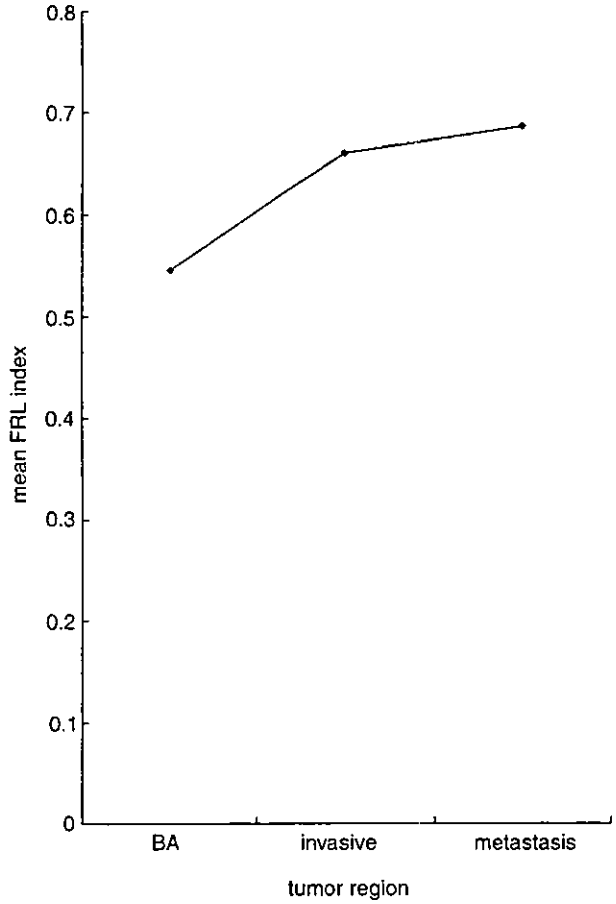

Figure 3 Mean fractional regional loss indices for the BA, invasive, and metastatic components in 10 chromosomal regions.

relatively frequent (91.7%) and heterogeneous (40.9%), and therefore indicative of late alterations required for malignant progression.

Discussion

Invasive and/or metastatic components contained more additional allelic losses than BA components. It has already been postulated that tumor cells evolved from BA to invasive and metastatic components because of the difference in histology between the lepidic pattern along the alveolar walls in BA components and invasiveness in other components.[7] Almost all metastases contained the same or more allelic losses when compared with invasive lesions in each individual tumor, although no significant difference was found in the mean FRL indices of the invasive and metastatic lesions. This suggests that tumor cells that showed noninvasive BA-type morphology evolved into invasive lesions and then to metastases, acquiring the invasive and metastatic phenotype through the process of clonal evolution occurring during multistep tumor progression. Eight cases (67%) actually showed accumulation of genetic alterations during morphological progression, but the tumor components examined thus far in the remaining four cases have shown genetic homogeneity or no obvious accumulation of genetic alterations despite great morphological divergence. This might be explained by the genetic alterations being present on loci other than those examined in this study. Although the additional allelic losses found in the metastases can be explained by the accumulation of genetic aberrations during the

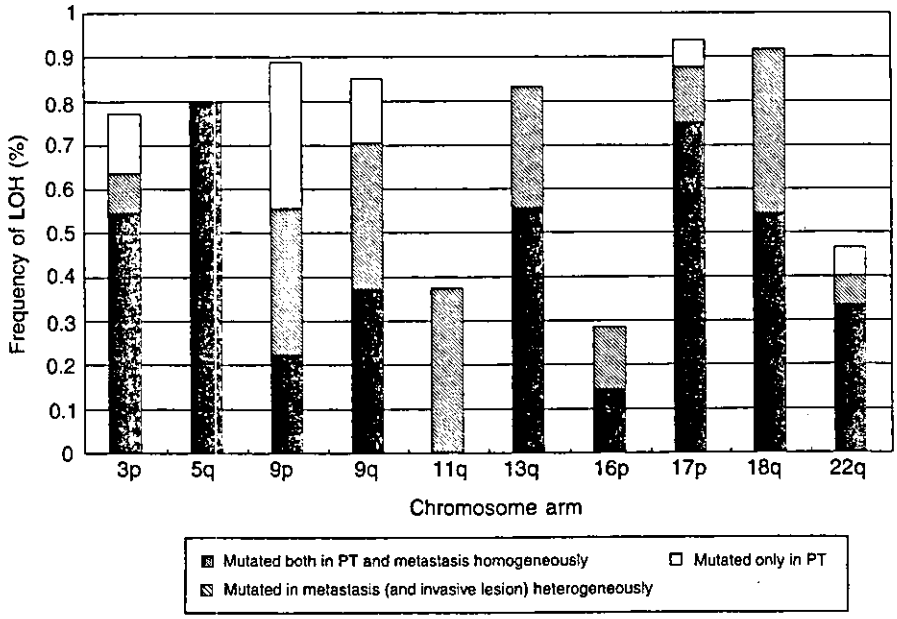

Figure 4 Prevalence and intratumor heterogeneity of allelic loss in primary tumors and lymph node metastases. PT, primary tumor.

course of tumor progression, genetic alterations that were not detectable in the metastases were present in the primary tumor. These findings indicate that the primary tumor progresses genetically even after the metastasis has occurred, that is, the predominant clone of some primary tumor components no longer represents the metastatic clones that we investigated.

Using multiple microdissected specimens within individual cases that included primary and metastatic tumors, we have shown highly frequent LOH at 5q and 17p even in the lowest-grade portions (ie, BA lesions) and found identical alleles to be deleted in all portions examined in each affected primary tumor and metastatic tumor, even though quite divergent histopathologically. These results suggest that LOH at 5q and 17p may be crucial steps in the early phase of the development to lymph node metastasis and that they are retained throughout successive clonal evolutions. A similar phenomenon was previously reported by Boland *et al*[23] based on an analysis of tumor progression schemes in colon cancers. They used multiple microdissected samples of colonic tumors showing cross-sections of the 'adenoma–carcinoma sequence' and detected a clear, abrupt occurrence of LOH at 5q at the transition phase from normal epithelium to adenoma. We observed LOH on at least one of the two loci on 5q and/or 17p uniformly in almost all cases examined (11/12; 92%). However, it is uncertain why such presumably early lesions are not present in all tumors. A number of cell types are thought to be potential precursors of lung cancers, and different initiation events may be involved depending on the differentiation pathway to which they are committed. Thus, certain cell types may not require inactivation of all putative tumor suppressor genes on 5q and 17p.

