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ORIGINAL ARTICLE

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A phase I study of carboplatin and etoposide administered in conjunction with dipyridamole, prochlorperazine and cyclosporine A

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Abstract *Purpose*: In recognition of the variety of available chemotherapeutic modulating agents and their potential to enhance the efficacy of platinum-based therapy, we embarked upon a phase I study to investigate the feasibility of combining fixed doses of carboplatinum (CBDCA) and etoposide (VP-16) with 24-h concurrent infusions of dipyridamole (DP), prochlorperazine (PCZ) and cyclosporine A (CSA) administered in escalating doses. *Methods*: Patients received intravenous VP-16 (200 mg/m^2) and CBDCA (300 mg/m^2), each over 30 min, starting at hour 6 of the modulator infusions. Resistance modulators were escalated sequentially to determine their respective maximally tolerated doses (MTDs). The pharmacokinetics (PK) of VP-16, CBDCA, and the three drug resistance (DR) modifiers were studied in eight patients. *Results*: A total of 59 patients were entered on study. The MTD was established at DP 5 mg/kg per day, PCZ 24 mg/h, and CSA 9.5 mg/kg per day. Dose-limiting toxicities included hypotension and severe sedation, presumably related to PCZ. No objective responses were seen. PK studies were performed when PCZ and DP doses were 24 mg/h and 3.3 mg/kg, and the CSA dose was either 8.5 mg/kg (five patients) or 9.5 mg/kg (three patients). The median clearance of VP-16 was 0.96 l/h per m^2 (range 0.8–1.5 l/h per m^2), which is lower than for VP-16 alone and similar to previously reported effects of CSA on VP-16 elimination. The median measured CBDCA AUC was 3.0 mg/ml · min (range 2.4–4.8 mg/ml · min). CBDCA AUC predicted by the Calvert formula using measured creatinine clearance underestimated the actual AUC in seven of the eight patients, in one case by as much as twofold. The median end of infusion PCZ and total DP plasma concentrations were 1.2 μ*M* (range 0.5–2.2 μ*M*) and 4.4 μ*M* (range 1.3–5.9 μ*M*), respectively, consistent with in vitro resistance modulatory levels. However, free DP was only 0.02 μ*M* (range 0.004–0.04 μ*M*). The median CSA level at 24 h of 1450 μg/l (range 1075–1640 μg/l) is in agreement with concentrations required for partial DR reversal in vitro, although it is much lower than levels achieved in our previous phase I study of CBDCA + CSA alone using similar doses of CSA. The CSA dose on the current trial was escalated beyond the MTD for the previous phase I study, suggesting that there may be an interaction between CSA and one of the other modulators. *Conclusion*: These results demonstrate that in vitro DR-reversing levels of two of the three agents used in this study can be achieved in vivo, and that this combination of DR modulators has significant effects on the pharmacokinetics of VP-16.

Key words Carboplatin · Etoposide · Drug resistance · Modulation

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Introduction

Resistance of malignant cells to chemotherapeutic agents is a serious problem in the development of effective therapies in clinical oncology. Mechanisms underlying such resistance have been found at multiple levels of the tumor cell and maneuvers to bypass these mechanisms have been under evaluation in the preclinical

and clinical arenas. Measures to decrease resistance to chemotherapeutic drugs have included administration of modulating agents which lack inherent cytotoxic potential but are capable of enhancing tumor cell killing [20, 24, 32]. Calcium channel blocking agents and calmodulin inhibitors have been reported to reverse resistance to certain drugs by enhancing drug accumulation in resistant lines [6, 32]. Phenothiazines, including trifluoperazine and prochlorperazine (PCZ), are examples of calmodulin inhibitors that have demonstrated the ability to reverse resistance to a variety of chemotherapeutic agents including vinca alkaloids [6], anthracyclines [5, 21], and cisplatin [15]. Dipyridamole (DP) has also been reported to increase the cytotoxic effect of several chemotherapeutic drug classes, although the exact mechanism(s) mediating this interaction are incompletely understood [6]. Inhibition of nucleoside transport is one possible mechanism responsible for the potentiating effect of DP upon antimetabolites [15]. However, DP has also been noted to augment the cytotoxic effects of doxorubicin [26], vinca alkaloids [7], and cisplatin [8] in preclinical systems. In addition, DP synergistically enhances the sensitivity of human ovarian carcinoma cells to etoposide (VP-16) in vitro [10].

