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Phase I Study of Intravenous PSC-833 and Doxorubicin: Reversal of Multidrug Resistance

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PSC-833 reverses multidrug resistance by P-glycoprotein at concentrations ≤1000 ng/ml. A phase I study of PSC-833 and doxorubicin was conducted to determine the maximum tolerated dose and to investigate pharmacokinetics. PSC-833 was intravenously infused as a 2-h loading dose (LD) and a subsequent 24-h continuous dose (CD). Doxorubicin was infused over 5 min, 1 h after the LD. The starting dose was 1 mg/kg for both LD and CD with 30 mg/m^2 doxorubicin; these dosages were increased to 2 and 10 mg/kg and 50 mg/m^2, respectively. Thirty-one patients were treated. Nausea/vomiting was controllable with granisetron and dexamethasone. Neutropenia and ataxia were dose limiting. Steady-state concentrations of PSC-833 >1000 ng/ml were achieved at a 2 mg/kg LD and a 10 mg/kg CD. *Ex-vivo* bioassay revealed that activity in serum for reversing multidrug resistance was achieved in all patients; IC_{50} of P-glycoprotein expressing 8226/Dox_6 in patients' serum was decreased from 5.9 to 1.3 μg/ml ($P<0.0001$) by PSC-833 administration. Doxorubicin clearance was 24.3±13.7 (mean±SD) liter/h/m^2, which was lower than the 49.0±16.9 liter/h/m^2 without PSC-833 ($P<0.0001$). The relationship between doxorubicin exposure and neutropenia did not differ between patients treated and not treated with PSC-833. The recommended phase II dose of PSC-833 was 2 and 10 mg/kg for LD and CD, respectively, which achieved a sufficient concentration in serum to reverse drug resistance, as confirmed by bioassay. The dose of doxorubicin should be reduced to 40 mg/m^2, not because of the pharmacodynamic interaction between PSC-833 and doxorubicin affecting hematopoiesis, but because of pharmacokinetic interaction.

Key words: PSC-833 — Doxorubicin — Pharmacokinetics — Pharmacodynamics — Multidrug resistance

Multidrug resistance is a phenomenon by which cancer cells show cross-resistance to various classes of anticancer agents including anthracyclines, vinca alkaloids, epipodophyllotoxins, paclitaxel, and irinotecan.[1–3] P-Glycoprotein, a product of multiple drug resistance gene 1 (*mdr-1*), is responsible for a large part of multidrug resistance, and P-glycoprotein is expressed in clinical samples of tumors obtained from patients with a variety of malignancies, both treated and untreated with anticancer agents.[1,4] It is a membrane transporter and acts as an efflux pump that excretes various anticancer drugs from cancer cells. Inhibiting the function of P-glycoprotein may increase the sensitivity of cancer cells to anticancer agents and improve the efficacy of chemotherapy. P-Glycoprotein is also expressed in normal tissues, including the biliary canalicular membrane of hepatocytes and the brush-border membrane of renal tubular cells, and acts as an active efflux transporter excreting drugs into bile and urine.[5] Furthermore, it is expressed in brain capillary endothelial cells and functions as a blood-brain barrier.[6,7] Therefore, P-glycoprotein is important not only in the resistance of cancer cells to anticancer agents, but also in the pharmacokinetics of the drugs.

In vitro and *in vivo* experiments showed that a variety of agents have activity to reverse the multidrug resistance of cancers. These agents include verapamil,[8] nifedipine,[9] quinidine,[10] quinine,[11] cyclosporin,[12] tamoxifen,[13] and toremifene.[14] Among these P-glycoprotein inhibitors of the first generation, verapamil and cyclosporin were the most active and most often tested in clinical studies.[2,12,15,16] Although they showed some ability to abolish the function of P-glycoprotein in clinical studies, the results of these studies did not fulfil the promise of the preclinical data.[15,16] Multiplicity and redundancy of the resistance mechanisms of cancers might partly explain the disappointing results of these clinical studies, but the weak activity in reversing drug resistance and the toxicities of these drugs also might be responsible for the negative results.[15,17–19]

PSC-833 ([3′-keto-Bmt[1]]-[Val[2]]-cyclosporin, valspodar) is a non-nephrotoxic and non-immunosuppressive deriva-

