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Phase I Study and Clinical Pharmacological Evaluation of Daily Oral Etoposide Combined with Carboplatin in Patients with Lung Cancer

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Twenty-eight patients with inoperable or relapsed lung cancer were given a combination of oral etoposide, administered once a day at doses ranging from 40 to 60 mg/m²/day (d) for 21 consecutive days, and carboplatin, administered intravenously over 1 h at doses ranging from 300 to 400 mg/m² on day 1 to determine the appropriate doses of this combination. In addition, pharmacokinetic and pharmacodynamic analyses were performed. All the patients had a performance status of 0 to 1. Serum etoposide and free platinum (Pt) concentrations were measured using high-performance liquid chromatography and atomic absorption, respectively. Myelosuppression, nausea and vomiting were the dose-limiting toxicities of this schedule. The maximum tolerated dose (MTD) was 50 mg/m²/d oral etoposide for 21 days and 400 mg/m² i.v. carboplatin on day 1. For heavily pretreated patients, the MTD was 40 mg/m²/d oral etoposide for 21 days and 350 mg/m² i.v. carboplatin on day 1. No cumulative increase in the area under the concentration-time curve (AUC) for oral etoposide over time was observed. There were significant correlations between the free Pt serum level (6, 8, 12, 24 h post-dose) and etoposide AUC level (days 1, 10 and 21) for graded hematological toxicity, and the percentage decreases and nadir counts of hemoglobin, leukocytes, neutrophils and platelets. Several pharmacodynamic models were developed to predict the hematological toxicity. In order to facilitate pharmacodynamic evaluations in future studies, a limited sampling model for oral etoposide was also developed and validated.

Key words: Oral etoposide — Carboplatin — Pharmacokinetics — Pharmacodynamics — Lung cancer

The combination of carboplatin and intravenous (i.v.) etoposide is active against small-cell lung cancer[1] and advanced non-small-cell lung cancer and less toxic than combination therapy with cisplatin and i.v. etoposide.[2]

Recent clinical trials have shown that long-term, low-dose oral etoposide administration is at least as effective as standard i.v. treatment regimens for the treatment of lung cancer.[3–5] Furthermore, introduction of oral etoposide into combination chemotherapy regimens may shorten the hospitalization period and thus reduce treatment costs. Therefore, it may be reasonable to consider substituting oral for i.v. etoposide for combined carboplatin and etoposide chemotherapy. However, the dose-limiting toxicity of each agent is myelosuppression; carboplatin induces thrombocytopenia, and chronic low-dose oral etoposide induces leukopenia. Therefore, in order to use this combination safely, hopefully in an ambulatory setting, we think it is vital to understand in detail the pharmacokinetic (PK)/pharmacodynamic (PD) profile of this regimen and to develop an efficient means of predicting its toxicity.

It has become increasingly apparent in recent years that PK/PD analysis is a prerequisite for conducting informative phase I/II trials and ensuring good clinical practice.[6,7] Although frequent blood sampling and detailed analysis are essential for therapeutic drug monitoring, facilities for frequent sampling in an outpatient clinic or community hospital are limited. One strategy for overcoming this problem is to apply limited sampling models (LSM) using stepwise multiple linear regression analysis.[8] Once an LSM has been set up, the area under the concentration-time curve (AUC) can be predicted easily by collecting a few blood samples and measuring the drug concentrations, and thus the risk of a patient developing toxicity can be predicted, providing the anticipated toxicity correlates with the AUC.[7]

The aims of this study were: 1) to determine the maximum tolerated dose (MTD) and most suitable dose for use in phase II studies of oral etoposide and i.v. carboplatin in combination, 2) to investigate the PK/PD profile of this combination, and 3) to develop LSMs for oral etoposide pharmacokinetics.

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PATIENTS AND METHODS

Eligibility and evaluation Eligible patients had histologically or cytologically confirmed lung cancer. Those with small-cell lung cancer were eligible if their disease was refractory or resistant to standard chemotherapy and radiotherapy regimens. All patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2. Prior to starting the protocol, at least 4 weeks had to have elapsed since the last course of chemotherapy or radiotherapy, and in the case of a mitomycin C-, and nitrosourea-containing regimen, at least 8 weeks had to have elapsed since the last course of chemotherapy. The patients were also required to have adequate bone marrow, hepatic and renal function, manifested as a white blood cell (WBC) count of $\geq 4{,}000/\mu l$, platelet count of $\geq 100{,}000/\mu l$, hemoglobin of ≥ 10 g/dl, total serum bilirubin of ≤ 1.5 mg/dl, serum transaminases $\leq 2 \times$ normal limit and serum creatinine ≤ 1.2 mg/dl. Measurable disease was not a prerequisite for entry into this study. Patients with massive pleural effusion, ascites and/or pericardial effusion were considered ineligible, and those with another concomitant malignancy and/or other serious medical or psychiatric disease were excluded. All patients gave their informed consent prior to commencing the study.

Histories were taken and physical examination and routine laboratory studies were carried out before treatment and weekly during therapy. The pretreatment and routine laboratory studies included complete blood cell and platelet counts, differential blood smear, serum total and direct bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase, total protein, albumin, creatinine, uric acid, electrolyte, calcium, phosphate and amylase levels, blood urea nitrogen (BUN), urinalysis and a fecal hemocult test for occult blood. Complete blood cell counts were also obtained every day if the WBC count declined to $\leq 1{,}000/\mu l$ and/or platelet count declined to $\leq 50{,}000/\mu l$. An electrocardiogram was obtained and the 24-h creatinine clearance determined before and after each course. A chest X-ray was obtained before treatment and weekly, and chest and brain computed tomography, abdominal ultrasonography and bone scintigraphy were performed before treatment and once a month during treatment or as clinically indicated.

