Table 1
Patient characteristics

	Number of patients
Number of treated	11
Sex	
Male	9
Female	2
Age (years)	
Median	73
Range	70–78
ECOG PS	
0	2
1	9
Histology	
Adenocarcinoma	6
Squamous cell carcinoma	5
Stage	
III A	2
III B	2
IV	3
Recurrent disease	4
Previous therapy	
None	7
Surgery and radiotherapy	2
Chemotherapy and radiotherapy	2

ECOG, Eastern Cooperative Oncology Group.

3.1. Toxicities

Hematologic toxicities. No hematologic toxicity was observed among patients treated at dose level 1 (25 mg/m^2/week). However, three of the five patients treated at dose level 2 (30 mg/m^2/week) experienced grade 3/4 neutropenia (Table 2). Of these, one patient had grade 4 neutropenia that lasted 4 days despite G-CSF support, and another experienced grade 3 febrile neutropenia and received antibiotics for 3 days. None of the patients required blood transfusion.

3.2. Non-hematologic toxicities

Treatment-related non-hematologic toxicities are summarized in Table 3. Nausea/vomiting, usually mild to moderate (grade 1 or 2), was the most frequent non-hematologic toxicity, and occurred in five patients (45%). Moderate liver dysfunction (elevation of ALT or AST) was also observed in six patients (55%). One patient experienced grade 3 diarrhea, but recovered within a few days. One patient had a single episode of grade 3 syncope and recovered with no sequelae; this patient also developed grade 2 chronic heart failure, which was considered unrelated to study treatment. Premedication with 8 mg of dexamethasone were performed for a few patients to prevent a hypersensitive reaction, however, no patient experienced allergic adverse events regardless of the administration of corticosteroids.

Table 3
Non-hematologic toxicities and maximum grade per patient

Toxicity	Dose level 1	Dose level 2
	Grade 1/2/3/4	Grade 1/2/3/4
Nausea/vomiting	2/0/0/0	2/1/0/0
Alopecia	0/1/0/0	1/0/0/0
Fatigue	0/0/0/0	1/1/0/0
Edema	0/0/0/0	0/1/0/0
Constipation	1/0/0/0	0/0/0/0
Diarrhea	1/0/0/0	1/0/1[a]/0
Neurosensory	0/1/0/0	0/0/0/0
Syncope	0/0/0/0	0/0/1/0
Vertigo	0/1/0/0	0/0/0/0
Liver (AST/ALT)	1/1/0/0	3/1/0/0
Electrolytes	2/0/0/0	2/0/0/0
Infection	1/0/0/0	0/0/1[a]/0
Chronic heart failure	0/0/0/0	0/1/0/0

[a] Dose-limiting toxicity.

3.3. Dose-limiting toxicities

The DLTs of this docetaxel regimen were neutropenia and diarrhea. Among five patients who received docetaxel 30 mg/m^2/week, one developed febrile neutropenia and grade 3 diarrhea, another experienced grade 4 neutropenia for more than 4 days, and the third did not receive day-8 treatment because of grade 3 neutropenia. According to the protocol definition, the MTD of this treatment was 30 mg/m^2.

Table 2
Hematologic toxicity by cycle

Dose level	No. pts.	No. cycles	Number of cycles with toxicity (NCI grade)									
			Neutropenia			Febrile neutropenia	Platelets			Hemoglobin		
			2	3	4		2	3	4	2	3	4
1	6	10	0	0	0	0	0	0	0	1	0	0
2	5	9	0	2[a]	1[a]	1[a]	0	0	0	0	0	0

3.4. Antitumor activity

Ten patients were reevaluated after two treatment cycles. One patient without disease progression was withdrawn from the study at the physician's discretion and was not reevaluated. Two patients (20%) treated at dose level 2 (30 mg/m^2/week) had a partial response to weekly docetaxel, neither of whom had received previous chemotherapy. Six of the 10 patients (3 at dose level 1, 3 at dose level 2) had stable disease. Two patients treated at dose level 1 (25 mg/m^2/week) were evaluated as progressive disease after the treatment.

4. Discussion

As reported by Langer et al., although response, toxicity, and survival rates of elderly lung cancer patients receiving platinum-based therapy were similar to those in younger patients, leukopenia and neuropsychologic toxicities occurred with higher frequency in those over 70 years of age [17]. Oshita et al. also emphasized caution in administering chemotherapy to elderly patients because of the high risk of myelosuppression, even if they appear to have normal organ function [18]. An Italian group reported that severe neutropenia (> grade 3, WHO criteria) was observed in 23% of patients receiving the vinorelbine/gemcitabine doublet and in 17% of those receiving vinorelbine alone. In addition, a few toxic deaths occurred with both regimens. These authors stated that the toxicity in elderly patients was not negligible, even in those receiving monotherapy [8].

Hainsworth et al. have reported results of two clinical trials of weekly docetaxel for elderly NSCLC patients [15,16]. In a phase I trial, the maximum-tolerated weekly dose of docetaxel was 43 mg/m^2, with fatigue and asthenia as DLTs [15]. In a subsequent phase II trial, grade 3 leukopenia was noted in only 8% of patients and none developed grade 4 myelosuppression, which was an encouraging result for elderly patients [16]. However, that trial also included 19 younger patients (< 70 years old) among 39 enrolled patients and patients with good PS and those with poor PS were mixed up regardless of their age. To our knowledge, there is no evidence that elderly patients with good PS and younger patients with poor PS can be regarded as the same patient population. We suspect there may be differences between those two groups, especially in the toxicity profile, and the results of the current study support that suspicion. Trials evaluating treatments in poor PS patients should be conducted separately from those in elderly patients.

In the current trial of weekly docetaxel for elderly NSCLC patients, the maximum tolerated weekly dose of docetaxel was 30 mg/m^2, with neutropenia and diarrhea as DLTs. Interestingly, the MTD and the toxicity profile of this study were markedly different from those in a previous phase I study, in which the MTD was 43 mg/m^2 and the DLTs were fatigue and asthenia. Despite the lower docetaxel dose in the current trial, myelosuppression was more severe than in the previous study. Three of the 11 (27%) enrolled patients had grade 3 or 4 neutropenia, which was a higher rate than in previous reports (8–14%). Of the three patients who experienced grade 3/4 neutropenia, two had been previously treated, one with chemotherapy and radiotherapy and the other with surgery and radiotherapy, which suggests no obvious relationship between occurrence of neutropenia and having received previous treatment. We also found no particular differences between characteristics of patients who did or did not experience neutropenia. No patients were administered immunosuppressive nor myelosuppressive agents including non-steroid antiinflammatory drugs during the study period. There were also no patients with chronic disease known to cause anemia. Although our small sample failed to demonstrate any clear trends, it should be emphasized that elderly patients themselves are at high risk of myelosuppression even with this weekly regimen.

Non-hematologic toxicities were relatively moderate in severity. Only two patients experienced grade 3 toxicity, and their symptoms were transient and reversible. This toxicity profile may owe to exclusion criteria that limited patients with severe comorbidities to enroll the present study. Syncope and chronic heart failure were observed in one patient, and while it was difficult to determine whether these effects were related to docetaxel, such symptoms are commonly seen in elderly patients. Fatigue, the major DLT in the previous study of weekly docetaxel, occurred in only a few patients.

According to the result of this phase I study, the recommended dose for future trials is 25 mg/m^2/week. The overall objective response rate of the treatment was 18%, however, no response had observed in patients treated at 25 mg/m^2/week. Although we cannot conclude the antitumor activity of this dosage in this small study, a phase II trial that evaluate the activity of weekly docetaxel at a dose of 25 mg/m^2/week should be performed before a large randomized trial.

5. Conclusion

In this phase I trial, the MTD of weekly administration of docetaxel to elderly NSCLC patients was 30 mg/m^2/week, with neutropenia and diarrhea as DLTs. The recommended dose for future trials is 25 mg/m^2/week. Although this treatment was generally well tolerated, 27% of patients experienced grade 3 or 4 neutropenia. The risk of myelosuppression still requires careful attention when using this regimen for elderly NSCLC patients.

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Original articles

Mutational analysis of the β-tubulin gene in lung cancer

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Abstract

Recently, several studies have suggested that a major mechanism of resistance to paclitaxel might involve mutations in the β-tubulin gene in tumor cells. To investigate the frequency of β-tubulin mutations in Japanese patients with small and non-small cell lung cancer, direct sequence analysis following reverse transcription-polymerase chain reaction (RT-PCR) of the β-tubulin gene was performed using total RNA from 20 lung cancer cell lines and 22 specimens from lung cancer patients. First-strand cDNA sequence analysis of the 42 samples showed silent mutations at codon 180 of the β-tubulin gene, which encodes the GTP-binding site of the protein, and codons 195 and 217. However, neither missense nor non-sense mutations affecting microtubule dynamics, within or near the GTP-binding site of the β-tubulin gene, were detected. These results indicate that β-tubulin gene mutations might not play a major role in the mechanism of resistance to paclitaxel in Japanese lung cancer patients. Further investigations are needed to clarify the mechanism of drug resistance.

