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SYNERGISTIC INTERACTION BETWEEN THE EGFR TYROSINE KINASE INHIBITOR GEFITINIB ("IRESSA") AND THE DNA TOPOISOMERASE I INHIBITOR CPT-11 (IRINOTECAN) IN HUMAN COLORECTAL CANCER CELLS

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Epidermal growth factor receptor [EGFR (HER1, erbB1)] is a receptor with associated tyrosine kinase activity, and is expressed in colorectal cancers and many other solid tumors. We examined the effect of the selective EGFR tyrosine kinase inhibitor (EGFR-TKI) gefitinib ("Iressa") in combination with the DNA topoisomerase I inhibitor CPT-11 (irinotecan) on human colorectal cancer cells. EGFR mRNA and protein expression were detected by RT-PCR and immunoblotting in all 7 colorectal cancer cell lines studied. Gefitinib inhibited the cell growth of the cancer cell lines *in vitro* with an IC_{50} range of 1.2–160 μM by 3,(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Lovo cells exhibited the highest level of protein and autophosphorylation of EGFR and were the most sensitive to gefitinib. The combination of gefitinib and CPT-11 induced supra-additive inhibitory effects in COLO320DM, WiDR and Lovo cells, assessed by an *in vitro* MTT assay. Administration of gefitinib and CPT-11 had a supra-additive inhibitory effect on WiDR cells and tumor shrinkage was observed in Lovo cell xenografts established in nude mice, whereas no additive effect of combination therapy was observed in COLO320DM cells. To elucidate the mechanisms of synergistic effects, the effect of CPT-11-exposure on phosphorylation of EGFR was examined by immunoprecipitation. CPT-11 increased phosphorylation of EGFR in Lovo and WiDR cells in time- and dose-dependent manners. This EGFR activation was completely inhibited by 5 μM gefitinib and gefitinib-induced apoptosis was enhanced by combination with CPT-11, measured by PARP activation although no PARP activation was induced by 5 μM CPT-11 alone. These results suggested that these modification of EGFR by CPT-11, in Lovo cells, is a possible mechanism for the synergistic effect of CPT-11 and gefitinib. These findings imply that the EGFR-TKI gefitinib and CPT-11 will be effective against colorectal tumor cells that express high levels of EGFR, and support clinical evaluation of gefitinib in combination with CPT-11, in the treatment of colorectal cancers.

Key words: *combination; gefitinib; "Iressa"; colorectal cancer; irinotecan*

Colorectal cancer is a major public health concern. Although chemotherapy appears to be of very limited value in advanced colorectal cancer, there have been many efforts to apply combination chemotherapy in patients with primary disease.[1–3]

The combination of fluorouracil and leucovorin used to be recognized as standard therapy for colorectal cancer, but the topoisomerase I inhibitor, irinotecan (CPT-11), has recently been demonstrated to be active against colorectal cancer that was resistant to prior therapy.[4,5] Moreover, the CPT-11/5-FU/LV combination has been approved as standard chemotherapy by the US FDA for metastatic colorectal cancer.[6] However, patients treated with CPT-11 plus bolus 5-FU/leucovorin have been found to have a 3-fold higher rate of treatment-induced or treatment-exacerbated death than patients treated with other arms of the respective studies.[7] We have therefore been seeking a new combination regimen containing CPT-11 and target-based drugs.

The development of target-based drugs, including receptor tyrosine kinase inhibitors (TKI), is one of the promising strategies for cancer chemotherapy.[8,9] Colorectal cancers express receptors of the type 1 tyrosine kinase family, including epidermal growth factor receptor (EGFR) and c-erbB-2,[10–12] and the EGFR has emerged as a central molecular target for modulation in cancer therapeutics. The correlation between high expression of EGFR and clinically aggressive malignant disease has made EGFR a promising target of therapy for many epithelial tumors, which represent approximately 2/3 of all human cancers. In solid cancers, including colorectal cancers, high EGFR expression correlates with poor prognosis.[11] Gefitinib ("Iressa") is an orally active, selective EGFR-TKI that blocks signal transduction pathways involved in the proliferation and survival of cancer cells and in other host-dependent processes promoting cancer growth.[13,14] In EGFR tyrosine kinase assays, gefitinib has an IC_{50} of 0.033 μM. Inhibition of c-erbB-2 and KDR occurs at doses 100-fold higher than for EGFR inhibition.[15] We have previously demonstrated that gefitinib exerts high growth-inhibitory activity against EGFR-positive tumors in a xenograft model,[16] and gefitinib is therefore expected to be a potent therapeutic agent against EGFR-positive colorectal cancers. In recent years, it has been shown that the combined treatment of established human colorectal cancer xenograft with anti-EGFR drug (cetuximab or gefitinib) and with topoisomerase I inhibitor, topotecan, increase the antitumor activity of these drugs.[17,18] The aim of the present study was to investigate the combination effect of gefitinib and CPT-11 and to elucidate the biochemical mechanism of synergistic interaction in colorectal cancers.

MATERIAL AND METHODS

Drugs and chemicals

Gefitinib (N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(morpholin-4-yl)propoxy] quinazolin-4-amine) was provided by AstraZeneca (Cheshire, UK). Gefitinib was dissolved in dimethyl sulfoxide (DMSO) for the *in vitro* study and suspended in 5% glucose, pH 6, for the *in vivo* study. CPT-11 was obtained from Yakult Honsha (Tokyo, Japan). CPT-11 was dissolved in 45 mg/ml solvitol (pH 3-4) for both the *in vivo* and *in vitro* studies.

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Animals

Female BALB/c nude mice, 6-weeks-old, were purchased from Japan Charles River Co., Ltd. (Atsugi, Japan). All mice were maintained in our laboratory under specific-pathogen-free conditions.

Cells and culture

Human colorectal cancer cell lines WiDR, LS-174T, COLO320DM, COLO320HSR, Lovo, SW480 and HCT116 were obtained from ATCC (Lockville, MD). Lovo cells, SW480 and HCT116 cells were maintained in HAM's F12 medium (GIBCO BRL, Grand Island, NY), Leibovitz's L-15 medium and McCoy's 5A medium (GIBCO BRL), respectively, all supplemented with 10% heat-inactivated fetal bovine serum (FBS). Other cell lines were maintained in RPMI1640 (Nikken Bio Med. Lab., Kyoto, Japan) supplemented with 10% FBS.

Growth-inhibition assay

We used the tetrazolium dye [3,(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, MTT] assay to evaluate the cytotoxicity of various drug concentrations. A 200 ml volume of an exponentially growing cell suspension (5×10^3–1.5×10^4 cells/ml) was seeded into a 96-well microtiter plate and 20 μl of each drug at various concentrations was added. After incubation for 72 hr at 37°C, 20 μl of MTT solution (5 mg/ml in phosphate buffered saline, PBS) was added to each well and the plates were incubated for a further 4 hr at 37°C. After centrifuging the plates at 200*g* for 5 min, the medium was aspirated from each well, and 180 μl of DMSO was added to each well to dissolve the formazan. Optical density was measured at 562 and 630 nm with a Delta Soft ELISA analysis program interfaced with a Bio-Tek Microplate Reader (EL-340, Bio-Metallics, Princeton, NJ). Each experiment was performed in 6 replicate wells for each drug concentration and carried out independently 3 or 4 times. The IC_{50} value was defined as the concentration needed for a 50% reduction in the absorbance calculated based on the survival curves. Percent survival was calculated as follows: (mean absorbance of 6 replicate wells containing drugs − mean absorbance of 6 replicate background wells)/(mean absorbance of 6 replicate drug-free wells − mean absorbance of 6 replicate background wells) × 100.

RT-PCR

Specific primers designed for EGFR CDS were used for detection of EGFR mRNA as described elsewhere.[16] First-strand cDNA was synthesized from the cells' RNA with an RNA PCR Kit (TaKaRa Biomedicals, Ohtsu, Japan). After reverse transcription of 1 μg of total RNA with Oligo(dT)-M4 adaptor primer, the whole mixture was used for PCR with 2 oligonucleotide primers (5′-AATGTGAGCAGAGGCAGGGA-3′,5′GGCTTGGTTTG-GAGCTTCTC-3′). PCR was performed with initial denaturation at 94°C for 2 min, 25 cycles of amplification (denaturation at 94°C for 30 sec, annealing at 55°C for 60 sec and extension at 72°C for 105 sec).

Immunoprecipitation and immunoblotting

The cultured cells were washed twice with ice-cold PBS, lysed in EBC buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40, 100 mM NaF, 200 mM Na orthovanadate and 10 mg/ml each of leupeptin, aprotinin and phenylmethylsulfonyl fluoride). The lysate was cleared by centrifugation at 20,000*g* for 5 min, and the protein concentration of the supernatant was measured by BCA protein assay (Pierce, Rockford, IL). For Immunoblotting, 20 μg samples of protein were electrophoretically separated on a 7.5% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). The membrane was probed with rabbit polyclonal antibody against EGFR (1005; Santa Cruz Biotech, Santa Cruz, CA), HER2/neu (c-18; Santa Cruz), phospho-EGFR specific for Tyr 845, Tyr 1045, and Tyr 1068 (numbers 2231, 2235 and 2234; Cell Signaling, Beverly, MA) and cleaved PARP (number 9544; Cell Signaling) as the first antibody, followed by horseradish peroxidase-conjugated secondary antibody. The bands were visualized by electrochemiluminescence (ECL, Amersham, Piscataway, NJ). For immunoprecipitation, 5×10^6 cells were washed, lysed in EBC buffer, and centrifuged. The resultant supernatants (1,500 μg) were incubated with the anti-EGFR antibody (1005) at 4°C overnight. The immunocomplex were absorbed onto protein A/G-Sepharose beads, washed 5 times with lysate buffer, denatured and subjected to electrophoresis on a 7.5% polyacrylamide gel followed by immunostaining probed with antiphosphotyrosine antibody (PY-20, BD Bioscience Clontech, Tokyo, Japan).

Combined effect of gefitinib and CPT-11 in vitro

The combined effect of gefitinib and CPT-11 on colorectal cancer cell growth was evaluated by the combination index (CI) analysis method.[6] For any given drug combination, CI represents the degree of synergy, additivity or antagonism. CI was expressed in terms of fraction-affected (F_a) values, which represents the percentage of cells killed or inhibited by the drug. Using the mutually exclusive ($\alpha=0$) or mutually nonexclusive ($\alpha=1$) isobologram equation, the F_a/CI plots for each cell line was constructed by computer analysis of the data generated from the median effect analysis. CI values were interpreted as follows: <1.0 = synergism; 1.0 = additive and >1.0 = antagonism.

Using the median-effect method, developed by Chou and Talalay, the dose-response curve was plotted for each drug and for multiple doses of a fixed-ratio combination by using the equation:

$$f_a/f_u = (D/D_m)^m,$$

where, D is the dose-administered, Dm is the dose required for 50% inhibition of growth, f_a is the fraction affected by dose D, f_u is the unaffected fraction and m is a coefffect curve. The dose-response curve was plotted by logarithmic conversion of the equation to determine the m and D_m values, and the dose D_x required for x percent effect $(f_a)_x$ was then calculated as

$$D_x = D_m[\,f_a]_x/(\,f_u)\,x]^{1/m}.$$

Thus, CI can be defined by the isobologram equation

$$CI = (D)_1/(D_x)^1 + (D)_2/(D_x)2 + \alpha(D)_1(D)_2/(D_x)_1(D_x)_2,$$

where $(D_x)_1$ is the dose of Drug-1 required to produce x percent effect alone, and $(D)_1$ is the dose of Drug 1 required to produce the same x percent effect in combination with Drug 2; similarly, $(Dx)_2$ is the dose of Drug 2 required to produce x percent effect alone and $(D)_2$ is the dose of Drug 2 required to produce the same x percent effect in combination with Drug 1. Theoretically, CI is the ratio of the combined dose to the sum of the single-drug doses at an isoeffective level. Consequently, CI values <1 indicate synergism, values >1 indicate antagonism and a value of 1 indicates additive effects. The CI values obtained from both the classical nonconservative ($\alpha=0$) and conservative ($\alpha=1$) isobologram equations are presented in this report.

Growth-inhibition assay in vivo

Experiments were performed in accordance with the United Kingdom Coordinating Committee on Cancer Research Guidelines for the welfare of animals in experimental neoplasia (second edition).

In vivo experiments were scheduled to evaluate the combined therapeutic effect on preexisting tumors of oral or intraperitoneal administration of gefitinib and intravenous injection of CPT-11. The dose of each drug was set based on the results of a preliminary experiment involving administration of each drug alone. Ten days before administration, 1×10^7 WiDR and COLO320DM or 2×10^6 Lovo cells were injected subcutaneously into the back of mice. Five or 6 mice per group were injected with tumor cells. Tumor bearing mice were either given gefitinib, 40 mg/kg/day *p.o.* on days 1–10, or CPT-11, 40 mg/kg/day *i.v.* on days 1, 5 and 9, or

both, or placebo (5%(w/v) glucose solution). Alternatively, gefitinib, 30 or 60 mg/kg, *i.p.* days 1–14, and *i.v.* CPT-11, 16.7 or 33.3 mg/kg, *i.v.* on days 1, 5 and 9, were administered to the mice. Tumor diameters were measured with calipers on days 1, 4, 7, 10, 14, 18 and 22 to evaluate the effects of treatment, and tumor volume was determined by using the following equation: tumor volume = $ab^2/2$ (mm^3) (where a is the largest diameter of the tumor and b is the shortest diameter). Day "x" denotes the day on which the effect of the drugs was estimated, and day "0" denotes the first day of treatment. All mice were sacrificed on day 22 after measuring their tumors.

Statistical analysis

Differences between the test groups were analyzed by 1-factor ANOVA followed by Fisher's protected least significant difference (PLSD). A value of $p<0.05$ was considered statistically significant.