Treatment plan Etoposide capsules were taken with at least 150 ml of water every morning (at 9:00 AM) after breakfast for 21 consecutive days (days 1 to 21). Etoposide is available in Japan as 25-, 50- and 100-mg capsules. Therefore, the actual dose administered was approximated to within a few milligrams of the required calculated dose, using the method described previously.[3] Etoposide was discontinued if the WBC count fell below $2{,}000/\mu l$, or platelet count fell below $5.0 \times 10^4/\mu l$. Carboplatin was diluted to 500 ml with isotonic saline solution and administered by i.v. infusion over 1 h (from 9:00 AM to 10:00 AM) on day 1 without administering prehydration or posthydration fluids. No premedication with antiemetics (domperidone, metoclopramide, granisitron) was allowed for this study. After 21 days, etoposide was discontinued for 1 week, and each patient was re-evaluated on day 28. Patients with obvious evidence of disease progression were removed from the study; those who were clinically stable or improved received a second course of treatment. In patients receiving more than one course, subsequent courses were initiated as soon as the WBC counts had risen to $\geq 4{,}000/\mu l$ and platelet counts had risen to $\geq 10.0 \times 10^4/\mu l$.

Five combined dose levels were chosen. The respective starting doses of etoposide and carboplatin were 40 mg/m^2/d orally for 21 days and 300 mg/m^2 i.v. on day 1 (300/40). These starting doses were selected on the basis of prior clinical experience at our institution[9] and published reports of studies using each agent singly.[3, 10] The doses were escalated until the MTD was reached. Dose escalation was not permitted in individual patients.

A minimum of 3 patients were treated at each dose level and the dose was escalated for the next group of patients if no unacceptable toxicity was observed. Toxicities were reported and classified using the WHO grading system.[11] If any of the first 3 patients at a given dose level developed unacceptable toxicity, defined as a leukocyte or platelet nadir reaching WHO grade 3, or non-hematological toxicity other than alopecia, reaching WHO grade 2, at least 3 additional patients were entered, up to a total of at least six at that dose level. The MTD of the regimen was defined as the dose level immediately below that at which either 2 patients of a cohort of ≤ 6 patients experienced WHO grade 3 or 4 non-hematological toxicity or 4 patients of a cohort of ≤ 6 patients experienced WHO grade 4 hematological toxicity.

Pharmacokinetic studies Twelve-milliliter blood samples were drawn from each patient before and 15 min, 30 min, and 1, 2, 3, 4, 6, 8, 12 and 24 h after i.v. carboplatin and taking oral etoposide (both starts at 9:00 AM) on day 1, and 5 ml blood samples were drawn from each patient before, 30 min, and 1, 2, 3, 4, 6, 8, 12 and 24 h after taking oral etoposide on days 10 and 21. A urine sample was obtained every 6 h until 24 h after the start of treatment. The blood samples were centrifuged to remove the red blood cells and both serum and urine samples were stored at -80°C for a maximum of 2 weeks until the assays were carried out. The concentration of etoposide in serum (2 ml) was measured using high-performance liquid chromatography (HPLC),[12] with a minimum detection limit of 0.1 μg/ml. A portion of serum (3 ml) was transferred to an Amicon Centrifree

tube (Amicon Co., Danvers, MA) and centrifuged; the ultrafiltrate was collected and its platinum (Pt) concentration determined using flameless atomic absorption spectrometry,[13] with a minimum detection limit of 25 ng/ml.

All the required pharmacokinetic parameters were calculated according to the statistical moment theory using the computer program MULTI.[14] The AUCs were calculated using the trapezoidal method from zero to the last time-point measured, and from there to infinity by exponential extrapolation using the elimination rate constant. Terminal half lives were calculated by using the equation $T_{1/2}=\ln(2)/k$, where k is the slope derived from a plot of ln (drug concentration) versus time for the terminal phase. CL_T for oral etoposide is an apparent clearance (CL_P/F=dose/AUC) where CL_P is measured plasma clearance (dose/AUC) and F (bioavailability) is assumed to be 1.0.

Pharmacodynamic studies Correlation analysis of each treatment course yielded estimates of the correlation coefficients for the pharmacokinetic parameters and observed toxicities. Pearson correlation coefficients were obtained for continuous variables, and Spearman correlation coefficients were calculated for those related to graded toxicity. Subsequently, stepwise forward multiple regression analysis was used to develop a linear pharmacodynamic model to predict the natural log-transform of nadir counts of leukocytes, neutrophils and platelets. For PD modeling, 15 courses (each of them was course 1) from 15 chronologically entered patients were used as the training data set. The F-test was used to select the optimal model, which was then validated using the test data set from 12 patients (14 treatment courses), to correlate the predicted and observed nadir counts.

Limited sampling model development Using the training data set, a separate univariate analysis was performed on the data obtained at each time-point to correlate the serum etoposide concentrations with the AUC. Subsequently, stepwise forward regression analysis was used to generate the best model, using three or fewer variables, for the training data set. The F-test was used to select the optimal model, which was then validated using the test data set, to correlate the predicted and observed AUC values. In this phase I study, we obtained 77 data series (the etoposide concentrations at 10 time-points constituting a data series). For day 1 modeling, 15 data series (each of them was from course 1) from 15 patients entered chronologically constituted the training data set and the subsequent 18 from 15 patients formed the test data set. For day 10 modeling, 12 data series (each of them was from course 1) from 12 patients entered chronologically constituted the training data set and the subsequent 12 from 9 patients formed the test data set. For day 21 modeling, 12 (each of them was from course 1) from 12 patients constituted the training data set and the subsequent 8 from 7 patients formed the test data set.

