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Phase I and pharmacokinetics studies of prochlorperazine 2-h i. v. infusion as a doxorubicin-efflux blocker

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Abstract. In an earlier phase I study, we reported that the maximal tolerated dose (MTD) of prochlorperazine (PCZ) given as a 15-min i. v. infusion was 75 mg/m^2. The highest peak plasma PCZ concentration achieved was 1100 ng/ml. The present study was conducted to determine if PCZ levels high enough to block doxorubicin (DOX) efflux in vitro could be achieved and sustained in vivo by increasing the duration of i. v. infusion from 15 min to 2 h. The treatment schedule consisted of i. v. prehydration with at least 500 ml normal saline (NS) and administration of a fixed standard dose of 60 mg/m^2 DOX as an i. v. bolus over 15 min followed by i. v. doses of 75, 105, 135, or 180 mg/m^2 PCZ in 250 ml NS over 2 h. The hematologic toxicities attributable to DOX were as expected and independent of the PCZ dose. Toxicities attributable to PCZ were sedation, dryness of mouth, anxiety, akathisia, hypotension, cramps, and confusion. The MTD of PCZ was 180 mg/m^2. Large interpatient variation in peak PCZ plasma levels (91–3215 ng/ml) was seen, with the plasma half-life ($t_{1/2}\alpha$) being approximately 57 min in patients given 135–180 mg/m^2 PCZ. The volume of distribution (V_d), total clearance (Cl_T), and area under the curve (AUC) were 350.1 ± 183.8 l/m^2, 260.7 ± 142.7 l m^2 h^{-1} and 1539 ± 922 ng ml h^{-1}, respectively, in patients given 180 mg/m^2 PCZ and the respective values for patients receiving 135 mg/m^2 were 48.9 ± 23.76 l/m^2, 33.2 ± 2.62 l m^2 h^{-1}, and 4117 ± 302 ng ml h^{-1}. High PCZ plasma levels (> 600 ng/ml) were sustained in all patients treated with 135 mg/m^2 PCZ for up to 24 h. DOX plasma elimination was biphasic at 135 and 180 mg/m^2 PCZ, and a > 10-ng/ml DOX plasma level was maintained for 24 h. Partial responses were seen in three of six patients with malignant mesothelioma, in two of ten patients with non-small-cell lung carcinoma, and in the single patient with hepatoma. Our data show that PCZ can be safely given as a 2-h infusion at 135 mg/m^2 with clinically manageable toxicities. The antitumor activity of the combination of DOX and PCZ needs to be confirmed in phase II trials.

Key words: Drug efflux – Doxorubicin – Multiple drug resistance – Modulation – Prochlorperazine

Introduction

Rapid drug efflux may be a major mechanism for tumor cell resistance to a variety of natural products used in cancer chemotherapy [8, 11, 27]. The presence of the multiple drug resistance (MDR) gene and the gene product (170-kDa P-glycoprotein), which presumably acts as the putative efflux pump, has been described in a variety of normal and malignant cells [2, 4, 6, 12, 23]. It has been reasoned that if rapid drug efflux is the major mechanism by which resistant cells reduce cellular retention of a cytotoxic agent, then blocking of this efflux by noncytotoxic drugs may enhance chemosensitivity. Several unrelated drugs such as verapamil (VPL), cyclosporine, quinidine, thaliblastine, tamoxifen, and phenothiazines have been used in vitro and in vivo to block drug efflux [1, 5, 7, 10, 14, 17–20, 25, 28, 37].

Earlier studies have shown that phenothiazines such as chlorpromazine (CPZ) and trifluoperazine (TFP) are potent blockers of doxorubicin (DOX) efflux [10, 14–16]. We have extensively studied and reported on the efflux-blocking activity of the phenothiazines CPZ, TFP, and prochlorperazine (PCZ) in DOX-resistant P388 cells and in a variety of human tumor cells obtained from ascites and pleural fluid or after enzymatic disaggregation of solid tumor biopsies [14–16]. Coincubation of tumor cells with 1 μ*M* (318 ng/ml) CPZ or PCZ (a noncytotoxic dose by

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itself) and DOX will increase the cell kill by 10% as compared with that observed in cells incubated with DOX alone [14, 15]. In laser flow-cytometric analysis of cellular DOX fluorescence, coincubation with 10 μM CPZ will increase the peak fluorescence value of DOX-resistant cells by a factor of 3–5 [14–16]. Thus, it is logical to assume that for blocking of DOX efflux, plasma levels higher than 1 μM (318 ng/ml) will have to be achieved in vivo. In an earlier in vitro study, we reported that TFP (10 μM) enhanced DOX retention in human lung tumor cells that were insensitive to the DOX-efflux blocking action of verapamil [16].

