improvement of serum markers of cholestasis.[21] Thus, IFN used with doxorubicin may bring about the partial improvement of cholestasis in patients with advanced HCC. However, the mechanism of reduction of serum bilirubin by this combination chemotherapy remains to be clarified. Marked improvement of total bilirubin by IFN-β and doxorubicin therapy in HCC patients might offer clinical proof of the novel characteristics of interferon.

Yang *et al.* reported the efficacy of gemcitabine and doxorubicin for patients with advanced HCC, with median survival of 4.6 months for all patients and median progression-free survival of 2.5 months.[22] Obi *et al.* reported the efficacy of combination therapy of systemic IFN-α and intra-arterial 5-FU for HCC patients with portal vein invasion, with the survival rate at 12 months being 34% and median survival time of 6.9 months.[23] The 1-year survival rate for CR or SD patients was 62.5% and that for all patients, including PD patients, was 45%, and the mean survival time for all patients was 10 months in the present study, although the number of the patients was small. The present findings suggested that IFN-β is more effective than gemcitabine or IFN-α for advanced HCC. This might explain the effectiveness of IFN-β injected into the tumor site in the liver directly through the catheter. To confirm the superior effects of intra-arterial IFN-β administration, further studies with more patients and longer treatment periods should be done.

All patients enrolled in the present study had extensively advanced HCC, with five cases including portal tumor thrombus Vp3. Patients with Child–Pugh grades A and B are also eligible for this combined chemotherapy regimen, but the dose and the interval of administration should be considered for patients with ascites or a serum level of total bilirubin at 3.0 mg/dL or more, such as Child–Pugh grade C.

Small amounts of IFN-β and doxorubicin do not tend to cause severe side-effects. Under the new enrollment criteria, HCC patients need only 2 or 3 days of hospital stay for port implantation, and outpatient therapy can be started immediately. Moreover, this one-shot intra-arterial injection therapy can be conducted within a short time to minimize restriction of the patient. Based on these findings, one-shot intra-arterial combination chemotherapy of IFN-β and doxorubin could be recommended for outpatient therapy of patients with advanced HCC.

In conclusion, for patients with progressive hepatocellular carcinoma, this preliminary study shows that combined IFN-β and doxorubicin intra-arterial chemotherapy has the potential of prolonging survival time while maintaining QOL in an outpatient clinic. This combination chemotherapy, with tolerable side-effects, has the potential of serving as an optimal treatment option for advanced HCC, by improving liver function and maintaining the QOL for outpatients.

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Original Article

Vitamin K2 inhibits the proliferation of HepG2 cells by up-regulating the transcription of *p21* gene

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Aim: Vitamin K2 has been reported to inhibit the growth of human hepatocellular carcinoma (HCC) *in vitro* and suppress hepatocarcinogenesis *in vivo*. However, its inhibitory mechanism has not yet been clarified.

Methods: Different concentrations of vitamin K2 (30, 10, 1, 0.1 and 0.01 μM) were added to the HCC cell line HepG2 to assess effects on cell growth. The effect of vitamin K2 on cell cycle progression was determined by flow-cytometric analysis. The expression of cell cycle regulatory proteins p21 and p27 was then examined by Western blot. Whether vitamin K2 regulates the gene expression through action on the *p21* promoter region was investigated by luciferase assay.

Results: Vitamin K2 inhibited the growth of HepG2 cells dose-dependently, and its inhibitory rate reached approximately 50% at the dose of 30 μM after 96 h treatment. After treatment with vitamin K2, the proportion of cells in G0–G1 phase increased, and in S phase decreased. Apoptotic cells were not detected. The expression of cell cycle regulatory protein p21 was induced by vitamin K2 treatment, but p27 was not. By the luciferase assay, vitamin K2 significantly activated the promoter of p21. Knock-down of p21 by siRNA reversed the growth inhibition of HepG2 cells by vitamin K2.

Conclusions: The findings suggest that vitamin K2 suppresses the proliferation of HCC cells by blocking the cell cycle G1/S progression through the transcriptional induction of *p21*.

Key words: hepatocellular carcinoma, HepG2, p21, vitamin K2

INTRODUCTION

VITAMIN K, AN essential vitamin, consists of different forms. Vitamin K1 (phylloquinone) is found in green leafy vegetables, vitamin K2 (menaquinone) is produced by the intestinal flora, and other vitamin K congeners such as vitamin K3 (menadione) and vitamin K5 are synthetic.[1,2] Vitamin K and its derivatives have been shown to possess cell growth inhibitory effects on a variety of human and murine cancer cell lines; however, the mechanisms of the inhibitory action have not yet been clarified.[3–7]

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The *in vivo* preventive effect of vitamin K2 on the development of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C and its *in vitro* inhibitory effect on the growth and invasiveness of HCC cells have been reported.[3,5,8] Some mechanisms of the inhibitory effect by vitamin K2 have been shown to be via protein kinase A activation[5] or cell cycle arrest.[6,7] The regulation of cell cycle progression was demonstrated at the transition from G1 to S phase and suggested to be the cause of the increased expression of cell cycle regulatory protein(s) or reduced expression of cyclin-dependent kinase 4 (Cdk4). Cell cycle regulatory proteins p21 and p27 are negative regulators of G1/S progression and play important roles in regulating tumor formation and progression in humans.[9–12] The transcription of the *p21* gene is directly activated by wild-type p53 tumor suppressor protein and could play a key role as a downstream mediator of the p53-induced cell growth arrest.[9] Here, we investigated the effect of vitamin K2 on p21 and p27 in HepG2 cells.

METHODS

Materials

VITAMIN K2 (MENATETRENONE, MK-4) was supplied from Eisai (Tokyo, Japan). The human wild-type full-sizes of *p21* and *p27* promoter-luciferase fusion plasmids, pWWP and p27PF, and Sp1 deletion mutant *p21* promoter plasmids, pWP101, pWP-mtSp1-3, pWP-mtSp1-4, and pWP-mtSp1-5-6, were as reported previously.[13–15] p21 Waf1/Cip1 siRNA (Human Specific) kit was purchased from Cell Signaling Technology (Beverly, MA, USA).

Cell culture

Human HCC cell line HepG2 was purchased from the American Type Culture Collection (ATCC). HepG2 cells were cultured in Dulbecco's modified Eagle's essential medium (DMEM; Gibco BRL, Grand Island, NY, USA) with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL) at 37°C in a humidified incubator with 5% CO_2.

Cell growth assays

Cells were seeded onto 96-well plates at a density of 2.5×10^3 cells. After 20 h culture, 100 μL of fresh medium containing different concentrations of vitamin K2 (30, 10, 1, 0.1 and 0.01 μM) was added to each well. Vitamin K2 was dissolved in 99% ethanol at the concentration of 10 mM, and then diluted with DMEM to the appropriate concentrations for experiments. Control cells were cultured in DMEM containing the corresponding concentration of ethanol to each dose of vitamin K2.

After 2-, 3- or 4-day culture with vitamin K2 treatment, the number of viable cells in each well was determined with the 3-(4,5-dimethyl-2-thiazolyl-2,5-diphenyl-2H-tetrazolium bromide) (MTT) assay (Roche, Nutley, NJ, USA) according to the manufacturer's instructions.[16]

For *p21* siRNA assay, 50 and 100 nM of *p21* siRNA were transfected to the HepG2 cells for 24 h, then 30 μM of vitamin K2 was added and the cells incubated for 96 h.

All experiments were carried out four times concurrently, and then repeated three times.

Cell cycle

Cell cycle distribution was determined by flow cytometric analysis of DNA content (Becton Dickinson, San Jose, CA, USA) after 96 h treatment of vitamin K2 at 10 or 30 μM. Cell suspensions were fixed overnight in 2 mL of 70% ice-cold ethanol and incubated with RNase at a concentration of 0.25 mg/mL at 37°C for 1 h. Cells were treated with propidium iodide (50 μg/mL) for 30 min in the dark. DNA histograms were analyzed using Lysis-II software (Becton Dickinson) to evaluate the cell cycle components.[16]

Western blotting

After 96 h culture with vitamin K2, HepG2 cells were washed twice with ice-cold phosphate-buffered saline (PBS), lysed and sonicated in RIPA buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 100 μg/mL phenylmethylsulfonyl fluoride, 45 μg/mL aprotinin, 100 mM sodium orthovanadate). The supernatant of the homogenate was used for protein determination with a BCA Protein Assay Kit (Pierce, IL, USA) and electrophoresis. Samples with equal amounts of total protein were electrophoresed on a 12.5% SDS-polyacrylamide gel under reducing conditions and blotted to polyvinylidene difluoride (PVDF) membrane by electroblotting. Actin was also used to confirm equal loading. The membranes were blotted with anti-p21 antibody or anti-p27 antibody.[16] Signals were developed with an ABC kit (Vector, Burlingame, CA, USA) and diaminobenzidine. The densities of the immunoreactive bands of p21 and p27 protein were estimated by NIH Image software.

