Cancer Chemother Pharmacol (2006) 57: 368–375
DOI 10.1007/s00280-005-0057-9

ORIGINAL ARTICLE

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Administration sequence-dependent antitumor effects of paclitaxel and 5-fluorouracil in the human gastric cancer cell line MKN45

Received: 25 March 2005 / Accepted: 24 May 2005 / Published online: 2 August 2005

Abstract *Background*: The clinical outcome of gastric cancer patients has been improved by combination of 5-fluorouracil (5-FU) and paclitaxel (PXL). However, the optimal schedule of this combination has not been determined. *Methods*: The efficacies of sequential administrations of 5-FU and PXL on the gastric cancer cell line MKN45 were investigated using a WST-8 colorimetric assay. The cell cycle distribution of each drug was evaluated by flow-cytometry. Furthermore, the mechanism of antitumor activity enhancement by the administration sequence was investigated by western blotting. *Results*: MKN45 cell growth was significantly inhibited by each drug in a dose- and time-dependent manner. The cytotoxicities of PXL followed by 5-FU were significantly greater than those of 5-FU followed by PXL. The flow-cytometric analysis revealed that PXL exposure caused viable cell accumulation in G2/M phase in a dose-dependent manner. Western blotting showed that PXL exposure followed by 5-FU up-regulated Chk1 and Wee1 protein expressions until PXL removal and 5-FU exposure, when these expressions gradually decreased to their basal levels. 14-3-3σ protein expression was significantly up-regulated upon PXL treatment followed by 5-FU. Interestingly, Mad2 protein expression with PXL treatment followed by 5-FU gradually increased after the PXL removal and 5-FU exposure. *Conclusions*: PXL followed by 5-FU administration may be the optimal sequence for treatment of gastric cancer. The enhanced viable cell accumulation after PXL pretreatment may be related to G2 arrest. After PXL removal and 5-FU exposure, the cells progressing to M phase may undergo cell death by mitotic catastrophe due to DNA damage caused by 5-FU exposure.

Keywords Gastric cancer · Paclitaxel · 5-Fluorouracil · Schedule dependency · Pharmacokinetic modulating chemotherapy (PMC)

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Introduction

Chemotherapy has been shown to confer benefits for survival and quality of life in a certain number of patients with advanced unresectable or metastatic gastric cancer. Although several intensive chemotherapeutic regimens, including FAMTX (5-fluorouracil (5-FU)/doxorubicin/methotrexate), etoposide/doxorubicin/cisplatin (EAP), epirubicin/leucovorin/5-FU (ELF), epirubicin/cisplatin/5-FU (ECF), and 5-FU/cisplatin (FP), have been proposed for the treatment of advanced gastric cancer, a standard regimen has not yet been established. Among these regimens, FP is considered to be the reference treatment for this disease. Its response rate (RR) and median survival time (MST) have been reported to be approximately 20–34% and 7.2–8.5 months, respectively [1–3].

Pharmacokinetic modulating chemotherapy (PMC), a combination of infused 5-FU and oral uracil/tegafur (UFT), has been shown to be highly effective and improve the prognosis in patients with advanced colorectal carcinoma [4–6]. Recently, we reported the efficacies of modified PMC, namely combinations of PMC with other anticancer agents such as paclitaxel (PXL), docetaxel, irinotecan, and cisplatin, for advanced gastric cancer in clinical trials [7]. In primary stage IV gastric cancer, the modified PMCs showed a RR as high as 31%, including one complete response (CR), and an MST of 10.5 months (range 4–26 months). These results are comparable to one of the landmark phase III trials, in which the DCF (docetaxel/cisplatin/5-fluorouracil)

regimen was reported to be associated with a significantly superior RR (39%) and MST (10.2 months) compared with FP [3]. Moreover, a preoperative modified PMC (PMC plus Taxotere (TXT)) for advanced gastric cancer resulted in significant downregulation of the primary tumor, and pathological evidence of chemotherapeutic effects on both the primary tumor and the regional lymph nodes was established [7]. Similarly, other studies have reported that combinations of 5-FU/PXL [8] and 5-FU/PXL/ Cisplatin (CDDP) [9, 10] are highly effective for gastric cancer.

However, the underlying cellular or molecular basis for the efficacy of the 5-FU/PXL combination has not yet been elucidated. Moreover, the impact of the administration schedule on the efficacy remains to be defined, especially against gastric cancer cells.

The aim of the present study was to examine the cytotoxic effects of different administration sequences of 5-FU and PXL in the human gastric cancer cell line MKN45 and to elucidate the biochemical mechanism of their synergistic interaction. Here, we focused on the administration sequence of 5-FU and PXL and used a fixed dose of PXL (a clinically relevant drug concentration) with varying doses of 5-FU (PMC therapeutic range).