Keywords: Paclitaxel; Small cell lung cancer (SCLC); Non-small cell lung cancer (NSCLC); Single nucleotide polymorphism (SNP); Multidrug resistance (MDR); Doxorubicin

1. Introduction

Although several studies have indicated that the multidrug resistance (MDR) phenotype mediated by the P-glycoprotein (Pgp) might be an important mechanism of resistance to paclitaxel [1,2], β-tubulin gene mutations in cancer cells have been recently shown to be closely associated with resistance. [3–6]. Cancer cell lines selected for resistance to paclitaxel by continuous exposure to the drug were reported to contain biochemically altered forms of β-tubulin and point mutations within or near the GTP-binding sites of the protein [3–5]. Furthermore, Monzo et al. reported a close correlation between point mutations in the β-tubulin gene in tumor samples from non-small cell lung cancer (NSCLC) patients and resistance to paclitaxel [6]. However, some of the mutations reported in that study were not located at the GTP-binding sites and their role in resistance remains unclear.

As there has been no previous report examining the β-tubulin gene in small cell lung cancer (SCLC), in the present study we conducted a mutational analysis of the of the β-tubulin gene (which contains GTP-binding sites) in SCLC and NSCLC.

2. Materials and methods

2.1. Cell lines

Ten SCLC and ten NSCLC cell lines were used in this study. The ten SCLC cell lines (Ms series) and seven of the NSCLC cell lines (Ma series) were kindly provided by Dr Masuda (Osaka Prefectural Habikino

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Hospital); cell line A549 (NSCLC) was purchased from Dainippon Pharmaceutical Co. Ltd.; cell lines PC-9 and PC-14 (NSCLC) were kind gifts of Dr Nishio (National Cancer Center Research Institute, Tokyo, Japan). All cell lines were cultured in RPMI 1640 containing 5% fetal bovine serum, penicillin G (100 units/ml), and kanamycin (0.1 mg/ml) at 37 °C in 10% CO_2–95% air atmosphere.

2.2. Tumor samples

Seventeen NSCLC tumor specimens and the surrounding normal lung tissues were obtained at Osaka City General Hospital from March 1996 to April 1999 from lung cancer patients who had not received prior chemotherapy. SCLC tumor samples were obtained from malignant pleural effusions of five SCLC patients at Kinki University Hospital from October 1999 to March 2000. None of the SCLC patients were treated with anti-microtubule agents prior to this study.

2.3. Isolation of total RNA and genomic DNA

Each lung cancer cell line (1.0×10^6 cells) or tumor specimen was homogenized using a QIAshredder (QIAGEN, Hilden, Germany). Total cellular RNA and genomic DNA were extracted from each sample with the RNeasy Mini Kit (QIAGEN) and the QIAamp DNA Mini Kit (QIAGEN), respectively. In case of the extraction of RNA, contaminant DNA in the samples was removed by treatment with RNase-free DNase (QIAGEN) for 15 min at room temperature. To confirm the elimination of contaminant DNA from each RNA extract and success in the extractions of genomic DNA, 1 μl of each extract was amplified via a set of human beta globin primers (Takara, Shiga, Japan) following the recommendation of the manufacturer. The PCR products from RNA or DNA extract were electrophoresed in 2% agarose gel and visualized with ethidium bromide staining under ultraviolet light. A corresponding band was observed in each PCR product of the DNA extract, but not in any of the RNA extracts.

2.4. RT and PCR

First-strand cDNA was synthesized by mixing 1 μg of total RNA with random hexameric oligonucleotide primers (Life Technologies, Inc., MD) and Superscript reverse transcriptase II (Life Technologies, Inc., MD). cDNA or genomic DNA extract from each sample was then subjected to PCR according to the manufacturer's recommendations. PCR and the subsequent sequence analysis were performed to detect point mutations in exon four of the class 1 β-tubulin gene, where frequent mutations had been reported in clinical samples [6]. The following previously published oligonucleotide primer pair for the β-tubulin gene [6] was used for PCR and cycle sequencing: forward 5′-AGAGAGCTGTGACTGCCTG-3′ and reverse 5′-TGAGTTCCGGCACTGTGAG-3′. After denaturing at 95 °C for 5 min, PCR amplification was performed in a PTC-225 thermal cycler (MJ Research, Inc., MA) for 35 cycles using the following conditions: denaturation at 95 °C for 1 min; annealing at 68 °C for 1 min; and extension at 72 °C for 2 min. The final extension was at 72 °C for 7 min. The expected size of the PCR products was 497 bp. PCR products were electrophoresed and visualized as mentioned above.

2.5. Isolation of DNA

2.5.1. Sequencing

PCR products were purified using the QIA Quick PCR purification kit (QIAGEN, Hilden, Germany) and sequenced by BigDye terminator chemistry (Roche Molecular Systems, Inc., NJ) using the ABI 310 automated sequencer (Perkin–Elmer, Inc., NJ). The same oligonucleotide primers used in the PCR were used as sequencing primers in this study. Purified PCR products were used as the templates in cycle sequencing reactions. All samples were sequenced on both strands. The sequencing data obtained were compared to the wild type β-tubulin sequences reported on the GenBank database (accession number; J00314).

3. Results

At first, direct nucleotide sequencing was performed using genomic DNA from tumor samples and cell lines; the raw sequencing data, however, revealed multiple non-specific nucleotide sequences which were thought to result from amplifications of human β-tubulin pseudogenes. Fig. 1 showed that a non-specific wave, thymine at codon 139 and cytosine at codon 140 besides the normal waves, which were identical with sequences of a human β-tubulin pseudogene, clone 46-β at the corresponding sites [15]. To avoid contamination of PCR products with sequences from pseudogenes, DNase treatment was performed before RT-PCR and DNA sequencing. Using this protocol, we succeeded in detecting nucleotide sequences only from the class 1 β-tubulin gene (Fig. 1B). The characteristics of the mutations detected in this study are summarized in Table 1. The presence of gene alternations was confirmed by direct sequencing of genomic DNA from the tumor samples.

Silent mutations in the β-tubulin gene were detected in seven of the 20 cell lines and in four of the 22 tumor specimens. Neither missense nor non-sense mutations within or near the GTP-binding sites of the class 1 β-tubulin gene were detected in this study. A het-

erozygous silent mutation at codon 217 (CTG to CTA) was observed in four SCLC and one NSCLC cell lines (Fig. 2A). Double heterozygous silent mutations at codon 180 (GTC to GTT) and codon 195 (AAT to AAC) were observed in two NSCLC cell lines and two tumor specimens. Two other NSCLC tumor specimens harbored the same two types of nucleotide alteration, but only either at codon 180 or codon 195 (Fig. 2B and C). All of four NSCLC patients whose tumor specimens contained the silent mutation at codon 180 and/or codon 195 had the same gene alternation in the corresponding normal tissue.

