of LV5FU2 versus FOLFOX4 versus oxaliplatin in patients whose tumors were resistant to IFL (irinotecan, fluorouracil, leucovorin), which was the standard chemotherapeutic regimen for advanced colorectal cancer in the United States at that time (study number: EFC4584). The response rates of the LV5FU2 group, FOLFOX4 group, and oxaliplatin group were 0%, 9.9% ($P = 0.0001$), and 1.3%, respectively, while the median time to progression (TTP) was 2.7 months, 4.6 months ($P = 0.0003$), and 1.6 months, respectively. Therefore, the FOLFOX4 group showed a significantly higher response rate and longer TTP than the LV5FU2 group. Based on these results, the FOLFOX4 regimen was approved as second-line treatment for patients resistant to IFL therapy in the United States in 2002 and in the EU in 2003.

In 2004, the Groupe d'Etude et de Recherche sur les Cancers de l'Ovaire et Digestifs (GERCOR) reported a controlled study (number V308) comparing the FOLFOX6 regimen (oxaliplatin+infusional 5-FU/LV) with the FOLFIRI regimen (CPT-11+infusional 5-FU/LV).[9] It was a crossover study, in which either FOLFOX6 or FOLFIRI was given first, and the regimen was switched if the patient's condition deteriorated. No difference was observed in the response rate (FOLFOX6 vs FOLFIRI: 54% vs 56%), TTP (median: 8.0 months vs 8.5 months), or survival time (median survival time: 21.5 months vs 20.6 months) between the two regimens when they were given as initial therapy. Oxaliplatin+5-FU/LV therapy and CPT-11+5-FU/LV therapy (FOLFIRI, Douillard, and AIO regimens) have since become the standard regimens for advanced (unresectable or recurrent) colorectal cancer[10] in Europe and the United States[11].

In Japan, on the other hand, the FOLFOX4 regimen (which is used in combination with infusional 5-FU/L-LV) has yet to be evaluated due to restrictions regarding the approved dosage and administration of 5-FU/L-LV. However, a CPT-11-based regimen was approved in 2001 for first-line treatment of advanced colorectal cancer. In the present study, we evaluated the efficacy and safety of second-line treatment with the FOLFOX4 regimen, which is the standard treatment for recurrent advanced colorectal cancer in most other countries worldwide.

Patients and Methods

Eligibility

Patients with histologically proven, unresectable, advanced, or metastatic colorectal cancer showing progression of disease after first-line treatment excluding oxaliplatin were eligible for the study if they met all of the following criteria: measurable disease; age ≥20 and ≤75 years; Eastern Cooperative Oncology Group performance status (ECOG) ≤2; life expectancy ≥3 months; adequate bone marrow, hepatic, and renal function; written informed consent given before enrollment in the study. The interval between the previous chemotherapy and the present therapy was more than 2 weeks for all patients.

Treatment Schedule

The chemotherapy schedules were as follows: 85 mg/m^2 intravenous (i.v.) oxaliplatin on day 1, and 100 mg/m^2 i.v. levoforinate (levo-leucovorin), 400 mg/m^2 i.v. bolus 5-FU and 600 mg/m^2 continuous intravenous infusion of 5-FU on days 1 and 2 every 2 weeks. Treatment was administered biweekly until progression of disease (PD), unacceptable toxicity, withdrawal of consent, physician's decision to terminate, or interruption of treatment for >14 days occurred.

Dose modifications were performed based on the hematological parameters and the degree of nonhematological toxicities. Chemotherapy was delayed until recovery if the neutrophils decreased to <1500/mm^3, platelets decreased to <75 000/mm^3, or significant persistent nonhematological toxicity occurred. The 5-FU dose was reduced to bolus 300 mg/m^2 and infusional 500 mg/m^2 if grade 3/4 diarrhea, stomatitis, nausea/vomiting, anorexia, dermatitis, grade 4 neutropenia, or grade 3/4 thrombocytopenia occurred. Oxaliplatin was also reduced to 65 mg/m^2 under the above conditions, exclud-

ing the occurrence of dermatitis, and in cases of persistent (15 days or longer) grade 2 neurotoxicity or temporary (8–14 days) grade 3 neurotoxicity. In cases of persistent (15 days or longer) grade 3 neurotoxicity or temporary grade 4 neurotoxicity, oxaliplatin was omitted from the regimen.

