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Effect of high-dose cyclosporine on etoposide pharmacodynamics in a trial to reverse P-glycoprotein (*MDR1* gene) mediated drug resistance

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Abstract *Purpose*: The consequences of using cyclosporine (CsA) therapy to modulate P-glycoprotein-mediated multidrug resistance include increased myelosuppression, hyperbilirubinemia, and altered disposition of the cytotoxin. The purpose of this study was to analyze further the relationship between the degree of leukopenia, and etoposide pharmacokinetic factors. *Methods*: Each patient initially received intravenously-administered etoposide alone (150–200 mg/m^2/d × 3). Later it was given in combination with CsA administered at escalating loading doses (range 2–7 mg/kg) as a 2 hour intravenous (IV) infusion followed by a 3 day continuous infusion, at doses ranging from 5 to 21 mg/kg/day. Serial plasma etoposide concentration-time samples were assayed by high-performance liquid chromatography (HPLC). The area under the curve (AUC) of unbound etoposide was calculated from the total plasma etoposide AUC using a previous published equation [22] where % unbound etoposide = (1.4 × total bilirubin) – (6.8 × serum albumin) + 34.4. The percent decrease in white blood cell (WBC) count and the total or unbound etoposide AUC relationship was fitted to a sigmoid Emax model adapted for paired observations, where:

$$\%\ \text{Decrease in WBC count} = E_{max} \times PDRV^{H+Z\times\delta} / (PDRV_{50} + Z \times \beta) + PDRV^{H+Z\times\delta}$$

In this equation, Z was the variable describing the two treatment groups (0 = no CsA and 1 = CsA). The fitted parameters were $PDRV_{50}$, the pharmacodynamic response variable (PDRV) producing 50% of the maximal response; parameter β, which describes the effect of the treatment group on the $PDRV_{50}$; parameter H (Hill constant), which defines the slope of the response curve and parameter δ, which describes the effect of the treatment group on parameter H. *Results*: CsA at a median concentration of 1,938 μg/ml resulted in a median increase in the total plasma etoposide AUC by 103% and the calculated unbound plasma etoposide AUC by 104%. This paralleled a 12% greater median percent decrease in WBC count during etoposide + CsA treatment (72% vs. 84%, $P = 0.03$). The percent decrease in WBC count and total or unbound etoposide AUC relationship was fitted to the sigmoid Emax model. The model using the unbound etoposide AUC described the data adequately ($r = 0.790$) and was precise, with a mean absolute error of 6.4% (95% confidence interval: −4.9, 7.8). The fitted parameter-estimates suggested that at equivalent unbound etoposide AUC values above 10 μg × h/ml, the sigmoid Emax model predicted a 5% greater WBC count suppression when CsA was added to the treatment regimen. *Conclusion*: These findings suggest that a small degree of the enhanced myelosuppression observed with CsA combined with etoposide might

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be attributable to inhibition of P-glycoprotein in bone marrow precursor cells. However, the majority of the effect observed appears to be due to pharmacokinetic interactions, which result in increases in unbound etoposide.

Key words Cyclosporine · Etoposide · Multidrug resistance · Pharmacokinetics · Pharmacodynamics

Introduction

Etoposide is a semisynthetic podophyllotoxin that is useful in the treatment of a variety of hematologic and solid tumors [18]. The best-characterized mechanism of cellular resistance to etoposide is P-glycoprotein (P-gp) mediated multidrug resistance (MDR), encoded by the *MDR1* gene. In tumor cells, P-gp mediated drug efflux results in decreases in intracellular drug accumulation and cell death [13]. MDR is associated with a broad cross-resistance to a variety of cytotoxic agents including the anthracyclines, the vinca alkaloids, the epipodophyllotoxins, the taxanes, and others [13]. Laboratory studies have shown that a number of drugs such as verapamil and cyclosporine (cyclosporin-A, CsA) may reverse MDR in vitro and in vivo by inhibition P-gp transport [6]. Myelosuppression is the dose-limiting toxicity of etoposide, that has been previously noted to correlate with drug exposure, especially unbound etoposide concentrations [20, 22, 24]. Such concentrations are determined by the patient's serum concentrations of albumin and bilirubin [22, 24]. In our previously reported Phase I clinical trial, we treated patients sequentially with etoposide alone, for one cycle, followed later with etoposide combined with CsA, and found that CsA produced a concentration-dependent decrease in renal and non-renal elimination of etoposide. High CsA concentrations ($>2{,}000$ ng/ml) increased systemic exposure to etoposide by approximately 80% [12]. A second important finding was that CsA caused a concentration-dependent reversible hyperbilirubinemia, a critical factor which determines the degree of etoposide protein binding. When etoposide doses were not reduced during CsA treatment, increased myelosuppression was observed, consistent with the observed pharmacokinetic changes, but perhaps also involving modulation of P-gp in normal bone marrow stem cells or other myeloid precursors [12, 14].