Cyclosporine A (CSA) is another modulating agent that has been evaluated extensively. It potentiates the activity of several antineoplastic agents including VP-16, carboplatinum (CBDCA) and cisplatin [22, 23, 30]. One mechanism postulated to underlie this effect is the ability of CSA to correct alterations in plasma membrane potentials found in resistant tumor cells [36]. In addition, CSA has been found to alter the expression of certain nuclear oncogenes (c-fos, c-myc) and may influence genes involved in the repair of cisplatin-induced DNA damage [12, 18], and to reverse, at least in part, P-glycoprotein-mediated drug efflux [23].

In recognition of the variety of chemotherapeutic modulating agents available and their potential to enhance the efficacy of platinum-based therapy, we designed this phase I study to investigate the feasibility of combining fixed bolus doses of CBDCA and VP-16 with 24-h infusional delivery of DP, PCZ and CSA administered in escalating doses. The goals of this study were as follows: (1) to define the maximal tolerated dose (MTD) and dose-limiting toxicities (DLTs) of a 24-h infusion of DP, PCZ and CSA when given in conjunction with fixed doses of CBDCA and VP-16 administered 6 h after the start of the modulators, and (2) to describe the pharmacokinetics of VP-16 and CBDCA when given in this combination and to assess in vivo systemic exposure to these modulators.

Materials and methods

A total of 59 patients were entered onto this protocol which had been reviewed and approved by the City of Hope Comprehensive Cancer Center Institutional Review Board. Eligible patients included those with histological or cytological documentation of neoplastic disease considered refractory to standard therapy or for whom no standard therapy existed. Patients were required to have a Karnofsky performance status of at least 60%, an estimated survival of at least 2 months, a hemoglobin greater than 10 g%, a leukocyte count of more than 3500/μl, and a platelet count of more than 150,000/μl. Total serum bilirubin could not exceed 1.5 mg/dl and serum transaminases were required to be less than three times the institutional upper limit of normal. The serum creatinine was required to be less than 1.5 mg/dl or creatinine clearance more than 60 ml/min. In patients with a history of pulmonary disease, the FEV_1 had to exceed 2 l and oxygen saturation had to exceed 92% with the patient breathing room air. Patients with measurable lesions were required to have baseline determinations performed with appropriate radiographic studies within 4 weeks of entry into the trial; pleural effusions, ascites, and bone metastases were not considered measurable. There were no limitations on the extent of exposure to prior radiotherapy or on the types and number of previously administered chemotherapies except that prior cisplatin exposure could not have exceeded 300 mg/m^2 (1200 mg/m^2 for CBDCA). All prior therapy had to have been completed at least 4 weeks before study entry.

Protocol exclusion criteria included pregnancy or the presence of concomitant nonmalignant disease that was either poorly controlled by therapy or of such severity that it was considered potentially unsafe for the patient to participate in the trial. Also excluded were patients with narrow angle glaucoma, Parkinson's disease, extrapyramidal disorders, seizure disorders, known hypersensitivity or neurological intolerance of phenothiazines or diphenhydramine, or who required chronic antipsychotic medication. Patients with symptomatic coronary artery disease, cardiac arrhythmias, venous thrombosis undergoing anticoagulant therapy, bronchodilator-dependent asthma, or chronic obstructive pulmonary disease were not eligible. Additionally, patients undergoing therapy with calcium channel blocking agents were ineligible unless these drugs could be withheld for at least 72 h prior to protocol treatment. All participants provided written, voluntary informed consent before therapy was initiated.

Treatment was administered over a 24-h period of a 21-day cycle. The biochemical modulating agents, DP, PCZ, and CSA were delivered by continuous intravenous infusion through a central venous access device over 24 h in a dose-escalating fashion as indicated in Table 1. Six hours after the modulator infusions began, VP-16 (200 mg/m^2) was administered as a 30-min intravenous infusion immediately followed by CBDCA (300 mg/m^2) also delivered over 30 min. (A fixed dose of CBDCA was utilized because at the time of the study's inception, the Calvert formula had not been fully validated.) Diphenhydramine (25 mg orally or 12.5 mg intravenously every 6 h for four doses beginning at time 0) was administered to prevent dystonic reactions. Due to the sedating effects of PCZ and diphenhydramine, patients were required to remain at bed rest during treatment. When G-CSF became commercially available, the protocol was amended to include its use at a dose of 5 μg/kg per day subcutaneously administered from the

Table 1 Dose-escalation scheme

Patient cohort	Dipyridamole (mg/kg/day)	Prochlorperazine (mg/h)	Cyclosporine (mg/kg/day)
1	1	0	0.0
2	1	18	0.0
3	1	18	7.5
4	2	18	7.5
5	2	21	7.5
6[a]	2	21	8.5
7	3.3	21	8.5
8	3.3	24	8.5
9	3.3	24	9.5
10	5	24	9.5
11	5	27	9.5

[a] G-CSF added to regimen during this cohort

day after chemotherapy was completed until the post-nadir absolute neutrophil count exceeded 10,000/μl.