RESULTS

EGFR and HER2 expression and EGFR autophsophorylation in colorectal cancer cells

We examined EGFR mRNA expression by RT-PCR analysis using 2 specific primers. Approximately 570 bp-long PCR products were amplified in all cell lines that exhibited expression of EGFR mRNA (Fig. 1*a*). Comparison of the protein expression levels of EGFR in colorectal cancer cells by immunoblotting (Fig. 1*b*) revealed high expression in Lovo and WiDR cells. EGFR protein was also detected in LS-174T, COLO320DM, COLO320HSR, HCT116 and SW480 cells, although the expression levels in COLO320DM and COLO320HSR are subtle. The highest expression level of phosphrylated EGFR measured by phospho-specific EGFR antibody (Tyr845, Tyr1045 and Tyr1068) was observed in Lovo cells (Fig. 1*b*). Because the function of EGFR is closely related to that of other HER families including HER2/neu, we also examined the protein level of HER2/neu. High expression of HER2/neu were observed in LS-174T, HCT-116 and SW480 (Fig. 1*b*).

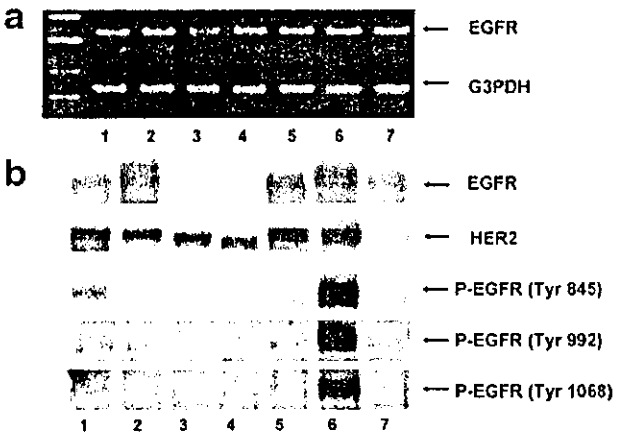


FIGURE 1 – EGFR expression in colorectal cancer cells. (*a*) Expression of EGFR mRNA in each cell line was detected by RT-PCR using specific primers designed for EGFR CDS. Expression of G3PDH mRNA was detected. Twenty-five cycles of PCR amplification were performed for each PCR product. Lanes 1–7 represent LS-174T, WiDR, COLO320DM, COLO320HSR, HCT116, Lovo and SW480 cells, respectively. (*b*) A 20 μg sample of total cell lysates was separated by 7.5% SDS-PAGE, transferred to PVDF membrane, and incubated with a specific anti-human EGFR, HER2/neu and phospho-EGFR (Tyr845, Tyr992 and Tyr1068).

Cellular sensitivity of colorectal cancer cells to gefitinib and CPT-11

The growth inhibitory effect of gefitinib and CPT-11 on colorectal cancer cells was examined by MTT assay. The IC_{50} values of gefitinib for the cell lines ranged from 1.2 μM (Lovo cells) to 160 μM (HCT116 cells) (Table I). No significant relationship was observed between EGFR expression levels and IC_{50} values among these cell lines. However, Lovo cells, which exhibited the highest EGFR expression and its phosphorylation, were the most sensitive to gefitinib. On the other hand, the IC_{50} values of CPT-11 for the cell lines ranged from 5.2 μM (Lovo) to 35 μM (SW480). The range of sensitivity to gefitinib was wider than to CPT-11.

In vitro *combined effect of gefitinib and CPT-11 on colorectal cancer cell lines*

Based on the results of the evaluation of *in vitro* growth-inhibition, 4 cell lines (WiDR, COLO320DM, Lovo, and SW480 cells) were selected for the *in vitro* combination study. Cells were treated with gefitinib or CPT-11 alone or in concomitant combination at fixed molar ratio for 72 hr. The ratios of gefitinib and CPT-11 were set based on the IC_{50} values of each cell line. Growth rate values are averages of data from at least 3 independent experiments. The effects of combinations of gefitinib and CPT-11 on cell growth are shown in Figure 2. CI values of <1, >1 and 1 indicate a supra-additive effect (synergism), antagonistic effect and additive effect, respectively. A low CI index was observed in WiDR, COLO320DM and Lovo cells over a wide range of inhibition levels. Synergistic effects were also observed in the relatively high F_a values in SW480 cells. These results suggest that gefitinib and CPT-11 had a synergistic effect on most of the colorectal cancer cell lines *in vitro*.

In vivo *combination effects of gefitinib and CPT-11*

In order to determine whether the combination of these 2 drugs is also synergistic against colorectal cancer *in vivo*, the growth-inhibitory effect of the combination was evaluated against the colorectal cancer cells in tumor xenografts. The growth inhibitory effect of gefitinib, 30 mg/kg, *i.p.* days 1–10, and CPT-11, 40 mg/kg, *i.v.* days 1, 5 and 9, on WiDR cells was evaluated (Fig. 3*a*,*b*). Administration of gefitinib or CPT-11 alone suppressed the tumor volume of WiDR cells with a T/C value of 73.9% and 69.2%, respectively, at day 22, (Fig. 3*c*), whereas gefitinib+CPT-11 suppressed WiDR tumors with T/C value of 51.8% at day 22, but this was not statistically significant (Fig. 3*d*, $p=0.164$ by 1-factor ANOVA). A 10% body weight loss was observed until day 15 in mice given the combination, but body weight recovered by day 22 (Fig. 3*e*). No growth inhibitory effect of single or combined therapy of CPT-11 and gefitinib in COLO320DM cells were observed (data not shown). In mice transplanted with Lovo cells, with a high EGFR expression level, marked tumor growth inhibition was achieved with gefitinib+CPT-11 (Fig. 3*f*). The T/C of the combination schedule at day 11 was 22.8% and significantly lower than in the control ($p<0.0012$ by Fisher's PSLD, Fig. 3*g*). A 10% maximum body weight loss until day 15 was also observed in mice treated with the combination (Fig. 3*j*).

Alternatively, the combined effect of oral administration of gefitinib and intravenous administration of CPT-11 was evaluated in mice transplanted with Lovo cells. Gefitinib, 30 or 60 mg/kg *p.o.* days 1–14, and CPT-11, 16.7 or 33.3 mg/kg *i.v.* days 1, 5 and 9, were administered (schedule 2, Fig. 4*a*), and greater growth inhibition was observed in mice treated with this combination, compared to the controls (Fig. 4*b*) A more marked growth-inhibitory effect was observed at a higher dose of CPT-11 (16.7 *vs.* 33.3 mg/kg), but there was no difference between 30 mg/kg and 60 mg/kg of gefitinib in the combination. The combination of gefitinib (30 and 60 mg/kg) and CPT-11 (33.3 mg/kg/*i.v.*) resulted in tumor reduction during treatment that was significant at day 15 (Fig. 4*c*). The T/C values imme-

TABLE 1 – *IN VITRO* GROWTH-INHIBITORY ACTIVITY OF GEFITINIB AND CPT-11 IN HUMAN COLORECTAL CANCER CELLS (MTT ASSAY)[1]

Cell line	gefitinib		CPT-11	
	IC_{50} (μM)	Concentration range (μM)	IC_{50} (μM)	Concentration range (μM)
WiDR	10 ± 1.1	0.83–53	33 ± 7.5	1.6–160
LS-174T	100.4 ± 10.1	N.D.	13	N.D.
COLO320DM	11 ± 3.8	0.63–100	11 ± 0.6	1.6–160
COLO320HSR	22	N.D.	5.5	N.D.
HCT116	177.0 ± 12.2	N.D.	11	N.D.
SW480	23 ± 0.6	1.6–10	35 ± 5.5	1.6–50
Lovo	1.2 ± 0.59	0.31–25	5.2 ± 0.82	0.16–10

[1]The IC_{50} value (μM) of each drug was measured by MTT assay, as described in the Materials and Methods. Each value is a mean ± SD of 3 or 4 independent experiments–N.D., not determined.

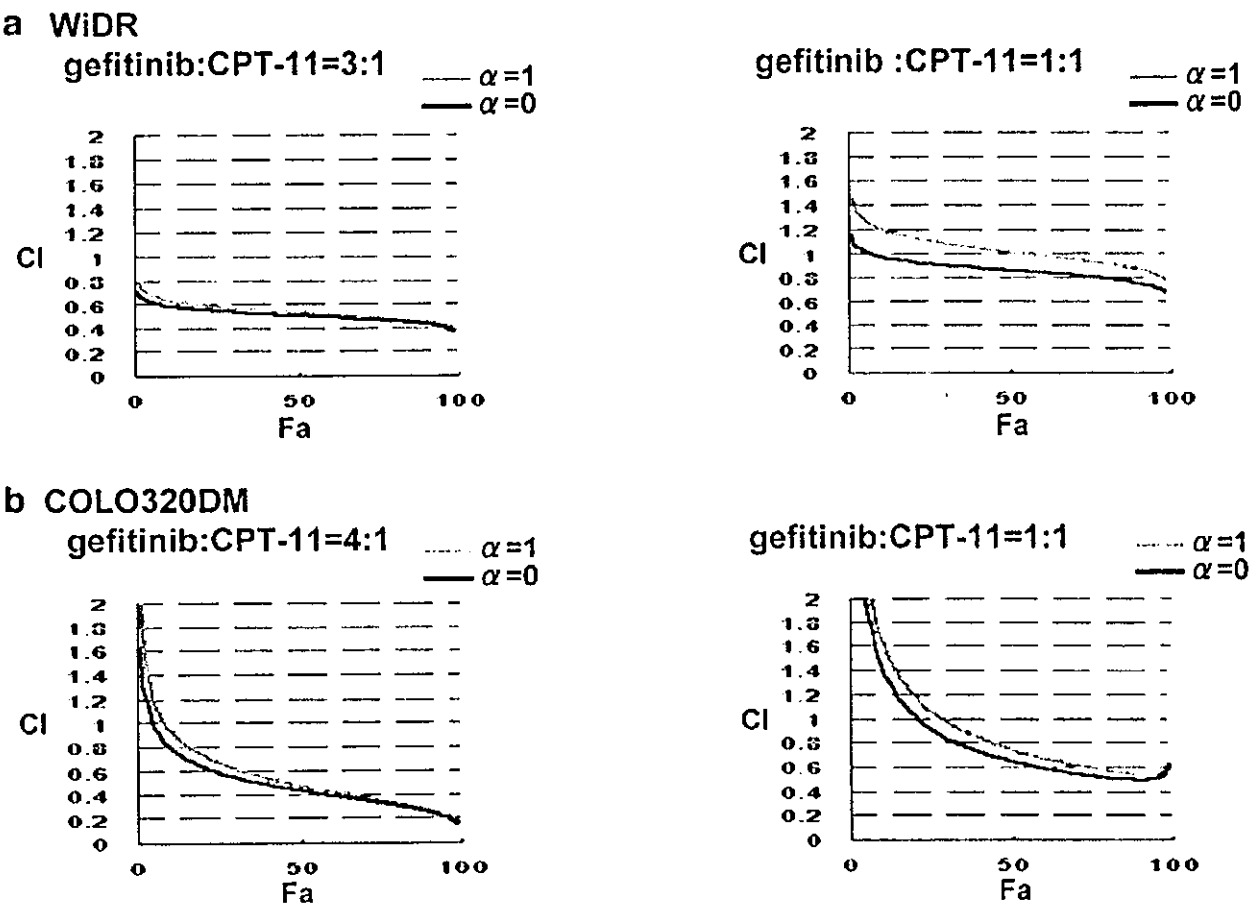


FIGURE 2 – Combination index (CI) plots of interactions between gefitinib and CPT-11. Cells were treated with gefitinib and CPT-11 alone and in combination at fixed molar ratios (molar ratios of gefitinib to CPT-11 of 3:1 and 1:1 [(*a*) WiDR], 4:1 and 1:1 [(*b*) COLO320DM], 1:2 and 1:5 [(*c*) Lovo], 1:1 [(d) SW480]. Using the mutually exclusive (CI) or mutually nonexclusive (CI′) isobologram equation, the affected fraction (F_a)-CI plot for each cell was constructed by computer analysis of the data generated from the median effect analysis. CI values <1 occurred over a wide range of inhibition levels, indicating synergy.

diately after the completion of treatment (at day 15) and at day 22 are summarized in Fig.4*d*. More severe body weight loss was observed, ~20% at day 15, in mice treated with 60 mg/kg of gefitinib alone or with CPT-11, suggesting that CPT-11 does not enhance the body weight loss induced by gefitinib. Body weight recovered by day 22 (Fig. 4*e*). No deaths of were observed during the treatment or observation period.