Pharmacodynamic models have been used to relate concentration-effect data to the hematologic toxicity of etoposide [10, 15, 24]. In this study we explore further the relationship of pharmacokinetic variables with the degree of etoposide-induced myelosuppression, and apply pharmacodynamic modeling techniques to show that a small degree of CsA potentiation of etoposide-induced leukopenia may be attributable to effects beyond increases in systemic exposure of etoposide, such as modulation of P-gp in some normal bone marrow cell precursors.

Materials and methods

Patient eligibility and treatment protocol

The treatment protocol for this trial has been previously reported [26]. Patients eligible for study included those who had signed informed consent, meeting Stanford University Medical Center and federal guidelines, to enter a phase I–II trial of CsA [Sandimmune I.V., Sandoz (now Novartis), East Hanover, N.J., USA] and etoposide (Vepesid, Bristol Laboratories, Evansville, Ind., USA) chemotherapy. The patients included those with a confirmed diagnosis of malignancy with no known curative treatment, measurable disease, no active central nervous system metastases, a Karnofsky performance status of greater than 50%, and no chemotherapy or radiation therapy given during the 4 weeks prior to study entry. Additional eligibility criteria included adequate renal, hepatic, and hematologic function as evidenced by: a 24 h urinary creatinine clearance >40 ml/min, a serum bilirubin <1.6 mg/dl, a serum aspartate aminotransferase (AST) level less than three times normal, a white blood cell (WBC) count $>3{,}400/\text{mm}^3$, and a platelet count $>100{,}000/\text{mm}^3$.

Drug treatments and pharmacokinetic studies were performed in the Clinical Research Center of Stanford University Medical Center. Pharmacokinetic studies were performed on the first day of etoposide treatment alone or when combined with CsA. Patients received firstly etoposide (150 or 200 mg/M^2 daily) for three consecutive days as a 2 h intravenous infusion in 0.5–1.0 l of 0.9% saline solution. The initial dose of etoposide was reduced to 150 mg/M^2 at the discretion of the investigator if the patient had a history of extensive prior radiation therapy or hematologic intolerance following chemotherapy. Treatment courses were repeated every 21 days, providing blood counts recovered to prescribed levels. Patients exhibiting disease progression or stabilization following one or two cycles of etoposide received CsA as an infusion in combination with etoposide (etoposide + CsA). The CsA was administered at escalating loading doses (range 2–7 mg/kg) given as a 2 h intravenous infusion followed by a 3 day continuous infusion at doses ranging from 5–21 mg/kg day. Detailed criteria for disease response, dose modification of etoposide, dose escalation of CsA, and toxicity grading have been previously published [26].

Complete blood counts were performed before treatment and every 10 to 12 days after a dose of etoposide. Prior to etoposide administration, a 24 h urinary creatinine clearance, and serum levels of AST, albumin and total bilirubin were determined. During courses of combined etoposide + CsA chemotherapy, urinary creatinine clearance and serum levels of creatinine and bilirubin were measured daily.

Etoposide sampling and analysis

For the determination of total plasma etoposide concentration, blood samples were collected in heparinized glass tubes at ten serial time points: prior to administration, at the end of infusion, and at 0.5, 1, 2, 4, 6, 12, 18 and 22 h after the end of infusion. Blood samples were centrifuged and plasma was separated. Plasma samples were then frozen at −70 °C in polypropylene-capped tubes until analysis. Total parent etoposide in plasma was extracted and assayed, with minor modifications applied to the high-performance liquid chromatography (HPLC) as previously described [8, 12]. The lower limit of quantification was 1 μg/ml. The intra-day and inter-day coefficient of variations at 10 μg/ml were 5.2% and 3.3% and at 2.5 μg/ml, were 9.2% and 9.7%, respectively.

