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Irinotecan pharmacokinetics/pharmacodynamics and *UGT1A* genetic polymorphisms in Japanese: roles of *UGT1A1*6* and **28*

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Objectives SN-38, an active metabolite of irinotecan, is detoxified by glucuronidation with UGT1A isoforms, 1A1, 1A7, 1A9, and 1A10. The pharmacogenetic information on *UGT1A* haplotypes covering all these isoforms is important for the individualized therapy of irinotecan. Associations between *UGT1A* haplotypes and pharmacokinetics/pharmacodynamics of irinotecan were investigated to identify pharmacogenetic markers.

Methods Associations between *UGT1A* haplotypes and the area under concentration curve ratio (SN-38 glucuronide/SN-38) or toxicities were analyzed in 177 Japanese cancer patients treated with irinotecan as a single agent or in combination chemotherapy. For association analysis, diplotypes of *UGT1A* gene segments [(*1A1*, *1A7*, *1A9*, *1A10*), and Block C (common exons 2–5)] and combinatorial haplotypes (*1A9-1A7-1A1*) were used. The relationship between diplotypes and toxicities was investigated in 55 patients treated with irinotecan as a single agent.

Results Among diplotypes of *UGT1A* genes, patients with the haplotypes harboring *UGT1A1*6* or **28* had significantly reduced area under concentration curve ratios, with the effects of *UGT1A1*6* or **28* being of a similar scale. A gene dose effect on the area under concentration curve ratio was observed for the number of haplotypes containing **28* or **6* (5.55, 3.62, and 2.07 for 0, 1, and 2 haplotypes, respectively, $P<0.0001$). In multivariate analysis, the homozygotes and double heterozygotes of **6* and **28* (**6/*6*, **28/*28* and **6/*28*) were significantly associated with severe neutropenia in 53 patients who received irinotecan monotherapy.

Conclusions The haplotypes significantly associated with reduced area under concentration curve ratios and neutropenia contained *UGT1A1*6* or **28*, and both of them should be genotyped before irinotecan is given to Japanese and probably other Asian patients. *Pharmacogenetics and Genomics* 17:497–504 © 2007 Lippincott Williams & Wilkins.

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Keywords: diplotypes, genetic polymorphism, haplotype, irinotecan, SN-38, UGT1A1

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Introduction

Irinotecan, an anticancer prodrug, is widely applied for colorectal, lung, stomach, ovarian, and other various cancers. It is activated by carboxylesterases to SN-38 (7-ethyl-10-hydroxycamptothecin), which shows antitumor activity by inhibiting topoisomerase I [1,2]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (*UGTs*) to form an inactive metabolite, SN-38 glucuronide (SN-38G) [3]. Dose-limiting toxicities of irinotecan are diarrhea and leukopenia [4], and reduced activity for SN-38G formation is closely related to severe toxicities [5]. Among UGT isoforms, UGT1A1 is abundant in both the liver and intestine and is thought to be mainly responsible for inactivation of SN-38 [3,6]. Genetic polymorphisms of *UGT1A1* result in reduced enzyme activity and increased toxicity by irinotecan. A significant association of *UGT 1A1*28*, a repeat polymorphism of the TATA box (-40_-39insTA) [3,7], with severe irinotecan-induced diarrhea/leukopenia was first reported in a retrospective study of Japanese cancer patients [8]. Subsequent pharmacogenetic studies in Caucasians have shown close associations of **28* with reduced glucuronidation of SN-38 and/or severe neutropenia/diarrhea [9–12]. These

studies have clearly indicated that **28* is a good genetic marker for individualized irinotecan therapy. On the basis of these observations, the Food and Drug Administration of the United States has approved an amendment of the label for Camptosar (irinotecan HCl) and added a warning to consider a reduction in the starting dose of irinotecan for **28* homozygous patients (NDA 20-571/S-024/S-027/S-028).

There is significant racial difference in *UGT1A1* polymorphisms among Asians, Caucasians, and Africans [13]. Although the association of *UGT 1A1*28* with toxicities by irinotecan was first described in Japanese patients, its frequency in Japanese is one-third of that in Caucasians. Another low-activity allele **6* [211G > A(G71R)], which is not detected in Caucasians or Africans, is as frequent as the **28* allele in Japanese. Moreover, the area under concentration curve (AUC) ratio of SN-38G to SN-38 was decreased in patients having **6* haplotypes [14].

In addition to UGT1A1, recent studies have suggested possible contributions to SN-38G formation by UGT1A7, 1A9, and 1A10 [15–17], which are expressed in the gastrointestinal tract, the liver and intestine, and extrahepatic tissues, respectively [18]. Altered activity resulted from genetic polymorphisms of these isoforms, including *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)], *1A9*22* (-126_-118T_9 > T_{10}), *1A9*5* [766G > A(D256N)], and *UGT1A10*3* [605C > T(T202I)], but clinical relevance of these polymorphisms is yet to be elucidated [16,19–24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–27]. Therefore, comprehensive investigation that covers these genes, along with linkages among the polymorphisms, is needed, in each ethnic population, to evaluate associations between the genetic polymorphisms and pharmacokinetics, as well as clinical outcomes of irinotecan therapy.

Recently, we have analyzed the segmental and block haplotypes of *1A8*, *1A10*, *1A9*, *1A7*, *1A6*, *1A4*, *1A3* and *1A1*, and the common exons 2–5 (Block C) in a Japanese population, including the 177 cancer patients treated with irinotecan, and showed close linkages between the haplotypes, that is, *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6*, and *1A7*3* and *1A1*28* [28]. Preliminary results of *UGT1A1* pharmacogenetics on 85 of these cancer patients were reported previously [14]. In the current study, we investigated the pharmacogenetics of irinotecan, focusing on diplotypes of the *UGT1A* complex covering *1A1*, *1A7*, *1A9*, *1A10*, and Block C (exons 2–5) of 177 patients, so as to elucidate haplotypes or genetic markers associated with altered glucuronidation of SN-38 and toxicities.

Methods

Patients and treatment schedule

Patients with cancers who started chemotherapy with irinotecan at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were eligible if they had not received irinotecan previously. Other eligibility criteria included bilirubin ≤ 2 mg/dl, aspartate aminotransferase (GOT) ≤ 105 IU/l, alanine aminotransferase (GPT) ≤ 120 IU/l, creatinine ≤ 1.5 mg/dl, white blood cell count ≥ 3000/μl, performance status of 0–2, and at least 4 weeks after the last chemotherapy (2 weeks for radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study, and written informed consent was obtained from all participants.

Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were according to approved usage in Japan; intravenous 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly. In terms of combination chemotherapy, the dose of irinotecan was reduced according to clinical protocols.