Statistical analysis In order to validate our model, we used the methods described previously.[15] The bias and precision of the model were measured by calculating the mean predictive error (MPE) and the root-mean-squared error (RMSE). All the statistical analysis was performed using a computer program (StatView II, Avacus Concepts, Inc., Berkeley, CA) on a Macintosh Powerbook 170 microcomputer.

RESULTS

Patients' characteristics Twenty-eight patients entered this trial between July 1992 and July 1993 at Hiroshima University Hospital. Their characteristics are summarized in Table I. Their median age was 58 years (range 42 to 75), 9 were female, and none had an ECOG performance status of 2. Sixteen patients had received no previous chemotherapy or radiotherapy and 3 of them received this combination chemotherapy in a post-surgical adjuvant setting.

Hematological toxicity The dose escalation scheme with the numbers of patients treated and treatment courses delivered are shown in Table II. One patient received 3 treatment courses (courses 1 to 3), 5 patients received 2 courses (courses 1 and 2) and 22 patients received one treatment course (course 1 only). Therefore, a total of 35 treatment courses was administered. The oral etoposide

Table I. Patient Characteristics

	No. of patients
Median age (range), years	58 (42–75)
Male/Female	19/9
Stage	
I/II/IIIA	2/2/2
IIIB	3
IV	19
ECOG performance status	
0	24
1	4
Previous therapy	
None	16[a]
Chemotherapy	8
Chemotherapy and radiotherapy	2
Radiotherapy	1
Surgery and chemotherapy	1
Histology	
Adenocarcinoma	19
Squamous cell	5
Small cell	3
Large	1

a) Three patients underwent surgical resection.

Table II. Toxicity

	Dose levels of carboplatin and etoposide				
	300/40[a]	350/40	400/40	400/50	400/60
Total No. of patients	6 (3)[b]	7 (3)	6 (3)	6 (4)	3 (3)
Total No. of courses	7	8	10	7	3
Delivered dose/planned dose (%)	89	86[c]	89	88	24
No. of courses with WHO grade ≥3 hematological toxicity					
Hb	2	1	3	2 (1)	0
WBC	1	3	3 (1)	3 (2)	0
Neutropenia	2	4 (1)	3 (1)	2 (1)	2 (2)
Plt	3 (1)	0	2 (1)	3 (1)	0
No. of courses with WHO grade ≥2 non-hematological toxicity					
Abnormal liver function	0	0	0	1	0
Nausea and vomiting	2	3	2	1 (1)	3 (3)
Pulmonary	0	1	1 (1)	0	0
Diarrhea	0	0	0	1 (1)	0

Abbreviations: WBC, white blood cell count; Plt, platelet count.
a) 300/40 denotes carboplatin 300 mg/m^2 i.v. on day 1 and etoposide 40 mg/m^2 orally for 21 days.
b) Number in parenthesis is the number of previously untreated patients.
c) One patient refused therapy on day 4 in the first course.

doses actually used were about 90% of those planned, up to the 400/60 dose level, when patients had to take a 100-mg capsule almost every day. At the 350/40 dose level, all the three heavily pretreated patients (those who received three or more courses of combination chemotherapy) experienced WHO grade 3 or higher neutropenia (Grade 3, two patients; Grade 4, one patient). Therefore, we considered that the MTD level for heavily pretreated patients was 350/40 and decided not to enter such patients into this protocol above the 400/40 dose level. The numbers of patients who experienced WHO grade 3 anemia/leukopenia/neutropenia/thrombocytopenia at each dose level were 2/0/1/2 at 300/40; 1/3/3/0 at 350/40; 3/3/2/1 at 400/40; 2/2/1/2 at 400/50 and 0/0/2/0 at 400/60 and the numbers of patients who experienced WHO grade 4 anemia/leukopenia/neutropenia/thrombocytopenia at each dose level were 0/1/1/1, 0/0/1/0, 0/0/1/1, 0/1/1/1 and 0/0/0/0. The median leukocyte/neutrophil nadir counts (/μl) at each dose level were 3500/1400 at 300/40; 2300/977 at 350/40; 3100/1690 at 400/40; 2100/1295 at 400/50 and 2800/630 at 400/60 and the respective median platelet nadir counts ($\times 10^4$/μl) were 14.7; 15.9; 12.9; 6.9 and 7.9. The median leukocyte/neutrophil nadir days at each dose level were: 20/22 at 300/40; 21/23 at 350/40; 19/19 at 400/40; 21/22 at 400/50 and 18/18 at 400/60, and the respective median platelet nadir days were: 22; 21; 17.5; 18 and 18.

Non-hematological toxicity The most severe non-hematological toxicity encountered was pulmonary toxicity. At the 400/40 dose level, a 50-year-old, previously untreated male patient died of respiratory insufficiency 25 days after starting the therapy. He had been diagnosed as having idiopathic interstitial pneumonia one year previously, which had been well controlled without steroid therapy, and therefore we considered him eligible for this phase I study. His WBC count was 1,700/μl (absolute neutrophil count, 570) on day 18, when started administering granulocyte colony-stimulating factor and stopped the etoposide. As he showed hypoxemia and a 3-fold increase of serum LDH on day 21, we diagnosed acute deterioration of interstitial pneumonia and administered high-dose methylprednisolone (1 g/d) for 3 days. A post-mortem examination revealed progressive adenocarcinomatous and diffuse bilateral subacute interstitial pneumonia. A 64-year-old heavily pretreated female patient had a bilateral multiple consolidation shadow on a chest roentgenogram with hypoxemia on day 24, but recovered completely within a week; she was treated with methylprednisolone, antibacterial and antifungal therapy. In the first case, we felt that the deterioration of interstitial pneumonia had not been caused solely by the combination chemotherapy, and thought that the patient's respiratory insufficiency had been due, at least partly, to progressive disease. In the second case, we thought that the respiratory insufficiency had been caused by drug-induced pneumonia, rather than infection, as the patient recovered rapidly after steroid treatment.