Patients were monitored with twice-weekly complete blood counts and weekly serum chemistry determinations, and prior to each cycle, all baseline laboratory studies were repeated. Treatment could be delayed up to 14 days to allow recovery to defined levels of hematopoietic function. Therapy was given until objective evidence of disease progression was documented, and toxicity was graded according to the NCI common toxicity guidelines. If patients required additional antiemetics they were allowed to receive metoclopramide or a 5-HT3 receptor, antagonist; drugs with sedative potential such as antihistamines (aside from diphenhydramine), corticosteroids, butyrophenones, or benzodiazepines were not allowed.

The doses of CBDCA and VP-16 were purposely administered at doses lower than those typically associated with dose-limiting myelosuppression in anticipation of the possibility that the modulatory agents might potentiate their effective concentrations, thereby reducing the risk of inducing excessive toxicity. The modulatory agents were escalated in a simultaneous fashion until a MTD for the combination was reached. Participants were enrolled in cohorts of three patients. If no grade 3 or 4 toxicity was manifest within a cohort, the dose of one of the modulators was escalated to the next dose level in the subsequent three patients while the other modulator doses were held steady. If any toxicity greater than grade 2 was observed at a dose level, the cohort was expanded to six patients. Dose escalation continued as long as there were no additional grade 3 toxicities in a cohort of six. The DLT was defined as the occurrence of two grade 3 toxicities in such an expanded cohort or any grade 4 toxicity. The study was terminated when six patients had been treated at a dose level in which no more than one grade 3 and no grade 4 toxicities were observed.

Patients who completed at least one cycle of therapy at the specified schedule were considered evaluable for toxicity, and those with measurable disease who completed at least two cycles of treatment were considered evaluable for response. A complete response was defined as the disappearance of all detectable tumor on two separate measurements (either radiographic or by clinical examination) at least 28 days apart. Partial response was considered a 50% or greater reduction of the sum of the products of the longest perpendicular diameters of all measurable lesions and the appearance of no new lesions, both conditions persisting on two separate measurements (either radiographic or by clinical examination) at least 28 days apart. Stable disease was defined by similar criteria except that indicator lesions decreased or increased by less than or equal to 25%. Progressive disease was defined as an increase in the product of perpendicular diameters of the indicator lesion(s) by more than 25% or the appearance of new lesions.

Pharmacokinetic studies were initiated at doses believed to be close to the MTD in order to describe the disposition of VP-16 and CBDCA in combination with infusional CSA, PCZ, and DP. Additional analyses were performed to determine the clinically achievable concentrations of these multidrug resistance modulators when given as simultaneous 24-h infusions. For the pharmacological studies, peripheral blood samples were obtained immediately prior to the combined modulator infusion, and at 1.5, 3.5, 6, 12, 16, and 24 h after the start of the VP-16 and CBDCA infusions. VP-16, PCZ, and DP plasma concentrations were determined in all samples by HPLC according to previously published methods with minor modifications [3, 4, 38]. Briefly, following organic extraction with chloroform (VP-16) or diethyl ether (PCZ and DP), drug concentrations in plasma were quantitated with reversed-phase separation and electrochemical detection. Unbound CBDCA and DP were separated from bound drug in peripheral blood samples by ultrafiltration of plasma using a 30-kDa cut-off filter (Amicon). Ultrafilterable CBDCA was determined by atomic absorption spectroscopy [1], and free DP concentrations were determined by reversed-phase HPLC with fluorescence detection [28]. CSA levels in whole blood were determined by fluorescence polarization immunoassay (FPIA) and HPLC. Pharmacokinetic data analyses for VP-16 and CBDCA were performed using ADAPT II software [2]. The predicted CBDCA AUC was calculated using the Calvert formula: AUC = dose/(GFR + 25), where GFR (glomerular filtration rate) was determined by 24-h urine collection [35].

Results

A total of 59 patients were registered and treated on this phase I protocol. The demographic features of the patient population are listed in Table 2. The median age was 50 years (range 30–72 years), and most patients had advanced cancer of the aerodigestive system with some compromise to their performance status. Prior therapy included radiotherapy in 26 patients and chemotherapy, biological therapy and/or hormone therapy in 44 patients.

