the other patient background factors described above. The multivariate analyses were performed by using JMP version 6.0.0 software (SAS Institute). The variables in the final models for both AUC ratio and neutropenia were chosen by forward and backward stepwise procedures at significance levels of 0.25 and 0.05, respectively.

Results

Patients and UGT1A haplotypes

Patient demographics and information on the treatment are summarized in Table 1. In addition to UGT1A1, UGT1A7, 1A9, and 1A10 were also reported to glucuronidate SN-38 [15–17]. In our previous study, haplotype analysis covering the *1A9* to *1A1* (5′–3′) gene segments was conducted, and the combinatorial diplotypes (*1A9-1A7-1A1*) of the patients were determined. It must be noted that close linkages between *1A9*22* and *1A7*1*, between *1A7*2* and *1A1*60*, and between *1A7*3* and *1A1*6* or *1A1*28* were observed as described previously [28]. To clarify the linkages between these segmental haplotypes (*1A9*, *1A7*, and *1A1*), we grouped the combinatorial (*1A9-1A7-1A1*) haplotypes into four categories (A–D) based on the *1A1* haplotypes (**1*, **6*, **60*, and **28*). Each group was further divided into the subgroups based on the previously defined Block 9/6 (including *1A9*, *1A7*, and *1A6)* haplotypes (Table 2). The frequency of Group B haplotypes (*B1-B4*) harboring *1A1*6* was 0.167 and higher than that of Group D haplotypes (*D1-D6*) with **28* (0.138) in this population.

Association of 1A9-1A7-1A1 diplotypes to SN-38G formation

When relationship between the *UGT1A* diplotypes (*1A9-1A7-1A1*) and the SN-38G/SN-38 AUC ratio was analyzed in the 176 cancer patients the AUC ratio for the diplotypes of *B2/B2*, *D2/A1*, and *D1/B2* was statistically significantly lower than the *A1/A1* diplotype (Fig. 2). These diplotypes harbored *1A1*6*, **28* or both. Significant gene–dose effects of *B2* (among *A1/A1*, *B2/A1*, and *B2/B2*) and *C3* (among *A1/A1*, *C3/A1*, and *C3/C3*) were also observed (Fig. 2). As no significant differences in AUC ratios were observed between *D1/A1* and *D2/A1*, *D1/C3* and *D2/C3*, and *D1/B2* and *D2/B2*, the haplotype combination *1A9*1-1A7*3* or *1A9*22-1A7*1* was not influential on the AUC ratio.

As the effect of diplotypes harboring *UGT1A1* polymorphism was prominent, we grouped the whole gene (*1A9-1A7-1A1*) diplotypes according to the *1A1* diplotypes (the upper part of Fig. 2). Patients with **6* or **28* (except for **28/*28*) haplotypes had significantly lower AUC ratios than the wild-type (**1/*1*), and significant gene–dose effects were observed for **28* (among **1/*1*, **28/*1*, and **28/*28*) and **6* (among **1/*1*, **6/*1* and **6/*6*). A significant additive effect of **6* and **28* on the decreased AUC ratio was also observed when the values for **28/*1* were compared with those for **28/*6* (Fig. 2 and Table 3).

Regarding other polymorphisms, a statistically nonsignificant tendency to decrease the AUC ratio was observed for **60*

Table 1 Characteristics of Japanese cancer patients in this study

		No. of participants	
Age			
Mean/range	60.5/26–78	177	
Sex			
Male/female		135/42	
Performance status	0/1/2	84/89/4	
Combination therapy and tumor type (initial dose of irinotecan; mg/m^2)			
Irinotecan monotherapy	Lung (100)	21	
	Colon (150)	28	
	Others (100)	7	
With platinum-containing drug[a]	Lung (60)	58[b]	48 [60][c]
	Stomach (70)	9	9 [80][c]
	Others (60)	5	5 [80][c]
With 5-fluorouracil (including tegafur)	Colon (100 or 150)	34	
	Others (90 or 100)	2	
With mitomycin-C	Stomach (150)	10	
	Colon (150)	1	
With amrubicin	Lung (60)	2	
Previous treatment			
Surgery	Yes/no	85/92	
Chemotherapy	Yes/no	97/80	
Radiotherapy	Yes/no	26/151	
Smoking history	Yes/no	29/148	

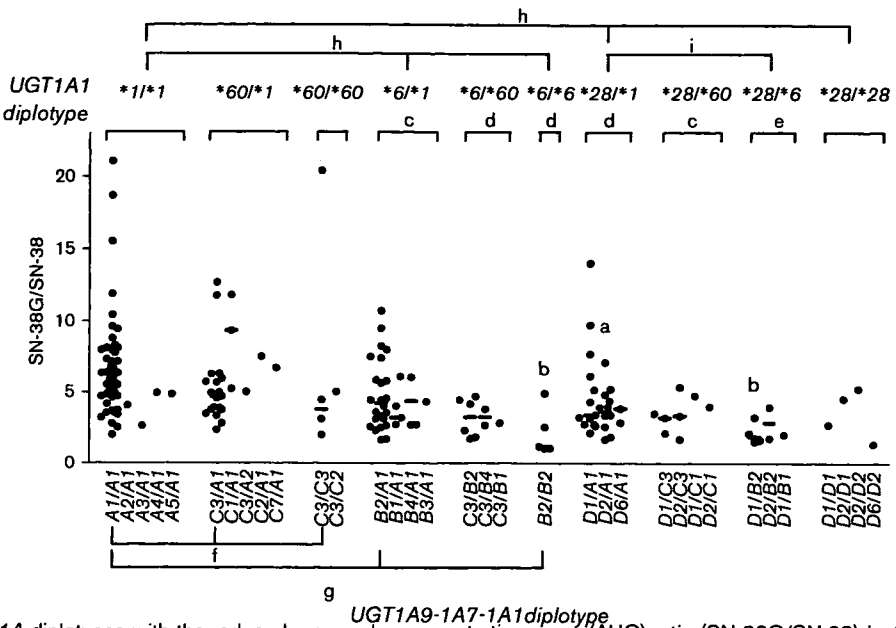
[a]Cisplatin, cisplatin plus etoposide or carboplatta.
[b]Two and eight patients received cisplatin and etoposide and carboplatin, respectively.
[c]Number of cisplatin-administered patients [initial dose of cinlatin (mg/m^2) is shown in brackets].

Table 2 Combinatorial haplotypes covering *UGT1A9*, *UGT1A7*, and *UGT1A1*

	Block haplotype[a]			Combination of segmental haplotypes	Cancer patients	
Haplotype	Block 9/6	Block 4	Block 3/1	1A9-1A7-1A1	*N*[b]	Frequency
A1[c]	**I*	**1*	**I*	**22-*1-*1*	189	0.534
	**I*	**3*	**I*			
A3	**III*	**1*	**I*	**1-*2-*1*	2	0.006
A2	**II*	**1*	**I*	**1-*3-*1*	1	0.003
A4	**IV*	**1*	**I*	**22-*3-*1*	1	0.003
A5				**T11-*1-*1*	1	0.003
B2[c]	**II*	**1*	**III*			
	**II*	**1*	**VI*	**1-*3-*6*	47	0.133
	**II*	**4*	**VI*			
B4	**IV*	**1*	**III*	**22-*3-*6*	6	0.017
B1	**I*	**1*	**III*	**22-*1-*6*	5	0.014
	**I*	**1*	**VI*			
B3	**III*	**1*	**III*	**1-*2- *6*	1	0.003
C3[c]	**III*	**3*	**IV*			
	**III*	**1*	**IV*			
	**III*	**3*	**V*	**1-*2-*60*	44	0.124
	**III*	**1*	**V*			
C1	**I*	**3*	**IV*	**22-*1-*60*	5	0.014
	**I*	**1*	**IV*			
C2	**II*	**3*	**IV*	**1-*3-*60*	2	0.006
C7	**VII*	**3*	**V*	**22-*2-*60*	1	0.003
D1	**I*	**1*	**IIa*	**22-*1-*28*	23	0.065
	**I*	**1*	**IIc*			
D2	**II*	**1*	**IIa*			
	**II*	**3*	**IIa*	**1-*3-*28*	22	0.062
	**II*	**1*	**IIc*			
D6	**VI*	**1*	**IIb*	**1-*2-*28*	4	0.011
				Total	354	1.000

[a]Block haplotypes described in Ref. [28] are shown for reference. *1A9* and *1A7* are included in block 9/6 and *1A1* is included in block 3/1.
[b]Number of chromosomes.
[c]Major combinatrial haplotypes.

Fig. 2



The association of *UGT1A* diplotypes with the reduced area under concentration curve (AUC) ratio (SN-38G/SN-38) in 176 Japanese cancer patients who received irinotecan. The whole gene (*1A9-1A7-1A1*) diplotypes are shown below the abscissa and the *UGT1A1* diplotypes are indicated in the upper part of the figure. Each point represents a patient value, and the median is indicated by a bar. Significant reductions in the AUC ratio were detected in the *B2/B2*, *D2/A1*, and *D1/B2* compared with *A1/A1* for the whole gene diplotypes [Kruskal–Wallis test ($P=0.0009$) followed by Dunnett's multiple comparison test]. As for the *1A1* diplotypes, significant reductions were detected in the **6/*1*, **6/*60*, **6/*6*, **28/*1*, **28/*60*, and **28/*6* compared with the **1/*1* group [Kruskal–Wallis test ($P<0.0001$) followed by Dunnett's multiple comparison test]. Gene–dose effects on the reduced AUC ratio were significant for **6* and **28* (Jonckheere–Terpestra test). A significant additive effect of **6* on the reduced AUC ratio by **28* was detected by comparing **28/*1* and **28/*6*. [a]$P<0.05$ and [b]$P<0.01$ against *A1/A1* group (Dunnett's multiple comparison test); [c]$P<0.05$, [d]$P<0.01$, and [e]$P\&<0.001$ against the **1/*1* group (Dunnett's multiple comparison test); [f]$P<0.05$, [g]$P<0.001$, and [h]$P<0.0001$ (Jonckheere–Terpestra test for gene–dose effect); [i]$P<0.01$ (Wilcoxon test).