End Points

The primary end point of the study was PFS, and the secondary end points were the objective response rate (RR), overall survival (OS), and adverse effects. During the 4 weeks before chemotherapy was commenced, all patients underwent the following studies: physical examination, complete blood cell count, hepatic and renal function tests, and chest and abdominal computed tomography scan or magnetic resonance imaging. Physical examination, hepatorenal function tests, and blood cell counts were performed every cycle. Patients were assessed before starting each 2-week cycle according to the National Cancer Institute — Common Toxicity Criteria (CTCAE ver. 3). Tumor evaluation was assessed every month for the first 3 months and then every 2 months according to the Response Evaluation Criteria In Solid Tumors (RECIST ver. 1.0). A complete response was defined as the disappearance of all known lesions and the absence of new lesions; a partial response (PR) was defined as a reduction of 30% or more in the sum of the maximum tumor lengths of up to 10 known lesions and the absence of new lesions; stable disease (SD) was defined as a reduction of <30% or an increase of <20% in the sum of the maximum tumor lengths of up to 10 known lesions and the absence of new lesions; progressive disease (PD) was defined as an increase of >20% in the sum of the maximum tumor lengths of up to 10 known lesions or the appearance of at least one new lesion. Treatment was continued until either disease progression or unacceptable toxicity occurred or the patient chose to discontinue treatment. All eligible patients were included in the response and survival analysis on an "intent-to-treat" basis.

Relative Dose Intensity

The relative dose intensity was calculated according to the following equation:

[(total actual administered dose/actual administration period)/(total planned administration dose/planned administration period)] × 100

Statistical Considerations

We examined whether the FOLFOX4 regimen could achieve a longer PFS in Japanese patients compared with other chemotherapeutic regimens, as observed in other countries. The null hypothesis median PFS was 3 months and the expected median PFS was 4.5 months. Registration was scheduled to continue for 12 months, and the patients were expected to be followed up for 6 months after the last registration. Assuming a one-sided alpha error of 0.05 and a beta error of 0.2, registration of 3.772 patients was needed per month, which amounted to 45.3 patients per year. This meant that 46 patients would be required. The number of patients was set at 50, taking into consideration possible ineligibility or exclusion of patients from the analysis. The 95% confidence intervals (CIs) for the response rate were estimated by the exact method. Cumulative proportions concerning survival were estimated by the Kaplan–Meier method, and the CIs were estimated by the Greenwood method. All statistical analyses were performed using the Stata ver. 10.1 software program (StataCorp, College Station, TX, USA).

Results

Characteristics of Patients

A total of 53 patients were enrolled in the study between August 2005 and July 2006. Four were ineligible and one did not receive the protocol therapy. Therefore the RR, OS, and PFS were evaluated in 48 patients, and toxicity was evaluated in all patients (n = 52), excluding the patient who had not received the protocol therapy.

The characteristics of the 48 patients are detailed in Table 1. The median age was 61.5 years (range, 34–75 years) and most of the patients (90%) had a PS of 0 according to the ECOG scale. Twenty-three were men and 25 were women. Well-differentiated adenocarcinoma was present in 18 patients (38%), while moderately differentiated adenocarcinoma was observed in 25 patients (53%) as the primary tumor. As first-line treatment, a CPT-11-containing regimen had been administered to 27 patients, 5-FU/LV to 7, UFT/LV to 7, hepatic arterial infusion of 5-FU to 2, intrapelvic arterial infusion of 5-FU to 1, and other regimens to 6 patients. The liver and lungs were the most common sites of metastases. One organ was involved in 18 patients, two in 16 patients, and three or more in 14 patients.

Tumor Response

A PR was observed in 10 patients. No complete response (CR) was observed. The overall response rate was 20.8% (95% CI, 10.5%–35.0%). Stable disease was obtained in 24 additional patients (Table 2). Therefore, the overall disease control rate (CR+PR+SD) was 70.8%. Multiple liver metastases in one patient and a

Table 1. Patients' characteristics (n = 48)