Induction of EGFR phosphorylation and enhanced gefitinib-induced PARP activation by CPT-11

To elucidate the synergistic effects of CPT-11 and gefitinib, we examined the effect of exposure of CPT-11 on EGFR phosphorylation in Lovo and WiDr cells. Phosphorylated EGFR was detected with anti-phosphotyrosine antibody (PY-20)

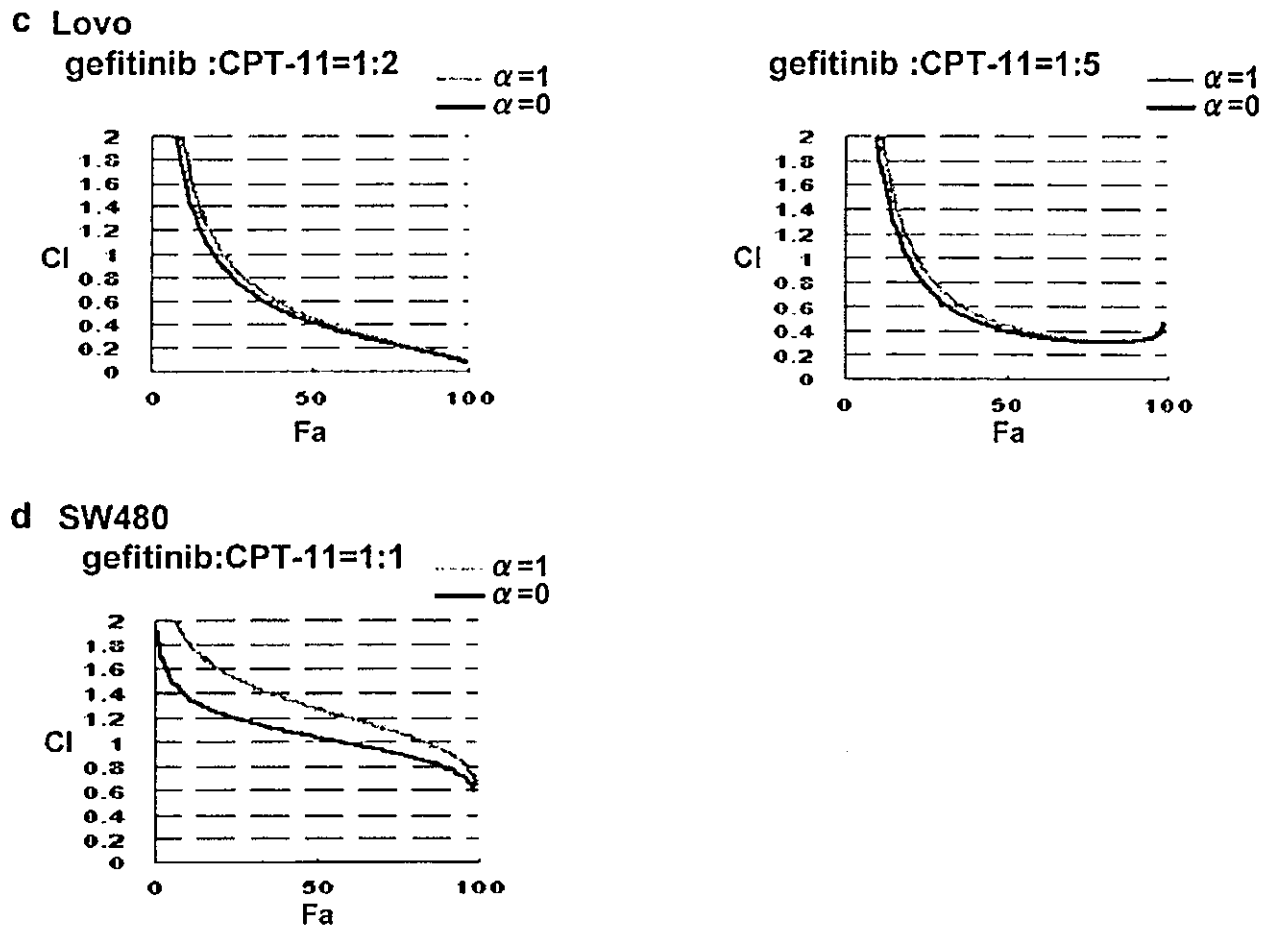


FIGURE 2 – CONTINUED.

against immunoprecipitated EGFR and increased phosphorylation of EGFR was observed after exposure to CPT-11 in Lovo cells in dose- and time- dependent manner (3–24 hr) (Fig. 5*a*). The dose-dependent activation of EGFR by CPT-11 was also obtained in WiDR cells (Fig. 5*b*). CPT-11-induced phosphorylation of EGFR was observed without ligand-stimulation. The EGFR activation was completely inhibited by 24 hd exposure of 5 μM gefitinib. gefitinib-induced apoptosis measured by PARP activation was enhanced by combination with CPT-11, although no PARP activation was induced by CPT-11 alone (Fig. 5*c*). These results suggest that the modification of EGFR by CPT-11 increases the cellular sensitivity to gefitinib, resulting the synergistic effect of CPT-11 and gefitinib. We also observed the effect of gefitinib on the expression and the activity of topoisomerase I by immunoblotting and decatenatnion assay. No modification of topoisomerase I by gefitinib was observed (data not shown).

DISCUSSION

Evidence has suggested that the new EGFR-targeting drug gefitinib is active against gastrointestinal malignancies as well as non-small cell lung cancer. Combination of gefitinib with cytotoxic drugs has been evaluated in the U.S. and Europe,[19,20] but combination with CPT-11 has not been evaluated. CPT-11 is a potent DNA-targeting drug in patients with colorectal cancer that is refractory to treatment with fluorouracil and leucovorin,[4,5] although a higher rate of treatment-induced toxicity was suspected in a retrospective analysis.[7] In preclinical study, Ciadiello *et al.*[17,18] reported that supra-additive combination effect of EGFR-targeting drug (cetuximab or gefitinib) and topoisomerase I inhibitor, topotecan was observed in human colorectal cancer GEO xenograft. We have therefore studied the synergistic potential for a new combination regimen containing CPT-11 and gefitinib. The synergistic potential of CPT-11 combined with gefitinib demonstrated in our study suggests that the gefitinib/CPT-11 combination is a promising regimen for colorectal cancer patients. Schedule 2, administration of oral gefitinib and intravenous CPT-11 designed in a xenograft model, was based on possible clinical administration of the drugs, and thus a treatment schedule consisting of intermittent *i.v.* CPT-11 and continuous gefitinib *p.o.* may be applicable to colorectal cancer in humans.

In xenograft models, body weight loss was observed when administered in combination as well as when each drug was administered alone. However, body weight loss rapidly recovered immediately after the completion of administration, and no deaths were observed. Diarrhea is the dose-limiting toxicity of CPT-11 in humans,[7] and it is also observed in patients treated with gefitinib.[21,22] However, no diarrhea or related phenomena were observed in the mouse model treated with combinations of these

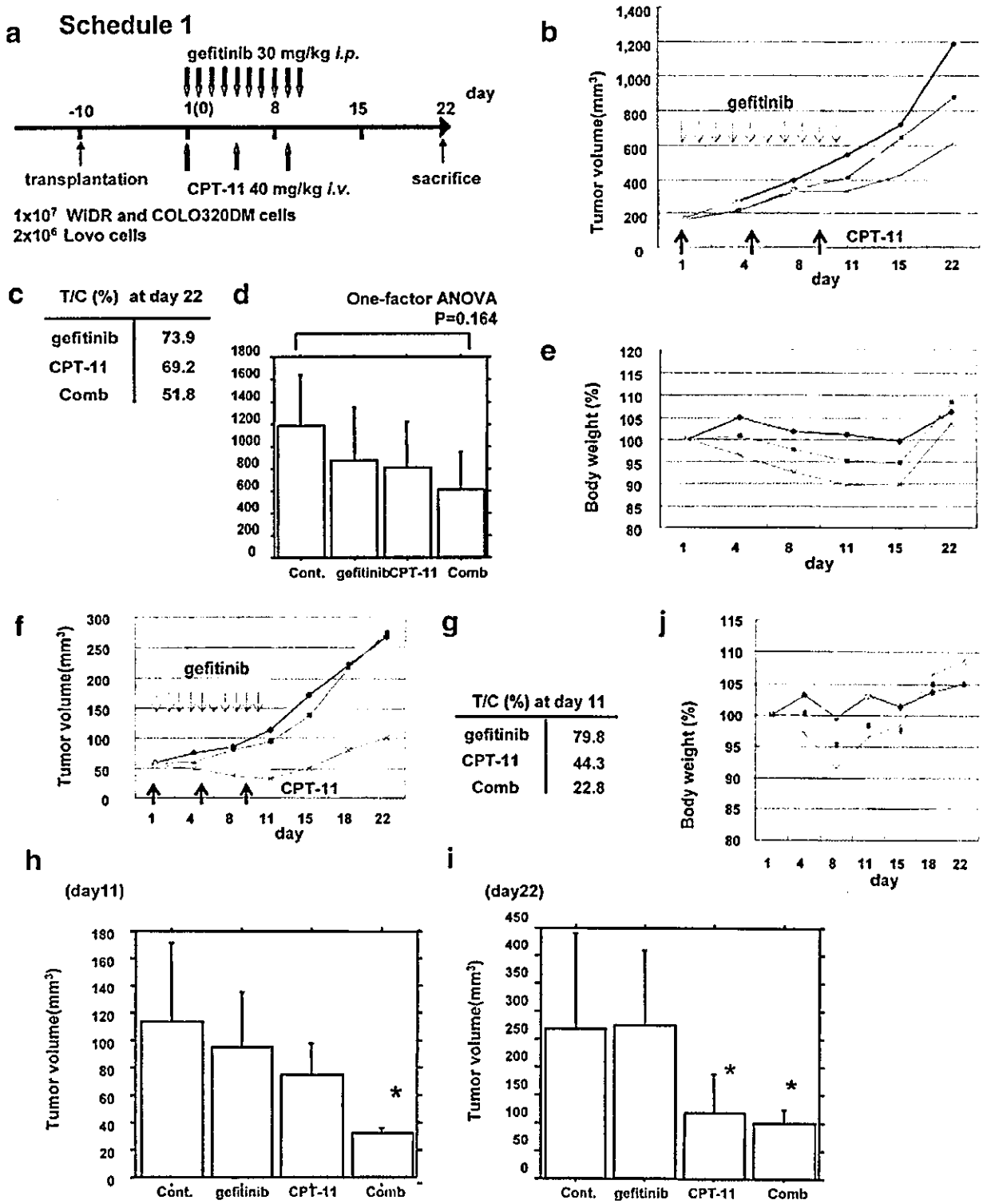
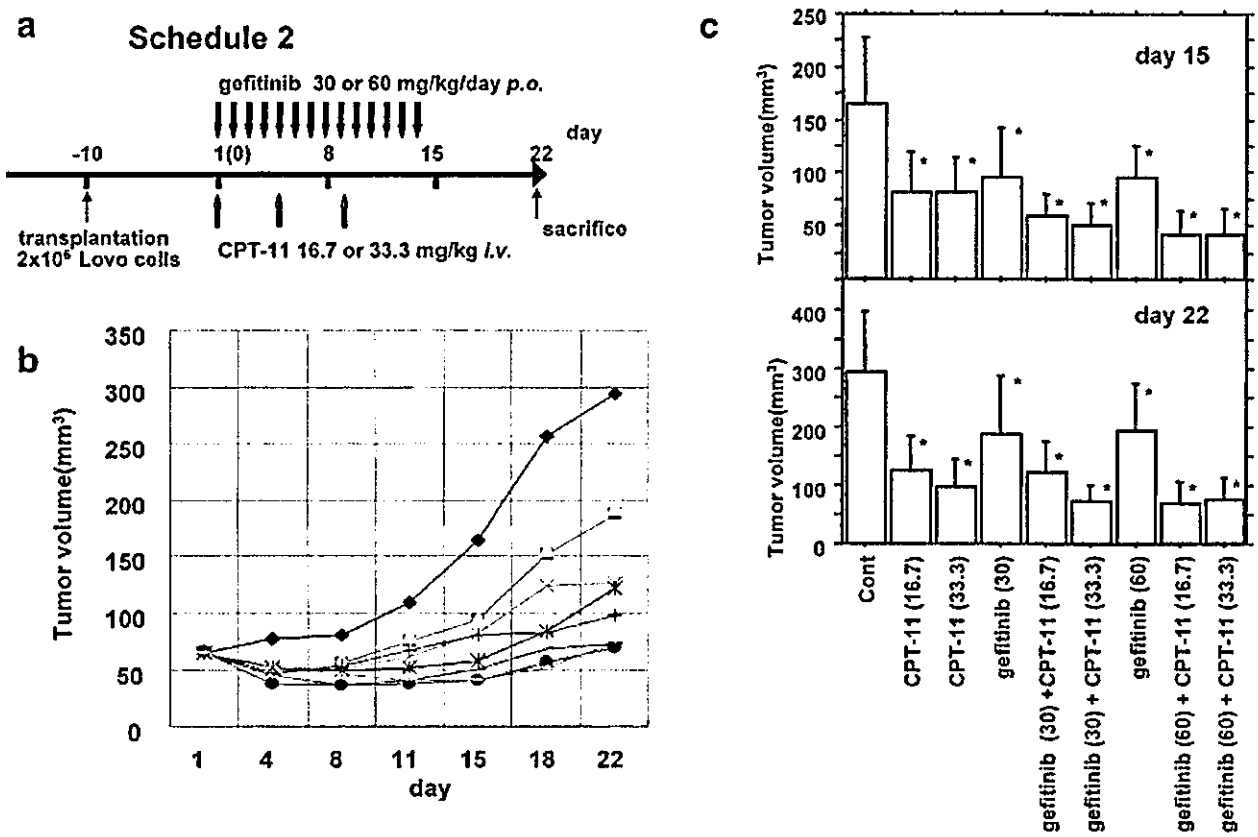


FIGURE 3 – *In vivo* combined effect of gefitinib and CPT-11 on WiDR and Lovo tumor xenografts. (*a*) Treatment schedule. (*b*) (WiDR) and F (Lovo), Tumor growth curves. Female nude mice bearing WiDR or Lovo xenografts were randomly allocated to treatment with 5% (w/v) glucose solution (diamond), gefitinib (square), CPT-11 (triangle), or the combination (×). Tumor volume was calculated as described in Material and Methods. Each data point represents the mean tumor volume of 5 mice. E (WiDR) and J (Lovo) Percent change in body weight in the gefitinib (hatched square) and combination (×) group. C (WiDR) and G (Lovo) Ratio of tumor volume in the control (C) to tumor volume in the treatment group (T) at day 22 and day 15. D (WiDR), H and I (Lovo) Histogram of mean tumor volume at day 11 and day 22 bars, *S.D.* Statistical analysis was performed by 1-factor ANOVA, followed by Fisher's PLSD between 2 groups, as described in the Material and Methods section. *Significant difference ($p<0.05$; Fisher's PLSD) compared to the control.