Genetic polymorphisms of UGT1As and pharmacokinetics

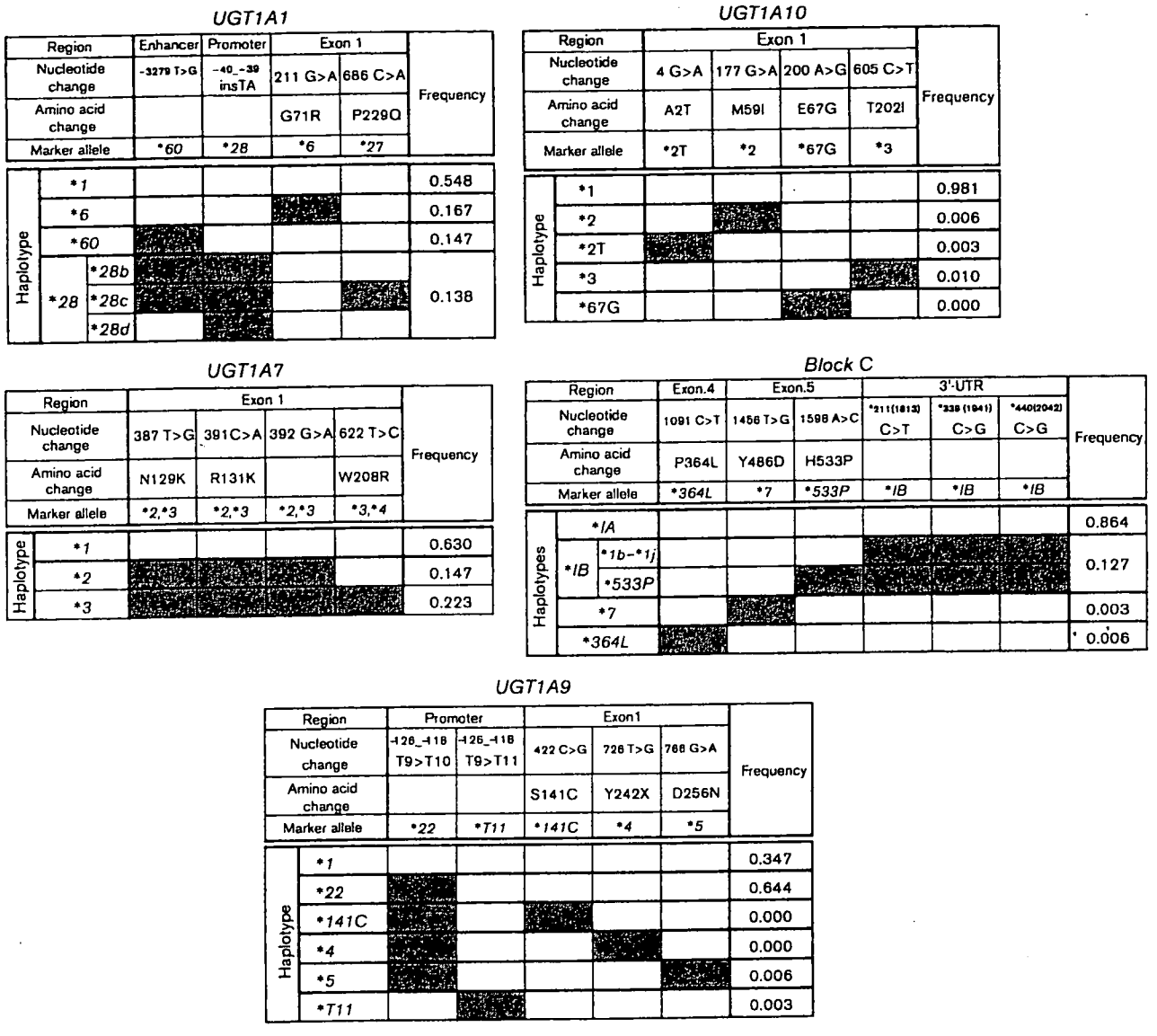
Detailed assay methods for genotypes of the *UGT1A* gene complex were reported previously [14,28]. In this study, we focused on the genetic variations in *UGT1A1*, *1A7*, *1A9*, and *1A10* and common exons 2–5, as they have been reported to contribute to the SN-38 glucuronidation. Haplotype analysis covering these regions was performed in our previous study [28], and haplotypes of each *UGT1A* segment [exon 1 for *1A1*, *1A7*, *1A9*, or *1A10*; and Block C (common exons 2–5)] are summarized in Fig. 1.

Pharmacokinetic analysis for irinotecan was performed as described previously [14]. Briefly, heparinized blood was collected before administration of irinotecan, as well as 0 and 20 min, and 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan, SN-38 and SN-38G were determined by the high-performance liquid chromatography [29], and AUC was calculated by the trapezoidal method using WinNonlin version 4.01 (Pharsight Corporation, Mountain View, California, USA). Associations between genotypes and the AUC ratio (AUC of SN-38G/AUC of SN-38) were evaluated in 176 patients.

Monitoring and toxicities

A complete medical history and data on physical examinations were recorded before the irinotecan therapy. Complete blood cell counts with differentials and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of irinotecan treatment. Toxicities were graded according to the Common Toxicity Criteria of National Cancer Institute version 2. Association of genetic factors with irinotecan toxicities was analyzed primarily in patients who received irinotecan as a single agent.

Fig. 1



Haplotypes of *UGT1A* gene segments (*UGT1A1*, *1A7*, *1A9*, *1A10*, and Block C) in 177 Japanese cancer patients. The tagging variations and haplotypes are shown. Variant alleles are indicated in grey. Definition of Block C haplotypes in our previous paper ([14]) (corresponding to Block 2) were slightly modified.

Statistical analysis

Statistical analysis on the differences in the AUC ratios (SN-38G/SN-38) among *UGT1A* genotypes was performed using the Kruskal–Wallis test, followed by nonparametric Dunnett's multiple comparison test, or with Wilcoxon test. Analysis of a gene–dose effect of each haplotype was performed using the Jonckheere–Terpestra test in the SAS system, version 5.0 (SAS Institute, Cary, North Carolina, USA). Relationship of *UGT1A* genetic polymorphisms to the toxicities of irinotecan was assessed by the χ^2 test via the use of using Prism version 4.0 (GraphPad Prism Software, San Diego, California, USA). The *P*-value of 0.05 (two-tailed) was set as a significant level, and the multiplicity adjustment was conducted for pharmacokinetics data with the false discovery rate [30].

To identify factors associated with the log-transformed AUC ratio of SN-38G/SN-38, multiple regression analysis was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, coadministered drugs, serum biochemistry parameters at baseline, and *1A9-1A7-1A1* and Block C haplotypes (five or more chromosome numbers) or '*1A1*6* or **28*'. For multiple regression analysis of neutropenia, variables included the absolute neutrophil count at baseline and the dosing interval, in addition to