Previous studies have shown allelic loss at the 17p loci to be involved at a relatively early stage of NSCLC,[8,24,25] and its loss may be associated with the genesis of NSCLC. *p53* is believed to play a role as a 'guardian' that maintains the integrity of the genome by participating in the DNA damage checkpoints in the cell cycle. Inactivation of *p53* has been reported to lead to increased frequency of mutations, chromosomal rearrangements, and abnormal chromosomal segregations.[26–29] Recent studies have suggested that the LOH at specific chromosomal loci, 1p, 3p, 5q, 9p, 17q, and 22q, is associated with a worse prognosis of NSCLC, although studies of patients from different populations have yielded conflicting results.[14,30–39] LOH at the *APC/MCC* gene cluster at chromosome 5q has been reported to correlate with poorer survival of patients with NSCLC.[38] In the present study, the tumors were small but were associated with lymph node metastasis, and a worse prognosis was assumed. Seven of nine informative cases (78%) showed allelic losses at 5q in all foci, including BA lesions. Our previous study also concluded that this deletion is a relatively early event in the progression of adenocarcinoma of the lung.[6] The high prevalence of 5q deletion in this study might indicate that 5q loss plays an important role in the progression of metastatic tumors and that it was determined in the early stage.

Although we showed frequent LOH at 18q in the tumors examined, approximately 40% of allelic losses were found in either metastatic lesions or invasive and metastatic lesions, not in all portions of each affected primary tumor and metastatic tumor examined. Therefore, LOH at 18q appears to have a role as late event in the metastatic progression of adenocarcinomas mixed BA and other subtypes of the lung. Shiseki *et al*[8] reported that loss at 18q plays an important role in the progression of NSCLC based on a comparison of stage I NSCLC and brain metastases. Lymph node metastases, most malignant portions, were shown to carry 18q deletions at even higher frequency than 5q or 17p deletions in the present study. However, it should be noted that accumulation of LOH at 18q occurred at various stages of tumor progression within individual tumors toward lymph node metastasis, that is, some LOH at 18q occurred in BA lesions, and some in metastatic lesions. This indicated a clear distinction from LOH at 5q and 17p, especially at 5q. Since approximately 60% of 18q deletions are present in all portions of individual tumors, it remains unclear whether LOH at 18q has a role both as an early event and a late event or acts at various steps in tumors that progress to lymph node metastasis.

In summary, we examined the topographical distribution of LOH on 10 chromosome arms, and the results suggest that tumor cells accumulate genetic alterations as they evolve from the BA lesions to the invasive and metastatic lesions. Early occurrence of 5q and/or 17p deletions and successive clonal expansion during the progression of individual tumors was inferred. By contrast, LOH at 18q seemed to be acquired at various stages during tumor progression to metastasis. Similar studies analyzing more genetic loci in a larger number of cases are warranted. Furthermore, since the lung cancers resulted from various genetic and epigenetic alterations, it would also be interesting to examine the topographical differences in other genetic or epigenetic changes, such as DNA methylation.

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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%