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	T/C (%) at day 15
gefitinib 30	57.6
gefitinib 60	58.4
CPT-11 16.7	49.6
gefitinib 30+CPT-11 16.7	35.4
gefitinib 60+CPT-11 16.6	25.1
CPT-11 33.3	49.3
gefitinib 30+CPT-11 33.3	30.9
gefitinib 60+CPT-11 33.3	25.3

	T/C (%) at day 22
gefitinib 30	63.7
gefitinib 60	65.7
CPT-11 16.7	43.0
gefitinib 30+CPT-11 16.7	41.6
gefitinib 60+CPT-11 16.6	23.6
CPT-11 33.3	33.2
gefitinib 30+CPT-11 33.3	24.9
gefitinib 60+CPT-11 33.3	25.3

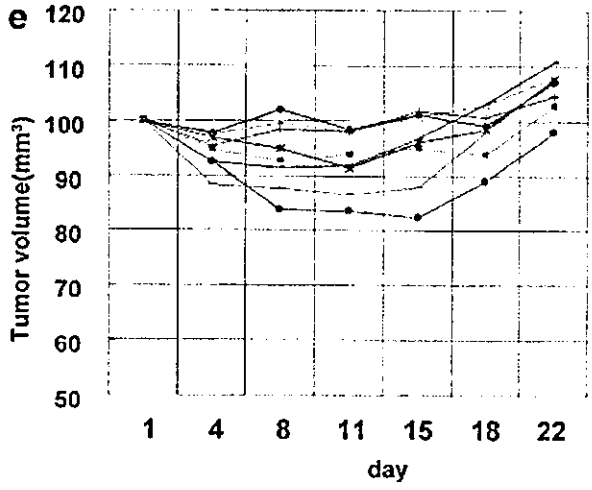

FIGURE 4 – The dose-dependent effect of combination therapy on Lovo cells *in vivo*. (*a*) Treatment schedule. (*b*) Significant growth-inhibition was observed in mice treated with the combination. Mice were allocated to 9 groups (6 mice/group) [closed diamond, 5%(W/V) glucose solution; ×, CPT-11 16.7 mg/kg; + CPT-11 33.3 mg/kg; square, gefitinib 30 mg/kg; star, gefitinib 30 mg/kg + CPT-11 16.7 mg/kg; blue line, gefitinib 30 mg/kg + CPT-11 33.3 mg/kg; open triangle, gefitinib 60 mg/kg; circle, gefitinib 60 mg/kg + CPT-11 16.7 mg/kg; light blue line, filled square, gefitinib 60 mg/kg + CPT-11 33.3 mg/kg]. (*c*) Mean tumor volumes and results of the statistical analysis at days 15 and 22, bars, *S.D.* *Significant difference ($p<0.05$) compared to the control. (*d*) T/C(%) at day 15 and 22. (*e*) Treatment-related body weight loss occurred in mice treated with gefitinib 60 mg/kg (triangle, circle, and light blue line).

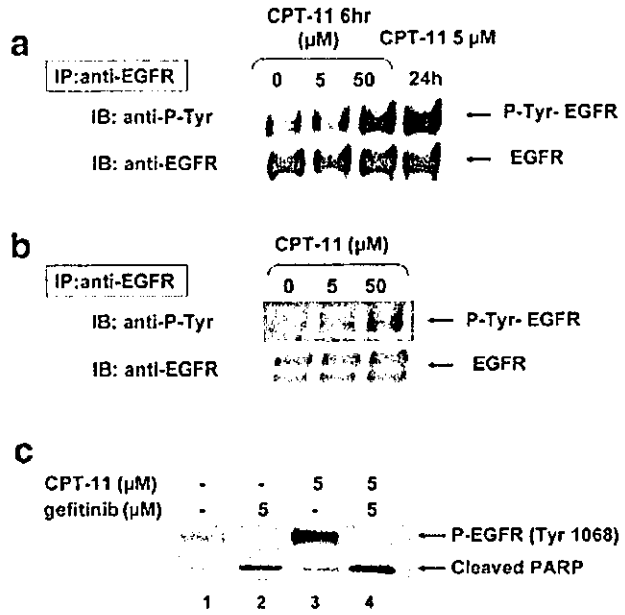

FIGURE 5 – The effect of CPT-11 on EGFR phosphorylation in WiDR cells. Lovo (*a*) and WiDR (*b*) cells (5×10^6) were treated with 5 or 50 μM CPT-11 for 6 hr. Additionally Lovo cells were treated with 5 μM CPT-11 for 24 hr. The 1,500 μg of total cell lysate was immunoprecipitated with an anti-EGFR antibody. Tyrosine-phosphorylated EGFR was determined with an anti-phosphotyrosine antibody and the membranes were reblotted by anti-EGFR antibody. (*c*) Lovo cells were treated with gefitinib or CPT-11 alone (lane 2 and 3) and in combination (lane 4) for 24 hr. A 20 μg of protein of each sample was analyzed by Western blotting using antiphospo-EGFR (Tyr 1068) and cleaved PARP antibody.

drugs. These results suggest that this regimen is intensive but can be tolerated, at least in mice.

The *in vitro* and *in vivo* experiments in our study demonstrated the synergistic potential of gefitinib – CPT-11 combination. We previously reported that topoisomerase I up-regulation by counter-part drugs was a possible mechanism for the synergy in an CPT-11 containing regimen.[23] On the other hand, the synergistic potential of gefitinib with topotecan, cisplatin, paclitaxel or radiation has been reported.[18,24–28] To elucidate the biochemical mechanism underlying the synergistic interaction between the gefitinib and CPT-11, the effect of CPT-11 on EGFR-phosphorylation was examined (Fig. 5). Increased phosphorylation of EGFR was observed after exposure to CPT-11 in dose and time-dependent manner in WiDR and Lovo cells. Since EGFR expression and phosphorylation were the major determining factors for sensitivity of the cells to gefitinib-induced growth-inhibition,[14] biochemical modulation of EGFR by CPT-11 might be responsible for the synergistic interaction between gefitinib and CPT-11. EGFR is induced and activated by cellular stress, such as oxidative stress and UV irradiation.[29–34] Ohmori *et al.*[22] demonstrated that increased autophosphorylation of EGFR was obtained in cisplatin-exposure in human lung cancer cells. A number of reports suggest that EGFR promotes cell survival through the activation of the ERK or the AKT pathway.[31,32] EGFR activation induced by these cellular stress may play a survival response against apoptosis.[31,32] In the present study, PARP activation by gefitinib was markedly enhanced by combination with CPT-11 at 5 μM exposure, which is comparable with IC_{50} value of CPT-11 in Lovo cells, although no PARP activation was observed by monotherapy of CPT-11. On the other hand, gefitinib does not modify the expression and the activation of topoisoerase I (data not shown). These result suggest that the inhibitory effect of gefitinib on the activated survival signal transduction induced by CPT-11 lead to synergistic effect. The findings of the present study suggest that biological modulation by various anticancer agents including DNA damaging agents will contribute to the synergistic effects of these anticancer agents and gefitinib in EGFR expressing tumor and support clinical evaluation of gefitinib in combination with DNA-targeting agents, especially CPT-11, in the treatment of colorectal cancers.

REFERENCES

1. Blijham G, Wagener T, Wils J, de Greve J, Buset M, Bleiberg H, Lacave A, Dalmark M, Selleslag J, Collette L, Sahmoud T. Modulation of high-dose infusional fluorouracil by low-dose methotrexate in patients with advanced or metastatic colorectal cancer: final results of a randomized European Organization for Research and Treatment of Cancer Study. J Clin Oncol 1996;14:2266–73.
2. O'Connell MJ, Klaassen DJ, Everson LK, Cullinan S, Wieand HS. Clinical studies of biochemical modulation of 5-fluorouracil by leucovorin in patients with advanced colorectal cancer by the North Central Cancer Treatment Group and Mayo Clinic. NCI Monogr 1987;185–8.
3. Focan C, Kreutz F, Focan-Henrard D, Moeneclaey N. Chronotherapy with 5-fluorouracil, folinic acid and carboplatin for metastatic colorectal cancer; an interesting therapeutic index in a phase II trial. Eur J Cancer 2000;36:341–7.
4. Shimada Y, Yoshino M, Wakui A, Nakao I, Futatsuki K, Sakata Y, Kambe M, Taguchi T, Ogawa N. Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. CPT-11 Gastrointestinal Cancer Study Group. J Clin Oncol 1993;11:909–13.
5. Cunningham D, Pyrhonen S, James RD, Punt CJ, Hickish TF, Heikkila R, Johannesen TB, Starkhammar H, Topham CA, Awad L, Jacques C, Herait P. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. Lancet 1998;352:1413–8.
6. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL, Miller LL. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer: Irinotecan Study Group. N Engl J Med 2000;343:905–14.
7. Rothenberg ML, Meropol NJ, Poplin EA, Van Cutsem E, Wadler S. Mortality associated with irinotecan plus bolus fluorouracil/leucovorin: summary findings of an independent panel. J Clin Oncol 2001;19:3801–7.
8. Modi S, Seidman AD. An update on epidermal growth factor receptor inhibitors. Curr Oncol Rep 2002;4:47–55.
9. Saijo N, Tamura T, Nishio K. Problems in the development of target-based drugs. Cancer Chemother Pharmacol 2000;46:S43–5.
10. Baselga J. The EGFR as a target for anticancer therapy: focus on cetuximab. Eur J Cancer 2001;37:S16–22.
11. Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001;37:S9–15.
12. Speer G, Cseh K, Winkler G, Takacs I, Barna I, Nagy Z, Lakatos P. Oestrogen and vitamin D receptor (VDR) genotypes and the expression of ErbB-2 and EGF receptor in human rectal cancers. Eur J Cancer 2001;37:1463–8.
13. Albanell J, Rojo F, Baselga J. Pharmacodynamic studies with the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839. Semin Oncol 2001;5:56–66.
14. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. Oncogene 2000;19:6550–65.
15. Woodburn JR, Morris CQ, Kelly H. EGF receptor tyrosine kinase inhibitors as anti-cancer agents-preclinical and early clinical profile of ZD1839. Cell Mol Biol Lett 1998;3:348–9.
16. Naruse I, Ohmori T, Ao Y, Fukumoto H, Kuroki T, Mori M, Saijo N, Nishio K. Antitumor activity of the selective epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) Iressa™ (ZD1839) in a EGFR-expressing multidrug resistant cell line in vitro and in vivo. Int J Cancer 2002;98:310–5.
17. Ciardiello F, Bianco R, Damiano V, De Lorenzo S, Pepe S, De Placido S, Fan Z, Mendelsohn J, Bianco AR, Tortora G. Antitumor activity of sequential treatment with topotecan and anti-epidermal

growth factor receptor monoclonal antibody C225. Clin Cancer Res 1999;5:909–16.
18. Ciardiello F, Caputo R, Bianco R, Damiano V, Pomatico G, De Placido S, Bianco A.R, Tortora G. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. Clin Cancer Res 2000;6:2053–63.
19. Sirotnak FM, Zakowski MF, Miller VA, Scher HI, Kris MG. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. Clin Cancer Res 2000;6:4885–92.
20. Slichenmyer WJ, Fry DW. Anticancer therapy targeting the erbB family of receptor tyrosine kinases. Semin Oncol 2001;28:67–79.
21. Kusaba H, Tamura T, Nakagawa K, Yamamoto N, Kudoh S, Negoro S, Takeda K, Tanigawara Y, Fukuoka M. A phase I intermittent dose-escalation trial of ZD1839 ('IRESSA') in Japanese patients with solid malignant tumors. Clin Cancer Res 2000;6:abs381.
22. Kris M, Ranson M, Ferry D, Hammond L, Averbuch S, Ochs J, Rowinsky E. Phase I study of oral ZD1839('IRESSA'): A novel inhibitor of epidermal growth factor receptor tyrosine kinase (EGFR-TKI)—Evidence of good tolerability and activity. Clin Cancer Res 1999;5:abs 99
23. Kanzawa F, Koizumi F, Koh Y, Nakamura T, Tatsumi Y, Fukumoto H, Saijo N, Yoshioka T, Nishio K. In vitro synergistic interactions between the cisplatin analogue nedaplatin and the DNA topoisomerase I inhibitor irinotecan and the mechanism of this interaction. Clin Cancer Res 2001;7:202–9.
24. Ohmori T, Ao Y, Nishio K, Saijo N, Arteaga CL, Kuroki T. Low dose cisplatin can modulate the sensitivity of human non-small cell lung carcinoma cells to EGFR tyrosine kinase inhibitor (ZD1839; 'Iressa') in vivo. Proc Am Assoc Cancer Res 2000;41:abs 3072.
25. Magne N, Fischel JL, Dubreuil A, Formento P, Marcie S, Lagrange JL, Milano G. Sequence-dependent effects of ZD1839 ('Iressa') in combination with cytotoxic treatment in human head and neck cancer. Br J Cancer 2002;86: 819–27.
26. Raben D, Helfrich BA, Chan D, Johnson G, Bunn PA Jr. ZD1839, a selective epidermal growth factor receptor tyrosine kinase inhibitor, alone and in combination with radiation and chemotherapy as a new therapeutic strategy in non-small cell lung cancer. Semin Oncol 2002; 29:37–46.
27. Sirotnak FM, Zakowski MF, Miller VA, Scher HI, Kris MG. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. Clin Cancer Res 2000;6:4885–92.
28. Williams KJ, Telfer BA, Stratford IJ, Wedge SR. ZD1839 ('Iressa'), a specific oral epidermal growth factor receptor-tyrosine kinase inhibitor, potentiates radiotherapy in a human colorectal cancer xenograft model. Br J Cancer 2002;86:1157–61.
29. Goldshmit Y, Erlich S, Pinkas-Kramarski R. Neuregulin rescues PC12-ErbB4 cells from cell death induced by H_2O_2. Regulation of reactive oxygen species levels by phosphatidylinositol 3-kinase. J Biol Chem 2001;276:46379–85.
30. Meves A, Stock SN, Beyerle A, Pittelkow MR, Peus D. H_2O_2 mediates oxidative stress-induced epidermal growth factor receptor phosphorylation. Toxicol Lett 2001;122:205–14.
31. Miyazaki Y, Hiraoka S, Tsutsui S, Kitamura S, Shinomura Y, Matsuzawa Y. Epidermal growth factor receptor mediates stress-induced expression of its ligands in rat gastric epithelial cells. Gastroenterology 2001;120:108–16.
32. Wang X, McCullough KD, Franke TF, Holbrook NJ. Epidermal growth factor receptor-dependent Akt activation by oxidative stress enhances cell survival. J Biol Chem 2000;275:14624–31.
33. Benhar M, Engelberg D, Levitzki A. Cisplatin-induced activation of the EGF receptor. Oncogene 2002;21:8723–31.
34. Kitagawa D, Tanemura S, Ohata S, Shimizu N, Seo J, Nishitai G, Watanabe T, Nakagawa K, Kishimoto H, Wada T, Tezuka T, Yamamoto T, et al. Activation of extracellular signal-regulated kinase by ultraviolet is mediated through Src-dependent epidermal growth factor receptor phosphorylation: its implication in an anti-apoptotic function. J Biol Chem 2002;277:366–71.

