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Prochlorperazine as a doxorubicin-efflux blocker: phase I clinical and pharmacokinetics studies*

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Summary. Doxorubicin (DOX) efflux in drug-resistant cells is blocked by phenothiazines such as trifluoperazine (TFP) and prochlorperazine (PCZ) in vitro. The present phase I study was conducted in 13 patients with advanced, incurable, nonhematologic tumors to determine whether PCZ plasma levels high enough to block DOX efflux could be achieved in vivo. The treatment schedule consisted of prehydration and i.v. administration of 15, 30, 50, and 75 mg/m^2 PCZ followed by a standard dose of 60 mg/m^2 DOX. The hematologic toxicities attributable to DOX were as expected and independent of the PCZ dose used. Toxicities attributable to PCZ were sedation, dryness of the mouth, cramps, chills, and restlessness. The maximal tolerated dose (MTD) of PCZ in this schedule was 75 mg/m^2. Pharmacokinetic analysis indicated a large interpatient variation in peak plasma PCZ levels that ranged from 95 to 1100 ng/ml. The three plasma half-lives of PCZ were: $t_{1/2\alpha}$ ($\pm$SE), 20.9$\pm$5.3 min; $t_{1/2\beta}$, 1.8$\pm$0.3 h; and $t_{1/2\gamma}$, 21.9$\pm$5.3 h. The volume of distribution (V_d), total clearance (Cl_T), and area under the curve (AUC) for PCZ were 2254$\pm$886 l/m^2, 60.2$\pm$13.5 l m^{-2} h^{-1}, and 1624$\pm$686 ng ml^{-1} h, respectively. DOX retention in tumor cells retrieved from patients during the course of therapy indicated the appearance of cells with enhanced DOX retention. The combination of DOX and high-dose i.v. PCZ appeared to be safe, well tolerated, and active in non-small-cell lung carcinoma.

Introduction

Inherent or acquired tumor-cell resistance due to enhanced drug efflux may be partially responsible for failure of chemotherapy [15]. Several trials [3, 4, 6, 9–12, 14, 20, 22–24, 26, 30] using efflux blockers to overcome resistance have been reported (Table 1). One of the reasons for the low response rates observed in these trials may be the inability to achieve in vivo concentrations of the efflux blocker high enough to block the efflux of the chemotherapeutic agent. In the present study, we chose prochlorperazine (PCZ) as an efflux blocker because our earlier data had shown that PCZ would enhance DOX retention in human tumor cells, whereas verapamil had no effect [18]. The greater antiemetic potency [7, 8, 25] and reduced antipsychotic and neurologic toxicities of PCZ as compared with trifluoperazine (TFP) also suggested the former as an excellent choice for blocking DOX efflux.

Patients and methods

Clinical studies. The eligibility criteria in general were similar to those used in our previous study [28]. However, in the present study, only patients with tumor accessible for repetitive analysis (pleural effusion) were included. All patients (Table 2) were hospitalized to receive the first course of treatment. Hydration with i.v. normal saline (NS) at 125 ml/h was started the night before PCZ therapy. PCZ was diluted in 50–100 ml NS and given i.v. over 15 min, followed by i.v. infusion of 60 mg/m^2 DOX for 15 min. If a patient had a neuromuscular reaction, diphenhydramine (50 mg) was given i.m. or p.o. every 6 h as needed. Subsequent courses were repeated every 21 days after i.v. prehydration with at least 500 ml NS over 2–4 h. Administration of the next cycle of therapy was delayed if WBC (<4,000/μl) or platelet counts (<100,000/μl) were low and was begun only after full hematologic recovery. Dose modification of DOX in the second and subsequent courses was based on clinical response and toxicity. In patients showing an objective response or clinical improvement associated with grade I or II toxicity (except alopecia, mucositis, nausea, and vomiting), identical doses of DOX and PCZ were continued. In patients developing mucositis (grade II–IV) or hematologic toxicity (grade III or IV), the subsequent dose of DOX was 15 mg/m^2 lower than the previous dose.

PCZ dose escalation was based on a modified Fibonacci scheme, and at least three patients were serially entered at each dose level. The

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Table 1. Clinical trials of drug-efflux blockers