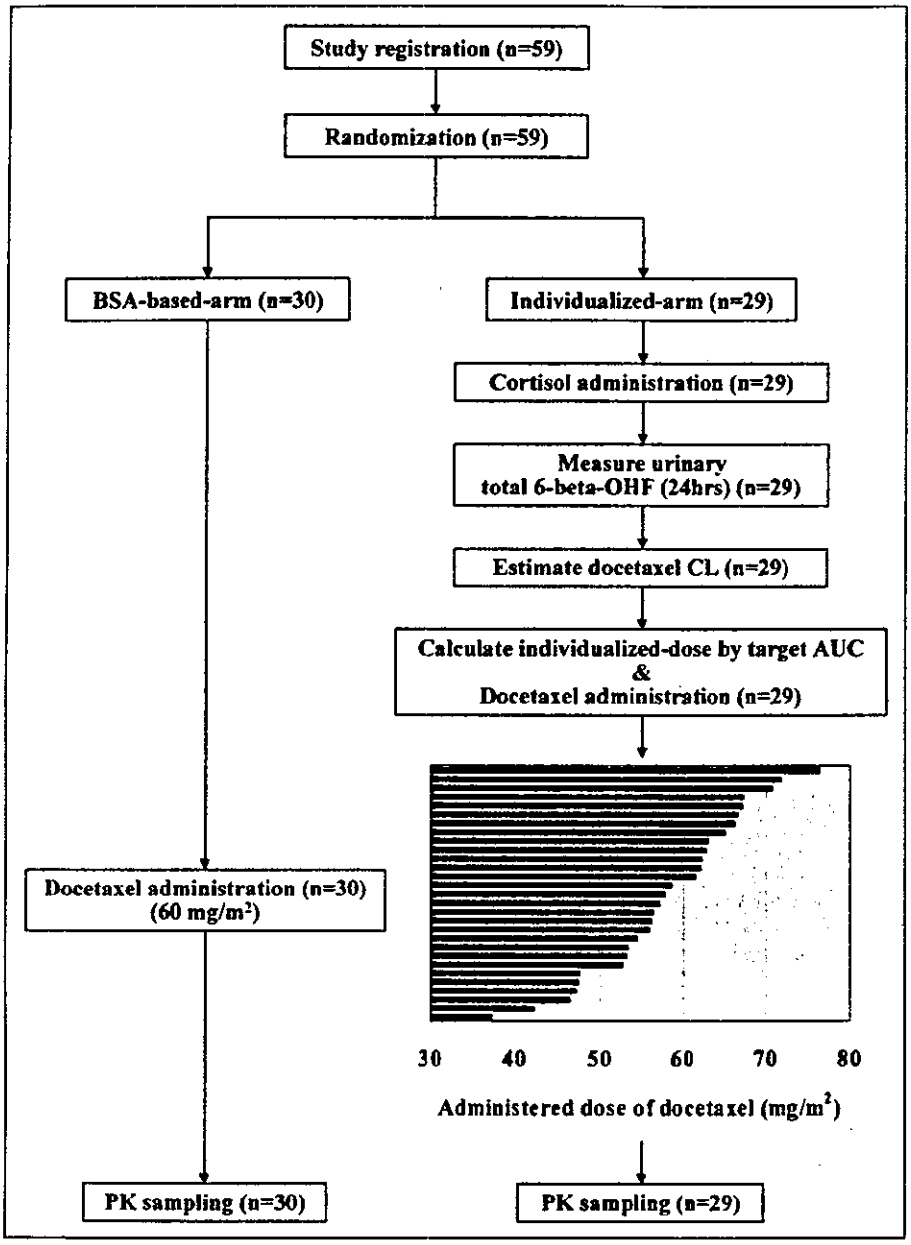

Fig 1. Study flow diagram and administered dose of docetaxel. PK, pharmacokinetic; AUC, area under the concentration-time curve; CL, clearance; 6-β-OHF, 6-beta-hydroxycortisol.

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

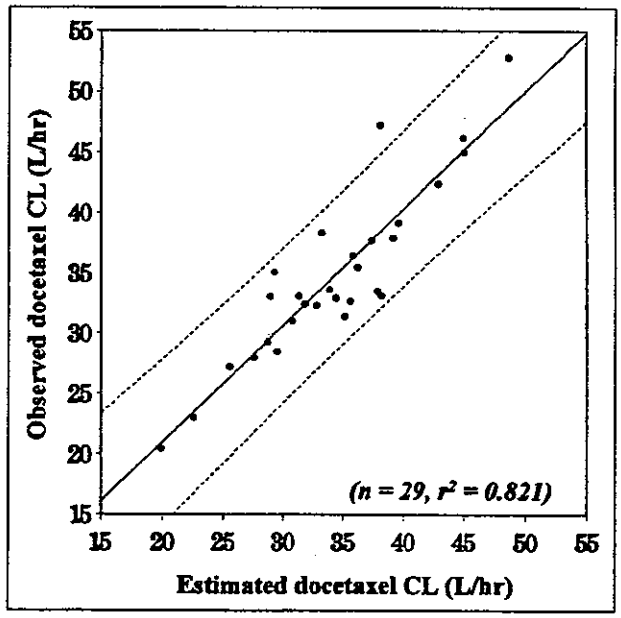

Fig 2. Correlation between the estimated and observed docetaxel clearance (CL) in the individualized arm (n = 29). (—) Linear regression line (r^2 = 0.821); (– – –) 95% CIs for individual estimates.

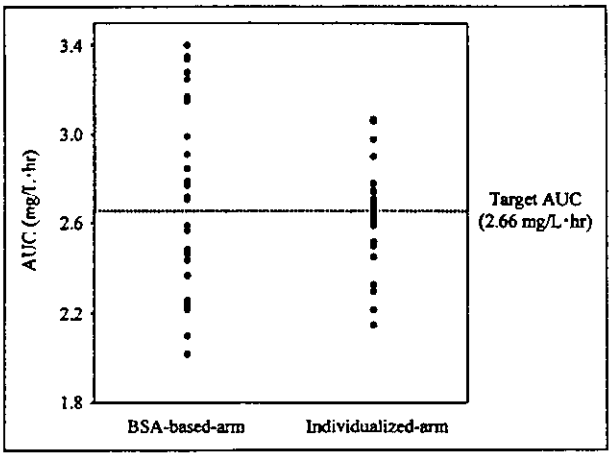

Fig 3. Comparison of area under the concentration-time curve (AUC) variability between the arms ($P < .01$; F test). BSA, body-surface area.

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.

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.

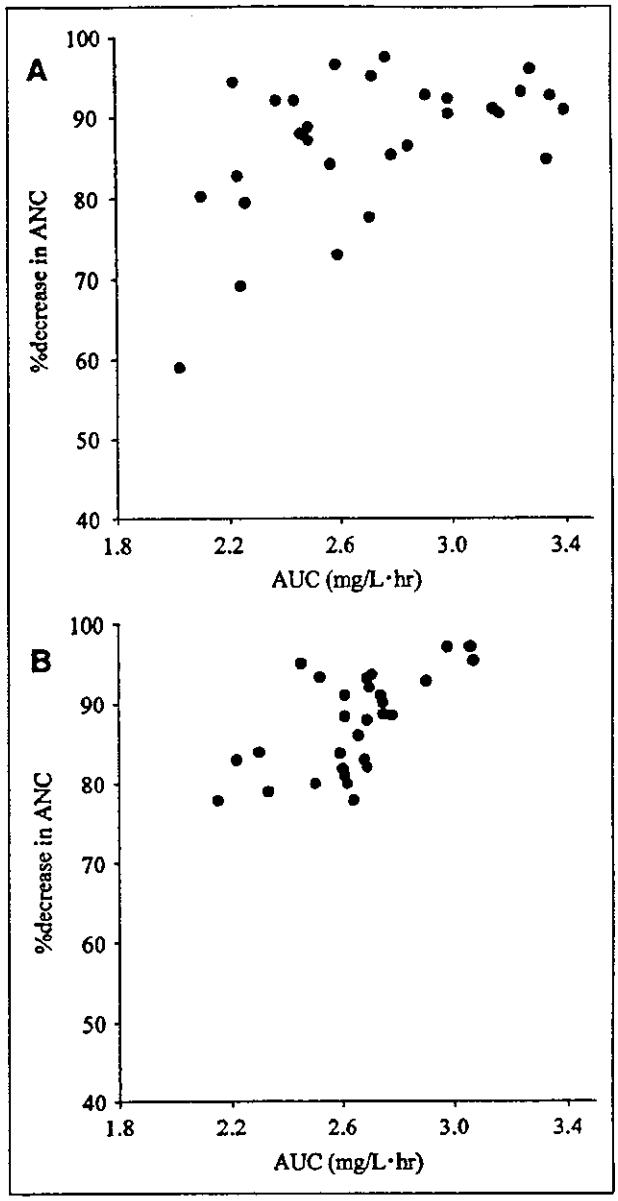

Fig 4. Correlation between area under the concentration-time curve (AUC) and percentage decrease in absolute neutrophil count (ANC) in each arm (A) body-surface area–based arm; (B) individualized arm.