At the 400/60 dose level, all the enrolled patients experienced severe nausea and vomiting. They needed i.v. antiemetic therapy (granisetron) every day and their food intake decreased markedly, necessitating i.v. fluid supplementation. We considered that dose escalation

Table III. Pharmacokinetic Parameters

Carboplatin (Free platinum)	AUC (μg h/ml)	$T_{1/2}$ (h)	CL_T (ml/min/m^2)	CL_R (ml/min/m^2)	V_{SS} (liter/m^2)
300 (mg/m^2) (n=6)	60.7±19.6 (32)	3.2±1.1 (35)	45.5±11.1 (25)	46.6±22.9 (49)	6.4±2.4 (37)
350 (n=7)	83.6±32.1 (38)	4.7±2.3 (48)	42.2±20.6 (49)	51.7±19.9 (39)	6.1±2.3 (38)
400 (n=20)	84.0±34.9 (38)	4.8±1.4 (28)	47.7±17.1 (34)	46.5±23.2 (44)	7.9±4.1 (51)
Etoposide p.o. (%)	**AUC**	**$T_{1/2}$**	**CL_T**	**CL_R**	**Urinary excretion**
50 (mg) d1 (n=12)	15.7±8.7 (56)	5.7±2.2 (40)	48.8±27.6 (57)	8.6±4.1 (48)	17.7±6.8 (39)
50 d10 (n=9)	17.4±6.2 (36)	6.9±3.4 (50)	36.1±9.7 (27)	8.7±2.3 (26)	23.2±8.5 (37)
50 d21 (n=8)	20.5±9.9 (49)	6.9±2.3 (34)	33.4±11.5 (34)	7.5±2.7 (37)	21.9±7.9 (36)
75 (mg) d1 (n=18)	18.0±6.7 (37)	6.4±2.6 (38)	45.5±15.5 (36)	8.2±2.5 (31)	17.2±6.8 (40)
75 d10 (n=14)	22.7±7.1 (31)	8.5±2.5 (30)	36.3±9.4 (26)	10.7±4.8 (45)	24.8±14.3 (58)
75 d21 (n=12)	20.8±7.5 (36)	6.9±2.0 (29)	36.1±12.4 (34)	10.0±2.3 (23)	26.6±9.1 (34)

Abbreviations: AUC, area under the concentration-time curve; $T_{1/2}$, elimination half-life; CT_T, total body clearance; CL_R, renal clearance; V_{SS}, volume of distribution. Number in parenthesis is the coefficient of variance (%).

above this dose level would not be applicable in an outpatient setting, and therefore we decided that the 400/60 dose level was intolerable and that the 400/50 dose level was the MTD.

Two patients experienced transient transaminase increases: one showed WHO grade 1 toxicity from day 35, which was resolved completely without treatment on day 45, and the other experienced WHO grade 2 toxicity from day 19, which was resolved completely on day 40. One patient at a dose level of 400/50 experienced moderate diarrhea, so we stopped etoposide administration on day 17.

Pharmacokinetics Of the 28 patients, 26 agreed to undergo blood/urine sampling for the PK study. One patient underwent PK sampling in courses 1 through 3, five patients did so in courses 1 and 2, and 20 patients did so in course 1 only. The patients were given the same oral dose of etoposide (50 or 75 mg) on days 1, 10 and 21 when we took blood samples. For carboplatin, we were able to perform complete PK sampling in 33 treatment courses from 26 patients (course 1 only, 20 patients; courses 1 and 2, 5 patients; courses 1, 2 and 3, 1 patient). For etoposide, we were able to perform complete PK sampling (blood/urine sampling on days 1, 10 and 21) in 18 treatment courses from 15 patients (course 1 only, 12 patients; courses 1 and 2, 2 patients; courses 1 and 3, 1 patient); days 1 and 10 sampling in 6 courses from 6 patients (course 1 only, 3 patients; course 2 only, 3 patients); days 1 and 21 sampling in 2 courses from 2 patients (course 1 only, 2 patients), and were able to perform day 1 only sampling in 7 courses from 7 patients (course 1 only, 6 patients; course 2 only, 1 patient).

The calculated carboplatin and oral etoposide PK parameters (means±SD) are listed in Table III. As to oral etoposide pharmacokinetics, we were able to detect etoposide in our HPLC system at 30 min after administration, with the peak serum concentration (C_{max}) occurring between 2 and 4 h after intake (50 mg etoposide, 2.53±1.04 h; 75 mg etoposide, 2.99±1.11 h). The mean (±SD) C_{max} of 50 mg etoposide was 2.12±0.89 (μg/ml) and that of 75 mg etoposide was 2.37±1.10 (μg/ml). Etoposide concentration was above 1 μg/ml between 1 and 8 h after intake. There was about 40% interpatient variability in the AUC values for both carboplatin and oral etoposide. When we examined the relationship between AUC of drug concentration levels of carboplatin or etoposide and concomitant medication, renal function (24-h creatinine clearance), albumin and total bilirubin concentrations at the PK sampling day, and prior chemotherapy in an attempt to explain the large interpatient variability, none of these factors showed statistically significant relationships that could explain the variability (data not shown). The mean (±SD) intra-patient variability in AUCs for oral etoposide was 21.1±9.3% (range: 14.4–37.5%). There were no significant differences between the PK parameters for each carboplatin dose. The AUC and urinary excretion of 50 mg oral etoposide on day 1 of combined treatment were significantly lower than those on days 10 (paired t test, P= 0.0498 and 0.0169, respectively) and 21 (paired t test, P =0.0051 and 0.0142, respectively). The total body clearance (apparent oral clearance) of 50 mg oral etoposide on day 1 tended to be higher than that on days 10 (paired t test, P=0.0472) and 21 (paired t test, P=0.0434). There was no significant difference between the day 1 elimination half-life ($T_{1/2}$) and that on day 10 or 21 (paired t test, P=0.2184, 0.171, respectively). The AUC, $T_{1/2}$ and urinary excretion of 75 mg oral etoposide on day 1 of combined treatment did not differ significantly from those on days 10 and 21 except for urinary excretion on

