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ORIGINAL ARTICLE

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Phase I study of E7010

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Abstract E7010 is a novel sulfonamide which was discovered using slow-growing colon 38 carcinoma cells as a screening model. E7010 exhibits a broad spectrum of antitumor activity against human tumor xenografts. The mechanism of action is by arresting the progression of cells in M phase of the cell cycle by inhibiting tubulin polymerization. The objective of this phase I study was to determine the maximum allowable dose (MAD), toxicity, and pharmacokinetics of single or 5-day repeated doses of E7010. In the single-dose study, E7010 was administered orally to 16 patients at doses ranging from 80 to 480 mg/m^2. The dose-limiting toxicity was peripheral neuropathy at a dose of 480 mg/m^2. Hematological and gastrointestinal toxicities were mild. In the 5-day repeated-dose study, 41 patients were given E7010 at doses ranging from 30 to 240 mg/m^2 per day. The dose-limiting toxicities were peripheral neuropathy and intestinal paralysis. Gastrointestinal toxicity was dose-dependent but not severe. Hematological toxicity was not dose-dependent. Pharmacokinetic analysis in the single-dose study showed a rapid increase in the plasma levels of the drug after administration, followed by disappearance with a $t_{1/2}$ of 4.4–16.6 h. The variation in area under the plasma concentration-time curve (AUC) between the patients was small and increased in a dose-dependent manner. Total drug recovery in urine 72 h after administration was 77.8 ± 11.4%, indicating that E7010 has favorable absorption and elimination profiles. The changes in the plasma levels of E7010 on day 5 in the 5-day repeated-dose study were almost the same as those on day 1, indicating that the drug did not accumulate. In the single-dose study, spinal cord metastasis exhibited a 74% reduction in a patient with uterine sarcoma and a minor response (MR) was observed in a pulmonary adenocarcinoma patient. In the 5-day repeated-dose study decreases in the tumor markers carcinoembryonic antigen (CEA) and squamous cell carcinoma antigen (SCC) were observed in a patient with stomach cancer and in a patient with recurrent uterine cervical carcinoma, respectively. The recommended phase II doses are 320 mg/m^2 for a single-dose study and 200 mg/m^2 per day for a 5-day repeated-dose study. Since the activity of E7010 is time-dependent, i.e. a certain concentration of E7010 is required for more than 12 h to suppress the growth of P388 leukemia cells, it is recommended that subsequent phase I/II studies be conducted using a divided dose schedule in order to maintain the blood level of E7010.

Key words E7010 · Sulfonamide · Antimitotic agent · Phase I study · Pharmacokinetics

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Introduction

More than 300 sulfonamide derivatives were synthesized to discover the agent E7010 which is active against solid tumors [1, 2]. The compounds were screened using a slow-growing colon 38 solid tumor model. E7010 has been found to have good antitumor activity, with a broad spectrum of activity against rodent solid tumors and various kinds of human tumor xenografts [3, 4]. E7010 is also active against various types of resistant cells such as vincristine-resistant, cisplatin-resistant and 5-fluorouracil-resistant P388 cells in vivo. The mechanism of action of E7010 is by arresting progression of

the cell cycle in M phase by inhibiting tubulin polymerization [5]. The binding site of E7010 to tubulin is the cholchicine binding site, which is different from that of vinca alkaloids.

E7010-resistant P388 leukemia cell lines were established by increasing the concentration of E7010 in vitro after *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) treatment. The resistant cell lines are not cross-resistant with other antitumor drugs, including vincristine. Interestingly, the cell lines are more sensitive to Taxol than the parent P388 leukemia cells. E7010 demonstrates a time-dependent antiproliferative effect. P388 leukemia cells should be exposed to E7010 for at least 12 h or more to exhibit the antiproliferative effect. This time-dependency seems to be derived from the reversibility of its binding to tubulin. ^{14}C-E7010 quickly dissociates from tubulin when excess unlabeled E7010 is added. This phase I study of E7010 was conducted because of the compound's good broad-spectrum antitumor activity and interesting characteristics such as activity against various types of resistant cells, a binding site different from that of vinca alkaloids and its reversible binding to tubulin. The single-dose study was conducted first. The 5-day repeated-dose study was started after the safety had been confirmed in the single-dose study.

Materials and methods

Patient selection

Eligible patients were those who had histologically or cytologically confirmed malignant solid tumors resistant to standard therapy, or for whom appropriate treatment did not exist. Patients were from 15 to 75 years of age, had an estimated life expectancy of 3 months or more and had a performance status of 0–2 (Criteria of the Japan Society for Cancer Therapy). All patients showed adequate organ function (WBC count ≥4000/μl, hemoglobin ≥9.5 g/dl, platelet count ≥100 000/μl, total bilirubin ≤1.5 mg/dl; SGOT and SGPT levels less than twice the upper normal limit, BUN and serum creatinine levels less than the upper normal limit). Eligible patients had to have recovered from toxicity of prior therapy and to have not received biological response modifiers, hormone preparations or antimetabolites for at least 2 weeks or other chemotherapy for at least 4 weeks prior to the study. Informed consent was obtained from the subjects or their guardians prior to enrollment.