Parameter	No. of patients	%
Sex		
Male	23	48
Female	25	52
Age (years)		
Median (range)	61.5 (34–75)	
Performance status (ECOG)		
0	43	90
1	4	8
2	1	2
Histology of primary tumor		
Well differentiated adenocarcinoma	18	38
Moderately differentiated adenocarcinoma	25	53
Poorly differentiated adenocarcinoma	1	2
Mucinous adenocarcinoma	1	2
Unknown	2	4
Affected organs		
Liver	28	60
Lung	24	51
Lymph node	14	30
Peritoneum	5	10
Intrapelvis	2	4
Primary site	19	40
Other(s)	6	13
Number of organs involved		
1	18	38
2	16	33
≥3	14	29
Prior chemotherapy		
CPT-11-containing regimen	27	56
5-FU/LV	7	15
UFT/LV	7	15
Hepatic arterial infusion using 5-FU	2	4
Intrapelvic arterial infusion using 5-FU	1	2
Other	4	8

ECOG, Eastern Cooperative Oncology Group; CPT-11, irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin; UFT, uracil and tegafur

Table 2. Tumor evaluation (RECIST ver. 1.0)

Response	No. of patients (%)
CR	0 (0.0)
PR	10 (20.8)
	20.8 (10.5–35.0)[a]
SD	24 (50.0)
PD	13 (27.1)
NE	1 (2.1)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable
[a]Objective response rate: CR+PR (95% confidence interval)

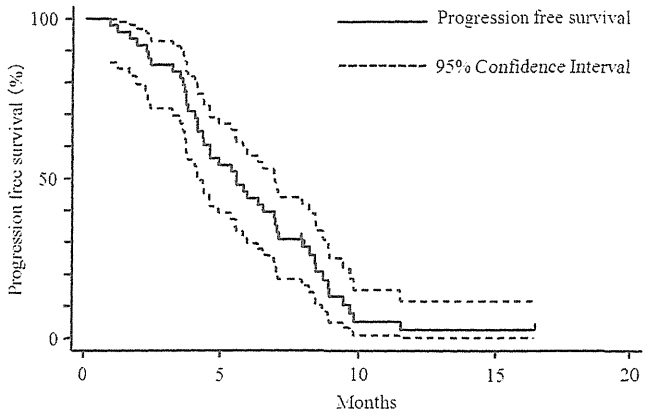

Fig. 1. Kaplan–Meier estimate for progression-free survival (PFS). The median PFS was 5.6 months (95% confidence interval, 4.1–7.0 months)

locally advanced primary tumor with multiple peritoneal disseminations in one patient were judged to be resectable after achievement of PR. Another patient who had SD with multiple peritoneal disseminations underwent surgery after nine treatment cycles.

Progression-Free Survival

After a median follow-up of 17.4 months, the median PFS was 5.6 months (95% CI, 4.1–7.0 months). The estimated 6-month and 1-year PFS were 43.8% (95% CI, 29.6%–57.1%) and 2.6% (95% CI, 0.2%–11.6%), respectively (Fig. 1).

Overall Survival

A total of 26 patients among the eligible 48 patients died due to progression of advanced colorectal cancer. At the time these analyses were carried out, the median OS was 19.6 months (95% CI, 11.4–24.3 months). The estimated 1-year and 2-year survival rates were 65.1% (95% CI, 49.3%–77.0%) and 36.3% (95% CI, 22.3%–50.5%), respectively (Fig. 2).

Toxicity and Tolerability

Toxicity data were available for 52 patients and 377 chemotherapy cycles (median = 8 cycles, range = 1–17). Frequently encountered nonhematological symptoms were peripheral neuropathy and gastrointestinal adverse effects, including diarrhea (Table 3). However, most of the nonhematological symptoms were grade 1 or 2. No grade 4 nonhematological toxicity was observed, while grade 3 peripheral neuropathy, fatigue, anorexia, and febrile neutropenia were noted in 3 (5.8%), 5 (9.6%), 3 (5.8%), and 3 out of 52 patients (5.8%), respectively. Grade 3 peripheral neuropathy was observed in the 7th, 8th, and 10th cycles. Other grade 3 nonhematological