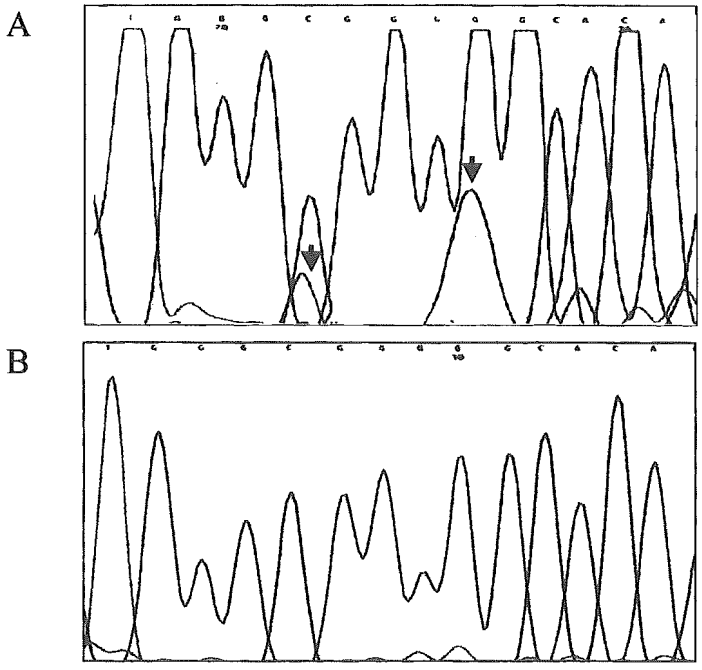

Fig. 1

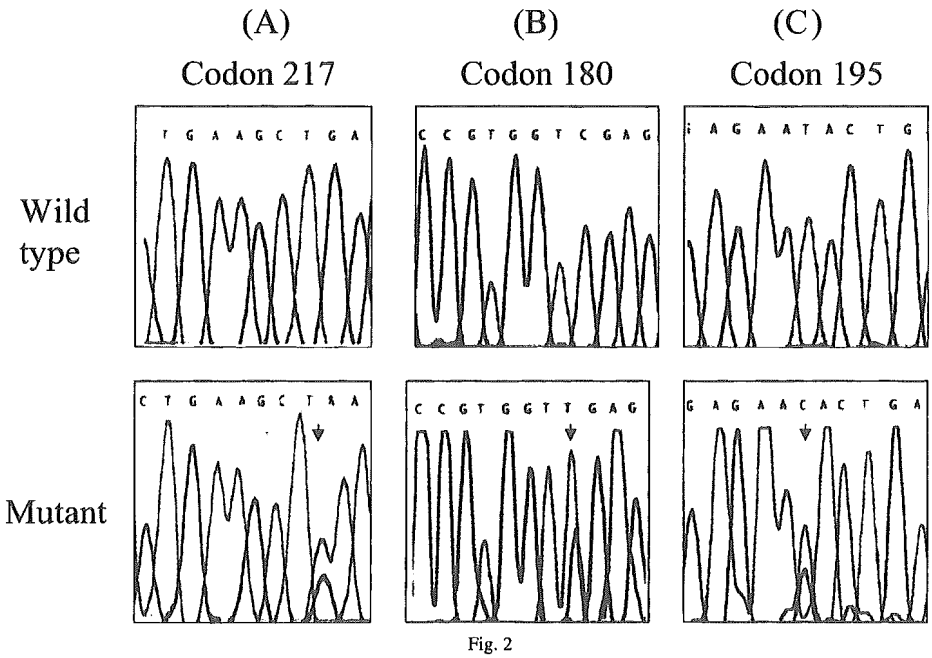

Fig. 2

Fig. 1. The results of sequence analysis using genomic DNA (A) and cDNA synthesized from RT-PCR following DNase treatment (B) in a NSCLC cell line. Arrows indicate non-specific nucleotide sequences found at codon 139 and 140 besides normal sequences. The left and right arrows indicate thymine and cytosine, respectively.

Fig. 2. Representative results of nucleotide sequence analysis. Wild type (top) and mutant (bottom) β-tubulin gene sequences at codons 217 (A), 180 (B), and 195 (C) are shown in the sense direction. The locations of the mutations are indicated with arrows.

Table 1
β-Tubulin mutations detected in lung cancer cell lines and tumor specimens

Codon	Nucleotide change	Amino acid change[a]	Cell lines		Tumor specimens		Total ($n = 42$)
			SCLC ($n = 10$)	NSCLC ($n = 10$)	SCLC ($n = 5$)	NSCLC ($n = 17$)	
217	CTG-CTA	Leu-Leu	4	1	0	0	5
180	GTC-GTT	Val-Val	0	0	0	1[b]	1
195	AAT-AAC	Asn-Asn	0	0	0	1[b]	1
180 & 195	GTC-GTT & AAT-AAC	Val-Val & Asn-Asn	0	2	0	2[b]	4
Total			4	3	0	4	11

[a] Leu: leucine, Val: valine, Asn: asparagin.
[b] Identical nucleotide changes were detected in normal tissues.

4. Discussion

Paclitaxel is a novel anticancer agent with activity in a broad range of epithelial cancers, including carcinomas of the ovary, breast, head, neck, bladder, lung, and prostate [7–10]. The drug acts by stabilizing microtubule assembly and reducing spindle microtubule dynamics. However, there are some patients who do not respond to paclitaxel even when it is used as first-line chemotherapy. Furthermore, most patients who initially respond to paclitaxel subsequently relapse and often become resistant to further treatment with paclitaxel due to the acquisition of drug resistance. The mechanism of resistance to paclitaxel might be related to a type of gene mutation that influences microtubule dynamics. To date, point mutations in the β-tubulin gene have been reported in human cancer cell lines selected by growing them in the presence of paclitaxel [11]. Furthermore, Monzo et al. reported the frequent occurrence of class 1 β-tubulin mutations in NSCLC patients and a close correlation between presence of a mutation and clinical resistance to paclitaxel [6]. In our study, mutational analysis of the β-tubulin gene in lung cancer cell lines and in clinical tumor specimens from patients with lung cancer revealed the presence of silent mutations at three different codons. Two of them, mutations at codons 180 and 190, were single nucleotide polymorphisms because the same gene alternations were found in the corresponding normal lung specimens. As silent mutations at codon 217 were detected only in SCLC cell lines, it was not possible to determine whether the altered nucleotides represented polymorphisms.

No study on the incidence of β-tubulin mutations in SCLC patients has been previously reported. Considering the clinical effectiveness of paclitaxel in the treatment of SCLC, the low incidence of β-tubulin mutations in SCLC patients found in the present study might be reasonable. Although the earlier study described above reported the frequent occurrence of missense mutations in the class 1 β-tubulin gene in NSCLC tumor specimens [6], no missense mutations were detected in our study. This discrepancy in the incidence of β-tubulin mutation in NSCLC patients might be explained by the following four points. First, in comparison to the previous report, our study may have had some bias due to the small number of NSCLC patients analysed ($n = 17$). Monzo et al. investigated the β-tubulin gene status in the tumors of 49 NSCLC patients and reported missense or non-sense mutations in 16 patients (32.7%). Second, the amplification and sequencing methodology in our study is different from that of Monzo et al., and the sensitivity of the assay may have been different. Third, the discrepancy might be explained by the different races of the patients examined in each study. In fact, differences in the incidence of mutations among races was reported on the incidence of K-ras mutations in NSCLC patients [12,13]. Similarily, the incidence of β-tubulin mutations among Japanese NSCLC patients might be lower than that among Caucasian patients. Therefore, alternative mechanisms of paclitaxel resistance, that do not involve β-tubulin mutations, may exist for Japanese NSCLC patients. Finally, the previous study may have overestimated the incidence of β-tubulin mutations as a result of the existence of pseudogenes, since genomic DNAs only were used to analyze the β-tubulin gene in tumor specimens [6]. To date, seven pseudogenes of the human class 1 β-tubulin gene have been reported [14–17]. These pseudogenes retain significant homology to the corresponding regions of the class 1 β-tubulin gene (Table 2), and the nucleotide sequences of the primers used in the previous study and ours were identical to sequences in some of these pseudogenes, especially in human β-tubulin pseudogene, clone 46p or 46q. Therefore, the existence of pseudogenes makes it difficult to analyze the nucleotide sequence of the true β-tubulin gene using genomic DNA alone as shown in Fig. 1. Most of the non-specific sequences found in PCR products using genomic DNA in the present study were

Table 2
Comparison of the DNA sequence of M40 to those of seven β-tubulin pseudogenes in the region investigated in this study

	Homology (%)	GenBank accession number
HUMTBB1P	80.4	J00315
HUMTBB7P	84.9	K00842
HUMTBB11P	79.7	J00316
HUMTBB14P	88.9	K00840
HUMTBB21P	94.2	K00841
HUMTBB46P	93.8	J00317
HUMTBB46Q	93.2	M24191

identical with those of human β-tubulin pseudogenes at corresponding sites. Furthermore, none of these non-specific sequences were found in sequencing analysis using DNA-free-RNA template on the corresponding samples. To prevent PCR amplification of pseudogenes, we performed direct sequencing of RT-PCR products following RNase-free DNase treatment, which resulted in the detection of single peaks on the automated sequencer, except in the case of significant gene alternations.

As an alternative mechanism of resistance, recent studies have reported that increased expression of specific β-tubulin isotypes was associated with resistance to paclitaxel [18,19]. In contrast, Blades et al. showed that increased expression of diverse isotypes of β-tubulin was not sufficient to confer resistance to paclitaxel [20]. Thus, at the present time, the contribution of the expression of diverse β-tubulin isotypes to paclitaxel resistance remains controversial.

Furthermore, the MDR phenotype mediated by the 170-kd Pgp efflux pump is thought to be one of the most generalized mechanisms of drug resistance to tubulin-binding agents [1,21]. In fact, paclitaxel is a good substrate for this pump [22,23] and several studies have demonstrated that the development of resistance to paclitaxel is associated with increased expression of the MDR1 gene [1,2]. However, the observation that paclitaxel is effective in many patients with cancers that are refractory to anthracycline-containing regimens suggests that MDR may not play an important role in the clinical resistance to paclitaxel [24].

5. Conclusion

This study shows that the frequency of missense mutations in the class 1 β-tubulin gene in Japanese lung cancer patients is lower than that previously reported in Caucasian lung cancer patients. Further clinical investigations, not only concerning mutation frequencies but also the diverse expression of β-tubulin isotypes and the overexpression of MDR in tumor cells, are warranted to clarify the mechanism of resistance to anti-microtubule agents in Asian patients.