Materials and methods

Cell lines and culture conditions

A p53 wild-human gastric cancer cell line, MKN45, [11] was obtained from the RIKEN Cell Bank (Ibaragi, Japan). MKN45 cells were grown in monolayer culture in RPMI 1640 (Sigma-Aldrich Inc., St. Louis, MO, USA), supplemented with fetal bovine serum (FBS; 10% (v/v); Gibco BRL, Tokyo, Japan), glutamine (2 mM), penicillin (100,000 U/L), streptomycin (100 mg/l), and gentamycin (40 mg/l) at 37°C in a 5% CO_2 environment.

For routine passages, cultures were spilt 1:10 when they reached 90% confluence, generally every 3 days. For all experiments, cells at the fifth to ninth passage were used. All experiments were performed with exponentially growing cells.

Anticancer agents

5-FU and PXL were obtained from Sigma-Aldrich Inc., reconstituted in distilled water at appropriate concentrations and stored at −20°C until use.

Experimental protocol concept

Although the IC_{50} value (drug concentration responsible for 50% growth inhibition) of each drug was usually used for combination studies, we followed their concentrations for clinical use as much as possible.

The 5-FU concentrations were chosen based on both our previous reports and drug information obtained from Kyowa Hakko Kogyo (Tokyo, Japan). Assays of plasma 5-FU in 23 patients receiving PMC revealed that the serum concentrations of 5-FU ranged from 88 to 1,323 ng/ml (approximately 0.1–10 μM) [12]. The drug information on 5-FU from Kyowa Hakko Kogyo indicated that the plasma concentration of 5-FU reaches 15.3 μg/ml (100 μM) after a bolus injection of 5-FU (500 mg/body) and 0.6 μg/ml (5 μM) during continuous infusion of 5-FU (60 mg/kg/48 h).

The PXL concentration was chosen based on plasma concentrations obtained from clinical use cited in the drug information for TAXOL INJECTION (paclitaxel) from Bristol Myers Squibb Company (Tokyo, Japan). This information indicated that the plasma concentration of PXL reaches 1–10 μg/ml (1–10 μM) after an injection and 0.05–0.1 μg/ml (50–100 nM) at 24 h after drip infusion of PXL (105–270 mg/m^2).

Drug concentrations, exposure times, and administration schedules

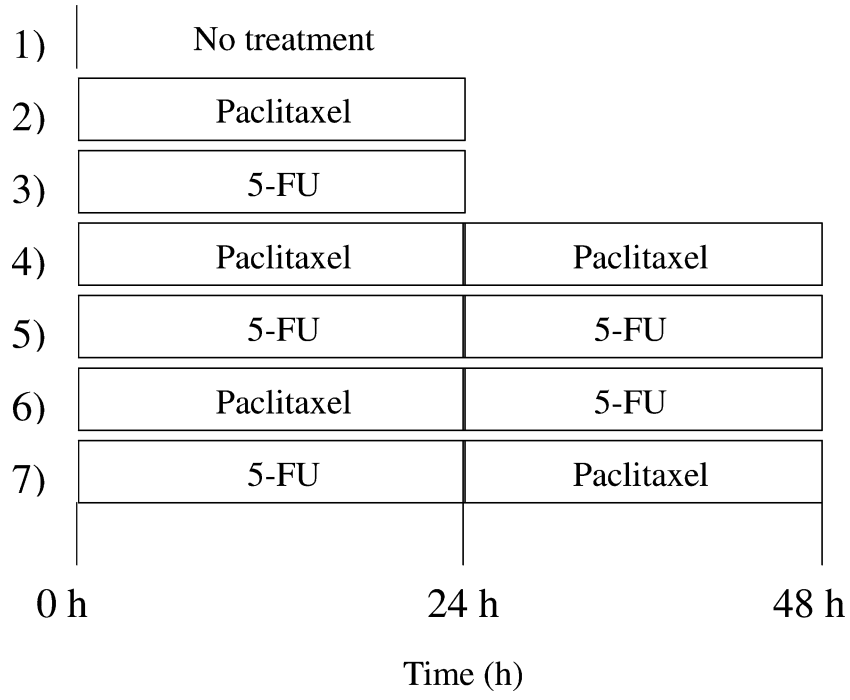
As mentioned above, we adopted clinically relevant concentrations of 5-FU and PXL in this study. Although we should ideally consider the doubling time of MKN45 cells before deciding the exposure time, we chose to use an exposure of 24 h for each drug for experimental simplicity. The final concentrations ranged from 0.1–1,000 μM for 5-FU and from 0.001–10 μM for PXL.

To test the cytotoxicity of each drug, MKN45 cells in the exponential growth phase were treated for 24 h with various concentrations of 5-FU or PXL. After discarding the media containing each drug and replacing it with fresh media, the cytotoxicity was evaluated using a WST-8 colorimetric assay.