Treatment		Efflux blocker	Dose	Tumor type	*n*	Response		Dose-limiting toxicities	Reference
Chemotherapy	Dose					PR	CR		
Vinblastine	1.5 mg/m^2 CI × 96 h	Verapamil	0.02 – 0.1 mg/kg loading dose, CI 0.036 – 0.18 mg kg^{-1}h^{-1} × 132 h	Nonhematologic tumors	17	0	0	Cardiac arrhythmia	[3]
Doxorubicin	50 mg/m^2 CI × 24 h	Verapamil	3rd day 0.15 µg/kg loading dose, 5 – 18 µg/kg/min × 3 days	Ovarian cancer	8	0	0	Cardiac arrhythmia	[22]
VAD	[a]	Verapamil	0.1 µg/kg loading dose, CI 0.15 – 0.45 mg/kg	NHL Multiple myeloma	8	3	0	Hypotension, cardiac arrhythmia	[10]
Doxorubicin	50 mg/m^2 bolus	Verapamil	120 – 240 mg q6h p.o. × 2 days	Nonhematologic tumors	13	1	0	Hypotension, cardiac arrhythmia	[24]
Vindesine	2 – 5 mg i.v.	Verapamil	80 mg p.o. t.i.d.	Nonhematologic tumors	12	3	0	Neurotoxicity, hypertension	[6]
Vincristine	1.5 mg/m^2 i.v.	Diltiazem	1.69 – 4.83mg kg^{-1}day^{-1}	ALL	6	1	0	Cardiac arrhythmia	[4]
Etoposide	100 – 200 mg/m^2 i.v. days 1,2 400 – 600 mg/m^2 p.o. days 3,4	Nifedipine	40 – 80 mg p.o. b.i.d.	Nonhematologic tumors	10	0	0	Hypotension, headache, dizziness	[23]
Doxorubicin	60 mg/m^2 CI × 96 h	Trifluoperazine	20 – 100 mg/day p.o., days 1 – 6	Nonhematologic tumors NHL, ANLL	36	6	1	Extrapyramidal	[20]
Bleomycin	30 units i.v., day 3	Trifluoperazine	3 – 12 mg p.o. b.i.d.	Nonhematologic tumors	17	2	2	Neuropsychiatric; and fatal interstitial pneumonitis in 1	[12]
Cisplatin	10 – 100 mg/m^2	Prochlorperazine	8 – 20 mg/h × 24 h	NA	28	NA	NA	Neuropsychiatric, dizziness, dry mouth	[9]
Epirubicin Vinblastine	90 mg/m^2 i.v. 0.1 mg/kg i.v.	Cyclosporin A	3 mg/kg i.v.	Nonhematologic tumors	24	1	0	Nausea-Vomiting	[30]
Vinblastine	2 mg/m^2 CI × 108 h	Cyclosporin A	1 – 6 mg kg^{-1}day^{-1} CI × 120 h	Nonhematologic tumors	27	NA	NA	Hypomagnesemia, hyperbilirubinemia	[26]
Doxorubicin Vincristine	50 mg/m^2 i.v. 1 mg/m^2 i.v.	Verapamil Tamoxifen	360 – 480 mg/day p.o. 80 – 100 mg/day p.o.	Small-cell lung cancer	58	20	14	Cardiac arrhythmia	[11]
Etoposide Epirubicin	100 mg/m^2 i.v. × 3 days 100 mg/m^2 i.v.	Tamoxifen Quinidine	80 – 100 mg/day 250 – 1000 mg p.o. b.i.d.	Breast cancer	31	NA	NA	Cinchonism	[14]

[a] VAD (vincristine, 0.4 mg/day × 4 days; DOX, 9 mg/m^2/day × 4 days; dexamethasone, 40 mg/day × 4 days; *n*, Number of patients evaluable; PR, partial response; CR, complete response; CI, continuous i.v. infusion; NA, not available; NHL, non-Hodgkin's lymphoma; ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia

Table 2. Patients' characteristics and course

Patient number	Age (years)/ sex (M/F)	PS	Primary tumor	Prior therapy			PCZ dose (mg/m^2)	Nadir in I cycle[a]			Number of courses	Best resp.	Time in weeks to PD	Survival[b] (months)
				CT	DOX	XRT		Hb	WBC	Plt				
1	70/F	3	Breast cancer	Yes	Yes	Yes	15	7.9	0.4	66	1	PD	3	1
2	51/M	3	Colon cancer	Yes	Yes	Yes	15	NA	NA	NA	1	PD	4	NA
3	80/F	3	NSCLC	No	No	No	15	12.5	3.0	423	2	PD	8	6
4	67/F	4	NSCLC	Yes	No	No	30	9.8	1.3	393	1	PD	3	2
5	65/F	4	NSCLC	No	No	No	30	11.7	3.0	376	4	PR	14+	8
6	71/M	3	Mesothelioma	No	No	No	30	12.6	2.4	238	1	PD	3	2
7	47/F	4	SCLC	Yes	Yes	Yes	50	12.2	1.6	205	2	PD	5	3
8	76/M	2	NSCLC	Yes	Yes	Yes	50	10.2	1.9	171	1	PD	3	2
9	65/M	2	Pancreas	Yes	No	Yes	50	11.6	1.6	607	2	PD	4	2
10	49/F	4	NSCLC	No	No	No	75	8.1	1.4	541	2	PD	4	3
11	62/F	3	NSCLC	Yes	No	Yes	75	7.8	3.0	243	1	NE	NA	2
12	75/F	1	NSCLC	No	No	Yes	75	8.7	0.7	135	9	IMP	32	11
13	55/F	3	Colon cancer	Yes	No	No	75	12.2	2.6	205	2	NE	NA	10