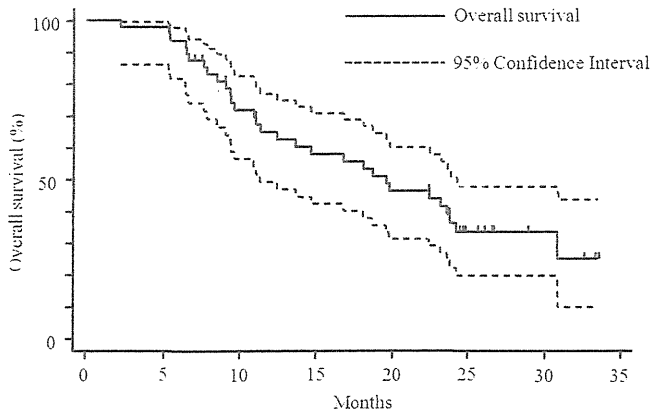

Fig. 2. Kaplan–Meier estimate for overall survival (OS). The median OS was 19.6 months (95% confidence interval, 11.4–24.3 months)

symptoms were vomiting in 2 patients and grade 3/4 neutropenia, constipation, nausea, stomatitis, hypersensitivity, or injection site reaction/extravasation. Hematological toxicities, including laboratory disorders, are summarized in Table 4. Grade 3/4 neutropenia and leukopenia and elevation of alanine aminotransferase and alkaline phosphatase were observed in 22 (43.1%), 6 (11.5%), 1 (1.9%), and 1 out of the 52 patients (1.9%), respectively.

Given the planned dose intensities of oxaliplatin at 85 mg/m^2 per 2-week cycle, 5-FU at 2000 mg/m^2 per cycle and levoforinate at 200 mg/m^2 per cycle, the relative dose intensities of each drug were 80.9%, 80.2% and 82.4%, respectively (Table 5). Adverse effects resulting in discontinuation of treatment were a longer than 14-day treatment delay due to neutropenia, febrile

Table 3. Nonhematological toxicity (n = 52)

Adverse effect	Toxicity grade					
	G0	G1	G2	G3	G4	G3+G4
Fever	43 (82.7)	7 (13.5)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Febrile neutropenia	49 (94.2)	0 (0.0)	0 (0.0)	3 (5.8)	0 (0.0)	3 (5.8)
Infection	50 (96.2)	0 (0.0)	1 (1.9)	1 (1.9)	0 (0.0)	1 (1.9)
Fatigue	22 (42.3)	17 (32.7)	8 (15.4)	5 (9.6)	0 (0.0)	5 (9.6)
Diarrhea	41 (78.8)	9 (17.3)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Nausea	27 (51.9)	14 (26.9)	10 (19.2)	1 (1.9)	0 (0.0)	1 (1.9)
Vomiting	42 (80.8)	3 (5.8)	5 (9.6)	2 (3.8)	0 (0.0)	2 (3.8)
Anorexia	17 (32.7)	17 (32.7)	15 (28.8)	3 (5.8)	0 (0.0)	3 (5.8)
Stomatitis	40 (76.9)	7 (13.5)	4 (7.7)	1 (1.9)	0 (0.0)	1 (1.9)
Peripheral neurotoxicity	18 (34.6)	19 (36.5)	12 (23.1)	3 (5.8)	0 (0.0)	3 (5.8)
Allergy	46 (88.5)	3 (5.8)	2 (3.8)	1 (1.9)	0 (0.0)	1 (1.9)
Alopecia	43 (82.7)	8 (15.4)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)
Rash	46 (88.5)	0 (0.0)	6 (11.5)	0 (0.0)	0 (0.0)	0 (0.0)
Hand–foot syndrome	48 (92.3)	2 (3.8)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Hyperpigmentation	48 (92.3)	2 (3.8)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)

Data are number of patients (%)

Table 4. Hematological toxicity

Adverse effect	Toxicity grade					
	G0	G1	G2	G3	G4	G3+G4
Leukopenia (n = 52)	10 (19.2)	12 (23.1)	24 (46.2)	6 (11.5)	0 (0.0)	6 (11.5)
Neutropenia (n = 51)	8 (15.7)	11 (21.6)	10 (19.6)	17 (33.3)	5 (9.8)	22 (43.1)
Thrombocytopenia (n = 52)	9 (17.3)	30 (57.7)	13 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anemia (Hb) (n = 52)	13 (25.0)	25 (48.1)	14 (26.9)	0 (0.0)	0 (0.0)	0 (0.0)
Total bilirubin (50)	38 (76.0)	10 (20.0)	2 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
ALT (n = 52)	35 (67.3)	14 (26.9)	2 (3.9)	1 (1.9)	0 (0.0)	1 (1.9)
AST (n = 52)	22 (42.3)	28 (53.9)	2 (3.9)	0 (0.0)	0 (0.0)	0 (0.0)
ALP (n = 50)	23 (46.0)	26 (52.0)	0 (0.0)	1 (2.0)	0 (0.0)	1 (2.0)
Creatinine (n = 52)	41 (78.9)	10 (19.2)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)