For combination studies, MKN45 cells were exposed to the first drug (5-FU or PXL) for 24 h. After discarding the media containing the first drug and replacing it with fresh media, the second drug was administered and incubated for 24 h. The cytotoxicity was evaluated as described above.

The drug-exposure schedules, which are summarized in Table 1, were as follows: (1) no treatment; (2) PXL (0.001, 0.01, 0.1, 1 or 10 μM) for 24 h; (3) 5-FU alone (0.1, 1, 10, 100 or 1,000 μM) for 24 h; (4) PXL alone (0.001, 0.01, 0.1, 1 or 10 μM) for 48 h; (5) 5-FU alone (0.1, 1, 10, 100 or 1,000 μM) for 48 h; (6) PXL (1 μM for 24 h) followed by 5-FU (0.1, 1, 10, 100 or 1,000 μM for 24 h); (7) 5-FU (0.1, 1, 10, 100 or 1,000 μM for 24 h) followed by PXL (1 μM for 24 h). The experiments were performed in triplicate for each time point, and the means ± SD were calculated.

Table 1 Schematic representation of the drug-exposure schedules



Growth inhibition assay

The cytotoxicity was evaluated using a WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H- tetrazolium, monosodium salt] colorimetric assay. MKN45 cells (5,000 cells/well) were seeded into 96-well cell plates (Becton Dickinson Labware, NJ, USA) in 100 μl of culture medium for 24 h prior to drug exposure, and then treated with various concentrations of 5-FU or PXL for various durations depending on the protocol.

After drug exposure for the indicated concentrations and times, the medium was discarded and replaced with 90 μl of fresh medium. Next, 10 μl of WST-8 reagent solution (Cell Counting Kit; Dojindo Laboratories, Japan) was added and incubated for 4 h at 37°C in an incubator.

Cell viability was determined colorimetrically by the optical density (OD) at a wavelength of 450 nm, as measured using a microplate reader (SoftMax, Molecular Devices Corporation, CA, USA). Cytotoxicity was evaluated using the Cell Counting Kit according to manufacturer's instructions.

Cell cycle analysis by flow-cytometry

The cell cycle distribution was determined by analyzing the DNA content after propidium iodide staining. Cells were treated with various concentrations of each drug for 24 h, and then harvested, fixed in 70% ethanol, incubated with 2 mg/ml RNase and stained with propidium iodide solution (50 μg/ml). The DNA content of approximately 1×10^6 stained cells was analyzed using a FACScan flow cytometer. The fractions of cells in the G0/G1, S and G2/M phases were analyzed using a DNA software program.

Protein extraction and western blot analysis

MKN45 cells were exposed to PXL, 5-FU or their combinations for 24 h each in various administration sequences. After drug treatment, the cells were homogenized in lysis buffer (Tris-buffered saline, pH 7.5, containing 1% Triton X-100) for 5 min on ice. After centrifugation at 15,000 rpm for 15 min at 4°C, the supernatants were collected and frozen at −20°C until analysis. The protein concentration was measured by the BCA protein assay (Pierce, Rockford, IL, USA). Lysates containing 10 μg total protein were mixed with an equal volume of 2x Laemmli loading buffer containing 2-mercaptoethanol and heated at 100°C for 5 min. The samples were electrophoretically separated in 12.5% gradient polyacrylamide gels containing 0.1% SDS at 25 mA for 2 h followed by semi-dry transfer to an Immun-Blot PVDF membrane (Bio-Rad Laboratories, Hercules, CA, USA) at 12 V for 2 h. The membranes were blocked with 5% skim milk in Tris-buffered saline, pH 7.5, containing 0.1% Tween 20 (TBS-T) for 1 h at room temperature, and then incubated with a primary antibody diluted in TBS-T/5% skim milk for 1 h at room temperature. The primary antibodies used were: a mouse monoclonal anti-hsMAD2 antibody (1:1,000 dilution; Transduction Laboratories, Lexington, KY, USA), a mouse monoclonal anti-Wee1 antibody (1:100 dilution; Santa Cruz Biotechnology Inc.), a mouse monoclonal anti-Chk1

antibody (1:100 dilution; Santa Cruz Biotechnology Inc.), a mouse monoclonal anti-14–3-3σ (1:100 dilution; Santa Cruz Biotechnology Inc.) and, a mouse monoclonal anti-actin antibody (clone C4; 1:3,000 dilution; ICN Biomedicals Inc., Aurora, OH, USA). After three washes in TBS-T, the blots were incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (Promega Corp., Madison, WI, USA) diluted 1:1,000 in TBS-T/5% skim milk for 1 h at room temperature. Following treatment with an enhanced chemiluminescence detection solution, the blots were exposed to an X-ray film for autoradiographic visualization of the bands. The films were scanned and the relative quantities of the protein bands were analyzed by densitometry using CS Analyzer version 2.0 (ATTO Corporation, Japan).