($P = 0.1134$). No significant effects on the AUC ratio were observed for Block C (exon 2–5) haplotypes or rare variations including *1A10* (**2T*, **2*, or **3*) and *1A9* (**5*, **T11*).

Multiple regression analysis of the area under concentration curve ratio

We further assessed the impact of *UGT1A* genetic factors on the AUC ratio by multiple regression analysis. First, we used the *1A9-1A7-1A1* and Block C haplotypes as genetic factors. The AUC ratio was significantly associated with the haplotypes *B2*, *D1*, and *D2* and serum biochemistry parameters indicating hepatic or renal function before treatment. The Groups B and D haplotypes harbor *1A1*6* and **28*, respectively. The dependency on specific *1A7* or *1A9* polymorphisms, however, was not obtained, considering the contributions of both *D1* and *D2*. As *1A1*6* and **28* are mutually exclusive and their effects are comparable, we grouped *1A1*6* and **28* into the same category in the final multiple regression model (Table 4). The final model confirmed the significant contribution of this genetic marker (**6* or **28*) to the AUC ratio.

Effects of the genetic marker '*6 or *28' on pharmaco kinetic parameters

Then, a dose effect of the genetic marker '**6* or **28*' on pharmacokinetic parameters was further analyzed (Fig. 3). Patients with one haplotype harboring either **6* or **28* (**6/*1*, **6/*60*, **28/*1*, and **28/*60*) had lower SN-38G/SN-38 AUC ratios (median, 3.62; interquartile range, 2.74–5.18) than patients without **6* or **28* (**1/*1*, **60/*1*, and **60/*60*) (5.55, 4.13–7.26), and patients with two haplotypes harboring **6* or **28* (**6/*6*, **28/*28*, and **28/*6*) had the lowest AUC ratio (2.07, 1.45–3.62) ($P < 0.0001$, Fig. 3a). Similarly, the number of the **6* or **28*-containing haplotypes affected the AUC ratios of SN-38 to irinotecan (Fig. 3b). When the correlations

Table 3 AUC ratio of SN-38 glucuronide to SN-38 for *UGT1A1* diplotypes

Diplotype	Number of patients	AUC ratio		*P*-value[a] (vs. **1/*1*)
		Median	Interquartile range	
**1/*1*	55	6.13	4.72–7.79	
**1/*60*	25	5.04	3.85–6.52	0.9803
**60/*60*	5	4.48	2.57–12.74	0.8141
**6/*1*	32	4.03	2.74–5.97	0.0126
**6/*60*	9	2.84	2.09–4.33	0.0021
**6/*6*	5	1.19	1.06–3.74	0.0012
**28/*1*	26	3.65	2.76–5.21	0.0040
**28/*60*	8	3.44	2.68–4.40	0.0261
**28/*6*	7	2.03	1.65–3.26	<0.0001
**28/*28*	4	3.65	2.05–4.92	0.2322

AUC, area under concentration curve.
[a]Dunnett's multiple comparison test.

between irinotecan dosage and the AUC of SN-38 were tested, different correlations were obtained according to the number of the haplotypes (Fig. 3c). The slope of regression line for one and two haplotypes harboring **6* or **28* was 1.4-fold and 2.4-fold greater, respectively, than that for the diplotype without **6* or **28*.

Associations of UGT1A1 genetic polymorphisms with toxicities

Association between genetic polymorphisms and toxicities was investigated in patients receiving irinotecan as a single agent. One patient was referred to another hospital 3 days after the first administration of irinotecan without evaluating toxicities and was lost in terms of follow-up. Therefore, association between genetic polymorphisms and toxicities was investigated in 55 patients. Six (11%) and 14 (25%) patients experienced grade 3 or greater diarrhea and neutropenia, respectively. As for the *1A9-1A7-1A1* diplotypes, a higher incidence of grade 3 or greater neutropenia was observed in *D1/B2* (*1A1*28/*6*) (100%, $n = 3$) than in *A1/A1* (11.8%, $n = 17$) ($P = 0.0088$, Fisher's exact test), indicating clinical impact of the genetic marker *1A1*6* or **28*. As for the dose effect of '**6 or *28*', incidences of grade 3 or 4 neutropenia were 14, 24, and 80% for 0, 1, and 2 haplotypes harboring these markers, respectively (Table 5). A significant association between '**6* or **28*'and neutropenia was also observed for 62 patients who received irinotecan in combination with cisplatin (Table 5). No association, however, was observed between diarrhea and the marker '**6* or **28*'.

Multivariate analysis for irinotecan toxicities

We further evaluated the effect of the genetic marker '**6* or **28*' on neutropenia in multivariate analysis, and confirmed a significant correlation of '**6* or **28*' with the nadir of absolute neutrophil counts (Table 6). Elevated alkaline phosphatase levels and the absolute neutrophil count at baseline were also significant.

Discussion

The association study with the *1A9-1A7-1A1* diplotypes revealed that the reduction in inactivation of SN-38, as well as neutropenia, was dependent on the Groups B and D haplotypes which corresponded to the *1A1*6* and **28* segmental haplotypes. Also, multivariate analyses clearly showed clinical significance of the genetic marker '**6* or **28*' for both pharmacokinetics and toxicity of irinotecan in Japanese patients (Tables 3 and 6). *UGT1A1*6* and **28* were mutually exclusive [14] and contributed to the reduction in glucuronidation of SN-38 to the same extent. Therefore, the activity of SN-38 glucuronidation in individuals depended on the number of the haplotypes harboring **6* or **28*. Although the role of *1A1*28* for irinotecan toxicity has been focused on [8–12], this study strongly suggests that **6* should be tested in addition to **28* before starting chemotherapy with irinotecan in Japanese patients.

The clinical importance of **6* for neutropenia by irinotecan was also supported by a recent report in Korean patients who received irinotecan and cisplatin [31]. Although no patients with irinotecan as a single agent were homozygous for **6* in our study, clinical significance of the double heterozygote, **6/*28*, was clearly demonstrated. Among patients treated with irinotecan in combination chemotherapy, the majority of patients received platinum agents in our study. A significant association of '**6* or **28*' with a higher incidence of grade 3 or 4 neutropenia was also observed in patients who received irinotecan and cisplatin (Table 5). These findings further support the necessity of testing '**6* or **28*' before irinotecan is given to patients.

As possible enhancement of toxicities by the **27* allele was suggested [8], we evaluated the effect of the **28c* haplotype, which had an additional single-nucleotide polymorphism [**27*; 686C > A(P229Q)] to the **28* allele (-40_-39insTA). In our cohort of patients, there were three **28c* heterozygotes (**28c/*1*) and one double heterozygote (**28b/*28c*). The values of the AUC ratio were within the range of variations of the **28* group, and no additional impact of **28c* was observed in relation to toxicities.

Although the decreasing trend of the AUC ratio for *1A1*60* (and combinatorial haplotype *C3*) was observed (Fig. 2), the contribution of *1A1*60* to toxicities was not clearly demonstrated in this study as reported in the Japanese retrospective study [32].

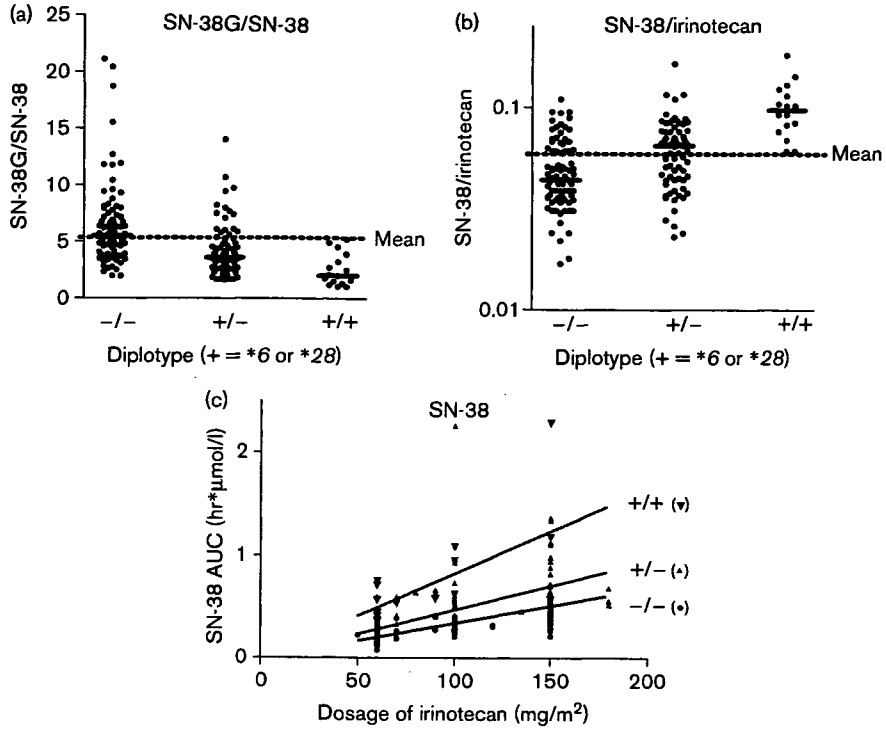
In addition to UGT1A1, recent studies have suggested possible contributions of UGT1A7, 1A9, and 1A10 to SN-38G formation [15–17]. An in-vitro study demonstrated that *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)] had reduced activity in terms of SN-38G formation [16]. Results of clinical studies, however, on the association between *1A7* polymorphisms and irinotecan toxicity/efficacy are inconsistent, whereas different populations with different combination therapies were used [19,20]. Furthermore, it was reported that the *UGT1A7* polymorphisms (**2* and **3*), which were linked to *1A9*1*, were associated with a lowered incidence

Table 4 Multiple regression analysis toward the AUC ratio (SN-38G/SN-38)[a]

Variable	Coefficient	F-value	*P*-value	R^2	Intercept	*N*
				0.410	0.8869	176
**6* or **28*	-0.189	70.2	<0.0001			
Age	0.005	8.88	0.0033			
Serum albumin level[b]	-0.136	9.92	0.0019			
Serum GOT and ALP[c]	0.070	8.88	0.0033			
Serum creatinine[d]	0.210	7.23	0.0079			

ALP, alkaline phosphatase; AUC, area under concentration curve.
[a]The values after logarithmic conversion were used as an objective variable.
[b]The absolute value (g/dl) before irinotecan treatment.
[c]Grade 1 or greater scores in both serum GOT and ALP before irinotecan treatment.
[d]Grade 1 or greater scores in serum creatinine before irinotecan treatment.