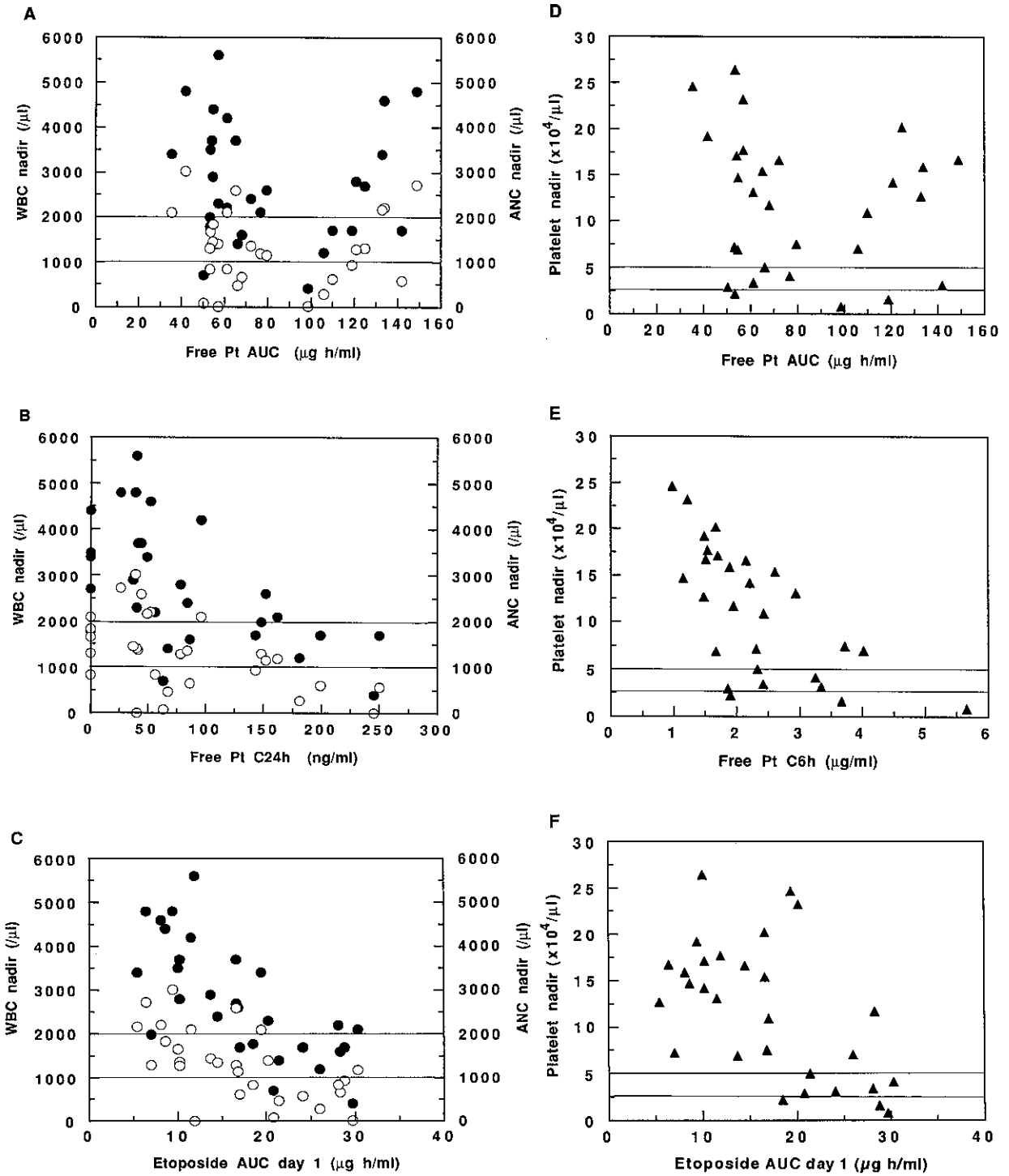

Fig. 1. Relationship between pharmacokinetic parameters and hematological toxicity: (A) free platinum AUC at day 1 and leucocyte/neutrophil nadir counts; (B) free platinum serum levels at 24-h post dose and leukocyte/neutrophil nadir counts; (C) etoposide AUC at day 1 and leukocyte/neutrophil nadir counts; (D) free platinum AUC at day 1 and platelet nadir counts; (E) free platinum serum levels at 6-h post dose and platelet nadir counts; (F) etoposide AUC at day 1 and platelet nadir counts. Solid circles indicate leukocyte counts and clear circles indicate neutrophil counts. Solid triangles indicate platelet counts. Horizontal lines indicate WHO grade 2/3 or 3/4 hematological toxicity borderlines.