PCZ is a potent phenothiazine with the advantages that it causes less neuropsychiatric toxicity than TFP and can be given i.v. It is an effective antiemetic [21] and may be used as an efflux blocker in vivo [34]. As it was important to determine if plasma levels of PCZ high enough to block DOX efflux in tumor cells could be achieved in vivo, we undertook earlier a phase I trial of escalating doses of PCZ given as a 15-min i.v. infusion followed by a standard dose of 60 mg/m^2 i.v. DOX [34]. Toxicities due to PCZ in this trial [34] were sedation, dryness of mouth, cramps, chills, and restlessness. By incorporating vigorous i.v. hydration, we were capable of reaching a higher maximal tolerated dose (MTD) of PCZ than that previously reported [21]. The MTD of PCZ in this trial [34] was 75 mg/m^2, with peak plasma levels ranging from 95–1100 ng/ml and the plasma half-life ($t_{1/2}\alpha$) being 20.9±5.3 min. From this study [34], we concluded that a combination of high-dose i.v. PCZ with DOX was safe and well tolerated and could possibly achieve short-term in vivo concentrations high enough to block DOX efflux. The present study was undertaken to define the MTD of i.v. PCZ given over 2 h and to determine if this schedule would allow us to sustain high plasma PCZ levels over a longer period.

Patients and methods

Clinical studies. The patients' eligibility criteria were similar to those used in our previous study [34], except that patients without a tumor accessible for repetitive analysis (e.g., pleural effusion) were also included. All patients with nonhematologic tumors judged to be refractory to standard therapies were eligible. Patients with breast, gastric, ovarian, and small-cell lung carcinomas, sarcomas, and lymphomas, were eligible only if prior DOX or DOX-containing combination chemotherapy had failed. Patients with other tumor types not previously treated with DOX were eligible. Other eligibility criteria were: a histologic/cytologic diagnosis of cancer, written informed consent, a WBC count of $>4 \times 10^9$/l, an absolute neutrophil count (ANC) of $>1.9 \times 10^9$/l, a platelet count of $>130 \times 10^9$/l, blood urea nitrogen (BUN) levels of <20 mg/dl, creatinine values of <1.2 mg/dl, normal hepatic function unless secondary to metastatic liver disease, and an age of over 18 years. Ineligibility criteria were: a myocardial infarction in the preceding 6 months or unstable angina; treatment with calcium channel blockers for cardiac disease or phenothiazines for psychiatric disorders; a normal left ventricular ejection fraction (LVEF) as determined by radionuclide multigated angiocardiography (MUGA) scan; a prior cumulative dose of DOX of >350 mg/m^2; bilirubin levels of >1.2 mg/dl; concurrent radiation therapy, chemotherapy, or hormonal therapy; and pregnancy.

All patients (see Table 1) were hospitalized to receive the first course of treatment. Hydration with normal saline (NS, i.v., 125 ml h^{-1}) was started the night before therapy. A fixed dose of DOX (60 mg/m^2) was given i.v. over 15 min followed by i.v. infusion of PCZ diluted in 250 ml NS over 2 h. Blood pressure was recorded pretherapy and every 30 min posttherapy for 4–6 h. If either the systolic or the diastolic blood pressure decreased >10 mmHg from the baseline value, 250 ml NS/h was infused until two consecutive hourly blood pressure (BP) recordings were similar to or higher than the baseline value. If a patient had a neuromuscular reaction, diphenhydramine (50 mg) was given i.m. or p.o. over 6 h as needed. Subsequent courses were repeated every 21 days. Administration of the next cycle of therapy was delayed if WBC ($<4.0\times10^9$/l) or platelet counts ($<100\times10^9$/l) were low and was begun only after full hematologic recovery. Patients who tolerated the first course well received the second and subsequent courses on an outpatient basis. Patients received at least 500 ml NS i.v. over 2 h before the start of therapy with DOX (15-min i.v. bolus) and PCZ given in 250 ml NS over 2 h. Following this, 500 ml NS was given i.v. over 2–4 h with careful monitoring of blood pressure. Dose modifications of DOX and PCZ in the second and subsequent courses were based on the clinical response and toxicity. The same level of PCZ was continued in subsequent courses if there was no grade III–IV nonhematologic toxicity and if stabilization or response of the tumor was seen. In patients with grade I or II toxicity (except alopecia, nausea, and vomiting) and in patients with grade I mucositis, identical doses of DOX were continued. In patients with mucositis (grades II–IV) or hematologic toxicity (grade III or IV), the subsequent dose of DOX was 15 mg/m^2 lower than the previous dose given. LVEF was assessed by radionuclide MUGA scan pretherapy and after every two courses.