Treatment plan

Capsules containing 10, 50, 100 or 200 mg E7010 were supplied by Eisai Co. Ltd (Tokyo 112-88, Japan) and administered orally after breakfast. The treatment schedule consisted of single or 5-day repeated doses. Since the antiproliferative effect of E7010 is time-dependent, i.e. a certain concentration of E7010 is required for more than 12 h to suppress the growth of P388 leukemia, the single-dose study was conducted first. The 5-day repeated-dose study was started after the maximum allowable dose (MAD) and dose-limiting toxicity (DLT) had been determined in the single-dose study. A starting dose of 80 mg/m^2 in the single-dose study was selected since this dose is approximately equal to 1/10 of the LD_{10} in mice (825 mg/m^2), which is lower than 1/3 of the toxic dose low (TDL) in beagles (2747 mg/m^2). The starting dose in the 5-day repeated-dose study was set at 30 mg/m^2 per day (1/5 of 150 mg/m^2), based on the following results: (a) anorexia and weight loss in one patient and nausea and anemia in another were the only toxicities that were related to E7010 out of seven patients given single doses up to 160 mg/m^2, and (b) the pharmacokinetic data suggest that E7010 does not accumulate in the plasma, even after 5-day continuous administration. The dose for the single-dose study was increased from 80 mg/m^2 to 160, 320, and 480 mg/m^2. In the 5-day repeated-dose study, the daily dose was increased from 30 mg/m^2 per day to 40, 60, 80, 120, 150, 180, 210 and 240 mg/m^2 per day.

Grading of toxicity was based on the Criteria for the Evaluation of the Clinical Effects of Solid Cancer Chemotherapy of the Japan Society for Cancer Therapy [6].

The dose was increased to the next level only after safety had been confirmed. When toxicity of grade 2 or greater, or toxicity associated with a change of more than one grade was observed, the incidence of toxicities, recovery time, and pharmacokinetics were carefully considered before adding another subject, increasing the dosage or continuing the study. The patients were observed before E7010 administration and weekly for 4 weeks after the start of administration. Objective and subjective factors, vital signs, hematology and blood chemistry were assessed, and urinalysis carried out. The MAD was defined as that dose at which two or more out of three patients experienced grade 2 or greater nonhematological toxicity (excluding nausea/vomiting and alopecia), and grade 3 or greater hematological toxicity.

For those patients with measurable disease, standard response criteria were utilized. A complete response was defined as the complete disappearance of all clinical evidence of cancer for at least 4 weeks and the absence of new lesions. A partial response was defined as a ≥50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions as compared with the baseline value for at least 4 weeks. A minor response (MR) was defined as a reduction of more than 25% and less than 50% in the sum of the area of all measurable lesions as compared with the baseline value for at least 4 weeks. Progressive disease was defined as a ≥25% increase in the sum of the areas of all measurable lesions, while stable disease was defined as that not meeting the criteria for a complete, partial response, MR or progressive disease.

Pharmacokinetic analysis

Blood samples for pharmacokinetic studies were collected in heparinized tubes prior to E7010 administration and 0.5, 1, 2, 4, 8, 24, 48 and 72 h after the administration in the single-dose study, and prior to E7010 administration and 0.5, 1, 2, 4 and 8 h after the first administration, before the second, third and fifth administrations, and 0.5, 1, 2, 4, 8, 24, 48 and 72 h after the fifth administration in the 5-day repeated dose study. After blood collection, heparinized blood was immediately centrifuged at 3000 rpm for 10 min and the plasma separated and frozen at −20 °C until assayed. Urine samples were collected prior to E7010 administration and for 0–8, 8–24, 24–48 and 48–72 h after administration in the single-dose study, and prior to E7010 administration and for 0–24 hours after administration from days 1 to 5, and for 24–48 and 48–72 h after the fifth administration in the 5-day study. Urine volume was measured accurately at the end of each collection period. Aliquots (10 ml) were stored at −20 °C until analyzed.

Quantification of E7010 in plasma and urine was conducted using high-performance liquid chromatography (HPLC). Plasma (250 μl) was mixed with 100 μl internal standard solution (4-ethoxy-*N*-[2-[(4-hydroxyphenyl)amino]-3-pyridyl]benzene-sulfonamide, 2.5 μg/ml) before adding 1.0 ml 0.1 *M* Britton Robinson buffer (pH 5.0) and 5.0 ml diethyl ether. Samples with higher concentrations were diluted for analysis. The mixtures were shaken for 10 min and then centrifuged at 3000 rpm for 5 min. The organic layer was removed and 0.4 ml 0.2 *N* HCl added. This mixture was shaken for 10 min and then centrifuged at 3000 rpm for 5 min. The upper organic layer was discarded and the aqueous layer analyzed by HPLC.

The HPLC system comprised an LC-9A pump (Shimadzu, Kyoto, Kyoto, Japan), a Lambda-Max 481 UV detector (Waters Associates, Milford, Mass., USA), a WISP-712B autosampler

(Waters Associates), and a C-R4AX integrator (Shimadzu). The analysis was conducted under the following conditions: column, YMC AM-312 C_{18} 5 μm (6.0 mm internal diameter × 150 mm); mobile phase, 60% CH_3CN/0.05 *M* acetate buffer (pH 5.5); flow rate, 1.2 ml/min; detection UV 270 nm; column temperature 35 °C; injection volume, 50 μl.