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).

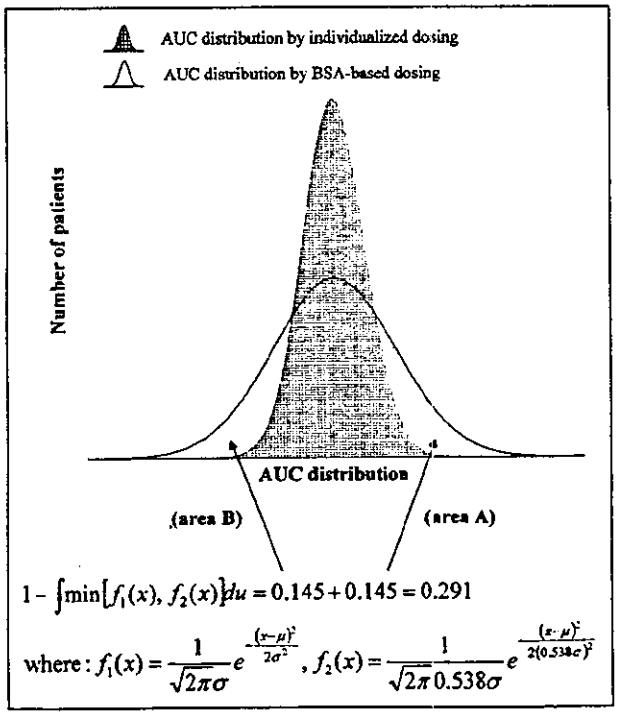

Fig 5. Simulated comparison of area under the concentration-time curve (AUC) distribution between body-surface area (BSA) –based dosing and individualized dosing when the variability of AUC is decreased 46.2% by individualized dosing applied using our method.

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.

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A Phase I/II Study Comparing Regimen Schedules of Gemcitabine and Docetaxel in Japanese Patients With Stage IIIB/IV Non-Small Cell Lung Cancer

Abbreviated Title: Gemcitabine and docetaxel for NSCLC

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JJCC in press

Original contribution

A Phase II Study of Topotecan in Patients with Relapsed Small-Cell Lung Cancer

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Abstract

An early phase II study of topotecan produced favorable results in a small number of untreated and previously treated patients with small-cell lung cancer (SCLC). This multicenter study was conducted in patients with relapsed SCLC at 19 medical institutions in Japan. Topotecan 1.0 mg/m^2/day was administered for 5 consecutive days every 3 weeks. Fifty-three patients were enrolled in the study. One patient was withdrawn before the commencement of study treatment, and 2 patients were unable to continue study treatment due to an interruption in the supply of study medication. The response rate was 26.0% in 13 of the 50 evaluable patients who were eligible and completed protocol-specified treatment and procedures. The median time to progression and overall survival were 133 days and 262 days, respectively. The most frequently reported toxicity was reversible myelosuppression, such as leukopenia, neutropenia, anemia (decreased hemoglobin), and thrombocytopenia. Nonhematological toxicity was also reported but the incidence of grade 3/4 symptoms was low. The results of this study indicate that topotecan is effective against relapsed SCLC with good tolerability.

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Key words: Chemotherapy, Camptothecin analogue, Topoisomerase I inhibitor, Myelosuppression, Granulocyte colony-stimulating factor

Introduction

Small-cell lung cancer (SCLC) is characterized by its high sensitivity to chemotherapy and radiotherapy. At present, combination chemotherapy with cisplatin/etoposide is used as the standard therapy for SCLC, and the response rate (RR) is as high as 81%-86% in previously untreated patients.[1,2] However, relapse inevitably follows in most responders, and the cancer progresses within 2 years in many patients. In order to improve survival in SCLC patients, new anticancer agents with a unique mechanism of action are needed.[3]

Topotecan is a semisynthetic camptothecin analogue. Preclinical data show that topotecan is particularly active against lung cancer with a broad spectrum of antitumor activity but without cross resistance to various anticancer agents.[4,5] The efficacy of topotecan alone has been reported in patients with SCLC treated in clinical studies conducted in the United States and Europe.[6-8] A phase I study in Japan was started in 1992 in patients with solid tumors.[9] The maximum tolerated dose was estimated to be 1.5 mg/m^2/day for a 5-consecutive-day dosing schedule with a dose-limiting toxicity (DLT) of leukopenia. Subsequently, an early phase II study was conducted between 1993 and 1997 in a small number of patients and produced favorable results in untreated and previously treated patients with SCLC. The RRs in untreated (n = 6) and previously treated (n = 15) patients were 33.3% and 26.7%, respectively. In this early phase II study in patients with SCLC, the starting dose was reduced to 1.0 mg/m^2/day after 1 death was reported at 1.2 mg/m^2/day. The response to topotecan, which was defined as a complete response (CR) or partial response (PR), was observed at both the 1.2 mg/m^2/day and 1.0 mg/m^2/day dose levels. Based on these safety and efficacy data from the early phase II

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study, 5 daily administrations of 1.0 mg/m²/day with a 3-week interval was selected as the recommended regimen for the phase II study in patients with SCLC, and the dose could be increased to 1.2 mg/m²/day if the starting dose was tolerated.

