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Phase I Pharmacokinetic Study of Cyclosporin A Combined with Doxorubicin[1]

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ABSTRACT

We performed a phase I trial of cyclosporin A (CsA) in combination with doxorubicin (dox) to determine the maximally tolerated dose (MTD) of the combination in man, to define the quantitative and qualitative toxicities of the combination, and to determine the pharmacokinetics of the two drugs when used together. CsA was administered as a continuous infusion for 6 days, and dox was administered as a single 10-min infusion 24 h after the initiation of CsA. The starting CsA infusion rate was 5 μg/kg/min, and the dox starting dose was 30 mg/m². Courses were administered every 4 weeks with first CsA and then dox being escalated in consecutive cohorts of patients until the MTD was determined. Twenty-three patients and 40 courses were evaluable for toxicity. Pharmacokinetic analysis was performed in 23 patients on the first course for whole blood CsA and plasma dox and doxorubicinol. The MTD of CsA was 6 μg/kg/min, and for dox it was 45 mg/m². Dose-limiting toxicity was neutropenia. Serum creatinine and creatinine clearance did not change over the infusion period. Bilirubin increased from a median of 10 μmol/liter at the initiation of the infusion to a median of 40.4 μmol/liter at the end of the infusion but returned to normal before the next cycle of therapy. Nausea and vomiting were common and marked, whereas thrombocytopenia was mild. Two patients, one with small cell lung cancer and one with breast cancer, had stable disease while receiving treatment for 5 and 6 months, respectively. Mean whole blood steady state concentrations of CsA were 2210 ng/ml during the infusion with total body clearance of 0.177 liter/h/kg. The area under the concentration × time curve (*AUC*) increased linearly with dose of dox, and total body clearance was independent of dose. The mean total body clearance was 2.46 liters/h/m², and terminal half-life was 49.6 h. The *AUC* for dox was greater and clearance was less than has been previously reported at the doses administered in this study. The ratio of *AUC* for doxorubicinol to *AUC* for dox was less than expected, suggesting that the metabolism and/or excretion of dox was decreased when administered with CsA. We conclude that dox can be combined with infusioned CsA but at a lower dose than when given alone. This may be due to altered metabolism and/or excretion of dox or increased bone marrow stem cell sensitivity to dox.

INTRODUCTION

A major obstacle to the successful systemic treatment of cancer is drug resistance (1). Antineoplastic drug resistance can be *de novo*, *i.e.*, intrinsic to the tumor cells without prior exposure to drug, or acquired, *i.e.*, developing after exposure to drugs. Several mechanisms of drug resistance have been identified, including MDR,[3] detoxification of potentially cytotoxic metabolites, decreased activation of prodrugs, increased production of target enzymes through gene amplification, the presence of salvage pathways, and enhanced DNA repair. Attempts to overcome these mechanisms can be divided broadly into two categories: development of new drugs including biological compounds or modulation of resistance to currently available agents to overcome the purported mechanism. Although drug resistance is complicated by the likelihood that no single mechanism is the sole factor in treatment failure, it is incumbent on investigators to address individual mechanisms as a first step in elucidating the role of that mechanism in the clinical setting.

Multidrug resistance is a major factor in the resistance to drugs such as the anthracyclines, VP-16, and the *Vinca* alkaloids (2, 3). It is usually associated with increased production of a 170-kD transmembrane protein, secondary to gene amplification, altered gene transcription, and altered mRNA translation for this protein. It confers on a cell the ability to extrude drug rapidly and thereby decrease the exposure of the cell to cytotoxic concentrations of drug. This glycoprotein is present in normal tissue, suggesting that it has an important function in man which may be related to the detoxification of a variety of xenobiotics (4, 5). Evidence for its presence in various malignant tumors has been presented (6–8).

The modulation of MDR has been explored experimentally, and it appears that several different drug categories are capable of reversing this form of resistance in experimental systems (9). These drugs include calcium channel blockers, calmodulin inhibitors, quinidine, and CsA. CsA is an appealing agent to consider in clinical trials of MDR modulation because there is extensive clinical experience in the use of the drug as an immunosuppressant, the concentrations required to reverse MDR in experimental models are achievable in man, and it is one of the most potent agents which reverses MDR *in vitro*. It has the added potential advantage that analogues of this compound, which are currently in preclinical development, may be more effective in modulation of MDR with little immunosuppressive effects and less toxicity (10–15). Thus, modulation of MDR with CsA is clinically feasible with potential for further development in the future.