Under these conditions, the limit of quantification was 0.05 μg/ml, and the retention times for E7010 and the internal standard were approximately 7.1 and 10.3 min, respectively. The standard curve for E7010 was linear ($r = 0.9999$) over the range 0.05–5 μg/ml. Endogenous substances did not interfere with the detection of E7010 or of the internal standard. E7010 concentrations were determined by comparing the ratio of the height of the E7010 peak with internal standard peak in the patient sample with a standard curve prepared simultaneously. Urine concentrations of E7010 were obtained using the same method as for plasma except that 500 μl of urine was used.

Results

Patient characteristics

The patient characteristics are summarized in Table 1. A total of 16 and 41 patients were enrolled in the single-dose and 5-day repeated-dose studies, respectively. Six patients were excluded from the repeated-dose study: in two patients the investigator decided not to administer E7010 because of rapid disease progression after enrollment, in one patient there was a protocol violation (6 mg/m^2 per day was administered, i.e. 1/10 the prescribed dose of 60 mg/m^2 per day), two patients were excluded because they died as a result of disease progression within 3 weeks of the completion of treatment, and one patient vomited 5 min after administration. Thus, the data for 51 patients (16 and 35 in the single-dose and 5-day repeated-dose studies, respectively) were used to evaluate the safety of E7010. In this study, patients could be enrolled at different doses. Two patients scheduled for the repeated-dose study were treated twice (one patient 30 and 40 mg/m^2 per day, one patient 80 and 120 mg/m^2 per day). Except for these patients, no other individual received a repeated course of E7010. More than half the patients in the single-dose study had lung cancer and all had undergone chemotherapy prior to the study, and more than half the patients in the repeated-dose study also had lung cancer. Gynecological cancer was the next most frequent type of cancer. Chemotherapy had been performed in 32 of the 35 patients.

Table 1 Patient characteristics

	Single administration	5-day repeated dose administration
Entered	16	41
Evaluable	16	35
M/F	10/6	18/17
Age (years)		
Median	65	57
Range	41–71	42–74
Performance status[a]		
0	0	8
1	4	22
2	12	5
Prior therapy		
Chemotherapy	16	32
Radiotherapy	8	15
Surgery	8	16
Immunotherapy	0	5
Diagnosis		
Lung cancer	11	17
Stomach cancer	0	2
Cancer of large intestine	0	2
Breast cancer	1	2
Carcinosarcoma of uterus	1	0
Uterine cervical cancer	1	6
Ovarian cancer	1	4
Invasive thymoma	1	0
Oropharyngeal cancer	0	1
Malignant pleural mesothelioma	0	1

[a] According to the Criteria for the Evaluation of the Clinical Effects of Solid Cancer Chemotherapy defined by the Japan Society for Cancer Therapy

Toxicity

The toxicities observed in the single-dose study are presented in Tables 2 to 4. Numbness of the extremities was observed at doses of 320 mg/m^2 (grades 1 and 2 in one patient each) and 480 mg/m^2 (grades 1, 2 and 3 in two, one and one patients, respectively). At 480 mg/m^2, grade 3 numbness was observed 1 day after E7010 administration, but rapidly improved to grade 2, but about 2 months were needed for it to improve from grade 2 to 1. The same patient developed grade 2 absent knee jerk reflex 2 days after E7010 administration which disappeared 3 days after onset. Neurologic toxicity was the DLT in the single-dose study (Table 2).

Hematological toxicity was mild and leukocytopenia and thrombocytopenia were rarely seen. Although reduced hemoglobin was observed in all groups, the incidence and severity did not appear to be dose-dependent (Table 3). Nausea and vomiting were dose-dependent. Anorexia, gastric discomfort and diarrhea were observed mainly at doses of 320 and 480 mg/m^2 per day. These gastrointestinal symptoms were mild and improved within 3 days of onset (Table 4).

The toxicities observed in the 5-day repeated-dose study are presented in Tables 5 to 7. Peripheral neuropathy, the DLT in the single-dose study, was observed in two of three patients at 30 mg/m^2 per day and two of four patients at 40 mg/m^2 per day, so caution was taken when increasing the dose. However, no numbness was observed at doses of 60 to 120 mg/m^2 per day. At higher doses, numbness was grade 1 in one of three patients at 150 mg/m^2 per day, grades 1 and 2 in one patient each of four patients at 180 mg/m^2 per day, grade 3 in one of four patients at 210 mg/m^2 per day, and grades 1 and 2 in two and one of six patients

Table 2 Neurologic toxicities in single-doze study

Dose (mg/m^2)	Patients (*n*)	Paresthesia Grade			Grade 2 or higher	Hypoactive or absent knee jerk reflex Grade		Grade 2 or higher
		1	2	3		1	2	
80	4	0	0	0	0/4	0	0	0/4
160	3	0	0	0	0/3	0	0	0/3
320	4	1	1	0	1/4	1	0	0/4
480	5	2	1	1	2/5	1	1	1/5

Table 3 Hematological toxicities in single-dose study. The numbers indicate the numbers of patients with abnormal values; the numbers in parentheses indicate the grade of toxicity according to the Criteria for the Evaluation of the Clinical Effects of Solid Cancer Chemotherapy defined by the Japan Society for Cancer Therapy