Toxicity

The first cohort of patients was treated with chemotherapy and DP as a single modulating agent. In the second cohort both DP and PCZ were administered with the cytotoxic therapy. This approach of introducing the modulators into the regimen sequentially was chosen to minimize the risk of toxicity; none of the six patients entered onto these dose levels had toxicities greater than grade 2 severity. A total of 12 patients were treated at the third dose level in which CSA was introduced along with the other two modulators; however, six of these

Table 2 Patient characteristics

Patients entered	59
Male	25
Female	34
Age (years)	
Median	50
Range	30–72
Performance status (%)	
100	12
80–90	37
60–70	10
Tumor type	
Gastrointestinal, adenocarcinoma	22
Lung (eight non-small-cell, one small-cell)	9
Unknown primary, adenocarcinoma	6
Breast	4
Sarcoma	4
Head and neck, squamous	2
Adrenal	2
Cervical	2
Mesothelioma	2
Prostate	1
Lymphoma	1
Melanoma	1
Bladder	1
Thyroid	1
Carcinoid	1
Prior therapy	
Surgery	58
Radiation	26
Systemic therapy	44

patients were considered inevaluable for a variety of reasons. One patient in this cohort experienced moderately severe oral mucosal breakdown considered to be related to progression of underlying head and neck cancer, and another patient experienced an exacerbation of underlying autoimmune hemolytic anemia. Thereby accurate grading of toxicities in these two patients was rendered impossible. Two patients experienced grade 2 hypotension and elected to stop therapy before the full 24 h of the protocol therapy was completed while a fifth patient inadvertently received the 24-h dose of PCZ over 12 h; all three were considered inevaluable. The sixth patient complained of palpitations shortly after therapy began, and was noted to have a supraventricular tachycardia; therapy was discontinued. Retrospectively, it was recognized that the patient had a prior history of arrhythmia rendering her ineligible for the study. Due to the problems described, it was elected to expand this cohort to a total of six evaluable patients, and none experienced greater than grade 2 toxicity.

A single patient in the fourth cohort experienced grade 3 nausea and vomiting. Therefore, the dose level was expanded to include six patients and no additional DLTs were observed. The fifth dose level consisted of three patients who all proceeded through treatment without significant toxicity. One of the first three patients treated at the sixth dose level experienced dyspnea that was attributed to progression of underlying pleural mesothelioma and was not considered evaluable. At this time the protocol was amended to include the use of G-CSF, so six additional patients were added to the cohort and no DLTs were observed. The seventh cohort of three patients proceeded through their therapy without experiencing any adverse events greater than grade 2 in severity. Two patients treated at the eighth dose level were considered inevaluable: one was hospitalized with recurrent urosepsis that was not considered related to the protocol therapy and the second experienced grade 2 hypotension and refused to complete the first cycle of therapy. Of the remaining six patients in this cohort, one experienced grade 3 hypotension. The ninth and tenth cohorts of patients completed therapy without experiencing significant adverse events. In the eleventh cohort of three patients, there were two grade 3 toxicities observed, hypotension in one and neurological (severe somnolence) in the other. Hence, three additional patients were accrued at dose level 10; no further grade 3 or 4 toxicities were observed. Since two of six patients experienced grade 3 toxicity at dose level 11, DLT was established at that dose. The MTD for the combination was dose level 10 with DP 5 mg/kg per day, PCZ 24 mg/h, and CSA 9.5 mg/kg per day in conjunction with CBDCA and VP-16.

Other toxicities experienced by patients were minor. While several patients in the earlier portion of the trial had brief grade 2 or 3 hematological toxicities, after the introduction of G-CSF, such events were less frequently observed. Gastrointestinal side effects such as nausea and vomiting were also minimal (aside from one patient with grade 3 nausea in cohort 11), and there were no significant hepatic or renal toxicities observed. While nearly all patients were sedated from the prochlorperazine infusion, most experienced this to a mild or moderate degree and remained easily arousable during treatment. Aside from these neurocortical effects, no adverse events of a neuro-sensory, -motor or -cerebellar nature were observed. Similarly, although hypotension of mild to moderate degree was experienced by several patients, no other cardiovascular toxicities were noted.

Responses

Of the 51 patients who were evaluable for response, a single patient with extensively pretreated large-cell lymphoma was noted to have a greater than 50% decrement in the size of nodal masses after the first cycle of treatment. However, when reevaluated after the second course of therapy, the patient had evidence of progressive disease. A further 17 patients were noted to have stabilization (2.1 months median progression-free survival) of their disease, and 25 patients experienced further progression of their cancer.