We have undertaken a phase I trial of CsA combined with dox to: (*a*) determine the MTD of the combination in man, (*b*) define the quantitative and qualitative toxicities of the combination, and (*c*) ascertain the pharmacokinetics of the two drugs when used together. We administered CsA as an infusion with the intent of achieving steady state whole blood levels of 2000 ng/ml of parent compound. This concentration was selected because *in vitro* studies suggested that 1000 ng/ml was sufficient to reverse MDR in cells expressing high levels of p-glycoprotein (16). A prolonged infusion of CsA was chosen because experimental data indicated that p-glycoprotein inhibition was reversed when CsA was removed *in vitro*.[4] Dox was administered after 24 h when steady state CsA levels were achieved. In this manner we reasoned that pharmacokinetic optimization of MDR modulation would be achieved.

MATERIALS AND METHODS

Patients. Eligibility criteria included histologically documented advanced or metastatic solid tumor refractory to standard therapy or for which no conventional therapy was available, 18 years or older, and Eastern Oncology

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[3] The abbreviations used are: MDR, multidrug resistance; MTD, maximally tolerated dose; V_{ss}, volume of distribution at steady state; CL_{th}, total body clearance; *AUC*, area under the concentration × time curve; C_{ss}, drug concentration at steady state; MRT, mean residence time; AUMC, area under the time × plasma concentration-time curve; NEUT, neutrophil count; CsA, cyclosporin A; dox, doxorubicin; doxol, doxorubicinol; R_{in}, infusion rate; VP-16, etoposide; AST, aspartate aminotransferase; *V1*, dox central distribution space; *V4*, doxol distribution space; MUGA, multigated nuclear angiogram.

[4] V. Ling, personal communication.

Cooperative Group performance status 0, 1, or 2. Prior radiotherapy and chemotherapy had to be completed at least 3 weeks (6 weeks for nitrosoureas and mitomycin C) prior to entry into the trial, and recovery from toxicity of prior therapy had to be complete before study entry. Patients with adequate organ function were eligible. Adequate organ function was defined as: granulocytes, $\geq 1.5 \times 10^9$/liter; platelets, $\geq 100 \times 10^9$/liter; creatinine, $\leq 1.5 \times$ normal; normal bilirubin; AST, $\leq 2 \times$ normal; and left ventricular ejection fraction, ≥50% as determined by MUGA scan. Written informed consent according to institutional and regulatory requirements was obtained prior to therapy. Ineligibility criteria included more than one prior anthracycline or intercalator; cumulative doses of ≥200 mg/m^2 dox, ≥85 mg/m^2 mitoxantrone, or ≥350 mg/m^2 epirubicin; a history of myocardial infarction within 1 year prior to entry; congestive heart failure; angina; uncontrolled hypertension; active cardiomyopathy or ventricular arrhythmias; prior therapy with a phase I agent; radiotherapy to the left hemithorax or mediastinum ≥4000 cGy or to ≥30% of the marrow bearing skeleton; surgery during the previous 14 days; more than one primary malignancy (except nonmelanoma skin cancer and *in situ* carcinoma of the cervix); and pregnancy.

Prior to therapy complete history, physical examination, tumor measurement, height, weight, and performance status were recorded. Baseline values of complete blood cell count, differential, platelet count, creatinine, uric acid, calcium, phosphorus, total protein, albumin, AST, alkaline phosphatase, bilirubin, and electrolytes were obtained. Urinalysis, electrocardiogram, MUGA scan, chest X-ray, and other imaging procedures to document measurable or evaluable disease were undertaken prior to initiation of therapy.

History, physical examination, clinical tumor measurement, and toxicity assessment were performed prior to each course. Complete blood count, creatinine, alkaline phosphatase, AST, and bilirubin were determined weekly. Complete blood counts were performed twice weekly when the dose escalation resulted in myelosuppression. The baseline biochemistry, urinalysis, and electrocardiogram were repeated prior to each course. MUGA scan was performed after every other cycle. Patients were withdrawn from study if disease progressed, disease was stable for 6 cycles of therapy, or at the patient's request.

Drug Treatment. CsA was supplied by Sandoz Canada (Montreal, Canada) and was constituted in 5% dextrose in water in glass bottles and protected from light. Nonpolyvinyl chloride containing i.v. tubing was used, and the 24-h dose was apportioned into three 8-h portions administered by infusion pump. The CsA infusion was maintained for 6 days. Dox, which was purchased from Adria Laboratories Canada (Mississauga, Canada), was administered as a 10-min infusion 24 h after initiation of the CsA infusion. The first cohort of patients was entered at 5 μg/kg/min (7.2 mg/kg/d) CsA with 30 mg/m^2 of dox. The study plan was to increase the CsA dose rate until the targeted steady state plasma level of 2000 ng/ml was achieved or the MTD was reached. This was then followed by increases in the dose of dox by 15-mg/m^2 increments. At least 3 new patients were treated at each dose level. Patients were entered on the next dose level only after 3 patients were treated and followed for 3 weeks at each new dose level and no evidence of dose-limiting toxicity was seen. Dose-limiting toxicity was defined as granulocyte nadir $\leq 0.5 \times 10^9$/liter and/or platelet count $\leq 50 \times 10^9$/liter and/or grade 3 or 4 other toxicity (using National Cancer Institute common toxicity criteria) and/or a decrease in left ventricular ejection fraction ≥10% from baseline. The MTD of CsA combined with dox was that dose rate of CsA and dose of dox which produced dose-limiting toxicity in at least one-half of the patients treated at that dose level.