Dose (mg/m^2)	Patients (*n*)	WBC	Platelets	Hemoglobin
80	4	1 (1)	1 (0)	2 (0, 1)
160	3	0	0	2 (2, 2)
320	4	0	0	1 (2)
480	5	0	0	1 (1)

Table 4 Gastrointestinal toxicities in single-dose study. The numbers indicate numbers of patients; numbers in parentheses indicate the grade of toxicity according to the Criteria for the Evaluation of the Clinical Effects of Solid Cancer Chemotherapy defined by the Japan Society for Cancer Therapy

Dose (mg/m^2)	Patients (*n*)	Nausea/ vomiting	Anorexia	Epigastric discomfort	Diarrhea
80	4	0	1 (2)	0	0
160	3	1 (1)	0	0	0
320	4	3 (1, 1, 2)	1 (1)	2 (1, 2)	0
480	5	2 (2, 3)	2 (2, 3)	1 (3)	3 (1, 1, 1)

at 240 mg/m^2 per day. These findings indicate that numbness was dose-dependent. Grade 3 numbness improved to grade 1 within about 2 weeks of onset in the patient at 210 mg/m^2 per day, but improved from grade 2 to 1 within 4 days of onset in the patient at 240 mg/m^2 per day. In the patients at 180 mg/m^2 per day, grade 2 numbness developed 28 days after completing E7010 administration but it was not bilateral numbness as had been observed previously, so the relationship between E7010 and this symptom remains unclear (Table 5).

Grade 1 intestinal paralysis was observed in one of the four patients at 180 mg/m^2 per day, Grade 4 in one of four patients at 210 mg/m^2 per day, and grades 1 and 4 in one each of six patients at 240 mg/m^2 per day. Grade 4 ileus observed in patients at 210 and 240 mg/m^2 per day developed on the final day of treatment and 2 days after completing treatment, respectively. However, it improved with intervention (limiting food and drink intake and Prostarmon-F therapy) within about 2 weeks of onset.

Grade 1 decreased or absent knee jerk reflex was observed in one of four patients at 120 mg/m^2 per day, and grade 2 was observed in two of six patients at 240 mg/m^2 per day. One patient given 240 mg/m^2 per day who had ileus 2 days after completing treatment developed grade 2 absent knee jerk reflex on the 5th day of administration, which had recovered by day 28 after onset. Another patient at 240 mg/m^2 per day who had grade 1 paresthesia on the 4th day of administration developed grade 2 absent knee jerk reflex on the 2nd day of administration. The symptom persisted for 39 days, but the patient experienced no inconveniences in daily life.

Leukopenia was observed in two of four patients at doses of 120, 180, and 210 mg/m^2 per day. However, it was not dose-dependent because only grade 0 leukopenia was observed in one of six patients at 240 mg/m^2 per day. Reduced hemoglobin was seen at doses of 30 to 240 mg/m^2 per day, but it too was not

Table 5 Neurologic toxicities in 5-day repeated-dose study

Dose (mg/m^2)	Patients (*n*)	Paresthesia				Hypoactive or absent knee jerk reflex			Intestinal paralysis				
		Grade			Grade 2 or higher	Grade		Grade 2 or higher	Grade				Grade 2 or higher
		1	2	3		1	2		1	2	3	4	
30	3	2	0	0	0/3	0	0	0/3	0	0	0	0	0/3
40	4	2	0	0	0/4	0	0	0/4	0	0	0	0	0/4
60	3	0	0	0	0/3	0	0	0/3	0	0	0	0	0/3
80	4	0	0	0	0/4	0	0	0/4	0	0	0	0	0/4
120	4	0	0	0	0/4	1	0	0/4	0	0	0	0	0/4
150	3	1	0	0	0/3	0	0	0/3	0	0	0	0	0/3
180	4	1	1	0	1/4	0	0	0/4	1	0	0	0	0/4
210	4	0	0	1	1/4	0	0	0/4	0	0	0	1	1/4
240	6	2	1	0	1/6	0	2	2/6	1	0	0	1	1/6

Table 6 Hematological toxicities in 5-day repeated-dose study. The numbers indicate the numbers of patients with abnormal values; numbers in parentheses indicate the grade of toxicity according to the Criteria for the Evaluation of the Clinical Effects of Solid Cancer Chemotherapy defined by the Japan Society for Cancer Therapy

Dose (mg/m^2/day)	Patients (*n*)	WBC	Hemoglobin
30	3	0	1 (1)
40	4	0	0
60	3	0	1 (1)
80	4	0	1 (3)
120	4	2 (1, 2)	1 (2)
150	3	0	1 (1)
180	4	2 (2, 3)	0
210	4	2 (2, 2)	0
240	6	1 (0)	1 (3)

dose-dependent. As described above, the hematological toxicities were mild (Table 6).

Gastrointestinal toxicities were dose-dependent, Grade 2 nausea and vomiting were recorded in one of three patients at 60 mg/m^2 per day, grade 1 in two of the three patients at 150 mg/m^2 per day, grade 1 in one of the four patients at 180 mg/m^2 per day, grades 2 and 3 in two and one of four patients at 210 mg/m^2 per day, and grades 1, 2 and 3 in two, two and one of six patients at 240 mg/m^2 per day. The patient at 210 mg/m^2 per day who developed grade 3 nausea and vomiting and the patient at 240 mg/m^2 per day who developed grade 2 nausea and vomiting both exhibited grade 2–3 anorexia. Epigastric discomfort and diarrhea were the other gastrointestinal toxicities observed. The gastrointestinal toxicities observed were not severe and were reversible (Table 7).