PCZ dose escalation was based on a modified Fibonacci scheme (with 1n = 15 mg/m^2; 1n, 2n, and 5n were studied in the earlier phase I study [34], and doses of 5n, 7n, and 12n were used in the current study) and at least three patients were serially entered at each dose level. The starting dose of PCZ was 75 mg/m^2 on the basis of our previous study [34]. The MTD was defined as the PCZ dose that produced in three patients nonhematologic grade III–IV toxicity prohibiting additional dose escalation. Since standard toxicity criteria were not specific enough for the anticipated toxicities, the following new criteria for grade III–IV toxicities were developed as described earlier [34]. Dose-limiting toxicities were defined as follows: central nervous system – somnolent for >50% of waking hours or coma, extrapyramidal reactions lasting for >6 h, or severe intolerable extrapyramidal reactions; cardiovascular system – symptomatic hypotension and/or a fall of ≥30 mmHg in systolic BP, multifocal premature ventricular contractions or ventricular tachycardia, symptomatic cardiac dysfunction, or a >15% fall in LVEF; hepatic – ALT/AST/alkaline phosphatase levels of ≥5.1 times normal, bilirubin values of ≥2.6 times normal; and allergic – bronchospasm requiring parenteral therapy and/or anaphylaxis or noncompliance. No patient was entered at a subsequent dose level until at least one of three patients entered at the prior dose level had recovered from toxicity. The PCZ dose was escalated to the next dose level in patients with progressive disease who had grade 0–II nonhematologic toxicities and were agreeable to dose escalation. Progressive disease, noncompliance, a cumulative DOX dose of 550 mg/m^2, and/or a ≥15% decrease in LVEF from the baseline level and/or clinical cardiac toxicity were reasons for patients being taken off the study. The criteria for response have previously been described [32].

Reagents and drugs. DOX (Adriamycin hydrochloride, NSC-123127; Adria Labs, Columbus, Ohio) and prochlorperazine edysilate (PCZ; Smith Kline and Beecham Laboratories, Philadelphia, Pa.) were purchased.

Pharmacokinetics studies. Peripheral blood (10 ml) was collected in sodium citrate-coated tubes at 5 min before as well as 5 and 10 min after DOX treatment and at 5, 10, 20, 40, and 60 min and 2, 3, 4, 6, 24, 48, and 72 h after PCZ administration. Blood was prevented from coming in contact with the rubber stopper during collection and mixing, and plastic pipets were used to transfer plasma. The blood was centrifuged at 1500 g for 15 min, and plasma was frozen at –20° C in polystyrene tubes (Falcon, catalog number 2097) for subsequent analysis.

Extraction and quantitation of PCZ and DOX. PCZ was extracted from plasma by modification of the method reported by Smith et al. [29]. In brief, 1 ml plasma was added to an octyl solid-phase extraction column that had previously been conditioned with one column volume wash with each of phosphoric acid, sodium carbonate, methanol, and water. After sample addition, the column was washed with water, one column volume of 0.5 ml methanol, and one column volume of sodium carbonate. Analytes were eluted from the column with 1 ml methanol, the solvent was evaporated under nitrogen at 50° C, the residue was dissolved in 0.25 ml mobile phase, and 50-µl aliquots were injected into an LDC/Milton Roy high-performance liquid chromatography (HPLC) unit equipped with a BAS electrochemical detector. Column (octyl, 4.6×150 mm; Keystone Scientific, Inc., Bellefonte, Pa.) elution was performed at a flow rate of 1.5 ml/min with an isocratic mixture of 70% acetonitrile, 0.08% phosphoric acid, and 0.08% diethylamine. The mobile phase was adjusted to pH 2.3 and kept at 50° C. The column eluant was monitored for change in electrical potential at +0.85 V. CPZ was used as an internal standard. The sensitivity limit of PCZ estimation was 10 ng/ml.

DOX in plasma was extracted and quantitated by a modification of the method described by Robert [26]. In brief, 1 ml plasma was added to an octadecyl solid-phase extraction column that had previously been conditioned with 100% methanol; a 1 : 1 (v/v) mixture of methanol : water, and 50 m*M* sodium phosphate. After sample addition, the column was washed with sodium phosphate (50 m*M*). Analytes were eluted with 4 ml 2 : 1 (v/v) chloroform/methanol, the organic phase was evaporated under nitrogen gas at 50° C, and the residue was dissolved in 150 µl chloroform/methanol. Then, 50 µl of this solution was analyzed in an LDC/Milton Roy HPLC unit equipped with a Kratos Spectroflow 980 fluorescence detector. A VYDAC phenyl column (0.4×30 cm) was used for elution with an isocratic mixture of 0.1 *M* ammonium formate (pH 4.0) and acetonitrile (75 : 25, v/v) at a flow rate of 3 ml/min. The eluant was monitored for fluorescence emission at 550 nm using an excitation wavelength of 230 nm. The sensitivity limit for DOX estimation was 25 ng/ml.

Plasma concentrations of drugs were fitted into a computer program using weighted nonlinear least-square regression analysis to the function $c = Ae^{-\alpha t} + Be^{-\beta t}\ldots$, where c is the plasma drug concentration, t is the time after dosing, A and B are hybrid coefficients, and α and β are hybrid exponents. On the basis of standard pharmacokinetic methods [38], these coefficients and exponents were used to calculate the initial distribution half-life, the total volume of distribution at steady state, and the total volume of clearance. The area under the curve was calculated by trapizoidal rule and terminal elimination.