Pharmacokinetic Studies. Each new patient at each new dose level underwent plasma sampling for pharmacokinetic evaluation. Blood was obtained from the arm opposite that of drug administration prior to drug infusion and at 30 and 60 min and 2, 4, 8, 10, 22, 24, 24.17, 24.33, 24.50, 24.67, 25, 26, 28, 30, 32, 34, 44, 48, 72, 96, and 120 h after the start of the CsA infusion. Beginning 24 h after the start of the CsA infusion, samples were collected for both CsA and dox determination. CsA samples were collected in EDTA-containing vacutainer tubes and frozen at −20°C until analysis of whole blood. Dox samples were collected in EDTA-containing vacutainer tubes and kept on ice until centrifuged, and plasma was separated and frozen at −20°C until analysis. Time until plasma separation was <30 min, and samples were protected from light throughout.

CsA concentrations in whole blood were determined using a solid phase extraction and sensitive high-pressure liquid chromatography assay reported previously (17). The lower limit of sensitivity was 25 ng/ml, and day to day variation averaged <8%. Dox and doxol plasma concentrations were determined using a solid phase extraction and a sensitive high-pressure liquid chromatography assay as reported previously (18). The lower limit of sensitivity for dox was 20 ng/ml, and for doxol it was 5 ng/ml. Coefficients of variation for the parent and metabolite determination were <10% at the lower limit of sensitivity, and day to day variation was 5%.

Data Analysis. All concentration *versus* time data were analyzed by customary compartmental model equations using the ADAPT II nonlinear fitting program (19) modified to run on a Macintosh computer. Optimal fit was defined via the minimized sum of squares according to the F test (20) and the Aikaike criteria (21).

Curve fitting of the CsA concentrations indicated that a one-compartment model adequately described the blood levels. The resulting fits permitted individual patient estimates to be obtained for the elimination rate constant, V_{ss}, and CL_{tb}. The CL_{tb} was identified from the R_o and the best computer estimate of the C_{ss}:

$$CL_{tb} = \frac{R_o}{C_{ss}} \quad \text{(A)}$$

The dox concentrations following administration could best be described by a triexponential equation indicative of a three-compartment model, while the appearance of the doxol was best fit by a one-compartment model. The computer program permitted the determination of AUC to infinity and the AUMC to infinity.

Dox CL_{tb} was then calculated as:

$$CL_{tb} = \frac{\text{Dose}}{AUC_{(0-\infty)}} \quad \text{(B)}$$

the dox MRT as:

$$\text{MRT} = \frac{AUMC_{(0-\infty)}}{AUC_{(0-\infty)}} \quad \text{(C)}$$

and dox V_{ss} as:

$$V_{ss} = \frac{[\text{dose} \times AUMC_{(0-\infty)}]}{[AUC_{(0-\infty)}]^2} \quad \text{(D)}$$

The ratio of doxol to dox AUC was determined as:

$$\frac{[\text{doxol}\, AUC_{(0-\infty)}]}{[\text{dox}\, AUC_{(0-\infty)}]} \quad \text{(E)}$$

Percentage decrease in NEUT was calculated as:

$$\%\ \text{decrease in NEUT} = \frac{\text{Pretreatment NEUT} - \text{nadir NEUT}}{\text{Pretreatment NEUT}} \times 100 \quad \text{(F)}$$

Analysis of variance was used to determine any differences in outcomes due to the changes in dox/CsA dosages. The relationship between pharmacokinetic and clinical variables was evaluated using Spearman's nonparametric correlation coefficient. Linear regression analyses with the logarithm of (nadir/baseline) WBC, neutrophils, and platelets as the dependent variables and dox AUC, doxol AUC, dox CL_{tb} as the independent variables were performed. A probability of <0.05 was considered significant.