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tive of cyclosporin D which can reverse the multidrug resistance caused by P-glycoprotein overexpression *in vitro* and *in vivo*. It was at least 10-fold more active than cyclosporin A on most P-glycoprotein-expressing tumor cell lines *in vitro*, and prolongation of survival times of mice bearing a multidrug-resistant tumor was documented *in vivo*.[20, 21] PSC-833 can completely inhibit P-glycoprotein of various cell lines *in vitro* at concentrations of 300 to 1000 ng/ml, and a target concentration of PSC-833 in clinical studies was considered to be 1000 ng/ml.[20, 21] Based on these data, clinical studies have been conducted using oral or intravenous administration of PSC-833 in combination with various anticancer agents.[22–26] Patients could tolerate the target concentration in these studies, and activity in patients' serum for reversing P-glycoprotein function was shown by the increased accumulation of daunorubicin in a P-glycoprotein-expressing cell line.[24] However, a large fluctuation of PSC-833 concentration was observed in studies using oral administration, with trough levels far below the target concentration,[22, 26] and activity in patients' serum to sensitize multidrug-resistant tumors has not yet been demonstrated. In this study, we conducted a phase I study of the intravenous infusion of PSC-833 in combination with doxorubicin to clarify the toxicity profile, to define the maximum-tolerated dose and the recommended dose, and to investigate the pharmacological profile of PSC-833 and doxorubicin in combination. We also demonstrated that serum of patients treated at the recommended dose of PSC-833 had sufficient activity to reverse the resistance to doxorubicin in a multidrug-resistant cancer cell line.

PATIENTS AND METHODS

Patients Eligibility criteria included patients with histologically or cytologically confirmed solid malignant tumors which were refractory to standard therapy or for which no effective therapy was available, who were 20 to 74 years of age, and had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of two or better. Prior chemotherapy and radiotherapy had to be completed at least 3 weeks (6 weeks for nitrosourea and mitomycin C) before entry into the study, and the total field of prior radiotherapy should encompass less than a third of hematopoietic bones. Patients should have a life expectancy of ≥8 weeks and adequate organ function, with leukocyte count ≥4000/mm^3 and <10 000/mm^3, hemoglobin ≥10.0 g/dl, platelet count ≥100 000/mm^3, creatinine ≤1.5 mg/dl, bilirubin ≤1.5 mg/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2× normal, creatinine clearance ≥50 ml/min, PaO_2 ≥70 mmHg, normal electrocardiogram, and left ventricular ejection fraction ≥50% as determined by ultrasonography. Patients who had been treated with a cumulative doxorubicin dose of ≥300 mg/m^2 and patients with a history of significant cardiovascular diseases were ineligible. Patients with an active infection, brain or leptomeningeal involvement, pregnancy, or a past history of central nervous diseases were also excluded from the study.

This phase I study was conducted in accordance with guidelines by the Ministry of Health and Welfare, Japan. Written informed consent according to institutional and regulatory requirements was obtained from all patients and the study was approved by the Institutional Review Board (IRB) of the National Cancer Center, Japan.

Treatment PSC-833 was administered as an intravenous infusion. A loading dose of PSC-833 was infused over 2 h in 100 ml of normal saline followed immediately by a continuous dose infused over 24 h in 500 ml of normal saline. Doxorubicin was dissolved in 20 ml of saline and intravenously infused over 5 min beginning 1 h after the end of the loading dose (1 h into the infusion of the continuous dose). The treatment was repeated every 3 weeks until disease progression or intolerable toxicity was observed.

Dose escalation was started at 1 mg/kg both for the loading dose and the continuous dose of PSC-833 in combination with 30 mg/m^2 of doxorubicin. In the first part of the dose escalation, PSC-833 dosages were increased with a fixed dose of doxorubicin (Table I). After the continuous dose was increased to 10 mg/kg at dose level VI, at which the target PSC-833 concentration of 1000 ng/ml was achieved in all patients, the dose of doxorubicin was increased by 10 mg/m^2 to 50 mg/m^2 with fixed doses of PSC-833. At least three patients were treated at one dose level and the dose was increased if none experienced dose-limiting toxicity. When dose-limiting toxicity was observed in one of three patients, the dose level was expanded to six patients. The dose escalation was continued until the maximum-tolerated dose was achieved at the point where two of the first three patients or two of the six patients experienced dose-limiting toxicity. The dose-lim-

Table I. Dose Levels

Dose level	Dose of PSC-833 (mg/kg)		Dose of doxorubicin (mg/m^2)	Number of patients
	Loading dose	Continuous dose		
I	1	1	30	4
II	1	2	30	3
III	2	4	30	3
IV	2	6	30	6
V	2	8	30	7
VI	2	10	30	3
VII	2	10	40	3
VIII	2	10	50	2

iting toxicities included grade 4 leukopenia and/or neutropenia lasting for 4 days or longer, grade 4 thrombocytopenia, and grade 3 or greater nonhematologic toxicity except for alopecia and hyperbilirubinemia. Hyperbilirubinemia was considered to be dose limiting when the serum bilirubin level was elevated to 10 mg/dl or higher. Intrapatient dose escalation was not performed.

Prophylactic anti-emetic drugs were not used until dose level IV. Three of the first four patients treated at dose level V developed grade 3 nausea or vomiting despite the prophylactic use of granisetron in two of those patients. Because of this observation, the protocol was amended to incorporate the intensive use of prophylactic anti-emetics. Granisetron 3 mg and dexamethasone 8 mg were administered intravenously twice a day on days 1 and 2. The use of granisetron and dexamethasone on days 3 to 5 was left to the discretion of attending physicians. PSC-833 is metabolized by CYP 3A4, and many pharmacokinetic interactions are known between drugs metabolized by CYP 3A4 and dexamethasone.[27] Because of the possible pharmacokinetic interaction between PSC-833 and dexamethasone, three patients were added to dose level IV to investigate the pharmacokinetics of PSC-833 and to confirm the safety of the combination of PSC-833 and dexamethasone. Further dose escalations were continued with the prophylactic use of granisetron and dexamethasone.