Luciferase assay

HepG2 cells (5×10^4 cells/dish) were seeded in 35 mm culture dish (Falcon, Lincoln Park, NJ, USA) in phenol red-free DMEM containing with 5% charcoal-dextran-stripped fetal bovine serum (FBS-CCS). The cells were transfected with 0.5 μg p21 or 2.5 μg p27 reporter vectors using a calcium-phosphate transfection kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Twenty-four hours later, culture media were changed to the fresh media with several concentrations (0, 10 and 30 μM) of vitamin K2, and incubated for 24 h. After incubation for 24 h, cells were harvested and lyzed with luciferase lysis buffer (Promega, Madison, WI, USA). Proteins were measured by BCA protein assay kit. Luciferase activity of each sample was measured by luciferase assay kit (Promega). The level of induction was calculated by dividing the mean luciferase activity of samples treated with vitamin K2 by the mean activity of untreated control samples. All experiments were carried out in triplicate and repeated at least three times.

Statistical analysis

The results are expressed as means ± SE. At least three separate experiments were performed for each data

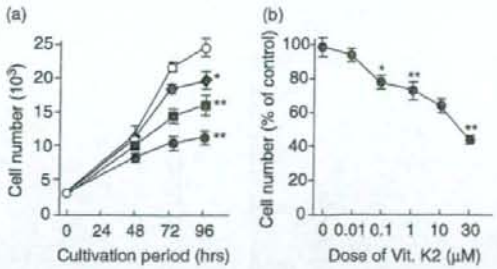

Figure 1 Vitamin K2 suppressed the proliferation of HepG2 cells in a dose-dependent manner. HepG2 cells were treated with various doses of vitamin K2 or the medium containing ethanol corresponding to 30 μM of vitamin K2 as the control. (a) Proliferation curves of HepG2 cells at various doses of vitamin K2 (1, 10 and 30 μM) for 48–96 h. ○, 0 μM; ◆, 1 μM; ■, 10 μM; ●, 30 μM. (b) Dose-dependent inhibition after vitamin K2 treatment for 96 h. Data are shown as mean ± SE of three independent experiments. $^*P < 0.05$; $^{**}P < 0.01$ *vs* control.

point. Statistical analyses were done using Student's unpaired *t*-test (two-tailed) or chi-squared test.

RESULTS

Effect of vitamin K2 on HepG2 growth

VITAMIN K2 SHOWED a dose-dependent inhibition on the proliferation of HepG2 cells (Fig. 1). The inhibitory effects by vitamin K2 after 96 h treatment were 53% and 32% at 30 μM and 10 μM, respectively. Even at 1 μM, vitamin K2 significantly inhibited the growth of HepG2 cells by about 20% after 96 h treatment ($P < 0.05$).

Effect of vitamin K2 on cell cycle progression

The cell cycle distribution of HepG2 cells was analyzed by flow cytometer after 4 days treatment with vitamin K2 (Fig. 2). The proportion of HepG2 cells in G0–G1, S and G2-M phases of the cell cycle were 58.5 ± 0.8%, 30.7 ± 1.6% and 10.9 ± 1.6%, respectively, with no treatment. After incubation with vitamin K2 for 96 h, the proportion of HepG2 cells in G0–G1 changed to 67.8 ± 1.7% and 72.8 ± 1.6%, and those in S phase to 21.0 ± 1.1% and 16.4 ± 2.7%, at 10 and 30 μM, respectively. The proportion of cells in G0–G1 phase increased, and those in S phase decreased significantly, as compared to the cells with no treatment ($P < 0.01$). In contrast, the proportion of cells in G2-M phase were 11.3 ± 2.7% and 10.9 ± 1.5% after vitamin K2 treatment at 10 and 30 μM, respectively, indicating that vitamin K2 did not affect the cell cycle phase from G2 to M. Apoptotic cells were not detected. These results showed that vitamin K2 inhibited the transition from G1 into S phase, resulting in G1 arrest.

Effect of vitamin K2 on expression of p21 and p27

To determine the molecular mechanism inducing G1 arrest by vitamin K2, the expression of cell cycle regulatory proteins p21 and p27 was examined by western blot. After 96 h treatment, p21 protein expression increased 1.77 ± 0.15 and 3.01 ± 0.16 fold by relative density at 10 and 30 μM of vitamin K2, respectively ($P < 0.01$). In contrast, p27 protein expression did not significantly increase (1.04 ± 0.09 at 10 μM and 0.93 ± 0.12 fold at 30 μM of vitamin K2; Fig. 3).

Effect of vitamin K2 on the activation of *p21* and *p27* promoters

Next, we investigated whether vitamin K2 regulates the gene expression through the action on the *p21* promoter region by use of the luciferase assay. The *p21* and *p27* reporter plasmids pWWP and p27PF were transiently transfected in HepG2 cells, and luciferase activities were examined. In HepG2 cells transfected with pWWP, the relative luciferase activity increased to 3.19 ± 0.32 and 6.04 ± 0.44 fold over than the control after vitamin K2 treatment of 10 and 30 μM, respectively (Fig. 4a). The relative luciferase activity did not increase in HepG2 cells transfected with p27PF (1.03 ± 0.11 fold at 10 μM and 0.9 ± 0.17 fold at 30 μM; Fig. 4b).

We further investigated the action sites of vitamin K2 on the promoter region of *p21* gene using several Sp1 deletion mutants of *p21* promoter. The luciferase activities did not significantly increase after the pWP101, pWP-mtSp1-3, pWP-mtSp1-4, and pWP-mtSp1-5-6 vectors were transfected (Fig. 4c). These results showed that four Sp1 sites between −101 to 0 in the *p21* promoter were not necessary, but the sequence between −2320 to −102 region was responsible for the induction of p21 by vitamin K2.

Effect of knock-down of p21 by siRNA on vitamin K2-induced growth inhibition

Next, we investigated whether the inhibition of p21 induction reversed the effect of vitamin K2 on HepG2 cells. The expression of p21 protein was suppressed after *p21* siRNA transfection (data not shown). After the knock-down of p21 by 50 nM and 100 nM *p21* siRNA,

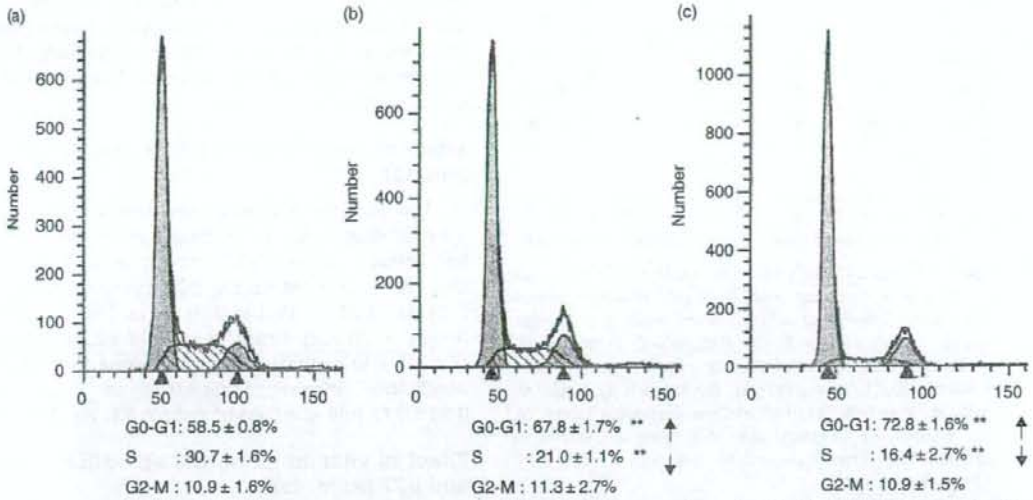

Figure 2 Effects of vitamin K2 on cell cycle progression. Cell cycle distribution was determined by flow cytometric analysis after 96 h treatment of vitamin K2. Each DNA histogram is a representative of three independent experiments at (a) 0, (b) 10 and (c) 30 μM of vitamin K2. The percentages of G0–G1, S and G2-M phase cells at each panel are means ± SE of three independent experiments. $^{**}P < 0.01$ *vs* control.