E7010 administration was discontinued in one patient (at 240 mg/m^2 per day) owing to eruptions and edema which appeared after the first dose on day 1.

Pharmacokinetics

In the single-dose study, the plasma levels of E7010 reached a peak (Cmax) approximately 3 h after E7010 administration, following which they rapidly decreased (Fig. 1, left side). The $t_{1/2}$ varied from about 4.4 to 16.6 h (Table 8). The AUC did not fluctuate widely between patients, and increased in a dose-dependent manner (Fig. 2). Total drug recovery in urine 72 h after administration was 77.8 ± 11.4% (unchanged form 0.7%, E7010 sulfate 54.0%, E7010 glucuronide 23.1%), indicating that E7010 has favorable absorption and elimination profiles (Fig. 3). The average protein binding rate was 98.6%.

Changes in plasma levels of E7010 in the 5-day repeated-dose study are shown in Fig. 1 (right side). The AUC of E7010 on day 5 was almost the same as on day 1, indicating that the drug did not accumulate. The sulfate and glucuronide forms of E7010 were mainly found as metabolites. Since the AUC of these metabolites showed no difference between day 1 and day 5, these metabolites did not accumulate. The 24-h AUC values on day 5 did not fluctuate widely between individuals, and the relationship between the AUC and dose was linear over the range 30 to 480 mg/m^2 (Fig. 2, Table 9).

Response

In the single-dose study, spinal cord metastasis exhibited a 74% reduction in a patient with uterine sarcoma given

Table 7 Gastrointestinal toxicities in 5-day repeated-dose study. Numbers in parentheses indicate the grade of toxicity according to the Criteria for the Evaluation of the Clinical Effects of Solid Cancer Chemotherapy defined by the Japan Society for Cancer Therapy

Dose (mg/m^2/day)	Patients (*n*)	Nausea/ vomiting	Anorexia	Epigastric discomfort	Diarrhea
30	3	0	0	0	1 (2)
40	4	0	0	0	0
60	3	1 (2)	1 (1)	0	0
80	4	0	0	0	0
120	4	0	1 (1)	0	0
150	3	2 (1, 1)	1 (1)	1 (1)	1 (2)
180	4	1 (1)	0	0	0
210	4	2 (2, 3)	1 (3)	2 (1, 3)	0
240	6	5 (1, 1, 2, 2, 3)	4 (1, 2, 2, 3)	2 (1, 2)	1 (2)

Table 8 Pharmacokinetic parameters for E7010 from the single-dose study (values are means ± SD)

Dose (mg/m^2)	*n*	C_{max} (μg/ml)	T_{max} (h)	AUC (0–24 h) (μg · h/ml)	$t_{1/2}$ (h)	CL/f (l/h/kg)
80	4	5.16 ± 1.02	3.50 ± 1.00	39.38 ± 7.60	11.98 ± 11.94	0.057 ± 0.013
160	3	9.73 ± 2.90	3.33 ± 1.15	76.10 ± 10.31	4.36 ± 1.24	0.067 ± 0.011
307	1	34.06	2.0	148.22	12.65	0.051
320	3	32.31 ± 1.30	1.67 ± 0.58	162.09 ± 36.53	12.39 ± 7.12	0.053 ± 0.010
480	5	23.48 ± 9.58	3.20 ± 1.10	188.88 ± 71.75	16.61 ± 6.07	0.087 ± 0.063

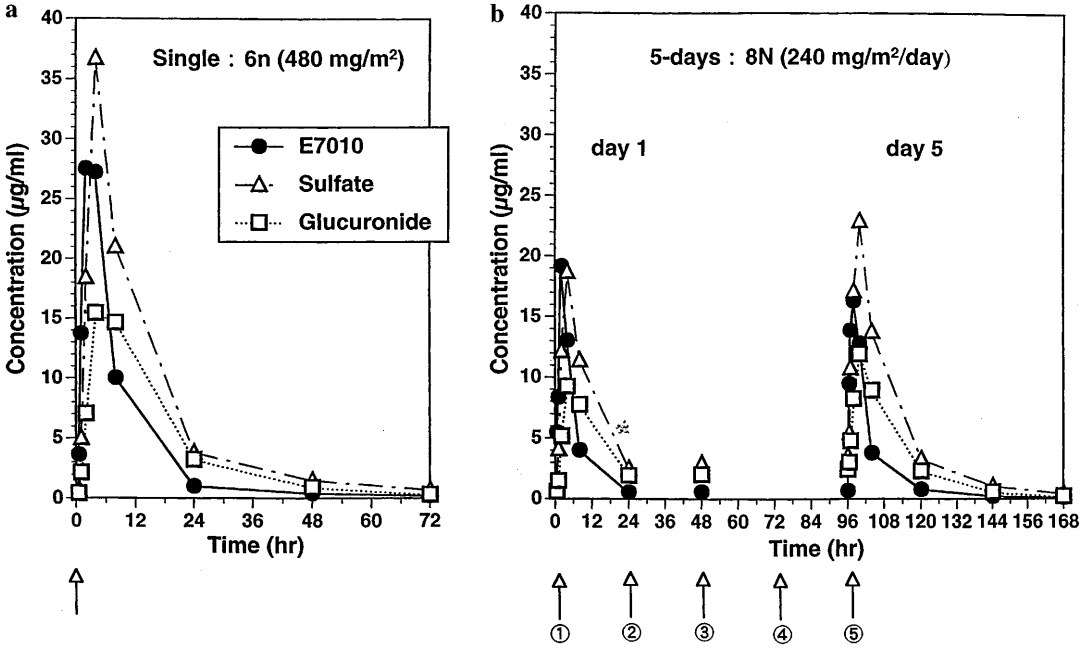

Fig. 1 Plasma concentration-time curves for E7010 and its metabolites after single (**a**) and 5-day (**b**) repeated administrations