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	

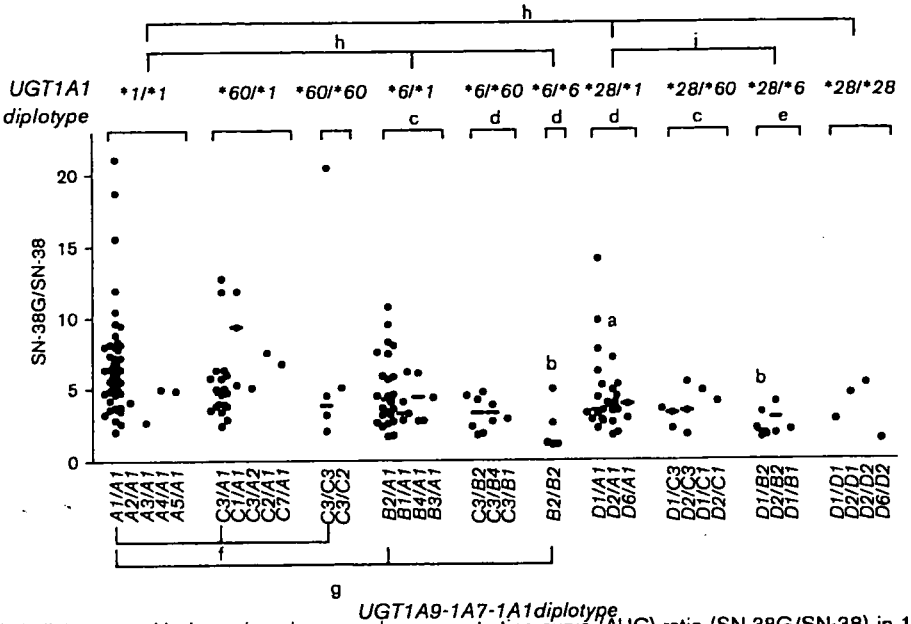
[a]Cisplatin, cisplatin plus etoposide or carboplatta.
[b]Two and eight patients received cisplatin and etoposide and carboplatin, respectively.
[c]Number of cisplatin-administered patients [initial dose of cinlatin (mg/m^2) is shown in brackets].

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.

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.

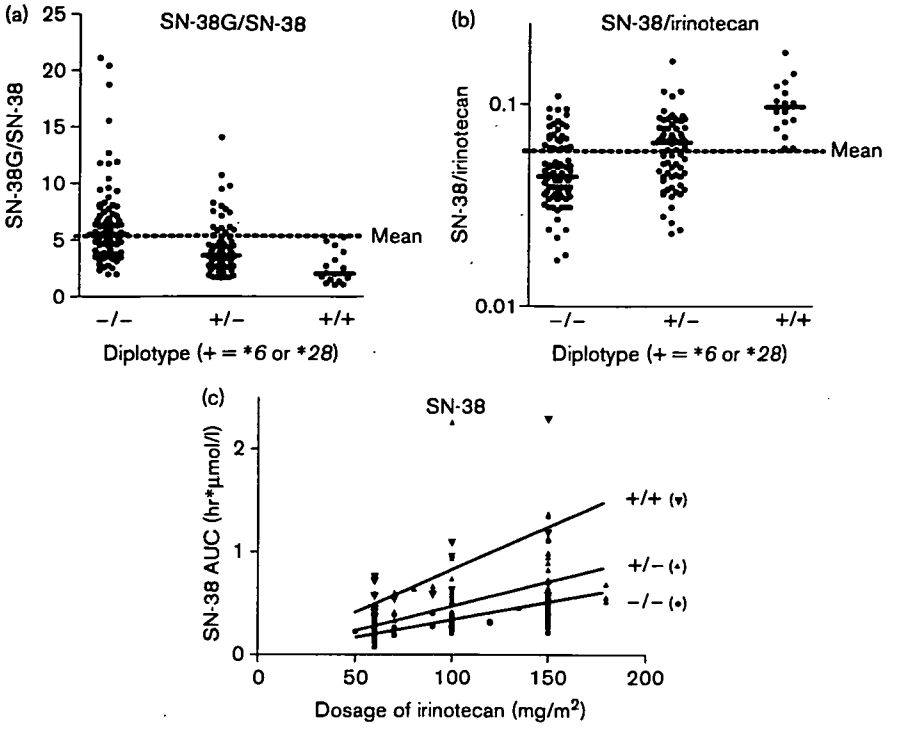
The clinical importance of **6* for neutropenia by irinotecan was also supported by a recent report in Korean patients who received irinotecan and cisplatin [31]. Although no patients with irinotecan as a single agent were homozygous for **6* in our study, clinical significance of the double heterozygote, **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

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	Coeffi-cient	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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Clinical Studies

Randomised phase III trial of carboplatin plus etoposide *vs* split doses of cisplatin plus etoposide in elderly or poor-risk patients with extensive disease small-cell lung cancer: JCOG 9702

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We compared the efficacy and the safety of a carboplatin plus etoposide regimen (CE) *vs* split doses of cisplatin plus etoposide (SPE) in elderly or poor-risk patients with extensive disease small-cell lung cancer (ED-SCLC). Eligibility criteria included: untreated ED-SCLC; age $\geqslant 70$ and performance status 0–2, or age <70 and PS 3. The CE arm received carboplatin area under the curve of five intravenously (IV) on day 1 and etoposide 80 mg m^{-2} IV on days 1–3. The SPE arm received cisplatin 25 mg m^{-2} IV on days 1–3 and etoposide 80 mg m^{-2} IV on days 1–3. Both regimens were given with granulocyte colony-stimulating factor support in a 21–28 day cycle for four courses. A total of 220 patients were randomised. Median age was 74 years and 74% had a PS of 0 or 1. Major grade 3–4 toxicities were (%CE/%SPE): leucopenia 54/51, neutropenia 95/90, thrombocytopenia 56/16, infection 7/6. There was no significant difference (CE/SPE) in the response rate (73/73%) and overall survival (median 10.6/9.9 mo; $P = 0.54$). Palliation scores were very similar between the arms. Although the SPE regimen is still considered to be the standard treatment in elderly or poor-risk patients with ED-SCLC, the CE regimen can be an alternative for this population considering the risk–benefit balance.
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Keywords: small-cell lung cancer; carboplatin; cisplatin; etoposide; elderly; poor-risk

Approximately half of patients with small-cell lung cancer (SCLC) are older than 70 years, and the proportion of elderly SCLC patients is continuously increasing in Japan (Morita, 2002). However, since many investigators have arbitrarily excluded elderly patients from clinical trials, no standard chemotherapeutic regimen has been established for elderly patients with SCLC. The Japan Clinical Oncology Group (JCOG) has reported that carboplatin plus etoposide (CE) is an active and less toxic regimen in elderly patients with SCLC (Okamoto *et al*, 1999). However, other clinical trials have indicated that the combination chemotherapy of reduced (Souhami *et al*, 1997) or split doses of cisplatin plus etoposide (SPE) (Murray *et al*, 1998; Westeel *et al*, 1998) can be safely and effectively administered in elderly or poor-risk patients with SCLC. Therefore, we conducted a phase III trial comparing CE with SPE in elderly or poor-risk patients with SCLC. Although elderly is not the same as poor-risk, many clinical trials for the elderly have included both types of patients. Therefore, we decided to include both elderly and poor-risk patients with SCLC at the time of proposal for this phase III trial.