Cyclosporine sampling and analysis

For CsA-level determinations, blood samples were collected in heparinized glass tubes, prior to the loading dose, at the end of the 2 h loading dose, before each etoposide dose, and at the end of the CsA infusion. These samples were frozen in polypropylene-capped

tubes and stored at −70 °C. The CsA plasma levels were analyzed by the use of a nonspecific fluorescence-polarization immunoassay method (TDx, Abbott Laboratories, North Chicago, Ill., USA) with a sensitivity of 40 ng/ml and an interassay coefficient of variation of less than 10% [11].

Pharmacokinetic and pharmacodynamic analyses

Total plasma etoposide was calculated from the area under the curve (AUC) extrapolated to infinity by the linear trapezoidal rule, for increasing plasma concentrations and by the logarithmic trapezoidal rule for decreasing plasma drug concentrations [4] (MKModel, Version 3.36, Biosoft, Cambridge, UK). The terminal elimination rate constant (Kz), used for extrapolations of the AUC to infinity (last concentration time point/Kz), was estimated by unweighted least squares regression analysis of the terminal phase of the etoposide plasma concentration-time data, using a minimum of three concentration-time points. The percent unbound etoposide was calculated using the equation of Stewart et al. [22] where: % unbound etoposide = (1.4 × total bilirubin) − (6.8 × serum albumin) + 34.4. The AUC of unbound etoposide was estimated by multiplying the % unbound by the total AUC.

Etoposide pharmacokinetic data used for analyzing the relationship between systemic exposure and the degree of leukopenia included the AUCs for total plasma and unbound etoposide, the total AUC greater than 2 μg/ml concentration, and the duration of time at greater than 2 μg/ml total etoposide concentration. The use of the latter two variables was based on previous observations that etoposide concentrations above 2 μg/ml contribute to etoposide-induced leukopenia [5]. Initial correlations between pharmacokinetic parameters to the degree of etoposide-induced leukopenia were assessed using Spearman's rank correlation coefficients.

The percent decrease in WBC count was calculated according to the following equation:

$$\%\ \text{Decrease} = [(\text{pretreatment count} - \text{nadir count})/\text{pretreatment count}] \times 100$$

The relationship between percent decrease in WBC count and etoposide pharmacodynamic response variable (PDRV) was modeled using the classical sigmoid Emax model [2, 16] as previously reported for etoposide [10, 15, 24].

Where the % decrease in blood cell count =

$$E_{max} \times PDRV^{H}/PDRV_{50} + PDRV^{H}$$

In this equation, E_{max} is the maximal effect; H is a parameter describing the steepness of the sigmoidal response curve; PDRV is the pharmacodynamic response variable (i.e., AUC or concentration) and $PDRV_{50}$ is the value of PDRV at 50% of the maximum effect. For this study, the above equation was modified to model differences in paired observations for overall group effect and specific treatment differences [7] (CsA and no CsA), where:

$$\%\ \text{Decrease in WBC count} = E_{max} \times PDRV^{H+Z\times\delta}/(PDRV_{50} + Z \times \beta) + PDRV^{H+Z\times\delta}$$

In this equation, E_{max} was fixed at 100% and Z was the variable describing the two treatment groups (0 = no CsA and 1 = CsA). The fitted parameters were $PDRV_{50}$, the PDRV producing 50% of the maximal response; parameter β, which describes the effect of the treatment group on the $PDRV_{50}$; parameter H (Hill constant), which defines the steepness of the response curve and parameter δ, which describes the effect of the treatment group on parameter H.

In this model, significant positive values for δ would then suggest that CsA would be additive to the value of H, which would reflect a steeper ascent to 100% effect, and significant positive values for parameter β would suggest that CsA would be additive to and contribute to a larger $PDRV_{50}$ value.