The clinical evaluation of a new anticancer agent alone for SCLC is usually carried out in previously treated patients. However, appropriate evaluation of efficacy is sometimes difficult when patients who are refractory to chemotherapy are included.[6] Recently, it has been proposed that the efficacy of a new anticancer agent should be evaluated in untreated patients (on the condition that the protocol specifies salvage therapy) or in previously treated patients who have responded to chemotherapy.[10,11] We conducted a phase II study of topotecan in the latter population. This study reports the efficacy and safety of topotecan in patients with advanced/relapsed SCLC.

Patients and Methods

Patients

A 5-day repeat dose study by intravenous infusion was conducted from January 1996 to January 1999 at 19 medical institutions. Patients with histologically or cytologically documented relapsed SCLC who met the following criteria were enrolled in this study: (1) The patient had been treated with one regimen of chemotherapy or radiotherapy and one regimen of chemotherapy; (2) the tumor responded to the first-line chemotherapy but recurred or progressed later; (3) the last chemotherapy was finished at least 8 weeks before commencing study treatment; and (4) the primary tumor was not surgically removed.
Complete histories and physical examinations were performed on all patients. The study was approved by each institutional review board and written informed consent was obtained from all patients.

To be eligible for inclusion in the study patients were required to be 15-75 years of age and have measurable disease, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2, a life expectancy of ≥ 3 months, and no active concomitant malignancy. Measurable disease was defined as the tumor demonstrated by conventional chest roentgenography or computed tomography (CT) of the whole body. In addition, all patients underwent a routine staging evaluation that consisted of standard radiologic studies (including CT of the abdomen and brain) as well as bone scanning.

Eligibility requirements also included the following: white blood cell (WBC) count ≥ 4000/μL and ≤ 12,000/μL, platelet count ≥ 100,000/μL, hemoglobin ≥ 9.5 g/dL, serum bilirubin < 1.5 mg/dL, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ twice the upper limit of normal, and serum creatinine < the upper limit of normal. Patients with severe drug hypersensitivity, interstitial pneumonia, pulmonary fibrosis, symptomatic brain metastasis, massive pleural effusion, ascites, or other severe complications were excluded from the study. Patients who were pregnant, nursing, or expressed a desire to become pregnant were also ineligible.

In this study, patients were hospitalized during the first treatment course. In the second and subsequent courses patients whose clinical course could be evaluated on an outpatient basis could be discharged temporarily.

Dosage and Administration

One course of treatment consisted of a 5-day repeat dosing of topotecan 1.0 mg/m²/day and a 16-day dose-free period. A sufficient amount of topotecan for 1 dose was dissolved in 100 mL of physiological saline and administered intravenously by drip infusion over a 30-minute period. No prophylactic antiemetics were used. Granulocyte colony-stimulating factor (G-CSF) was not administered routinely, but was used as needed according to the published guidelines.[12]

Subsequent courses were given after it was confirmed that the WBC count was ≥ 4000/μL and the platelet count was ≥ 100,000/μL. If G-CSF was given, it was confirmed that the WBC count was ≥ 4000/μL and the platelet count was ≥ 100,000/μL at least 48 hours after the end of administration of G-CSF. When WBC or platelet counts did not return to the above level, the treatment-free period could be extended up to 6 weeks.

Dose reductions/escalations were based on the lowest leukocyte count detected at weekly determinations. If leukopenia or thrombocytopenia was ≤ grade 2 (WBC count ≥ 2000/μL, platelet count ≥ 50,000/μL) at weekly determinations after the first course, the dose for the subsequent courses could be increased to 1.2 mg/m²/day at the judgment of the investigator. If grade 4 leukopenia or thrombocytopenia (WBC count < 1000/μL, platelet count < 30,000/μL) occurred after study treatment, the dose was reduced to 0.8 mg/m²/day in subsequent courses. More than 3 courses were given unless disease progression was observed.

Evaluation

In order to assess response and adverse effects, the following tests were done once a week during treatment: complete blood count, AST, ALT, alkaline phosphatase, lactate dehydrogenase, bilirubin, creatinine, blood urea nitrogen, serum electrolytes, urinalysis, and chest roentgenography.

Antitumor effects were evaluated according to the criteria established by the Japan Society for Cancer Therapy and the Japan Lung Cancer Society.[13] The investigator rated clinical symptoms at least once a week as grade 0-4 according to the grading scale established by the Japan Society for Cancer Therapy.[14] The severity of other symptoms was assessed on the 5-point scale: 0 = no symptom; 1 = mild; 2 = moderate; 3 = severe; and 4 = very severe.

Time to progression (TTP) was defined as the time from the commencement of study treatment to progressive disease (PD) or death. Patients who did not show progression were censored. Survival was defined as the time from the commencement of study treatment until death due to any cause. Patients lost to follow-up were censored on the date of last contact with the investigator.

The following parameters were examined in the study: tumor findings; laboratory tests (hematology, clinical chemistry, urinalysis, tumor markers); clinical findings (body temperature, body weight, PS, subjective symptoms/objective signs); and electrocardiography. Laboratory tests and other examinations were carried out just before the commencement of study treatment, at least

Table 1 Baseline Characteristics

Baseline Characteristics	Number of Patients (n = 50)
Sex	
Male	34
Female	16
Median Age, Years (Range)	65.5 (42–75)
ECOG Performance Status	
0	11
1	30
2	9
Previous Therapy	
Chemotherapy only	19
Chemotherapy + radiotherapy	29
Chemotherapy + radiotherapy – others	1
Chemotherapy – others	1
Previous Chemotherapy	
Platinum/Etoposide	30
Including irinotecan HCl	10
Others	10
Site of Lesion Evaluated	
Primary tumor	30
Metastatic tumor	
Lymph node	26
Brain	12
Liver	8
Lung	7
Adrenal	5
Hydrothorax	2
Kidney	2
Perivertebra	1

Abbreviation: ECOG – Eastern Cooperative Oncology Group

once a week, and whenever necessary after the commencement of study treatment. The measurable disease determined by the investigator was examined just before the commencement of study treatment and once a week, if possible, or at least every 4 weeks after the commencement of study treatment.