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Point mutations in the topoisomerase I gene in patients with non-small cell lung cancer treated with irinotecan

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Abstract

Reverse transcription polymerase chain reaction (RT-PCR) single-strand conformation polymorphism analysis was used to detect topoisomerase I (topl) mutations in total RNA from 16 specimens that were excised during surgery from eight patients with non-small cell lung cancer (NSCLC) who had received preoperative chemotherapy consisting of irinotecan (CPT-11) and cisplatin. PCR single-strand conformation polymorphism and subsequent DNA sequencing analysis showed two nucleotide substitutions resulting in Trp736stop (TGG to TGA) and Gly737Ser (GGT to AGT) in one tumor specimen. The mutations were located near a site in topl that was previously reported to harbor a mutation in the human lung cancer cell line PC7/CPT, which was selected for CPT resistance. These results demonstrate that mutations in topl occur after chemotherapy with CPT-11 in NSCLC patients and suggest that development of resistance to CPT-11 in some patients may involve mutation of topl. However, the significance of topl mutations to CPT resistance needs to be further investigated.

Keywords: Topoisomerase I; Mutation; Irinotecan; Drug resistance

1. Introduction

Combination chemotherapy consisting of irinotecan (CPT-11) and cisplatine (CDDP) is one of the standard therapies for patients with small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [1,2]. Furthermore, CPT-11, either alone or in combination with drugs such as 5-fluorouracil and leucovorin, also plays a vital role in the treatment of advanced colorectal cancer patients [3]. However, tumors in about half of cases are resistant to CPT-11 in the first treatment and those tumors that initially respond to CPT-11 eventually acquire resistance. To overcome the problem of drug resistance and improve the clinical outcome of patients, we need to elucidate the mechanism of resistance to CPT-11 in tumor cells. Cancer cell lines selected for resistance to topoisomerase I (topl) inhibitors, which are cytotoxic in parental cells, have recently been shown to contain point mutations in several regions of the topl gene [4–9]. These mutations are thought to be associated with the observed resistance to the topl inhibitor. However, previous studies have failed to find topl gene mutations in specimens obtained from cancer patients [10,11]. In this study, we report point mutations in the topl gene in a tumor specimen obtained from a patient with locally advanced NSCLC who had already received combination chemotherapy containing CPT-11.

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2. Material and methods

2.1. Patients and tumor tissues

The present study concerns paired samples of normal and tumor tissue, and eight pairs of specimens from a primary lung tumor and its corresponding normal tissue were obtained surgically from eight patients with NSCLC at the Osaka General Medical Center from December 1994 to April 1996. All patients were determined to be clinical stage IIA and diagnosed histologically as having lung cancer (three adenocarcinoma, four squamous cell carcinoma, and one large cell carcinoma), before they were enrolled in a clinical trial of induction chemotherapy [12]. The characteristics of the patients are shown in Table 1. All patients received two cycles of preoperative chemotherapy consisting of CPT 60 mg/m^2 (day 1, 8, 15) and CDDP 80 mg/m^2 (day 1). Surgical removal of the residual tumor was carried out 2–4 weeks after last administration of CPT. Tumor response was determined according to WHO criteria and clinical stage was assigned using the staging system of the US Department of Veterans Affairs Lung Study Group [13]. This study was approved by the ethical committee of the Osaka General Medical Center, and a written informed consent was obtained from each patient in the study. Samples were immediately frozen in liquid nitrogen and stored at −70 °C until further analysis. The identity of each tissue sample was confirmed microscopically. Specimens were free of necrosis or damage that might have been caused during preparation or storage.

2.2. RNA preparation and reverse-transcription polymerase chain reaction (RT-PCR)

Histological analysis of tumor samples obtained by surgery showed that they consisted mainly of NSCLC cells. Total RNA was then isolated from the samples by acid guanidine thiocyanate–phenol–chloroform extraction [14]. First-strand cDNA was synthesized by mixing 1 g of total RNA with random hexameric oligonucleotide primers (Life Technologies, Inc, MD) and Superscript reverse transcriptase II (Life Technologies). Target sequences of the human topl gene were amplified for 35 cycles with specific primers according to a protocol recommended by the manufacturer (Takara Ex Taq; Takara Shuzo, Shiga). Oligonucleotide primers for PCR and nucleotide sequencing were designed based on the published nucleotide sequences of the topl gene available in the GenBank database (accession numbers M60705 and M60706). The target sequence contained the four mutation sites (at cordons 361, 363, 722 and 729) that have been found in CPT-resistant tumor cells [4–6,8]. The name and nucleotide sequence of each primer, and the location in the topl gene of the amplified DNA fragment is as follows: KT1s, 5′-GAGAGGATTGCTAACT-3′, nucleotides 1253–1268; KT1a, 5′-GGAGGAGGAGAAGGAACCTTGGCATC-3′, nucleotides 1376–1401; KT2s, 5′-CGAGAGGAAATAAACAGATTGCCC-3′, nucleotides 2333–2357; and KT2a, 5′-GGCCCAGGCAAACTTCTCCCGCTGG-3′, nucleotides 2452–2476. All oligonucleotide primers were labeled with Rhodamine X (Takara, Japan). The expected lengths of the products obtained by amplification with KT1 and KT2 primers were expected to be 149 and 144 bp, respectively. A 10 μl volume of each PCR product was separated on a 2% agarose gel, which was subsequently stained with ethidium bromide and photographed.

Table 1
Patient characteristics

Total	8
Age (median)	45–47 (62)
Gender (male)	7
Clinical stage IIIA	
cT2N2M0	6
cT3N2M0	2
Histology	
Adenocarcinoma	3
Squamous cell	4
Large cell	1

2.3. Single strand conformation polymorphism (SSCP) analysis

Gene alterations in topl were examined by non-radiolabeled PCR-SSCP using the FM-BIO system (Takara). The PCR products described above were labeled with Rhodamine X and then mixed with an equal volume of 95% formamide and denatured at 95% for 5 min. Then, the denatured products were chilled on ice to stabilize the single-stranded DNA and separated on 5% polyacrylamide gels according to conditions recommended by the manufacturer (Takara).

2.4. DNA sequence analysis

PCR products were purified using the QIA Quick PCR purification kit (QIAGEN, Hilden, Germany) and then used as the templates in cycle sequencing reactions using the Autoload Solid Phase Sequencing Kit (Amersham Pharmacia Biotech, Japan), and analyzed by A.L.F.red II DNA sequencer (Pharmacia Biotech, Japan). The same oligonucleotide sequencing primers used in PCR were applied for sequencing analysis in this study. Each sample was sequenced on both strands. To establish that the Ma10 cells had a normal topl gene and could be used as a normal control in SSCP

Table 2
Results of preperative chemotherpay and topo1 mutation analysis

Patients histology	Response	Aberrant shift (SSCP)	DNA sequence
Ad	NC[a]	–	
Ad	NC	–	
Ad	NC	–	
Sq	NC	–	
La	NC	+	TGG 736 TGA GGT 737 AGT
Sq	PR[b]	–	
Sq	PR	–	
Sq	PR	–	

Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma.
[a] No change.
[b] Partial response.

analysis, we sequenced the Ma10 topl gene as described above for the tumor samples. All SSCP data obtained with the tumor samples was compared with those obtained with the Ma10 cells.

3. Results

Pairs of tumor samples and corresponding normal tissues from eight patients with NSCLC were studied. Four out of eight patients showed a partial response to preoperative chemotherapy; none of the patients achieved a complete response (Table 2). All of tissue specimens were obtained by surgery after two cycles of systemic chemotherapy consisting of CPT-11 and CDDP. Reverse transcription of the RNA isolated from the samples, followed by PCR-SSCP analysis was performed to detect mutations in two regions of the topl gene where point mutations had previously been reported in cell lines resistant to topl inhibitors [4,8,15]. A NSCLC cell line, Ma10, was used as a normal control, since direct sequencing of PCR products from this cell line did not reveal any mutations in the two regions analyzed here. By SSCP analysis an altered migrating band corresponding to the KT2 region, in addition to the normal band, was reproducibly observed in a tumor specimen obtained from one patient (Fig. 1), whose tumor did not respond to the preoperative chemotherapy (Table 2). The altered migration pattern of DNA from the tumor sample was not observed in the corresponding normal tissue. No abnormally migrating SSCP bands in the KT1 region were observed in any of the tumor or normal specimens. DNA sequencing analysis of the sample showing the abnormal migrating band in SSCP analysis revealed two serial nucleotide substitutions at cordons 736 (TGG to TGA) and 737 (GGT to AGT), which resulted in a nonsense, Trp736stop and a missense mutation, Gly737Ser (Fig. 2). Since the SSCP analysis in the sample showed only one mutant band in addition to a pair of wild band, these serial nucleotide substitutions were thought to reside on the same allele. To distinguish true mutations from experimental artifacts resulting from the amplification process, we repeated each experiment twice, starting from the first step of the RT reaction.