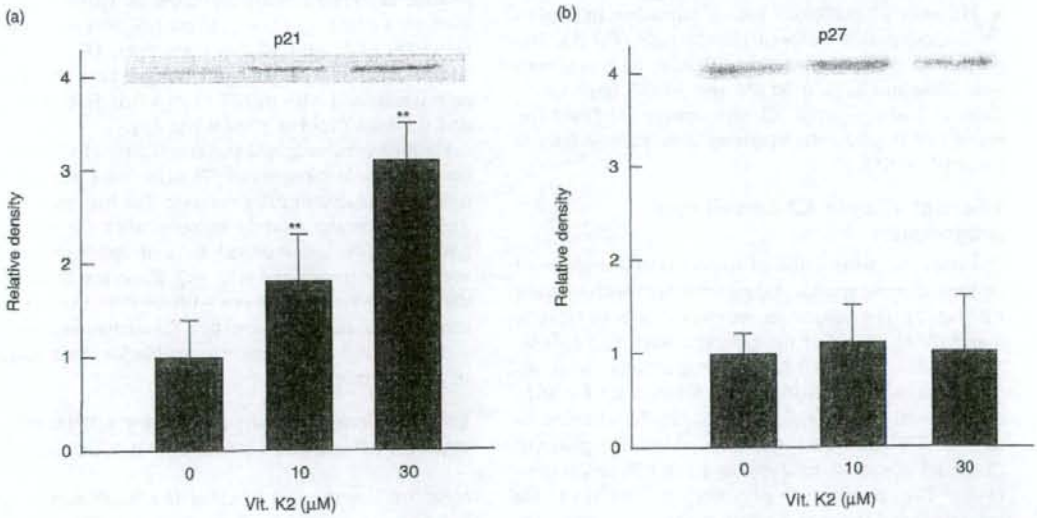

Figure 3 Vitamin K2 induced the expression of p21 protein, but not p27 protein. Expression of (a) p21 protein and (b) p27 protein was evaluated by western blot after 96 h treatment of vitamin K2 at 0, 10 and 30 μM, and the densities of immunoreactive bands were measured by NIH Image software. Data are shown as means (bars, SE) of three independent experiments. $^{**}P < 0.01$ *vs* control.

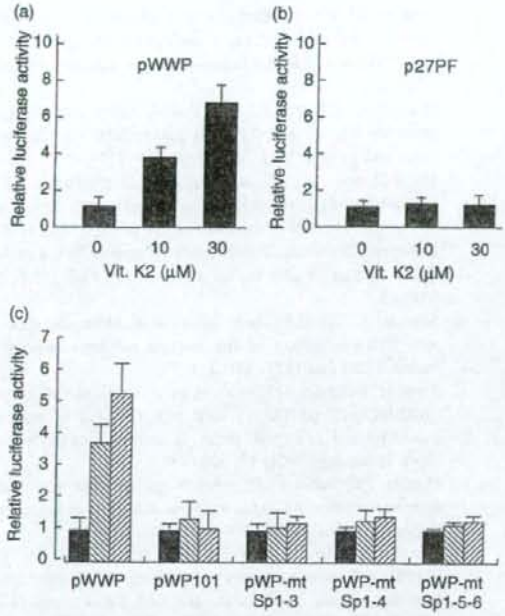

Figure 4 Vitamin K2 activated the p21 gene promoter, but not p27 gene promoter. pWWP and Sp1 deletion mutant vectors were used as luciferase reporter plasmids for p21 gene promoter, and p27PF was used for p27 gene promoter. HepG2 cells were transfected with 0.5 μg of p21 or 2.5 μg of p27 promoter vectors. After incubation with indicated concentrations of vitamin K2 for 24 h, cells were harvested and relative luciferase activities were measured. (a) pWWP, (b) p27PF and (c) Sp1 deletion mutant p21 vectors. ■, 0 μM; ▨, 10 μM; ▨, 30 μM.
Data are shown as means (bars, SE) of three independent experiments.

the cell growth inhibited by vitamin K2 at 30 μM recovered from 46.9 ± 1.8% to 54.9 ± 2.8% and 73.0 ± 9.1%, respectively (Fig. 5). These findings suggest that p21 is necessary for the growth inhibition of HepG2 cells by vitamin K2.

DISCUSSION

VITAMIN K2 HAS been reported to inhibit the growth of HepG2 cells in dose-dependent manner.[5,6] The inhibitory mechanism of vitamin K2 has been shown to cause the arrest of cell cycle progression.[7,17,18] By FACScan method, the G1–S block has been reported to be induced by vitamin K2 treatment in HepG2 cells.[3,6] The present results demonstrating the growth inhibition through G1 arrest induced by vitamin K2 are consistent with these previous observations.

In the eukaryotic cell cycle, several positive and negative factors regulate cell cycle progression.[9] Among the positive cell cycle regulators, the key players are a family of protein kinases termed cyclin-depedent kinase (Cdk). Cdks play important roles in promoting the transition from G1 to S phase by the phosphorylation of the retinoblastoma protein (pRB).[9] The negative cell cycle regulators, of which the important proteins are p21 and p27, appear to function as broad specific inhibitors of Cdk complexes.[9] Vitamin K2 has been reported to inhibit the growth of HCC cell line PLC/PRF/5 by inducing Cdk4.[17] Overexpression of p21 has been reported to suppress the growth of MG63 osteosarcoma cells and induce the differentiation of carcinoma cells.[18] p21 and p27 also play important roles in cell-cycle arrest induced by cyclooxygenase-2 inhibitor (etodolac) or PPAR-γ ligand (troglitazone) in human hepatoma cells.[15,16] The p21 expression was reported to be decreased in HCC.[19] The increased expression of p21 after vitamin K2 treatment has been shown by immunoblot in HepG2 cells, but at a higher concentration (500 μM) than the clinically used dose.[6] In this study, we confirmed the induction of p21 protein in HepG2 cells by vitamin K2 at the concentration of clinically used dose (30 μM), by Western blot, and furthermore the induction of *p21* gene by luciferase promoter assay. In addition, the suppression of vitamin K2-induced p21 by siRNA reversed the growth inhibitory effects of vitamin K2. These results

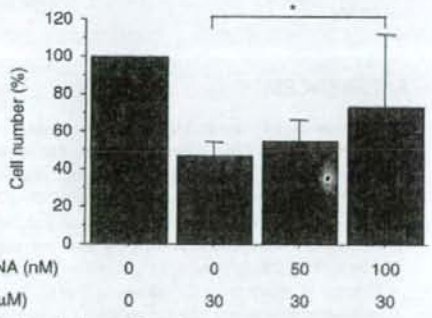

Figure 5 Knock-down of p21 by siRNA reversed the growth inhibitory effects of vitamin K2. p21 siRNA was transfected to HepG2 cells at the concentration of 50 and 100 nM. Twenty-four hours later, vitamin K2 (30 μM) was added and incubated for 96 h. Cell numbers (%) are shown as means (bars, SE) of three independent experiments. $^*P < 0.05$.

show that p21 plays a key role in the growth inhibition of HepG2 cells by vitamin K2. However, the induction of p27 was not observed by either western blot or luciferase reporter assay. No previous study has reported on the induction of p27 by vitamin K2. Thus, p27 seems to play no important roles on the growth inhibition of HCC induced by vitamin K2. These findings suggest that vitamin K2 suppresses the proliferation of HepG2 cells by the mechanism of G1 arrest via the induction p21 cell cycle regulator through the activation of *p21* gene promoter.

p21 was thought to be a target gene of vitamin K2 and vitamin D3.[18] Some medications such as butyrate, trichostatin A and vesnarione have been reported to directly bind to the promoter region and activate the transcription of *p21* through Sp1 sites in several cancer cell lines.[11,12,20] The Sp1 site is also the binding site of vitamin D3 in the *p27* gene promoter.[15] In the present study, the Sp1 site in −101 to 0 region of *p21* promoter was not responsible for vitamin K2 action. The upstream region from −102 to −2320 was essential for p21 induction by vitamin K2. Thus, the Sp1 site seems to be not necessary for interaction with vitamin K2. It is very important to analyze the detailed mechanism of the action of vitamin K2 on the *p21* promoter in future.