Parameter-estimates were determined using unweighted nonlinear regression analyses (PCNONLIN, version 4.2, SCI Software, Lexington, Ky.). In the modeling, since the number of fitted parameters in relation to the number of observations was limited, we selected to estimate the parameters in serial phases. In the first phase, the data were initially fitted to the model to determine initial estimates for all four parameters. During the second phase, we serially assessed a final estimate for each parameter, while fixing the other parameters. Firstly, we estimated $PDRV_{50,}$ while fixing H, β, and δ at the initial estimates. Then, this process was repeated for β, then H, and finally δ.

Statistical analyses

Patients' clinical and pharmacokinetic data were tested for normal distribution using the Wilk-Shapiro statistic and were found to be not normally distributed (data not shown) [21]. Thus they were summarized as medians, and variability around the median was presented as the 25th to 75th percentile (interquartile) ranges. Paired patient data were compared using the Wilcoxon sign-rank test. The Mann-Whitney *U* test was used for within-group (unpaired data) comparisons. The a priori level of significance was $P < 0.05$ (two-tailed).

Initial correlations between pharmacokinetic parameters to the degree of etoposide-induced leukopenia were assessed using Spearman's rank correlation coefficients. The performance of the pharmacodynamic model was evaluated by determining precision and bias. Bias, expressed as the mean error of the estimates, is the mean of (predicted value–actual value). Since the mean error can have negative or positive values, it is used to assess whether the model has a tendency to under- or over- predict actual values. The 95% confidence intervals (95% CI) for mean error, that include zero indicate no significant bias [16]. Precision was expressed as the mean absolute error of the estimates, which is the absolute value of (predicted value–actual value). Significance of the effect of estimates of parameters δ and β in the model were determined by 95% CI. In the model, intervals that include zero would indicate that these parameters had no significant effect.

Results

Fifteen patients (eight male, seven female) were studied during paired courses of etoposide and etoposide + CsA chemotherapy. At study entry, the median age of the patients was 54.6 years (range 36–73) with a median Karnofsky performance status of 88% (range 70–100%). All but one had solid tumors. Five patients had liver metastases, one had had a right hepatic lobe resection and recurrent liver metastases, and one patient had biopsy-proven alcohol-related liver cirrhosis. In all but one patient, previous therapy had been myelotoxic, and had included chemotherapy in ten patients, four had had chemotherapy plus radiation therapy (which had included the pelvis or mediastinum), one had received radiation treatment which included the mediastinum, and one patient had undergone hormonal therapy.

Patient-characteristics, from the time of initiation of etoposide alone to etoposide + CsA treatment courses, showed no significant differences in Karnofsky performance status, serum levels of albumin, bilirubin or creatinine, 24 h urinary creatinine clearance values, or body weight. The median value for CsA concentration was 1,938 μg/ml (range 297–5,073) and peak serum bilirubin concentration was 1.6 mg/dl (range 0.2–4.5) during the 3 day infusion of CsA.

Table 1 lists the median values for pharmacokinetic, laboratory, and WBC count data during the first day of treatment with etoposide or etoposide + CsA. The CsA administration resulted in an average increase in total plasma etoposide AUC of 103% when adjusted for differences in dosing ($P = 0.001$). Despite patients having received a smaller median etoposide dose when combined with CsA, their calculated unbound plasma etoposide AUC increased by 104%. Other concentration-dependent pharmacokinetic data showed a similar trend, which correlated with the 50% decline in median etoposide clearance during CsA administration. The increase in systemic exposure to etoposide resulted in a lower median WBC count nadir during etoposide plus CsA treatment (1.1 vs. 2.5×10^3 cells/mm^3, $P = 0.003$) and a greater median percent decrease in WBC count (72% vs. 84%, $P = 0.03$) when compared with treatment with etoposide alone.

Table 2 lists the values for the Spearman's rank correlation coefficients for the relationship between the pharmacokinetic data and the degree of etoposide-induced leukopenia. The AUC for the unbound drug showed the highest correlation for etoposide alone or when combined with CsA, with r-values of 0.395 and 0.490, respectively. The total etoposide variables showed poor correlations with the degree of leukopenia ($r = 0.186$ for etoposide AUC alone and $r = 0.095$ for etoposide AUC + CsA).