Table 9 Pharmacokinetic parameters for E7010 from the 5-day repeated-dose study (values are means ± SD)

Dose (mg/m^2)	*n*	C_{max} (μg/ml)	T_{max} (h)	AUC (0–24 h) (μg · h/ml)	$t_{1/2}$ (h)	CL/f (l/h/kg)
30	3	2.65 ± 1.93	3.67 ± 3.79	15.76 ± 3.93	3.93 ± 1.29	0.041 ± 0.016
40	3	3.95 ± 1.65	2.00 ± 1.73	15.49 ± 4.57	5.12 ± 2.81	0.057 ± 0.024
60	3	5.60 ± 2.23	2.17 ± 1.76	32.47 ± 4.27	4.31 ± 0.45	0.034 ± 0.011
80	4	6.73 ± 1.75	2.75 ± 1.50	39.45 ± 10.20	4.04 ± 1.60	0.044 ± 0.019
120	4	10.27 ± 4.40	2.50 ± 1.00	65.98 ± 19.72	4.67 ± 0.69	0.046 ± 0.016
150	3	13.45 ± 5.01	3.50 ± 3.97	91.06 ± 33.40	5.11 ± 0.18	0.037 ± 0.014
180	5	11.75 ± 2.21	3.20 ± 1.10	73.56 ± 24.39	3.95 ± 0.92	0.056 ± 0.028
210	5	17.43 ± 4.09	2.80 ± 1.10	109.28 ± 39.40	4.62 ± 0.43	0.041 ± 0.012
240	6	21.07 ± 5.17	2.08 ± 1.11	120.10 ± 24.64	4.62 ± 0.84	0.040 ± 0.014

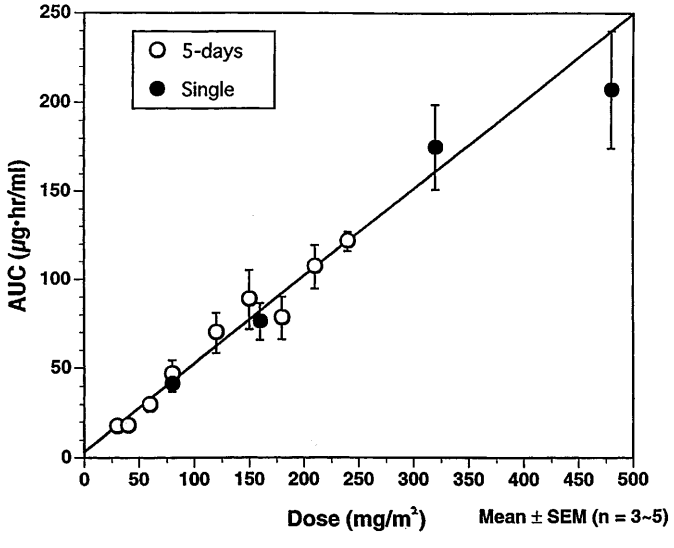

Fig. 2 Relationship between dose and AUC

160 mg/m^2. MR was observed in a patient with pulmonary adenocarcinoma given 480 mg/m^2.

In the 5-day repeated-dose study, a decrease in the tumor marker CEA from 515.5 to 245.4 ng/ml was observed in a patient with stomach cancer given 120 mg/m^2 per day, and inhibition of tumor growth and a decrease in the tumor marker SCC from 9.1 to 6.2 ng/ml were observed in a patient with progressive, recurrent uterine cervical carcinoma given 240 mg/m^2 per day.

Discussion

The objective of this study was to determine the MAD, toxicity and pharmacokinetics of single and 5-day repeated doses of E7010. Hematological and gastrointestinal toxicities were mild in the single-dose study. Grade 2 or greater neurologic toxicity was seen in one of four patients at 360 mg/m^2 per day and in two of five patients at 480 mg/m^2 per day. Although these results do not