PS, performance status; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; CT, chemotherapy; XRT, radiation therapy; PD, progressive disease; PR, partial response; IMP, improvement; NE, not evaluable

[a] Hg, mg/dl; WBC and Plt (platelets), × 10^9/µl

[b] Survival from DOX + PCZ therapy

Table 3. Nonhematologic toxicities encountered after one course[a]

	PCZ dose (mg/m^2)			
	15 (n = 3 patients)	30 (n = 3 patients)	50 (n =3 patients)	75 (n =4 patients)
Nausea	1	0	1	0
Vomiting	1	0	1	0
Hypotension of ≥10 mmHg	0	0	1	1
Chills	0	0	1	2
Sedation:				
Grade I–II	0	3	3	3
Grade III	0	0	0	1
Confusion	0	0	1	0
Dryness of mouth	0	0	1	2
Muscle spasm	0	0	0	1
Noncompliance	0	0	0	2

[a] The cumulative DOX dose and corresponding decrease in LVEF in 3 patients were as follows: patient 12, 270 mg/m^2 and 20%; patient 5, 285 mg/m^2 and 25%; and patient 7, 390 mg/m^2 and 15%

starting dose of PCZ was 15 mg/m^2. The MTD was defined as the PCZ dose that produced nonhematologic grade III–IV toxicity prohibiting additional dose escalation in three patients. Dose-limiting toxicities were defined as follows: central nervous system – somnolence 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 blood pressure, multifocal premature ventricular contractions or ventricular tachycardia, symptomatic cardiac dysfunction, a decrease of >15% in the left ventricular ejection fraction (LVEF); hepatic – alanine aminotransferase/aspartate aminotransferase/alkaline phosphatase values of ≥5.1 times normal, bilirubin levels of ≥2.6 times normal; allergic – bronchospasm requiring parental 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. PCZ dose escalation was not done in the same patient. The same level of PCZ was continued in subsequent courses if there was a lack of grade III–IV nonhematologic toxicity, if stabilization or response of the tumor occurred, and if the total dose of DOX had not exceeded 550 mg/m^2 (450 mg/m^2 in patients with prior mediastinal radiation therapy). Progressive disease (PD), noncompliance, a cumulative DOX dose of 550 mg/m^2, and a decrease of ≥15% in LVEF from baseline levels and/or clinical cardiac toxicity were reasons for patients' being taken off the study. Criteria for response were standard [28], except for patients with only evaluable cancer, in whom improvement was defined as a definite decrease in tumor size as agreed upon by a minimum of two observers.

Reagents and drugs. Propidium iodide (PI) and RNase type 1 A (Sigma, St. Louis, Mo.), Adriamycin hydrochloride (DOX, NSC-123 127; Adria Labs, Columbus, Ohio), daunorubicin hydrochloride (Cerubidine, DNR, NSC-821 151; Wyeth Labs, Philadelphia, Pa.), prochlorperazine edysilate (PCZ), and chlorpromazine hydrochloride (CPZ), Smith Kline and Beecham Labs, Philadelphia, Pa.) were purchased.

Laboratory studies. Cells from pleural effusion were recovered by centrifugation for 10 min at 200 g and layered on a 70% preformed Percoll (Pharmacia, Piscataway, N. J.) gradient. Cells were recovered by centrifugation at 400 g for 20 min. Cytospin preparations before and after gradient separation were made in a Shandon Cytospin tabletop centrifuge (Shandon Inc., Pittsburg, Pa.), fixed in a fresh solution of methanol: acetic acid (3 : 1, v/v), and stained by the regressive Papanicolaou staining procedure. For cellular DOX-retention studies, a laser flow-cytometric procedure previously reported by us was used [17]. The PI/hypotonic citrate method [16] was used for determination of cellular DNA content (ploidy and cell-cycle distribution). Peripheral blood (10 ml) collected in sodium ethylenediaminetetraacetate (EDTA)-coated tubes was centrifuged at 200 g for 15 min, and plasma was frozen in plastic tubes for subsequent analysis. For extraction and quantitation of PCZ, the method described in BAS application note 41 (Bioanalytical Systems, Inc., West Lafayette, Ind.) was followed. DOX in plasma was extracted and quantitated by high-pressure liquid chromatography [28, 29].

Statistical analysis. Pharmacokinetic analyses included all data measured between time 0 and 48 h. Missing time points were estimated using the G3GRID procedure in the SAS Graphics package (SAS/Graph Software, 1990; SAS Institute, Inc., Cary, N. C.). The data were ordered by highest initial value and interpolation was performed on the surface of response by time and order; the JOIN interpolation option was chosen since it constrains interpolated values to lie within the range of the initial values. Five data points were estimated for the PCZ data set and seven were estimated for the DOX data set. Interpolated data were analyzed using the NLIN procedure in the SAS statistical package (SAS/STAT Users Guide, Release 6.03 Edition, 1988; SAS Institute, Inc., Cary, N. C.). The Marquardt method was used, with estimates being weighted inversely to the level of the response. Pharmacokinetic parameters for both drugs were estimated from the NLIN output according to an established procedure reported earlier [27].