Statistical analysis. The data were analyzed using the BMDPAR statistical package [9]. The estimates were weighted inversely to the level of response. If convergence failed for any member of a dose group, then prior estimates and ridge values were calculated from the means and standard errors of the converged values. Convergence failed in ten cases because of unstable parameter estimates. A common approach, to "ridge" the estimates by supplying for each estimate a ridge value equal to the residual mean square error divided by an estimate of the parameters standard error, was followed. This value was then added to the diagonal of the matrix used in the iterative algorithm applied to estimate the parameters. In our use of the ridge method, estimates of the residual mean square, the estimated standard error of the parameter(s), and a prior estimate of the parameter were substituted. The ridge values and prior estimates were calculated from the averages of these values among dose-group members whose values converged to a stable solution. Pharmacokinetic parameters for both drugs were estimated from the BMDP output according to established procedures [9, 38]. Statistical tests on pharmacokinetic data were carried out using the Wilcoxon nonparametric rank-sum test. Statistical significance was set at $P < 0.05$.

Results

Clinical data

Table 1 lists the characteristics of the subjects entered into this study. Acute toxicities were evaluable in all patients. The dose of PCZ was escalated in one patient. Patient 1 was given 75 and 105 mg/m^2 PCZ. Patient 12, who died while on study, had no immediate toxicities during the first course of therapy with PCZ and DOX. On the 4th day posttherapy,

Table 1. Patients' characteristics

Patient number	Age (years), sex	Diagnosis	Prior surgery	Prior RT	Prior CT/IL2	PS
1	77 M	Lung adenocarcinoma	+	–	+ (D)	3
2	47 F	Breast cancer	+	+	+ (P)	3
3	76 M	Lung squamous carcinoma	–	+	+ (D)	3
4	62 M	Lung adenosquamous carcinoma	–	+	–	2
5	62 M	Peritoneal mesothelioma[a]	–	–	+	1
6	67 M	Lung adenocarcinoma	+	–	+ (P)	1
7	50 M	Lung adenocarcinoma	–	–	–	2
8	69 M	Pleural mesothelioma	–	–	– (P)	2
9	53 F	Lung adenocarcinoma	–	–	–	2
10	50 M	Renal cancer	+	–	IL-2	1
11	75 F	Hemangiopericytoma	+	+	+ (D)	1
12	53 M	Renal cancer	+	+	IL-2	1
13	48 M	Renal cancer	–	–	+	1
14	47 F	Pleural mesothelioma	–	–	–	1
15	74 M	Lung adenocarcinoma	+	+	+	3
16	66 F	Lung adenocarcinoma	–	+	+	4
17	58 F	Hepatoma	+	+	+	3
18	36 M	Lung adenocarcinoma	+	+	+	1
19	47 M	Renal cancer	+	+	IL-2	1
20	71 M	Pleural mesothelioma	–	–	–	1
21	63 M	Pleural mesothelioma	–	–	–	1
22	67 M	Pleural mesothelioma	+	–	–	1
23	38 M	Lung adenocarcinoma	–	–	–	3

CT, Chemotherapy; D, DOX; IL-2, interleukin 2; P, pirarubicin (4′*O*-tetrahydropyranyladriamycin); RT, radiation therapy

[a] Prior lymphoma treated with chlorambucil

Table 2. Hematologic toxicity encountered in the first course of 60 mg/m² DOX at each dose level of PCZ

PCZ dose (mg/m²)	Hemoglobin (g/dl)	WBC (×10⁹/l)	ANC (×10⁹/l)	Platelets (×10⁹/l)
75 (n = 3)	12.7 (0.2–14.9)	2.5 (1.9–2.9)	1.274 (0.551–2.523)	112.0 (36.0–171.0)
105 (n = 2)	11.8 (11.2–12.7)	3.1 (2.6–3.2)	0.788 (0.2–1.798)	198.0 (137.0–401.0)
135 (n = 15)	11.0 (7.3–14.6)	3.15 (0.7–17.6)	1.972 (0.070–15.136)	268.5 (31.0–703.0)
180 (n = 4)	12.7 (8.3–13.8)	2.9 (2.1–5.2)	1.133 (0.651–2.4)	315.0 (125.0–549.0)
All patients (n = 23)	11.2 (7.3–14.9)	2.3 (0.7–17.6)	1.274 (0.070–15.136)	251.0 (31.0–703.0)

Data represent median values; ranges are given in parentheses. WBC, White blood cell count; ANC, absolute neutrophil count

Table 3. Nonhematologic toxicities

PCZ dose (mg/m²)	Number of patients' courses	Sedation Grade 1 2 3 4	Urinary retention	Anxiety	Akathisia	Muscular cramps	Hypotension Grade 1 2 3 4	Nausea 1	Vomiting 1	Refused to continue
75	3	1 1 1 0	1	0	0	0	0 0 0 0	2	2	0
	4	2 1 1 0	1	0	0	0	0 0 0 0	2	2	0
105	3	3 0 0 0	1	0	0	0	0 0 0 0	0	0	1
	11	11 0 0 0	4	0	0	0	0 0 0 0	0	0	1
135[a]	15[b]	13 0 2 0	1	1	2	1	0 0 2 1	2	1	0
	42[b]	34 0 8 0	5	3	4	1	0 0 7 1	3	1	0
180	4[c]	3 0 1 0	0	1	3	1	0 0 1 0	2	2	2
	7[c]	6 0 1 0	0	2	4	3	0 0 1 0	2	2	2