The relationship between the etoposide AUC and the percentage decrease in WBC count was fitted to the sigmoid Emax model, modified to discriminate between differences in paired observations for overall group effect and specific treatment differences [7]. The use of the total plasma etoposide AUC as a variable in the model, described the degree of myelosuppression poorly, as evidenced by a large sum of square residuals and a poor correlation between predicted and actual % decrease in WBC values (sum of squared residuals = 4,883, $r = 0.45$). However, when the calculated unbound etoposide plasma AUC (time 0 to infinity) was used in the model, the sum of squared residuals in the regression decreased when compared with the use of the total etoposide AUC (from 4,883 to 1,665), along with a better correlation between predicted and observed % decrease in WBC values ($r = 0.790$, Table 2, Fig. 2).

Table 2 Spearman's rank correlation coefficients of pharmacokinetic data and etoposide-induced leukopenia during treatment with and without cyclosporin-A (CsA) (m^2 square meter body surface area, *AUC* area under the curve)

Parameter	Etoposide	Etoposide/CsA
AUC (Total, 0 to infinity)	0.186	0.095
AUC (Total, ≥2 μg/ml)	0.095	0.095
Time (h, ≥2 μg/ml, total)	0.141	0.165
Clearance (total, l/h/m^2)	0.236	0.172
AUC (Unbound, 0 to infinity)	0.395	0.490
AUC (Unbound, ≥2 μg/ml)	0.297	0.154

Table 2 outlines the results of the predictive performance calculations for the model using the AUC for unbound etoposide. The model was not biased, since the 95% CI of the mean error included zero (95% CI: −3.0, 2.7), and was precise, with a mean absolute prediction error of 6.4% (95% CI = 4.9, 7.8). The 95% CI of the estimate for the regression parameter β, ranged from −1.23 to 1.31. Since this range included the value zero, this suggested that CsA had no significant influence on the AUC_{50} value. The parameter δ had an estimate of 0.228 (95% CI = 0.219 to 0.236) suggesting CsA significantly increased the value of H, the Hill coefficient, suggesting a steeper response curve for etoposide plus CsA (Fig. 1).

When the sigmoid Emax model was used to calculate the percent WBC decrease for etoposide with and without CsA at equivalent unbound etoposide AUC values for comparison, the addition of CsA led to a predicted median 5% greater WBC count suppression (74% versus 79%, $P = 0.0007$), that is depicted in Fig. 3.

Table 1 Comparison of pharmacokinetic data and etoposide-induced leukopenia with and without cyclosporin-A (CsA) treatment (m^2 square meter body surface area, *AUC* area under the curve, *WBC* white blood cell)

	Etoposide	Etoposide/CsA	*P* value[a]
Pharmacokinetic data			
Dose (mg/m^2)	200 (150–200)	150 (150–200)	0.060
AUC (per 100 mg/m^2 dose)	74 (65–93)	150 (103–176)	0.001
AUC (0 to infinity)	144 (103–185)	220 (147–352)	0.003
AUC (≥2 μg/ml)	143 (98–171)	199 (136–359)	0.016
Time (h ≥2 μg/ml)	17.0 (12.0–22.5)	23.5 (18.4–27.0)	0.002
Clearance (l/h/m^2)	1.35 (1.08–1.53)	0.67 (0.57–0.97)	0.001
AUC (unbound, 0 to infinity)	12.9 (10.9–17.7)	26.0 (14.9–33.3)	0.002
AUC (unbound ≥2 μg/ml)	12.2 (8.2–16.4)	24.5 (15.5–34.8)	0.007
Percent unbound	10.3 (6.8–11.4)	12.7 (6.7–16.1)	0.029
Laboratory data			
WBC nadir (×10^3/mm^3)	2.5 (1.0–2.8)	1.1 (0.5–1.9)	0.003
% WBC decrease	72 (60–83)	84 (72–94)	0.030
Albumin (mg/dl)**	3.6 (3.5–4.1)	3.8 (2.9–4.2)	0.563
Bilirubin (mg/dl)**	0.3 (0.3–0.4)	1.0 (0.5–2.2)	0.001

[a] Wilcoxon sign-rank, 2-sided

** Values obtained during the first day of etoposide treatment. Etoposide and etoposide/CsA values are given as median (25th to 75th percentile range)