Fig. 3



Effects of the genetic marker of *UGT1A1* '*6 or *28' on the area under concentration curve (AUC) ratios of SN-38G/SN-38 (a) and SN-38/irinotecan (b), and SN-38 by irinotecan dosage (c) in 176 Japanese cancer patients after irinotecan treatment.

Table 5 Association of *UGT1A1*6* and *28 with irinotecan toxicities

Diplotype (+ = *6 or *28)	Number of patients	Diarrhea (grade 3)	Neutropenia (grade 3 or 4)
Irinotecan monotherapy			
−/−	21	3 (14.3%)[a]	3 (14.3%)
+/−	29	2 (6.90%)	7 (24.1%)
+/+	5	1 (20.0%)	4 (80.0%)
P-value[b]		0.8500	**0.0117**
P-value[c]		0.3889	**0.0124**
With cisplatin			
−/−	35	1 (2.9%)	20 (57.1%)
+/−	20	2 (10.0%)	14 (70.0%)
+/+	7	1 (14.3%)	7 (100%)
P-value[b]		0.1747	**0.0315**
P-value[c]		0.3886	0.0863

[a]Percentage of the patient number in each diplotype is indicated in parentheses.
[b]Chi-squared test for trend.
[c]Fisher's exact test, (−/− and +/−) vs. +/+.

Table 6 Multiple regression analysis of the nadir of absolute neutrophil counts in the patients with irinotecan monotherapy

Variable	Coefficient	F-value	*P*-value	R^2	Intercept	*N*
				0.3942	643	53
Serum ALP[a]	−349.9	12.2	0.0010			
Neutrophil count before irinotecan treatment	0.2466	13.5	0.0006			
*6 or *28	−369.1	6.40	0.0146			

[a]Grade 1 or greater scores of serum ALP before irinotecan treatment.

of diarrhea in the irinotecan/capecitabine regimen, in which diarrhea was a major toxicity [20]. A highly frequent allele *1A9*22* with an insertion of T into the nine T repeats in the promoter region (-126_-118T_9 > T_{10}) was shown to have an enhanced promoter activity in an in-vitro reporter assay [21], whereas 1A9 protein expression levels did not change in the clinical samples [22]. Rare variations, *1A9*5* [766G > A(D256N)] and *UGT1A10*3* [605C > T(T202I)], were shown to cause reduced activity *in vitro*, but their clinical importance is still unknown [23,24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–28].

Our study also revealed close linkages between *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6* or *28 [28]. This fact makes it difficult to draw firm conclusions about the effects of *1A7*3* and *1A9*22* themselves. It is, however, reasonable to conclude that the degree of neutropenia depends on the activity of UGT1A1, because UGT1A1 is a major UGT1A enzyme in the liver and plays a primary role for regulating plasma concentrations of SN-38.

Taken together, for practical application to individualized irinotecan therapy, genotyping of *UGT1A1*6* and *28 would be beneficial and necessary in Japanese cancer patients to avoid severe adverse reactions. The frequency

of homozygotes for '**6* or **28*' (namely, **6/*6*, **6/*28*, and **28/*28*) is approximately 10%, which is comparable to the frequency of **28* homozygotes in Caucasian populations. In our study, it may be difficult to establish definite guidelines for dose reductions of irinotecan for patients homozygous for '**6* or **28*'. Considering, however, 2.4-fold steep relationship between the dose of irinotecan and the AUC of SN-38 for patients homozygous for '**6* or **28*' compared with patients without '**6* or **28*' (Fig. 3c), the dose for patients homozygous for '**6* or **28*' should be reduced to a half of the dosage recommended for other patients. Prospective studies are necessary to confirm the validity of the recommendation for dose reduction in Japanese cancer patients homozygous for '**6 or *28*'.

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Detection of unsuspected distant metastases and/or regional nodes by FDG-PET in LD-SCLC scan in apparent limited-disease small-cell lung cancer

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Summary We retrospectively investigated the clinical usefulness of fluorodeoxyglucose positron emission tomography (FDG-PET) for evaluation of patients with limited-disease small-cell lung cancer (LD-SCLC) diagnosed by conventional staging procedures. Sixty-three patients received whole body FDG-PET scans after routine initial staging procedures. The findings of FDG-PET scans suggesting extensive-stage disease were confirmed by other imaging tests or by the patient's clinical course. FDG-PET scan findings indicated distant metastases in 6 of 63 patients. Metastatic disease was confirmed in five of these six patients (8%, 95% confidence interval: 3–18%). FDG-PET scan also detected regional lymph node metastases even in nine patients (14%) in whom computed tomography images had been negative, including contralateral lymph node metastasis in three patients. FDG-PET scan detected additional lesions in patients diagnosed as having LD-SCLC by conventional staging procedures. The therapeutic strategies were changed in 8% of patients based on the results of FDG-PET. FDG-PET scan is recommended as an initial staging tool for patients with this disease.

1. Introduction

Small-cell lung cancer (SCLC) accounts for 15–20% of all lung cancers. SCLC shows more aggressive biological behaviour than non-small cell lung cancer (NSCLC). A clinical two-stage system proposed by the Veterans Administration Lung

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Study Group (VALSG) distinguishes limited-disease (LD) and extensive-disease (ED) in SCLC [1]. LD is defined as limited to one hemithorax, including mediastinal, contralateral hilar and ipsilateral supraclavicular lymph nodes, while ED represents tumour spread beyond these regions. Approximately two-thirds of patients with SCLC are diagnosed as having ED at the initial staging. The current standard care for LD-SCLC is a combination of chemotherapy and chest irradiation. With current treatment, patients with LD have a median survival of 23–27 months [2,3], compared to 10–12 months for those with ED [4]. Therefore, accurate pretreatment staging is important for patients with SCLC in order to determine the appropriate therapy.

Conventional staging procedures for lung cancer consist of computed tomography (CT) of the chest and upper abdomen, bone scan, and CT scan or magnetic resonance imaging (MRI) of the brain. Recently, fluorodeoxyglucose positron emission tomography (FDG-PET) was introduced as a staging tool for NSCLC. According to the guidelines of the American Society of Clinical Oncology, PET scan is recommended for survey occult locoregional lesions and distant metastases in patients with NSCLC [5]. Two separate prospective studies demonstrated that FDG-PET detected unsuspected distant metastases in 24% of patients with apparent stage III NSCLC [6,7]. Another study showed that FDG-PET changed or influenced management decisions in 67% of patients with NSCLC. PET plays an important role in staging of NSCLC [8]. However, previous PET studies of SCLC involved only a relatively small number of patients [9–17]. In a prospective study, FDG-PET was performed for 24 patients diagnosed as having LD-SCLC by conventional staging procedures [9]. Based on FDG-PET findings, two of these 24 patients were upstaged to ED. Bone metastases were found in one patient, and contralateral supraclavicular lymph node metastasis in another. Larger studies are required to confirm the role of FDG-PET in the staging of LD-SCLC. In this study, we retrospectively investigated the usefulness of FDG-PET to detect distant metastases or unsuspected regional nodal metastases in patients with LD-SCLC diagnosed by conventional staging procedures.

2. Patients and methods

2.1. Patients

Seventy patients were newly diagnosed as having LD-SCLC by conventional staging procedures at the National Cancer Center Hospital East between July 2003 and December 2006. Conventional staging procedures included history and physical examination, chest radiography, CT scan of the chest, CT scan or ultrasound (US) of the abdomen, bone scan, and CT scan or MRI of the brain. CT scan and MR images were enhanced with contrast media. LD is defined in this study as disease limited to one hemithorax, including mediastinal, contralateral hilar and supraclavicular lymph nodes, ipsilateral pleural effusion, and pericardial effusion, while ED represents tumour spread beyond these manifestations [18]. This study included 63 patients who received whole body FDG-PET scan after the routine initial staging procedures. Fifty-seven were male and the remaining 6 were female. Median age was 64 years, range 48–80 years. Forty-two patients received FDG-PET before commencement of chemotherapy. The remaining 21 patients received FDG-PET 1 to 11 days (median: 4 days) after commencement of chemotherapy. Forty-four and 19 patients received CT scan and US of the abdomen, respectively.

2.2. FDG-PET scan

FDG-PET scans were performed before March 2005 (patients No. 1–25), and FDG-PET/CT scans were performed after April 2005 (patients No. 26–63). Three hundred MBq of F-18 FDG were intravenously injected after at least 6 h of fasting. Acquisition was initiated 60 min after the injection. FDG-PET imaging was performed using a GE Advance Scanner (General Electric Medical System, Milwaukee, WI), whose axial field of view was 15.2 cm and spatial resolution 4.9 mm of full-width-half-maximum. Scans were performed using two-dimensional acquisition mode from the thigh to the skull base with seven bed positions. Each bed position was composed of 1 min of transmission scanning and 5 min of emission scanning.

FDG-PET/CT imaging was performed using a GE Discovery LS Scanner (General Electric Medical System, Milwaukee, WI) or a GE Discovery ST Scanner (the same manufacturer). The PET component of the GE Discovery LS Scanner was the same as that of the GE Advance Scanner. For the PET component of the GE Discovery ST Scanner, the axial field of view was 15.7 cm and the spatial resolution was 6.2 mm of full-width-half-maximum. PET scans were performed with both scanners using 2-dimensional acquisition mode from the thigh to the skull base with 7 bed positions. Each bed position was composed of 4 min of emission scanning. The CT component of both PET/CT scanners was a 16-row multidetector CT scanner and CT images were acquired with a tube voltage of 140 kV, and the tube current was automatically set using the auto-exposure control function so that the number of standard deviations of noise was limited to 10. Attenuation correction of PET images was performed using the data from CT images.