[a] Patient 8 had received a prior cumulative dose of 470 mg/m² pirarubicin and, after showing a 64% left ventricular ejection fraction (LVEF) as determined by a MUGA scan pretherapy, developed congestive heart failure and an LVEF of 35% posttreatment with 135 mg/m² DOX + PCZ

[b] One patient had ear congestion and palpitation in all three courses

[c] One patient had ear congestion and palpitation and one patient each had dry mouth and weakness

he developed fever, chills, postobstructive pneumonia, a WBC of 2.8×10⁹/l, an ANC of 2.7×10⁹/l, a platelet count of 165×10⁹/l, and fatal respiratory arrest.

In Table 2, hematologic toxicities seen during the first course of PCZ administration are listed. The mean (and range) WBC recorded for patients at all PCZ dose levels was 2.3×10⁹/l (0.7–17.6×10⁹/l), with four grade I and ten grade II toxicities as well as one grade III and one grade IV toxicity being observed. The median (and range) ANC was 1.274×10⁹/l (0.07–15.13×10⁹/l). Two patients had thrombocytopenia. The WBC and ANC nadirs appeared independent of the PCZ dose used. There was no indication of cumulative hematopoietic toxicity. One of the patients who had earlier received 470 mg/m² pirarubicin had clinical cardiotoxicity manifesting as congestive heart failure. None of the other 22 patients had cardiotoxicity as determined by LVEF criteria or by clinical evaluation.

The nonhematologic toxicities were related to the PCZ dose delivered (Table 3). Three patients required administration of diphenhydramine for muscle cramps. Transient hypotension occurred in four patients and was corrected by the i.v. administration of NS. All toxicities except myelosuppression and alopecia were transient and reversed within 12–24 h. At 180 mg/m² PCZ, akathisia was noted after the first cycle in three of four patients treated. The dose of PCZ was reduced from 180 to 135 mg/m² in one patient (patient 21) due to grade III sedation and grade III hypotension. Two patients with akathisia refused subsequent therapy with PCZ. Although 180 mg/m² PCZ appeared to be a dose that could be safely given, the frequency of the toxicities observed prevented further escalation of PCZ in this schedule.

Table 4 lists the response to therapy and the survival of patients entered on this protocol. Three of six patients with malignant mesothelioma, two of ten patients with non-small-cell lung cancer, and one patient with a hepatoma had a partial response (PR).

Pharmacokinetics

The pharmacokinetic analysis of DOX and PCZ was carried out on plasma samples of patients given 135 or 180 mg/m² PCZ and 60 mg/m² DOX. The plasma concentration versus time curve generated for DOX in patients receiving 135 or 180 mg/m² PCZ is shown in Fig. 1. DOX elimination was biphasic at both PCZ doses. Figure 2 shows the plasma PCZ levels achieved in patients given 135 or 180 mg/m² PCZ by 2-h infusion. The highest peak plasma

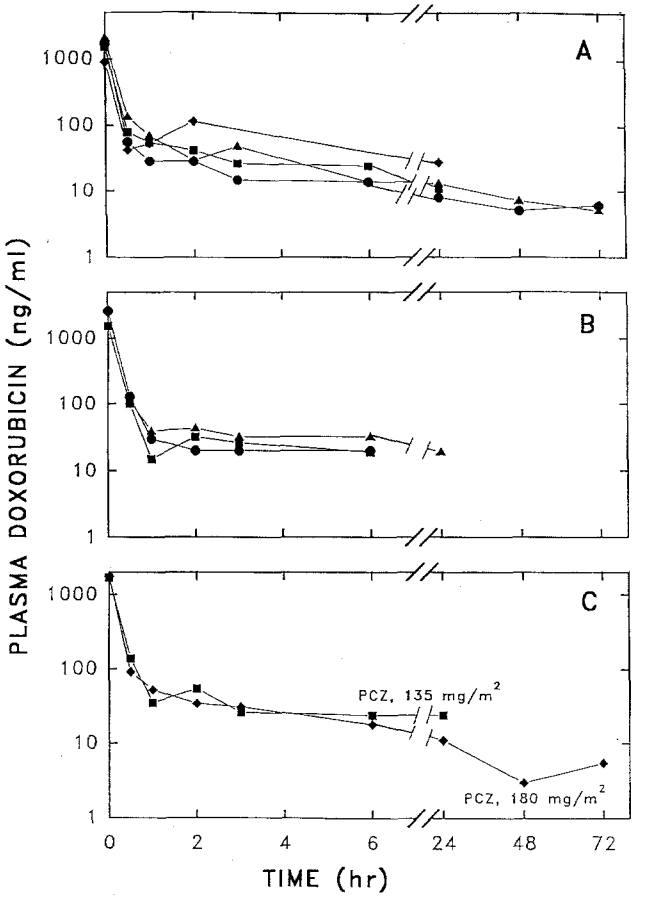

Fig. 1A–C. Plasma DOX concentration as a function of time. DOX (60 mg/m²) was given as a 15-min i.v. bolus followed by a 2-h i.v. infusion of PCZ at 135 (**A**) and 180 mg/m² (**B**). Identical symbols represent the plasma drug concentration of the same patient. **C** Computer-generated composite plasma DOX decay as determined after the administration of 135 and 180 mg/m² PCZ