4. Discussion

The Japan Clinical Oncology Group and other cooperative study groups in Japan have demonstrated that combination chemotherapy consisting of CPT-11 and CDDP should be the standard therapy for patients with advanced NSCLC and extensive disease due to SCLC [1,2]. Furthermore, CPT-11 has proved to be a promising agent for patients with various cancers, including colorectal cancer [3]. However, most tumors ultimately acquire resistance to CPT-11 even if they responded well initially. Unfortunately, the main mechanism of resistance to the drug in such cancer patients remains unclear.

This is the first report of a mutation in the topl gene detected in a clinical sample. In this study, eight tumor specimens and eight corresponding normal tissues were examined for mutations in the topl gene; one of the tumor specimens was shown to harbor two point mutations resulting in Trp736stop or Gly737Ser on the same allele, and consequently the latter mutation would not be translated. It means that the tumor tissue would contain a truncated topl protein in addition to the wild type. The predominant expression of the truncated topo1 protein and lack of expression of the normal allele is expected to be highly resistant to CPT-11 in the tumor tissue, and actually, a previous investigation on a highly resistant cells to camptotecin revealed presence of mutant protein and lack of expression of the normal allele although showing the presence of both allele in genomic DNA of the cell [4]. Therefore, in the present study, the normal allele of the cancer cells must be

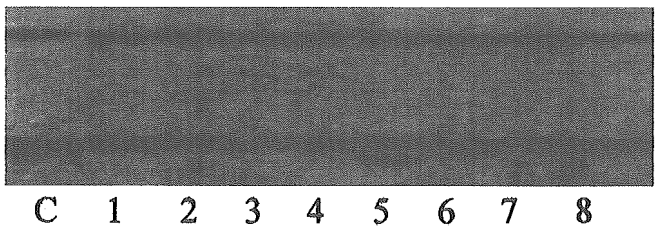

Fig. 1. PCR–SSCP analysis of the KT3 site of the topl gene in tumor specimens. A shift in mobility, compared with the pattern observed for normal control cells, was observed in a tumor sample obtained from patient 51 (Lane 5). Lane C, Ma10 cell (normal control); Lanes 1–8, tumor samples.

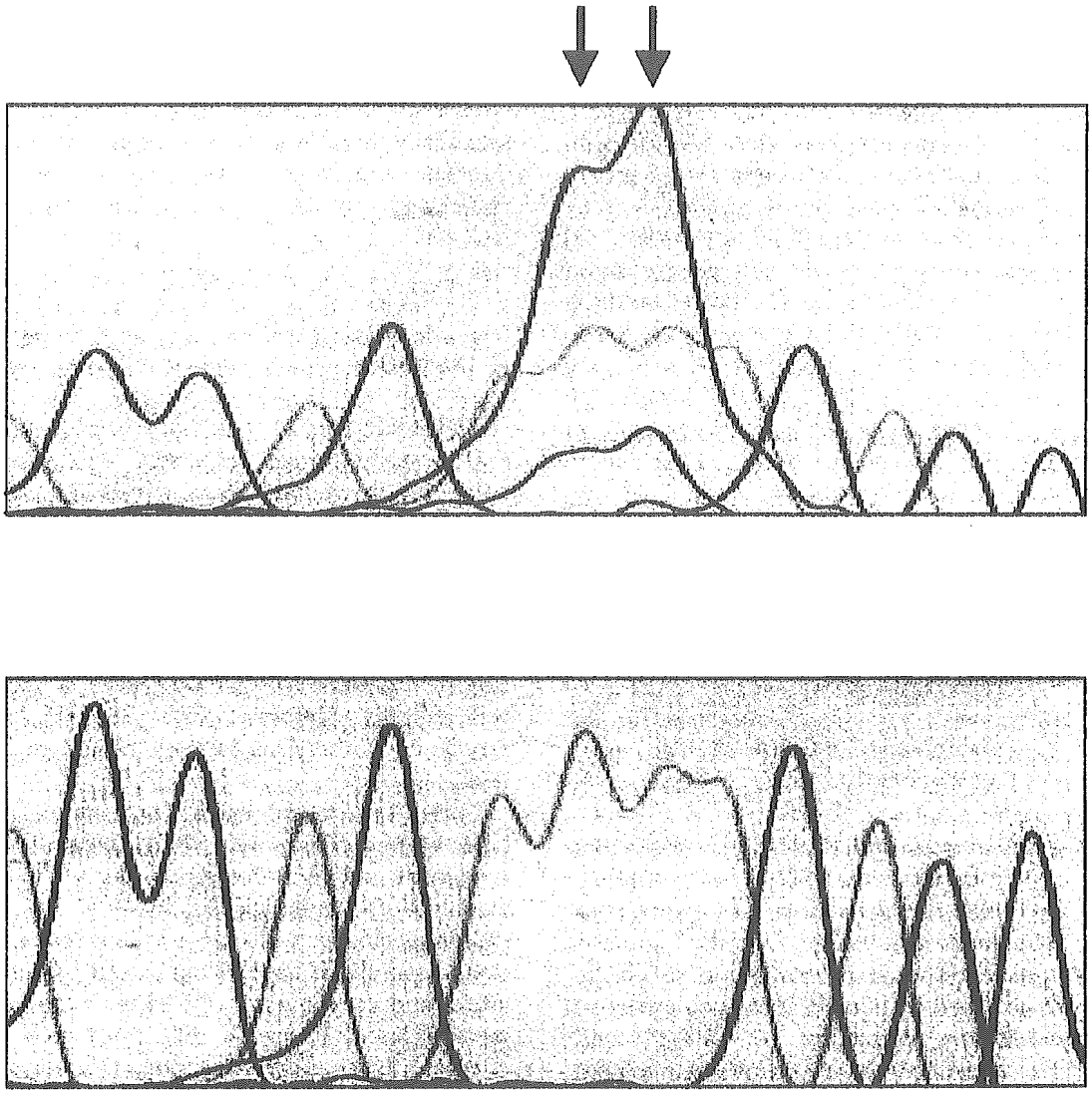

Fig. 2. The DNA sequences of the KT3 site in a tumor sample from patient 51 (top) and a normal control cell (bottom) are shown in the sense direction. The locations of the mutations in cordons 736 and 737 are indicated with arrows.

silent and the normal allele detected in SSCP and DNA sequencing analysis come from the interstitial component. To date, mutations in topl have been reported in several cancer cell lines, which are resistant to topl inhibitors [4–9]. These mutations are reviewed in Fig. 3. The mutations have been proposed to cause alterations in the topl protein, which could result in decreased topl catalytic activity or impaired CPT-topl binding, and develop resistance to CPT in cancer cells [4,8,9,15,16]. Although in the present study, Trp736stop was identified, a catalytic tyrosine, codon722 was conserved in the mutant cells, and then the truncated topo1 protein might be catalytically active. However, cleavage activity or CPT binding activity of the truncated topo1 might be reduced according to the previous report [4], and consequently might be resistant to CPT-11 in the tumor tissue. Actually, CPT resistance mutations have been reported even further away from the catalytic tyrosine, Phe361Ser [5] and Gly363Cys [6]. Those mutation sites were thought to be important in respect of CPT binding site on the enzyme. The presence of the cancer cells which express the truncated topo1 protein and lack of expression of the normal allele in the tumor tissue might cause the inefficiency of the agent in the patient. Therefore, Trp736stop identified here may play

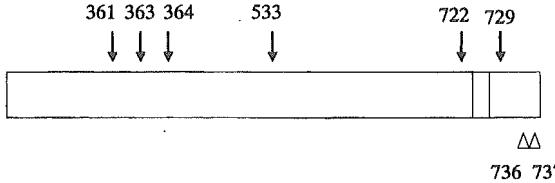

Fig. 3. A review of reports of mutations in the topl gene from cancer cell lines. Each arrow or number indicate the site of a mutation previously reported. The two arrow heads show the sites of the two mutations found in the present study. The sequence that encodes the catalytic region of the topl protein is shown as a closed square.

a role in the patient's resistance to preoperative chemotherapy with CPT-11 (Table 2).

Several researchers have failed to detect mutations in the topl gene in clinical specimens [10,11,21]. In previous studies, however, few patients were treated with CPT-11 before analysis; thus, the discrepancy between our findings and those obtained in previous studies may be due to the fact that we subjected all patients to chemotherapy with CPT-11 before conducting mutational analysis. We have also reported a point mutation in the topoisomerase II (top2) gene in a tumor specimen obtained after etoposide-containing chemotherapy [22]. Most previous studies that used the PCR-SSCP method to search for a mutation in the topl gene in clinical specimens failed to do so. However, the failure to detect topl gene mutations in clinical specimen may be due to the fact that SSCP analysis was not carried out under the appropriate conditions.