Image reconstruction was performed using an ordered subsets expectation maximization (OSEM) algorithm with subset and iteration values of 14 and 2, respectively.

2.3. Image interpretation

All PET and CT images were interpreted by experienced radiologists and physicians. The 4.25 mm-thick images of axial, coronal and sagittal planes on hard copy films were reviewed. Uptake stronger than mediastinal blood pool activity was diagnosed as malignancy by the visual estimation. Symmetrical activities observed in both hilar regions were considered to be benign reactive changes. Any discrepancies between the radiologist and physician were resolved by discussion. The findings detected by FDG-PET were confirmed by other image tests or observation of the clinical course. FDG-PET was conducted after conventional staging procedures. CT, US and bone scans were interpreted without the FDG-PET findings. However, FDG-PET scan was interpreted in comparison with CT findings, while PET/CT findings were interpreted independently.

Table 1 Discrepancy between FDG-PET and conventional staging procedures (distant metastases)

Patient no.	Age (years)	Gender	CT N	PET N	PET M	Interval between conventional staging procedures and FDG-PET (days)	Comments
2	61	Male	2	2	1	20	Multiple bone metastases (PET)
6	68	Male	2	2	1	7	Lymph node metastasis around the cardia (PET)
47	61	Male	3	3	1	28	Multiple bone metastases (PET)
55	68	Male	2	2	1	20 (CT) and 14 (bone scan)	Liver, axillary lymph node, and iliac bone metastases (PET)
59	52	Male	3	3	1	13	Adrenal, cervical and mandibular lymph node metastases (PET)
63	59	Male	3	3	1	18 (CT) and 11 (bone scan)	Multiple bone and liver metastases (PET)

FDG, fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; N, node; M, metastasis.
* Diagnosis of lymph node metastasis was not confirmed by other imaging modalities or observation of the clinical course.

3. Results

3.1. Detection of distant metastasis

FDG-PET showed results different from those of conventional staging procedures in 17 of 63 patients. PET scan demonstrated findings suggesting distant metastases in 6 of 63 patients (Table 1). The median interval between conventional staging procedures and FDG-PET was 16 days (range: 7–28). Abnormal uptake was observed around the cardia in one of these six patients (No.6). A repeat FDG-PET study demonstrated a longer uptake stripe indicating radiation-induced oesophagitis and the diagnosis could not be established, as there was a remaining possibility of physiological uptake in the oesophagus. The diagnosis of metastatic disease was confirmed in the remaining five patients (8%, 95% confidence interval (CI): 3–18%). Among these five patients, four had bone metastases, two had liver metastases, one had adrenal metastasis, and two had lymph node metastases in the cervical or axillary region. The therapeutic strategy for these five patients was changed and they received only chemotherapy without thoracic radiotherapy. One patient (No. 47) had shown negative findings on bone scintigraphy four weeks before the FDG-PET study, but PET scan demonstrated increased FDG uptake in bones throughout the body. MRI of the spine confirmed the diagnosis of multiple bone metastases (Fig. 1). A repeat bone scan after three months detected obvious multiple bone metastases in No. 2 patient. Two hepatic lesions, as well as the primary tumour, mediastinal and hilar lymph nodes, had all increased in size after two cycles of chemotherapy in patient No. 55. A hepatic lesion, as well as the primary tumour, had decreased in size after two cycles of chemotherapy in patient No. 63. These hepatic lesions were compatible with liver metastases. Abnormal uptake by the right adrenal gland disappeared on repeat PET/CT after four cycles of chemotherapy in patient No. 59. Abnormal uptake in primary and mediastinal lesions was extremely decreased in this patient. The right adrenal gland lesion was compatible with metastasis.

FDG-PET detected liver metastasis in one of 44 patients staged by CT scan of the abdomen (No. 55), and liver or adrenal metastasis in two of 19 patients staged by US (Nos. 59 and 63). Liver and adrenal metastases not detected by US were small, such that the CT part of PET/CT could not detect them as metastases. Ratios of upstaging by FDG-PET between initial CT scan and US of the abdomen were not statistically significant (1/44 versus 2/19, $P = 0.214$).

3.2. Detection of regional lymph node metastases

FDG-PET scans detected regional lymph node metastases that had been negative on CT scans in nine patients (14%) (Table 2). The median interval between CT of the chest and FDG-PET was 19 days (range: 7–34). FDG-PET scans newly detected ipsilateral supraclavicular lymph node metastasis in four patients, contralateral lymph node metastasis in three, and mediastinal lymph node metastasis in two. These nine patients all underwent curative chemoradiotherapy, and abnormal FDG uptake in mediastinal and/or supraclavicular lymph nodes disappeared or decreased on repeat PET scans after chemoradiotherapy. These lymph nodes were considered positive for metastasis.

CT scan detected swollen mediastinal lymph nodes without abnormal FDG uptake in two patients. One patient had a past history of pulmonary tuberculosis complicated by pulmonary fibrosis. The swollen pretracheal lymph node was considered negative for metastasis because the node size remained unchanged after four cycles of chemotherapy although the primary tumour shrank. This case showed false positive findings on CT whereas FDG-PET correctly diagnosed the extent of disease (No. 43). The other patient had atelectasis of the right middle lobe due to the primary tumour. Superior mediastinal and subcarinal lymph nodes were considered to be metastatic on CT, but abnormal FDG uptake was absent. After three cycles of chemotherapy the

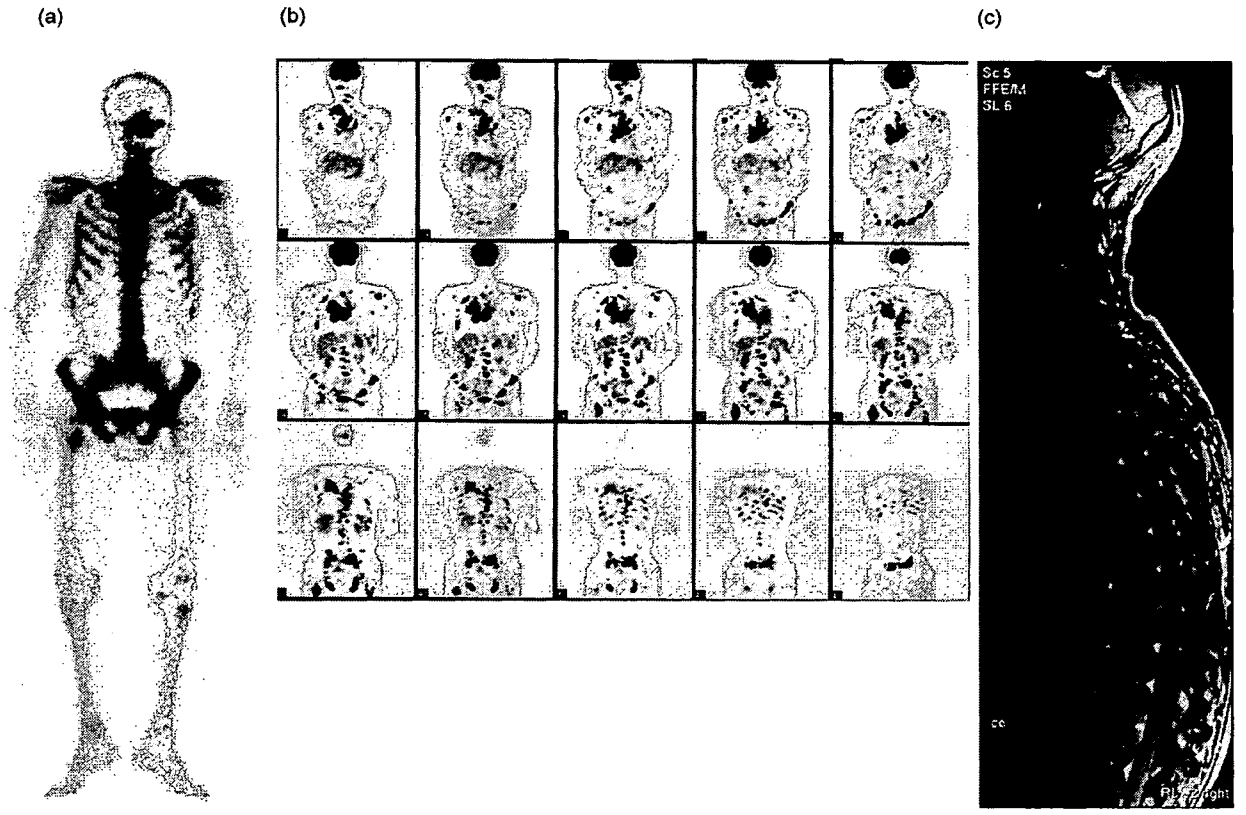

Fig. 1 A 61-year-old man with small-cell lung cancer. Bone scintigraphy was negative for osseous metastasis (a). However, PET scan demonstrated increased FDG uptake in bones throughout the body (b). MRI of the spine confirmed multiple bone metastases (c).

mediastinal lesion showed no change although the primary tumour had decreased in size and atelectasis of the right middle lobe was improved. The mediastinal lymph nodes were considered negative for metastasis (No. 61).