Statistical analysis

The results are expressed as the means ± SD. The Mann–Whitney *U* test was used for comparisons among unpaired groups. *P* values of less than 0.05 were considered statistically significant.

Results

Growth inhibition of MKN45 cells by 5-FU and PXL

To elucidate the optimal administration sequence for PXL/5-FU combinations, we first evaluated the time-dependent and dose-dependent cell viabilities after exposure to each drug individually. The cytotoxic effects of 5-FU and PXL on MKN45 cells were assessed after 24 h drug exposure, followed by the WST-8 colorimetric assay. The concentrations of each drug used were based on our previous studies and reported plasma levels in clinical use [12]. Growth inhibitory effects were observed for treatment with either 5-FU or PXL alone in dose- and time-dependent manners (Fig. 1). Moreover, the MKN45 cell growth was inhibited at the clinically used concentrations of PMC (0.1–10 μM) and the clinically relevant concentrations of PXL (0.001–10 μM).

Cytotoxic effects of different 5-FU and PXL administration sequences

5-FU is clinically administered as either a bolus injection or a continuous infusion. Our PMC consists of infused 5-FU and oral UFT, and results in various concentrations of 5-FU [12]. In contrast, PXL is usually administered as a 3-h drip infusion. The concentrations of 5-FU vary more than those of PXL due to the various administration methods. Taking these considerations into account, PXL was used at a fixed concentration of 1 μM, while 5-FU was used at serial concentrations of 0.1–1,000 μM for the sequential studies. The cytotoxicities of PXL (1 μM) followed by 5-FU (0.1, 1, 10 and 100 μM) were significantly greater than those of 5-FU (0.1, 1, 10 and 100 μM) followed by PXL (1 μM) ($p < 0.05$) (Figure 2). The cytotoxicities of 5-FU (0.1 and 1 μM) followed by PXL (1 μM) were poorer than that of PXL (1 μM) alone, indicating that lower levels of 5-FU pretreatment adversely affect the subsequent PXL cytotoxicity. The sequential combinations of 5-FU (10 and 100 μM) followed by PXL (1 μM) were as cytotoxic as PXL (1 μM) alone and showed better cytotoxicities than 5-FU (10 and 100μM) alone. In contrast, the sequential combinations of PXL (1 μM) followed by 5-FU (0.1, 1, 10, and 100 μM) significantly enhanced the antitumor activity compared with the reverse sequential combinations, PXL (1 μM) alone and 5-FU (0.1, 1, 10, and 100 μM) alone. Regarding the clinical use of PXL/5-FU combinations, these results suggest that PXL pretreatment enhances the subsequent 5-FU cytotoxic-

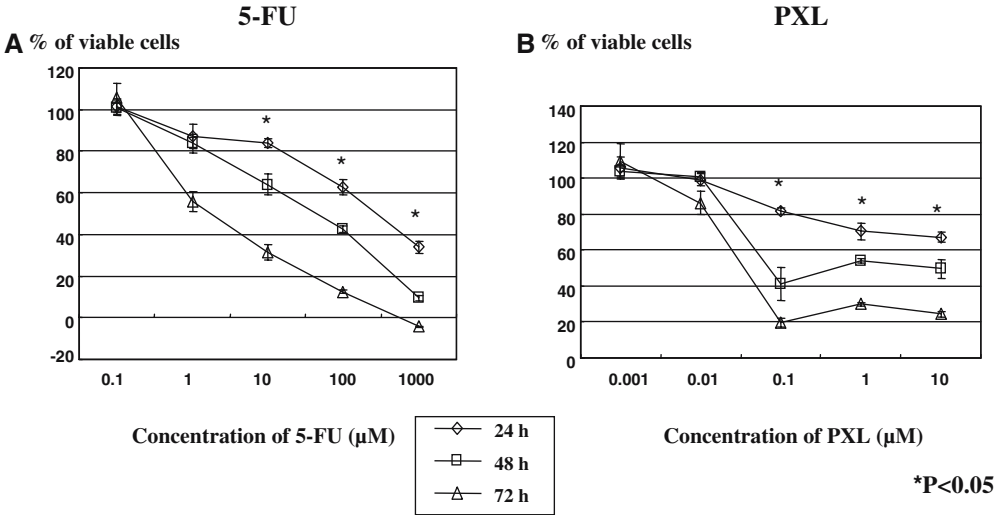


Fig. 1 Cytotoxicities of 5-FU and PXL in MKN45 cells. Cells were treated with different concentrations of the anticancer drugs for 24 h (**a**), 48 h (**b**) and 72 h (**c**), and the cell growth was determined using a WST-8 colorimetric assay. The results are expressed as percentages of cell growth relative to untreated control cells. The data represent the means ± SD of three experiments