In conclusion, our results indicate that vitamin K2 activates the promoter region and enhances the transcription of *p21* gene, which then suppresses the proliferation of HCC cells. Although future study is still needed to investigate the detailed mechanism of the effect of vitamin K2 on cell growth inhibition, these findings suggest that vitamin K2 might be a useful treatment for HCC.

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A Phase I Study of Combination Therapy of the Oral Fluorinated Pyrimidine Compound S-1 with Low-dose Cisplatin Twice-a-week Administration (JFMC27-9902 Step2) in Patients with Advanced Gastric Cancer Using a Continual Reassessment Method

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Objective: We conducted a Phase I study to evaluate the safety and efficacy of a combination of S-1 with semi-weekly low-dose cisplatin in patients with unresectable/recurrent gastric cancer to determine the recommended dose (RD) for a subsequent Phase II study.
Methods: S-1 was administered orally at 80–120 mg/body/day based on body surface area. One cycle consisted of the consecutive administration of S-1 for 28 days followed by 14 days rest. Three dose levels, 7.5, 10, and 15 mg/m^2/day, were set for cisplatin, which was administered twice-a-week for 4 weeks followed by 2 weeks of rest in each cycle. Dose-limiting toxicity (DLT) data were continually monitored to enable decisions regarding cisplatin dose escalation and deescalation based on a new dose-finding algorithm using a continual reassessment method (CRM). The CRM target toxicity level to estimate the RD was set at 20%.
Results: Eight and five patients were treated at cisplatin dose levels of 10 and 15 mg/m^2/day, respectively. Two DLTs occurred at both dose levels. On the basis of this data, the CRM estimated the RD to be 10 mg/m^2/day of cisplatin. Three patients of eight patients treated with 10 mg/m^2/day of cisplatin exhibited a confirmed partial response during the treatment period.
Conclusion: For future trials examining the safety and efficacy of daily S-1 with semi-weekly cisplatin in patients with unresectable/recurrent gastric cancer, we found a cisplatin RD of 10 mg/m^2/day.

Key words: S-1 – low-dose cisplatin – continual reassessment method – gastric cancer – Phase I clinical study

INTRODUCTION

The oral dihydropyrimidine dehydrogenase inhibiting fluoropyrimidine S-1 is both safer and more effective than other therapies for the treatment of recurrent and/or unresectable gastric cancer (1–3), and S-1 has been incorporated into standard gastric cancer treatment regimens in Japan. A variety of S-1 based combination chemotherapies have been studied to establish a more effective treatment regimen that minimizes the occurrence of adverse events. In particular, combinations of cisplatin (CDDP) (4–8), docetaxel (9,10), paclitaxel (11), or irinotecan (12) with S-1 have shown promise for the treatment of gastric cancer through Phase

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I or II study. Two regimens combining different doses of CDDP with S-1 were examined: a high-dose regimen with 60 mg/m^2 CDDP once in 5 weeks (7) or 75 mg/m^2 once in 4 weeks (8) or a low-dose regimen involving weekly 25 mg/m^2 or less administration of CDDP (4–6). Additionally, Hyodo et al. (4) conducted a dose-finding Phase I study and determined that weekly administration of 20 mg/m^2 CDDP was optimal when combined with 70 mg/m^2 of S-1 administered daily for 2 weeks followed by a 1-week washout period. With this regimen, 58% of patients responded to therapy, but 54% of patients experienced Grade 2 gastrointestinal toxicity during the first two cycles. This is particularly concerning because oral anticancer agents such as S-1 must achieve high concentrations to be maximally effective, and gastrointestinal toxicity including nausea and anorexia severely limits this. A lower dose of CDDP administered more frequently may be as effective with less gastrointestinal toxicity.

The Japanese Foundation for Multidisciplinary Treatment of Cancer carried out a Phase I clinical trial (JFMC27-9902) examining the safety of escalating low doses of CDDP given in combination with a fixed dose of S-1 five times per week (5). Among the doses of CDDP examined, 1, 2, 3, 4, or 6 mg/m^2, 4 mg/m^2 given 5 days per week was optimal. However, the dosing schedule would require a large degree of hospitalization that is not compatible with the current medical and economic environment in Japan. The reality of this situation suggests that a new Phase I/II study is needed to determine an optimal twice-a-week CDDP administration schedule that maintained or increased regimen efficacy while minimizing adverse events (13). Data from the previous Phase I trial was used as a baseline for the development of a twice-a-week CDDP dosing schedule in the current Phase I study (JFMC27-9902 Step2), and we adopted a new dose-finding algorithm using a continual reassessment method (CRM) (14,15) to estimate a recommended dose (RD) for the new dosing schedule. Data from the present study will be used to develop a Phase II study to examine the efficacy of a novel CDDP, S-1 combination regimen for the treatment of unresectable and/or recurrent gastric cancer.

PATIENTS AND METHODS

Trial Eligibility

Patients with a histologic diagnosis of unresectable or recurrent gastric cancer and a performance status (PS) of Eastern Cooperative Oncology Group of 0 to 1 were eligible for study participation. Additional eligibility criteria included: (i) age ranging from 20 to 75 years, (ii) no anti-tumor therapy within 28 days prior to enrollment except for hormone-therapy and immunotherapy completed 2 weeks before enrollment into this trial and postoperative adjuvant chemotherapy not using CDDP completed 1 month before enrollment into this trial, (iii) life expectancy longer than 12 weeks, (iv) adequate bone marrow function (Hb $\geq$9.0 g/dl, white blood cells between 4000 and 12 000/μl, platelets $\geq$100 000/μl), and (v) sufficient organ function (total bilirubin $\leq$1.5 mg/dl, GOT and GPT $\leq$2.5 times the upper normal level, alkaline phosphatase $\leq$ two times the upper normal level, and blood urea nitrogen (BUN) and serum creatinine $\leq$ the upper normal level). Informed consent was obtained from each patient before enrollment. Each institutional review board for human experimentation approved the protocol of this study.

Treatment Regimen

S-1 was administered orally twice daily after a meal at one of three initial doses based on body surface area (BSA): (i) BSA $<$1.25 m^2, 80 mg per day, (ii) 1.25 m^2 $\leq$ BSA $<$ 1.5 m^2, 100 mg day per day, and (iii) 1.5 m^2 $\leq$ BSA, 120 mg per day. One cycle consisted of twice daily S-1 administration for 28 consecutive days followed by 14 days of withdrawal. CDDP in 100 ml of normal saline was given as a 1-h intravenous injection twice a week for 4 weeks on days 1, 4, 8, 11, 15, 18, 22, and 25, followed by 2 weeks of withdrawal in each cycle. Three CDDP dose levels, 7.5 mg/m^2 per day (Level 1), 10 mg/m^2 per day [Level 2; starting dose (see below)], and 15 mg/m^2 per day (Level 3), were chosen. These doses were determined using data from the previously conducted Phase I trial JFMC27-9902 (5). No patient was given hydration to protect against nephrotoxicity.

Patients underwent at least two consecutive cycles of combination therapy. Patients remained in the study unless (i) dose-limiting toxicities (DLTs) (within the first cycle) or Grade 4 hematological or Grade 3 or 4 non-hematological toxicities (after the first cycle) occurred, (ii) objective evidence of tumor progression appeared, or (iii) the patient refused to continue the treatment. Additionally, therapy was discontinued if hematologic toxicities including Grade 2 or greater leukopenia, neutropenia and thrombocytopenia, Grade 2 or greater non-hematologic toxicities (except alopecia), and a deterioration in PS of two or more during each treatment cycle were observed. If the toxicity causing treatment discontinuation was Grade 3 or greater leukopenia, neutropenia or Grade 2 or greater thrombocytopenia, the S-1 dose was reduced from 80, 100, and 120 mg per day to 50, 80, and 80–100 mg per day, respectively. The dose of CDDP was not modified during the first and second cycles.

Assessments of Toxicity and Efficacy

Adverse events were evaluated according to the National Cancer Institute— Common Toxicity Criteria version 2.0. DLT was defined for this study as the occurrence of any of the following observed within the first cycle of treatment: (i) Grade 3 or 4 leukopenia for 3 days or more, (ii) Grade 3 or 4 neutropenia along with fever (febrile neutropenia), (iii) Grade 3 or 4 thrombocytopenia, (iv) Grade 3 or greater non-hematologic toxicity, excluding alopecia, nausea/vomiting, and general fatigue, (v) total treatment interruption lasting $>$3 weeks, or (vi) patient's refusal to continue treatment due to adverse events or related matters. The assessment of

tumor response was based on the RECIST criteria. In addition, the tumor response data were reviewed extramurally. The protocol was approved by the Protocol Review Committee of the Japanese Foundation for Multidisciplinary Treatment of Cancer (JFMC).