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PATIENTS AND METHODS

Patient selection

Eligibility criteria included patients with histologically or cytologically confirmed SCLC who were $\geqslant 70$ years of age and had an Eastern Cooperative Oncology Group performance status (PS) of 0–2, or who were <70 years in age and had a PS of 3. Additional criteria consisted of extensive disease (ED), chemotherapy-naive, evaluable or measurable disease, expected survival $\geqslant 2$ months, adequate organ functions (leucocyte count $\geqslant 4000\,\mathrm{mm}^{-3}$, platelet count $\geqslant 100\,000\,\mathrm{mm}^{-3}$, haemoglobin level $\geqslant 9.0\,\mathrm{g\,dl}^{-1}$, AST/ALT $\leqslant 2\times$ upper limit of normal range, total bilirubin $\leqslant 1.5\,\mathrm{mg\,dl}^{-1}$, creatinine $\leqslant 1.5\,\mathrm{mg\,dl}^{-1}$, 24-h creatinine clearance (Ccr) $\geqslant 50\,\mathrm{ml\,min}^{-1}$, and PaO2 $\geqslant 60$ mmHg), no symptomatic pericardial or pleural effusion requiring drainage, no active concomitant malignancy, no senile dementia, and written informed consent. Exclusion criteria included brain metastases requiring radiotherapy, superior vena cava (SVC) syndrome requiring radiotherapy, serious medical or psychiatric illness, or pregnancy or lactation. Staging procedures included chest X-ray, computed tomography (CT) scan of the chest, CT scan or magnetic resonance

imaging (MRI) of the brain, CT scan or ultrasound of the abdomen, isotope bone scanning, and bone marrow aspiration or biopsy.

Treatment protocol

Patients were randomised to either the CE arm or the SPE arm. The CE regimen consisted of carboplatin area under the curve (AUC) of five intravenously (IV) on day 1 and etoposide 80 mg m^{-2} IV on days 1, 2, and 3. The SPE regimen consisted of cisplatin 25 mg m^{-2} IV on days 1, 2, and 3 and etoposide 80 mg m^{-2} IV on days 1, 2, and 3. Cycles were repeated every 3–4 weeks for up to four courses. In our previous phase II study using the CE regimen for elderly patients with SCLC, carboplatin AUC of 5 on day 1 and etoposide 100 mg m^{-2} on days 1, 2, and 3 were administered every 4 weeks (Okamoto *et al*, 1999). However, because grade 3 or 4 neutropenia occurred in 91% of the patients, in the current phase III trial we decided to reduce the etoposide dosage to 80 mg m^{-2} on days 1, 2, and 3, and repeat the cycle every 3–4 weeks instead of every 4 weeks. Twenty-four-hour Ccr was substituted for glomerular filtration rate (GFR) in Calvert's formula. Antiemetic prophylaxis with 5-HT3 antagonists plus dexamethasone was used at the treating physician's discretion. According to the Japanese approved guideline, prophylactic use of recombinant human granulocyte colony-stimulating factor (G-CSF) was recommended for daily administration after day 4 until the leucocyte (neutrophil) count exceeded 10 000 (5000) mm^{-3}. If the leucocyte (neutrophil) count decreased to less than 3000 (1500) mm^{-3}, then G-CSF was restarted. However, the actual use of G-CSF was left at the discretion of the treating physician. Subsequent courses of chemotherapy were initiated when leucocyte count $\geqslant$3000 mm^{-3}; platelet count $\geqslant$75 000 mm^{-3}; Cr$\leqslant$1.5 mg dl^{-1}; AST/ALT$\leqslant$2.5 $\times$ upper limit of normal range; and either PS$\leqslant$2 and age$\geqslant$70 years, or PS$\leqslant$3 and age$<$70 years were satisfied both after day 21 and two or more days after the discontinuation of G-CSF. If the above criteria were not satisfied by the first day of the next course, treatment was withheld until full recovery. If more than 6 weeks passed from day 1 of the last course, the patient was removed from protocol treatment. Dose modifications were made based only on grade 4 haematologic toxicities. If grade 4 leucopenia or neutropenia lasting 4 days or more was present, or grade 4 thrombocytopenia occurred, the doses for the next course were carboplatin AUC of 4 on day 1, cisplatin 20 mg m^{-2} for 3 days, and etoposide 60 mg m^{-2} for 3 days. If the same haematologic toxicity was observed after dose reduction, the patient was removed from protocol treatment. If grade 3 or 4 non-haematologic toxicities, except for nausea/vomiting and hyponatraemia, occurred, the patient was removed from protocol treatment even if the toxicities improved thereafter.

Responders after four courses were not allowed to receive further chemotherapy until progressive disease (PD) developed. Although post-protocol treatment was left at the discretion of the physician, crossover treatment was prohibited.

Evaluation

Tumour responses were evaluated according to World Health Organization criteria (World Health Organization, 1979). Toxicities were evaluated according to JCOG Toxicity Criteria (Tobinai *et al*, 1993), which are similar to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC ver 1) for the grading of toxicities.

Palliation score

Study-specific eight-item palliation scores were completed by patients before treatment and 3 weeks after the third course of chemotherapy. The attending physicians were not allowed to complete the scores. The items consisted of cough, pain, anorexia, shortness of breath, well-being, nausea, diarrhoea or constipation, and sleep. The items were scored as not at all present (0), a little (1), moderate (2), and very much (3). The sum of the total score for all eight items was compared between the baseline and post-treatment assessments. If the post-treatment score was below the baseline score, the palliation score for that patient was judged as having shown improvement.

Study design and statistics

This trial was designed as a multicentre, prospective, randomised phase III trial. 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. The primary endpoint was overall survival (OS). In this study, the experimental arm was the CE arm and the control was the SPE arm. The MST of our previous phase II trial for elderly patients with extensive disease small-cell lung cancer (ED-SCLC) using the CE regimen was 10.1 months. The MST of the SPE regimen for a similar population was not available at the time of the study proposal. Although Westeel and co-workers in 1998 and Murray and co-workers in 1998 reported an excellent MST of SPE plus concurrent chest radiotherapy for elderly or frail patients with limited disease (LD)-SCLC, an MST of the SPE regimen for elderly or frail patients with ED-SCLC was not available at that time. The only data available on the CAV/PE regimen for elderly or poor-risk patients with SCLC using reduced cisplatin (60 mg m^{-2} IV on day 1) were reported by Souhami and co-workers in 1997 and the MST of that study was 5.9 months. Therefore, for statistical calculations in the current phase III trial, we used the MST value of the Souhami trial for the control arm instead of the MST of the SPE regimen. In addition, an individualised AUC-based dosing strategy of carboplatin was expected to have greater efficacy and less toxicity compared with the SPE regimen at that time. This trial was designed as a superiority trial and the planned sample size was 110 patients in each arm for 80% power to detect a 0.67 hazard ratio for CE to SPE in OS at an alpha of 0.025 (one sided) (Schoenfeld and Richter, 1982). Patients were randomised to receive either CE or SPE with a minimisation method for balancing centre, PS (0–1 *vs* 2–3) and age ($\geqslant$70 years *vs* $<$70 years).

Survival distributions were compared by unstratified log-rank test. Proportion of improvement in palliation score was evaluated by Fisher's exact test. The change in each symptom score by treatment arm was evaluated by the Wilcoxon rank-sum test. The relationship between the interval of each chemotherapy course and the two regimens was evaluated by the Wilcoxon rank-sum test. Multivariate analysis was performed using Cox's proportional hazards model to evaluate the importance of seven clinically selected variables (treatment arm, PS, age, sex, lactate dehydrogenase level, alkaline phosphatase level, and leucocyte count) as prognostic factors. All *P*-values in this report are two sided, excluding *P*-values for OS and progression-free survival (PFS).