4. Discussion

SCLC tends to disseminate early in the disease course and displays a more aggressive clinical behaviour than NSCLC. Local treatment modalities alone such as radiotherapy or surgery are not effective in prolonging survival beyond a few weeks. Systemic chemotherapy is the mainstay of treatment for patients in all stages of SCLC. A combination of chemotherapy and thoracic irradiation can promote long-term survival for patients diagnosed as having limited disease and recent clinical trials of chemoradiotherapy for LD-SCLC obtained 5-year survival rates of 24–26% [2,3]. However, thoracic irradiation might cause severe radiation pneumonitis, resulting in respiratory failure and/or treatment-related death. Furthermore, thoracic irradiation might also cause oesophagitis which worsens patient quality of life. Accurate clinical staging is important to determine the indications for chemoradiotherapy in SCLC. Our study demonstrated that FDG-PET scan detected unsuspected distant metastases in 8% of patients with LD-SCLC based on conventional staging procedures and that the detection of these new lesions changed their therapeutic strategies. Furthermore, FDG-PET scan detected regional lymph node metastases which had not been visualized on CT scan in 14% of patients. The radiation field could be appropriately set to cover the positive nodes based on the PET study results. Our results reconfirmed those of a previous preliminary study with a smaller number of patients [9].

Is the rate of the detection of unsuspected distant metastases (8%) clinically significant? Previous studies demonstrated that FDG-PET scan detected unsuspected distant metastases in 24% of patients with stage III NSCLC [6,7]. Compared to this result, the impact of FDG-PET on the staging of SCLC seems to be weaker. SCLC tends to have more obvious distant metastases than NSCLC, because of the aggressive biological behaviour of SCLC. Therefore, FDG-PET might detect unsuspected distant metastases at a relatively low rate. The most common region for unsuspected PET-detected metastasis in NSCLC was the abdomen, with 53% of pateints having adrenal, liver, and other lesions [6]. In our study, FDG-PET detected bone metastases in four of five patients who were upstaged from LD to ED. These lesions might reflect metastasis to the bone marrow, although no pathological evidence was obtained, because neither bone marrow biopsy nor aspiration cytology was routinely conducted for the initial clinical staging.

Our retrospective analyses have several limitations. We did not confirm histologically regional lymph node or distant metastases detected by FDG-PET or CT. These lesions were not routinely biopsied and most metastatic lesions were chemosensitive and radiosensitive. Our confirmation was inevitably based on observation of the clinical course.

Table 2 Disagreement between FDG-PET and conventional staging procedures (regional lymph node metastases)

Patient no.	Age (years)	Gender	CT N	PET N	PET M	Interval between CT scan of the chest and FDG-PET (days)	Comments
1	63	Male	3	3	0	8	Contralateral supraclavicular lymph node metastasis (PET)
5	64	Female	1	2	0	34	Subcarinal lymph node metastasis (PET)
16	71	Male	3	3	0	7	Contralateral supraclavicular lymph node metastasis (PET)
20	69	Male	3	3	0	20	Ipsilateral supraclavicular lymph node metastasis (PET)
25	60	Male	3	3	0	27	Ipsilateral supraclavicular lymph node metastasis (PET)
30	66	Male	2	2	0	7	Pretracheal lymph node metastasis (PET)
33	72	Male	3	3	0	13	Ipsilateral supraclavicular lymph node metastasis (PET)
41	49	Female	3	3	0	19	Contralateral supraclavicular lymph node metastasis (PET)
43	73	Male	2	0	0	34	False-positive pretracheal lymph node metastasis (CT)
56	48	Female	3	3	0	11	Ipsilateral supraclavicular lymph node metastasis (PET)
61	74	Male	2	0	0	27	False-positive superior mediastinal and subcarinal lymph nodes (CT).

FDG, fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; N, node; M, metastasis.

We employed no special strategies to reduce the bias of PET readers. PET readers might have reported in such a way as to reduce or increase the impact of PET. One-third of patients received FDG-PET after commencement of chemotherapy. However, the median interval between commencement of chemotherapy and FDG-PET was 4 days (range: 1–11 days). We considered the chemotherapy to have had no effects on the findings of FDG-PET in such a short time after the initiation of chemotherapy.

FDG-PET is expected to have the potentially to both up- and downstage patients with SCLC as well as NSCLC. A previous study demonstrated that FDG-PET correctly downstaged ED to LD in three of 120 patients with SCLC [10]. These three patients had adrenal swelling on CT scan, but these lesions were negative on FDG-PET. On the other hand, FDG-PET correctly upstaged LD to ED in 10 of 120 patients with SCLC. It seems that SCLC seldom has a solitary distant metastasis because of its aggressive clinical behaviour. Most ED-SCLC has multiple, not solitary, or obvious distant metastasis. Furthermore, the health insurance system does not allow patients who obviously have metastatic lung cancer to receive FDG-PET in Japan. Therefore, we did not include patients with ED-SCLC in our analysis. Needless to say, FDG-PET is considered to be useful in patients with possible, but not evident, distant metastasis on other imaging tests, such as a solitary adrenal swelling.

According to the VALSG system, LD-SCLC is defined as a tumour confined to one hemithorax and regional lymph nodes [1]. Contralateral hilar or contralateral supraclavicular nodal involvement was classified as ED. According to the International Association for the Study of Lung Cancer (IASLC) consensus report, the classification of LD-SCLC includes bilateral hilar and/or supraclavicular nodal involvement, and ipsilateral pleural effusion [18]. A previous retrospective study demonstrated that the IASLC staging criteria for SCLC patients had a higher prognostic impact than VALSG criteria [19]. Therefore, we adopted the IASLC staging criteria for SCLC in our study.

In conclusion, FDG-PET scans detected unsuspected distant metastases in five of 63 patients with LD-SCLC (95% CI: 3–18%) and these findings resulted in a change of therapeutic strategies in these five patients. FDG-PET scans also detected contralateral supraclavicular lymph node metastases that had been negative on CT scans in three other

patients. These additional findings facilitated setting appropriate irradiation fields. FDG-PET scan is recommended as an initial staging tool in patients with apparent LD-SCLC.

Conflict of interest

The authors certify that there are no potential conflicts of interest.

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original article

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Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan

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Background: To compare the efficacy and toxicity of three platinum-based combination regimens against cisplatin plus irinotecan (IP) in patients with untreated advanced non-small-cell lung cancer (NSCLC) by a non-inferiority design.
Patients and methods: A total of 602 patients were randomly assigned to one of four regimens: cisplatin 80 mg/m^2 on day 1 plus irinotecan 60 mg/m^2 on days 1, 8, 15 every 4 weeks (IP) carboplatin AUC 6.0 min × mg/mL (area under the concentration–time curve) on day 1 plus paclitaxel 200 mg/m^2 on day 1 every 3 weeks (TC); cisplatin 80 mg/m^2 on day 1 plus gemcitabine 1000 mg/m^2 on days 1, 8 every 3 weeks (GP); and cisplatin 80 mg/m^2 on day 1 plus vinorelbine 25 mg/m^2 on days 1, 8 every 3 weeks (NP).
Results: The response rate, median survival time, and 1-year survival rate were 31.0%, 13.9 months, 59.2%, respectively, in IP; 32.4%, 12.3 months, 51.0% in TC; 30.1%, 14.0 months, 59.6% in GP; and 33.1%, 11.4 months, 48.3% in NP. No statistically significant differences were found in response rate or overall survival, but the non-inferiority of none of the experimental regimens could be confirmed. All the four regimens were well tolerated.
Conclusion: The four regimens have similar efficacy and different toxicity profiles, and they can be used to treat advanced NSCLC patients.
Key words: carboplatin, cisplatin, gemcitabine, irinotecan, non-small-cell lung cancer, paclitaxel, randomized phase III study, vinorelbine

introduction

Nearly 60 000 patients in Japan died of lung cancer in 2004, and the mortality rate is still increasing [1]. Even old-generation cisplatin-based chemotherapy provides a survival benefit and symptom relief in patients with inoperable non-small-cell lung cancer (NSCLC) [2]. Several anticancer agents including irinotecan, paclitaxel, docetaxel, gemcitabine, and vinorelbine, were developed in the 1990s and most of them have mechanisms of action that differ from those of the old-generation agents [3–7]. The combinations of platinum and these new agents developed in the 1990s are more useful against advanced NSCLC than old-generation combination chemotherapy, and doublets of platinum and new-generation anticancer agents are considered standard chemotherapy regimens for advanced NSCLC, although no consistent standard regimens have yet been established [8–17].

Two phase III studies comparing cisplatin plus irinotecan (IP) with cisplatin plus vindesine for advanced NSCLC have been conducted in Japan [18, 19]. Fukuoka et al. [20] reported the results of a combined analysis of the 358 eligible stage IV patients in these studies. They carried out a multivariate analysis using the Cox regression model with adjustment for well-known prognostic factors, and the Cox regression analysis demonstrated that treatment with IP was one of significant independent favorable factor. Based on their data, we selected IP for the reference arm in our study.

The Ministry of Health, Labour and Welfare of Japan approved the prescription of paclitaxel, gemcitabine, and

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vinorelbine for NSCLC in 1999 and requested a phase III study to confirm the efficacy and safety of these agents. The Japanese investigators and the pharmaceutical companies decided to conduct a four-arm randomized phase III study for NSCLC, the so-called FACS, Four-Arm Cooperative Study. The purpose of the study was to compare the efficacy and toxicity of three platinum-based combination regimens, carboplatin plus paclitaxel (TC), cisplatin plus gemcitabine (GP), cisplatin plus vinorelbine (NP), with IP as the reference arm.

patients and methods

patient selection

Patients with histologically and/or cytologically documented NSCLC were eligible for participation in the study. Each patient had to meet the following criteria: clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy, such as malignant pleural effusion, pleural dissemination, malignant pericardiac effusion, or metastatic lesion in the same lobe), at least one target lesion >2 cm, no prior chemotherapy, no prior surgery and/or radiotherapy for the primary site, age 20–74 years, Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, adequate hematological, hepatic and renal functions, partial pressure of arterial oxygen (paO_2) ≥60 torr, expected survival >3 months, able to undergo first course treatment in an inpatient setting, and written informed consent. The study was approved by the Institutional Review Board at each hospital. Written informed consent was obtained from every patient.