Monitoring Information with regard to a complete history, physical examination, and tumor measurement was recorded prior to the therapy. The baseline values of complete blood cell counts with differential and platelet counts were measured and measurements were repeated three times a week after treatment. Values for serum total protein, albumin, total bilirubin, total cholesterol, uric acid, urea nitrogen, creatinine, AST, ALT, alkaline phosphatase, γ-GTP, LDH and glucose were obtained prior to the therapy and repeated weekly after the treatment. Among blood chemistry studies, total bilirubin, AST, ALT, γ-GTP, LDH and alkaline phosphatase studies were repeated three times in the first week. ECG was performed prior to the treatment and on day 2 of each cycle. Ejection fractions were monitored with ultrasonography before each cycle of treatment. Toxicities were graded according to the common toxicity criteria and antitumor responses were assessed using the response criteria of ECOG.

Pharmacokinetics Blood sampling for determinations of pharmacokinetics of PSC-833 was conducted before and at the end of the loading dose infusion, at 1, 2, 4, 8, and 12 h into the continuous dose infusion, at the end of the continuous dose infusion, and 24 and 48 h after the end of infusion. For the pharmacokinetic study of doxorubicin, blood was drawn just before the administration of doxorubicin and 5 (end of infusion), 10, 20, and 35 min, and 1, 2, 3, 4, 7, 11, 23, 47 and 71 h after the start of the doxorubicin infusion. To investigate the effect of PSC-833 on doxorubicin pharmacokinetics, doxorubicin pharmacokinetics of study patients treated with PSC-833 were compared to those of control patients who did not receive PSC-833. The control patients were treated with doxorubicin as a single agent or in combination chemotherapy in a separate IRB-approved pharmacokinetic study. This control group consisted of 17 females and 9 males who were eligible for the phase I study of PSC-833 in combination with doxorubicin with regard to age, PS and hepatorenal function. Doxorubicin at doses ranging from 20 to 70 mg/m^2 was administered to the control patients by a 5-min infusion using the same schedule as the phase I study of PSC-833 and doxorubicin. Blood for studies of the pharmacokinetics of doxorubicin was obtained before doxorubicin administration, 5 (end of infusion), 15, and 30 min, and 1, 3, 4, 7, 24, 48 and 72 h after the beginning of the doxorubicin infusion.

PSC-833 concentrations in whole blood were determined using a radioimmunoassay kit (ANAWA Laboratorien AG, Zurich, Switzerland).[23] The assay for doxorubicin and its metabolite, doxorubicinol, was conducted by using, with slight modification, a high-performance liquid chromatography method which was described previously.[28] Pharmacokinetic analysis was performed using a non-compartment method. The elimination constant was calculated by linear regression of the terminal log-linear part of the time-concentration curves. However, the elimination constant of PSC-833 could not be determined due to the limited number of samples after the end of the infusion. The steady-state concentration of PSC-833 was calculated as the area under the time-concentration curve (AUC) from 1 h after the administration of doxorubicin to the last measured concentration of PSC-833 during the infusion divided by the time duration.

Pharmacodynamics For the pharmacodynamic analysis, the surviving fraction of neutrophils was calculated by dividing the neutrophil count at the nadir by the pretreatment count, and relationship between the AUC of doxorubicin and the surviving fraction was investigated. When the effect of the PSC-833 concentration on doxorubicin pharmacodynamics was evaluated, the relationship between the AUC of doxorubicin vs. the surviving fractions of neutrophils in the control patients treated with doxorubicin alone was compared to that of patients treated with doxorubicin and PSC-833.

***Ex-vivo* bioassay of activity for reversal of resistance** The activity of patients' serum in reversing the resistance to doxorubicin of a multidrug-resistant cell line was tested using an *ex-vivo* bioassay. For this purpose, blood was drawn before the PSC-833 infusion, just before the doxorubicin administration during the PSC-833 infusion, and at the end of the infusion of the continuous dose of PSC-833. The bioassay was performed using 8226/Dox_6, a doxorubicin-resistant cell line of human myeloma RPMI

8226.[29, 30] 8226/Dox_6 and RPMI 8226 were kind gifts from Dr. William S. Dalton (University of Arizona Cancer Center, Tucson, AZ). The method for the bioassay has been described in detail elsewhere (Uchiyama-Kokubu, N. *et al.*, in preparation). In brief, the cells were exposed to doxorubicin at various concentrations for 3 h in patients' serum. For the serum obtained at the end of the PSC-833 infusion, the amount of doxorubicin added to the serum was the difference between the plasma concentration of doxorubicin measured by HPLC and the final concentration required for the assay. We intended to perform the bioassay using whole serum, but the final serum concentration was 75% because of the volume of doxorubicin solution added. Cell-growth was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and an IC_{50} value reflecting the concentration of doxorubicin producing 50% inhibition of tumor growth was calculated. The activity for reversing doxorubicin resistance in patients' serum during treatment with PSC-833 was expressed as the dose-modifying factor, which was calculated by dividing the IC_{50} of doxorubicin in serum before the infusion of PSC-833 by the IC_{50} in serum during the infusion.