Study Design and Statistical Analyses

The RD was estimated using a CRM proposed by O'Quigley and Shen (15). We adapted this approach, because a CRM defines the RD more precisely than a conventional '3 + 3' cohort design, thus the CRM was considered to suit the objective of this study. Dose escalations and deescalations for consecutive patient cohorts and the size of each cohort were based on the dose-finding CRM algorithm and clinical judgment. Skipping from Levels 1 to 3 was not allowed in the CRM calculations. The target toxicity level of the CRM to estimate the RD was set at 20%, which is the minimum value typically used in Phase I trials (15). Prior to starting the trial, participating clinicians predicted possible DLT occurrence probabilities of dose levels 1, 2, and 3 as 10% (5–30%), 20% (10–40%), and 40% (20–70%), respectively, based on the previous Phase I trial (JFMC27-9902) data (5). The ranges in the parentheses represent the pretrial clinician uncertainty of the DLT occurrence probability at each of the three dose levels. We determined the starting dose level in this trial to be Level 2, with the first two enrolled patients (first patient cohort) treated at this level. According to the pre-specified dose-escalation rule, if no DLT was observed in the first two patients, one patient enrolled in the second cohort was treated at Level 3. The projected sample size for the Phase I study was expected to require 10–16 patients, taking the simulation studies performed by O'Quigley et al. (14) into account.

In the CRM calculations, sensitivity analysis for parameters in the dose-toxicity model was performed. Four clinical scenarios for DLT occurrence probabilities at the three dose levels were established based on the pretrial prediction of the probabilities by the clinicians. We used scenarios (i) 10, 20, and 40%, (ii) 5, 10, and 20%, (iii) 30, 40, and 70%, and (iv) 5, 30, and 60% for DLT occurrence probabilities at Levels 1, 2, and 3, respectively. We considered bringing the Phase I to an early close when clear separation of the confidence intervals for the three dose levels appeared. This decision was also made according to clinical judgment. In addition, the trial was designed to be halted at the end of the Phase I if the selected regimen at the RD was insufficiently active when the hypothesis that the response rate (RR) at the RD is over 60% was statistically rejected (16). The Independent Data and Safety Monitoring Committee (IDSMC) independently reviewed the interim analysis and monitored protocol compliance, safety, and on-schedule study progress. The IDSMC considered stopping the trial from clinical as well as statistical points of view.

RESULTS

Patient Characteristics

A total of 13 patients were enrolled from the three sites (Osaka City University Hospital, Kochi Health Sciences Center, and Tohoku Employees' Pension Welfare Hospital) from December 2003 to March 2006. Eight and five patients were treated at Levels 2 and 3, respectively, and their baseline clinical characteristics are summarized in Table 1. The total number of cycles administered ranged from one to five and one to four for patients treated at Levels 2 and 3, respectively. Treatment was discontinued in two patients at Level 2 and two patients at Level 3 during the first cycle of therapy due to the occurrence of DLT. Treatment was discontinued in two patients at Level 2 and one patient at Level 3 during the second cycle, caused by the progression disease. Treatment was discontinued in three and two patients at Levels 2 and 3, respectively, due to the initiation of subsequent therapy. One patient at Level 2 underwent surgery after obtaining a partial response within the second cycle.

Toxicity

Hematologic and non-hematologic toxicities for the 13 patients observed during the first cycle are detailed in Table 2. Grade 3/4 toxicities were observed more frequently in patients treated at Level 3. Two patients treated at Level 3

Table 1. Patient characteristics

	Level 2 ($n = 8$)	Level 3 ($n = 5$)
Gender		
Male	6	4
Female	2	1
Age		
30–49	1	1
50–59	2	0
60–69	4	3
70–75	1	1
Performance status (baseline)		
0	6	4
1	2	1
Diagnosis		
Unresectable	7	5
Recurrent	1	0
Hepatic metastasis		
Negative	1	4
Positive	7	1
Lymph node metastasis		
Negative	3	0
Positive	5	5

Table 2. Hematological and non-hematological toxicities

Adverse event	Level 2 ($n = 8$)				Level 3 ($n = 5$)			
	Grade				Grade			
	1	2	3	4	1	2	3	4
Hematological								
Leukopenia	1	2	0	0	0	1	1	0
Neutropenia	1	2	1	0	0	1	1	0
Anemia	2	3	0	0	3	2	0	0
Thrombocytopenia	4	1	0	0	2	0	0	0
Non-hematological								
Anorexia	2	0	0	0	1	1	2	0
Nausea	0	0	0	0	0	1	1	0
Vomiting	0	1	0	0	0	0	0	0
Diarrhea	0	0	0	0	1	0	0	0
Hand-foot skin reaction	0	0	0	0	1	0	0	0
Fatigue	1	0	0	0	3	0	0	0
ALT/AST	2	1	0	0	2	0	0	0
Creatinine	0	1	0	0	0	0	0	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

suffered Grade 3 anorexia during the first cycle, causing the two DLTs observed on Level 3 therapy. Grade 2 gastrointestinal toxicity (vomiting) was observed in only one patient (12.5%) at Level 2. Additionally, one patient suffered Grade 2 anorexia in the second cycle (data not shown). Therefore, two (25%) out of the eight patients at Level 2 experienced Grade 2 gastrointestinal toxicities through the completion of the first and the second cycle.

Dose Escalation/Deescalation

The first two patients treated at Level 2 did not experience any DLT. According to the pre-specified dose-escalation rules of this study, the dose level for the second patient cohort was escalated to Level 3. Although the one patient in the second cohort did not experience any DLT, one of two patients in the third cohort experienced DLT (anorexia, Grade 3). At this point, the CRM was invoked and estimated the DLT occurrence probabilities as 3.1, 8.7, and 24.9% at Levels 1, 2, and 3, respectively. Thus, in the forth cohort, one patient was still assigned to Level 3, and this patient did not experience any DLT. The next single patient enrolled in the fifth cohort suffered DLT (anorexia, Grade 3), and the recalculated DLT occurrence probabilities estimated using the CRM were 5.9, 13.8, and 32.4% at Levels 1, 2, and 3, respectively. Consequently, the estimated Level 3 DLT probability considerably exceeded the target level, and the next patient cohort was treated at Level 2. In the subsequent six patients treated at Level 2, two DLTs (total treatment

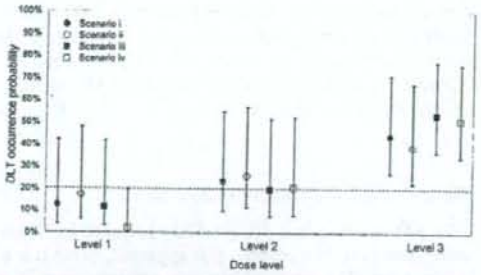

Figure 1. The probabilities of dose-limiting toxicity occurrence with 90% confidence intervals estimated at Levels 1, 2, and 3, based on dose-limiting toxicity data observed the 13 patients. These estimates were performed using the following four clinical scenarios: (i) 10, 20, and 40%, (ii) 5, 10, and 20%, (iii) 30, 40, and 70%, and (iv) 5, 30, and 60%, for DLT occurrence probabilities *a priori* predicted for Levels 1, 2, and 3, respectively.

interruption lasting >3 weeks, patient's refusal to continue treatment due to Grade 2 toxicity) were observed. Figure 1 shows the probabilities of DLT occurrence at Levels 2 and 3, estimated using all DLT data observed in the 13 patients under the 4 clinical scenarios used for sensitivity analysis. When the results of the sensitivity analysis and the observed toxicities at each dose level were considered, the IDSMC suggested that combination therapy at Level 2 was acceptable in terms of safety.

Response to Treatment

The clinical responses of nine patients who did not experience any DLT on the combination therapy were assessed (Table 3). Three patients treated at Level 2 responded to treatment during the Phase I study, and they all exhibited a confirmed PR during treatment. The upper bound of the 95% confidence interval of the RR at Level 2 was 75.5%. Thus, the hypothesis that the RR at the RD is over 60% was not rejected, and these data indicate that the combination therapy regimen is effective and supports proceeding to the Phase II trial.