Pharmacokinetics

Based upon previous clinical experience, it was assumed that the MTD for CSA would be close to 8.8 mg/kg per 24 h. Therefore, collection of pharmacokinetic data was initiated starting at a CSA dose of 8.5 mg/kg per 24 h. A total of eight patients were studied at CSA doses of either 8.5 mg/kg (five patients) or 9.5 mg/kg (three patients). The doses of PCZ and DP in all eight patients were 24 mg/h and 3.3 mg/kg per day, respectively. The median plasma concentration versus time profile for VP-16 is shown in Fig. 1. The plasma pharmacokinetics of VP-16 were best described by a two-compartment model, with first-order elimination. The median VP-16 plasma clearance and volume of distribution in the eight patients were 0.96 l/h per m^2 (range 0.8–1.5 l/h per m^2) and 10.8 l/m^2 (range 6.1–16.9 l/m^2), respectively. As shown in Fig. 1, the clearance of VP-16 determined in this study was slower than has been previously reported for VP-16 alone [19].

The pharmacokinetics of unbound CBDCA were best described by a one-compartment model with first-order elimination. Fig. 2 illustrates the relationship between measured CBDCA AUC and the AUC predicted using the Calvert formula in the seven patients who had complete 24-h urinary creatinine clearances. The median measured unbound CBDCA AUC was 3.0 mg/ml · min, while the median predicted AUC was 2.4 mg/ml · min. As depicted in Fig. 2, the actual CBDCA AUC was underestimated by the Calvert formula in six of the seven patients by a median of 18%. The median unbound CBDCA clearance was 104.2 ml/min per m^2 (range 62.8–123.7 ml/min per m^2).

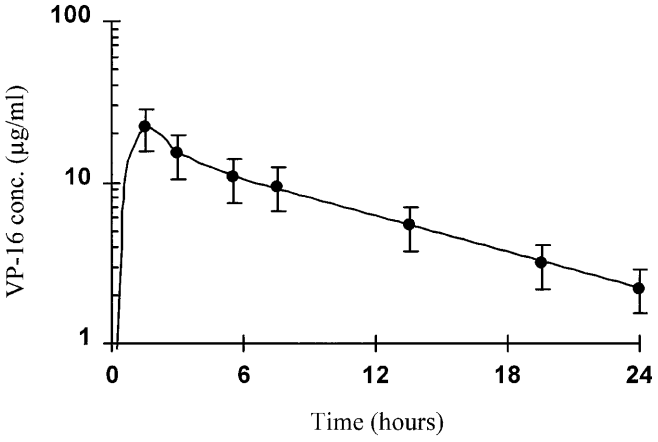

Fig. 1 Plasma concentration versus time curve for VP-16 derived using the mean pharmacokinetic parameters from patients receiving VP-16 along with continuous infusions of CSA, DP, and PCZ. The points represent means and the bars represent the standard error

To determine the clinically achievable concentrations of CSA, PCZ, and DP when given as simultaneous 24-h continuous infusions, the concentrations of the resistance modulators were determined in patients studied near the MTD. Table 3 shows the end of infusion concentrations for each of the three resistance-modulating agents used in the trial. The median end of infusion CSA concentration as measured by FPIA was 1450 μg/l (range 1075–1640 μg/l), while the median level measured in the same samples by HPLC was 856 μg/l (range 749–1136 μg/l). End of infusion PCZ concentrations ranged from 0.5 to 2.2 μ*M*, with a median of 1.2 μ*M*. The median end of infusion total DP level was 4.4 μ*M* (range 1.3–5.9 μ*M*); however, the median free DP level was only 0.02 μ*M* (0.004–0.04 μ*M*).

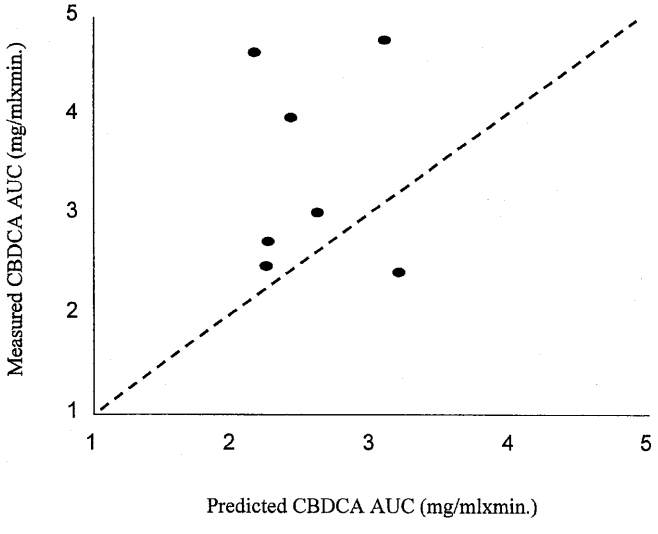

Fig. 2 Plot of the ultrafilterable CBDCA area-under-the-curve predicted by the Calvert formula using urinary creatinine clearance as the measure of GFR versus the actual measured free CBDCA area-under-the-curve. The dashed line represents the line of identity