Table II. Characteristics of Patients

Total number of patients	31
Sex (female/male)	16/15
Age (median)	58
(range)	28–69
PS (0/1/2)	7/21/3
Diseases	
Breast cancer	8
Colorectal cancer	5
Gastric cancer	2
Non-small cell lung cancer	2
Ovarian cancer	2
Others	12
Prior therapy	
Surgery	22
Radiotherapy	13
Chemotherapy	29

RESULTS

Toxicities A total of 31 patients were treated with 75 courses of doxorubicin combined with PSC-833 (Table II). Before the entry into this study, all but two patients had received prior chemotherapy of 1 to 4 (median 2) regimens. Nineteen patients had received anthracyclines, and six other patients had been treated with other multidrug resistance-related anticancer agents, including vindesine and irinotecan.

At dose level V, grade 4 neutropenia was observed in three of seven patients, and a patient with carcinoma of the ampulla of Vater developed grade IV leukopenia, neutropenia and anemia complicated by liver abscess (Table III). This patient died from septic shock and disseminated intravascular coagulation 7 days after treatment. Neutropenia lasting for 4 days or longer was dose limiting in two patients treated at dose level VIII, which was deemed the maximum-tolerated dose. Thrombocytopenia was mild, and grade 3 thrombocytopenia was observed only in the patient who died from septic shock complicated by disseminated intravascular coagulation (Table III).

No grade 3 or greater nausea or vomiting was observed without prophylactic anti-emetics until dose level V, where three of the first four patients experienced grade 3 nausea or vomiting despite the prophylactic use of granisetron in two of these patients (Table IV). After the incorporation of intensive prophylaxis with dexamethasone (8 mg twice a day) and granisetron (3 mg twice a day), no grade 3 or worse nausea or vomiting was observed at any dose.

Table III. Hematological Toxicity

Dose level	No. patients	No. cycles	Grade															
			Leukopenia				Neutropenia				Thrombocytopenia				Anemia			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
I	4	16	0	3	1	0	0	3	0	0	0	0	0	0	1	3	0	0
II	3	3	0	2	1	0	0	2	1	0	0	0	0	0	3	0	0	0
III	3	6	1	0	1	0	1	0	1	0	1	0	0	0	1	2	0	0
IV	6	22	2	3	1	0	1	4	0	0	0	0	0	0	2	3	1	0
V	7	11	0	2	3	1	1	1	1	3	2	0	1	0	2	4	0	1
VI	3	4	0	1	2	0	0	0	3	0	0	0	0	0	1	2	0	0
VII	3	6	1	1	1	0	0	1	0	1	2	1	0	0	1	2	0	0
VIII	2	4	0	0	1	1[a)]	0	0	0	2[a)]	0	1	0	0	0	2	0	0

a) Dose-limiting toxicity.

Table IV. Nonhematological Toxicity

Dose level	No. patients	No. cycles	Grade																	
			Nausea				Vomiting				Anorexia				Hyperbilirubinemia				Ataxia			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
I	4	16	2	1	0	0	1	2	0	0	2	1	0	0	0	1	0	0	0	0	0	0
II	3	3	3	0	0	0	2	0	0	0	2	1	0	0	0	1	0	0	0	0	0	0
III	3	6	2	0	0	0	1	0	0	0	2	0	0	0	0	3	0	0	0	0	0	0
IV	6	22	1	1	0	0	1	3	0	0	0	2	0	0	0	3	0	0	0	0	0	0
V	7	11	0	2	2	0	0	2	1	0	1	2	2	0	0	0	1	1	0	0	0	0
VI	3	4	2	1	0	0	1	1	0	0	2	1	0	0	0	1	0	0	0	0	0	0
VII	3	6	1	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0
VIII	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1[a]	0

a) Dose-limiting toxicity.

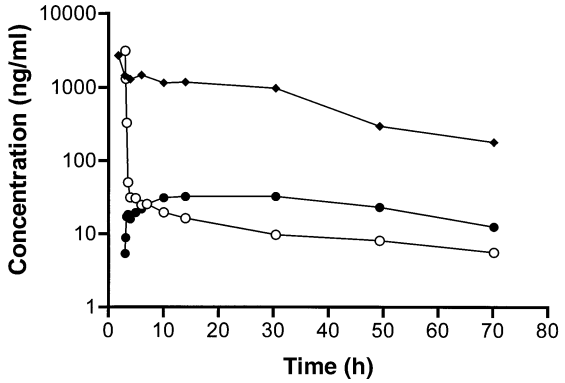

Fig. 1. Time-concentration curves of PSC-833, doxorubicin and doxorubicinol in a patient treated with a loading dose and a continuous dose of PSC-833 at 2 and 10 mg/kg, respectively, in combination with doxorubicin at 40 mg/m^2. ◆ PSC-833, ○ doxorubicin, ● doxorubicinol.