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ORIGINAL REPORT

Randomized Pharmacokinetic and Pharmacodynamic Study of Docetaxel: Dosing Based on Body-Surface Area Compared With Individualized Dosing Based on Cytochrome P450 Activity Estimated Using a Urinary Metabolite of Exogenous Cortisol

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose
Docetaxel is metabolized by cytochrome P450 (CYP3A4) enzyme, and the area under the concentration-time curve (AUC) is correlated with neutropenia. We developed a novel method for estimating the interpatient variability of CYP3A4 activity by the urinary metabolite of exogenous cortisol (6-beta-hydroxycortisol [6-β-OHF]). This study was designed to assess whether the application of our method to individualized dosing could decrease pharmacokinetic (PK) and pharmacodynamic (PD) variability compared with body-surface area (BSA) –based dosing.

Patients and Methods
Fifty-nine patients with advanced non–small-cell lung cancer were randomly assigned to either the BSA-based arm or individualized arm. In the BSA-based arm, 60 mg/m^2 of docetaxel was administered. In the individualized arm, individualized doses of docetaxel were calculated from the estimated clearance (estimated clearance = 31.177 + [7.655 × 10^{-4} × total 6-β-OHF] − [4.02 × alpha-1 acid glycoprotein] − [0.172 × AST] − [0.125 × age]) and the target AUC of 2.66 mg/L · h.

Results
In the individualized arm, individualized doses of docetaxel ranged from 37.4 to 76.4 mg/m^2 (mean, 58.1 mg/m^2). The mean AUC and standard deviation (SD) were 2.71 (range, 2.02 to 3.40 mg/L · h) and 0.40 mg/L · h in the BSA-based arm, and 2.64 (range, 2.15 to 3.07 mg/L · h) and 0.22 mg/L · h in the individualized arm, respectively. The SD of the AUC was significantly smaller in the individualized arm than in the BSA-based arm ($P < .01$). The percentage decrease in absolute neutrophil count (ANC) averaged 87.1% (range, 59.0 to 97.7%; SD, 8.7) in the BSA-based arm, and 87.4% (range, 78.0 to 97.2%; SD, 6.1) in the individualized arm, suggesting that the interpatient variability in percent decrease in ANC was slightly smaller in the individualized arm.

Conclusion
The individualized dosing method based on the total amount of urinary 6-β-OHF after cortisol administration can decrease PK variability of docetaxel.

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INTRODUCTION

Many cytotoxic drugs have narrow therapeutic windows despite having a large interpatient pharmacokinetic (PK) variability. The doses of these cytotoxic drugs are usually calculated on the basis of body-surface area (BSA). Although several physiologic functions are proportional to BSA, systemic exposure to a drug is only partially related to

this parameter.[1-3] Consequently, a large interpatient PK variability is seen when doses are based on BSA. This large interpatient PK variability can result in undertreatment with inappropriate therapeutic effects in some patients, or in overtreatment with unacceptable severe toxicities in others. Understanding interpatient PK variability is important for optimizing anticancer treatments. Factors that affect PK variability include drug absorption, metabolism, and excretion. Among these factors, drug metabolism is regarded as a major factor causing PK variability. Unfortunately, however, no simple and practical method for estimating the interpatient variability of drug metabolism is available. If drug metabolism in each patient could be predicted, individualized dosing could be performed to optimize drug exposure while minimizing unacceptable toxicity.

Docetaxel is a cytotoxic agent that promotes microtubule assembly and inhibits depolymerization to free tubulin, resulting in the blockage of the M phase of the cell cycle.[4] Docetaxel has shown promising activity against several malignancies, including non–small-cell lung cancer, and is metabolized by hepatic CYP3A4 enzyme.[5-15]

Human CYP3A4 is a major cytochrome P450 enzyme that is present abundantly in human liver microsomes and is involved in the metabolism of a large number of drugs, including anticancer drugs.[16-18] This enzyme exhibits a remarkable interpatient variation in activity as high as 20-fold, which accounts for the large interpatient differences in the disposition of drugs that are metabolized by this enzyme.[19-22] Several noninvasive in vivo probes for estimating the interpatient variability of CYP3A4 activity have been reported and include the erythromycin breath test, the urinary dapsone recovery test, measurement of midazolam clearance (CL), and measurement of the ratio of endogenous urinary 6-beta-hydroxycortisol (6-β-OHF) to free-cortisol (FC).[23-27] The erythromycin breath test and the measurement of midazolam CL are the best validated, and both have been shown to predict docetaxel CL in patients.[28,29] However, neither probe has been used in a prospective study to validate the correlations observed, or to test their utility in guiding individualized dosing.

We developed a novel method for estimating the interpatient variability of CYP3A4 activity by urinary metabolite of exogenous cortisol. The total amount of 24-hour urinary 6-β-OHF after cortisol administration (total 6-β-OHF) is significantly correlated with docetaxel CL, which is metabolized by the CYP3A4 enzyme. We also illustrate the possibility that individualized dosing to optimize drug exposure and decrease interpatient PK variability could be performed using this method.[30]

We conducted a prospective, randomized PK and pharmacodynamic (PD) study of docetaxel comparing BSA-based dosing and individualized dosing based on the interpatient variability of CYP3A4 activity, as estimated by a urinary metabolite of exogenous cortisol. The objective of this study was to assess whether the application of our method to individualized dosing could decrease PK and PD variability of docetaxel compared with BSA-based dosing.

PATIENTS AND METHODS

Patient Selection

Patients with histologically or cytologically documented advanced or metastatic non–small-cell lung cancer were eligible for this study. Other eligibility criteria included the following: age $\geq$ 20 years; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; 4 weeks of rest since any previous anticancer therapy; and adequate bone marrow (absolute neutrophil count [ANC] $\geq$ 2,000/μL and platelet count $\geq$ 100,000/μL), renal (serum creatinine level $\leq$ 1.5 mg/dL), and hepatic (serum total bilirubin level $\leq$ 1.5 mg/dL, AST level $\leq$ 150 U/L, and ALT level $\leq$ 150 U/L) function. Written informed consent was obtained from all patients before enrollment onto the study.

The exclusion criteria included the following: pregnancy or lactation; concomitant radiotherapy for primary or metastatic sites; concomitant chemotherapy with any other anticancer agents; treatment with steroids or any other drugs known to induce or inhibit CYP3A4 enzyme[17]; serious pre-existing medical conditions, such as uncontrolled infections, severe heart disease, diabetes, or pleural or pericardial effusions requiring drainage; and a known history of hypersensitivity to polysorbate 80. This study was approved by the institutional review board of the National Cancer Center.

Pretreatment and Follow-Up Evaluation

On enrollment onto the study, a history and physical examination were performed, and a complete differential blood cell count (including WBC count, ANC, hemoglobin, and platelets), and a clinical chemistry analysis (including serum total protein, albumin [ALB], bilirubin, creatinine, AST, ALT, gamma-glutamyltransferase, alkaline phosphatase [ALP], and alpha-1 acid glycoprotein [AAG]) were performed. Blood cell counts and a chemistry analysis except for AAG were performed at least twice a week throughout the study. Tumor measurements were performed every two cycles, and antitumor response was assessed by WHO standard response criteria. Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

Study Design

This study was designed to assess whether the application of our method to individualized dosing could decrease PK and PD variability compared with BSA-based dosing. The primary end point was PK variability and the secondary end point was PD variability (ie, toxicity). In our previous study involving 29 patients who received 60 mg/m^2 of docetaxel, the area under the concentration-time curve (AUC) was calculated to be 2.66 $\pm$ 0.91 (mean $\pm$ standard deviation [SD]) mg/L $\cdot$ h.[30] We assumed that the variability of AUC, represented by the SD, could be reduced by 50% in the individualized arm compared with that in the BSA-based arm, and that AUC would be normally distributed. The required sample size was 25 patients per arm to detect this difference with a two-sided F test at α = .05 and a power of 0.914.

Patients were randomly assigned to either the BSA-based arm or individualized arm (Fig 1). In the BSA-based arm, each patient received a dose of 60 mg/m^2 of docetaxel. In the individualized arm, individualized doses of docetaxel were calculated from the estimated docetaxel CL after cortisol administration and the target AUC (described in the Docetaxel Administration section).

Cortisol Administration and Urine Collection

In the individualized arm, 300 mg of hydrocortisone (Banyu Pharmaceuticals Co, Tokyo, Japan) was diluted in 100 mL of 0.9%

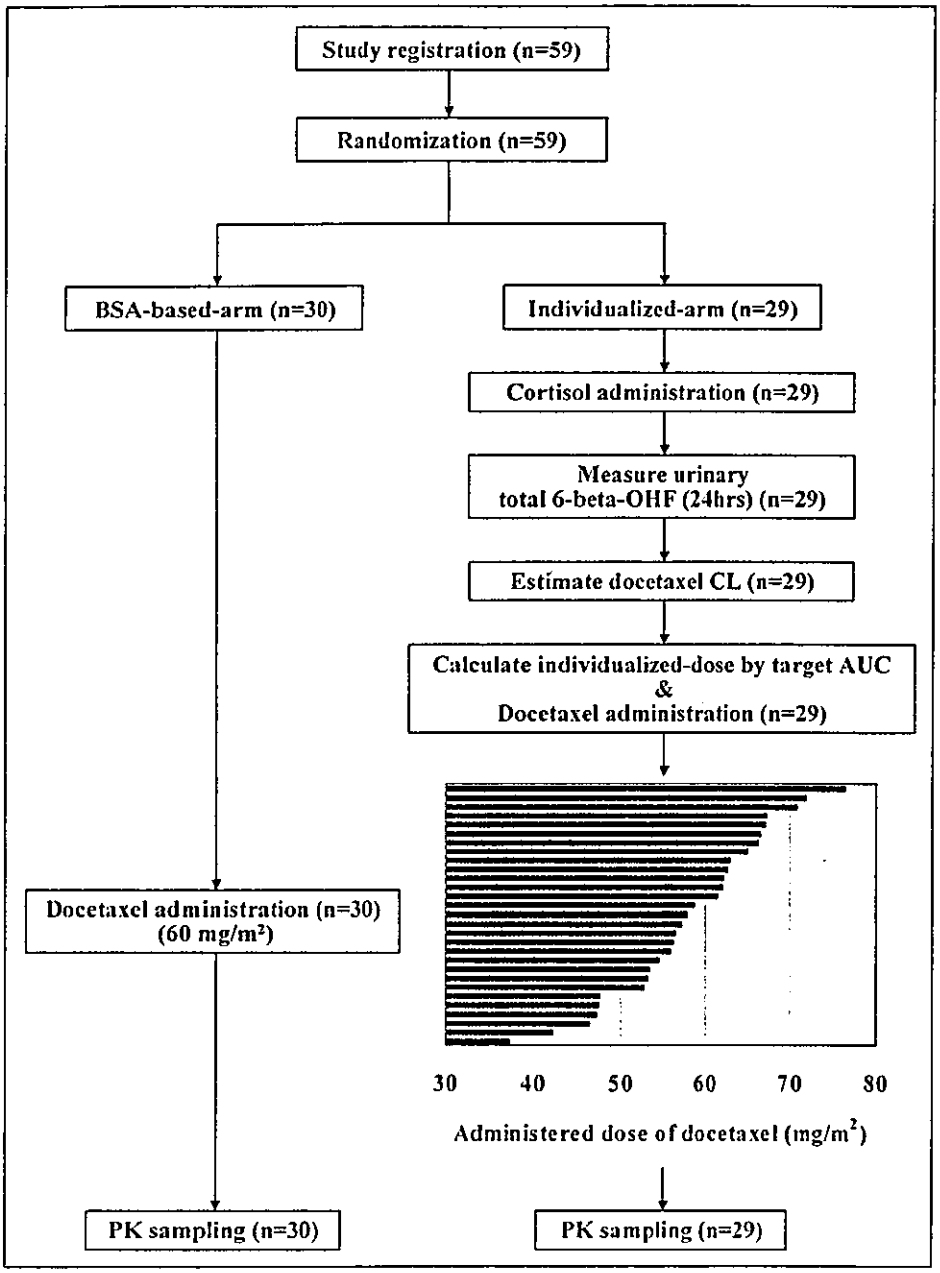

Fig 1. Study flow diagram and administered dose of docetaxel. PK, pharmacokinetic; AUC, area under the concentration-time curve; CL, clearance; 6-β-OHF, 6-beta-hydroxycortisol.

saline and administered intravenously for 30 minutes at 9 AM on day 1 in all patients to estimate the interpatient variability of CYP3A4 activity. After cortisol administration, the urine was collected for 24 hours. The total volume of the 24-hour collection was recorded, and a 5-mL aliquot was analyzed immediately.

Docetaxel Administration

Docetaxel (Taxotere; Aventis Pharm Ltd, Tokyo, Japan) was obtained commercially as a concentrated sterile solution containing 80 mg of the drug in 2 mL of polysorbate 80. In the BSA-based arm, a dose of 60 mg/m^2 of docetaxel was diluted in 250 mL of 5% glucose or 0.9% saline and administered by 1-hour intravenous infusion at 9 AM to all patients.