Results

Clinical toxicity and course

Acute toxicities were evaluable in all patients (Table 3). Patient 10 tolerated the first course, and the second course was followed a day later by a seizure and a fatal respiratory arrest. An autopsy confirmed lung carcinoma with extensive metastases in the meninges and brain. Nadir blood counts after the first course were available in 12 patients; after a dose of 60 mg/m^2 DOX, the median (range) WBC was 1600/μl (400–3000/μl), with 2 grade I, 2 grade II, 5 grade III, and 2 grade IV toxicities. The median (range) absolute granulocyte count (AGC) was 680/μl (0–1716/μl). Only one patient had thrombocytopenia. The WBC and AGC nadirs appeared independent of the PCZ dose used. The median (range) WBC counts in irradiated and nonirradiated patients were 1600/μl (400–3000/μl) and 2500/μl (1300–3000/μl), respectively. Both episodes of grade IV leukopenia occurred in irradiated patients. Six patients received the second course, two patients each received the third and the fourth courses, and one patient received nine courses (Table 2). There was no indication of cumulative hematopoietic toxicity. None of the patients had clinical cardiotoxicity; however, cardiac toxicity by radionuclide LVEF criteria [1] was noted in three patients (Table 3), in two of whom cardiotoxicity was the basis for removal from the study. These two patients received cumulative doses of DOX lower than the standard recommended maximal doses but higher than the doses found to be cardiotoxic in some patients. The nonhematologic toxicities were related to the PCZ dose given (Table 3). Three patients required administration of diphenhydramine for muscle spasms. Hypotension responded to administration of i. v. NS. Chills were associated with hypotension in two patients. All toxicities were transient and reversed within 12–24 h.

At 75 mg/m^2 PCZ, multiple adverse reactions (grade III sedation, hypotension, chills, and muscle spasms) were

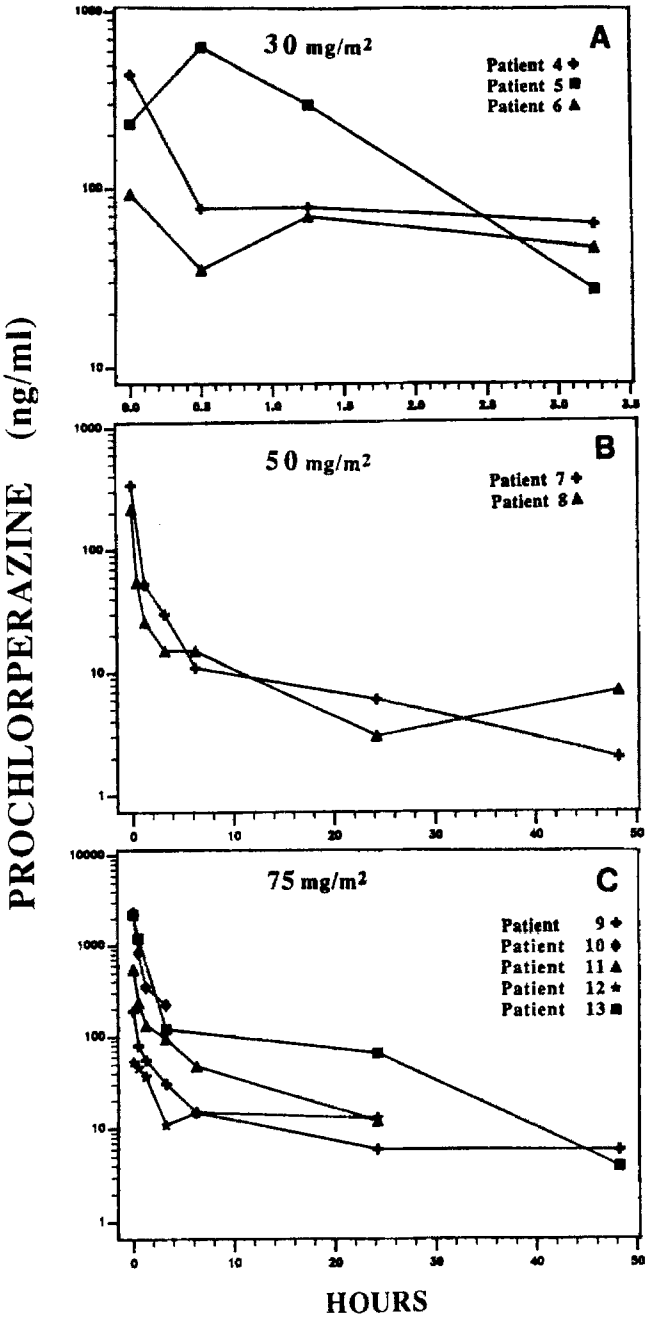

Fig. 1 A–C. Plasma levels of PCZ in patients given **A** 30, **B** 50, or **C** 75 mg/m² as a 15-min i. v. infusion. Pt 9 received 50 mg/m²

noted after the first cycle in two of the four patients treated. The other two patients tolerated the therapy, showing only grade I/II sedation. In addition, two patients who experienced grade III (patient 13) and grade I sedation (patient 11) refused subsequent therapy. Although 75 mg/m² PCZ appeared to be a dose that could safely be given, the frequency of adverse reactions and noncompliance prevented further escalation of PCZ in this schedule.