DISCUSSION

We conducted a dose-finding Phase I trial in 13 patients with unresectable or recurrent gastric cancer to determine the RD of a regimen combining S-1 and low-dose CDDP with twice-a-week administration (JFMC27-9902 Step2). We found that the intensity of CDDP per week at the RD level

Table 3. Tumor response

Dose level	CR	PR	SD	PD	NE
2	0	3	0	3	2
3	0	0	1	2	2

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, non-evaluable.

of this regimen (10 mg/m^2, twice-a-week) was identical to that identified by the previous JFMC27-9902 study. (4 mg/m^2, five times per week) (5). In the JFMC27-9902 trial, three and five patients were treated with 4 mg/m^2 CDDP, five times-a-week (the dosage is identical to Level 2 in the current study) or 6 mg/m^2 CDDP, five times-a-week (corresponding to Level 3 in the current study), respectively. Although none of the three patients treated with 4 mg/m^2 CDDP experienced a DLT, two of the five treated with 6 mg/m^2 CDDP suffered Grade 3 anorexia. Thus, the JFMC27-9902 research team selected a dose of 4 mg/m^2 CDDP for future research. Additionally, Grade 1 anorexia was observed in two out of the three patients treated with 4 mg/m^2 CDDP, but no Grade 2/3/4 anorexia occurred in JFMC27-9902 at this treatment level. In the current JFMC27-9902 Step2 study, Grade 1 anorexia was observed in two out of the eight patients on Level 2 treatment, but Grade 2/3/4 anorexia was not observed in the first cycle. During the twice-a-week administration of 10 mg/m^2 CDDP, two patients (25%) experienced Grade 2 gastrointestinal toxicities during the two cycles. An additional study reported that with the weekly administration of 20 mg/m^2 CDDP, 54% patients suffered Grade 2 gastrointestinal toxicity during the two cycles (4). These results suggest that the mild gastrointestinal adverse events caused by twice-a-week CDDP administration with S-1 may provide adequate safety. Additionally, no other Grade 3/4 non-hematological toxicities were observed at the RD level in the current study.

The present study showed three (37.5%) of the eight patients were assessed at the RD acquired PR. The overall RR in the 13 patients was 23.1%. Therefore, twice-a-week CDDP administration with S-1 may seem to provide lower efficacy, compared with the weekly CDDP administration with S-1 proposed by Hyodo et al. (RR: 61%) (4), the high-dose CDDP administration with S-1 (RR: 74%) (7), and the S-1 mono-therapy (RR: 44–54%) (1–3). However, because the present study was a Phase I trial examining the efficacy of the regimen in such a small number of patients, the estimation of RR was not necessarily reliable. The examination of the RR at the RD is underway in a larger population of patients in the subsequent Phase II trial. With respect to the CDDP concentrations achieved, one report showed that twice-a-week administration of 7 mg/m^2 CDDP maintained a serum CDDP concentration comparable to that attained by 5 weekly doses of 3.5 mg/m^2 CDDP. Additionally, the CDDP concentration attained by the administration of 10 mg/m^2 twice-a-week might be equal to that attained by 4 mg/m^2 five times per week (17). In the ongoing JFMC27-9902 Step2 Phase II study, the pharmacokinetics of CDDP at the RD level determined here (10 mg/m^2, twice-a-week) will be compared with those determined with the 4 mg/m^2, five times per week regimen used in JFMC27-9902.

We applied the CRM to determine a final recommended treatment dose for future Phase II trial(s). In the present study, it took 28 months to enroll all the 13 patients. Although CRM designs have not been used so often because of their longer study duration compared with conventional study designs (18), the long time period for patient enrollment of this study was also due to low patient enrollment rate. The assessment of DLT was carried out during the first treatment course consisting of 6 weeks. Thus, if patients had been treated in cohorts of one and all patients had been consecutively enrolled with no gap, it would have taken at most 20 months to complete a study. In addition, Goodman et al. (18) reported that if one assigns more than one subject at a time to each dose level, the study duration can be reduced by >50% compared with the one-patient/cohort CRM design. Due to the relatively small number of patients enrolled in this study, the confidence intervals for the probability of DLT events at the three treatment levels are not clearly separable. Such uncertainty, however, is typical for Phase I dose-finding trials (19). However, we further performed a sensitivity analysis to estimate the DLT occurrence probabilities under a variety of assumptions to more clearly define the dose-toxicity relationship, and this analysis suggested that treatment Level 2 most closely approximated the study treatment goals. The robust results obtained through the sensitivity analysis supports the validity of the dose recommendation we reached. However, we must continue monitoring both the toxicity and efficacy of the combination regimen in the Phase II trial. A study design simultaneously monitoring both efficacy and toxicity, as proposed by Thall and Cook (20,21), may be useful in this context. Given the small number of patients we studied, despite the CRM algorithm, it is essential that the safety of the combination therapy be evaluated further with a larger patient population. As described in the study protocol, the RR of the RD will be further examined during the subsequent Phase II trial, and we will also monitor toxicity using CRM to reconfirm the safety of the RD (13). The sample size for the Phase II was set at 42.

In conclusion, we demonstrated that the combination regimen consisting of S-1 40 mg/m^2 twice daily for days 1–28 and CDDP 10 mg/m^2 on days 1 and 4 per week for 4 weeks followed by a 2-week washout period should be evaluated further in a Phase II trial in patients with unresectable or recurrent gastric cancer.

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Conflict of interest statement
None declared.

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Continuous Infusion of 5-fluorouracil with Versus without Low-dose, Consecutive Administration of Cisplatin in Advanced Colorectal Cancer. A Prospective Randomized Phase II Study

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Recently, the treatment of advanced gastric cancer by continuous infusion of 5-fluorouracil (5-FU) with low-dose cisplatin (CDDP) has improved efficacy without severe toxicities. The possible effectiveness of 5-FU+low-dose CDDP for colorectal cancer (CRC) is intriguing. One hundred fifty-five patients with far-advanced CRC including at least one measurable lesion were enrolled in a prospective randomized clinical trial funded by the Japanese Foundation for Multidisciplinary Treatment of Cancer. These patients were assigned to the two arms to assess the value of low-dose CDDP when added to a continuous intravenous infusion of 5-FU at a dose of 300 mg/m^2/24 hrs in a one-week cycle consisting of 5 days of treatment and 2 days of rest for at least 12 weeks. CDDP was given intravenously at a dose of 3 mg/m^2 on days 1-5 and days 8-12, and then at a dose of 7 mg/m^2 twice a week. Three patients were excluded from the trial. The response rate in the 5-FU+low-dose CDDP arm (n=75) was significantly higher than that in the 5-FU arm (n=77) (25.3% vs. 11.7%; P = 0.037). There was no significant difference in the median overall survival time between the 5-FU+low-dose CDDP arm and the 5-FU arm (479 and 491 days, respectively). Grades 3/4 toxicities occurred infrequently in both arms. The quality of life was almost the same between the arms. Low-dose CDDP improved the response rate while keeping toxicities within clinically acceptable limits. However, this combined treatment did not confer a survival advantage over treatment with continuous infusion of 5-FU alone for patients with far-advanced CRC; that might be attributable to the short CDDP administration setting of 12 weeks.

Key Words: 5-fluorouracil, Low-dose cisplatin, Colorectal cancer

Phase III trials in patients with colorectal cancer (CRC) demonstrated that continuous infusion of 5-fluorouracil (5-FU) resulted in a significantly higher response rate and reduced myelosuppression compared to bolus 5-FU (1-3). However, there were no statistically significant differences in overall survival between the two treatments. Recently, continuous infusion of 5-FU combined with low-dose and consecutive administration of cisplatin (CDDP) for gastric cancer has been widely used in Japan, and its high efficacy and low toxicity have been recognized (4-6). CDDP enhances the anticancer effect of 5-FU by the following mechanism: CDDP inhibits methionine uptake into tumor cells and decreases the volume of methionine pools in the cells. In response to the lack of methionine pools, the cells may increase methionine biosynthesis and the pools of folate cofactors. The increased 5,10-methylenetetrahydrofolate enhances 5-FU's cytotoxicity by increasing the reduction of thymidylate synthase (TS) to form a ternary complex, in which a 5-FU

metabolite fluorodeoxyuridylate, TS, and CH_2-H_4 folate are tightly bound together (7-9). The Japanese Foundation for Multidisciplinary Treatment of Cancer (JFMC) planned to investigate whether or not the low-dose, consecutive administration of CDDP in association with continuous infusion of 5-FU could confer tumor-suppression and survival advantages on patients with CRC.