Results

Patient Disposition

Fifty of the 53 eligible patients completed protocol-specified treatment and procedures. One of the 3 patients who did not complete the study received no study treatment, and the 2 remaining patients were unable to continue study treatment due to an interruption in the supply of study medication.

Table 2 Results

	Number of Patients (n = 50)
Overall Response Rate	13 (26%)*
Complete response	0
Partial response	13 (26%)
Stable Disease	21 (42%)
Progressive Disease	11 (22%)
Unknown	5 (10%)

*90% CI, 16.1%–38.1%

Patient Characteristics

Table 1 shows the baseline characteristics of the 50 evaluable patients. Thirty-four patients were male, and approximately half of the patients (n = 24) were in their 60s. Eleven patients had an ECOG PS of 0, 30 had a PS of 1, and 9 had a PS of 2. As previous chemotherapy, combination therapy with a platinum preparation and etoposide was used in the majority of patients, and combination regimens including irinotecan were used in 10 patients. The majority of sites for evaluation were primary tumor, lymph nodes, and metastatic lesion in the brain.

The median number of courses was 2 (range, 1-7), and the mean and median total doses were 15.0 mg/m^2 ± 7.8 mg/m^2 and 13.38 mg/m^2 (range, 5.0-35.0 mg/m^2), respectively. The dose of topotecan was reduced in the second or subsequent courses in 5 patients.

Efficacy

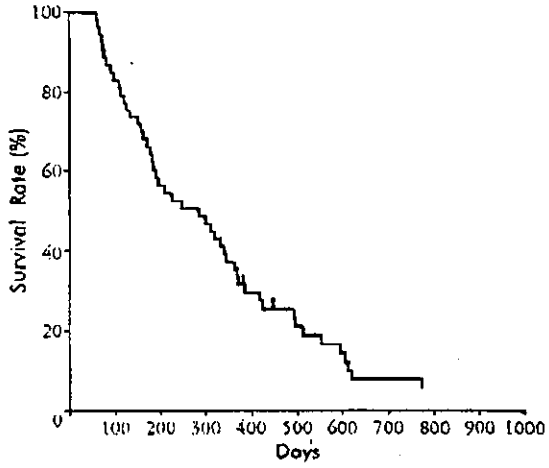
The antitumor effect of topotecan was evaluated by the investigator and confirmed by the extramural evaluation committee. The results are shown in Table 2. A PR was observed in 13 of 50 evaluable patients, stable disease in 21, and PD in 11. The response rate, percentage of CRs and PRs, was 26.0% with a 90% confidence interval (CI) of 16.1%-38.1%. The median number of courses given to the responders who achieved a PR was 4 (range, 3-6).

The median TTP was 133 days (95% CI, 70-178 days). Eleven patients were assessed as PD because of an increase in tumor size in the first course (n = 4) or second course (n = 7). The median survival time was 262 days (95% CI, 177-339 days). The survival curve is shown in Figure 1.

Safety

After excluding 1 eligible patient who did not receive any treatment with topotecan and 2 eligible patients who could not receive the prescribed number of courses because of the recall of the clinical supply, 50 patients were evaluable for safety. Table 3 shows the incidence of adverse drug reactions. The most frequently reported adverse drug reactions were anorexia (62%),

Figure 1 Survival Curve



nausea/vomiting (58%), fever (46%), fatigue (28%), and alopecia (34%). Grade 3 or 4 symptoms included anorexia (10%), nausea/vomiting (8%), and alopecia (6%). Two patients had a fever of ≥ 38°C and grade 3/4 neutropenia in 1 day, while 12 patients developed a fever of ≥ 38°C after the onset of grade 3/4 neutropenia in 1 course of treatment. Fifty-six percent of the patients evaluable for safety were treated with antibiotics. The incidence of diarrhea (1 of the DLTs of irinotecan, a drug in the same class as topotecan) was 20% and all events were grade 1/2.

Frequently observed hematological toxicities were anemia (92%), leukopenia (100%), neutropenia (100%), and thrombocytopenia (90%). Grade 3/4 abnormalities were decreased hemoglobin (46%), leukopenia (72%), neutropenia (92%), and thrombocytopenia (40%). All of these effects were reversible and resolved or showed a tendency toward resolution. Eight patients required a red blood cell transfusion, and 3 patients required a platelet transfusion. There were no deaths whose causality was attributed to study treatment.

Discussion

Topotecan is a semisynthetic camptothecin analogue. It is a topoisomerase I inhibitor with less toxicity than camptothecin. Irinotecan has a similar mechanism of action, and its efficacy against SCLC as monotherapy has been reported.[15] A randomized phase III trial in Japan suggested that the combination of irinotecan and cisplatin produced survival superior to the standard etoposide/cisplatin regimen in patients with extensive-stage SCLC.[16]

In this study of topotecan, the RR in previously treated patients with SCLC was 26.0%, which was similar to that reported for irinotecan alone (33.3%) in the same population.[15] In previous studies, the RR of topotecan alone for recurrent SCLC ranged from 19% to 33%.[6-8] In the present study, topotecan was shown to be effective at 1.0 mg/m²/day, which is lower than the dose used in previously reported studies.[6-8] In this study, the median duration of response and the median time to response were 49 days and 28 days, respectively, which are similar to those reported for irinotecan (50 days and 28 days, respectively).[15] These findings suggest that the relatively low dose of topotecan has a promising efficacy against relapsed SCLC.