Figure 3 shows the plasma concentration versus time profiles for PCZ and DP in the eight patients studied. As depicted in Fig. 3, PCZ concentrations rose slowly but continuously during the 24-h infusion. Once the infusion ended, clearance of PCZ was very slow. In contrast to PCZ, plasma concentrations of DP rose quickly, reaching steady-state levels within 20 h, and then decreased more rapidly following the end of the infusion. Interestingly, DP concentrations dropped precipitously upon initiation of the VP-16 infusion in all patients studied. As shown in Fig. 4, total DP concentrations decreased transiently after the VP-16 was started, free DP levels remained unchanged, and the percentage of unbound DP increased by fivefold. These data suggest that there is a protein binding interaction in which VP-16 displaces DP from its protein-binding sites in plasma.

Discussion

Many studies have been carried out to investigate the ability to administer biochemical modulators with chemotherapy in an attempt to overcome resistance. However, these trials generally have focused upon the use of a single modulator. In recognition of the array of mechanisms underlying chemotherapy resistance, this study was undertaken to assess the feasibility of administering multiple modulators (PCZ, DP and CSA) concurrently with CBDCA and VP-16. Simultaneously, we evaluated the pharmacokinetic effects resulting from the combination.

Our results indicate that this combination of modulators can be safely delivered with minimal adverse effects. The DLTs observed included both hypotension and severe sedation, presumably both related to the effects of PCZ, although hypotension might also have been related to the VP-16 infusion. While other investigators have reported hyperbilirubinemia as a DLT related to the combination of CSA and VP-16 [39], we did not observe any hepatotoxicity. In prior experience within our institution, nephrotoxicity was noted as a dose-limiting consequence resulting from the administration of CSA as a modulator of CBDCA [22], but no such renal toxicity was observed in this trial. Stewart et al. administered epirubicin with multiple resistance modulators (metronidazole, tamoxifen, DP, ketoconazole and CSA) and reported several toxicities including moderate nausea and vomiting, headache and cardiovascular effects [33]. As a result, they concluded that further attempts using this regimen were probably not indicated. In comparison, gastrointestinal toxicity was minimal in our experience, presumably due to the antiemetic effect of the PCZ infusion. Likewise, only mild headache (probably secondary to DP) was reported by our patients. Perhaps the better tolerance experienced in our patient population was related to the fact that the modulators were delivered intravenously, rather than orally as in the trial by Stewart et al.

Table 3 Clinically achievable plasma concentrations of anticancer drug resistance-reversing agents

Anticancer drug	Modulator	Current study	Concentration for reversal	Reference
Cisplatin	CSA	1.4 μg/ml (FPIA) 0.86 μg/ml (HPLC)	5 μg/ml (complete) 1–2 μg/ml (partial)	12
	PCZ	1.2 μ*M*	1–10 μ*M*[a]	25
	DP	4.4 μ*M* (total) 0.02 μ*M* (free)	20 μ*M*	11
VP-16	CSA	1.4 μg/ml (FPIA) 0.86 μg/ml (HPLC)	1–2 μg/ml	23
	PCZ	1.2 μ*M*	5 μ*M*[a]	13
	DP	4.4 μ*M* (total) 0.02 μ*M* (free)	2–20 μ*M*	9

[a] Related phenothiazine compound, trifluoperazine

While a single patient with lymphoma had a transient decrease in the size of nodal disease, it rapidly regrew and hence did not fulfill the definition for a confirmed partial response. Therefore, further evaluation of this regimen would be necessary before any conclusions could be drawn regarding its efficacy.

Perhaps the most significant results arising from this study concern the pharmacokinetic findings. Multiple clinical investigations of drug resistance reversal have demonstrated the effects of various resistance modulators on the pharmacokinetics of anticancer agents [14, 19, 29]. In most cases, the drug resistance modulator decreases the clearance of the agent being modulated, resulting in higher than expected drug levels. Both interference with hepatic drug metabolism and inhibition of P-glycoprotein in liver and kidneys have been suggested as possible sources of these interactions. However, as a result, it has often been difficult to separate the effects of drug resistance modulation from the effects of increased anticancer agent exposures due to pharmacokinetic drug interactions in the clinical trials.