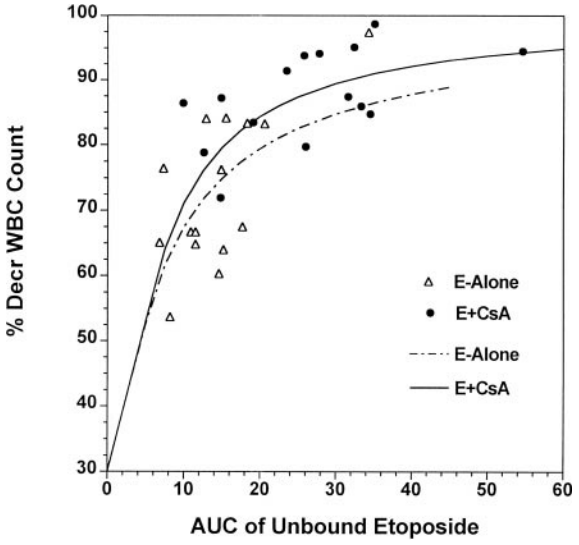

Fig. 1 Sigmoid Emax model fit of the AUC (0 to infinity) of unbound etoposide and % decrease in WBC count

Discussion

Our previous phase I trial demonstrated an enhanced degree of leukopenia when CsA was added to etoposide chemotherapy for the modulation of P-gp mediated MDR. These observations suggested that when CsA was combined with etoposide, it may have produced an enhanced leukopenia by three potential mechanisms: (1) a pharmacokinetic effect due to a P-gp mediated renal and hepatic etoposide excretion and/or decreased cytochrome P-450 metabolism, (2) hyperbilirubinemia, a dose-dependent CsA effect, leading to increased unbound etoposide concentrations and (3) enhanced bone marrow cytotoxicity by CsA-modulation of P-gp expression in normal bone marrow [12, 13].

Since etoposide is a low clearance drug, one would anticipate that its total clearance would increase after it is displaced from its binding site, with unbound clearance staying the same if intrinsic clearance is not altered [23]. However, total clearance was significantly reduced in patients given CsA, this indicating a significant alteration in the intrinsic clearance of etoposide. In this current analysis, when pharmacokinetic and laboratory data of patients who were receiving etoposide alone or combined with CsA, was compared with those of patients who experienced an equal to or greater than 80% decrease in WBC count, the data showed that patients with the greater degrees of leukopenia had higher unbound etoposide AUC values. These higher values, as estimated from the equation of Stewart et al. [22], are the result of lower serum albumin and/or higher serum bilirubin concentrations.

To analyze these effects further, we fitted the relationship between the etoposide AUC and the percentage decrease in WBC count to the sigmoid Emax model. The use of the total plasma etoposide AUC as a pharmacodynamic response factor in the model described the degree of myelosuppression poorly. The use of the unbound etoposide AUC in the model allows adjustment for the effect of CsA on etoposide clearance and protein binding due to increases in serum bilirubin concentration or decreases in serum albumin, thus allowing a comparison of effects of CsA beyond changes in pharmacokinetics alone. When the unbound etoposide plasma AUC was used in the model, an improved correlation between predicted and actual % decrease in WBC values ($r = 0.790$, Table 2) was observed. The estimate for the parameter δ, for group effect (with or without CsA), indicated CsA significantly increased the value of H, the Hill coefficient, suggesting a steeper response curve for etoposide + CsA, which is depicted in Fig. 1. Thus, at equivalent unbound etoposide AUC values, the sigmoid Emax model predicated a 5% greater WBC count suppression when CsA was added to the treatment regimen (Fig. 3).

Expression of P-gp in bone marrow stem cells raises a potential concern for additive myelosuppression, when modulators of MDR are used in combination with drugs which are known to substrates of P-gp [14]. Delayed recovery from myelosuppression was not observed in the etoposide + CsA cycles, suggesting that the increased myelosuppression was the result of toxicity to myeloid precursors, but not at the level of bone marrow stem cells.