level recorded was 3.215 μg/ml in a patient given 180 mg/m² PCZ.

The individual and composite mean values of PCZ and DOX for plasma half-lives ($t_{1/2}\alpha$), steady-state volume of distribution (V_d), total clearance (Cl_T), and area under the curve (AUC) are given in Tables 5 and 6. The data for both drugs were fitted into the two-compartment model. Significant differences in V_d, Cl_T, and AUC at the two doses of PCZ (135 and 180 mg/m²) were observed. However, due to the large interpatient variation observed at each dose and the small number of patients involved, no firm conclusion could be drawn. The important observation in this study was that plasma PCZ levels high enough to block DOX efflux in vitro were achieved in some of the patients on this protocol by administration of 135 and 180 mg/m² PCZ. In spite of the initially identical decay of PCZ, the elimination phase appeared to be slower for PCZ at 135 mg/m² (Fig. 2C) and the retention of PCZ was higher in patients treated at this dose. The average steady-state plasma concentration, C_α [38], for doses of 135 and 180 mg/m² PCZ were estimated at 1.32 and 0.32 μg/ml for DOX and 6.2 and 3.1 μg/ml for PCZ, respectively. The elimination half-life of PCZ was >24 h, but an exact value could not be estimated.

Table 4. PCZ dose, clinical response, and survival

Histology	Patient number	PCZ dose (mg/m²)	Number of courses	Best response[a]	Survival from PCZ therapy (months)
Breast cancer	2	75	1	PD	0.5
NSCLC	1	75	2	PD	15
	1[b]	105	3	PD	–
	3	75	2	PD	1.5
	4	105	2	PD	2.5
	6	135	3	SD	9.5
	7	135	2	PD	1.5
	9	135	6	SD	12
	15	135	1	PD	3
	16	135	1	PD	0.5
	18	135	3	PR (3)	15
	23	180	2	PR (5)	17
Meso-thelioma	5	105	8	PR (8)	43[d]
	8	135	2	SD	13
	14	135	1	SD	9.5[d]
	20	180	1	SD	7
	21	180	2	PR (4)	8
	21[c]	135	4	PR (4)	–
	22	180	2	PR (3)	9.5
Renal cancer	10	135	3	PD	9.5
	12	135	1	NE	0.1
	13	135	1	PD	1.5
	19	135	6	SD	38[d]
Hepatoma	17	135	5	PR (4)	8
Hemangio-pericytoma	11	135	1	PD	3

PD, Progressive disease; SD, stabilized disease; PR, partial response; NE, not evaluable

[a] The duration of response in months is indicated in parentheses

[b] Received 75 mg/m² in the first course

[c] 180 mg/m² PCZ in two courses resulted in grade II sedation and hypotension. The dose was reduced to 135 mg/m² in subsequent courses

[d] Patient was alive

Significant differences in DOX pharmacokinetics were observed in patients treated at the two different PCZ doses (135 and 180 mg/m²), suggesting that PCZ may alter the disposition of DOX (Table 6). Administration of 135 mg/m² PCZ resulted in higher plasma retention of DOX as indicated by the greater $t_{1/2}\gamma$ value (Fig. 1C), the low Cl_T value, the high V_d value, and the increased AUC value as compared with administration of 180 mg/m² PCZ (Table 6).

Discussion

The prolongation of survival in common adult cancers such as those arising in the lung, breast, and gastrointestinal tract may be feasible if rates of complete response (CR) can be increased [3, 4]. As one of the major reasons for the currently low CR rates may involve cellular resistance, the response to chemotherapy may be increased by overcoming cellular drug resistance [31]. Tumor cells expressing multidrug resistance (MDR) retain less DOX than sensitive