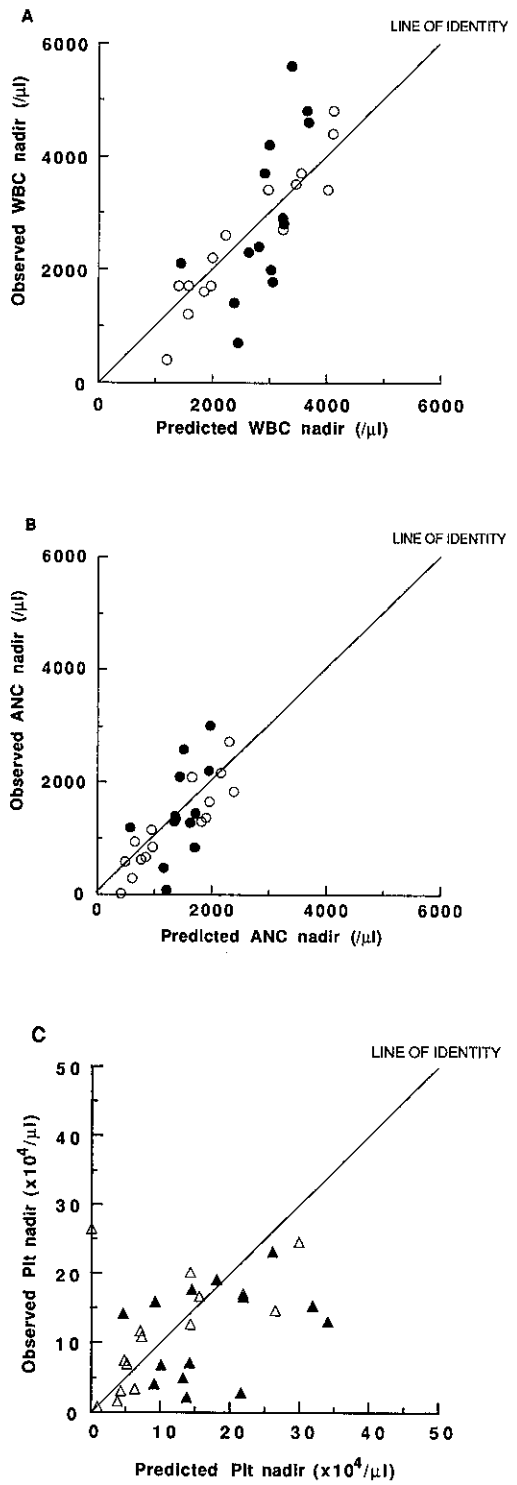

Fig. 2. Relationship between predicted nadirs and actually observed nadirs of leukocytes (A), neutrophiles (B) and platelets (C). Prediction was performed by our developed pharmacodynamic models. Clear circles and triangles indicate training data set, and solid circles and triangles indicate test data set.

day 1, which was significantly lower than that on day 21 (paired t test, $P=0.0373$).

Pharmacodynamics Of the 35 treatment courses given, we considered that 29 courses from 22 patients were evaluable for determining PD correlations between AUC or drug concentrations and clinical outcome. With these evaluable treatment courses, the patients received oral etoposide for at least 10 days and the mean ($\pm$SD) percentage of the delivered to the planned dose was 90.3 $\pm$15.3%.

There were significant (Spearman and Pearson, $P<0.05$) correlations between etoposide AUC on days 1, 10, and 21 and Hb, leukocyte/neutrophil, platelet WHO graded toxicity, the percentage decrease in the count of each blood cell component and the natural log-transform of each nadir count. It should be noted here that not only etoposide AUC on day 10 (data not shown) or 21, but also that on day 1, indicate roughly but clearly the risk of a patient developing leukopenia or thrombocytopenia. Patients appeared to experience WHO grade 3 or higher hematological toxicity when their etoposide AUC on day 1 exceeded approximately 20 μg h/ml (Fig. 1). As to carboplatin, there were significant correlations between drug level (4, 6, 8, 12, and 24 h post dose) and all these hematological toxicities except for Hb. However, we did not observe any correlation between carboplatin AUC and hematological toxicities.

We then performed PD model development to describe the relationships between PK parameters and hematological toxicities in this patient population by stepwise regression analysis, as described in "Patients and Methods." Although etoposide is known to bind to albumin and bilirubin in serum, the concentration of neither was correlated with hematological toxicity in our study population. In addition, 24-h creatinine clearance did not correlate well with hematological toxicities (data not shown). Therefore, we did not include these variables in our pharmacodynamic model. The variables entered were: etoposide AUCs on days 1, 10 and 21; carboplatin (CBDCA) AUC; serum Pt level (μg/ml) 6 h (CBDCA C_6) and 24 h (CBDCA C_{24}) after the start of carboplatin infusion; natural log-transform pretreatment counts of leukocytes, neutrophils or platelets. The reason for having chosen CBDCA C_6 and CBDCA C_{24} was that those two variables were the best correlated with hematological toxicities.