treatment schedule

All patients were randomly assigned to one of the four treatment groups by the central registration office by means of the minimization method. Stage, PS, gender, lactate dehydrogenase (LDH) and albumin values, and institution were used as adjustment variables. The first group received the reference treatment, 80 mg/m^2 of cisplatin on day 1 and 60 mg/m^2 of irinotecan on days 1, 8, and 15, and the cycle was repeated every 4 weeks. The second group received 200 mg/m^2 of paclitaxel (Bristol-Myers K.K., Tokyo, Japan) over a 3-h period followed by carboplatin at a dose calculated to produce an area under the concentration–time curve of 6.0 min × mg/mL on day 1 and the cycle was repeated every 3 weeks. The third group received 80 mg/m^2 of cisplatin on day 1 and 1000 mg/m^2 of gemcitabine (Eli Lilly Japan K.K., Kobe, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. The fourth group received 80 mg/m^2 of cisplatin on day 1 and 25 mg/m^2 of vinorelbine (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. Each treatment was repeated for three or more cycles unless the patient met the criteria for progressive disease or experienced unacceptable toxicity.

response and toxicity evaluation

Response was evaluated according to the Response Evaluation Criteria in Solid Tumors, and tumor markers were excluded from the criteria [21]. Objective tumor response in all responding patients was evaluated by an external review committee with no information on the treatment group. Toxicity grading criteria in National Cancer Institute Common Toxicity Criteria Ver 2.0 were used to evaluate toxicity.

quality of life assessment

Quality of life (QoL) was evaluated by means of the Functional Assessment of Cancer Therapy—Lung (FACT-L) Japanese version and the QoL Questionnaire for Cancer Patients Treated with Anticancer Drugs (QoL-ACD), before treatment, immediately before the second cycles of chemotherapy, and 3 and 6 months after the start of treatment [22–24].

statistical analysis and monitoring

The primary end point of this study was overall survival (OS), and the secondary end points were response rate, response duration, time to progressive disease (TTP), time to treatment failure (TTTF), adverse event, and QoL. The 1-year survival rate of the control group in this study was estimated to be 43% based on the data in published papers, and the 1-year survival rate in the other treatment group was expected to be 50%. The lower equivalence limit for 1-year survival rate was set as '−10%'. The criterion for the non-inferiority of each treatment was a lower limit of the two-sided 95% confidence interval (CI) of the 1-year survival rate of treatment minus that of control larger than the lower equivalence limit. Because the non-inferiority of each treatment versus the control was to be evaluated independently, a separate null hypothesis was stated for each treatment, and for that reason no multiple comparison adjustment was included in the study. Based on the above conditions and binomial distribution, 135 patients were needed per arm for a one-sided Type I error of 2.5% and 80.0% power. In view of the possibility of variance inflation due to censoring, the sample size was set at 600 (150 per arm).

Central registration with randomization, monitoring, data collection, and the statistical analyses were independently carried out by a contract research organization (EPS Co., Ltd, Tokyo, Japan).

results

patient characteristics

From October 2000 to June 2002, a total of 602 patients were registered by 44 hospitals in Japan. All patients had been followed up for >2 years, and 447 patients had died as of June 2004. Of the 602 patients registered, 151 were allocated to the reference treatment, IP, and 150, 151, and 150 patients were allocated to TC, GP, and NP, respectively. Since 10 patients did not receive chemotherapy and 11 patients were subsequently found to be ineligible, 592 patients were assessable for toxicity and 581 patients were assessable for efficacy. Four patients did not receive chemotherapy due to electrolytic disorder, fever, symptomatic brain metastases, and rapid tumor progression in IP, two patients due to refusal and pneumonia in TC, four patients due to lower WBC counts (two patients), rapid tumor progression, and nephritic syndrome in NP. Two patents were ineligible due to wrong stage in IP, two patients were wrong stage and one patient had double cancer in TC, two patients were wrong diagnosis, one patient had massive pleural effusion, one patient received prior chemotherapy in GP, one patient had no target lesions in NP. Age, gender, PS, stage, and LDH and albumin values were well balanced in each arm (Table 1). Fewer patients with adenocarcinoma and more patients with squamous cell carcinona were, however, entered in three experimental arms than in IP.

objective tumor response and response duration

Objective tumor response is shown in Table 2. Forty-five partial responses occurred in the 145 assessable patients in the reference arm, IP, for an objective response rate of 31.0% with a median response duration of 4.8 months. The response rate and median response duration were 32.4% and 4.0 months in TC, 30.1% and 3.5 months in GP, and 33.1% and 3.4 months in NP. The response rates in TC, GP, and NP were not statistically different from the rate in IP according to the results of the χ^2 test.

Table 1. Patient characteristics and treatment delivery

	Cisplatin + irinotecan	Carboplatin + paclitaxel	Cisplatin + gemcitabine	Cisplatin + vinorelbine
Assessable patients	145	145	146	145
Gender (male/female)	97/48	99/46	101/45	101/44
Age, median (range)	62 (30–74)	63 (33–74)	61 (34–74)	61 (28–74)
PS (0/1)	44/101	44/101	45/101	45/100
Histology				
Adenocarcinoma	121	104	108	109
Squamous cell carcinoma	16	31	29	29
Others	8	10	9	7
Stage (IIIB/IV)	31/114	28/117	30/116	26/119
No. of cycles				
Mean ± SD	3.0 ± 1.3	3.5 ± 1.5	3.2 ± 1.2	3.1 ± 1.3
Median	3	3	3	3
Range	1–7	1–10	1–7	1–8

PS, performance status; SD, standard deviation.

Table 2. Survival, TTP, TTTF, response rate, and response duration

	N	Median survival, months	1-year survival (%)	Difference in 1-year survival from IP	2-year survival (%)	TTP (median), months	TTTF (median), months	Response rate (%)	Response duration (median), months
Cisplatin + irinotecan	145	13.9	59.2	–	26.5	4.7	3.3	31.0	4.8 ($n = 45$)
Carboplatin + paclitaxel	145	12.3	51.0	−8.2% (95% CI −19.6% to 3.3%)	25.5	4.5 ($P = 0.355$)[a]	3.2 ($P = 0.282$)[a]	32.4 ($P = 0.801$)[b]	4.0 ($n = 47$)
Cisplatin + gemcitabine	146	14.0	59.6	0.4% (95% CI −10.9% to11.7%)	31.5	4.0 ($P = 0.170$)[a]	3.2 ($P = 0.567$)[a]	30.1 ($P = 0.868$)[b]	3.5 ($n = 44$)
Cisplatin + vinorelbine	145	11.4	48.3	−10.9% (95% CI −22.3% to 0.5%)	21.4	4.1 ($P = 0.133$)[a]	3.0 ($P = 0.091$)[a]	33.1 ($P = 0.706$)[b]	3.4 ($n = 48$)

[a]Compared with IP by the generalized Wilcoxon test.

[b]Compared with IP by the χ^2 test.

CI, confidence interval; IP, cisplatin plus irinotecan; TTP, time to progressive disease; TTTF, time to treatment failure.

OS, TTP disease, and TTTF

OS and TTP are shown in Figure 1. Median survival time (MST), the 1-year, and 2-year survival rate in IP were 13.9 months, 59.2%, and 26.5%, respectively. The MSTs, 1-year, and 2-year survival rates were, respectively, 12.3 months, 51.0%, and 25.5% in TC; 14.0 months, 59.6%, and 31.5% in GP; and 11.4 months, 48.3%, and 21.4% in NP. The lower limits of the 95% CI of the difference in 1-year survival rate between IP and TC (−19.6%), GP (−10.9%), and NP (−22.3%) were below −10%, which was considered the lower equivalence limit (Table 2). Thus, the results did not show non-inferiority in three experimental regimens compared with reference treatment. Median TTP and median TTTF were 4.7 and 3.3 months, respectively in IP. Median TTP and TTTF were, respectively, 4.5 and 3.2 months in TC, 4.0 and 3.2 months in GP, and 4.1 and 3.0 months in NP. There were no statistical differences in either TTP or TTTF in TC, GP, or NP, compared with IP according to the results of the generalized Wilcoxon test (Table 2).

hematologic and non-hematologic toxicity

In IP, 47.6% and 83.7% of patients developed grade 3 or worse leukopenia and neutropenia, respectively (Table 3). The incidences of grade 3 or worse leukopenia (33.1%, $P = 0.010$) and neutropenia (62.9%, $P < 0.001$) were significantly lower in GP than in IP. The incidence of grade 3 or worse leukopenia (67.1%, $P < 0.001$) was significantly higher in NP than in IP. Grade 3 or worse thrombocytopenia developed in 5.4% of the patients in IP, and the incidence was significantly higher in GP (35.1%, $P < 0.001$). The incidence of febril neutropenia in IP was 14.3%, and was significantly lower in GP (2.0%, $P < 0.001$).