The interim analysis was performed after half of the planned number of patients had been enrolled in March 2002, with adjustment for multiplicity by the alpha-spending function (DeMets and Lan, 1994) with an O'Brien-Fleming type boundary. Because the interim analysis did not meet the prespecified stopping criteria, the study was continued and the planned accrual of 220 patients was randomised in this trial.

RESULTS

Patient characteristics

Between August 1998 and February 2004, a total of 220 patients were registered from 24 institutions. Baseline characteristics were well balanced between the arms. Median age was 74 years, 92% were 70 years or older, 88% were male, and 74% had a PS of 0 or 1 (Table 1). One patient in the CE arm was found to have LD after the completion of protocol chemotherapy due to protocol violation, and this patient was considered ineligible (Figure 1).

Delivery of treatment

Reasons for termination of treatment are listed in Figure 1, and there were no major differences between the arms. Of the patients, 63% in the CE arm and 67% in the SPE arm completed four courses, and 11% in the CE arm and 8% in the SPE arm did not complete treatment because of toxicity or complications. Treatment-related death (TRD) occurred in four patients; three patients in the CE arm and one in the SPE arm. All TRDs of patients who were ⩾70 years old with a good pretreatment PS (all PS 1) were associated with neutropenic infection, which occurred after the first course of chemotherapy. Although the median interval of chemotherapy was slightly more prolonged in the CE arm than in the SPE arm, total delivered courses were similar between the arms (Table 2). One patient in the SPE arm never received chemotherapy due to the occurrence of delirium after registration. Dose reduction was more frequently observed in the CE arm than in the SPE arm: 29% *vs* 10%, $P<0.01$. Course delay, G-CSF delivery and total courses with G-CSF delivery were similar between the arms.

Toxicity and palliation score

Toxicities are listed in Table 3. Grade 3 or 4 leucopenia and neutropenia occurred in 54 and 95% of the CE arm *vs* 51 and 90% of the SPE arm, respectively. Grade 3 or 4 thrombocytopenia occurred more frequently in the CE arm than in the SPE arm: 56 *vs* 16%, $P<0.01$. Gastrointestinal toxicities including nausea or vomiting and diarrhoea were mild in both arms. There were few grade 3 or 4 toxicities and no remarkable differences between the arms. Other non-haematologic toxicities were similarly distributed between the arms. Grade 3–4 hyponatraemia, mainly caused by syndrome of inappropriate antidiuretic hormone (SIADH) secretion, occurred in 14–16% of the patients. More importantly, thrombocytopenia occurred more frequently in the CE arm, but none of the patients in either arm showed grade 3 or 4 bleeding. Only one patient in the CE arm showed grade 2 bleeding. Because no grading of febrile neutropenia was listed in JCOG toxicity criteria, the rate of the toxicity was not investigated in this study.

Baseline and post-treatment palliation scores were evaluated in 220/220 (100%) and 208/220 (95%) patients, respectively. We handled missing values by imputing the worst score. Improvement was achieved in 69 (63%) patients in the CE arm *vs* 61 (56%) patients in the SPE arm, although the difference was not statistically significant ($P=0.34$). Similarly, there were no statistical differences in the change of each symptom score between the arms (Table 4).

Objective tumour response, PFS and OS

The objective response rate of 73% was quite similar between the arms. Five CRs and 75 PRs were observed in each arm (Table 5). Progression-free survival curves and OS curves are shown in Figure 2A and B. Ninety-seven percent of the patients had progressed or died at the time of final analysis. Progression-free survival was quite similar between the arms ($P=0.20$, one sided).

Table 1 Patient characteristics

	CE ($n=110$)	SPE ($n=110$)	*P*-value
Age (years)			
Median (range)	74 (56–86)	73.5 (55–85)	0.34
⩾70 years old (%)	102 (93)	100 (91)	0.81
Sex (male/female)	95/15	98/12	0.68
ECOG PS, 0–1/2/3	81/21/8	81/19/10	0.80
⩾5% weight loss	26	38	0.18
LN metastasis			
Contralateral mediastinum	71	59	0.13
Supraclavicular	89	79	0.15
Distant metastasis			
Liver	30	30	1.0
Lung	31	30	1.0
Brain	18	18	1.0
Bone	25	17	0.23
Adrenal	13	7	0.24
Bone marrow	12	12	1.0

CE, carboplatin plus etoposide; ECOG, Eastern Cooperative Oncology Group; LN, lymph node; PS, performance status; SPE, split doses of cisplatin plus etoposide.

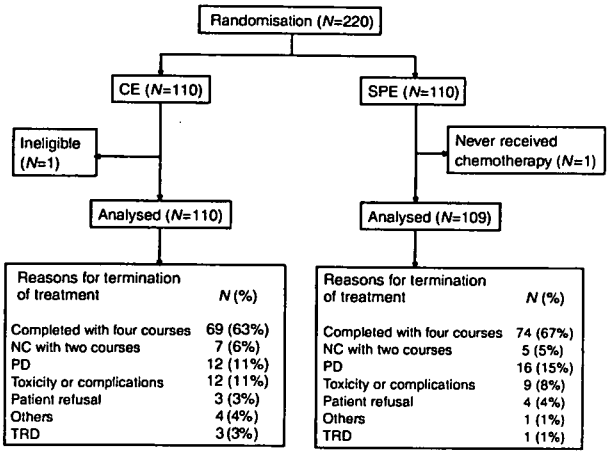

Figure 1 Flow diagram of randomised phase III trial of CE *vs* SPE in elderly or poor-risk patients with extensive disease SCLC.

Table 2 Compliance and drug delivery

	CE ($n=110$)	SPE ($n=109^a$)	*P*-value
Median interval of each chemotherapy (days) (range)			
1–2	27 (14–35)	23 (20–37)	0.02[b]
2–3	25 (21–56)	22 (20–35)	0.07[b]
3–4	27 (21–36)	24 (21–38)	0.05[b]
Total delivered courses/projected courses	353/440 (80%)	360/436 (83%)	
Dose reduction	32 (29%)	11 (10%)	<0.01[c]
Course delay	45 (41%)	40 (37%)	0.58[c]
G-CSF delivery	81 (74%)	84 (77%)	0.64[c]
No. of courses with G-CSF delivery/number of total courses	183/354 (52%)	203/362 (56%)	

CE, carboplatin plus etoposide; G-CSF, granulocyte colony-stimulating factor; SPE, split doses of cisplatin plus etoposide. [a]One patient never received chemotherapy due to delirium after registration. [b]Wilcoxon rank-sum test. [c]Fisher's exact test.