Dose-limiting grade 3 ataxia was observed at dose level VIII. Although grade 4 hyperbilirubinemia was observed in the patient who died from biliary infection, hyperbilirubinemia was transient and clinically insignificant in the other patients. Usually it was not associated with an elevation of transaminases.

An asymptomatic decrease of the ejection fraction was documented in a patient treated at dose level IV. The ejection fraction in the patient was 64% before treatment and decreased to 58% after the first course and to 49% 2 weeks after the second course; this value then returned to 58% in 3 weeks.

Pharmacokinetics Time courses of the concentration of PSC-833, doxorubicin and doxorubicinol in a representative patient treated at dose level VII are shown in Fig. 1. A stable concentration of PSC-833 during infusion of the continuous dose was achieved.

Table V. Pharmacokinetic Parameters of Doxorubicin and Doxorubicinol

Dose level	No. patients	PSC-833		Doxorubicin dose (mg/m^2)	Doxorubicin				Doxorubicinol		$\frac{AUC_{doxol}}{AUC_{dox}}$[e]
		LD[a] (mg/kg)	CD[b] (mg/kg)		AUC_t[c] (ng×h/ml)	Half life (h)	Clearance (liter/h/m^2)	V_{dss}[d] (liter/m^2)	AUC_t (ng×h/ml)	Half life (h)	
I–VI	26	1–2	1–10	30	1051±528	49.6±29.0[f]	24.8±15.0[f]	830±388[f]	1236±509[g]	35.0±15.2[h]	1.3±0.5[g]
VII	3	2	10	40	1197±54	51.8±1.8	24.4±1.2	1259±127	1946±1205	34.3±6.8	1.6±1.0
VIII	2	2	10	50	2207±425	41.7±0.4	18.5±3.5	727±150	4860±909	32.4±11.2	2.2±0.0
Total	31					49.2±26.4[i]	24.3±13.7[i]	867±380[i]		34.7±13.9[j]	1.4±0.6[k]
Control	2	0	0	20	378±167	35.2±40.0	41.5±29.8	894±921	369±1	37.6±4.0	1.1±0.5
Control	8	0	0	30	479±171	19.2±13.5[l]	56.0±24.3[l]	536±190[l]	363±194[l]	47.1±9.0[l]	1.1±0.5[l]
Control	3	0	0	40	802±172	28.9±8.4	38.4±7.4	1016±225	660±104	34.5±5.1	0.9±0.2
Control	9	0	0	50	819±177	29.8±9.9	48.1±10.2	1242±453	676±213	41.9±5.1	0.9±0.2
Control	4	0	0	70	904±500	31.8±13.7[m]	50.9±13.8[m]	1346±376[m]	1036±672	36.8±1.8[m]	1.3±0.1
Total	26					27.3±14.3[f]	49.0±16.9[f]	992±490[f]		41.5±7.3[f]	0.9±0.3[f]

Parameters are mean value±SD.

a) LD, loading dose; *b*) CD, continuous dose; *c*) AUC_t, area under the concentration-time curve to the last measured point; *d*) V_{dss}, distribution volume at the steady state; *e*) AUC_{doxol}/AUC_{dox}, the ratio of doxorubicinol AUC_t to doxorubicin AUC_t; *f*) data for 24 patients; *g*) data for 23 patients; *h*) data for 21 patients; *i*) data for 29 patients; *j*) data for 26 patients; *k*) data for 28 patients; *l*) data for 7 patients; *m*) data for 3 patients.

Pharmacokinetic parameters of doxorubicin and doxorubicinol are listed in Table V, together with control data (without PSC-833). Doxorubicin clearance in all patients treated with PSC-833 (24.3±13.7 liter/h/m^2, mean±SD) was significantly lower than that in patients treated without PSC-833 (49.0±16.9 liter/h/m^2, $P<0.0001$ by Mann-Whitney U test). Accordingly, when the AUC of doxorubicin was compared among patients treated with the same dose (30 mg/m^2), the AUC of doxorubicin in the presence of PSC-833 was two times greater than that of controls ($P=0.004$). The relationship between the steady-state concentration of PSC-833 and the clearance of doxorubicin is shown in Fig. 2. The doxorubicin clearance of patients treated without PSC-833 is plotted on the ordinate line. The doxorubicin clearance was decreased when the PSC-833 concentration was increased to around 500 ng/ml. The distribution volume of doxorubicin at the steady state did not differ with regard to combination with PSC-833, and the half life of doxorubicin in patients treated with PSC-833 was longer than that in the controls (49.2±26.4 vs. 27.3±14.3 h, $P=0.0007$).