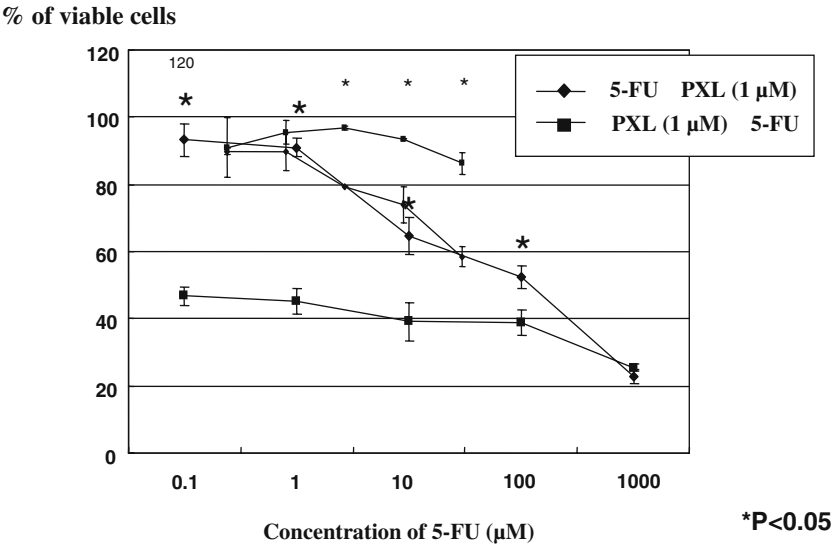


Fig. 2 Administration sequence-dependent cytotoxicities of 5-FU and PXL in MKN45 cells. Cells were treated with either 5-FU (0.1, 1, 10, 100 or 1,000 μM for 24 h) followed by PXL (1 μM for 24 h) or paclitaxel (1 μM for 24 h) followed by 5-FU (0.1, 1, 10, 100 or 1,000 μM for 24 h), and the cell growth was determined using a WST-8 colorimetric assay. The data represent the means ± SD of three experiments

ity, whereas 5-FU pretreatment suppresses the subsequent PXL cytotoxicity, in the context of their clinically used concentrations. These findings led us to further investigate the cell cycle distributions by flow-cytometry and cell cycle-related protein expressions by western blotting.

PXL- and 5-FU-induced cell cycle effects

There were no significant differences in the cell cycle distributions among the various concentrations of 5-FU after a 24 h exposure. However, significant accumulation of cells in G2/M phase was observed in a dose-dependent manner after exposure to PXL for 24 h (Fig. 3).

Influence of different drug administration sequences on cell cycle-related proteins

PXL exposure for 24 h produced a two- to threefold increase in the percentage of cells in G2/M phase compared with the control cells. We mainly investigated G2/M and M phase checkpoint-related proteins by western blotting (Chk1, 14-3-3σ, Wee1 and Mad2). We also determined the concentration of each drug that showed equivalent efficacy for viable cell inhibition at 24 h and significant differences in the sequential effects on cell viability.

Pre-exposure to PXL for 24 h was accompanied by increased Chk1 protein expression. After PXL removal and subsequent 5-FU exposure, Chk1 protein expression was prolonged for 12 h and then returned to its basal level at 24 h. However, upon sequential administration of 5-FU followed by PXL, neither 5-FU exposure for 24 h or the subsequent PXL exposure affected Chk1 protein expression (Fig. 4a, c).

PXL pretreatment for 24 h increased Wee1 protein expression to a much higher level than 5-FU pretreatment for 24 h. After the initial drug removal and subsequent drug exposure, Wee1 protein expression gradually decreased to its basal level (Fig. 4a, b).

14-3-3σ Protein expression was significantly upregulated by PXL treatment followed by 5-FU exposure compared to 5-FU treatment followed by PXL