Table 3 Toxicities (JCOG Toxicity Criteria, Worst Grade of Any Course)

	CE					SPE					
	Grade					Grade					
Toxicity	**1**	**2**	**3**	**4**	**3+4 (%)**	**1**	**2**	**3**	**4**	**3+4 (%)**	***P*-value**
Haematologic											
Leucopenia	5	45	46	13	(54)	8	43	49	7	(51)	0.79
Neutropenia	0	5	46	58	(95)	4	7	41	57	(90)	0.22
Anaemia	9	58	32	—	(29)	20	45	27	—	(25)	0.54
Thrombocytopenia	20	18	29	32	(56)	16	15	12	5	(16)	<.01
Non-haematologic											
Nausea/vomiting	40	24	2	—	(2)	46	28	3	—	(3)	0.68
Diarrhoea	8	9	1	0	(1)	11	3	1	0	(1)	1.0
Bilirubin	—	31	0	0	(0)	—	16	1	0	(1)	0.50
AST	47	9	3	0	(3)	30	8	6	0	(6)	0.33
ALT	40	9	2	0	(2)	38	8	4	0	(4)	0.45
Creatinine	10	2	0	0	(0)	27	3	1	0	(1)	0.50
Hyponatraemia	38	11	7	11	(16)	46	20	6	9	(14)	0.58
PaO2	39	21	7	1	(10)	44	23	2	1	(4)	0.22
Fever	15	15	0	0	(0)	21	16	0	0	(0)	—
Infection	12	15	5	3	(7)	16	7	5	1	(6)	0.78
Bleeding	8	1	0	0	(0)	4	0	0	0	(0)	—
Neurologic-sensory	2	1	0	—	(0)	3	2	0	—	(0)	—
Alopaecia	67	22	—	—		66	15	—	—		

CE, carboplatin plus etoposide; JCOG, Japan Clinical Oncology Group; PaO_2, partial pressure of oxygen; SPE, split doses of cisplatin plus etoposide.

Table 4 Palliation score

	CE		SPE		
	Change from baseline		Change from baseline		
Symptom	**Mean (s.d.)**	**Median (range)**	**Mean (s.d.)**	**Median (range)**	***P*[a]**
Cough	−0.38 (1.16)	0 (−3 to 3)	−0.54 (1.06)	0 (−3 to 3)	0.51
Pain	−0.19 (1.00)	0 (−3 to 3)	−0.19 (0.96)	0 (−3 to 3)	0.96
Anorexia	−0.07 (1.16)	0 (−3 to 3)	0.08 (1.22)	0 (−3 to 3)	0.37
Shortness of breath	−0.05 (1.02)	0 (−2 to 3)	−0.31 (0.95)	0 (−3 to 3)	0.12
Well-being	−0.15 (1.13)	0 (−3 to 3)	−0.02 (1.14)	0 (−3 to 3)	0.48
Nausea	0.16 (0.84)	0 (−2 to 3)	0.26 (0.80)	0 (−1 to 3)	0.21
Diarrhoea or constipation	0.05 (1.07)	0 (−3 to 3)	0.04 (0.99)	0 (−3 to 3)	0.69
Sleep	−0.15 (1.08)	0 (−3 to 3)	−0.04 (0.89)	0 (−3 to 2)	0.10
Total	−0.80 (6.04)	−2 (−12 to 22)	−0.71 (5.35)	−1 (−15 to 21)	0.32

CE, carboplatin plus etoposide; s.d., standard deviation; SPE, split doses of cisplatin plus etoposide. [a]Wilcoxon rank-sum test.

The MST was 5.2 months in the CE arm *vs* 4.7 months in the SPE arm. OS was very similar between the arms ($P = 0.54$, one sided). The MST and 1-year survival rate was 10.6 months and 41% in the CE arm *vs* 9.9 months and 35% in the SPE arm.

Second-line chemotherapy

According to an *ad-hoc* survey (not pre-specified in the protocol), 130 (59%) patients (68 (62%) patients in the CE arm and 62 (56%) in the SPE arm) received second-line chemotherapy after relapse and the regimens were almost equally distributed between the arms. The same regimen as the initial chemotherapy, platinum-based combinations, and irinotecan regimens with or without other agents were administered in 17 (15%), 48 (44%), and 40 (36%) patients in the CE arm *vs* 10 (9%), 44 (40%), and 40 (36%) in

Table 5 Therapeutic response (WHO)

	CE	**SPE**	**Total**
CR	5	5	10
PR	75	75	150
NC	17	11	28
PD	11	16	27
NE	2	3	5
Total	110	110	220
Response rate	73%	73%	
95% CI	63–81%	63–81%	

CE, carboplatin plus etoposide; CI, confidence interval; CR, complete response; NC, no change; NE, not evaluable; PD, progressive disease; PR, partial response; SPE, split doses of cisplatin plus etoposide; WHO, World Health Organization.

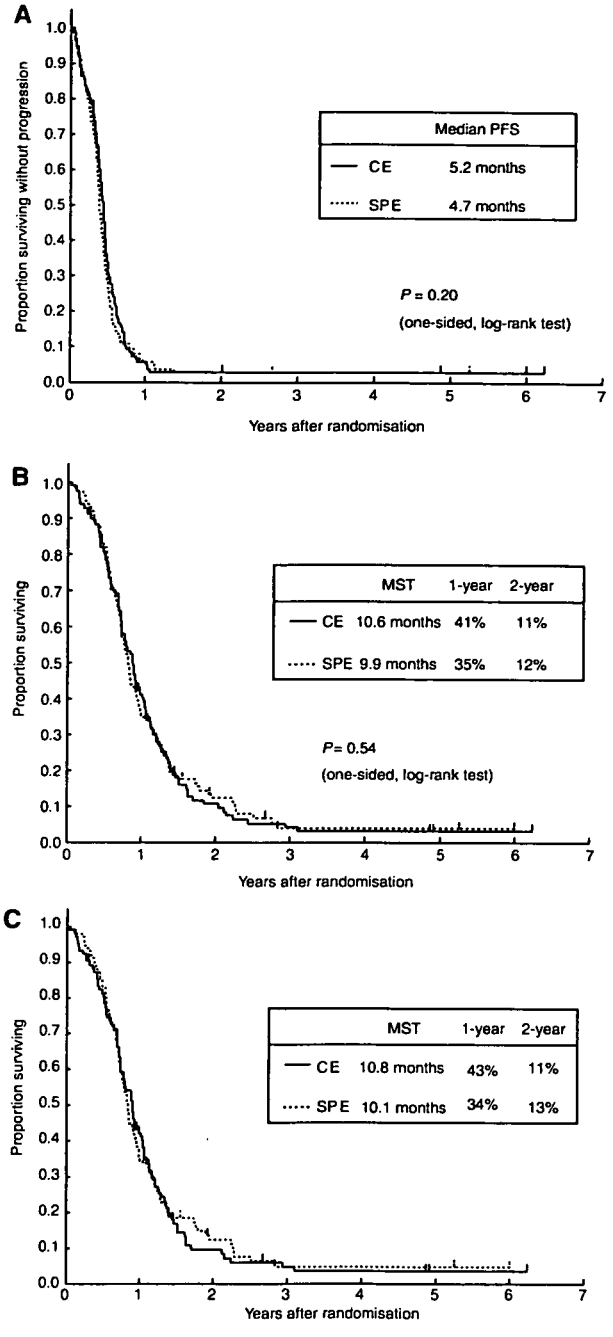

Figure 2 (**A**) PFS curves ($n=220$). (**B**) OS curves ($n=220$). (**C**) Survival curves of the patients ⩾70 years of age with a PS of 0–2 ($n=202$).

the SPE arm. Other chemotherapy regimens included topotecan monotherapy, amrubicin monotherapy, or other regimens.