RESULTS

Twenty-three patients were entered into the trial, and 40 cycles were evaluable for toxicity. Pharmacokinetic parameters were determined during 23 courses. The patient characteristics are listed in Table 1. The dose rates of CsA used in this study were 5 μg/kg/min (7.2 mg/kg/d) and 6 μg/kg/min (8.64 mg/kg/d), and the dose levels of dox were 30, 40, and 45 mg/m^2. Dose level of CsA and dox are listed in Table 2. Twelve, 7, 7, and 14 courses of treatment were administered to 6, 5, 5, and 9 patients at dose levels 1, 2, 3, and 4, respectively. Dox dose escalation from 30 to 45 mg/m^2 was performed according to protocol and then decreased to 40 mg/m^2 when it was determined that dose-limiting toxicity was reached at 45 mg/m^2. The MTD was 45 mg/m^2

Table 1 *Patient characteristics*

N = 23; mean age, 51.0 years; range, 37–74 years.

Characteristic	No. of patients
Sex (M/F)	12/11
Eastern Oncology Cooperative Group performance status	
0	1
1	16
2	6
Primary site	
Breast	7
Colon	5
Kidney	5
Lung	2
Other	5
Prior therapy	
Chemotherapy	17
Anthracycline	8
Hormone	7
Immunotherapy	2
Radiotherapy	7
Surgery	23

Table 2 *Hematological toxicity during first treatment course*

Dose Level	Drug dose (CsA:dox; μg/kg/min: mg/m²)	No. of patients	Median WBC nadir (range)	Median NEUT nadir (range)	Median platelet nadir (range)
1	5,30	6	4.55 (2.2–5.1)	3.09 (1.1–3.38)	288 (197–362)
2	6,30	5	7.9 (6.7–11.8)	6.1 (4.62–10.58)	302 (155–539)
3	6,40	3	1.8 (1.6–2.3)	0.69 (0.6–1.17)	132 (106–151)
4	6,45	9	0.8 (0.4–11.5)	0.62 (0.6–9.09)	133 (61–297)

dox when combined with CsA at a dose rate of 6 μg/kg/min. Dose reduction of dox was required in 2 patients from 45 to 40 mg/m² after 4 cycles for one patient and after one cycle for the other. Thirteen patients received one course of therapy only, 8 received 2 courses, one received 5 courses, and one received 6 courses. One patient with small cell lung cancer which recurred after a complete response to cyclophosphamide, dox, and vincristine alternating with etoposide and cisplatin remained stable for 5 months during treatment. One patient with breast cancer who had received prior cyclophosphamide, epirubicin, and fluorouracil treatment and progressed remained stable for 5 months during treatment.

Hematological toxicity is summarized in Table 2. There was no significant change in nadir WBC, neutrophils, or platelets when the CsA dose rate was increased from 5 to 6 μg/kg/min. There was a significant decrease in WBC and neutrophil nadir when the dox dose was increased. Dose-limiting toxicity was neutropenia with 5/7 cycles of dox 40 mg/m², and 12/14 cycles at 45 mg/m² were associated with neutropenia ($\leq 1000 \times 10^6$/liter). The median duration of neutropenia ($\leq$1000) was 4 and 8 days at 40 and 45 mg/m², respectively. Thrombocytopenia was mild and did not limit therapy. No courses of therapy were delayed because of hematological toxicity. Sepsis requiring hospitalization and antibiotics occurred in one patient at 6 μg/kg/min CsA with 45 mg/m² dox. Fever without documented infection was observed in 3 patients.

Nonhematological toxicity, summarized in Table 3, did not appear to be dose related. Grade 2 to 3 nausea and vomiting occurred starting on day 2 of CsA treatment and persisted until the cessation of the infusion despite antiemetic therapy with prochlorperazine, lorazepam, and Decadron. Weight gain secondary to fluid retention was common but reversed when the CsA infusion was stopped. In three patients who had effusions or massive edema and creatinine clearances of 1 ml/s, treatment was stopped prematurely because of a decrease in renal function. In each case renal function returned to baseline soon after cessation of CsA infusion. Serum bilirubin, creatinine, and creatinine clearance at the initiation of therapy, on the last day of CsA infusion, and at the end of the first cycle of therapy are shown in Table 4. Serum alkaline phosphatase and AST did not change from day 1 to day 6. There was a small increase in mean serum creatinine and a decrease in creatinine clearance from day 1 to 6 during the first cycle which was not clinically significant. Bilirubin elevations occurred in patients receiving infusion rates of either 5 or 6 μg/kg/min CsA and increased during the infusion period but returned to normal after cessation of the CsA. Fig. 1 shows the time course for bilirubin elevation and decline during cycle 1 for all patients. No significant change in alkaline phosphatase or AST was observed (data not shown).