Grade 2 or worse nausea, vomiting, anorexia, and fatigue occurred in 60.5%, 51.0%, 65.3%, and 38.8%, respectively, of the patients in IP. The incidences of grade 2 or worse nausea (TC: 25.0%, $P < 0.001$, NP: 47.3%, $P = 0.022$), vomiting (TC: 22.3%, $P < 0.001$, NP: 36.3%, $P = 0.011$), and anorexia (TC: 32.4%, $P < 0.001$, NP: 49.3%, $P = 0.005$) were significantly lower in TC and NP than in IP. Grade 2 or worse diarrhea was

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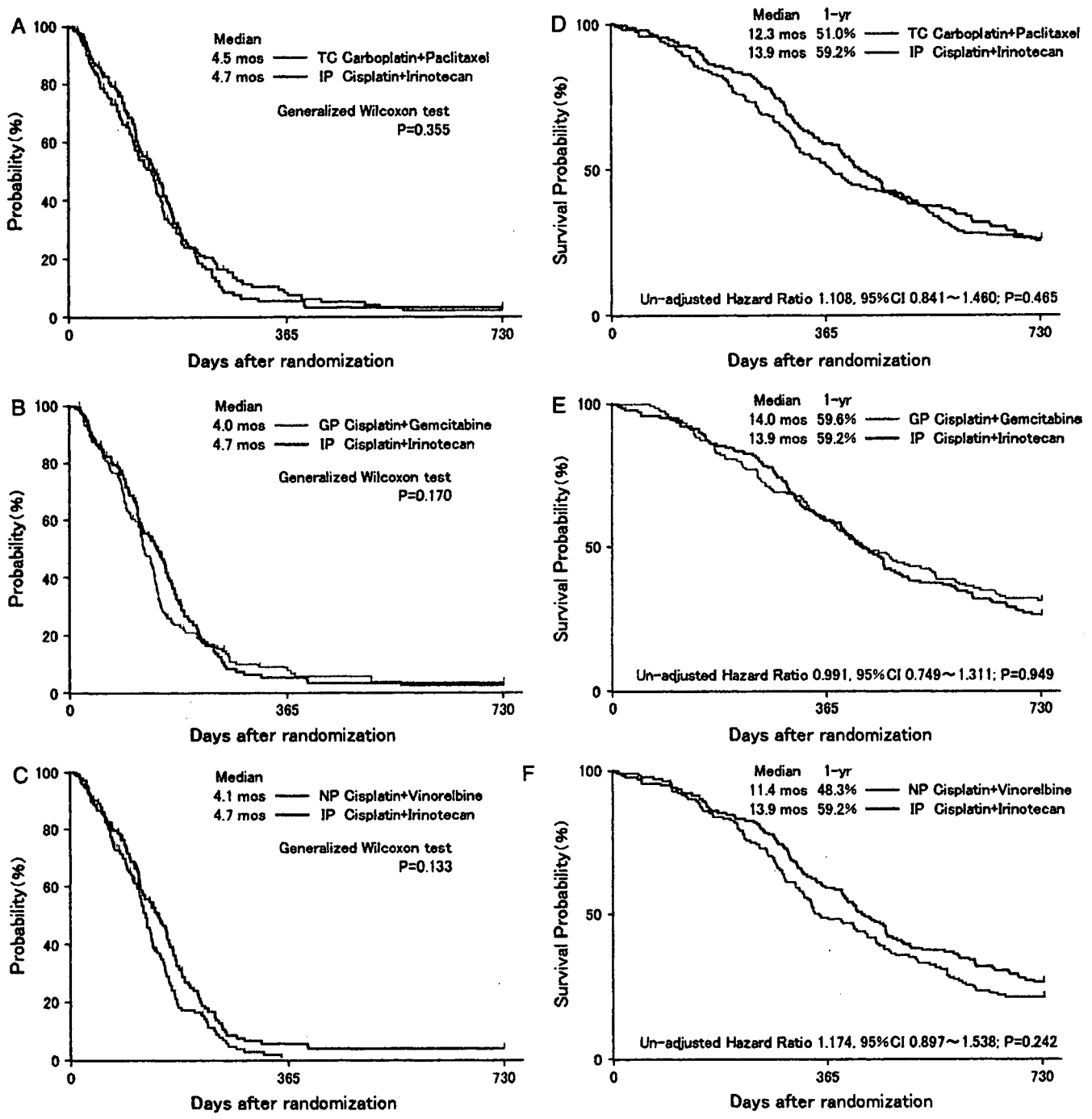

Figure 1. Overall survival (OS) and time to progressive (TTP) disease. TTP and OS in the carboplatin plus paclitaxel (TC) (A, D), cisplatin plus gemcitabine (GP) (B, E), and cisplatin plus vinorelbine (NP) (C, F) were not statistically significantly different from the values in the cisplatin plus irinotecan.

significantly less frequent in TC (6.8%), GP (8.6%), and NP (11.6%) than in IP (48.3%, $P < 0.001$). The incidences of grade 2 or worse sensory neuropathy (16.9%, $P < 0.001$), arthralgia (21.6%, $P < 0.001$), and myalgia (17.6%, $P < 0.001$) were significantly higher in TC than in IP. Grade 2 alopecia occurred in 30.6% of the patients in IP, and its incidence was significantly higher in TC (44.6%, $P = 0.013$) and significantly lower in GP (15.2%, $P = 0.001$) and NP (8.9%, $P < 0.001$). Grade 2 injection site reactions were more frequent in NP (26.7%) than in IP (4.8%, $P < 0.001$).

A total of five patients died of treatment-related toxicity: three in IP (cerebral hemorrhage, interstitial pneumonia, acute circulatory failure/disseminated intravascular coagulation: 2.0%), one in TC (acute renal failure: 0.7%), and one in NP (pulmonary embolism: 0.7%).

second-line treatment

Data on second-line treatment, but not third-line or later treatment, was available in this study, and they showed that

Table 3. Toxicity

	IP ($n = 147$)[a]			TC ($n = 148$)			GP ($n = 151$)			NP ($n = 146$)		
	Grade (%)			Grade (%)			Grade (%)			Grade (%)		
	2	3	4	2	3	4	2	3	4	2	3	4
Leukocytes	42	43	5	39	42	3	40	31[a]	2[a]	25	51[b]	16[b]
Neutrophils	11	39	45	5	19	69	21	40	23[a]	5	16	72
Hemoglobin	42	24	7	42	13[a]	2[a]	44	22	5	43	25	5
Platelets	6	5	1	9	11	0	22	35[b]	0[b]	3	1[a]	0[a]
Febrile neutropenia	–	14	0	–	18	0	–	2[a]	0[a]	–	18	0
Nausea	32	29	–	14[c]	11[c]	–	35	23	–	33[c]	14[c]	–
Vomiting	38	13	0	17[c]	5[c]	0[c]	34	14	0	29[c]	7[c]	0[c]
Anorexia	30	33	2	15[c]	17[c]	1[c]	31	26	1	29[c]	20c	1[c]
Fatigue	27	12	1	26	2	1	17[c]	3[c]	0[c]	23[c]	3[c]	0[c]
Diarrhea	33	15	1	4[c]	3[c]	0[c]	7[c]	2[c]	0[c]	8[c]	4[c]	0[c]
Constipation	27	7	0	30	8	0	33	9	0	40[d]	14[d]	0[d]
Neuropathy, motor	1	0	0	1	1	1	0	0	0	0	0	0
Neuropathy, sensory	1	0	0	14[d]	3[d]	0[d]	0	0	0	0	0	0
Alopecia	31	–	–	45[d]	–	–	15[c]	–	–	9[c]	–	–
Arthralgia	2	0	0	20[d]	2[d]	0[d]	0	0	0	1	0	0
Myalgia	1	0	0	16[d]	2[d]	0[d]	0	0	0	1	1	0
Injection site reaction	5	0	–	5	0	–	5	0	–	27[d]	0[d]	–
Pneumonitis	0	1	1	0	1	0	0	0	0	0	1	0
Creatinine	8	1	0	2[c]	0[c]	0[c]	7	0	0	8	1	0
AST	7	1	1	5	1	0	6	3	0	1	3	0
Fever	2	0	0	5	1	0	1	0	0	1	0	0
Treatment-related death	3 (2.0%)			1 (0.7%)			0			1 (0.7%)		

[a]Incidence of grade 3 or 4 toxicity significantly ($P < 0.05$) lower than that with IP.
[b]Incidence of grade 3 or 4 toxicity significantly ($P < 0.05$) higher than that with IP.
[c]Incidence of grade 2 or worse toxicity is significantly ($P < 0.05$) lower than that with IP.
[d]Incidence of grade 2 or worse toxicity significantly ($P < 0.05$) higher than that with IP.
GP, cisplatin plus gemcitabine; IP, cisplatin plus irinotecan; NP, cisplatin plus vinorelbine; TC, carboplatin plus paclitaxel.
AST, aspartate aminotransferase; –, no category in the criteria.

60%–74% of the patients received chemotherapy and 6%–9% received thoracic irradiation as second-line treatment (Table 4). The percentages of patients in each treatment group who received second-line chemotherapy were not significantly different ($P = 0.081$).

quality of life

The details of the QoL analysis will be reported elsewhere. No statistically significant difference in global QoL was observed among the four treatment groups based on either the FACT-L Japanese version or the QoL-ACD. Only the physical domain evaluated by QoL-ACD was significantly better in TC, GP, and NP than in IP.

discussion

Many randomized phase III studies have compared platinum-plus-new-agent doublets in NSCLC, but, this is the first to evaluate the efficacy of an irinotecan-containing regimen in comparison with other platinum-plus-new-agent doublets in NSCLC [14–17]. Although non-platinum-containing chemotherapy regimens are used as alternatives, doublets of platinum and a new-generation anticancer agent, such as TC, GP, and NP, are considered standard chemotherapy regimens for advanced NSCLC worldwide [13–17, 25]. Although the non-inferiority of none of the three experimental regimens could be confirmed in this study, no statistically significant differences in response rate, OS, TTP, or TTTF were observed between the reference regimen and the experimental regimens. All four platinum-based doublets have similar efficacy against advanced NSCLC but different toxicity profiles. Nevertheless, IP was still regarded as the reference regimen in this study because the non-inferiority of none of the three experimental regimens could be confirmed.

OS in this study was relatively longer than previously reported. The estimated 1-year survival rate in the reference arm was 43%, but the actual 1-year survival rate was 59.2%, much higher than expected. The MSTs reported for patients treated with TC, GP, and NP in recent phase III studies have ranged from 8 to 10 months, and in the present study they were 12.3, 14.0, and 11.4 months, respectively [14–17]. One reason for the good OS in this study was the difference in patient selection criteria, for example exclusion of PS2 patients. Ethnic differences in pharmacogenomics have also been indicated as a possible reason for the good OS in this study [26]. The OS in IP in this study, however, was better than in previous Japanese studies [18, 19]. TTP in this study ranged from 4.0 to 4.7 months, and was similar to the TTP of 3.1–5.5 months reported in the literature [15, 16]. OS not TTP was longer in this study

Table 4. Second-line treatment

	Cisplatin + irinotecan	Carboplatin + paclitaxel	Cisplatin + gemcitabine	Cisplatin + vinorelbine	
Number of patients	145	145	146	145	
Chemotherapy	107 (74%)	87 (60%)	101 (69%)	95 (66%)	$P = 0.081$
Docetaxel	39	25	50	51	
Gefitinib	11	9	18	12	
Paclitaxel	15	14	7	11	
Gemcitabine	24	28	17	28	
Vinorelbine	9	12	2	9	
Irinotecan	15	4	3	3	
Thoracic irradiation	8	10	13	10	

than previously reported, and higher 2-year survival rates, 21.4%–31.5%, were observed in the minimum 2-year follow-up in this study. Second-line or later treatments may affect survival, because docetaxel has been established as standard second-line chemotherapy for advanced NSCLC [27, 28]. Gefitinib is also effective as second-line or later chemotherapy for advanced NSCLC, especially in Asian patients, never smokers and patients with adenocarcinoma [29–32].