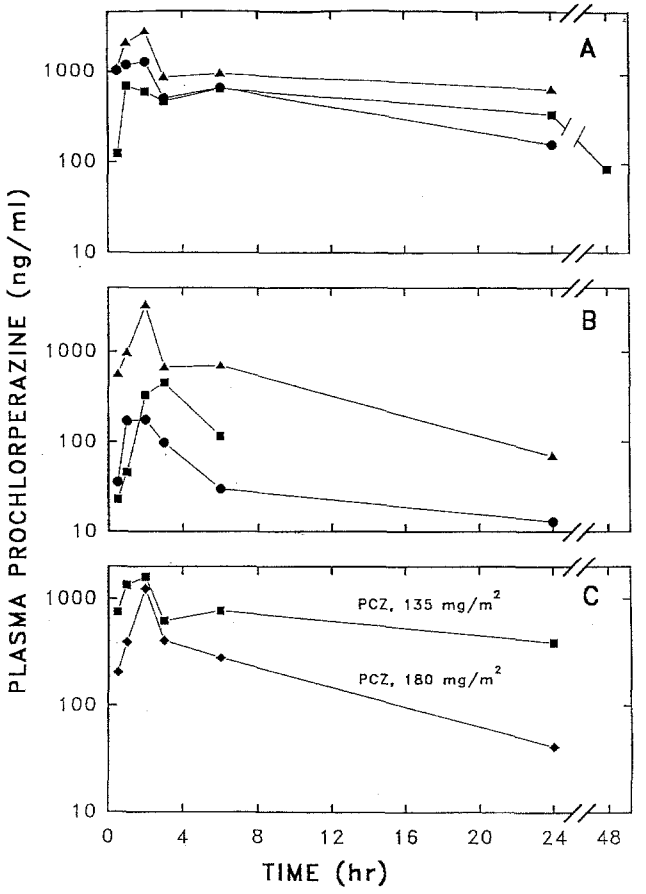

Fig. 2A–C. Plasma concentration of PCZ as a function of time. PCZ at 135 (**A**) and 180 mg/m² (**B**) was given as a 2-h i.v. infusion immediately after the administration of 60 mg/m² DOX as an i.v. bolus over 15 min. Identical symbols represent the plasma drug concentration of the same patient. **C** Computer-generated composite plasma PCZ decay as determined after the administration of 135 and 180 mg/m² PCZ

Table 5. PCZ pharmacokinetics[a]

PCZ dose	$t_{1/2}\alpha$ (min)	V_d[b] (l/m²)	Cl_T (1 m^{-2} h^{-1})	AUC (ng ml^{-1} h)
135 mg/m²:	104.2	96.3	38.4	3514
	30.2	22.1	30.4	4436
	38.4	38.2	30.7	4400
Composite (n = 3)[c]	57.6±23.4	48.9±23.7	33.2±2.6[d]	4117±302[d]
180 mg/m²:	54.1	534.4	695.0	337
	61.7	193.8	287.5	929
	52.4	53.7	67.7	3350
Composite (n = 3)[c]	56.1±2.8	260.7±142.7	350.1±183.7[d]	1539±922[d]

[a] All patients were given 60 mg/m² DOX
[b] At steady state
[c] Data represent mean values ± SE
[d] Statistically significant test, $P < 0.05$

cells, and MDR cells can be made to retain higher intracellular levels of DOX by coincubation with a number of noncytotoxic efflux blockers [10, 14, 22, 25, 28]. These laboratory observations provided the rationale for a number of clinical trials of efflux blockers reviewed earlier [34]. The use of verapamil (VPL) as a DOX-efflux blocker was reported to yield some PRs. One of the reasons for the low response rate may have involved the inability to achieve the requisite in vivo concentration of VPL due to its dose-limiting cardiac toxicity [7, 22]. Studies with cyclosporine as an efflux blocker of DOX [1], daunorubicin [18], and etoposide [19] have shown that plasma cyclosporine levels adequate for blocking efflux can be achieved. However, the cyclosporine combinations resulted in increased toxicities such as transient hyperbilirubinemia and myelosuppression. The latter may have been due to increased plasma levels of daunorubicin, etoposide, and DOX. Another reason for the increased myelosuppression noted with cyclosporine and DOX may have involved a 3-fold increase in the active metabolite doxorubicinol [1].

Our earlier studies suggest that PCZ may be more effective than VPL in blocking DOX efflux in vitro in common adult tumors such as lung and breast cancers [16]. The present study shows that increasing the duration of PCZ infusion from 15 to 120 min will result in the tolerance of doses of PCZ higher than those previously reported [21, 34]. The toxicity profile of a higher-dose 2-h i.v. infusion of PCZ is similar to that previously reported by us for bolus PCZ except that chills were not seen [34]. The rarity of extrapyramidal side effects (EPS) was a pleasant surprise and the reasons are unknown. EPS are common with prolonged oral therapy with phenothiazines, including PCZ, and may be due to dopamine depletion and/or dopamine receptor blockade in the brain. A single i.v. dose may not induce prolonged dopamine receptor blockade. Another possible explanation is that PCZ metabolite(s) rather than PCZ itself may cause EPS. Altered PCZ disposition is due either to massive dose escalation or to infused PCZ reaching the brain prior to metabolism in the liver and gut. Patient 17, who had developed EPS manifesting as torticollis after receiving oral PCZ as an antiemetic for prior chemotherapy, tolerated five courses of high-dose i.v. PCZ without developing EPS.