Data are number of patients (%)

Table 5. Relative dose intensity

Agent	Mean	SD	Median	Min	Max
Oxaliplatin	80.9	14.8	83.1	43.8	102.4
Levoforinate	82.4	13.7	83.2	52.2	102.4
5-FU (all)	80.2	14.8	81.3	46.4	102.4

neutropenia, or fatigue in 5 patients, an allergic reaction/hypersensitivity in 2 patients, neuropathy in 2 patients, pulmonary fibrosis in 1 patient, and a hearing disorder in 1 patient.

Discussion

In comparison with previous phase III studies the present study showed favorable efficacy, with a 20.8% response rate and 5.6-month median PFS for FOLFOX4. In this study, the null-hypothesis median PFS was 3.0 months and the expected median PFS was 4.5 months. The achieved median PFS was significantly longer than the null-hypothesis median PFS, and longer than the expected median PFS of 4.5 months. In a salvage setting, FOLFOX4 has been shown to be beneficial in the treatment of patients with advanced colorectal cancer showing progression of disease after the IFL regimen.[7] In this second-line study, FOLFOX4 achieved an objective response rate of 9.9% and a median TTP of 4.6 months. Patients treated with FOLFOX4 experienced a higher incidence of clinically significant toxicities than those treated with LV5FU2 or oxaliplatin alone, but these toxic effects were predictable and manageable. Similar to our present results, a GERCOR study[9] of second-line treatment with FOLFOX6 achieved a 15% response rate and a 4.2-month median PFS.

The high response rate seen in our patients may be explained by the fact that 56% of these patients had previously received a CPT-11-based regimen as first-line treatment. In fact, the response rate was 32% in patients receiving the CPT-11-based regimen as prior therapy and only 12% in those who did not (data not shown). Moreover, the median OS of the second-line therapy was 19.8 months, similar to the 20-month median OS achieved in a GERCOR study[9] conducted as first-line therapy for advanced colorectal cancer. There seems to be a discrepancy between the OS of 19.8 months and PFS of 5.6 months. One reason for this favorable OS may be that the majority of the patients who survived past 5.6 months continued to receive further therapy, with 36 patients receiving third-line therapy and 22 patients receiving fourth-line therapy. In addition, in Japan a novel biological agent, bevacizumab, a recombinant humanized antivascular endothelial growth factor (VEGF) monoclonal antibody, was approved for advanced colorectal cancer in 2006. A total of 18 patients received bevacizumab-containing regimens after second-line therapy. Therefore, this improved OS in our study may have been partly dependent on the sequential use of bevacizumab, which was shown to produce statistically significant increases in the response rate and survival in first- and second-line settings in combination with CPT-11- or oxaliplatin-based regimens.[12,13] With regard to another novel targeted agent, cetuximab, which inhibits epidermal growth factor receptor (EGFR), a randomized phase II study showed the efficacy of cetuximab either in combination with CPT-11 or in monotherapy as a second-line treatment after a CPT-11-based regimen.[14] However, it is unclear as to whether cetuximab contributed to the improvement in OS seen here, as only four of the patients received cetuximab-containing regimens after second-line therapy. Taken together, these results suggest that second-line FOLFOX alone or in combination with biologic agents may improve survival in patients with advanced colorectal cancer.

The toxicity profiles in our study were generally predictable and manageable. Grade 3/4 neutropenia was the most common hematologic toxicity, occurring in 43.1% of the patients, and febrile neutropenia was detected in only 5.8%. However, the incidence of grade 3/4 toxicities other than neutropenia was lower than that expected for the FOLFOX regimen based on earlier phase II/III studies. In a number of trials with oxaliplatin-based therapies, neurotoxicity was the most frequently encountered adverse effect leading to discontinuation of treatment. In our study, grade 3/4 neurotoxicity was restricted to a limited number of patients (5.8%). In addition, only a low percentage of patients (22.9%, 11/48) experienced toxicities leading to discontinuation of treatment (data not shown).

Park et al.[15] reported good tolerability and modest activity for second-line FOLFOX4 for advanced colorectal cancer patients with CPT-11 failure in the Korean population. Taken together, these data suggest that the efficacy of FOLFOX4 as second-line therapy for advanced colorectal cancer in Asian people might not be different from that observed in phase II/III studies in Western populations.