The current study confirms the pharmacokinetic drug interaction previously observed between CSA and VP-16. Lum et al. first demonstrated that coadministration of CSA and VP-16 decreases VP-16 clearance by 38%, resulting in significantly longer elimination half-lives and increased VP-16 systemic exposures [19]. In the present study, we found that when combined with CSA, DP,

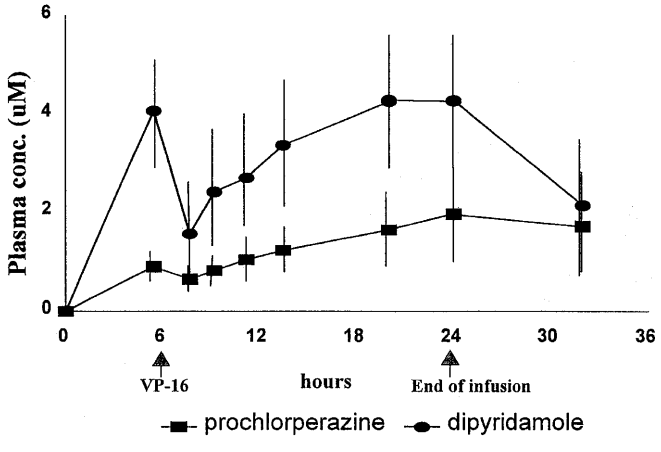

Fig. 3 Plasma concentration versus time curves for total DP concentrations (*solid circles*) and PCZ (*solid squares*). The error bars represent the standard deviation

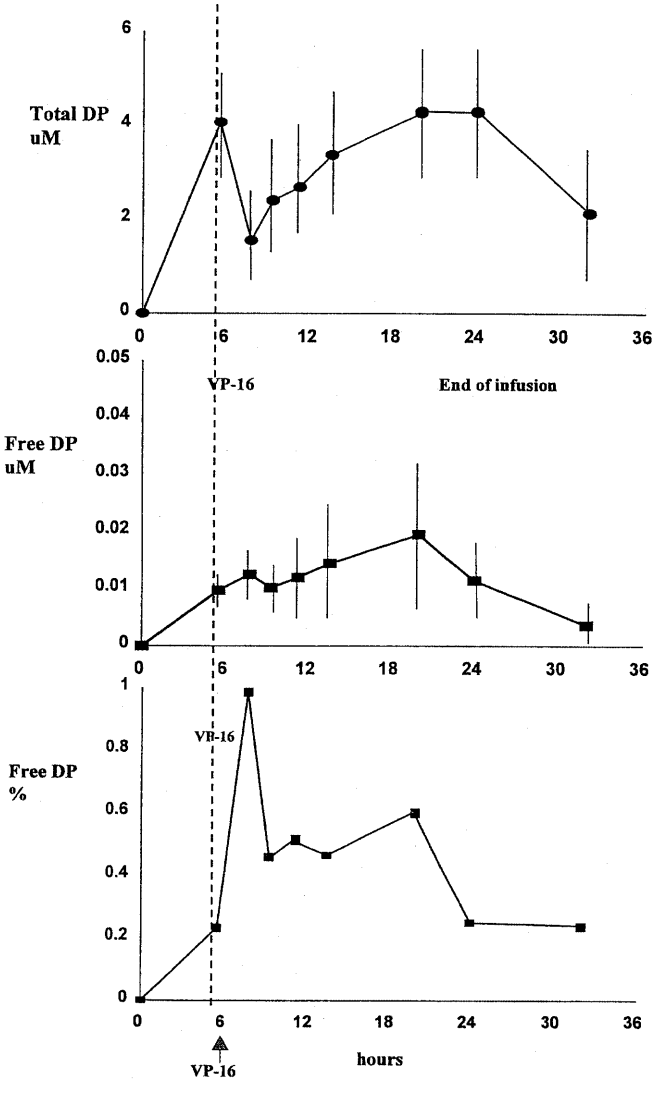

Fig. 4a–c Total and unbound plasma levels of DP (**a** total dipyridamole concentrations, **b** unbound levels, **c** unbound dipyridamole as a percentage of the total). The error bars represent the standard deviation

and PCZ, VP-16 clearance was 27% slower than previously reported for VP-16 alone, but similar to that for VP-16 when combined with CSA alone. Although the number of patients studied was small, our data suggest that the other two modulators used here do not have a dramatic effect on VP-16 clearance.