All 13 patients underwent thoracocentesis prior to therapy and at least once posttherapy. In addition, paracentesis was done in one patient before therapy. Inspiration and expiration chest X-rays were performed after each thoracocentesis procedure. There were two episodes of pneumothorax in two patients. One of them was symptomatic and required the placement of a chest tube. Tetracycline sclerosis was done before removal of the chest tube. The pneumothorax resolved without chest-tube placement in the second patient. Of the remaining 11 patients, elective chest-tube sclerosis was required in only 1. Patient 13 had a decrease in ascites and survived for 10 months. Two subjects (patients 5 and 12) showed tumor regression after two and four courses of therapy, respectively, associated with symptomatic relief and control of malignant effusions.

Pharmacokinetics

The plasma levels of PCZ achieved after 15-min i. v. infusions of 30, 50, and 75 mg/m² PCZ are shown in Fig. 1. The mean (± SE) peak plasma concentrations achieved after the administration of 30, 50, and 75 mg/m² PCZ were 393 ± 161, 278 ± 61, and 1151 ± 474 ng/ml, respectively. There was a large interpatient variation in the peak plasma levels achieved, and the coefficient of variation was 22%–40%. In patient 5 (Fig. 1 A), there was a marked increase in the plasma PCZ level measured at 30 min. A similar phenomenon (erratic initial distribution phase) has been described by Olver et al. [21]. The plasma-decay curves showed a gradual drop in PCZ levels within the first few hours of drug administration, and at between 10 and 50 h a plasma level ranging between 2 and 100 ng/ml was maintained (Fig. 1 B, C). Plasma samples collected and analyzed only during the first 4 h in four patients were excluded from further analysis for terminal half-life determination. Data from patient 10 was also excluded from further analysis because steady-state levels were nearly 1 log higher than those seen in other patients. PCZ pharmacokinetics in the remaining five patients was best fitted into a three-compartment model (Table 4). The mean terminal plasma half-lives were: $t_{1/2\alpha}$, 20.9 ± 5.3 min; $t_{1/2\beta}$, 1.8 ± 0.3 h; and $t_{1/2\gamma}$, 21.9 ± 5.3 h. The volume of distribution (V_d) was 2254 ± 886 l/m², the total clearance (Cl_T) was 60.2 ± 13.5 l m^{-2} h^{-1}, and the AUC was 1624 ± 686 ng ml^{-1} h.

The pharmacokinetics of DOX in six patients studied (Table 5, Fig. 2) was similar to that reported earlier [2, 13]. Since DOX concentrations were not sampled between 6 and 12 h, it was not possible to estimate the $t_{1/2\beta}$ value. The DOX pharmacokinetic data (Table 5) was best fitted into a two-compartment model. The half-lives of DOX were: $t_{1/2\alpha}$, 15.1 ± 1.3 min and $t_{1/2\gamma}$, 25.1 ± 4.2 h. The V_d was 4075 ± 1341 l/m², the Cl_T was 107.7 ± 25.2 l m^{-2} h^{-1}, and the AUC was 737 ng ml^{-1} h.

Laboratory studies

DOX or DNR retention and the effect of an efflux blocker (PCZ, CPZ) was studied in single-cell suspensions of tumor cells isolated on gradients from pleural effusion of 7 of 13 subjects (patients 3–6 and 11–13). Pleural fluid obtained from patient 3 and enriched for tumor and mononu-