While in previous studies specimens were examined retrospectively, the present study was conducted prospectively, and the regimen of the chemotherapy and the timing of surgery were consistent among all patients.

SSCP analysis did not reveal the presence of abnormally migrating bands in several tumor specimens obtained from patients who did not respond to the CPT-11-containing regimen (Table 2). This finding suggested that an alternative mechanism may be responsible for resistance to CPT-11 in these patients. For instance, reduced RNA expression of the topl gene has been reported to correlate with resistance to CPT in several tumor cell lines [17–19]. It is thought that low levels of topl protein reduce the availability of the target of topl inhibitors, thereby decreasing the sensitivity of cells to such agents. On the other hand, CPT-11 and its active form, SN-38, have been shown to serve as substrates for the breast cancer resistance peptide (BCRP) [20]. Kawabata et al. reported that intracellular SN-38 accumulation was reduced in the cell line PC-6/SN2-5H, which was approximately 34-fold more resistant to SN-38 than the parental cell line PC-6, and that BCRP mRNA was expressed at higher levels, in proportion to the degree of SN-38 resistance [20]. That report suggested that BCRP is involved in CPT-11 resistance in clinical specimens.

Further investigations are needed to verify the clinical association between topl gene mutations and resistance to CPT-11 in cancer patients.

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IRINOTECAN PLUS CISPLATIN COMPARED WITH ETOPOSIDE PLUS CISPLATIN FOR EXTENSIVE SMALL-CELL LUNG CANCER

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ABSTRACT

Background Irinotecan hydrochloride, a topoisomerase I inhibitor, is effective against small-cell lung cancer. In a phase 2 study of irinotecan plus cisplatin in patients with extensive small-cell lung cancer, there was a high response rate and a promising median survival time.

Methods We conducted a multicenter, randomized, phase 3 study in which we compared irinotecan plus cisplatin with etoposide plus cisplatin in patients with extensive (metastatic) small-cell lung cancer.

Results The planned size of the study population was 230 patients, but enrollment was terminated early because an interim analysis found a statistically significant difference in survival between the patients assigned to receive irinotecan and cisplatin and those assigned to receive etoposide and cisplatin; as a result, only 154 patients were enrolled. The median survival was 12.8 months in the irinotecan-plus-cisplatin group and 9.4 months in the etoposide-plus-cisplatin group ($P=0.002$ by the unadjusted log-rank test). At two years, the proportion of patients surviving was 19.5 percent in the irinotecan-plus-cisplatin group and 5.2 percent in the etoposide-plus-cisplatin group. Severe or life-threatening myelosuppression was more frequent in the etoposide-plus-cisplatin group than in the irinotecan-plus-cisplatin group, and severe or life-threatening diarrhea was more frequent in the irinotecan-plus-cisplatin group than in the etoposide-plus-cisplatin group.

Conclusions Irinotecan plus cisplatin is an effective treatment for metastatic small-cell lung cancer. (N Engl J Med 2002;346:85-91.)

THE usual chemotherapy for extensive small-cell lung cancer is etoposide plus cisplatin or this combination in alternation with a regimen of cyclophosphamide, doxorubicin, and vincristine.[1-4] In preliminary studies, irinotecan hydrochloride, a topoisomerase I inhibitor, was effective against small-cell lung cancer,[5] and a phase 2 study of irinotecan plus cisplatin yielded a rate of complete response of 29 percent and an overall response rate of 86 percent (median survival, 13.2 months) in patients with extensive small-cell lung cancer.[6] For these reasons, we conducted a randomized, phase 3 study to compare irinotecan plus cisplatin with etoposide plus cisplatin in patients with extensive small-cell lung cancer.

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METHODS

Patients

To be included in the study, patients had to have cytologically or histologically confirmed small-cell lung cancer; extensive disease (defined by distant metastasis, contralateral hilar-node metastasis, or both; those with pleural effusion alone were excluded); no prior radiotherapy, chemotherapy, or surgery; measurable lesions; an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2; a life expectancy of at least three months; an age of 70 years or less; and adequate organ function. Staging of the tumor was based on the results of physical examination, chest radiography, fiberoptic bronchoscopy with biopsy and cytologic examination, computed tomography (CT) of the chest and the brain, ultrasonography or CT of the abdomen, radionuclide bone scanning, bone marrow aspiration or biopsy, and other tests as needed. Adequate organ function (adequate function of the bone marrow, liver, and kidney) was defined as indicated by a leukocyte count of at least 4000 per cubic millimeter, a platelet count of at least 100,000 per cubic millimeter, a hemoglobin level of at least 9.5 g per deciliter (5.9 mmol per liter), aspartate aminotransferase and alanine aminotransferase levels no higher than 100 IU per milliliter, a serum creatinine level no higher than 1.2 mg per deciliter (106 μmol per liter), and a creatinine clearance of at least 60 ml per minute.

The exclusion criteria were infection, diarrhea, ileus, interstitial pneumonitis, pulmonary fibrosis, uncontrolled diabetes mellitus, myocardial infarction within the preceding three months, massive pleural or peritoneal effusion, symptomatic brain metastases requiring whole-brain irradiation or administration of corticosteroids, a paraneoplastic syndrome, an active synchronous cancer, a metachronous cancer within three disease-free years, and pregnancy or breast-feeding.

Treatment Assignment and Drug Administration

The patients were randomly assigned to receive either a combination of irinotecan and cisplatin or a combination of etoposide and cisplatin by the minimization method of balancing the groups according to the institution and the patients' performance status. Randomization was performed at the Japan Clinical Oncology Group

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*Other participating institutions and investigators are listed in the Appendix.

(JCOG) data center according to the order in which information on enrollments was received by telephone or fax. The regimen of irinotecan and cisplatin consisted of four four-week cycles of 60 mg of irinotecan per square meter of body-surface area on days 1, 8, and 15 and 60 mg of cisplatin per square meter on day 1. The regimen of etoposide and cisplatin consisted of four three-week cycles of 100 mg of etoposide per square meter on days 1, 2, and 3 and 80 mg of cisplatin per square meter on day 1. Both regimens required hydration and administration of antiemetic drugs. If the leukocyte count fell below 2000 per cubic millimeter or the neutrophil count fell below 1000 per cubic millimeter, recombinant human granulocyte colony-stimulating factor was administered until the leukocyte or neutrophil count was restored. Because not all patients received the planned dose intensity (due to toxicity), we considered the planned intensity of cisplatin to be 15 mg and 26.7 mg per square meter per week in the irinotecan-plus-cisplatin group and the etoposide-plus-cisplatin group, respectively.

Dose Modifications and Modifications in the Treatment Schedule

Toxic effects were graded according to the JCOG Toxicity Criteria,[7] in which a grade of 1 indicates a mild effect, grade 2 a moderate effect, grade 3 a severe effect, and grade 4 a life-threatening effect. Administration of irinotecan was skipped on day 8 or 15 if the leukocyte count was 2000 per cubic millimeter or less, if the platelet count was 50,000 per cubic millimeter or less, or if there was diarrhea. Administration of subsequent cycles of irinotecan was allowed when the leukocyte count reached at least 3500 per cubic millimeter, the platelet count reached at least 100,000 per cubic millimeter, and the diarrhea had subsided. The dose of irinotecan in subsequent cycles was reduced by 10 mg per square meter from the planned dose if there were grade 4 hematologic toxic effects or if grade 2 or 3 diarrhea developed. Treatment was discontinued in patients with grade 4 diarrhea.

Etoposide was discontinued if the leukocyte count was 3500 per cubic millimeter or less, if the platelet count was 75,000 per cubic millimeter or less, or if the serum creatinine level was 1.5 mg per deciliter (132.6 μmol per liter) or higher. In patients with grade 4 hematologic toxic effects, the doses of etoposide and cisplatin in subsequent cycles were reduced to 75 percent of the planned doses. In both study groups, the dose of cisplatin was reduced to 75 percent of the planned dose in patients with grade 2 renal toxic effects. Subsequent cycles of treatment were suspended entirely in patients with grade 2 hepatic toxic effects until the results of liver-function tests were normal. Treatment was terminated in patients with renal toxic effects rated grade 3 or higher, pulmonary toxic effects rated grade 2 or higher, or hepatic toxic effects rated grade 3 or higher.

A second randomization to evaluate subsequent thoracic radiotherapy as a means of inhibiting local relapse was canceled because of an inadequate number of eligible patients.