Patients and Methods

Patient Selection

Patients between 20 and 80 years of age with a histologic diagnosis of unresectable, noncuratively operated, or recurrent CRC were eligible. Enrollment in the study envisaged an Eastern Cooperative Oncology Group performance status (PS) of 2 or less, a life expectancy of 12 weeks or more, and a measurable disease. Subjects who had received prior adjuvant treatment for CRC were allowed to participate provided they completed the treatment at least 28 days before enrolling in the study. Other eligibility requirements included adequate bone marrow function (Hb 9.0 g/dl or more, white blood cells between 4,000 and 12,000 /μl, neutrophils 2,000/μl or more, platelets 100,000/μl or more), total bilirubin 2.0 mg/dl or less, AST (GOT) and ALT (GPT) 100 IU/l or less, BUN 25 mg/dl or less, serum creatinine 1.5 mg/dl or less, and creatinine clearance 50 ml/min or more. Patients with psychiatric or medical problems rendering them unable to give informed consent were ineligible, as were patients with a serious concurrent, uncontrolled medical condition. The study was approved by the ethics and scientific committees of each participating institution. Each patient provided written informed consent before being randomly assigned to a treatment arm.

Treatment Regimens

Patients were randomized to receive either continuous infusion of 5-FU with low-dose, consecutive administration of CDDP or continuous infusion of 5-FU alone. The latter was considered a control arm against the combination chemotherapy arm in this study to allow us to observe the effect of adding CDDP. In the 5-FU+low-dose CDDP arm, 5-FU was given at a dose of 300 mg/m²/24 hrs as a continuous intravenous infusion by a balloon pump (Baxter Infuser Multiday Type 2C1080KJ, Baxter International Inc., Deerfield, IL, USA) via a subcutaneous reservoir connected to a central venous infusion catheter for 5 consecutive days followed by 2 days of rest. The cycle was repeated every 7 days. CDDP was given at a dose of 3 mg/m² as a 1 hr IV infusion on days 1-5 and 8-12, and then CDDP was given at a dose of 7 mg/m² as a 1 hr IV infusion twice a week at 2- or 3-day intervals. In the 5-FU arm, the 5-FU regimen was the same as that in the combination therapy. Both regimens were continued for at least 12 weeks. Hydration to protect against nephrotoxicity was not given to any patient. No prophylactic administration of anti-emetic agents or granulocyte colony-stimulating factor (G-CSF) was allowed. The patients in both arms were admitted to the hospital for at least two weeks to implant the subcutaneous reservoir, to get practize in the management of the infusional balloon pump, and to observe the adverse effects of the regimens. The rest regimens were carried out on an outpatient basis.

Assessment of Toxicity

Blood counts and biochemical profiles were performed at least once a week. We monitored patients for the occurrence of nonhematologic toxicities such as appetite loss, nausea/vomiting, stomatitis, diarrhea, skin pigmentation, eczema, hand-foot syndrome, and general fatigue. Toxicity during each course was evaluated according to the National Cancer Institute - Common Toxicity Criteria version 2.0.

Dose Modification and Regimen Interruption, Resumption, and Cessation

Doses of 5-FU and CDDP were modified in accordance with the following guidelines. When white blood cells decreased to less than 3000/μl or platelets decreased to less than 75,000/μl, when nonhematologic toxicities reached Grade 2 or higher, or when PS 3/4 occurred, the dose of 5-FU was reduced from 300 mg/m² to 200 mg/m² per day, the dose of CDDP during the first 2 weeks was reduced from 3 mg/m² to 2 mg/m² per day, and the dose of CDDP at 3 weeks or later was reduced from 7 mg/m² to 3 mg/m². Should these dose modifications not reduce the toxicities to their previous levels, the regimen was then interrupted to be resumed as soon as the patient recovered from the adverse effects of the previous doses. The regimen was stopped if the treatment interruption lasted more than 2 weeks, if the disease progressed, if unacceptable levels of toxicity occurred, or if the patient declined further participation.

Evaluation of Response Rate and Survival Data

Lesions noted at baseline and every 4 weeks during treatment were measured by computed tomography, ultrasonography, magnetic resonance imaging,

colonoscopy, and/or barium enema radiography. Objective responses were classified according to the World Health Organization criteria for primary and metastatic lesions as follows. Complete response (CR) was defined as the disappearance of all cancerous lesions for at least 4 weeks. Partial response (PR) required a reduction of 50% or greater in the sum of the cross-product of the maximum perpendicular diameters of all measurable lesions lasting for at least 4 weeks. Progression of disease (PD) was determined if there was a 25% or more increase in the sum of the cross-product of the maximum perpendicular diameters of all measurable lesions, or if a new lesion appeared. Legions not meeting the criteria for response or progression were considered to have stable disease (SD). Patient eligibility and response to treatment were reviewed extramurally. The extramural review was conducted by three clinical oncologists and one radiologist from institutions not participating in the study. Progression-free survival (PFS) meant survival from the date of registration until progression. Overall survival (OS) was measured from the date of registration to the date of death. The follow-up time was measured from the date of registration to the last contact or death. The Response Evaluation Criteria in Solid Tumors (RECIST) was not employed here, because the evaluation method in this protocol had been established before the RECIST criteria were opened to the public in 2000.

Evaluation of Quality of Life (QOL)

The Japanese Quality of Life Research Group has developed a QOL questionnaire suitable for patients who receive chemotherapy (10) the so-called QOL Questionnaire for Cancer Patients Treated with Anticancer Drugs (QOL-ACD). The JFMC has modified the QOL-ACD. This modified version is a 26-item questionnaire covering four categories: daily activity (Question numbers (Q#) 1-6), physical condition (Q# 7-13), mental and psychological status (Q# 14-18), and social activities (Q# 19-23). Q# 24 is a global QOL, which asks about overall QOL. Q# 25 is a face scale. The last item, Q# 26, asks whether or not the patient intends to continue the present treatment. All questionnaires answers,except Q# 24, range from 1 to 5, with higher scores indicating better status. For Q# 24, the patients marked their overall sense of well-being using a linear analog scale, which was transformed to a scale of 0 to 100, with higher scores indicating better status. Patients' QOL was assessed at the base line and 2, 4, 8, and 12 weeks after the day the first treatment was given. In this study, the total score of Q#1 to 23 (with a range of 23 to 115), the linear analog scale of overall QOL (0 to 100), the face scale (1 to 5), and the intention to continue the present treatment (1 to 5) were compared between the 5-FU+low-dose CDDP arm and the 5-FU arm at each assessment time.

Statistical Considerations

The primary endpoint in this trial was response rate. Secondary endpoints were duration of response (DR), PFS, OS, toxic effect, and QOL.

Statistical analyses were carried out at the JFMC data center using Statistical Analysis System software (version 8.2., SAS Institute, Cary, NC, USA). An intent-to-treat analysis was applied. Background factors were compared using the Fisher's exact method and the Mann-Whitney U-test. Objective response was examined using Fisher's exact method. The cut-off dates were August 31, 2004, for overall survival, with a 31-month median potential follow-up time for the entire cohort. DR, PFS, and OS curves were generated by the Kaplan-Meier method, and the log-rank test was used to compare the curves. The Cochran-Mantel-Haenszel test was used to compare proportions of toxicities. QOL scores were analyzed using a repeated measures, mixed effects model (SAS Mixed procedure). All P-values reported were two-tailed. Statistical significance was set at a level of 0.05 except for background or QOL assessments. For background assessment, $P < 0.15$ was considered statistically significant. For QOL assessment of each point, $P < 0.0125$ (Bonferroni correction) was considered significant. The statistical analyses and their interpretations were approved by the JFMC clinical trial committee.