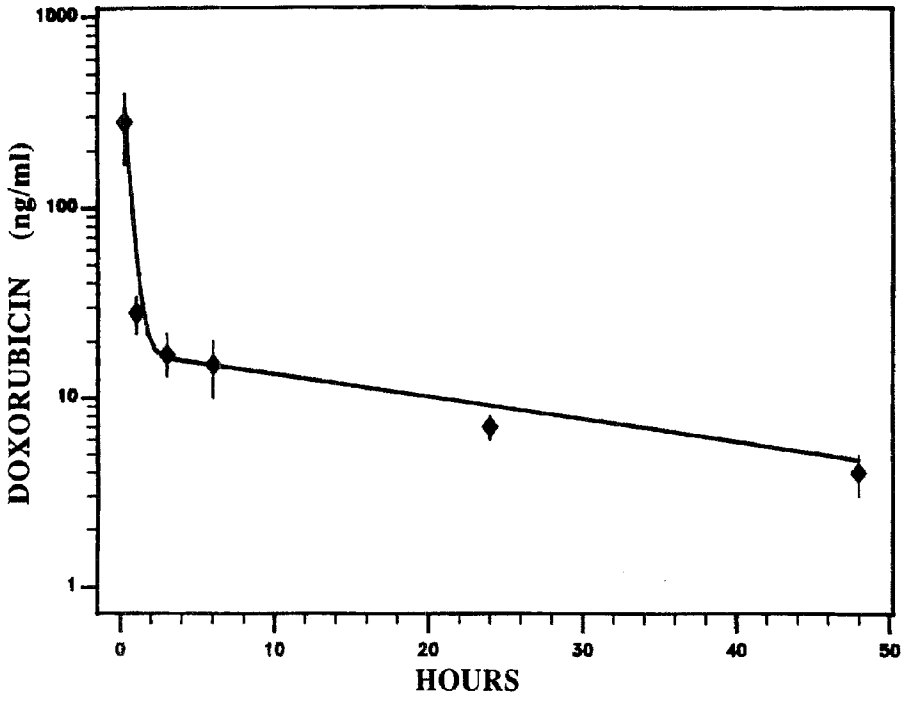

Fig. 2. Plasma levels of DOX in six patients given 60 mg/m^2 by 15-min i. v. infusion after 15 min of PCZ infusion

Table 4. PCZ pharmacokinetics

	Patient number	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	V_d (l/m^2)	Cl_T (l m^{-2} h^{-1})	AUC (ng ml^{-1} h)
Dose 50 mg/m^2:							
	7	20.8	1.8	18.1	1466	56.3	888
	9	14.1	2.7	30.1	3985	91.7	545
Composite (n = 2)[a]		17.4 ± 3.4	2.2 ± 0.5	24.1 ± 6.0	2726 ± 1259	73.9 ± 17.7	716 ± 171
Dose 75 mg/m^2:							
	8	12.7	1.9	38.1	4278	86.1	871
	11	15.5	1.9	10.9	778	49.3	1521
	13	41.2	0.8	12.5	315	17.5	4295
Composite (n = 3)[a]		23.1 ± 9.1	1.6 ± 0.4	20.5 ± 8.8	1940 ± 1400	50.9 ± 19.8	2229 ± 1049
All patients (n = 5)[a]		20.9 ± 5.3	1.8 ± 0.3	21.9 ± 5.3	2254 ± 886	60.2 ± 13.5	1624 ± 686

[a] Data represent mean values ± SE

Table 5. DOX pharmacokinetics[a]

Patient number	$t_{1/2\alpha}$ (min)	$t_{1/2\gamma}$ (h)	V_d (l/m^2)	Cl_T (l m^{-2} h^{-1})	AUC (ng ml^{-1} h)
2	16.4	19.3	2955	106.4	546
7	18.6	21.7	4892	156.1	384
8	11.9	19.0	1168	42.5	1411
9	16.9	34.7	1193	203.9	294
11	10.3	14.9	1651	76.6	783
13	16.4	40.8	3590	61.0	983
Composite (n = 6)	15.1 ± 1.3	25.1 ± 4.2	4075 ± 1341	107.7 ± 25.2	737 ± 170

[a] All patients were given 60 mg/m^2 DOX

clear cells on a Percoll gradient had two distinct populations with a DNA index of 1.0 (90%) and 2.0 (10%). Dot plots of cellular DOX retention measured at 1 day prior to (Fig. 3A) and 29 days after the first course of therapy (Fig. 3B) in this patient showed extensive heterogeneity in forward-angle light scatter and DOX retention (on a log scale). However, in the posttherapy sample (Fig. 3B), a significant increase in DOX retention of the major subpopulation (arrow) was evident.

In patient 6, tumor cells (DNA index, 2.0) retrieved from Percoll gradients of pleural effusion showed high DOX retention and PCZ did not further increase DOX