In conclusion, the results of this study demonstrated that FOLFOX4 had good tolerability and efficacy for second-line treatment of Japanese patients with advanced colorectal cancer who did not respond, or whose disease progressed, after first-line therapy including CPT-11. This indicates that FOLFOX4 represents a promising regimen for second-line therapy for advanced colorectal cancer in the Japanese population.

Contributors

Y.M. was the principal investigator. S.T., Y.E., and Y.K. were responsible for the conception of the study and study design. Y.O., H.H., N.S., H.S., F.F., T.T., M.K., M.Y., T.M., and M.I. provided patients. Y.E., E.O., Y.K., K.S., H.S., and Y.O. did the review. Y.E., Y.K., Y.M., and H.S. collected and collated the data. S.T. and Y.E. analyzed the data. S.T., Y.O., Y.K., and Y.M. interpreted the data. Y.O. wrote the manuscript, which was approved by all authors.

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消化器がんの副作用対策としてのエレンタールの効用

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はじめに

粘膜傷害はがん化学療法における最も頻度の高い有害事象のひとつである。特に口腔内の粘膜傷害である口内炎はその重症度に関わらず患者に苦痛をもたらし、QOLや治療意欲の低下をきたす。また、化学療法による骨髄抑制をきたしている患者では二次感染の要因となるため口内炎対策は化学療法を継続し、治療効果を担保するためにはきわめて重要である。

口内炎を引き起こしやすい抗がん剤としてはメソトレキセートやフルオロウラシル（5-FU）などが代表的である。大腸癌化学療法では5-FUの急速および持続投与を含むFOLFOXやFOLFIRIが標準的レジメンあり、口内炎の発生率は40％前後と報告[1,2]されている。したがって、大腸癌はがん化学療法における口内炎対策が欠かせない癌腫のひとつである。

筆者らはグルタミンの粘膜保護作用[3]に着目し、化学療法中に口内炎を発生した大腸癌患者に対し、l-グルタミンを80g中1932mg含有する成分栄養剤エレンタール（1パック80g、300kcal）を投与する前向きパイロットスタディを行った。本研究では、エレンタールの口内炎の予防・治療効果を検討するとともにがん化学療法時の有害事象対策としての栄養学的介入法の意義について考察する。

1. 対象と方法

1）対象症例

2008年5月より2009年4月までにmFOLFOX6またはFOLFIRIベースの化学療法施行中（前コース）にGrade 1からGrade 3の口内炎（CTCAE v 3.0）を認めた大腸癌患者12例を対象とした。

2）エレンタール投与法

2週1コースの化学療法中（day1からday14）通常の経口摂取に加え、1日に成分栄養剤エレンタール1パック/80g以上を可能な限り経口摂取する。エレンタールの経口投与に際してはフレーバーやゼリー化による摂取法を紹介した。2コース以上のエレンタール投与を原則とした（図1）。

3）評価項目

エレンタール投与2コースにおけるエレンタール摂取日数、摂取量、口内炎発生の有無および程度、好中球減少の有無と程度を評価した。前コースの口内炎の程度と好中球減少の有無および程度を対照に比較し、エレンタール摂取日数や摂取量との関連性について検討した。