The AUC and steady-state concentrations of PSC-833 were increased with dose escalations of PSC-833, and clearance was unchanged (Table VI). There were good linear relationships between the total dose of PSC-833 vs. AUC ($r^2=0.55$, $P<0.0001$) and between the continuous dose of PSC-833 vs. the steady-state concentration ($r^2=0.85$, $P<0.0001$). The steady-state concentration of PSC-833 in eight patients treated with 2 mg/kg loading dose and 10 mg/kg continuous dose (dose levels VI–VIII) ranged from 954 to 1927 ng/ml and exceeded the target concentration of 1000 ng/ml in all but one patient. Dexamethasone for the prophylaxis of emesis was incorporated into the treatment after 17 patients were treated. Because PSC-833 is metabolized by CYP 3A4,[27] possible pharmacokinetic interaction between PSC-833 and dexamethasone was sought but no difference in PSC-833 clearance was observed between patients who did and did not receive dexamethasone (0.26±0.11 liter/h/kg vs. 0.28±0.08 liter/h/kg).

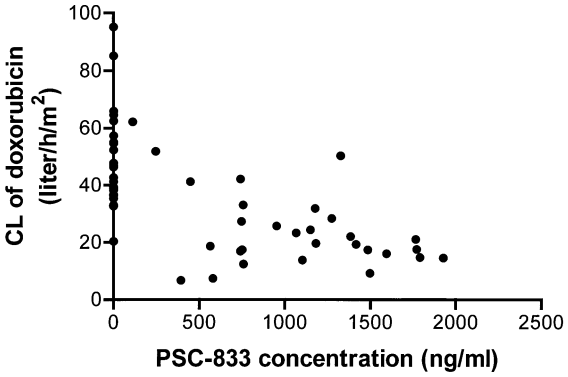

Fig. 2. Relationship between PSC-833 concentration and doxorubicin clearance. Data of doxorubicin clearance in control patients treated without PSC-833 are plotted on the ordinate line.

Pharmacodynamics There was a negative sigmoidal relationship with large variability between the surviving fraction of neutrophils and the AUC of doxorubicin (Fig. 3). Although neutropenia was dose limiting at 50 mg/m^2 of doxorubicin, which was lower than the conventional dose without PSC-833, the pharmacodynamic relationship between the doxorubicin AUC and neutropenia was not altered by combination with PSC-833. Comparison of neutropenia in the control patients who were treated with doxorubicin as a single agent to that in patients treated with doxorubicin and PSC-833 showed no difference in distri-

Table VI. Pharmacokinetic Parameters of PSC-833

Dose level	No. patients	PSC-833		Pharmacokinetic parameters			
		LD[a] (mg/kg)	CD[b] (mg/kg)	C_{max}[c] (ng/ml)	AUC_t[d] (μg×h/ml)	C_{ss}[e] (ng/ml)	Clearance (liter/h/kg)
I	4	1	1	1288±589	7.8±4.1	190±104	0.28±0.15
II	3	1	2	1088±359	26.7±11.6	474±96	0.18±0.03
III	3	2	4	2331±638	36.0±9.8	688±107	0.25±0.04
IV	6	2	6	2174±652	53.2±16.6	1147±328	0.24±0.08
V	7	2	8	1734±575	54.7±19.3	1277±434	0.29±0.11
VI–VIII	8	2	10	2495±1311	58.2±14.8	1383±352	0.32±0.08

Parameters are mean value+SD.
a) LD, loading dose; *b*) CD, continuous dose; *c*) C_{max}, the maximum concentration; *d*) AUC_t, area under the concentration-time curve to the last measured point; *e*) C_{ss}, steady-state concentration.

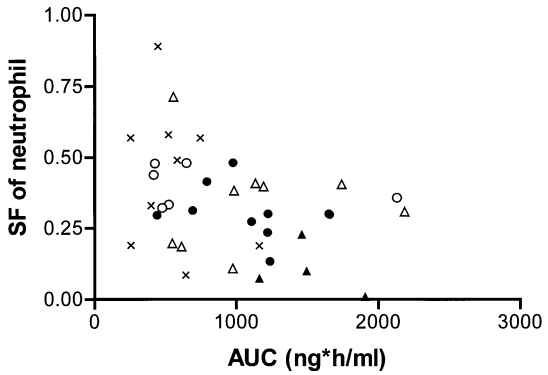

Fig. 3. Pharmacodynamic relationship between doxorubicin AUC and the surviving fraction (SF) of neutrophils according to the concentration of PSC-833. Data for the PSC-833 concentration of 0 ng/ml are from control patients treated with doxorubicin as a single agent. No difference in distribution in the scatterplot was observed by the addition of PSC-833 or by varying the concentration of PSC-833. PSC-833 concentration: × 0 ng/ml, ○ <500 ng/ml, △ 500–1000 ng/ml, ● 1000–1500 ng/ml, ▲ 1500–2000 ng/ml.