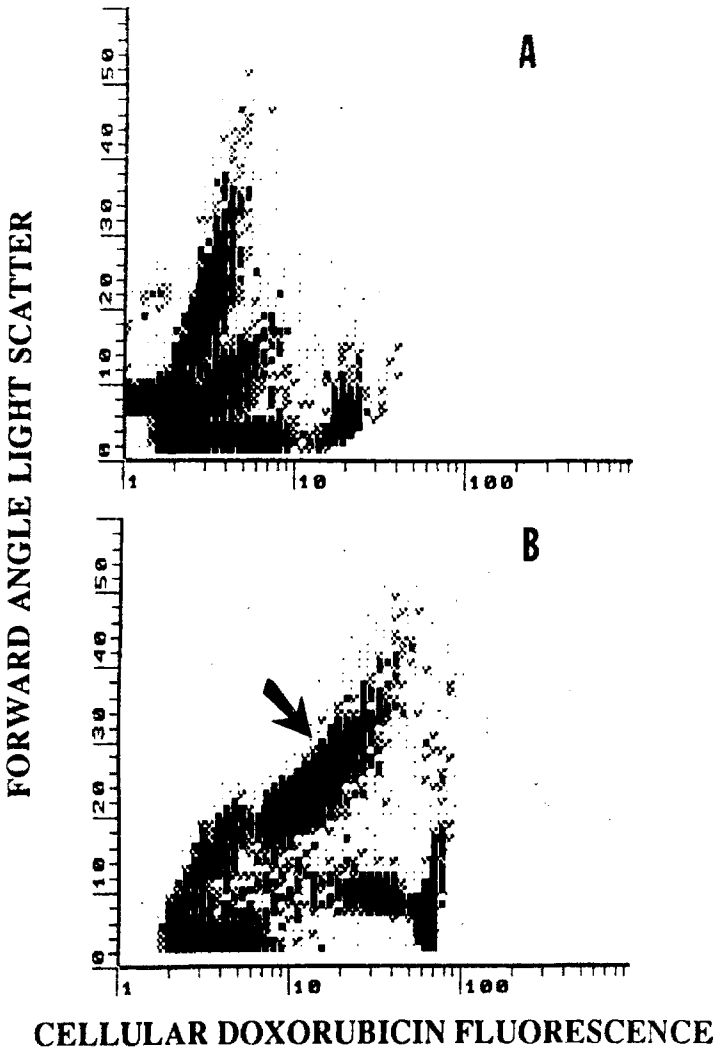

Fig. 3 A, B. Dot plots of cellular DOX fluorescence in cells from the pleural effusion of patient 3. The *vertical axis* records forward-angle light scatter, which approximates cell size, and the *horizontal axis* records on a 3-decade log scale cellular DOX fluorescence. **A** Cells from the pretherapy sample. **B** Cells obtained 1 h after the start of the PCZ infusion. Cells recovered on Percoll gradient were incubated with 3.55 μM DOX for 2 h. The *arrow* in **B** points to a population of large cells with enhanced DOX fluorescence

retention. In a sample obtained after 24 days of therapy, DOX retention was heterogeneous (two major populations) and coincubation with PCZ had no major effect on DOX retention. In the pleural effusion of patient 11, 30% of the cells had a DNA index of 1. A major subpopulation with a DNA index of 1.5 also had large S and G_2 components, indicating a large number of proliferating cells. In vitro DOX retention in cells obtained from this pleural effusion showed a broad distribution, and in the presence of PCZ, DOX retention was enhanced in one of the subpopulations.

Several samples of pleural effusion were retrieved during the course of disease from patient 12. DNR-retention histograms (Fig. 4B) revealed a predominant population showing retention intermediate between that observed for DOX-sensitive murine leukemic P388 cells and DOX-resistant P388/R-84 cells before and after incubation with efflux blocker (Fig. 4A). A sample obtained after 75 min from the start of therapy (Fig. 4C) showed enhanced DOX retention as compared with that observed in cells from the pretherapy sample (Fig. 4B).

Discussion

Several clinical protocols (Table 1) using drugs such as verapamil and cyclosporin were recently developed with the aim of blocking drug efflux, enhancing cytotoxic drug retention, and thus reducing resistance. Verapamil is potentially cardiotoxic, whereas cyclosporin may cause hepatotoxicity and increased myelosuppression. In the present study, we used the phenothiazine, PCZ, because it is non-cardiotoxic and on the basis of our laboratory data [18] showing that in human tumor cells from pleural fluid, phenothiazines block DOX or DNR efflux and significantly enhance cellular retention, whereas verapamil has no effect on drug retention under similar conditions.

In one of the pioneering studies, Miller et al. [20] gave TFP orally in divided doses 24 h before DOX and continuing for 6 consecutive days. The MTD of TFP was 60 mg/day and the dose-limiting factor was extrapyramidal toxicity [20]. The combination of DOX and TFP was well tolerated, and 7 of the 36 patients achieved a PR or CR [20]. In a subsequent report, Hait et al. [12] used TFP to enhance the antitumor activity of bleomycin. Of the 19 patients on the study receiving at least 2 weeks of treatment, 2 were unevaluable, and 4 of the remaining