The toxicity profile of each treatment differed and the toxicity of all four regimens was well tolerated. Overall QoL was similar in the four platinum-based doublets. Only physical domain QoL evaluated by the QoL-ACD was statistically better in TC, GP, and NP than in IP. This finding is presumably attributable to the fact that diarrhea is a statistically less frequent adverse effect of TC, GP, and NP than of IP.

In conclusion, all four platinum-based doublets had similar efficacy for advanced NSCLC but different toxicity profiles. All the four regimens can be used to treat advanced NSCLC patients in clinical practice.

appendix

Institutions of the FACS Cooperative Group: National Hospital Organization (NHO) Hokkaido Cancer Center, Tohoku University Hospital, Yamagata Prefectural Central Hospital, Niigata Cancer Center Hospital, Tochigi Cancer Center, NHO Nishigunma National Hospital, Saitama Cancer Center, National Cancer Center Hospital East, Chiba University Hospital, National Cancer Center Hospital, Tokyo Medical University Hospital, Japanese Foundation for Cancer Research, Kanagawa Cancer Center, Yokohama Municipal Citizen's Hospital, Kanagawa Cardiovascular and Respiratory Center, Aichi Cancer Center Hospital, Prefectural Aichi Hospital, Nagoya City University Hospital, NHO Nagoya Medical Center, Nagoya University Hospital, Gifu Municipal Hospital, NHO Kyoto Medical Center, Osaka City General Hospital, Osaka City University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, NHO Toneyama Hospital, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Kinki University School of Medicine, Rinku General Medical Center Izumisano Municipal Hospital, Kobe Central General Hospital, The Hospital of Hyogo College of Medicine, Hyogo Medical Center for Adults, Tokushima University Hospital, Kagawa Prefectural Central Hospital, NHO Shikoku Cancer Center Hospital, Hiroshima University Medical Hospital, NHO Kyushu Cancer Center Hospital, Kyushu University Hospital, National Nagasaki Medical Center, Nagasaki Municipal Hospital, Nagasaki University Hospital of Medicine and Dentistry, Kumamoto Chuo Hospital, Kumamoto Regional Medical Center, NTT West Osaka Hospital.

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Susceptibility to Lung Cancer and Genetic Polymorphisms in the Alcohol Metabolite-related Enzymes Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 in the Japanese Population

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BACKGROUND. It is believed that acetaldehyde plays an important role in alcohol-related carcinogenesis; although current epidemiologic studies have provided inconsistent findings on the association between alcohol consumption and the risk of lung cancer.

METHODS. To clarify the hypothesis that genetic polymorphisms in alcohol-metabolizing enzymes may influence susceptibility to lung cancer, the authors conducted a hospital-based case-control study and examined genetic polymorphisms in the alcohol dehydrogenase 3, aldehyde dehydrogenase 2 ($ALDH_2$), and cytochrome P450 2E1 genes in 505 patients with histologically confirmed lung cancer and in a group of 256 noncancer controls who provided complete cigarette and alcohol consumption histories. Genotyping was conducted by polymerase chain reaction-restriction fragment-length polymorphism assay.

RESULTS. A significant association was noted between alcohol consumption and lung cancer risk. Thus, using the median value for the controls as the cut-off point, the odds ratios (OR) for light and heavy drinkers were 1.76 and 1.95, respectively (P for trend = .012), compared with nondrinkers. In addition, there was a significant trend toward increased risk of lung cancer in drinkers with $ALDH_2$ variant alleles (P for trend <.0001). The adjusted OR for heavy drinkers was 6.15 compared with nondrinkers. Regarding associations between histologic type and genotypes, the $ALDH_2$ variant allele was significantly less common in patients who had adenocarcinoma compared with controls.

CONCLUSIONS. The current observations suggested a positive association between alcohol consumption and the risk of lung cancer: Drinking may increase the risk, especially among individuals who have the variant $ALDH_2$ alleles. ***Cancer* 2007;110:353–62.** *2007 American Cancer Society.*

KEYWORDS: lung cancer, alcohol consumption, case-control study, genetic polymorphism, alcohol dehydrogenase 3, aldehyde dehydrogenase 2, cytochrome P450 2E1.

Epidemiologic studies have provided inconsistent results regarding the associations between alcohol consumption and the risk of lung cancer. In general, therefore, the involvement of alcohol in lung cancer etiology has been regarded with skepticism, with any indication of an association being attributed in most instances to confounding factors, such as cigarette smoking.[1] It indeed is difficult to separate the effects of alcohol and smoking because, the 2 tend to be

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correlated, but this problem does not automatically exclude the possibility that there is a separate alcohol effect. A panel of experts commissioned by the World Cancer Research Fund and the American Institute for Cancer Research in 1997, after reviewing the epidemiologic evidence, concluded that alcohol intake *possibly* may increase lung cancer risk.[2] Although the mechanism by which alcohol may cause cancer remains obscure, many epidemiologic studies have identified chronic alcohol consumption as a significant risk factor for cancers of the oral cavity, pharynx, larynx, and esophagus in humans.[3] When investigating the role of alcohol-related carcinogenesis, most studies have concentrated on the type of alcoholic beverage consumed and the amount of daily intake, but this does not fully explain the variance in individual susceptibility to alcohol-related cancer.

Recent reports strongly implicate acetaldehyde, the first metabolite of ethanol, rather than alcohol itself, as responsible for the risk of developing alcohol-related cancers. It has been reported that acetaldehyde causes mutations by DNA adduct formation and inhibition of DNA repair. Moreover, drinking or inhaling acetaldehyde has mutagenic and carcinogenic effects and induced nasal and laryngeal carcinomas in experimental animals.[4–8]

Ethanol is primarily (80%) oxidized to acetaldehyde by alcohol dehydrogenase (*ADH*), and most of this acetaldehyde is then eliminated by aldehyde dehydrogenase (*ALDH*). However, ethanol and acetaldehyde also are metabolized through the microsomal ethanol-oxidizing system and the microsomal acetaldehyde-oxidizing system, and cytochrome P450 2E1 (*CYP2E1*) is a major contributor to those systems.[9,10] *CYP2E1* has high oxidation activity and is induced by long-term alcohol intake. These enzymes exhibit wide interindividual variability in their activity, suggesting that the variation may be caused by genetic polymorphisms.

There are several *ADH* subtypes, some of which have genetic variants with altered kinetic properties. ADH_3 is polymorphic, and the enzyme encoded by the ADH_3^1 allele metabolizes ethanol to acetaldehyde 2.5 times faster than that encoded by the ADH_3^2 allele.[11] $ALDH_2$ is a key enzyme in the elimination of acetaldehyde. In individuals with $ALDH_2^2$, a variant allele that is prevalent among East Asians (eg, 50% prevalence in Japan[12]), the activity of this enzyme is extremely low. The *CYP2E1* variant allele, which is detectable by *Rsa*I digestion (termed the c2 variant), corresponds to higher activity ethanol metabolism and is associated with greater alcohol consumption.[13–15] Individuals who have 1 or more ADH_3^1, $ALDH_2^2$, and *CYP2E1* c2 alleles accumulate more acetaldehyde in the blood after drinking ethanol and may be at increased risk for various alcohol-related diseases at similar levels of alcohol intake as individuals who do not carry these alleles. Because the ADH_3 variant allele is common in whites, and the $ALDH_2$ and *CYP2E1* variant alleles are found at high frequency in Asians, research on these genes is most advanced regarding alcohol-related diseases and alcohol metabolism.

The association between genetic polymorphisms in these enzymes and susceptibility to some types of cancer has been reported in case-control studies. The ADH_3^1 and $ALDH_2^2$ alleles are associated closely with alcohol-related cancers in the upper aerodigestive tract,[16–21] and systemic acetaldehydemia has been considered responsible for carcinogenesis in this locality. However, to our knowledge, there are no reports on associations between polymorphisms of *ALDH* and lung cancer risk. In relation to *ADH*, a negative association between genetic variation in ADH_3 and lung cancer has been reported recently.[22] *CYP2E1* is responsible primarily for the bioactivation of many low-molecular-weight, tobacco-specific carcinogens, including certain nitrosamines, such as *N*-nitrosodimethylamine and *N*-nitrosonornicotine. It is possible that the *CYP2E1* c2 variant not only may increase the blood concentration of acetaldehyde but also may activate these carcinogens more strongly. Activated nitrosamines have been linked to the development of numerous cancers. However, results from studies that evaluated the role of *CYP2E1* polymorphisms in relation to lung cancer have been discrepant.[23–28] Because previous investigations did not adjust for alcohol consumption and/or did not have sufficient power to distinguish the risk from alcohol consumption, these inconsistent findings may have been caused by variations in *CYP2E1* enzyme activity induced by ethanol.

We conducted a hospital-based case-control study to evaluate whether ADH_3, $ALDH_2$, or *CYP2E1* polymorphisms are associated with lung carcinogenesis. The primary endpoint of the current study was to clarify the association between each genetic polymorphism and the risk of lung cancer, controlling for the amount of alcohol consumed and smoking habits. Furthermore, associations between alcohol consumption and lung cancer risk in individuals with variant alleles, again controlling for smoking, and associations between these polymorphisms and histologic characteristics were evaluated.

MATERIALS AND METHODS

Participants

This study was approved by the Institutional Review Board and the Ethics Committee of the National