Evaluations

All the patients underwent weekly evaluations that included an assessment of symptoms, a physical examination, chest radiography, a complete blood count, blood-chemistry studies (including measurements of aspartate aminotransferase and alanine aminotransferase, lactate dehydrogenase, bilirubin, serum creatinine, blood urea nitrogen, total protein, serum albumin, serum electrolytes, and calcium), and urinalysis. Tumor response was evaluated according to World Health Organization criteria[8] and was assessed by chest radiography or chest CT and by the same tests used initially to stage the tumor. A complete response was defined as the disappearance of all clinical and radiologic evidence of tumor for at least four weeks; a partial response was defined as a decrease of 50 percent or more in the sum of the products of the longest perpendicular diameters of all measurable lesions for at least four weeks; and progressive disease was defined as an increase of more than 25 percent in the sum of the products of the perpendicular diameters of all measurable lesions or the appearance of new lesions. All other circumstances were considered to indicate no change. All the observed responses were reviewed by an extramural panel at regular study-group meetings. A planned quality-of-life study[9] was terminated because of poor compliance.

Study Design and Statistical Analysis

This trial was designed as a multicenter, prospective, randomized phase 3 study. The study protocol was approved by the Clinical Trial Review Committee of JCOG and the institutional review board of each participating institution before the initiation of the study, and all the patients provided written informed consent before randomization in accordance with the policies of JCOG in effect in 1995, when enrollment began. The primary end point was overall survival, and the secondary end points were the rates of complete and overall response, progression-free survival, sites of relapse, and toxicity. The sample size initially planned was 230 patients from 54 participating sites, with 115 patients in each group. The planned duration of accrual was 3 years, and the planned follow-up time was 1.5 years. This sample size was designed to provide the study with 80 percent power to detect an improvement of 9 months in the median survival of the patients in the etoposide-plus-cisplatin group and an improvement of 13 months in the median survival of patients in the irinotecan-plus-cisplatin group (hazard ratio, 0.69) with a one-sided type I error of 0.05.

All comparisons of patients' characteristics, prognostic variables, response rates, and rates of toxic effects were performed with Fisher's exact test, except for age, for which the t-test was used. Survival was measured as the date of randomization to the date of death or the date of the most recent follow-up. Progression-free survival was measured as the date of randomization to the date of the first observation of disease progression or the date of death from any cause if there had been no progression. If there was no progression and if the patient had not died, data on progression-free survival were censored as of the date that the absence of progression was confirmed. If a patient died without information on progression, data on progression-free survival were censored as of the last date on which progression could be ruled out by review of follow-up forms. Survival curves were calculated by the Kaplan–Meier method[10] and compared with use of the log-rank test.

Two interim analyses were planned, with adjustment for multiple comparisons taken into account by the method of Lan and DeMets.[11] The O'Brien–Fleming type alpha spending function was used. The first interim analysis was planned for the date on which half the planned number of patients had been enrolled, and the second for the date on which all the patients had been enrolled. The boundaries were calculated with the use of computer programs provided by Reboussin et al.[12] The current study was designed and conducted on the basis of one-sided testing, but the results are presented with two-sided P values. Unadjusted P values are reported because of the conservative spending function used.

All patient-information forms were collected and managed at the data center. In-house interim monitoring was performed at the data center to ensure the submission of data, the eligibility of the patients, compliance with the protocol, safety, and progress of the study on schedule. The monitoring reports were submitted to and reviewed by an independent monitoring committee semiannually.

RESULTS

Enrollment in the study began in November 1995. The first interim analysis, performed in August 1998, suggested a difference in overall survival between the two study groups; the monitoring committee therefore recommended that the second interim analysis be performed earlier than planned. The second analysis, performed in December 1998, found a significant dif-

ference in overall survival between the two groups ($P<0.001$), and the monitoring committee therefore recommended termination of the study. Enrollment was discontinued and the study was terminated in January 1999.

Between November 1995 and November 1998, 154 patients were enrolled in the study at 27 sites among the 54 institutions planned, with 77 randomly assigned to receive irinotecan and cisplatin and 77 randomly assigned to receive etoposide and cisplatin (Table 1). All the enrolled patients were included in the analyses of survival, progression-free survival, and tumor response. However, two patients in the irinotecan-plus-cisplatin group were given no chemotherapy, one because of rapid progression of disease and the other because of an acute gastric ulcer that was diagnosed immediately after randomization. Both of these patients were excluded from the analysis of toxicity. The average follow-up time was 16.8 months in the irinotecan-plus-cisplatin group and 11.7 months in the etoposide-plus-cisplatin group. None of the patients were lost to follow-up.

Table 1. Characteristics of the Patients.

Characteristic	Irinotecan plus Cisplatin (N=77)	Etoposide plus Cisplatin (N=77)	P Value
Age (yr)			0.12
Median	63	63	
Range	30–70	41–70	
Sex			0.25
Male	63	69	
Female	14	8	
ECOG performance status*			0.61
0	10	9	
1	61	58	
2	6	10	
Weight loss during previous 6 mo			0.76
<5% of body weight	55	56	
5–10% of body weight	14	11	
>10% of body weight	8	10	
Lymph-node metastasis			
Contralateral mediastinal			1.00
Absent	51	50	
Present	26	27	
Supraclavicular			0.24
Absent	64	57	
Present	13	20	
Distant metastasis†			0.43
Absent	6	10	
Present	71	67	
Liver	14	13	
Lung	18	20	
Brain	10	17	
Bone	28	18	
Adrenal gland	5	5	
Bone marrow	5	4	

*ECOG denotes Eastern Cooperative Oncology Group.

†Some of the patients had distant metastasis to more than one site.

Toxicity

Hematologic toxic effects are shown in Table 2. JCOG grade 3 or 4 leukopenia and neutropenia and grade 3 or 4 thrombocytopenia were more frequent in the etoposide-plus-cisplatin group than in the irinotecan-plus-cisplatin group. Grade 3 or 4 diarrhea occurred in 16.0 percent of the irinotecan-plus-cisplatin group and in none of the etoposide-plus-cisplatin group ($P<0.001$). Grade 1 or 2 diarrhea was also frequent in the irinotecan-plus-cisplatin group. The incidence of nausea and vomiting and other nonhematologic toxic effects did not differ significantly between the two groups.

Major deviations from the protocol were the failure to reduce the dose of chemotherapy despite the presence of grade 4 neutropenia (in six patients in the irinotecan-plus-cisplatin group and seven in the etoposide-plus-cisplatin group), administration of irinotecan despite the presence of grade 1 or 2 diarrhea (in nine patients in the irinotecan-plus-cisplatin group), continuation of the study treatment despite grade 2 or 3 pulmonary toxic effects (in three patients in the irinotecan-plus-cisplatin group and six in the etoposide-plus-cisplatin group), and continuation of the study treatment despite grade 3 hepatic toxic effects (in one patient in the irinotecan-plus-cisplatin group and three in the etoposide-plus-cisplatin group).

There were four treatment-related deaths, three in the irinotecan-plus-cisplatin group and one in the etoposide-plus-cisplatin group. In the irinotecan-plus-cisplatin group, a 63-year-old man died of bleeding from a metastatic site in the lung, a 62-year-old man died of sepsis associated with neutropenia and diarrhea, and a 64-year-old woman died of pneumonia associated with neutropenia. In the etoposide-plus-cisplatin group, a 69-year-old man died of radiation pneumonitis after completion of subsequent thoracic radiotherapy.

Delivery of Treatment

There were no significant differences between the two groups in the delivery of treatment (Table 3). The proportion of patients who received the planned four cycles of chemotherapy was approximately 70 percent in each group. More patients in the etoposide-plus-cisplatin group (38 percent) than in the irinotecan-plus-cisplatin group (29 percent) completed their assigned study treatment with no modifications in the doses or delivery schedule. The dose intensity (the actual dose delivered as a proportion of the planned dose) was 80.4 percent for irinotecan and 95.3 percent for cisplatin in the group assigned to receive irinotecan