Results of previous clinical trials suggest that the pharmacokinetics of unbound CBDCA are unchanged when given with CSA [22]. The results of the current trial, however, indicate that CBDCA clearance was slower than expected based on the pretreatment urinary creatinine clearance. As a result, the measured unbound CBDCA AUC was higher than the predicted AUC calculated using the Calvert formula. The Calvert formula was originally derived using [^{51}Cr]-EDTA clearance as an index of GFR. Due to the inconvenience of using a radiolabeled tracer, most clinicians now use either urinary creatinine clearance or calculated creatinine clearance by the method of Cockroft and Gault as a measure of GFR in the Calvert equation. Recently, it has been suggested that either of the latter two measures of GFR systematically over- or underestimate creatinine clearance, and, as a result, lead to over- and under-dosing of CBDCA [27]. It is possible that by using urinary creatinine clearances as the index of GFR in the Calvert formula, CBDCA clearances in the present study were over-predicted, leading to lower predicted AUCs. Therefore, it cannot be definitively concluded that the three modulators used here have an effect on CBDCA pharmacokinetics.

Previous clinical experience with PCZ as a drug resistance modulator is limited. Sridhar et al. [31] have shown that PCZ doses of 135 mg/m^2 can be given safely as a 2-h infusion when preceded by 60 mg/m^2 doxorubicin. Due to the long terminal elimination half-life of PCZ, plasma concentrations in this study were maintained above 600 ng/ml (3 μM) for 24 h despite the shorter infusion schedule. Since previous in vitro data have demonstrated that PCZ levels of 1–10 μM are required for reversal of doxorubicin resistance [17], we have found that PCZ levels required for partial modulation of drug resistance are achievable in patients, confirming the results of Sridhar et al.

Trifluoperazine (TFP), like PCZ, is a calmodulin inhibitor that has been demonstrated to reverse cytotoxic drug resistance in vitro. Non-toxic concentrations of TFP (5–10 μM) have been found to increase the cytotoxicity of doxorubicin, VP-16, and cisplatin in vitro [13, 25]. In a previous clinical trial combining TFP with doxorubicin [21], it was found that maximal TFP plasma levels of 130 ng/ml are achievable in vivo without undue toxicity. However, the TFP levels measured in patients are approximately tenfold lower than those shown to be modulatory in vitro.

DP has been previously investigated in the clinic for its ability to potentiate the cytotoxicity of 5-fluorouracil [27], VP-16 [12], and cisplatin [11, 16]. Plasma total DP concentrations in the range of 6–7 μM have been achieved in patients receiving continuous intravenous infusions [27], while in patients given intraperitoneal infusions, intraperitoneal concentrations of >25 μM have been seen [10]. In vitro studies have identified that DP levels in the range of 2–20 μM are necessary for reversal of drug resistance. Based on these in vitro data, and the results of clinical trials, it has been hypothesized that modulatory levels of DP are clinically achievable.

Prior to the current study, most published data regarding clinically achievable DP levels have been limited to total drug concentrations. However, DP is extensively protein-bound and, therefore, it is likely that <1–2% of total measured drug is available as free drug in vivo [34]. We found that although total DP levels measured in our patients approached in vitro modulatory levels (4.4 μM), free drug levels were >200-fold lower. Interestingly, Howell et al. [9] have demonstrated that DP is 25-fold less potent in vitro when cells are incubated in human plasma versus standard tissue culture medium. These authors found that 96% of DP is non-protein-bound in tissue culture medium, compared to <15% free drug when plasma is used.

There is now extensive clinical experience with CSA as a drug resistance modifier. We have recently reported the results of a trial of infusional CSA combined with CBDCA in patients with advanced solid tumors [22]. Although the CSA doses used in the previous trial were identical to those used here, the mean CSA levels were 33% lower on the current study using three modulators in combination. This might suggest that there was an interaction between CSA and one of the other drugs used on this trial which led to an increase in CSA clearance, and this might also explain why patients on the current study were able to tolerate higher CSA doses with no evidence of renal toxicity. However, a definitive comparison between our prior trial and the present study cannot be made due to differences in the patient populations and treatment regimens. Despite the lower than expected plasma levels, the CSA concentrations achieved here were within the range of those required for reversal of multidrug resistance [12].

Drug resistance remains a critical problem in both hematology and solid tumor oncology, and strategies to overcome acquired and inherent resistance to cytotoxic agents have been under investigation for over a decade. Despite hundreds of clinical trials involving thousands of patients, an effective approach to drug resistance modulation has yet to be identified. It is clear that better modulating agents or combinations of existing agents are needed. In an effort to identify the maximum achievable doses and tolerability of a three-agent resistance-reversing regimen, we sequentially escalated CSA, DP, and PCZ in combination with fixed doses of VP-16 and CBDCA. The current trial demonstrates that this particular combination of resistance modulators is safe. Moreover, in the case of CSA and PCZ, drug concentrations that are required for reversal of resistance are achievable in vivo. However, we have also shown that DP is not a good candidate for clinical drug resistance modulations due to its extensive protein binding, resulting in very low free drug levels.

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