In the individualized arm, individualized dose of docetaxel was calculated from the estimated CL and the target AUC of 2.66 mg/L · h using the following equations:

$$\text{Estimated CL (L/h/m}^2) = 31.177 + (7.655 \times 10^{-4} \times \text{total-6-}\beta\text{-OHF [}\mu\text{g/d]}) - (4.02 \times \text{AAG [g/L]}) - (0.172 \times \text{AST [U/L]}) - (0.125 \times \text{age [years]})^{30}$$

$$\text{Individualized dose of docetaxel (mg/m}^2) = \text{estimated docetaxel CL (L/h/m}^2) \times \text{target AUC (2.66 mg/L} \cdot \text{h)}$$

At least 2 days after cortisol administration, individualized doses of docetaxel were diluted in 250 mL of 5% glucose or 0.9% saline and administered by 1-hour intravenous infusion at 9 AM to each patient. The doses of docetaxel in subsequent cycles of treatment were unchanged, and no prophylactic premedication to protect against docetaxel-related hypersensitivity reactions was administered in either of the treatment arms.

PK Study

Blood samples for PK studies were obtained from all of the patients during the initial treatment cycle. An indwelling cannula was inserted in the arm opposite that used for the drug infusion, and blood samples were collected into heparinized tubes. Blood samples were collected before the infusion; 30 minutes after the start of the infusion; at the end of the infusion; and 15, 30, and 60 minutes and 3, 5, 9, and 24 hours after the end of the infusion. All blood samples were centrifuged immediately at 4,000 rpm for 10 minutes, after which the plasma was removed and the samples were placed in polypropylene tubes, labeled, and stored at −20°C or colder until analysis.

PK parameters were estimated by the nonlinear least squares regression analysis method (WinNonlin, Version 1.5; Bellkey Science Inc, Chiba, Japan) with a weighting factor of 1 per year.[2] Individual plasma concentration-time data were fitted to two- and three-compartment PK models using a zero-order infusion input and first-order elimination. The model was chosen on the basis of Akaike's information criteria.[31] The peak plasma concentration (C_{max}) was generated directly from the experimental data. AUC was extrapolated to infinity and determined based on the best-fitted curve; this measurement was then used to calculate the absolute CL (L/h), defined as the ratio of the delivered dosage (in milligrams) and AUC.

To assess PD effect of docetaxel, the percentage decrease in ANC was calculated according to the following formula: % decrease in ANC = (pretreatment ANC − nadir ANC)/(pretreatment ANC) × 100.

Measurements

The concentration of urinary 6-β-OHF was measured by reversed phase high-performance liquid chromatography with UV absorbance detection according to previously published methods.[30,32,33]

Docetaxel concentrations in plasma were also measured by solid-phase extraction and reversed phase high-performance liquid chromatography with UV detection according to the previously published method.[30,34] The detection limit corresponded to a concentration of 10 ng/mL.

Statistical Analysis

Fisher's exact test or χ^2 test was used to compare categoric data, and Student's t test was used for continuous variables. The strength of the relationship between the estimated docetaxel CL and the observed docetaxel CL was assessed by least squares linear regression analysis. The interpatient variability of AUC for each arm was evaluated by determining the SD and was compared by F test. Biases, or the mean AUC value in each arm minus the target AUC (2.66 mg/L · h), were also compared between the arms by Student's t test.

A two-sided P value of ≤ .05 or less was considered to indicate statistical significance. All statistical analyses were performed using SAS software version 8.02 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

Between October 1999 and May 2001, 59 patients were enrolled onto the study and randomly assigned to either the BSA-based arm (n = 30) or the individualized arm (n = 29). All 59 patients were assessable for PK and PD analyses. The pretreatment characteristics of the 59 patients are listed in Table 1. The baseline characteristics were well balanced between the arms except for three laboratory parameters: ALB, AAG, and ALP. These three parameters were not included in the eligibility criteria. The majority of patients (95%) had a performance status of 0 or 1. Twenty (67%) and 16 (55%) patients had been treated with platinum-based chemotherapy in the BSA-based arm and individualized arm, respectively. Only two patients in the individualized arm had liver metastasis, and most of the patients had good hepatic functions.

Individualized Dosing of Docetaxel

In the individualized arm, the total amount of 24-hour urinary 6-β-OHF after cortisol administration (total 6-β-OHF) was 9,179.6 ± 3,057.7 μg/d (mean ± SD), which was similar to the result of our previous study.[30] The estimated docetaxel CL was 21.9 ± 3.5 L/h/m^2 (mean ± SD), and individualized dose of docetaxel ranged from 37.4 to 76.4 mg/m^2 (mean, 58.1 mg/m^2; Fig 1).

PK

Docetaxel PK data were obtained from all 59 patients during the first cycle of therapy, and PK parameters are listed in Table 2. Drug levels declined rapidly after infusion and could be determined to a maximum of 25 hours. The concentration of docetaxel in plasma was fitted to a biexponential equation, which was consistent with previous reports.[30,35-38] The mean alpha and beta half-lives were 9.2 minutes and 5.0 hours in the BSA-based arm and 9.2 minutes and 7.4 hours in the individualized arm, respectively.

In the BSA-based arm, docetaxel CL was 22.6 ± 3.4 L/h/m^2 (mean ± SD), and AUC averaged 2.71 mg/L · h (range, 2.02 to 3.40 mg/L · h). In the individualized arm, docetaxel CL was 22.1 ± 3.4 L/h/m^2, and AUC averaged 2.64 mg/L · h (range, 2.15 to 3.07 mg/L · h). The least squares linear regression analysis showed that the observed docetaxel CL was well estimated in the individualized arm (r^2 = 0.821; Fig 2).

The SDs of AUC in the BSA-based arm and in the individualized arm were 0.40 and 0.22, respectively, and the ratio of SD in the individualized arm to that in the BSA-based arm was 0.538 (95% CI, 0.369 to 0.782). The biases from the target AUC in the BSA-based arm and in the individualized arm were 0.047 (95% CI, −0.104 to 0.198) and −0.019 (95% CI, −0.102 to 0.064), respectively, with no significant difference. The interpatient variability of

Table 1. Patient Characteristics

Characteristic	BSA-Based Arm No. of Patients	%	Individualized Arm No. of Patients	%	P
Enrolled	30		29		
Eligible	30	100	29	100	
Age, years					.62
Median	61		62		
Range	52-73		45-73		
Sex					
Male	25	83	19	66	.14
Female	5	17	10	34	
ECOG PS					
0	7	23	1	3	.08
1	22	73	26	90	
2	1	3	2	7	
Prior treatment					
None	4	13	4	14	.99
Surgery	11	37	9	31	.65
Radiotherapy	13	43	10	34	.49
Chemotherapy	21	70	18	62	.52
Platinum-based regimens	20	67	16	55	.37
Site of disease					
Lung	23	77	28	97	.10
Liver	0	0	2	7	.24
Pleura	8	27	12	41	.23
Bone	7	23	9	31	.71
Extrathoracic lymph nodes	0	33	10	34	.93
Laboratory parameters					
ALB, g/L					.02
Median	38		35		
Range	26-45		24-44		
AAG, g/L					.04
Median	1.00		1.25		
Range	0.28-2.15		0.64-2.54		
AST, U/L					.67
Median	21		22		
Range	10-40		7-41		
ALT, U/L					.88
Median	18		18		
Range	6-54		4-45		
ALP, U/L					.03
Median	249		324		
Range	129-540		185-986		

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status, ALB, serum albumin; AAG, alpha-1-acid glycoprotein; ALP, serum alkaline phosphatase.

AUC was significantly smaller in the individualized arm than in the BSA-based arm ($P < .01$; Fig 3).

PD

In both arms, neutropenia was the predominant toxicity related to docetaxel treatment, and 28 of 30 (93%) patients in the BSA-based arm and 25 of 29 (86%) patients in the individualized arm had grade 3 or 4 neutropenia.

Table 2. Docetaxel PK Parameters

Parameters	BSA-Based Arm (n = 30)	Individualized Arm (n = 29)
C_{max}, μg/mL	0.36-2.70	0.99-2.41
$t_{1/2}$ alpha*, minutes	9.2 ± 3.3	9.2 ± 2.7
$t_{1/2}$ beta*, hours	5.0 ± 4.8	7.4 ± 11.7
CL* L/h	37.6 ± 6.3	34.8 ± 7.1
CL* L/h/m²	22.6 ± 3.4	22.1 ± 3.4
AUC		
Mean mg/L · h	2.71	2.64
Range mg/L · h	2.02-3.40	2.15-3.07
Median	2.65	2.66
SD	0.40	0.22

Abbreviations: PK, pharmacokinetic; BSA, body-surface area; CL, clearance; AUC, area under concentration-time curve; SD, standard deviation.
*Data represent mean ± SD.

Nonhematologic toxicities, such as gastrointestinal and hepatic toxicities (ie, hyperbilirubinemia, aminotransferase elevations), were mild in both arms.

PD effects shown as the percentage decrease in ANC are listed in Table 3. The percentage decrease in ANC for the BSA-based arm and individualized arm were 87.1% (range, 59.0 to 97.7%; SD, 8.7) and 87.5% (range, 78.0 to 97.2%; SD, 6.1), respectively, suggesting that the interpatient variability in the percentage decrease in ANC was slightly smaller in the individualized arm than in the BSA-based arm (Fig 4). The response rates between the two arms were similar; five of 30 (16.7%) and four of 29 (13.8%) patients

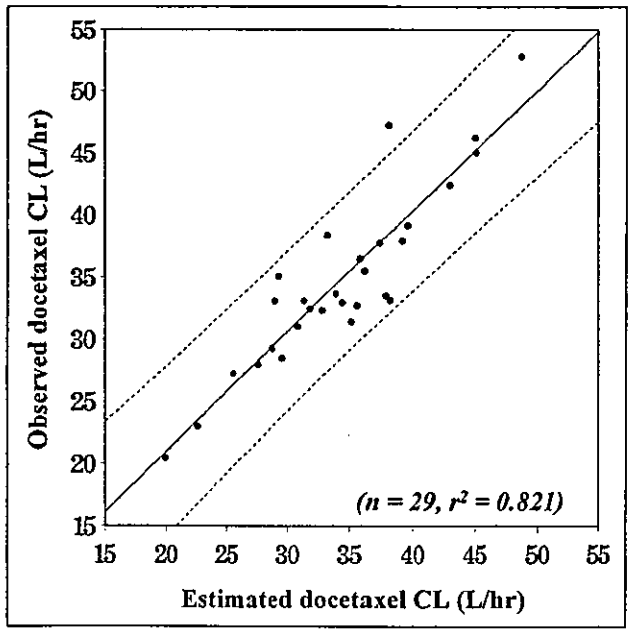

Fig 2. Correlation between the estimated and observed docetaxel clearance (CL) in the individualized arm (n = 29). (—) Linear regression line (r^2 = 0.821); (- - - -) 95% CIs for individual estimates.

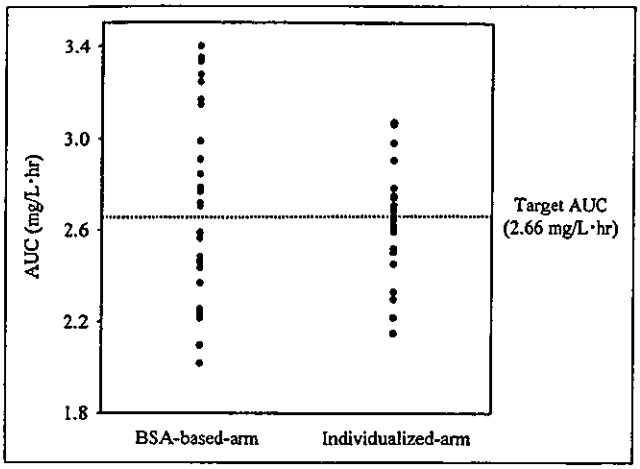

Fig 3. Comparison of area under the concentration-time curve (AUC) variability between the arms ($P < .01$; F test). BSA, body-surface area.

achieved a partial response in the BSA-based arm and individualized arm, respectively.

DISCUSSION

In oncology practice, the prescribed dose of most anticancer drugs is currently calculated from BSA of individual patients to reduce the interpatient variability of drug exposure. However, PK parameters, such as CL of many anticancer drugs, are not related to BSA.[2,39-43] Although PK parameters of docetaxel are correlated with BSA, individualized dosing based on individual metabolic capacities could further decrease the interpatient variability.[43]

CYP3A4 plays an important role in the metabolism of many drugs, including anticancer agents such as docetaxel, paclitaxel, vinorelbine, and gefitinib. This enzyme exhibits a large interpatient variability in metabolic activity, accounting for the large interpatient PK and PD variability. We have developed a novel method of estimating the interpatient variability of CYP3A4 activity by urinary metabolite of exogenous cortisol. That is, the total amount of 24-hour urinary 6-β-OHF after cortisol administration was highly correlated with docetaxel CL. We conducted a prospective randomized PK and PD study of docetaxel to evaluate whether the application of our method to individualized dosing could decrease PK and PD variability compared with BSA-based dosing.