Subset analysis and multivariate analysis

Subset analysis was performed according to PS and age (Table 6). There were no differences in OS between the arms in any subset; thus, an interaction between treatment and PS is unlikely. The survival curves of the patients ⩾70 years of age with a PS of 0–2 are shown in Figure 2C, and the survival curves were very similar with that of original overall populations. Even in the multivariate analysis with seven selected baseline variables, there was no difference in OS between the arms. High lactate dehydrogenase level was most strongly associated with poor prognosis (Table 7).

Table 6 Subset analysis – overall survival

Subgroup	Number of patients (%)	MST (months) CE	MST (months) SPE
PS 0–1	162 (74)	10.9	10.1
PS 2–3	58 (26)	8.3	8.1
<70 years and PS 3	18 (8)	7.1	6.9
⩾70 years and PS 0–2	202 (92)	10.8	10.0

CE, carboplatin plus etoposide; MST, median survival time; PS, performance status; SPE, split doses of cisplatin plus etoposide.

Table 7 Multivariate analysis with baseline prognostic factors

Variables	*P*-value	Hazard ratio	95% CI
Treatment arm (CE vs. SPE)	0.99	0.99	0.75–1.33
Alkaline phosphatase level (normal *vs* abnormal)	0.97	0.99	0.68–1.46
Lactate dehydrogenase level (⩾ × 1.5 *vs* < × 1.5)	<0.001	1.69	1.23–2.26
Leucocyte count (⩾ 10000/mm^3 *vs* < 10000/mm^3)	0.06	1.82	0.99–3.36
Age (⩾75 years *vs* <75 years)	0.77	1.05	0.78–1.41
PS (2–3 *vs* 0–1)	0.41	1.15	0.82–1.61
Sex (female *vs* male)	0.13	0.70	0.45–1.11

CE = carboplatin plus etoposide; SPE = split doses of cisplatin plus etoposide; PS = performance status; CI = confidence interval.

DISCUSSION

Until recently, there was no standard chemotherapeutic regimen for elderly SCLC patients. Two phase III (Medical Research Council Lung Cancer Working Party, 1996; Souhami *et al*, 1997) and two randomised phase II trials (Pfeiffer *et al*, 1997; Ardizzoni *et al*, 2005) have shown that suboptimal chemotherapies, such as oral etoposide monotherapy or attenuated doses of combination chemotherapy, may lead to reduced survival in elderly or poor-risk SCLC patients when compared with standard doses of combination chemotherapies. The CE regimen, which has acceptable toxicities and reproducible efficacy, has been used in elderly or poor-risk patients with SCLC worldwide, although there have been substantial differences in toxicities and efficacy between the reported phase II trials. Four trials demonstrated both favourable toxicities and efficacy (Carney, 1995; Evans *et al*, 1995; Matsui *et al*, 1998; Okamoto *et al*, 1999) and three showed somewhat disappointing results because of suboptimal doses of oral etoposide (Larive *et al*, 2002), greater inclusion of patients with poor prognostic factors (Samantas *et al*, 1999), and deterioration of comorbidities as a result of chemotherapy (Quoix *et al*, 2001). No phase III trial evaluating the role of the CE regimen in this population has been reported until now.

This is the first phase III trial comparing carboplatin-based CE and cisplatin-based SPE regimens in elderly or poor-risk patients with ED-SCLC. In addition, this is also the largest randomised trial specifically designed for elderly or poor-risk SCLC patients. Although there was no significant difference in the palliation scores, response rate, and OS between the arms, the efficacy of

both regimens was promising, as this study included only elderly or poor-risk patients with SCLC. Most toxicities were tolerable and the treatment compliance was also favourable in both arms. Approximately two-thirds of the patients received all four cycles of treatment. The CE arm in the current trial had more pronounced thrombocytopenia, which was considered manageable because none of the patients in the CE arm showed grade 3 or 4 bleeding, and the CE arm had a slightly prolonged course interval and a slightly greater incidence of dose reduction. However, in our opinion, these toxicities are less meaningful in clinical practice. More importantly, the CE regimen does not require hydration and can be given in an outpatient setting. Based on the results of this study, many JCOG members prefer the CE regimen to the SPE regimen and consider it to be more suitable for the control arm of future phase III trials.

The MST of each regimen (10.6 months for CE *vs* 9.9 months for SPE) was promising considering that this study included only elderly or frail patients with ED-SCLC. However, some retrospective studies have shown that fit elderly patients who have adequate organ functions, a good PS, and no comorbidity are able to tolerate intensive chemotherapy well and show a similar therapeutic response and survival rate as younger patients (Siu *et al*, 1996; Yuen *et al*, 2000). In fact, in this trial the MST of fit elderly patients $\geqslant 70$ years of age with a PS of 0–1 was 10.9 months for the CE arm and 10.1 months for the SPE arm. In contrast, the MST of patients with a PS of 3 was only approximately 7 months. Furthermore, the group of fit elderly patients comprised 74% of the patients in this study. Therefore, the favourable survival rates in our trial may be attributable to patient selection. In other words, one limitation of this study is that the results of this trial cannot be extrapolated to frail elderly with a poor PS and/or comorbid illness because of the likelihood of greater inclusion of fit elderly patients in this trial.

Although the total dose in both the CE and SPE arms was slightly lower than the standard regimen, 92% of the patients showed grade 3 or 4 neutropenia, and dose reduction and course delay occurred frequently. However, the MST of both regimens was comparable with that of non-elderly or non-selected patients with ED-SCLC in historical reports (Noda *et al*, 2002; Niell *et al*, 2005). These findings suggest that both regimens are not suboptimal, but are near-full and effective doses for elderly or poor-risk patients with ED-SCLC. The CE arm in the current trial had a slightly prolonged course interval and a slightly greater incidence of dose reduction when compared to the SPE regimen. However, 95% of the patients showed grade 3 or 4 neutropenia and 56% showed grade 3 or 4 thrombocytopenia. Therefore, we believe that the dose escalation of the CE regimen may be difficult in this trial.

It remains unclear whether the elderly are able to tolerate a single modest dose of cisplatin (60–80 mg m^{-2} IV) on day 1. We feel that a fit elderly person who passes strict eligibility criteria can receive a modest dose of cisplatin IV on day 1. However, the more common situation is of elderly patients who have comorbidity and a poor PS, and cannot tolerate a standard single dose of cisplatin. Westeel *et al* (1998) and Murray *et al* (1998) reported that split doses of cisplatin were safely and effectively administered in elderly or frail patients with LD-SCLC. The SPE regimen appeared to be an appropriate treatment for elderly patients with SCLC who cannot tolerate a standard single dose of cisplatin. However, it remains unclear whether fit elderly patients in our trial can tolerate a standard single dose of cisplatin, and if so, it also remains unclear whether fit elderly patients who receive a standard single dose of cisplatin are able to achieve a more improved survival than those who receive SPE. Unfortunately, no randomised study comparing a single standard dose of cisplatin with SPE has been reported in fit elderly patients with SCLC.