Results

Patient Characteristics

A total of 155 patients from 27 institutions were enrolled in this study between May 2000 and April 2003. Three patients judged ineligible by extramural reviewers were excluded from the analysis. Therefore, 152 patients were analyzed in this study. Two patients, one in each arm, did not receive any protocol treatment after moving to a nonparticipating study site in one case and after a rapid worsening of general status in the other. They were kept in the statistical analyses of antitumor effect and survival data because the results were obtained on an intent-to treatment basis. However, data regarding both these patients were excluded from adverse-effect analysis. The demographic characteristics of the patients are listed in Table I. There were no

Table I - Comparison of clinical characteristics at baseline between 5-FU+low-dose CDDP arm and 5-FU arm

Characteristics	Treatment arm		*P*-value
	5-FU+low-dose CDDP	5-FU	
No. of patients	75	77	
Age median (range)	64 (41-79)	63 (39-79)	0.245
Sex (Male : Female)	49:26	51:26	1.00
Performance status			0.859
0	57	58	
1	16	15	
2	2	4	
Primary tumour location			0.697
Colon	45	41	
Rectum	29	35	
Colon+Rectum	1	1	
Tumour status			0.751
Residual tumour after noncurative operation	40	38	
Recurrent tumour after potentially curative operation	32	35	
Unresectable tumour	3	4	
Site of disease			0.750
local abdominal mass	18	19	
local recurrent mass	9	9	
liver	46	45	
lung	23	26	
brain	2	3	
lymph node	26	22	
peritoneal dissemination	9	12	
skin	0	1	
bone	4	7	
other	5	5	
ascites	5	1	
pleural effusion	0	2	
Number of organs involved			0.309
1	32	38	
2	26	18	
3 or more	17	21	
Differentiation			0.174
Well differentiated	36	23	
Moderately differentiated	32	46	
Poorly differentiated	5	5	
Mucinous carcinoma	2	2	
Adenocarcinoma (cytology)	0	1	
Previous chemotherapy			0.857
Yes	20	22	
No	55	55	

5-FU, 5-fluorouracil. CDDP, cisplatin.

statistical differences in background between the arms, such as age, sex, PS, primary tumor location, tumor status, site of disease, number of organs involved, histological differentiation, or previous chemotherapy. The treatments results after the 12-week regimen are shown in Table II. Although there was no statistical significance between the post-regimen treatments in the two arms, somewhat more patients in the 5-FU arm were treated with 5-FU+low-dose CDDP than in the 5-FU+low-dose CDDP arm.

Table II - Comparison of treatments after protocol regimen between 5-FU+low-dose CDDP arm and 5-FU arm

Characteristics	Treatment arm		*P*-value
	5-FU+low-dose CDDP	5-FU	
Chemotherapy			0.370
oral fluoropyrimidine	13	11	
S-1 (oral DIF)	3	0	
S-1+CDDP(+irinotecan)	1	1	
infusional 5-FU	2	0	
5-FU+low-dose CDDP	7	17	
5-FU+leucovorin	5	4	
5-FU+irinotecan	5	6	
irinotecan	7	5	
irinotecan+CDDP	3	5	
hepatic arterial infusion (5-FU or 5-FU+CDDP)	6	8	
Radiotherapy	4	5	1.000
Immunotherapy	5	3	0.492
Surgery			0.354
Hepatectomy	2	1	
Pneumonectomy	1	3	
Local mass resection	0	1	
Gastrointestinal Bypass	0	2	

5-FU, 5-fluorouracil. CDDP, cisplatin.
DIF, dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine

Compliance

The completion rates of the defined 12-week treatment were 78.7% (59/75) in the 5-FU+low-dose CDDP arm and 71.4% (55/77) in the 5-FU arm (P = 0.351). The major reasons for stopping the regimen were worsening of general status (43.8%; 7/16) and PD (18.8%; 3/16) in the 5-FU+low-dose CDDP arm; in the 5-FU arm, the reasons were PD (54.6%; 12/22), worsening of general status (18.2%; 4/22), and patients' and their families' intents not to continue the regimen (18.2%; 4/22). Catheter troubles, such as occlusion, occurred only in two patients (1.3%).

The median total administration of CDDP in the 5-FU+low-dose CDDP arm was 250 mg/body (maximum; 1330 mg/body). The median total 5-FU administration in the 5-FU+low-dose CDDP arm was 29.3 g/body (maximum 148.5 g/body), and that in the 5-FU arm was 30.0 g/body (maximum, 191.3 g/body); there was no statistical difference in total 5-FU administration between the arms (P = 0.802). Doses were modified for 14 patients (18.7%) in the 5-FU+low-dose CDDP arm and for 3 patients (3.9%) in the 5-FU arm; there was a statistical difference in the dose modification rate between the two arms (P = 0.004).

Efficacy

A statistical difference was observed between the response rate of the 5-FU+low-dose CDDP arm and that of the 5-FU arm (25.3% vs 11.7%; P = 0.037)(Tab. III). No statistical differences between the arms were seen in DR or PFS (Tab. III). The median survival time (MST) of the 5-FU+low-dose CDDP arm was 15.7 months (479 days: 95% confidence interval (CI), 363-593 days) and that of the 5-FU arm was 16.1 months (491 days: 95% CI, 330-596 days); there was no statistical difference between the two arms (Fig. 1, Table III)(P = 0.582).

Toxicity

In the 5-FU+low-dose CDDP arm, neutropenia, anemia, and nausea were the most common effects of toxicity. However, grade 3/4 toxicities were infrequent. The rates of grade 3 neutropenia, anemia, and nausea were 8.1%, 6.8% and 4.1%, and grade 4 toxicity was observed only in thrombocytopenia (1.4%). In the 5-FU arm, grade 3/4 toxicities occurred at very low rates (0 to 3.9%). Statistical differences between the toxicity profiles of the arms were observed in neutropenia, anemia, and nausea (Tab. IV). Anti-emetic agents were

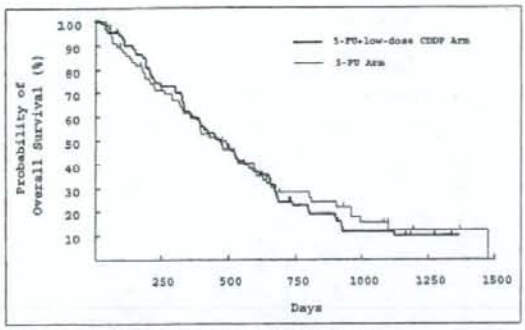

Fig. 1 - Kaplan-Meier curve of overall survival.

given to 31 (41.3%) patients in the 5-FU+low-dose CDDP arm and to 14 (18.2%) patients in the 5-FU arm; this difference was statistically significant (P = 0.002). G-CSF was given to 2 (2.7%) patients in the 5-FU+low-dose CDDP arm, whereas no patients in the 5-FU arm needed to use the agent.

QOL

The rates at which the questionnaire was answered completely and correctly were 78.5% in the 5-FU+low-dose CDDP arm and 86.4% in the 5-FU arm. The total scores in the 5-FU+low-dose CDDP arm tended to be lower than those in the 5-FU arm prior to and during the 12-week regimen, but the difference was not statistically significant. There were no statistically significant differences between the arms in the linear analog scale or the face scale during the 12-week regimen. The intention to continue treatment in the 5-FU+low-dose CDDP arm gradually decreased, whereas that of the 5-FU group was maintained. However, no statistically significant differences were observed except at 4 weeks from randomization (Fig. 2).

Discussion

The effectiveness of chemotherapeutic treatment for CRC has improved substantially in the past decade. In advanced CRC patients with unresectable metastatic lesions treated with 5-FU/leucovorin with oxaliplatin regimen (FOLFOX) or by 5-FU/leucovorin with irinotecan regimen (FOLFILI), response rates and MST of 39-56% and 14.8-21.5 months, respectively, have been reported (11-13). However, high rates of the grade 3/4 toxicities (53-74%) and some therapy-related deaths by these regimens have also been reported (11-

Table III - Comparison of chemotherapeutic effects between 5-FU+low-dose CDDP arm and 5-FU arm

Characteristics	Treatment arm		*P*-value
	5-FU+low-dose CDDP	5-FU	
Total No. of patients	75	77	
Efficacy			0.037
CR	0	0	
PR	19 (25.3%)	9 (11.7%)	
SD	36 (48.0%)	35 (45.5%)	
PD	15 (20.0%)	25 (32.5%)	
NE	5 (6.7%)	8 (10.4%)	
Median duration of response (95% CI)	226 days (185-232)	148 days (91-336)	0.494
Median progression-free survival (95% CI)	178 days (141-256)	131 days (92-197)	0.282
Median overall survival (95% CI)	479 days (363-593)	491 days (330-596)	0.582

5-FU, 5-fluorouracil. CDDP, cisplatin. CR, complete response. PR, partial response. SD, stable disease. PD, progresstive disease. NE, not evaluable. CI, confidence interval.