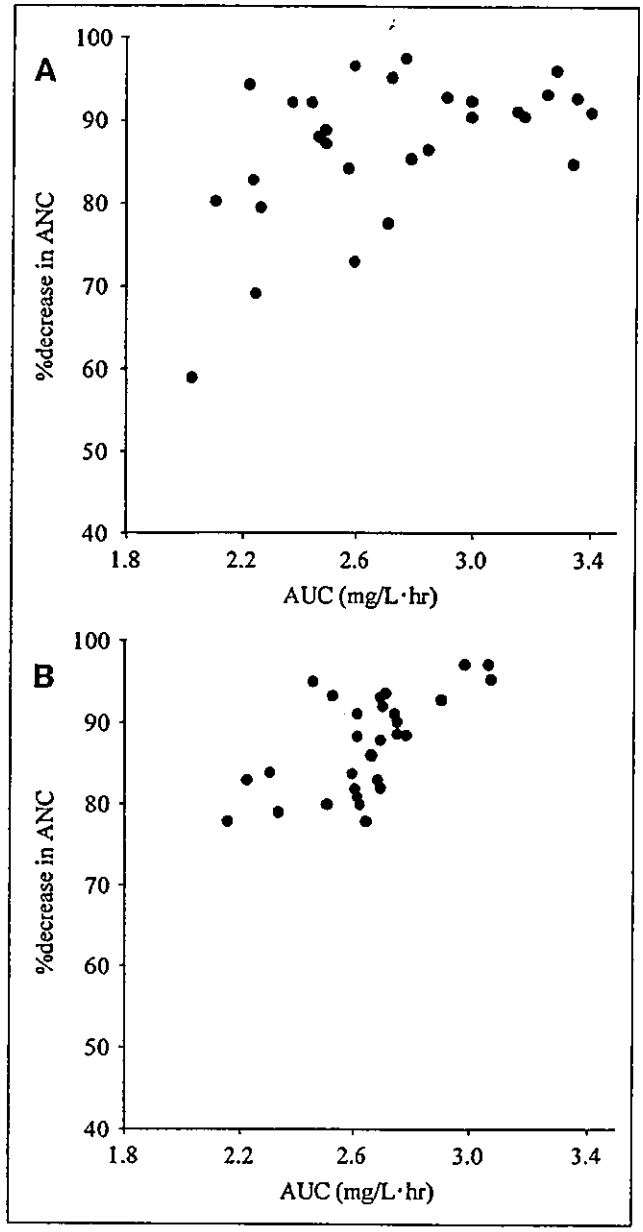

Fig 4. Correlation between area under the concentration-time curve (AUC) and percentage decrease in absolute neutrophil count (ANC) in each arm. (A) body-surface area–based arm; (B) individualized arm.

Table 3. Percentage Decrease in ANC

Parameters	BSA-Based Arm (n = 30)	Individualized Arm (n = 29)
Percentage decrease in ANC, %		
Mean	87.1	87.4
Range	59.0-97.7	78.0-97.2
Median	89.7	88.4
SD	8.7	8.1

Abbreviations: ANC, absolute neutrophil count; BSA, body-surface area; SD, standard deviation.

The study by Hirth et al[28] showed a good correlation between the result of the erythromycin breath test and docetaxel CL, and the study by Goh et al[29] showed a good correlation between the midazolam CL and docetaxel CL. In our study, we prospectively validated the correlation between docetaxel CL and our previously published method using the total amount of urinary 6-β-OHF after

cortisol administration in the individualized arm. As shown in Fig 2, the observed docetaxel CL was well estimated, and the equation for the estimation of docetaxel CL developed in our previous study was found to be reliable and reproducible. The target AUC in the individualized arm was set at 2.66 mg/L · h. This value was the mean value from our previous study, in which 29 patients were treated with 60 mg/m^2 of docetaxel. Individualized doses of docetaxel ranged from 37.4 to 76.4 mg/m^2 and were lower than expected.

The SD of AUC in the individualized arm was about 46.2% smaller than that in the BSA-based arm, a significant difference; this result seems to indicate that the application of our method to individualized dosing can reduce the interpatient PK variability. Assuming that the variability of AUC could be decreased 46.2% by individualized dosing applying our method, overtreatment could be avoided in 14.5% of BSA-dosed patients by using individualized dosing (Fig 5, area A), and undertreatment could be avoided in another 14.5% of these patients (Fig 5, area B). We considered that neutropenia could be decreased with patients in area A by individualized dosing. However, it is unknown whether the therapeutic effect of docetaxel could be improved in the patients in area B by individualized dosing because no significant positive correlation has been found between docetaxel AUC and antitumor response in patients with non–small-cell lung cancer.[43] In this study, seven of 30 (23.3%) and two of 30 (6.7%) patients in the BSA-based arm were included in area A and B, respectively (Figs 3 and 5).

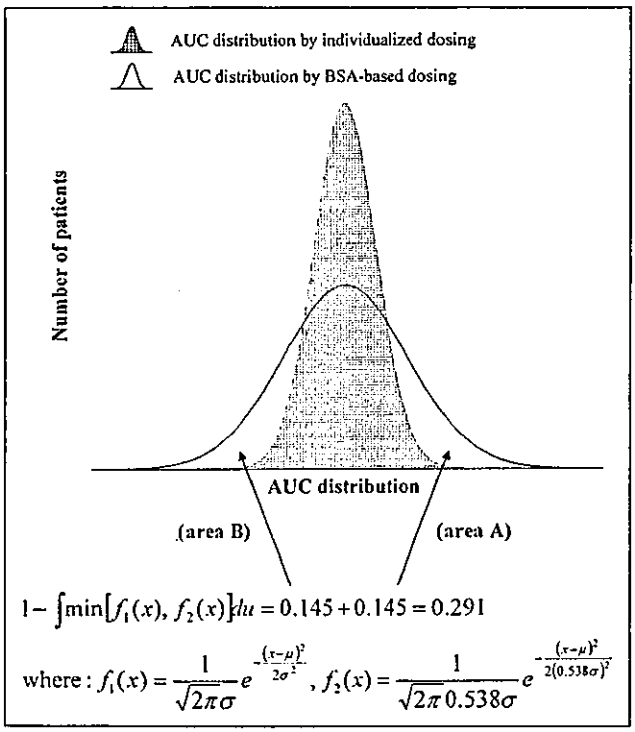

Fig 5. Simulated comparison of area under the concentration-time curve (AUC) distribution between body-surface area (BSA) –based dosing and individualized dosing when the variability of AUC is decreased 46.2% by individualized dosing applied using our method.

As shown in Figure 4, the percentage decrease in ANC was well correlated with AUC in both arms, which was similar to previous reports.[37,43] It was also indicated that the interpatient variability in the percentage decrease in ANC was slightly smaller in the individualized arm than in the BSA-based arm; however, this difference was not significant. The response rates between the two arms were similar. Although the interpatient PK variability could be decreased by individualized dosing in accordance with our method, the interpatient PD variability such as toxicity and the antitumor response could not be decreased. Several reasons could be considered.

With regard to toxicity, the pretreatment characteristics of the patients in this study were highly variable. More than half of the patients in each arm had previously received platinum-based chemotherapy, and more than 30% had received radiotherapy. The laboratory parameters (ie, ALB, AAG, and ALP) were not balanced across the arms, although they were not included in the eligibility criteria (Table 1). These variable pretreatment characteristics and unbalanced laboratory parameters may have influenced the frequency and severity of the hematologic toxicity as well as the pharmacokinetic profiles. The antitumor effect may have been influenced by the intrinsic sensitivity of tumors, the variable pretreatment characteristics, and the imbalance in laboratory parameters. Non–small-cell lung cancer is a chemotherapy-resistant tumor. The response rate for docetaxel ranges from 18% to 38%,[5] and no significant positive correlation between docetaxel AUC and antitumor response has been found. We considered it quite difficult to control the interpatient PD variability by controlling the interpatient PK variability alone. Although we did not observe any outliers in either arm, such as the two outliers with severe toxicity observed in the study by Hirth et al,[28] our method may be more useful for identifying such outliers. If we had not excluded patients with more abnormal liver function or a history of liver disease by the strict eligibility criteria, the results with the two dosing regimens may have been more different, and the interpatient PD variability, such as the percentage decrease in ANC, may have been smaller in the individualized arm than in the BSA-based arm. Furthermore, the primary end point of this study was PK variability, evaluated by the SD of AUC in both arms, and the sample size was significantly underpowered to evaluate whether the application of our method to individualized dosing could decrease PD variability compared with BSA-based dosing.

For the genotypes of CYP3A4, several genetic polymorphisms have been reported (http://www.imm.ki.se/CYPalleles/); however, a clear relationship between genetic polymorphisms and the enzyme activity of CYP3A4 has not been reported. Our phenotype-based

individualized dosing using the total amount of urinary 6-β-OHF after cortisol administration produced good results. However, this method is somewhat complicated, and a simpler method would be of great use. We analyzed the expression of CYP3A4 mRNA in the peripheral-blood mononuclear cells of the 29 patients in the individualized arm. No correlation was observed between the expression level of CYP3A4 mRNA and docetaxel CL or the total amount of urinary 6-β-OHF after cortisol administration (data not shown).

In conclusion, the individualized dosing of docetaxel using the total amount of urinary 6-β-OHF after cortisol administration is useful for decreasing the interpatient PK variability compared with the conventional BSA-based method of dosing. This method may be useful for individualized chemotherapy.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

REFERENCES

1. Sawyer M, Ratain MJ: Body surface area as a determinant of pharmacokinetics and drug dosing. Invest New Drugs 19:171-177, 2001
2. Gurney H: Dose calculation of anticancer drugs: A review of the current practice and introduction of an alternative. J Clin Oncol 14: 2590-2611, 1996
3. Ratain MJ: Body-surface area as a basis for dosing of anticancer agents: Science, myth, or habit? J Clin Oncol 16:2297-2298, 1998
4. Ringel I, Horwitz SB: Studies with RP 56976 (Taxotere): A semisynthetic analogue of taxol. J Natl Cancer Inst 83:288-291, 1991
5. Cortes JE, Pazdur R: Docetaxel. J Clin Oncol 13:2643-2655, 1995
6. Fossella FV, Lee JS, Murphy WK, et al: Phase II study of docetaxel for recurrent or metastatic non-small-cell lung cancer. J Clin Oncol 12:1238-1244, 1994
7. Fossella FV, Lee JS, Shin DM, et al: Phase II study of docetaxel for advanced or metastatic platinum-refractory non-small-cell lung cancer. J Clin Oncol 13:645-651, 1995
8. Gandara DR, Vokes E, Green M, et al: Activity of docetaxel in platinum-treated non-small-cell lung cancer: Results of a phase II multicenter trial. J Clin Oncol 18:131-135, 2000
9. Kunitoh H, Watanabe K, Onoshi T, et al: Phase II trial of docetaxel in previously untreated advanced non-small-cell lung cancer: A Japanese cooperative study. J Clin Oncol 14:1649-1655, 1996
10. Fossella FV, DeVore R, Kerr RN, et al: Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens: The TAX 320 Non-Small Cell Lung Cancer Study Group. J Clin Oncol 18:2354-2362, 2000
11. Shepherd FA, Dancey J, Ramlau R, et al: Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 18:2095-2103, 2000
12. Hudis CA, Seidman AD, Crown JP, et al: Phase II and pharmacologic study of docetaxel as initial chemotherapy for metastatic breast cancer. J Clin Oncol 14:58-65, 1996
13. Trudeau ME, Eisenhauer EA, Higgins BP, et al: Docetaxel in patients with metastatic breast cancer: A phase II study of the National Cancer Institute of Canada-Clinical Trials Group. J Clin Oncol 14:422-428, 1996
14. Chan S, Friedrichs K, Noel D, et al: Prospective randomized trial of docetaxel versus doxorubicin in patients with metastatic breast cancer: The 303 Study Group. J Clin Oncol 17:2341-2354, 1999
15. Marre F, Sanderink GJ, de Sousa G, et al: Hepatic biotransformation of docetaxel (Taxotere) *in vitro*: Involvement of the CYP3A subfamily in humans. Cancer Res 56:1296-1302, 1996
16. Nelson DR, Koymans L, Kamataki T, et al: P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 6:1-42, 1996
17. Lin JH, Lu AYH: Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet 35:361-390, 1998
18. Parkinson A: An overview of current cytochrome P450 technology for assessing the safety and efficacy of new materials. Toxicol Pathol 24:45-57, 1996
19. Shimada T, Yamazaki H, Mimura M, et al: Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 270:414-423, 1994
20. Guengerich FP: Characterization of human microsomal cytochrome P450 enzymes. Annu Rev Pharmacol Toxicol 29:241-264, 1989
21. Guengerich FP, Turvy CG: Comparison of levels of human microsomal cytochrome P450 enzymes and epoxide hydrolase in normal and disease status using immunochemical analysis of surgical samples. J Pharmacol Exp Ther 256: 1189-1194, 1991
22. Hunt CM, Westerkam WR, Stave GM: Effects of age and gender on the activity of human hepatic CYP3A. Biochem Pharmacol 44: 275-283, 1992
23. Watkins PB, Turgeon DK, Saenger P, et al: Comparison of urinary 6-beta-cortisol and the erythromycin breath test as measures of hepatic P450IIIA (CYP3A) activity. Clin Pharmacol Ther 52:265-273, 1992
24. Kinirons MT, O'Shea D, Downing TE, et al: Absence of correlations among three putative *in vivo* probes of human cytochrome P4503A activity in young healthy men. Clin Pharmacol Ther 54:621-629, 1993
25. Hunt CM, Watkins PB, Saenger P, et al: Heterogeneity of CYP3A isoforms metabolizing erythromycin and cortisol. Clin Pharmacol Ther 51:18-23, 1992
26. Thummel KE, Shen DD, Podoll TD, et al: Use of midazolam as a human cytochrome P450 3A probe: II. Characterization of inter- and intra-individual hepatic CYP3A variability after liver transplantation. J Pharmacol Exp Ther 271:557-566, 1994
27. Thummel KE, Shen DD, Podoll TD, et al: Use of midazolam as a human cytochrome P450 3A probe: I. In vitro-in vivo correlations in liver transplant patients. J Pharmacol Exp Ther 271: 549-556, 1994
28. Hirth J, Watkins PB, Strawderman M, et al: The effect of an individual's cytochrome CYP3A4 activity on docetaxel clearance. Clin Cancer Res 6:1255-1258, 2000
29. Goh BC, Lee SC, Wang LZ, et al: Explaining interindividual variability of docetaxel pharmacokinetics and pharmacodynamics in Asians through phenotyping and genotyping strategies. J Clin Oncol 20:3683-3690, 2002
30. Yamamoto N, Tamura T, Kamiya Y, et al: Correlation between docetaxel clearance and estimated cytochrome P450 activity by urinary metabolite of exogenous cortisol. J Clin Oncol 18:2301-2308, 2000
31. Yamaoka K, Nakagawa T, Uno T: Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. J Pharmacokinet Biopharm 6:165-175, 1978
32. Nakamura J, Yakata M: Determination of urinary cortisol and 6 beta-hydroxycortisol by high performance liquid chromatography. Clin Chim Acta 149:215-224, 1985
33. Lykkesfeldt J, Loft S, Poulsen HE: Simultaneous determination of urinary free cortisol and 6 beta-hydroxycortisol by high-performance liquid chromatography to measure human CYP3A activity. J Chromatogr B Biomed Appl 660:23-29, 1994
34. Vergniol JC, Bruno R, Montay G, et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatogr 582:273-278, 1992
35. Taguchi T, Furue H, Niitani H, et al: Phase I clinical trial of RP 56976 (docetaxel) a new anticancer drug. Gan To Kagaku Ryoho 21:1997-2005, 1994
36. Burris H, Irvin R, Kuhn J, et al: Phase I clinical trial of Taxotere administered as either a 2-hour or 6-hour intravenous infusion. J Clin Oncol 11:950-958, 1993
37. Extra JM, Rousseau F, Bruno R, et al: Phase I and pharmacokinetic study of Taxotere (RP 56976; NSC 628503) given as a short

intravenous infusion. Cancer Res 53:1037-1042, 1993

38. Pazdur R, Newman RA, Newman BM, et al: Phase I trial of Taxotere: Five-day schedule. J Natl Cancer Inst 84:1781-1788, 1992