TABLE 2. TOXIC EFFECTS, ACCORDING TO STUDY GROUP AND JCOG GRADE OF TOXICITY.*

TOXIC EFFECT	IRINOTECAN PLUS CISPLATIN (N=75)						ETOPOSIDE PLUS CISPLATIN (N=77)						P VALUE†
	GRADE 1	GRADE 2	GRADE 3	GRADE 4	GRADE 3 OR 4	GRADE 4	GRADE 1	GRADE 2	GRADE 3	GRADE 4	GRADE 3 OR 4	GRADE 4	
	no. of patients				% of patients		no. of patients				% of patients		
Hematologic													
Neutropenia	8	17	30	19	65.3	25.3	0	5	21	50	92.2	64.9	<0.001
Leukopenia	16	38	17	3	26.7	4.0	5	30	35	5	51.9	6.5	0.002
Anemia	10	38	20	—	26.7	—	9	43	23	—	29.9	—	0.72
Thrombocytopenia	6	9	1	3	5.3	4.0	19	13	14	0	18.2	0	0.02
Nonhematologic													
Diarrhea	19	21	8	4	16.0	5.3	8	5	0	0	0	0	<0.001
Nausea or vomiting	26	28	10	—	13.3	—	36	23	5	—	6.5	—	0.18
Infection	17	9	3	1	5.3	1.3	23	9	1	2	3.9	2.6	0.72
Decrease in arterial oxygen pressure	20	5	1	1	5.0‡	2.5‡	27	9	2	1	5.8‡	1.9‡	1.0
Increase in alanine aminotransferase	30	7	3	0	4.0	0	28	6	2	1	3.9	1.3	1.0
Increase in aspartate aminotransferase	30	5	0	0	0	0	24	2	1	1	2.6	1.3	0.5
Fever	12	17	1	0	1.3	0	14	16	2	0	2.6	0	1.0
Increase in bilirubin	—	16	0	0	0	0	—	20	0	0	0	0	—
Increase in creatinine	15	4	0	0	0	0	16	5	0	0	0	0	—
Peripheral neuropathy	4	0	0	—	0	—	10	1	0	—	0	—	—

*Two patients in the irinotecan-plus-cisplatin group were not included in the analysis of toxicity because they received no chemotherapy (one because of disease progression and one because of acute gastric ulcer). JCOG denotes Japan Clinical Oncology Group. Dashes indicate that there was no scale of grading in the JCOG toxicity criteria. A grade of 1 indicates a mild effect, a grade of 2 a moderate effect, a grade of 3 a severe effect, and a grade of 4 a life-threatening effect.

†P values are for the comparison between the two study groups of the incidence of grade 3 or 4 toxic effects.

‡Data were missing for 35 patients in the irinotecan-plus-cisplatin group and 25 patients in the etoposide-plus-cisplatin group.

plus cisplatin and was 83.9 percent for etoposide and 84.6 percent for cisplatin in the group assigned to receive etoposide plus cisplatin. The actual intensity of the dose of cisplatin in the etoposide-plus-cisplatin group was 1.58 times that in the irinotecan-plus-cisplatin group (22.6 vs. 14.3 mg per square meter per week, respectively).

Rates of Response

The rate of complete response and the overall response rate were 2.6 percent (95 percent confidence interval, 0.3 to 9.1 percent) and 84.4 percent (95 percent confidence interval, 74.4 to 91.7 percent), respectively, in the irinotecan-plus-cisplatin group and 9.1 percent (95 percent confidence interval, 3.7 to 17.8 percent) and 67.5 percent (95 percent confidence interval, 55.9 to 77.8 percent), respectively, in the etoposide-plus-cisplatin group (Table 4). The rate of overall response in the irinotecan-plus-cisplatin group was significantly higher than that in the etoposide-plus-cisplatin group (P=0.02).

Overall Survival

As of March 2001, when the final analysis was conducted, the median overall survival was 12.8 months (95 percent confidence interval, 11.7 to 15.2) in the irinotecan-plus-cisplatin group and 9.4 months (95 percent confidence interval, 8.1 to 10.8) in the etoposide-plus-cisplatin group; 70 patients in the irinotecan-plus-cisplatin group and 74 in the etoposide-plus-cisplatin group died (P=0.002 by the log-rank test) (Fig. 1). The rate of overall survival in the irinotecan-plus-cisplatin group was 58.4 percent (95 percent confidence interval, 47.4 to 69.4 percent) at one year and 19.5 percent (95 percent confidence interval, 10.6 to 28.3 percent) at two years; in the etoposide-plus-cisplatin group, the rates of overall survival at these time points were 37.7 percent (95 percent confidence interval, 26.8 to 48.5 percent) and 5.2 percent (95 percent confidence interval, 0.2 to 10.2 percent). The risk of death in the irinotecan-plus-cisplatin group relative to that in the etoposide-plus-cisplatin group was 0.60 (95 percent confidence interval, 0.43 to 0.83). Similar results were obtained in analyses that adjusted for age, sex, performance status, and weight loss and in an analysis that excluded the 23 patients randomly assigned to the radiotherapy portion of the study, which was canceled.

Progression-free Survival

The median known progression-free survival was 6.9 months (95 percent confidence interval, 6.1 to 7.3)

TABLE 3. NUMBER OF CYCLES OF TREATMENT AND ACTUAL DOSES DELIVERED.

VARIABLE	IRINOTECAN PLUS CISPLATIN (N=77)		ETOPOSIDE PLUS CISPLATIN (N=77)	
	no. of patients (%)		no. of patients (%)	
Cycles				
0	2 (2.6)		0	
1	6 (7.8)		2 (2.6)	
2	8 (10.4)		13 (16.9)	
3	8 (10.4)		7 (9.1)	
4	53 (68.8)		55 (71.4)	
	delivered dose (mg/m²/wk)	% of planned dose	delivered dose (mg/m²/wk)	% of planned dose
Agent				
Cisplatin	14.3	95.3	22.6	84.6
Irinotecan	36.2	80.4	—	—
Etoposide	—	—	83.9	83.9

TABLE 4. OBJECTIVE TUMOR RESPONSE.*

RESULT	IRINOTECAN PLUS CISPLATIN	ETOPOSIDE PLUS CISPLATIN
	no. of patients (%)	
Response		
Complete	2 (2.6 [0.3–9.1])	7 (9.1 [3.7–17.8])
Partial	63 (81.8)	45 (58.4)
Overall	65 (84.4 [74.4–91.7])†	52 (67.5 [55.9–77.8])
No change	2 (2.6)	16 (20.8)
Progression	3 (3.9)	9 (11.7)
Not evaluated	5 (6.5)	0
Not treated	2 (2.6)	0

*Values in brackets are 95 percent confidence intervals.

†P=0.02 for the comparison with the rate of overall response in the etoposide-plus-cisplatin group. The absolute difference between the two groups in overall response rate was 16.9 percent (95 percent confidence interval, 3.7 to 30.1 percent).

in the irinotecan-plus-cisplatin group and 4.8 months (95 percent confidence interval, 4.3 to 5.5) in the etoposide-plus-cisplatin group. The rate of known progression-free survival in the irinotecan-plus-cisplatin group was 65.3 percent (95 percent confidence interval, 54.3 to 76.3 percent) at six months and 12.5 percent (95 percent confidence interval, 4.9 to 20.1 percent) at one year; in the etoposide-plus-cisplatin group the rates of progression-free survival at these time points were 35.6 percent (95 percent confidence interval, 24.8 to 46.3 percent) and 7.9 percent (95 percent confidence interval, 1.8 to 14.0 percent), respectively (P=0.003 by the log-rank test) (Fig. 2). Progression was known to have occurred in 68 patients in the irinotecan-plus-cisplatin group and 75 patients in the etoposide-plus-cisplatin group. The relative risk of disease progression in the irinotecan-plus-cisplatin group as compared with that in the etoposide-plus-cisplatin group was 0.61 (95 percent confidence interval, 0.44 to 0.84). The estimates of progression-free survival, however, may be biased because information on progression was not monitored continuously and because there were 10 instances of early censoring because of death without data on progression (8 instances in the irinotecan-plus-cisplatin group and 2 in the etoposide-plus-cisplatin group).

DISCUSSION

The current standard chemotherapy for extensive small-cell lung cancer — a regimen of etoposide and cisplatin or this combination alternating with a combination of cyclophosphamide, doxorubicin, and vincristine — yields a median survival of 8 to 10 months and a 2-year survival rate of 10 percent. In this phase 3 study, 77 patients with metastatic small-cell lung cancer who were treated with irinotecan plus cisplatin had a median survival of 12.8 months, whereas the group that received etoposide plus cisplatin had a median survival of 9.4 months (P=0.002). The overall rates of survival in these two groups at two years were 19.5 percent and 5.2 percent, respectively.

Myelosuppression was the most frequent toxic effect in both groups and was more frequent in the etoposide-plus-cisplatin group than in the irinotecan-plus-cisplatin group. There was, however, a significantly higher incidence of grade 3 or 4 diarrhea among the patients who received irinotecan than among those who received etoposide.

The three treatment-related deaths in the irinotecan-plus-cisplatin group occurred during the first or second cycle of treatment and were attributed to hematologic toxic effects of the first cycle. Severe hematologic toxic effects, as well as diarrhea, during the initial cycles of chemotherapy should therefore be managed carefully. All cases of grade 1 to 4 diarrhea occurred during the first and second cycles of irinotecan-plus-cisplatin treatment, but early suspension of treatment prevented death associated with diarrhea in all but one case, which involved a protocol violation in which the patient was given irinotecan on day 8 of the first cycle despite the presence of grade 1 diarrhea. We administered loperamide hydrochloride or a Chinese herbal drug such as hange-shashin-to to ameliorate the diarrhea at the discretion of the attending physicians.

The proportion of patients who received all four