The median pharmacokinetic parameters derived from the curve fits for CsA and dox for all 23 patients (according to dose levels of CsA and dox) are summarized in Table 5. There was no significant difference in CsA CL_{tb} or C_{ss} at the 4 dose levels studied. Dox CL_{tb} varied from 0.76 to 5.56 liters/h/m² with no detectable difference between 30, 45, and 45 mg/m² dose levels. Dox MRTs ranged from 24.3 to 138 h with a median of 58.2 h and were similar at each dose level (data not shown). The $AUC_{0\text{-INF}}$ for dox and doxol showed a 10-fold variation over the dose range studied. No significant difference in dox or doxol AUC between dose levels of dox were observed. The median ratio of AUC_{doxol}/AUC_{dox}, based on area calculations to time infinity, was 0.255. If the dox and doxol areas were truncated to the last times at which concentrations were available for both compounds, the median ratio of areas was 0.143. Fig. 2 shows the dox and doxol concentrations for patients receiving the 6:40 CsA:dox treatment regimen. The dox and doxol data for each patient were simultaneously fit to the composite compartmental model shown. Thereby, estimates were obtained for all individual mass transfer constants and the $V1$. However, since the $V4$ could not be unambiguously differentiated because the fraction of dox converted to doxol (f) was also unknown, $V4$ was assigned the value of 1 liter/m². This strategy permitted a fitted estimate to be obtained for f. The solid line in the figure represents the average of the concentrations associated with individual patient fitted lines, and the dotted lines represent ±1 SD.

Relationships among the primary pharmacokinetic parameters and the clinical variables were explored. The association between % change in serum creatinine, creatinine clearance, bilirubin, and neutrophils and CsA CL_{tb} and dox CL_{tb} were determined. The Spearman's nonparametric correlation coefficients for this analysis were not significant for the selected parameters other than a significant inverse relationship between % change in serum creatinine and the % change

Table 3 *Cycle 1 nonhematological toxicity (N = 23)*

Toxicity	Grade[a] 1	2	3	4
Alopecia	4	11	0	0
Anemia	1	5	2	1
Bilirubin	0	3	2	4
Creatinine	3	4	0	0
Diarrhea	2	0	0	0
Edema	0	3	0	0
Fever	0	2	0	0
Nausea	3	14	3	0
Headache	1	2	0	0
Sepsis	0	0	0	1
Stomatitis	2	1	0	0
Vomiting	4	11	0	1

[a] National Cancer Institute common toxicity criteria.

Table 4 *Biochemical toxicity (N = 23)*

Toxicity	Baseline	Day 6	End of Course 1
Creatinine (μmol/liter)	84.3 (17.3)[a]	101.2 (30.9)	88.8 (22.5)
Creatinine clearance (ml/s)	1.56 (0.45)	1.25 (0.41)	1.33 (0.46)
Bilirubin (μmol/liter)	10.0 (4.1)	40.4 (38.8)	10.3 (4.8)

[a] Mean (SD).

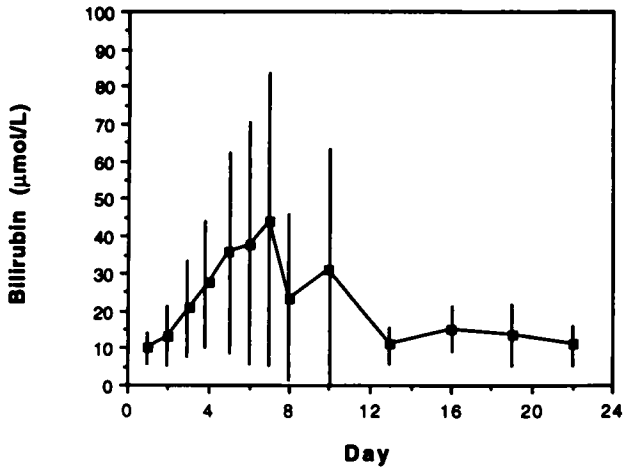

Fig. 1. Mean (*points*) bilirubin concentrations (±SD, *bars*) as a function of time.

in neutrophils (P = 0.006). In particular, there was no association between CsA CL_{tb} or C_{ss} and % change in bilirubin, WBC, or NEUT. Similarly, there was no relationship between dox CL_{tb} and change in bilirubin. Analysis using linear regression with logarithm of (nadir/baseline) WBC, neutrophils, or platelets as the dependent variables and dox *AUC*, doxol *AUC*, or dox CL_{tb} as independent variables revealed a borderline significant association between dox CL_{tb} and neutrophils (P = 0.04, not adjusted for multiple testing). No other significant associations were found.