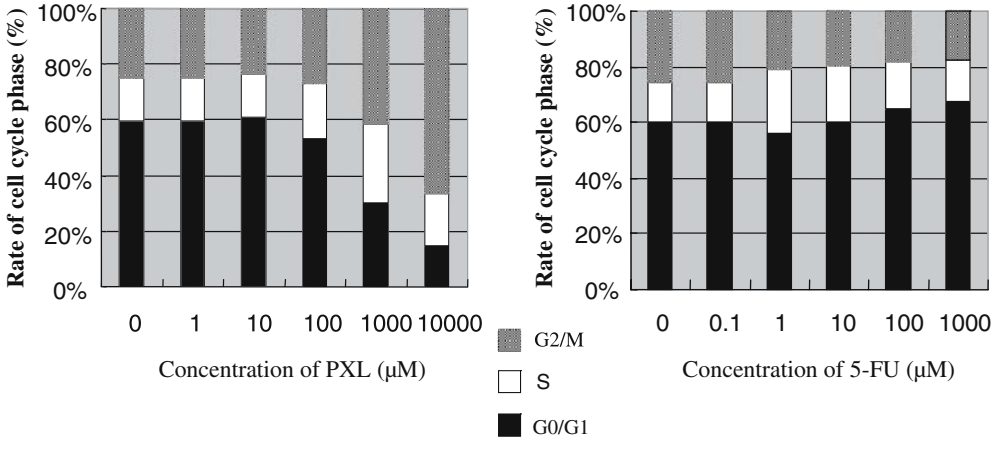


Fig. 3 Effects of 5-FU and PXL on the cell cycle distribution. Cells were treated with either 5-FU (0.1, 1, 10, 100 or 1,000 μM) or PXL (0.01, 0.1, 1, 10 or 100 μM) for 24 h. The cells were then harvested and the cell cycle distributions were analyzed by flow-cytometry. The results from three separate experiments were averaged, and the percentages of cells in the G1, S and G2/M phases are shown in a *vertical stacked bar graph* format for each drug

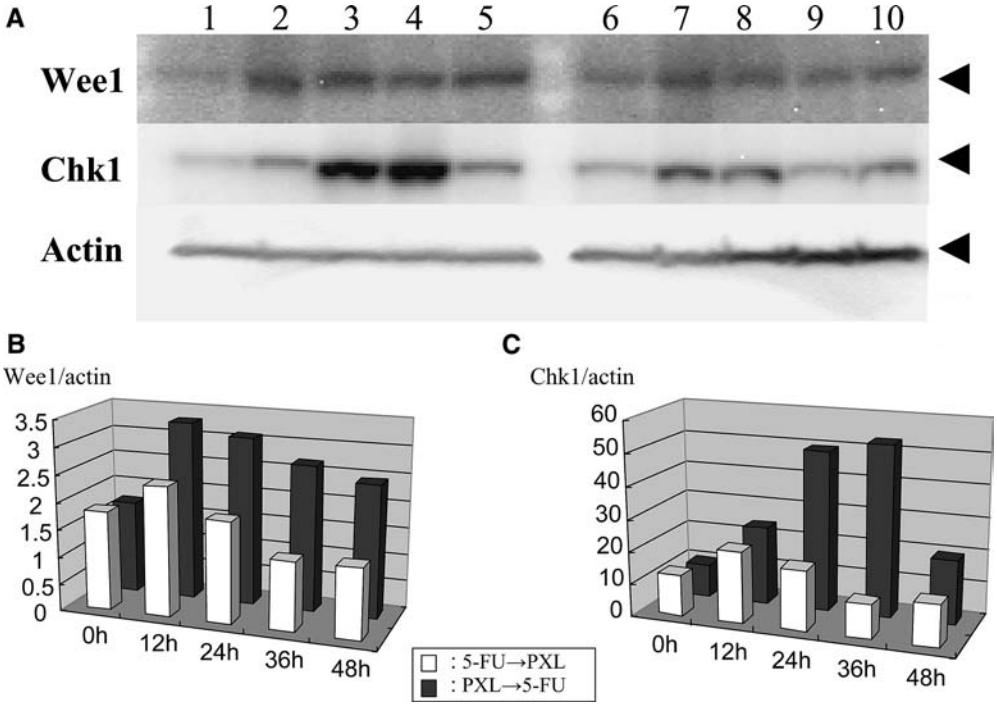


Fig. 4 Effects of PXL (1 μM for 24 h) followed by 5-FU (10 μM for 24 h) or 5-FU (10 μM for 24 h) followed by PXL (1 μM for 24 h) on Chk1 and Wee1 protein expressions over time (0–48 h). Equal amounts of protein (10 μg) were resolved by SDS-PAGE and analyzed by western blotting. Immunoblots of Chk1, Wee1 and the universally used control protein actin are shown in Fig. 4a. The lanes are: *1* PXL (0 h); *2* PXL (12 h); *3* PXL (24 h); *4* PXL (24 h) followed by 5-FU (12 h); *5* PXL (24 h) followed by 5-FU (24 h); *6* 5-FU (0 h); *7* 5-FU (12 h); *8* 5-FU (24 h); *9* 5-FU (24 h) followed by PXL (12 h); *10* 5-FU (24 h) followed by PXL (24 h). Figure 4b, c shows the semi-quantitative Chk1 and Wee1 protein expression patterns. Similar results were obtained in three separate experiments

exposure. In more detail, 14-3-3σ protein expression was increased by pre-exposure to PXL at 12 h, and this expression level was maintained after PXL removal and subsequent 5-FU exposure (Fig. 5a, b).

Mad2 protein expression was increased by PXL treatment for 24 h. Following PXL removal and subsequent 5-FU exposure for 24 h, Mad2 protein expression showed a further increase. In contrast, 5-FU treatment followed by PXL exposure did not affect Mad2 protein expression (Fig. 5a, c).