The natural log-transform of the nadir counts of leukocytes (WBC_n(/μl)), neutrophils (ANC_n (/μl)) and platelets (Plt_n ($\times 10^4$/μl)) were estimated as follows: ln $WBC_n = 8.58 - 2.407$[CBDCA C_{24}] $- 0.03$[VP-16 AUC d1]; ln $ANC_n = 8.072 - 4.226$[CBDCA C_{24}] $- 0.039$[VP-16 AUC d1]; ln $Plt_n = 1.428$[ln Pre Plt] $- 0.45$[CBDCA C_6] $- 1.137$, where VP-16 AUC d1 was the etoposide AUC (μg h/ml) on day 1. The estimated correlations

Table IV. Stepwise Regression Development of the Limited Sampling Strategy

Model	Time (h)			Coefficient				MPE±SE (%)	RMSE (%)	R^2 (Test)
	T_1	T_2	T_3	K_1	K_2	K_3	K_4			
A1	2	8	—	1.47	16.48	—	0.68	3.2±4.4	16.9	0.94
A2	2	8	24	2.13	11.52	20.97	1.19	2.0±3.7	13.4	0.97
A′	2	8	12	1.34	9.54	12.18	0.92	1.6±2.2	8.4	0.95
B1	0.5	8	—	6.32	11.49	—	6.94	−0.3±3.4	8.0	0.76
B2	0.5	8	24	4.64	9.24	17.14	6.38	−1.6±1.9	6.5	0.87
B′	2	12	24	2.39	20.46	19.56	2.12	−1.7±2.4	8.0	0.97
C1	1	6	—	1.78	15.59	—	−2.48	1.3±5.4	17.8	0.84
C2	1	6	24	1.90	9.67	28.50	0.44	0.3±2.3	7.6	0.99
C′	1	3	12	0.52	4.06	22.24	−0.40	0.1±0.9	3.1	0.92

The model is AUC (μg h/ml) $=K_1\ C(T_1)+K_2\ C(T_2)+K_3\ C(T_3)+K_4$, where $C(T_i)$ is the etoposide concentration at T_i ($i=1$, 2, 3) hours after oral intake. Models A1, A2, A′ are for day 1; models B1, B2, B′ for day 10; models C1, C2, C′ for day 21.

were 0.94 between the observed and predicted WBC_n, 0.87 between the observed and predicted ANC_n and 0.83 between the observed and predicted Plt_n. The MPE±SE (%) for the leukocyte, neutrophil and platelet nadirs were 14.7±14.0, 285±269, and 17.6±14.3, respectively. The respective RMSE (%) values for the nadirs of each count were 54.4, 1048, 54.5 respectively. To validate these models, we applied them to the test data set. Fig. 2 depicts the relationship between the observed and our PD model-predicted nadir counts.

Of the 28 patients, response data from 21 were evaluable. Two patients achieved a partial response (one with previously untreated stage IV adenocarcinoma and one with stage IV recurrent small-cell carcinoma), both of whom were treated with the 400/40 dose regimen. However, the relationship between AUCs or drug concentrations and tumor response was not significant. Furthermore, nausea/vomiting pulmonary toxicity, hepatotoxicity, and diarrhea could not be explained with reference to the PK variables.

Limited sampling model development As the etoposide AUC showed some threshold for hematological toxicity (Fig. 1), we developed an LSM for oral etoposide to facilitate PD studies in future phase II studies. The etoposide concentrations at each of the 10 time-points were correlated with the total AUC, using the training data set. As with a previously reported LSM,[8] we restricted all our models to three (or fewer) time-points. First, we performed LSM development for oral etoposide AUC on day 1, and a model using the 2- and 8-h time-points was obtained (Model A1 in Table IV). When we considered the use of a model with three time-points, the best obtained was that using 2-, 8- and 12-h post-dose data (Model A′ in Table IV). However, as obtaining a blood sample 12 h after oral intake may be impractical for patients treated in the morning, we tried stepwise regression analysis using variables other than those at 12 h post-dose. The best model with three time-points obtained was that using 2-, 8- and 24-h post-dose data (Model A2 in Table IV). We then performed LSM development for day 10 and day 21 oral etoposide AUC (Model B1, B2 and B′ for day 10; model C1, C2 and C′ for day 21, Table IV). Each of these models was applied to the test data set and the predicted and observed AUCs were compared (Fig. 3). As shown in Table IV and Fig. 3, there was little bias, and the precision of the model was satisfactory.

DISCUSSION

In this combination phase I study, we investigated a novel combination of i.v. carboplatin and chronic low-dose oral etoposide in patients with lung cancer. The MTD of this regimen was 50 mg/m^2/d oral etoposide for 21 days and 400 mg/m^2 i.v. carboplatin on day 1. But, we recommend a regimen of 40 mg/m^2/d oral etoposide for 21 days and 350 mg/m^2 i.v. carboplatin on day 1 for heavily pre-treated patients.

Long-term daily administration of low-dose oral etoposide has proved a useful treatment modality, due to its efficacy profile background (possible schedule-dependency of its anti-neoplastic effect) and relative ease of administration, which may decrease overall treatment costs and shorten the hospitalization period. When designing this combination chemotherapy regimen, we chose carboplatin as a partner for oral etoposide because of its relative ease of administration, in the hope we could use this combination in an outpatient setting. Moreover, as both these drugs are myelosuppressive, we carried out dose escalation carefully and precisely using both 25-mg (available only in Japan) and 50-mg etoposide capsules and performed detailed PK/PD analysis.

The PK parameters of carboplatin (ultrafilterable Pt) that we observed differed from the data quoted in a