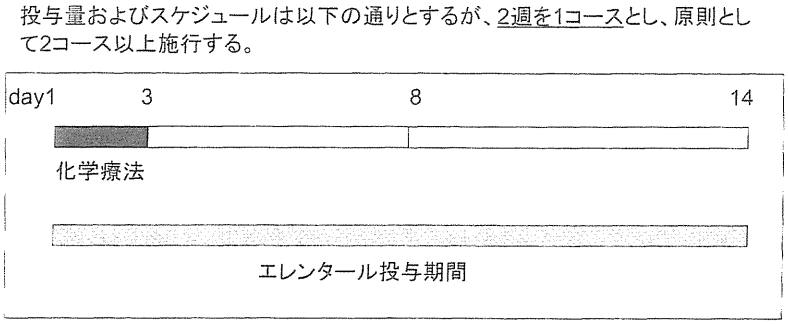

図1　化学療法のスケジュールとエレンタールの投与法

2. 結　果

1) 症例の概要とエレンタール摂取量

症例の概要とエレンタール摂取量を表1に示す。症例は男性7例、女性5例で、平均年齢は67歳(59歳から84歳)であった。前コースにおける口内炎の程度はGrade 3が2例、Grade 2が5例とGrade 1が5例であった。化学療法はmFOLFOX6とFOLFIRIの4コースごとの交替療法であるFIREFOX + bevacizumabが9例、FOLFIRI + bevacizumabが2例とFIREFOXが1例であった。口内炎がGrade 3の2例とGrade 2の1例では5-FU投与量を1レベル減量した。化学療法再開基準は一般的な臨床試験に準じた。すなわち、口内炎はgrade 1以下に回復、その他の非血液毒性として下痢がない、出血がない、grade 1以下の蛋白尿(bevacizumab併用の場合)、grade 1以下の末梢神経症状(oxaliplatin使用例)であり、血液毒性としてgrade 1以下の好中球減少、grade 1以下の血小板減少である。ただし、症例5は化

表1　症例の概要とエレンタール摂取量

症例	レジメン	減量	性・年齢	口内炎 Grade	エレンタール服用量 1コース目・2コース目	併用薬剤
症例1	BV+FIREFOX	5-FU	M・68	3	9日/720g・7日/560g	
症例2	BV+FOLFIRI	5-FU	M・75	3	7日/560g・6日/480g	
症例3	BV+FIREFOX	無	M・59	2	10日/800g・7日/560g	
症例4	BV+FOLFIRI	無	F・67	2	9日/800g・7日/560g	MA-S
症例5	BV+FIREFOX	5-FU	F・82	2	6日/480g・7日/560g	MA-S
症例6	BV+FIREFOX	無	F・84	2	6日/480g・5日/400g	
症例7	BV+FIREFOX	無	M・73	2	3日/240g・3日/240g	
症例8	BV+FIREFOX	無	F・59	1	9日/720g・6日/480g	MA-S
症例9	FIREFOX	無	M・66	1	7日/400g・8日/640g	
症例10	BV+FIREFOX	無	F・62	1	6日/400g・7日/560g	MA-S
症例11	BV+FIREFOX	無	M・44	1	4日/320g・4日/320g	
症例12	BV+FIREFOX	無	M・61	1	3日/240g・3日/240g	MA-S

BV: bevacizumab、FIREFOX: mFOLFOXとFOLFIRIの交替療法、MA-S: マーズレンS顆粒

学療法再開時 grade 2 の口内炎を認めたが、本人希望により 5-FU を減量することで施行した。エレンタール摂取日数は1コース目が3日から10日、平均6.6日、2コース目が3日から8日、平均5.8日で、摂取量としては1コース目が240gから800g、平均513g、2コース目が240gから640g、平均467gであった。なお、12例中5例にl-グルタミンとアズレンスルフォン酸ナトリウムを主成分とする商品名マーズレンS顆粒(MA-S)(l-グルタミンとして1.98g/日)を併用した(表1)。

2) 口内炎改善効果および好中球減少に及ぼす効果

口内炎 Grade 3 の1例はエレンタール投与により1コース目は grade 1、2コース目は認めなかった。Grade 3 の他の1例は1コース目が grade 2、2コース目には grade 1 と軽減した。Grade 2 の5例では1コース目は grade 0 が1例、grade 1 が3例、grade 2 が1例であった。2コース目には3例には口内炎を認めず、他の2例は grade 1 と軽減した。Grade 1 の5例では、1コース目に3例が、2コース目には4例が grade 0 とエレンタールの口内炎予防効果がみられた(表2)。

前コースでは口内炎 grade 3 の1例と grade 2 の1例に grade 3 の好中球減少を、その他2例に grade 2、別の2例に grade 1 の好中球減少を伴っていたが、エレンタール投与によりほとんどの症例で grade 1 以下に軽減した(表2)。なお、2コース目の化学療法は grade 2 の口内炎(症例2)と grade 2 の好中球減少(症例6)によりそれぞれ7日の延期となった。また、併用した MA-S と口内炎軽減効果に一定の関連性は認めなかった。

3) 口内炎予防効果とエレンタール摂取量

口内炎の grade が2段階改善した場合を著効、1段階改善を有効、改善がみられない場合を無効とし、エレンタール摂取量と口内炎の予防効果を検討した。1コース目の著効2例、有効7例、無効3例のエレンタール摂取量はそれぞれ760g、549gおよび267gであり、2コース目までの著効5例、有効6例、無効1例の1コー