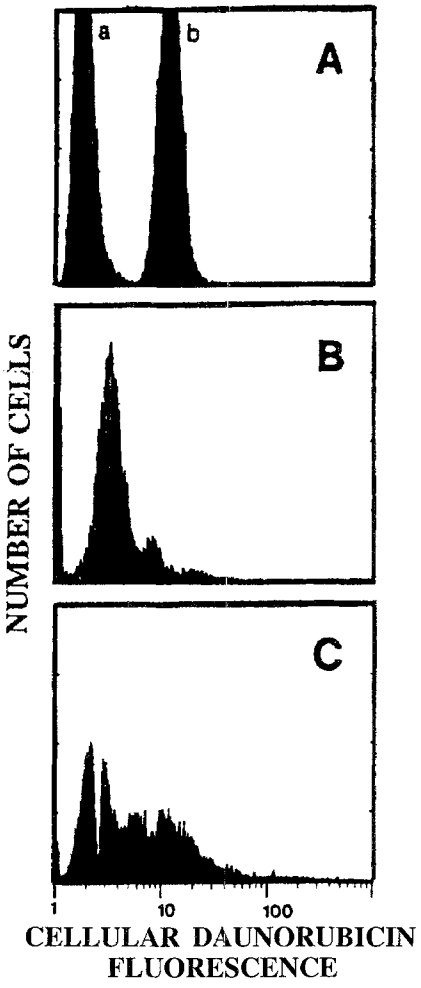

Fig. 4 A–C. Histograms of cellular DNR fluorescence in cells from pleural fluid of patient 12. **A** DNR retention in resistant P388/R-84 cells alone (*a*) or in the presence of an efflux blocker, 25 μM PCZ (*b*). **B** In cells retrieved from the pleural effusion prior to the start of therapy, a major subpopulation showed DNR retention intermediate between that observed for the P388/R-84 cells in the presence versus the absence of PCZ (**A**). **C** A sample obtained after 75 min from the start of therapy (15 min PCZ followed by 15 min DNR) shows the presence of cells with enhanced DOX retention

17 patients achieved a PR or CR. For a clinical protocol seeking to block DOX efflux with a blocking agent (e.g., PCZ, cyclosporin, verapamil), it is important that peak plasma levels sufficient to block efflux and enhance cellular retention be achieved. Thus, one of the major aims of the present study was to escalate the PCZ dose so as to achieve in vivo levels similar to those known to block DOX efflux in vitro and to enhance chemosensitivity. The bolus schedule for PCZ administration was chosen as it was most likely to result in the maximal achievable peak concentration. Should the peak plasma concentration achieved in the bolus schedule be lower than the levels required for efflux blocking, then other schedules would be less likely to be useful. As shown in the present study, i.v. administration of 75 mg/m^2 PCZ over 15 min in combination with 45–75 mg/m^2 DOX was tolerated safely. Importantly, we found that the peak PCZ concentration achieved in some patients was >1 μg/ml, a level high enough to block DOX efflux in vitro. The high PCZ plasma concentration was achieved during the $t_{1/2\alpha}$ phase of DOX, the time during which cellular efflux of DOX was most likely to occur. Lower levels of PCZ were sustained during the $t_{1/2\beta}$ and $t_{1/2\gamma}$ phases of DOX. The latter may be one of the factors influencing the drug's cytotoxicity. On the basis of these considerations, in a follow-up study we are using 2-h infusion of PCZ to block DOX efflux. Pharmacokinetic data from this study are being analyzed to provide a better understanding of the interpatient variation in PCZ levels.

In the present Phase I trial, we reached an MTD of PCZ higher than that previously reported [21] due to the amelioration of hypotension by i.v. hydration. We observed neither hepatotoxicity nor irreversible extrapyramidal side effects. The toxicities attributable to PCZ were transient sedation, chills, restlessness, cramps, and hypotension. The hematologic and mucosal toxicities of DOX were unaltered. The cardiotoxicity of DOX is well recognized [5, 19] and additional studies are needed to determine whether the cumulative cardiotoxicity of DOX may be enhanced by PCZ.

Our data suggest that the i.v. schedule of PCZ administration results in some toxicities different from those reported for chronic p.o. PCZ therapy. The i.v. administration of PCZ following hydration results in sedation but not in major extrapyramidal toxicities. Due to potential toxicities such as hypotension and sedation, the first course of this combination should be delivered in the hospital. If patients tolerate the first course well, the second course and subsequent courses can be given on an outpatient basis. The i.v. hydration can also be reduced for the second course and subsequent courses if no major changes in blood pressure have occurred during the previous administration of PCZ and DOX. Although this was a phase I study, we noted activity for this combination in non-small-cell lung cancer. Detailed studies on the MDR-1 (P-glycoprotein) gene expression the anthracycline retention, and the chemosensitivity of lung-tumor cells from patients treated on this protocol are being published separately (Ramachandran et al. 1993 [24 a]).

It is noteworthy that in several of our patients (especially patient 12), DOX retention in tumor cells after therapy with the efflux blocker was significantly higher than that in cells obtained before the start of therapy. One can postulate that exposure to PCZ in vivo did block efflux and thus enhance DOX retention in cells retrieved after 1 h of DOX and PCZ administration. However, the clinical significance of this observation needs to be tested by screening of tumor cells from patients on PCZ/DOX protocols and correlation of increased retention either with greater chemosensitivity in soft-agar assay or with significantly enhanced clinical response. Our ongoing studies are focused on this aspect of the PCZ-based modulaton of cellular DOX retention.

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