The DLT of topotecan was myelosuppression,[6-8] while myelosuppression and diarrhea were the DLTs of irinotecan.[17] In this phase II study, grade 3/4 neutropenia was reported in 92% of the patients, and neutropenic fever occurred in 24%. Neutropenia and fever were immediately alleviated by using G-

Table 3 Adverse Drug Reactions > 2% in 50 Safety Evaluable Patients

Adverse Drug Reaction	Number of Patients by Grade				
	All Grades	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal System					
Anorexia	31 (62%)	11	15	5	–
Nausea/vomiting	29 (58%)	19	6	4	–
Diarrhea	10 (20%)	7	3	–	–
Abdominal pain	3 (6%)	2	1	–	–
Constipation	2 (4%)	1	1	–	–
Stomatitis	4 (8%)	4	–	–	–
Body as a Whole (General)					
Fever	23 (46%)	13	10	–	–
Fatigue	14 (28%)	7	7	–	–
Weight loss	7 (14%)	7	–	–	–
Skin and Appendages					
Alopecia	17 (34%)	13	1	3	–
Urinary System					
Hematuria	3 (6%)	3	–	–	–
Hematology					
Anemia	46 (92%)	10	13	20	3
Leukopenia	50 (100%)	3	11	34	2
Neutropenia	50 (100%)	1	3	22	24
Thrombocytopenia	45 (90%)	17	8	15	5
Clinical Chemistry					
AST	8 (16%)	7	1	–	–
ALT	10 (20%)	9	1	–	–
Total bilirubin	5 (10%)*	4	–	–	–
Urinalysis					
Urinary protein†	4 (8%)	4	–	–	–

Adverse drug reactions were graded according to criteria established by the Japan Society for Cancer Therapy.[14]
*1 patient < grade 1.
†The number of evaluable patients was 49 for urinary protein.
Abbreviations: AST = aspartate aminotransferase; ALT = alanine aminotransferase

Original article

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Phase I pharmacokinetic trial of the selective oral epidermal growth factor receptor tyrosine kinase inhibitor gefitinib ('Iressa', ZD1839) in Japanese patients with solid malignant tumors

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Background: This phase I dose-escalating study investigated the tolerability and toxicity of the selective epidermal growth factor receptor tyrosine kinase inhibitor gefitinib ('Iressa', ZD1839) in Japanese patients with solid tumors. Thirty-one patients were included.

Patients and methods: Patients initially received a single oral dose of gefitinib followed by 10–14 days of observation. Oral gefitinib was subsequently administered on 14 consecutive days, every 28 days. Dose escalation was from 50 mg/day to a maximum of 925 mg/day or dose-limiting toxicity (DLT).

Results: Most adverse events were mild (grade 1/2); the most frequent were an acne-like rash and gastrointestinal effects. Two of six patients at 700 mg/day had DLT; no further dose escalation occurred. C_{max} was reached within 3–7 h and exposure to gefitinib increased with dose. Mean terminal half-life following multiple dosing was 50.1 h (range 27.8–79.7 h). A partial response (duration 35–361 days) was observed in five of the 23 patients with non-small-cell lung cancer over a range of doses (225–700 mg/day), and seven patients with a range of tumors had disease stabilization (duration 40–127 days).

Conclusions: In conclusion, gefitinib showed a favorable tolerability profile in Japanese patients. The safety profile, pharmacokinetic parameters and antitumor activity observed in our study are comparable to those observed in patients from the USA and Europe.

Key words: efficacy, EGFR inhibitor, gefitinib, 'Iressa', phase I trial, tolerability, ZD1839

Introduction

Specific inhibition of epidermal growth factor receptor (EGFR) function is an attractive therapeutic target in anticancer treatment. Potential new therapies are under development that modulate the activation of this signal transduction pathway, resulting in inhibition of mitogenesis and other cancer-promoting processes [1]. The extracellular ligand-binding region of the EGFR has been targeted by monoclonal antibodies such as cetuximab [2], while agents that target the intracellular tyrosine kinase region include small-molecule tyrosine kinase inhibitors (TKIs) such as gefitinib ('Iressa', ZD1839) [3] and erlotinib [4]. Advantages of these compounds compared with standard chemotherapy include their ability to inhibit specific deregulated pathways in cancer cells with minimal effects on normal cell function. This class of agents may therefore offer antitumor activity with a better-tolerated adverse event profile than traditional agents.

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The rationale for targeting the EGFR comprises several key points. Activation of the EGFR tyrosine kinase has been found to be a key factor in cell proliferation and has been implicated in the control of cell survival, decreased apoptosis and increased metastasis [5]. Furthermore, the EGFR is expressed or highly expressed in a wide variety of human solid tumors, and high-level expression has been associated with advanced disease, development of a metastatic phenotype and poor prognosis [6, 7].

Gefitinib is an orally active, selective EGFR-TKI that blocks signal transduction pathways implicated in the proliferation and survival of cancer cells and other host-dependent processes promoting cancer cell growth. Early preclinical studies indicated that, *in vitro*, gefitinib potently inhibited EGFR tyrosine kinase activity at low concentrations that did not significantly affect other kinases tested [8]. Preclinical toxicology studies showed gefitinib to have a favorable tolerability profile over 6 months of oral dosing in animals, with mechanism-based, dose-dependent reversible effects on the skin, cornea, kidney, liver and ovary [9]. This range of toxicity is explained by the fact that EGFR signal transduction is involved in the normal physiology of these organs. Gefitinib has been shown to inhibit growth of a range of human tumor cell lines expressing EGFR (IC_{50} 0.2–0.4 μmol/l) when used as a single

agent, and to potentiate the activity of cytotoxic agents [10]. It also showed antitumor activity in various xenograft models [10, 11]. Gefitinib was well tolerated in healthy volunteers and demonstrated a terminal half-life of 28 h, which suggests that once-daily oral administration is appropriate [12].

Our study was performed to investigate the safety and pharmacokinetics of gefitinib in Japanese patients and to enable a comparison between Japanese patients and non-Japanese patients participating in a parallel study in the USA and Europe [13] in accordance with regulatory requirements. It was principally designed to evaluate the tolerability of increasing oral doses of gefitinib in Japanese patients with solid malignant tumors, using an intermittent dosing schedule to ensure the patients' safety.