表2 治療効果

症例	口内炎 前コース	口内炎 1コース目	口内炎 2コース目	好中球減少 前→1コース→2コース	備考
症例1	3	1	0	2→1→0	
症例2	3	2	1	3→1→1	摂取障害改善
症例3	2	0	0	2→0→0	
症例4	2	1	0	無	MA-S
症例5	2	1	0	2→1→0	MA-S
症例6	2	1	1	3→2→1	
症例7	2	2	1	無	
症例8	1	0	0	1→0→1	MA-S
症例9	1	0	0	無	
症例10	1	0	0	1→0→0	MA-S
症例11	1	1	0	無	
症例12	1	1	1	無	MA-S

数値は有害事象 grade、MA-S: マーズレン S 顆粒併用

スあたりの摂取量はそれぞれ608g、433gおよび240gと摂取量依存性の口内炎予防効果がみられた（表3）。

表3　口内炎治療効果とエレンタール摂取量

効果	1コース目	2コース目
著効	2例/760g	5例/544g（608g）
有効	7例/549g	6例/440g（433g）
無効	3例/267g	1例/240g（240g）

著効：2 grade改善、有効：1 grade改善、
無効：gradeの改善なし
（　）：1+2コースの平均エレンタール投与量

3. 考　察

がん化学療法時の口内炎の発生機序として抗がん剤により生じた活性酸素によるDNA傷害や種々の転写因子の活性化やサイトカイン等の産生によるアポトーシスの誘導によって引き起こされること、また抗がん剤による免疫機能低下が原因となる細菌・真菌への感染により引き起こされることが明らかにされている[4]。

口内炎の発生は、口腔粘膜上皮細胞の細胞周期と関連し、化学療法開始後5日から10日ほどで出現する。口腔粘膜は通常7日から14日サイクルで再生しているため、粘膜傷害が出現してから回復までには2週間から3週間を要する。当然、抗がん剤の種類や投与量、治療サイクル、患者の状態によって発現頻度、程度や回復までの期間は異なる。

口内炎対策としては予防が重要である。口腔内ケア等一般的な予防法に加え、いったん口内炎が発生した後ではその程度に応じた抗がん剤投与量や投与スケジュールの調整が必要となる。薬剤強度の低下は当然治療効果に反映する。したがって、薬剤強度をできる限り低下させることなく治療を継続するためには有効な口内炎予防法の確立が求められる。

本パイロットスタディでみられたエレンタールの用量依存性の口内炎予防効果は十分にその可能性を期待させる。その作用機序として筆者らが当初期待したのはグルタミンの粘膜保護作用であった。しかし、エレンタールの摂取量は期待したほど多くはなく、l-グルタミンとして14日間で6gから15gと比較的少量の摂取量であった。さらに、併用したMA-S（2g連日服用、l-グルタミンとして1日1.98g摂取）と口内炎軽減効果に関連がなかったことを考えると口内炎予防効果におけるグルタミンの役割はそれほど大きくないことが推測される。2007年Americal Cancer Society の Clinical Practice Guidelines for Prevention and Treatment of Mucositis[5] では、l-グルタミンの全身投与は化学療法施行時における口内炎を含むGI mucositis対策として推奨されていない。しかし、Choiら[6]は5-FU/LV治療の進行・再発大腸癌患者に対する高用量（30g/日）経口グルタミンの口内炎予防効果を報告しているように、glutamineの効果については引き続き検討の余地が残されている。

興味深いことに、今回筆者らが検討したエレンタール（アミノ酸製剤）を用いた栄養学的介入法は、好中球減少を抑制する可能性が示唆され、がん化学療法における有害事象対策としての栄養学的介入法の可能性を期待させる結果である。いくつかの臨床試験で食欲不振は他の有害事象の発生を助長することが示されており[7]、がん化学療法における栄養学的介入の重要性を示唆している。好中球減少の抑制に関しては、エレンタールの主要な成分であるグルタミンやアルギニンなど個々のアミノ酸の持つ免疫能賦活作用[8]の可能性が考えられるが、経口グルタミン単独での好中球減少の抑制は期待が薄い[9]。今後アミノ酸分析を含めた栄養学的評価を詳細に行うことで栄養状態と好中球減少および口内炎予防効果との関連性が明らかになるものと期待される。

2004年、FDAは造血幹細胞移植を必要とす