DISCUSSION

We undertook a phase I trial of CsA combined with dox in order to define a schedule and the doses of both drugs which would reverse multidrug resistance and still be clinically tolerable. It was our premise that whole blood CsA levels of at least 2000 ng/ml would be necessary to reverse resistance because *in vitro* concentrations required to reverse resistance in cell lines were in approximately 1000 ng/ml. Since CsA distributes into the RBC, we predicted that whole blood levels of 2000 ng/ml would achieve 1000 ng/ml in plasma (22–24). Whether higher blood concentrations of CsA would increase the magnitude of p-glycoprotein inhibition is not clinically clear. Furthermore, we reasoned that such concentrations of CsA should be present for at least 5 half-lives of dox since the inhibition of the p-glycoprotein pump by CsA may be reversible when CsA is removed *in vitro*. Since dox undergoes extensive hepatic metabolism, with biliary excretion, and CsA had the potential to interfere with either of these steps of dox disposition or reverse p-glycoprotein function in normal tissue, significant drug interaction (25) was possible. Hence, we selected a low starting dose of dox with the plan of escalating to the maximally tolerated dose after achieving the target CsA blood level. As it was thought that patients entered into this trial would have already progressed while receiving anthracycline-based treatments or had tumors unresponsive to anthracyclines, a course of treatment with dox alone was not given initially.

The characteristics of the 23 patients entered into the study listed in Table 1 are typical of phase I trial populations. Six patients had no prior chemotherapy, 7 had one prior chemotherapy, 8 had 2 prior chemotherapies and 2 had 3 prior chemotherapies. Of note, 8 patients had prior anthracycline therapy. An infusion rate of 6 μg/kg/min CsA achieved the targeted whole blood CsA level of 2000 ng/ml, but only 40–45 mg/m^2 of dox could be given with this infusion of CsA (Table 2). Although sepsis requiring hospitalization occurred in only one patient, significant neutropenia occurred at a dox dose of 45 mg/m^2 in patients with minimal prior chemotherapy. Thus, the dose of 40 mg/m^2 was evaluated and found to be tolerable. Possible explanations for the increased granulocytopenia seen when CsA was combined with low doses of dox include a drug interaction between CsA and dox (*vide infra*) and/or the reported presence of p-glycoprotein on CD34+ marrow precursor (26).

The major nonhematological toxicity consisted of nausea and vomiting (Table 3). This appeared to be related to CsA because it persisted throughout the infusion but resolved rapidly when CsA administration was stopped. Standard antiemetic therapy used in this trial did not effectively ameliorate this side effect. Although edema was not a major problem *per se*, weight gain was common during the infusion of CsA. This was rapidly reversed after the infusion was completed with an associated diuresis. The decreased blood flow associated acutely with CsA administration may underlie this clinical observation. Furthermore, the need to stop treatment in patients with preexisting edema or effusions because of an associated decrease in renal function is consistent with this effect of CsA and indicated that such patients should not be treated with this combination. The effects on serum creatinine and creatinine clearance were small and reversible (Table 4), demonstrating that the high drug doses used do not cause clinically important nephrotoxicity. However, patients with edema and effusion with borderline renal function should not be considered for this treatment.The increase in bilirubin that we observed is similar to that observed by Yahanda *et al.* (27) using a higher CsA infusion rate for a shorter duration. Despite the frank jaundice observed, it was readily reversed (Table 4, Fig. 1). The mechanism of this effect may be due to inhibition of bilirubin excretion and is consistent with the reports (28) suggesting that at high concentrations of bilirubin p-glycoprotein may extrude bilirubin. However, other possible actions of CsA on bilirubin metabolism and bile flow cannot be ruled out.

The observation that 2 patients, one with small cell cancer of the lung and one with breast cancer, were stable while receiving this treatment is intriguing. Both had shown progression prior to starting

Table 5 *Median pharmacokinetic parameters*

Drug dose (CsA:dox; μg/kg/min:mg/m^2)	CsA CL_{tb} (liters/h/kg)	CsA Css (μg/mL)	Dox CL_{tb} (liters/h/m^2)	Dox MRT (h)	AUC_{dox} (μg/h/ml)	AUC_{doxd} (μg/h/ml)	$\frac{AUC_{doxol}}{AUC_{dox}}$
5:30	0.096	2.99	1.35	72.3	22.1	4.99	0.196
(6)[a]	(0.093–0.164)[b]	(1.84–3.90)	(1.09–4.14)	(26.8–117)	(7.24–27.5)	(1.42–8.98)	(0.177–0.406)
6:30	0.268	1.61	1.85	49.0	16.2	4.13	0.336
(5)	(0.172–0.333)	(1.22–2.57)	(1.69–3.16)	(26.1–13.6)	(9.50–17.8)	(2.29–5.57)	(0.140–0.512)
6:40	0.151	2.20	3.17	64.8	13.2	4.04	0.308
(3)	(0.147–0.183)	(1.78–2.44)	(1.19–5.56)	(30.3–93.1)	(7.19–33.6)	(2.62–8.44)	(0.198–0.472)
6:45	0.159	2.27	2.42	48.3	20.0	2.10	0.100
(9)	(0.107–0.221)	(1.63–3.28)	(0.76–4.65)	(24.3–138)	(9.68–59.3)	(0.87–6.16)	(0.057–0.634)
Total	0.164	2.21	1.86	58.2	16.1	4.08	0.225
(23)	(0.093–0.333)	(1.22–3.90)	(0.76–5.56)	(24.3–138)	(7.19–59.3)	(0.872–8.98)	(0.057–0.636)

[a] Number of patients.
[b] Range.