39. Mathijssen RHJ, Verweij J, de Jonge MJ, et al: Impact of body-size measures on irinotecan clearance: Alternative dosing recommendations. J Clin Oncol 20:81-87, 2002

40. De Jongh FE, Verweij J, Loos WJ, et al: Body-surface area-based dosing does not increase accuracy of predicting cisplatin exposure. J Clin Oncol 19:3733-3739, 2001

41. Gurney HP, Ackland S, Gebski V, et al: Factors affecting epirubicin pharmacokinetics and toxicity: Evidence against using body-surface area for dose calculation. J Clin Oncol 16:2299-2304, 1998

42. Loos WJ, Gelderblom H, Sparreboom A, et al: Inter- and intrapatient variability in oral topotecan pharmacokinetics: Implications for body-surface area dosage regimens. Clin Cancer Res 6:2685-2689, 2000

43. Bruno R, Hille D, Riva A, et al: Population pharmacokinetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998

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Randomised phase II study of docetaxel/cisplatin *vs* docetaxel/irinotecan in advanced non-small-cell lung cancer: a West Japan Thoracic Oncology Group Study (WJTOG9803)

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Docetaxel plus cisplatin and docetaxel plus irinotecan are active and well-tolerated chemotherapy regimens for advanced non-small-cell lung cancer (NSCLC). A randomised phase II study compared their efficacy and toxicity in 108 patients with stage IIIb/IV NSCLC, who were randomised to receive docetaxel 60 mg m^{-2} and cisplatin 80 mg m^{-2} on day 1 (DC; $n=51$), or docetaxel 60 mg m^{-2} on day 8 and irinotecan 60 mg m^{-2} on day 1 and 8 (DI; $n=57$) every 3 weeks. Response rates were 37% for DC and 32% for DI patients. Median survival times and 1- and 2-year survival rates were 50 weeks (95% confidence interval: 34–78 weeks), 47 and 25% for DC, and 46 weeks (95% confidence interval: 37–54 weeks), 40 and 18% for DI, respectively. The progression-free survival time was 20 weeks (95% confidence interval: 14–25 weeks) with DC and 18 (95% confidence interval: 12–22 weeks) with DI. Significantly more DI than DC patients had grade 4 leucopenia and neutropenia ($P<0.01$); more DC patients had grade $\geqslant 2$ thrombocytopenia ($P<0.01$). Nausea and vomiting was more pronounced with DC ($P<0.01$); diarrhoea was more common with DI ($P=0.01$). Three treatment-related deaths occurred in DC patients. In conclusion, although the DI and DC regimens had different toxicity profiles, there was no significant difference in survival.
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Keywords: combination chemotherapy; doublets; irinotecan; cisplatin; docetaxel; non-small-cell lung cancer; carboplatin

Unfortunately, non-small-cell lung cancer (NSCLC) is a member of the group of neoplastic diseases that is relatively chemoresistant. Recent meta-analyses show that cisplatin-based chemotherapy improves survival (Non-Small Cell Lung Cancer Collaborative Group, 1995), and it is considered a standard treatment for NSCLC, Most cisplatin-based regimens have substantial toxicities that require close monitoring and supportive care. Thus, there is a need to develop active and less toxic chemotherapy regimens that include new active compounds with novel mechanisms of action.

In the 1990s, several new, active therapies with single-agent response rates of 15–30% became available for NSCLC, including irinotecan, docetaxel, paclitaxel, vinorelbine, and gemcitabine. Because irinotecan and docetaxel were approved for NSCLC earlier than the other drugs in Japan, development of regimens containing irinotecan or docetaxel is more advanced. Docetaxel 60 mg m^{-2} showed good antitumour activity against advanced NSCLC (Kunitoh *et al*, 1996), and the combination of docetaxel plus cisplatin (DC) is one of the most effective regimens for advanced NSCLC (Rodriguez *et al*, 2001; Schiller *et al*, 2002). Studies in Japan included a phase II study in which DC yielded a response rate of 42% (Okamoto *et al*, 2002), and a phase III study in which DC was associated with better survival than the vindesine and cisplatin (VC) combination (Kubota *et al*, 2002).

Irinotecan demonstrated activity similar to that of VC in stage IIIb/IV NSCLC (Negoro *et al*, 2003), and significant longer overall survival time than VC in stage IV NSCLC (Fukuoka *et al*, 2000). We reported a phase I study of docetaxel plus irinotecan (DI) in patients with advanced NSCLC, in which a promising response rate of 48% and the median survival time of 48 weeks were achieved with acceptable toxicities (Masuda *et al*, 2000). Thus, DI appeared to be a promising non-cisplatin-containing regimen.

Based on the above findings, we conducted a randomised trial of DC *vs* DI in patients with advanced NSCLC to compare the respective response rates, survival data, and toxicity profiles of the two regimens. This was a multicentred phase II study.

PATIENTS AND METHODS

Patients

Patients enrolled in this trial had histologically or cytologically confirmed stage IIIb or IV NSCLC. Patients with stage IIIb disease who were not candidates for thoracic radiation and patients with stage IV disease were eligible if they had not received previous therapy, had measurable disease, and had a life expectancy of at least 3 months. Additional entry criteria were age $\geqslant 20$ years, performance status of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) scale, adequate bone marrow function (leucocyte

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count 4000–12 000 μl^{-1}, haemoglobin concentration $\geqslant 9.5\,g\,dl^{-1}$ platelet count $\geqslant 100\,000\,\mu l^{-1}$), kidney function (creatinine $\leqslant$ upper limit of normal, 24-h creatinine clearance $\geqslant 60\,ml\,min^{-1}$), liver function (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leqslant 2.0$ times the upper limit of normal, total bilirubin $\leqslant 1.5\,mg\,dl^{-1}$), and pulmonary function ($PaO_2 \geqslant 60$ torr). Patients with active concomitant or a recent (<3 years) history of any malignancy, symptomatic brain metastases, past history of drug allergy reactions, complication by interstitial pneumonia, watery diarrhoea, ileus, treatment with nonsteroidal anti-inflammatory drugs, or other serious complications, such as uncontrolled angina pectoris, myocardial infarction within 3 months, heart failure, uncontrolled diabetes mellitus or hypertension, massive pleural effusion or ascites, or serious active infection were excluded. All patients gave written informed consent, and the institutional review board for human experimentation approved the protocol.

Study evaluations

Pretreatment studies included a complete medical history and physical examination, chest X-ray, electrocardiography, computed tomography (CT) scan of the brain and chest, CT or ultrasound examination of the abdomen, and bone scintigraphy. Blood and blood chemistry studies included complete blood cell count, liver function test, serum electrolytes, serum creatinine, and blood urea nitrogen. Chest X-ray, blood and blood chemistry analyses, and urinalysis were repeated weekly.

Randomisation and treatment schedule

Patients were randomly assigned to receive the DC regimen or the DI regimen by a minimisation method using stage (IIIB/IV) and treatment institution. The DC regimen was consisting of docetaxel 60 mg m^{-2} on day 1 and cisplatin 80 mg m^{-2} on day 1, and the DI regimen was consisting of docetaxel 60 mg m^{-2} as a 60-min intravenous infusion on day 8 and irinotecan 60 mg m^{-2} as a 90-min intravenous infusion on days 1 and 8 (Figure 1). Both regimens were repeated every 3 weeks. Participating researchers at each institution decided the amount of fluid replacement and the type of antiemetic therapy to administer. Standard antiemetic treatment in the DC arm consisted of 5-HT_3 receptor antagonist plus 16 mg dexamethasone intravenously on day 1, before cisplatin administration. In the DI arm, standard antiemetic treatment consisted of 5-HT_3 receptor antagonist intravenously before chemotherapy administration on days 1 and 8. Patients received at least two treatment cycles, and those with a complete or partial response after two cycles had treatment continued until there was evidence of disease progression, intolerable toxicity, or patient refusal.

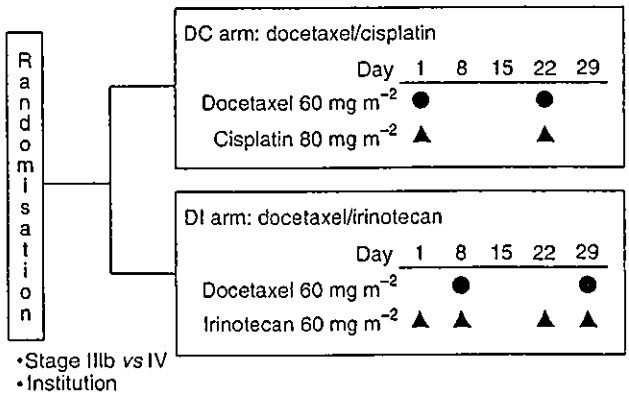

Figure 1 *Treatment schema*: after stratification by stage and institution, enrolled patients were randomly allocated to receive docetaxel plus cisplatin (DC) or docetaxel plus irinotecan (DI).

Dose modifications

Toxicity assessment was based on the National Cancer Institute–Common Toxicity Criteria version 2.0. Dose levels and treatment schedule were modified to avoid severe adverse effects. Patients receiving DI had the day-8 docetaxel and irinotecan doses postponed to day 15 if any of the following toxicities was present on day 8: leucocyte count $<3000\,\mu l^{-1}$, platelet count $<100\,000\,\mu l^{-1}$ diarrhoea consisting of bloody or watery stools, or increased to two or more diarrhoea within 24 h, abdominal pain rated mild or worse, hepatic toxicity $\geqslant$ grade 3, or fever >38°C. If these toxicities occurred on day 15 after skipping the day-8 treatment, DI was stopped in that course.

Patients could receive the next treatment course only if the following criteria were met: leucocyte count $\geqslant 4000\,\mu l^{-1}$, platelet count $\geqslant 100\,000\,\mu l^{-1}$ AST/ALT <2.0 times the upper limit of normal, total bilirubin $\leqslant 1.5\,mg\,dl^{-1}$ serum creatinine $\leqslant$ the upper limit of normal, ECOG PS $\leqslant 2$, neurotoxicity $\leqslant$ grade 1, no diarrhoea or oedema. However, if more than 6 weeks passed before these criteria were satisfied, the patient was removed from the study.

Dose modification criteria for each drug are shown in Table 1. If during the previous course, grade 4 leucopenia, grade 4 neutropenia lasting $\geqslant 3$ days, or grade 4 thrombocytopenia had occurred, doses of all drugs were reduced by 10 mg m^{-2}. Doses of both cisplatin and docetaxel were reduced by 10 mg m^{-2} in subsequent cycles if chemotherapy induced grade $\geqslant 2$ neurotoxicity. Moreover, dose of docetaxel was reduced by 10 mg m^{-2} if grade $\geqslant 2$ hepatic toxicity or grade $\geqslant 3$ stomatitis had occurred. Dose of cisplatin was reduced by 20/mg/m^2 if grade $\geqslant 2$ renal toxicity occurred. Dose of irinotecan was reduced by 5 mg m^{-2} if grade $\geqslant 2$ hepatic toxicity had occurred and by 10 mg m^{-2} if grade $\geqslant 2$ diarrhoea or cancellation of day-8 treatment had occurred.

Evaluation of response and survival

Tumour response was classified according to World Health Organization (WHO) criteria (World Health Organization, 1979). Complete response was defined as complete disappearance of all measurable and assessable disease for at least 4 weeks, Partial response was a $\geqslant$50% decrease in the sum of the products of the two IL largest perpendicular diameters of all measurable tumours lasting at least 4 weeks and without appearance of any new lesions. No change was defined as a <50% decrease or a <25% increase of tumor lesions for at least 4 weeks with no new lesions.

Table 1 Dose modification criteria

Toxicities in previous cycle	Decrease in docetaxel dose (mg/m^{-2})	Decrease in cisplatin dose (mg/m^{-2})	Decrease in -irinotecan dose (mg/m^{-2})
Grade 4 neutropenia lasting $\geqslant 3$ days, leucopenia or thrombocytopenia	10	10	10
Grade $\geqslant 2$ neurotoxicity	10	10	—
Grade $\geqslant 2$ renal toxicity	—	20	—
Grade $\geqslant 2$ hepatic toxicity	10	—	5
Grade $\geqslant 3$ stomatitis	10	—	—
Grade $\geqslant 2$ diarrhoea	—	—	10
Cancellation of day-8 treatment	—	—	10