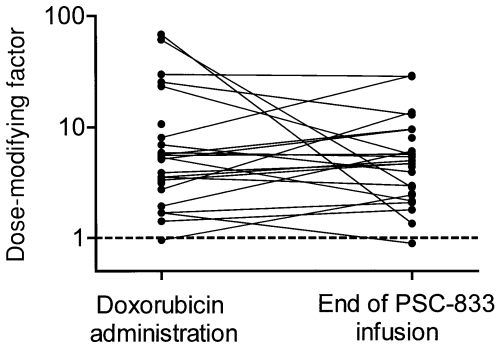

Fig. 4. Dose-modifying factors of patients' serum at the administration of doxorubicin and the end of PSC-833 infusion. Dose-modifying factors are the ratio of IC_{50} of doxorubicin in serum before the PSC-833 infusion to IC_{50} in serum during the infusion. Dose-modifying factors greater than unity indicate that sensitivity to doxorubicin of a multidrug-resistant cell line, 8226/Dox_6, is increased by PSC-833 in serum.

bution in the scatterplot between the AUC of doxorubicin vs. the surviving fraction of neutrophils (Fig. 3). Similarly, there was no difference in the pharmacodynamic relationship according to the concentration of PSC-833.

Bioassay The activity of patients' serum for reversing the resistance to doxorubicin of the P-glycoprotein-expressing 8226/Dox_6 cell line was investigated when sufficient samples were obtained. In the patients' serum before the administration of PSC-833, the median IC_{50} of the sensitive parent cell line, 8226S, was 0.5 μg/ml (0.2–1.4 μg/ml, n=28), while the median IC_{50} of the resistant cell line, 8226/Dox_6, was 5.9 μg/ml (1.2–19.3 μg/ml, n=28). The sensitivity of 8226/Dox_6 was increased to a median IC_{50} of 1.3 μg/ml (0.2–8.3 μg/ml, n=26) when patients' serum at the time of doxorubicin administration and during PSC-833 infusion was used (P<0.0001 by Wilcoxon signed-rank test, n=24). The increased sensitivity was maintained to the end of the PSC-833 infusion, at which point the median IC_{50} was 1.1 μg/ml (0.4–7.8 μg/ml, n=28). The IC_{50} value of doxorubicin in 8226/Dox_6 cells ranged from 1.2 to 19.3 μg/ml, suggesting that the IC_{50} value would vary from patient to patient. To correct the interpatient variability of the IC_{50} before treatment, a dose-modifying factor was calculated for each patient by dividing the IC_{50} in the serum before the PSC-833 infusion by the IC_{50} during the infusion. The dose modifying factor at the time of doxorubicin administration ranged from 1.0 to 68.2 (median 4.6, n=24). These values were unchanged at the end of the PSC-833 infusion (median 4.7, range 0.9–29.1, n=25, Fig. 4).

One partial response was observed at the highest dose level in a patient with ovarian cancer who had received doxorubicin prior to entry into this study.

DISCUSSION

This study showed that PSC-833 could be administered in combination with doxorubicin with acceptable toxicity. Neutropenia was dose limiting when the dose of doxorubicin was increased to 50 mg/m^2. The only clinically significant toxicity attributable to PSC-833 was grade 3 ataxia observed in a patient treated at the highest dosage. Isolated hyperbilirubinemia was observed throughout the dose escalation but was transient and clinically insignificant in all but one patient who had carcinoma of the ampulla of Vater and died from biliary infection complicated with grade 4 neutropenia. These toxicity profiles were in accordance with other studies of PSC-833 in combination with doxorubicin or other anticancer agents in which observed toxicities were essentially those of anticancer agents and in which neurological toxicities were observed only at very high dosages.[22–26] The inhibitory action of PSC-833 on hepatocyte transporters may be the principal mechanism for the hyperbilirubinemia. Bilirubin is excreted into bile from hepatocytes via membrane transporters which include multidrug resistance protein 2 (MRP2),[31, 32] and PSC-833 was reported to inhibit not only P-glycoprotein, but also MRP2.[33–35] Also, in previous reports, the inhibition of the transporters by PSC-833 and cyclosporin resulted in hyperbilirubinemia.[18, 19, 24] Similarly, the inhibitory action of PSC-833 on P-glycoprotein, which plays a key role as the blood-brain barrier,[36–39] may explain the ataxia noted in this and other studies.[24] This also might be

related to the observation in this study that the severity of emetic reactions to the combination of doxorubicin and PSC-833 was more than that to doxorubicin as a single agent without PSC-833 or in combination with other anticancer agents.

A decrease in the ejection fraction was observed in a patient after two courses of treatment, although it resulted in no symptoms and was clinically insignificant. He had not been treated with anthracyclines before entry into this study. The cumulative dose of doxorubicin was 60 mg/m^2 when the decrease of ejection fraction developed, suggesting that the combination of doxorubicin with PSC-833 might increase the cardiotoxicity of doxorubicin. Enhanced cardiotoxicity of doxorubicin as a result of P-glycoprotein modulation was suggested because the inhibition of P-glycoprotein function in mice by either the disruption of the *mdr1a* gene or the administration of PSC-833 increased the accumulation of doxorubicin and its cardiotoxic metabolite, doxorubicinol, in the heart.[40, 41] Cardiac functions should be carefully monitored when doxorubicin is combined with P-glycoprotein-modifying agents. However, the cumulative dose of doxorubicin that can be used in combination with PSC-833 could not be estimated in this study.