Patients and methods

Trial design

We conducted an open, multicenter, non-randomized, phase I, dose-escalating study, recruiting patients at four centers in Japan. Our primary objective was to investigate the tolerability and toxicity of single and multiple oral doses of gefitinib in patients with solid malignant tumors. Secondary objectives included assessment of pharmacokinetics and antitumor activity. In addition, we compared our pharmacokinetic results with those from patients taking part in a parallel study in the USA and Europe.

Patient eligibility

We enrolled patients with solid malignant tumors that were resistant to standard therapies or for which the investigator believed no appropriate treatment was available. Tumors were among those known to commonly express or overexpress EGFR, but patients were not selected on the basis of individual EGFR status. Patients aged 20–74 years with a life expectancy of ≥3 months and World Health Organization (WHO) performance status of ≤2 were eligible for inclusion. Exclusion criteria have been described previously [13].

Prior to initiation of the study, we recorded information on each patient's background and treatment history and conducted assessments, including a physical examination, vital signs, performance status, clinical laboratory tests (hematology, blood biochemistry, urinanalysis, fecal test), ophthalmologic assessments and 12-lead ECG with measurement of PR intervals. All patients gave written informed consent and the study was conducted in accordance with 'Good Clinical Practice for Trials on Drugs' [14] and the 'Declaration of Helsinki' [15].

Treatment

In the first part of the trial, patients received a single oral dose of gefitinib, followed by 10–14 days of observation. If drug exposure was well tolerated, patients progressed to the second part of the study and received the same dose repeated daily for 14 days, followed by 14 days of observation (one cycle), based on advice from the Efficacy and Safety Evaluation Committee. A parallel study in the USA and Europe was conducted in a similar dose-escalation manner (except that the single dose was given only at the 50 mg dose level) and we compared, on an ongoing basis, pharmacokinetic data following single and multiple dosing in this study with data from our study to determine whether similar dose dependency was observed in Western and Japanese patients and to consider whether prior single dosing to establish safety was necessary at each dose level.

The 50 mg starting dose was chosen on the basis of preclinical animal toxicology studies and two clinical studies in healthy volunteers. The Western volunteer studies at single doses up to 100 mg/day showed that the maximum plasma concentration (C_{max}) and the area under the plasma concentration–time curve from 0 to 24 h (AUC_{0-24}) increase linearly with dose. Provided the disposition of gefitinib in our patient population is similar to that in the healthy Caucasian volunteers, C_{max} and AUC_{0-24} following the initial single 50 mg dose will be ~17 ng/ml and 220 ng·h/ml, respectively. These values represent approximately one-third and one-fifth, respectively, of the exposure at the no-effect dose in rats, the most sensitive species, in the 28-day toxicology studies (2 mg/kg/day). Following multiple dosing at 50 mg for 14 consecutive doses, predictions from the Western volunteer data suggest that the steady-state C_{max} and AUC_{0-24} will be ~35 ng/ml and 500 ng·h/ml, respectively. These values represent approximately one-fifteenth and one-tenth, respectively, of the exposure at the NOAEL (no observed adverse effect level; 10 mg/kg/day) in the 28-day toxicology studies in rats. The single dose of 50 mg/day caused no clinically significant adverse effects in the volunteers.

We planned to escalate the dose to 100, 150, 225, 300, 400, 525, 700 and 925 mg/day, with the option to omit dose levels following consideration of the results of the parallel USA/European study. We initially entered four patients' at each dose level, but if National Cancer Institute-Common Toxicity Criteria (NCI-CTC 2.0) grade 3 or 4 drug-related toxicity occurred in one of these patients, we enrolled two additional patients. Dose-limiting toxicity (DLT) was defined during the first treatment period as any grade 3/4 drug-related adverse effect, significant corneal epithelial change or PR interval (measured by 12-lead ECG) prolongation attributed to gefitinib (as prolongation of the PR interval was noted in preclinical animal toxicity studies). The dose at which DLT occurred in more than two patients was defined as the maximum tolerated dose (MTD). Dose escalation took place following a review of safety data when all patients at a dose level reached day 28 or had been removed from the trial due to drug-related toxicity, and following consideration of the results from the Western study.

Appropriate supportive care measures and symptomatic treatment were permitted, as was prophylactic use of antiemetics during the second and subsequent cycles, but not during the first 28 days. Any grade 3/4 nausea that was not readily managed with antiemetics was classed as DLT.

Following completion of the first 14 days of treatment and 14 days of observation, patients demonstrating clear clinical benefit could remain on gefitinib (14-day treatment period, every 28 days) if there was no drug-related DLT and if they fulfilled eligibility criteria. Gefitinib treatment was discontinued in the event of ocular toxicity, cardiac conduction defects, disease progression, any DLT, withdrawal of consent or if it was in the patient's best interest to discontinue treatment. Following withdrawal, patients were monitored for 30 days for reversibility of drug-related adverse effects or occurrence of new adverse effects. No intra-patient dose escalation or reduction was allowed.

Safety assessments

We recorded the incidence, type and severity of adverse effects at each dose level. If grade 3/4 myelosuppression was observed, we carried out hematology assessments at least every 2 days until values returned to grade 1/2. All clinical laboratory tests were conducted at screening, pre-first dose, 24 h after the single dose, on days 1, 3, 8, 15, 22 and 29, and at withdrawal. Ophthalmologic assessments, including slit-lamp examination, were carried out at screening, 48 h after the single dose, on days 8, 15 and 22 and at withdrawal. The other safety parameters were also reassessed periodically throughout the trial.

Pharmacokinetic analysis

During the first cycle, plasma concentrations of gefitinib were determined from blood samples (4 ml) using liquid–liquid extraction and high-performance liquid chromatography with mass spectrometric detection [16]; pharmacokinetic parameters were calculated by standard methods. Blood samples were taken pre-first dose, and at 1, 3, 5, 7, 12, 24, 48, 72, 96, 120 and 144 h after administration of the single dose. The same sampling times were used following the last dose of the multiple dosing administration (i.e. day 14) and samples were also taken pre-dose on days 1, 3, 7 and 10 during multiple dosing.