Discussion

Clinically, the RR to PXL as a single agent has been reported to be 15–24% for the treatment of gastric

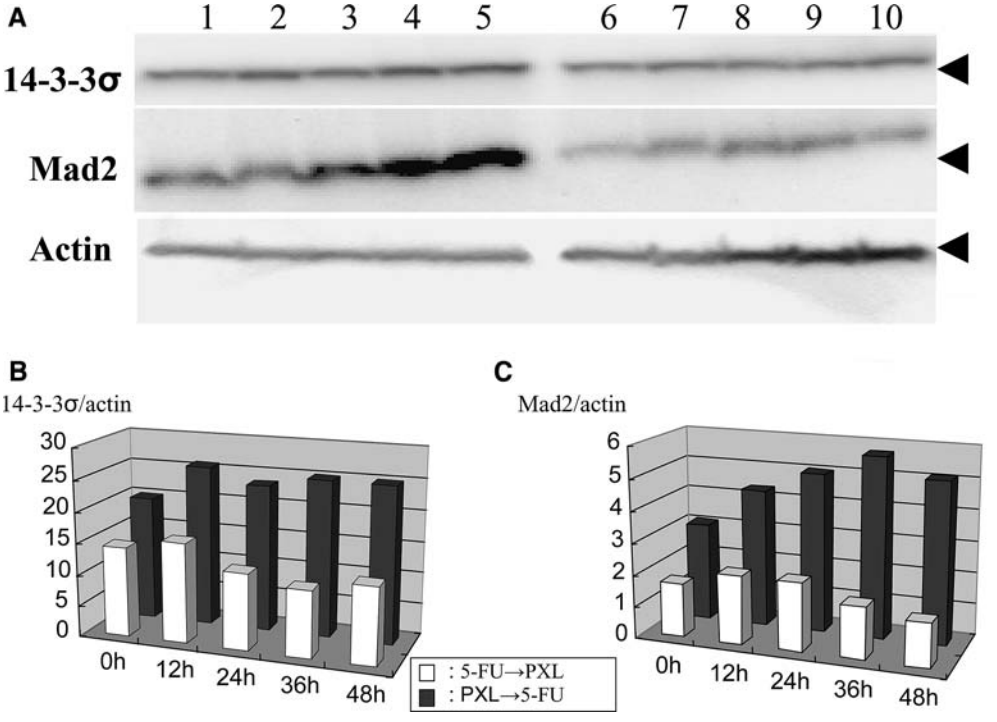


Fig. 5 Effects of PXL (1 μM for 24 h) followed by 5-FU (10 μM for 24 h) or 5-FU (10 μM for 24 h) followed by PXL (1 μM for 24 h) on 14-3-3σ and Mad2 protein expressions over time (0–48 h). Equal amounts of protein (10 μg) were resolved by SDS-PAGE and analyzed by western blotting. Immunoblots of 14-3-3σ, Mad2, and the universally used control protein actin are shown in Fig. 5a. The lanes are: *1* PXL (0 h); *2* PXL (12 h); *3* PXL (24 h); *4* PXL (24 h) followed by 5-FU (12 h); *5* PXL (24 h) followed by 5-FU (24 h); *6* 5-FU (0 h); *7* 5-FU (12 h); *8* 5-FU (24 h); *9* 5-FU (24 h) followed by PXL (12 h); *10* 5-FU (24 h) followed by PXL (24 h). Figure 5b, c shows the semi-quantitative 14-3-3σ and Mad2 protein expression patterns. Similar results were obtained in three separate experiments

cancer [13–15]. The overall response rate (OR) and MST of the PXL/5-FU combination were 65.5% (CR 24.1%; RR 41.4%) and 12 months, respectively [8]. The OR and MST of the PXL/5-FU regimen in combination with CDDP were 51% and 14 months, respectively [9, 10]. However, there are few publications regarding the molecular basis of PXL/5-FU combination therapy against human cancers, despite its excellent clinical outcome.

In breast and epidermoid carcinoma cell lines, 5-FU was shown to inhibit paclitaxel-induced mitotic arrest and apoptosis using any schedule, such as concomitant and sequential combinations, compared with PXL alone [16–18]. In contrast, sequential administration of PXL followed by 5-FU was found to be highly cytotoxic to a breast cancer cell line, since the initial PXL exposure did not interfere with the subsequent 5-FU metabolism or thymidylate synthase inhibition, and the delayed 5-FU exposure did not inhibit either the PXL-mediated mitotic arrest or apoptosis [19]. Moreover, in lung, breast, ovarian, and colon cancer cell lines, the cytotoxic interaction of PXL and 5-FU was definitely schedule-dependent, and the maximum cytotoxic effects were obtained in the four cancer cell lines when PXL preceded 5-FU [20].

However, no information is available regarding the combined effects of 5-FU and PXL on gastric cancer cells in vitro. Here, we demonstrated that PXL followed by 5-FU was more cytotoxic to the gastric cancer cell line MKN45 than 5-FU followed by PXL, PXL alone and 5-FU alone. Our results were consistent with the latter results cited above [19, 20] and could explain that PXL pretreatment may make more sensitive to 5-FU.