In vitro experiments suggested that a PSC-833 concentration of 1000 ng/ml was sufficient for reversal of the multidrug resistance of cancer cells,[20, 21] and we escalated the dose of PSC-833 to achieve a target concentration of 1000 ng/ml. The target concentration was exceeded through a steady-state concentration of 2 mg/kg loading dose and 10 mg/kg continuous dose of PSC-833. In this and other studies, a stable concentration was achieved by continuous intravenous infusion in contrast to oral administration, with which trough levels decreased to far below the target concentration.[22, 24, 26] When PSC-833 concentrations effective for the reversal of multidrug resistance from *in vitro* experiments are extrapolated to concentrations in patients, caution should be paid to the difference in protein concentrations between *in vitro* culture systems and human bodies. The usual concentration of serum for *in vitro* experiments is 10 to 15% while that in blood is 100%. Protein binding of PSC-833 in plasma was 97 to 98%, suggesting that most of the PSC-833 in blood might be pharmacologically inactive.[42] Actually, an 8-fold decrease in the inhibitory effect on drug transport by PSC-833 in tissue culture medium was observed when 100% fetal bovine serum was used instead of the usual 10% concentration.[43] In this study, however, we demonstrated that serum from patients treated with PSC-833 did increase the sensitivity to doxorubicin of the doxorubicin-resistant cell line. Although the expression of MRP2 or other transporters in the doxorubicin-resistant cell line was not extensively investigated, we believe that the inhibition of P-glycoprotein by PSC-833 was the main mechanism of reversal of the resistance to doxorubicin.

To seek pharmacokinetic interaction between doxorubicin and PSC-833, pharmacokinetic data for doxorubicin in combination with PSC-833 were compared to those of doxorubicin administered without PSC-833. The co-administration of PSC-833 decreased the systemic clearance of doxorubicin by 50%. Although this was not a crossover study, we selected control patients who fulfilled the eligibility criteria of this study with regard to age, PS, hepatic and renal function. There was no difference in these variables, as well as in sex, weight and body surface area, between the patients treated in this phase I study and the control patients (data not shown). Furthermore, the administration schedule of doxorubicin was the same in the two groups. Therefore, we believe that the control data were comparable to the data obtained in this study. A decrease in the clearance and an increase in the AUC of doxorubicin and other anticancer agents by the co-administration of PSC-833 also have been documented in other studies.[22–24, 26] P-Glycoprotein and other transporters are found at the biliary canalicular membrane of hepatocytes and excrete xenobiotics, and the inhibition of excretory pumps by PSC-833 may be the principal mechanism for the pharmacokinetic interaction.[44]

The recommended doxorubicin dose in combination with PSC-833 in this study was 40 mg/m^2, which was half of the conventional dose.[45] This was in accordance with the results of a dose-finding study of orally administered PSC-833 and doxorubicin where the doxorubicin dose was decreased to 35 mg/m^2.[22] P-Glycoprotein is also expressed in hematopoietic stem cells, and the possible potentiation of myelosuppression by PSC-833 had been a concern.[46] However, the reduction of the dosage of doxorubicin appears to result from the pharmacokinetic interaction between doxorubicin and PSC-833, not from the increased sensitivity of hematopoietic cells to doxorubicin. The combination with PSC-833 doubled the AUC of doxorubicin in this study and another study.[26] The 50% reduction of the doxorubicin dose corresponded to the change in the doxorubicin AUC in combination with PSC-833. Furthermore, the pharmacodynamic relationship between doxorubicin AUC and neutropenia was not changed by combination with PSC-833 (Fig. 3). The extent of neutropenia was not increased, as the concentration of PSC-833 was increased when the AUC of doxorubicin was taken into account.

Altered doxorubicin pharmacokinetics, increased central nervous system toxicities, and augmented emetic reactions indicated that pharmacological activity of PSC-833 could be achieved in patients. However, clinical benefits of PSC-833 depend on the selectivity of modulation. The extent of modulation in tumors should be greater than that in normal tissues. In recent preliminary reports of randomized trials, PSC-833 failed to improve complete remission rates or survival when used in combination chemotherapy against

leukemia.[47, 48] Further clinical studies should be conducted to determine if PSC-833 overcomes the drug resistance of other cancers or when used in other regimens.

In conclusion, PSC-833 at 2 and 10 mg/kg for the loading dose and the continuous dose, respectively, was safely combined with doxorubicin at 40 mg/m^2. Activity to reverse the resistance to doxorubicin was achieved in patients' serum at those doses of PSC-833. Because the addition of PSC-833 doubled the AUC of doxorubicin, the dosage of doxorubicin should be reduced accordingly, but no augmentation of neutropenia was observed when the change in doxorubicin pharmacokinetics was considered.

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