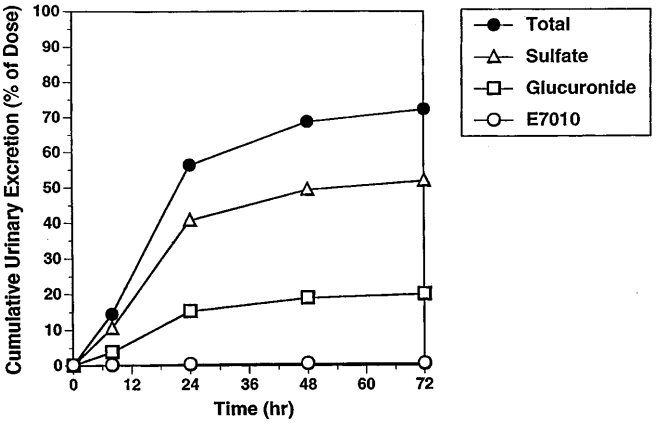

Fig. 3 Mean ($n = 3$) cumulative urinary excretion of E7010 and its metabolites after a single 480 mg/m^2 dose

meet the definition of MAD (the dose at which two or three patients out of three experience grade 2 or greater toxicity), numbness may become progressively worse after repeating a course and it was determined that neurotoxicity was the DLT and the MAD was 480 mg/m^2. The AUC increased in a dose-dependent manner and absorption was at least 80%, indicating a favorable pharmacokinetic profile. Furthermore, a 74% reduction in spinal cord metastasis was seen in a patient with uterine sarcoma given 160 mg/m^2 and an MR was observed in a patient with pulmonary adenocarcinoma given 480 mg/m^2. Based on these results, the recommended dose for a single-dose administration phase II study is 320 mg/m^2.

In the 5-day repeated-dose study, ileus was observed in one of four patients at 210 mg/m^2 per day and in one of six patients at 240 mg/m^2 per day. The patient at 210 mg/m^2 per day who had ileus developed grade 3 numbness and grade 3 gastrointestinal toxicities (nausea/vomiting, epigastric discomfort, anorexia). The patient at 240 mg/m^2 per day who had ileus developed grade 2 absent knee jerk reflex and grades 2–3 gastrointestinal toxicities. Hematological toxicity and gastrointestinal toxicity were mild. Although these results did not indicate the MAD, ileus is a grade 4 toxicity, and the numbness may become progressively worse after repeating the course, so it was determined that ileus and neurotoxicity were DLTs and the MAD was 240 mg/m^2 per day. Pharmacokinetic analysis indicated that the drug did not accumulate during the 5 days, and that the relationship between the AUC and dose was linear over the range 30 to 480 mg/m^2 per day. Antitumor activity was indicated by decreases in the tumor markers CEA and SCC in a patient with stomach cancer and one with uterine cervical carcinoma, respectively. Based on these results, the recommended dose for a 5-day repeated-dose administration phase II study is 200 mg/m^2 per day.

The peripheral neuropathy caused by E7010 seems to be similar to that caused by vincristine and Taxol because paresthesia was the major symptom and the numbness of the extremities was symmetrical [7, 8]. However, it is interesting to note that the location of the onset of the numbness was different from that found with vincristine and Taxol: the symptom was observed first in the toes and then the fingers. It has been reported that numbness develops simultaneously in the toes and fingers with Taxol [9] and that the paresthesia appears initially in the fingers and then the feet with vincristine [10].

In the 5-day repeated-dose study, four patients showed grade 1 paresthesia at low doses, two each at 30 and 40 mg/m^2 per day. Of these four, one who had been previously treated extensively with chemotherapy, including cisplatin, developed grade 1 numbness in the upper limb at doses of 30 and 40 mg/m^2 per day. Another patient who developed grade 1 paresthesia in the oral cavity at 30 mg/m^2 per day had received chemotherapy, including Taxotere. Another patient who developed grade 1 paresthesia in the hands at 40 mg/m^2 per day had been previously given cisplatin and vindesine. Thus, it seems that patients who had received chemotherapy tended to develop paresthesia. Similar results have been reported for Taxol [11]. Neurotoxicity usually develops in patients treated with Taxol at a dose of 250 mg/m^2 [12, 13]. However, neuropathy is observed at a lower dose (135 mg/m^2) when Taxol is administered to patients who have previously been treated with cisplatin.

In the present study peripheral neuropathy was the DLT. While paresthesia (grade 2 or more) was observed at doses of 320–480 mg/m^2 in the single-dose study, it was not observed up to a total dose of 750 mg/m^2 (150 mg/m^2 per day $\times$ 5) in the 5-day repeated-dose study. This indicates that the appearance of peripheral neuropathy depends on the amount of the single dose and not on the total dose. Hence, it seems that the severity of peripheral neuropathy may be milder if each daily dose of E7010 is divided into two or three smaller doses.

The cytostatic effect of E7010 depends upon the actual amount of time it is in contact with the cancer cells, so it is desirable that the blood concentration be maintained at high levels. The plasma levels decreased relatively rapidly in the present study compared with preclinical findings. Therefore, it may be necessary to administer E7010 twice or three times daily in order to maintain a sufficient plasma level (Fig. 1). As mentioned above, peripheral neuropathy may not develop if E7010 is administered twice or three times -daily. Thus, it is important to carry out 5-day repeated-dose phase I/II studies using a twice-daily or three times -daily regimen in order to investigate the safety of this method of administration and dosage, and to evaluate the antitumor effect of E7010.

Since E7010 exhibited antitumor activity at a dose of 160 mg/m^2 (single administration), we believe that its activity would be observed more frequently for higher or 5-day-repeated doses. However, while antitumor activity was observed in one patient given a single dose of 480 mg/m^2 and in two patients given 5-day repeated doses of 120 mg/m^2 per day or 240 mg/m^2 per day, the activity was not as strong as expected. In the present

phase I study, E7010 was administered only in one course. The antitumor activity of tubulin-interacting agents such as Taxol [14, 15], Taxotere [16, 17] and Navelbine [18, 19] appears after repeating the course. Therefore, the antitumor activity of E7010 may be observed in more patients if the courses were repeated, for example as two 5-day repeated administrations with 3-weeks between.