To understand the influence of each drug to p53 wild MKN45 on the cell cycle, we performed a flow-cytometric analysis. The results revealed that 5-FU could increase the G1/S phase cell rate and prevent entry into G2/M phase, whereas PXL caused accumulation of cells in G2/M phase. Since p53 is implicated in the control of the cell cycle, it is conceivable that the status of p53 may modulate cell sensitivity to anticancer drug. But the role of the p53 protein for responsiveness to paclitaxel is discussed controversially for different tumor types (20.21.22.23). Prior studies have demonstrated an increased sensitivity to paclitaxel in p53 mutant cells endowed with a defective G1 checkpoint and an increase in the percentage of cells in G2/M phase of the cell cycle [21, 22]. And furthermore, several previous studies suggest that the greatest cytotoxic effects of these antimitotic agents are observed when the majority of cells are arrested in mitosis [23, 24]. It therefore may be suggested that the mechanism for the antagonistic effect of 5-FU treatment followed by PXL is merely to prevent the majority of cells from accumulating in G2/M phase of the cell cycle.

In light of the fact that the synergistic effect of PXL treatment prior to 5-FU may be related to the G2/M or M checkpoints, we performed a western blotting analysis to determine the expression patterns of cell cycle-related proteins (Chk1, Wee1, 14-3-3σ and Mad2) in each treatment regimen. The G2/M checkpoint proteins Chk1 and Wee1 were upregulated after PXL exposure, but returned to their basal levels after PXL removal and 5-FU exposure. 14-3-3σ protein was upregulated after pre-exposure to PXL and further increased after PXL removal and 5-FU exposure. These findings suggest that this administration sequence induces significantly higher G2 checkpoint protein expressions than the reverse administration sequence. Mad2 protein expression was increased by pretreatment with PXL, and subsequently increased to a higher level after PXL removal and 5-FU exposure compared to 5-FU treatment followed by PXL exposure. Previous reports showed that overexpression of Mad2 led to an increased sensitivity to vincristine [25] and cisplatin [26] in nasopharyngeal carcinoma cells. Recent observations have revealed that cells with spindle checkpoint abnormalities are insensitive to Taxol, a spindle poison [27]. Furthermore, suppression of the spindle checkpoint function by BubR1 or Mad2 RNA interference in DNA-damaged cells led to escape from catastrophic death and then to abnormal mitosis [28]. It is therefore conceivable that Mad2 expression induced by paclitaxel might be sensitizer of 5-FU and furthermore RNA interference studies directed against Mad2 gene would be strengthened to examine the increased efficacy of 5-FU treatment.

Importantly, our results were observed at the clinically available concentrations of PXL and 5-FU. In particular, 5-FU concentrations were based on the PMC therapeutic dose, indicating that this basic study can directly explain the efficacy of the PXL/5-FU combination in modified PMC (PMC with PXL) as well as the above clinical trials.

Regarding the efficacy of PXL followed by 5-FU, we further showed that the cytotoxic activity at lower 5-FU concentrations was greater than that at high concentrations. Since the PMC regimen consists of a higher level of 5-FU by infusion once a week and a lower level of 5-FU by oral UFT for 5–7 days per week, the various 5-FU concentrations in PMC provide dual antitumor effects of 5-FU, depending on the dose: (a) G1/S arrest and apoptosis at higher 5-FU concentrations and (b) G2/M arrest and mitotic catastrophe at lower 5-FU concentrations [12]. This significant cytotoxicity of PXL followed by lower concentrations of 5-FU may be dependent on cell death by mitotic catastrophe in G2/M arrested cells due to the PXL pre-exposure.

Modified PMCs, such as PMC combined with PXL or taxotere, have demonstrated not only a high RR of 31%, including one complete response, but also an excellent MST of 10.5 months for stage IV gastric cancer [7]. Therefore, the combination of PMC and PXL may be a better regimen than the conventional 5-FU/PXL combination. Moreover, PXL followed by continuous 5-FU or an oral 5-FU derivative may be more optimal than PXL followed by bolus 5-FU, since either continuous 5-FU or an oral 5-FU derivative produce lower 5-FU plasma concentrations than bolus 5-FU.

In conclusion, we have demonstrated that the cytotoxicity of PXL followed by 5-FU was greater than that of 5-FU followed by PXL. PXL pretreatment plays an important role in sensitization to 5-FU cytotoxicity, whereas 5-FU pretreatment does not increase the subsequent PXL cytotoxicity. These data suggest that modifying the administration schedule of 5-FU/PXL may increase the efficacy with improved toxicity. However, the concern of our study is that these data are only generated one cell line but consistent with several previous reports, further investigation of other gastric cancer cell line would be required.

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