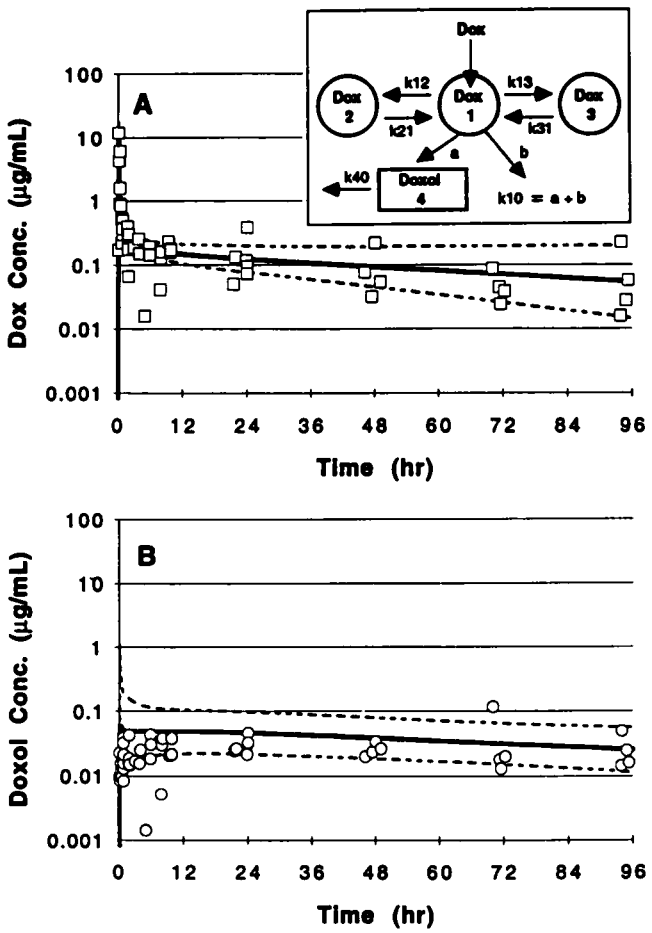

Fig. 2. Plot of dox (□) and doxol (○) in patients being treated with CsA (6 μg/kg/min) and dox (40 mg/m²). *Solid lines*, means of the individual patient fits to the composite model shown. *Dotted lines* enclose the region associated with the mean ± 1 SD. The parameters for the solid lines are: $V1$ = 1.33 liters/m²; $V4$ = 1 liters/m² (assigned value); $k12$ = 5.12/h; $k21$ = 1.18/h; $k13$ = 9.73/h; $k31$ = 0.068/h; $k10$ = 1.13/h; $k40$ = 0.041/h; $f = a/k10$ = 0.0082.

this therapy. The tumor in the patient with small cell lung cancer had recurred after treatment with anthracycline, and disease in the patient with breast cancer had progressed during treatment with anthracycline containing combination chemotherapy. Neither patient had biopsies for p-glycoprotein because this was not a component of the phase I trial. Nevertheless, the stability of disease with very low but maximally tolerated doses of dox is encouraging.

The infusion schedule of CsA which we chose resulted in a median C_{ss} for CsA of 2210 ng/ml when dox was administered and was maintained during the full period of the infusion (Fig. 2). However, we observed a wide interindividual variation in C_{ss} for CsA (Table 5) similar to other reports of CsA pharmacokinetics in patients undergoing transplantation (24). At this time the relationship between blood CsA level or CsA duration of exposure and *in vivo* inhibition of p-glycoprotein function is unclear. If the magnitude of inhibition of P-glycoprotein is dose dependent, then higher blood levels may be necessary. Thus, the clinical impact of the wide variation of CsA C_{ss} on resistance modulation must await results of phase II studies relating response to C_{ss} for CsA. The CsA CL_{tb} of 0.177 liters/h/kg was less than reported in patients undergoing transplantation. This may be due to several factors, including the difference in the underlying medical condition, the age of the patient, and the coadministration of other drugs. Nevertheless, this raises the possibility that dox may alter the disposition of CsA. Further studies will be necessary to delineate the significance of this observation.