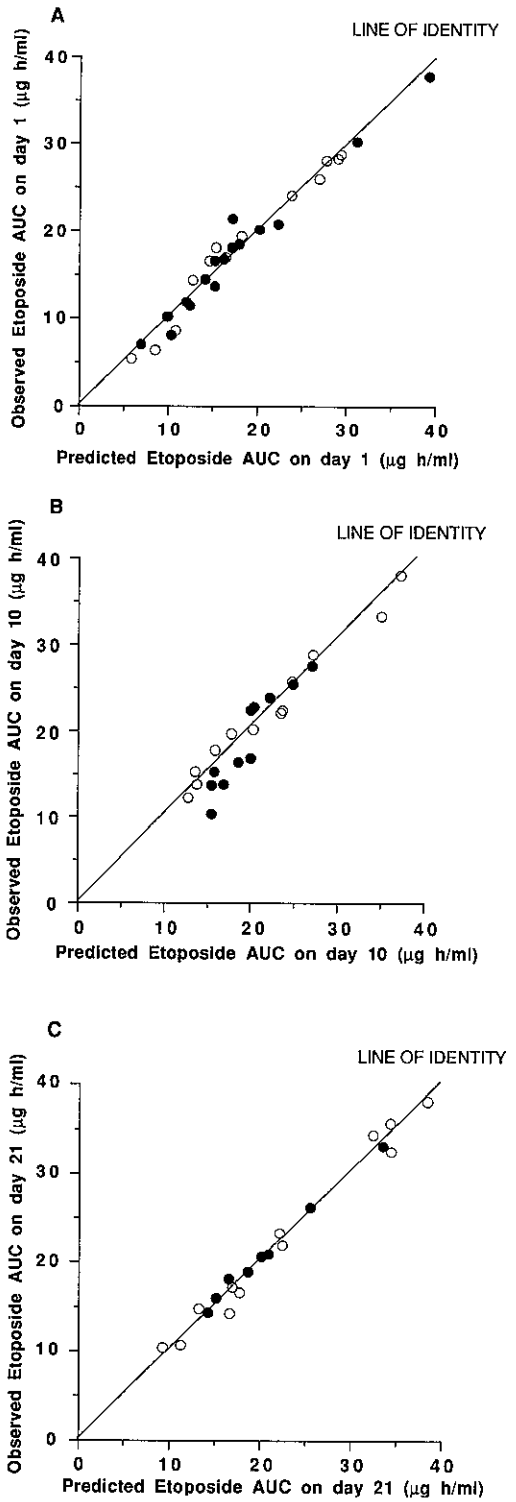

Fig. 3. Validation of limited sampling model for oral etoposide AUC on training and test data set. Prediction was performed by our developed limited sampling models A2 (A), B2 (B) and C2 (C). Clear circles indicate training data set, and solid circles indicate test data set.

previous review[16] in that CL_T and volume of V_{SS} were lower in our study. These discrepant data and the finding that there were no significant differences among the PK parameters for each carboplatin dose may have been due to the small sample size and high interpatient variability; the coefficient of variance was around 40% in our study. These discrepancies may also be explained by a PK interaction between carboplatin and etoposide, although Newell *et al.* did not observe any effect of carboplatin co-administration on etoposide pharmacokinetics in four patients (8 courses) who received IV etoposide.[17]

Unlike many previous studies,[16] we were unable to show a clear relationship between 24-h creatinine clearance and carboplatin AUC (Pearson's $r=0.05$). When we substituted 24-h creatinine clearance for the predicted creatinine clearance proposed by Jelliffe,[18] we also found no relationship (data not shown), thus excluding the possibility of error in our 24-h creatinine clearance measurements. Co-administration of oral etoposide may alter carboplatin PK, but the precise reason for this lack of relationship between carboplatin AUC and 24-h creatinine clearance remains to be elucidated.

Unlike carboplatin pharmacokinetics, PK parameters of etoposide in the present study coincided well with those reported in a previous review.[19]

A recent report from the Pediatric Branch of the National Cancer Institute has described that the pharmacokinetics of daily oral 6-mercaptopurine (6-MP) were so variable from day to day that therapeutic monitoring on any given day of treatment of a patient with acute lymphoblastic leukemia provided no useful information with regard to what the kinetics of 6-MP would be in the same patient on a different day.[20] Although the interpatient variabilities in the AUC of carboplatin and oral etoposide were fairly large (coefficient of variation about 40%), the intrapatient variability in the AUC of oral etoposide was fairly small (coefficient of variation about 20%) in our present study. Therefore, we tried to develop a PD model using the PK values on day 1. Although our PD model, in its present form, was not satisfactory for precise hematological toxicity prediction because of large bias and low precision reflected by large MPE and RMSE, we think we will be able to improve it after we have collected more patients' PK/PD data in future.

Considering the fact that our proposed LSM for etoposide AUC proved to be a highly predictive (Fig. 3) and easily implemented model, if a patient's predicted AUC is above the threshold level (etoposide AUC, 20 μg h/ml), then the physician can follow him/her up in hospital; if not, follow-up can be conducted in an outpatient setting by taking a few blood samples on day 1, 10 or 21.

Although many researchers have found relationships between carboplatin AUC and both the toxic and therapeutic PD consequences of carboplatin,[21-23] we were

unable to prove such relationships. This may be explained partly by the fact that this study adopted a combination chemotherapy regimen, although Belani *et al.* observed a clear relationship between the AUC of carboplatin, but not that of etoposide, and thrombocytopenia using a combination regimen of both drugs.[24] As their report did not include a detailed PK analysis of both drugs, we cannot compare our results with theirs. Therefore the precise reason for the discrepancy remains to be elucidated.

A recent study by Jones *et al.* demonstrated a clear relationship between the AUC of carmustine and acute lung injury.[25] The two patients who experienced pulmonary toxicity in our study showed a high etoposide AUC, but this is also an indicator of hematological toxicity. Therefore, we were unable to identify a specific risk factor for pulmonary toxicity among the PK variables studied. To our knowledge, etoposide-induced pulmonary toxicity has not been reported, but we think it should be borne in mind as a possibility when long-term low-dose oral etoposide treatment is used.

As we observed a partial response in one patient with small cell lung cancer, who had relapsed after 3 courses of carboplatin and conventional 3-day IV etoposide treatment, we believe this i.v. carboplatin and oral etoposide combination is promising. The therapeutic activity of this regimen and the feasibility of our proposed etoposide threshold levels for toxicities and LSM will be verified in forthcoming phase II studies.

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