The lack of hepatotoxicity was not surprising, since PCZ-induced hepatotoxicity is idiosyncratic and not dose-related. The dose-limiting toxicities of PCZ were sedation and akathisia. In selected compliant patients, the MTD of i.v. PCZ may be ≥180 mg/m². There was noncompliance in two of four patients treated with PCZ at 180 mg/m², a dose level that resulted in lower sustained PCZ levels than did 135 mg/m². A potential difference in the pharmacokinetics of DOX at the two different PCZ doses would be almost impossible to determine, given the variability in drug levels seen, the small number of patients studied, and the observation that the PCZ levels were actually lower at the 180-mg/m² dose. Thus, in the absence of pharmacokinetics done on the same patients or on different groups of patients in the presence and absence of PCZ, it is impossible to tell whether or not there is any effect of PCZ on the metabolism of DOX.

Table 6. DOX pharmacokinetics[a]

Dose of PCZ	$t_{1/2}\alpha$ (min)	$t_{1/2}\gamma$ (h)	V_d[b] (l/m^2)	Cl_T (1 m^{-2} h^{-1})	AUC (ng ml^{-1} h)
135 mg/m^2:	5.5	22.4	876	27.2	2208
	6.5	38.5	1979	35.6	1685
	6.9	36.5	2155	40.9	1465
	5.3	19.8	1077	37.7	1592
Composite (n = 4)[c]	6.1 ± 0.3	29.3 ± 4.8[d]	1522 ± 319[d]	35.4 ± 2.9[d]	1737 ± 163[d]
180 mg/m^2:	4.7	2.4	618	176.0	337
	4.9	3.7	620	117.2	929
	6.0	2.4	352	102.1	3350
Composite (n = 3)[c]	5.2 ± 0.4	2.8 ± 0.4[d]	530 ± 89[d]	131.8 ± 22.5[d]	480 ± 73[d]
All patients (n = 7)	5.7 ± 0.3	17.9 ± 5.9	1097 ± 265	76.7 ± 21.3	1199 ± 270

[a] All patients were given 60 mg/m^2 DOX
[b] At steady state
[c] Data represent mean values ± SE
[d] The P values were ≤0.05 according to Student's t-test and were statistically significant

Dispite these limitations, the significant observation in this study was that a sustained plasma level of PCZ adequate to block DOX efflux was achieved in all patients treated with 135 mg/m^2. On the basis of the clinical toxicity profile and the pharmacokinetic data, we suggest 135 mg/m^2 PCZ with concurrent i. v. hydration as a dose that is safe in most patients and results in mild to moderate sedation. This PCZ dose may achieve and sustain PCZ levels of about 600 ng/ml for up to 24 h, which may be sufficient to block DOX efflux in vivo. Since the tissue PCZ levels may be higher than the plasma levels, potentially DOX-efflux-blocking levels of PCZ may be achieved in tumor tissue. In contrast to cyclosporine blocking, modulation of MDR with PCZ is feasible without causing hyperbilirubinemia [1, 19]. Transient sedation will occur after treatment with 135 mg/m^2 PCZ. However, the drug-induced antiemetic effects and sedation may be not only acceptable but, in most cases, highly desirable [13].

We noted activity (three PRs in six patients) in mesothelioma, a disease in which nearly half the tumors express P-glycoprotein [24]. There is a need for confirmation of our results by a phase II trial in mesothelioma [33]. We have also noted that neoadjuvant chemotherapy may be more effective than salvage chemotherapy in mesothelioma [32, 33] and lung cancer [31, 35], which raises the possibility that tumors recurrent after either resection or radiation therapy may also express MDR. Given these considerations, trials seeking to overcome MDR may be more effective when neoadjuvant chemotherapy rather than salvage therapy is used for terminal cancer patients. The low PR rate in non-small-cell lung cancer (NSCLC, two of ten patients) may be due to mechanisms of resistance other than that of MDR, for which PCZ as an efflux blocker may have no role. The inherent tumor heterogeneity that is well known in NSCLC [16] and multiple sites of metastases may also influence this outcome. Stage III (locoregional) NSCLC responds to chemotherapy [31, 35]. In contrast, stage IV (systemic metastases) NSCLC does not respond to identical chemotherapy [30], which is consistent with data indicating that MDR is influenced by the site of metastasis [36]. Liver tumor cells have high P-glycoprotein expression [36], and we observed a PR in a hepatoma patient. Hence, patients with primary and secondary hepatic tumors need to be studied with DOX-efflux-blocking combinations. Despite the limitations cited, the data presented herein can be used to design phase II trials of DOX and PCZ combinations in tumors such as mesothelioma, breast carcinoma, lung carcinoma, hepatoma, and ovarian carcinoma and to design phase I trials of i. v. PCZ in combination with cyclophosphamide, vincristine, etoposide, and DOX in small-cell lung carcinoma and in combination with DOX, vincristine, cyclophosphamide, and prednisone in lymphoma as well as combination of PCZ and DOX in lymphomas and leukemias. High-dose PCZ may also increase the antitumor effects of combinations using MDR-related drugs such as DOX, vincristine, and etoposide.

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