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References

1. Yoshino H, Ueda N, Niijima J, Sugumi J, Kotake Y, Okada T, Koyanagi N, Asada M, Yoshimatsu K, Kitoh K (1992) E7010, a novel sulfonamide antitumor agent. I. Discovery and structure activity relationships. Proc Am Assoc Cancer Res 33: A3082
2. Yoshino H, Ueda N, Niijima J, Sugumi J, Kotake Y, Okada T, Koyanagi N, Asada M, Yoshimatsu K, Kitoh K (1992) Novel sulfonamides as potential, systemically active antitumor agents. J Med Chem 35: 2496
3. Koyanagi N, Nagasu T, Fujita F, Watanab T, Tsukahara K, Funahashi Y, Fujita M, Taguchi T, Yoshino H, and Kitoh K (1992) E7010, a novel sulfonamide antitumor agent. II. In vivo antitumor activity. Proc Am Assoc Cancer Res 33: A3083
4. Koyanagi N, Nagasu T, Fujita F, Watanab T, Tsukahara K, Funahashi Y, Fujita M, Taguchi T, Yoshino H, Kitoh K (1994) In vivo tumor growth inhibition produced by a novel sulfonamide, E7010, against rodent and human tumors. Cancer Res 54: 1702
5. Yoshimatsu K, Lijima A, Asada M, Kitoh K (1992) E7010, a novel sulfonamide antitumor agent. III. Mechanism of action and cell-kill kinetics. Proc Am Assoc Cancer Res 33: A3084
6. Furue H, Hara Y, Imai Y, Kimura T, Koyama Y, Kurihara M, Majima H, Nakao I, Niitani H, Ogawa M, Onoshi T, Saito T, Sakai Y, Sakano T, Sakuma A, Takaya O, Tominaga S, Yokoyama M, Japan Society for Cancer Therapy (1993) Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. J Jpn Soc Cancer Ther 28: 101
7. Casey EB, Jelliffe AM, Le Quesne PM, Millett YL (1973) Vincristine neuropathy: clinical and electrophysiological observations. Brain 96: 69
8. Rowinsky EK, Chaudhry V, Cornblath DR, Donehower RC (1993) Neurotoxicity of taxol. Monogr Natl Cancer Inst 15: 107
9. Wiemk PH, Schwartz EL, Strauman JJ et al (1987) Phase I clinical and pharmacokinetic study of taxol. Cancer Res 47: 2486
10. Sandler SG, Tobin W, Henderson ES (1969) Vincristine-induced neuropathy. A clinical study of fifty leukemic patients. Neurology 19: 367
11. Cavaletti G, Bogliun G, Marzorati L, Zincone A, Marzola M, Colombo N, Tredici G (1995) Peripheral neurotoxicity of taxol in patients previously treated with cisplatin. Cancer 75: 1141
12. Holmes FA, Walters RS, Theriault RL, et al (1991) Phase II trial of taxol, and an active drug in metastatic breast cancer J Natl Cancer Inst 83: 1797
13. Rowinsky EK, Burke PJ, Karp JE, et al (1989) Phase I and pharmacokinetic study of taxol in refractory acute leukemias. Cancer Res 49: 4640
14. Seewaldt VL, Greer BE, Cain JM, Figge DC, Tamini FK, Brown WS, Miller SA (1994) Paclitaxel (Taxol) treatment for refractory ovarian cancer: phase II clinical trial. Am J Obstet Gynecol 170: 1666
15. Seidman AW, Reichman BS, Crown JPA, Yao TJ, Currie V, Hakes TB, Hudis CL, Gilewski TA, Baselga J, Forsythe P, Lepore J, Marks L, Fain K, Souhrada M, Onetto N, Arbuck S, Norton L (1995) Paclitaxel as second and subsequent therapy for metastatic breast cancer: activity independent of prior anthracycline response. J Clin Oncol 13: 1152
16. Chevallier B, Fumoleau P, Kerbrat P, Dieras V, Roche H, Krakowski I, Azli N, Bayssas M, Lentz MA, Glabbeke V (1995) Docetaxel is a major cytotoxic drug for the treatment of advanced breast cancer: a phase II trial of the clinical screening cooperative group of the European Organization for Cancer and Treatment of Cancer. J Clin Oncol 13: 314
17. Fossella FV, Lee JS, Shin DM, Calayag M, Huber M, Perez-Soler R, Murphy WK, Lippman S, Benner S, Glisson B, Chasen M, Hang WK, Raber M (1995) Phase II study of docetaxel for advanced or metastatic platinum-refractory non-small-cell lung cancer. J Clin Oncol 13: 645
18. Depierre A, Lemarie E, Dabouis G, Garnier G, Jacoulet P, Dalphin C (1991) A phase II study of Navelbine (Vinorelbine) in the treatment of non-small-cell lung cancer. Am J Clin Oncol 14: 115
19. Gasparini G, Caffo O, Barni S, Frontini L, Testolin A, Guglielmi RB, Ambrosini G (1994) Vinorelbine is an active antiproliferative agent in pretreated advanced breast cancer patients: a phase II study. J Clin Oncol 12: 2094