There are some problems with the design in this study. The hypothesis was that carboplatin would improve survival, and the design of the trial was a superiority design with survival as the primary end point. However, this hypothesis was based on two possible misconceptions. First, carboplatin could be better dosed and might be more efficacious than cisplatin in SCLC. Unfortunately, this hypothesis could not be sustained on the basis of the available literatures. A number of clinical trials have indicated that carboplatin-based combination chemotherapy has a similar or slightly reduced efficacy compared with cisplatin-based combination chemotherapy against various tumours (Go and Adjei, 1999; Hotta *et al*, 2004). Therefore, our trial should have been designed as a non-inferiority trial. However, if this trial were planned as a non-inferiority trial, a total sample size would be about 500 to 1000 patients, with equal expected survival and a non-inferiority margin for hazard ratio ranging from 1.2 to 1.3. Second, the cisplatin dose in the control arm was an attenuated dose. Souhami *et al* (1997) used reduced dose of cisplatin (60 mg m^{-2} IV on day 1) and Murray *et al* (1998) used a single course of a split cisplatin dose in their studies. These regimens were completely different from the control arm in the present study. A standard dose of cisplatin given in 3 days is the best way of giving standard cisplatin (30 mg m^{-2} IV on days 1–3) with etoposide (130 mg m^{-2} IV on days 1–3), according the North Central Cancer Treatment Group (Maksmiuk *et al*, 1994). Had standard SPE been used for the control arm, better survival might have been achieved with increased toxicities. Another problem with the design was the inclusion of patients with a PS of 3, even if they were less than 70 years old. This made the target population heterogeneous. The number of such patients actually recruited was quite small, so emphasising the inappropriateness of their inclusion. A further limitation of this study may be a long accrual period of five-and-a-half years. Because our oncologists might have been afraid of the risk of TRD or increased toxicities in frail elderly with a poor PS and/or comorbid illness, more fit elderly patients were selectively registered and consequently the accrual rate was very slow.

In our trial, although both regimens were well-tolerated and efficacy was promising, over 90% of the patients in both arms showed grade 3 or 4 neutropenia, which may be justified and acceptable for a clinical trial involving elderly or poor risk patients with ED-SCLC, because only 6% of the patients showed grade 3 or 4 infection and TRD occurred in only four (1.8%) patients. Because all TRD occurred after the first course of chemotherapy, careful monitoring and management is necessary, particularly in the first course, if CE or SPE are administered to elderly or frail patients. Several retrospective analyses (Findlay *et al*, 1991; Radford *et al*, 1992) and a prospective study (Timmer-Bonte *et al*, 2005) have shown that standard-dose chemotherapy without G-CSF support causes more risk of early death and sepsis in the older population. Moreover, the American Society of Clinical Oncology (ASCO) guideline recommends the use of prophylactic G-CSF in patients at higher risk for chemotherapy-induced infection, such as those having a poor PS, older age, or comorbid illness (Smith *et al*, 2006). In this trial, the prophylactic use of G-CSF was recommended, but the actual use was left to the discretion of the treating physician because the use of G-CSF leads to increased drug cost. Although G-CSF was administered in only 54% of the total courses, we believe that the prophylactic use of G-CSF with CE regimen should be recommended in a new trial or clinical practice.

In conclusion, although the SPE regimen is still considered to be the standard treatment for elderly or poor-risk patients with ED-SCLC, the CE regimen can be an alternative for this population considering the risk-benefit balance. Based on the results of our trial, a phase III trial of the CE regimen *vs* amrubicin monotherapy, supported by a pharmaceutical company, is now ongoing in elderly patients with ED-SCLC in Japan, and a comparative trial of the CE regimen *vs* carboplatin plus irinotecan regimen (Okamoto *et al*, 2006) is being discussed for a future trial in our group.

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Appendix

This study was coordinated by the Japan Clinical Oncology Group (N Saijo, Chairperson) and was performed with the cooperation of the following institutions and investigators: Tochigi Cancer Center Hospital, Tochigi (K Mori, M Noda, T Kondo, and Y Kamiyama); National Nishi-Gunma Hospital, Gunma (S Tsuchiya, Y Koike, K Satoh, A Tohi, and K Kaira); Gunma Cancer Center Hospital, Gunma (K Minato); Saitama Cancer Center Hospital, Saitama (H Sakai, K Kobayashi, and R Kuroki); National Cancer Center, Central Hospital, Tokyo (T Tamura, Y Ohe, H Kunitoh, I Sekine, H Nokihara, and H Murakami); National Cancer Center Hospital East, Chiba (R Kakinuma, K Kubota, H Ohmatsu, K Gotoh, and S Niho); National International Medical Center, Tokyo (Y Takeda, S Izumi, A Kawana, M Kamimura, and M Iikura); Toranomon Hospital, Tokyo (K Kishi, and M Kawabata); Kanagawa Cancer Center Hospital, Kanagawa (K Yamada, I Nomura, F Oshita, and M Ikehara), Yokohama Municipal Citizen's Hospital, Kanagawa (K Watanabe, H Kunikane, H Okamoto, A Nagatomo, and H Aono); Niigata Cancer Center Hospital, Niigata (A Yokoyama, H Tsukada, M Makino, T Shinbo, S Kinebuchi, J Tanaka, M Tango, and T Ohara); Gifu City Hospital, Gifu (T Sawa, M Miwa, T Ishiguro, M Sawada, and T Yoshida); Aichi Cancer Center Central Hospital, Aichi (K Yoshida, and T Hida); Aichi Cancer Center Aichi Hospital, Aichi (H Saitoh, and M Okuno); Osaka City University Medical School, Osaka (S Kudoh, S Kyoh, H Kamoi, N Yoshimura, T Kodama, K Ohtani, S Shiraishi, S Nomura, S Enomoto, H Matsuura, and R Wake); Kinki University Medical School, Osaka (T Nogami, N Yamamoto, S Sakai, K Kodama, K Akiyama, J Tsurutani, K Tamura); Osaka Prefectural Adult Disease Center, Osaka (S Nakamura, F Imamura, M Yoshimura, S Yamamoto, K Ueno, H Ohmiya, H Matsuoka, and H Uda); Osaka Prefectural Respiratory and Allergy Medical Center, Osaka (M Furukawa, T Yamadori, T Takimoto, and T Hirashima); National Kinki Central Thoracic Disease Center, Osaka (S Minami, N Naka, T Kawaguchi, and H Ishikawa); National Toneyama Hospital, Osaka (Y Okano); Osaka City General Medical Center, Osaka (N Takifuji, and M Miyazaki); Kobe City Central Hospital, Kobe (T Nishimura, Y Okazaki, D Kinose, H Fujii, S Takakura, and M Hayashi); Sasebo City General Hospital, Nagasaki (J Araki); Kumamoto Regional Medical Center, Kumamoto (H Senba, T Seto, and S Fujii).

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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