Dox CL_{tb} and AUC in our patient population showed a wide interpatient variation similar to that of CsA but was less than reported previously (18, 29–35). The wide interpatient variability and the small numbers of patients treated at each dose levels do not allow us to detect a significant difference in AUC with increasing dose. There was a marked decrease in dox CL_{tb} in our study when compared to that published previously. It is estimated that clearance of dox was 10-fold less than predicted. Furthermore, there was a lower than expected median ratio of AUC_{doxol}/AUC_{dox} (mean 0.255) when compared to 7 reported series (range, 0.326–0.658). Areas ratios based on extrapolations to time infinity may be sensitive to the estimated terminal half-life. However, even when truncated areas were used, based on calculations up to the last time at which concentrations were available for dox and doxol, the median ratio was only 0.143. Furthermore, as illustrated in Fig. 2, there was no evidence that the terminal doxol half-life was longer than that for dox and, thus, no compelling indications that the doxol area to infinity had been underestimated. The decreased ratio may be due to decreased dox excretion or metabolism, decreased doxol formation, altered doxol excretion or metabolism, or a combination of these factors. Because renal excretion of dox and its metabolites accounts for <10% of the total drug over 5 days (36), it is unlikely that the lower than expected dox CL_{tb} and AUC_{doxol}/AUC_{dox} are related to an effect on renal excretion. The elevation of bilirubin with CsA could be proposed as another factor contributing to the decreased dox clearance since dox undergoes biliary excretion. Decreased biliary excretion of dox and doxol metabolites may explain in part the observed result. However, if one assumes that decreased biliary excretion is the sole factor, it follows that the biliary excretion of doxol metabolites must be affected to a lesser degree than that of dox metabolites for which there is no evidence. Furthermore, the lack of association between dox CL_{tb} and peak bilirubin or % change bilirubin suggests that hyperbilirubinemia is not sufficient in itself to explain the decreased dox CL_{tb}. On the other hand, a decreased formation of doxol is consistent with decreased dox CL_{tb}, suggesting that the interaction of CsA with dox is at a metabolic step. In order to delineate the interaction between CsA and dox further, we analyzed our data for an association between C_{ss} or CL_{tb} of CsA and dox CL_{tb}. No such association was found. The failure to find such associations may be due to patient-specific effects of CsA on the metabolism of dox. We do not have intrapatient information at different CsA levels to identify such relationships. At present our results are consistent with CsA altering either dox metabolism or excretion or both. Nevertheless, the fact that we observed substantial neutropenia at low dox doses is consistent with the higher exposure to dox because of lower clearance.

The exploratory analysis of relationship between pharmacokinetic parameters and clinical parameters identified two significant associations. A negative association between % change NEUT and % change serum creatinine was found. It is probable that this result occurred by chance because there is no underlying biological basis one can propose for such an association. The association between log (nadir/baseline) neurophils and dox CL_{tb} is consistent with the clinical observation that a low dose of dox causes significant neutropenia. The decrease in dox CL_{tb} would lead to increased exposure to drug with its attendant toxicity. The lack of such a relationship between dox AUC and neutropenia may be due to the number of patients available for analysis. Additional clinical and pharmacokinetic information are being collected in ongoing phase II trials of this combination to explore this relationship further.

Others have combined cyclosporin with chemotherapeutic agents which are associated with the MDR phenotype (37–39). Lum *et al.* (37) using VP-16 reported a similar increase in bilirubin as we observed. Furthermore, there appears to be a decrease in VP-16 clear-

ance in the presence of CsA. The recent report by Sonneveld *et al.* (38) of CsA combined with vincristine, dox, and dexamethasone in patients with multiple myeloma holds promise in the therapy of patients refractory to conventional treatment. Interestingly, the investigators report a AUC_{doxol}/AUC_{dox} which is inverse to that observed in our study and in previous reports (18, 29–35). This raises the question of whether the continuous infusion of dox with its lower peak levels of dox in plasma could circumvent any inhibition of dox metabolism. This is particularly intriguing since the dose of the dox used in this trial was the same as that used when given without CsA. Our results taken together with the observations of Sonneveld *et al.* suggest that the interaction between dox and CsA is clinically important from the point of toxicity. Infusion of dox may be a means of circumventing this interaction, but the impact on antitumor efficacy of bolus *versus* infusion is not clear.

In conclusion, we have performed a phase I trial combining the MDR-reversing agent CsA with dox in a pharmacologically designed dose schedule. We observed that this treatment is tolerable with little nephrotoxicity at prolonged high blood levels of CsA but was associated with reversible hyperbilirubinemia. The unexpected marked neutropenia associated with low doses of dox may be due to altered dox metabolism and excretion. Ongoing studies with 6 μg/kg/min CsA and 40 mg/m^2 dox will determine whether such pharmacological modulation can increase dox uptake into patient tumors and lead to an improved therapeutic index.

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