Pharmacokinetic studies of daunorubicin in rats have shown that the addition of verapamil produced an eightfold increase in the plasma AUC of daunorubicin and a

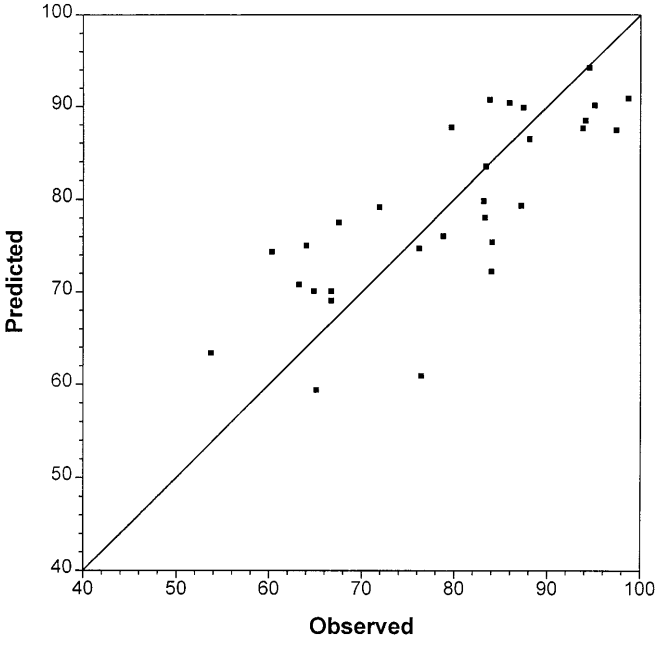

Fig. 2 Comparison of the % decrease in the WBC count for etoposide with and without CsA, calculated from the sigmoid Emax model, using each patient's actual unbound etoposide AUC values from the etoposide-alone treatment, to provide paired equal unbound etoposide AUC values for comparison

two-fold increase in the exposure to normal tissues, including the heart, lungs, liver, and bone marrow [17]. The potential for the interaction of modulators of MDR and cytotoxins transported by P-gp to increase toxicity has been substantiated by Horton et al. [9]. In this study, the use of verapamil in combination with vincristine led to greater cytotoxin concentrations in the liver, kidney and small intestine of tumor-bearing mice, and resulted in an increase in the toxicity-related death rate. These laboratory studies could not show whether the enhanced toxicity was due to the effect of the modulator on the pharmacokinetics of the cytotoxin or to the effect of the modulator directly on normal tissue. More recently, mice with disrupted P-gp genes have been shown to have alterations in the pharmacokinetics and normal tissue toxicology of MDR-related cytotoxins [1]. This genetically altered animal model may permit differentiation between P-gp mediated pharmacokinetic versus pharmacodynamic effects of MDR modulators.

This is the first study to use pharmacodynamic modeling to describe the effect of a modulator of MDR on the pharmacodynamic effect of an antineoplastic agent transported by P-gp when each patient serves as his/her own control, i.e., receives treatment with and without the MDR modulator. Few studies have been performed to date using pharmacodynamic modeling to assess the effect of a modulator on the response of an antineoplastic agent. Trump et al. [25] found that the sigmoid Emax model would describe the degree of potentiation of 5-fluorouracil-induced leukopenia when combined with the modulator dipyridamole, an inhibitor of nucleoside transport. A number of investigators have similarly demonstrated a correlation between pharmacokinetic data and pharmacodynamic response, such as efficacy or toxicity, in a number of antineoplastic drugs [19]. The correlation between pharmacokinetic parameters and etoposide-induced hematologic toxicity has been described in previous studies [20, 23]. These studies have shown that parameters such as the unbound etoposide AUC, steady state concentrations, or time above a threshold cytotoxic concentration correlate with the degree of myelosuppression [10, 15, 20, 23]. Using the experience of the above studies, we were able to modify the classical sigmoid Emax pharmacodynamic model to allow quantification of the effect of a modulator on the effect of the antineoplastic agent.

The use of the equation reported by Stewart et al. [22] in the study of our patients may have led to overestimation of unbound etoposide concentrations. In their previous report, this equation had biased predictions (overestimations) of percent unbound etoposide concentrations in patients with bilirubin concentrations <1.5 mg/dl. This error averaged 2.4% (95% CI of 1.2, 3.6). This overestimation would have been a greater factor in our etoposide-alone patients, where all treatments were associated with bilirubin concentrations <1.5 mg/dl, in contrast to only 50% of courses with etoposide + CsA treatment. Thus, our results probably underestimated the degree of enhanced leukopenia observed during CsA therapy. In addition, although the model performed well (Table 2 and Figs. 2 and 3), predictive accuracy may have been improved by more frequent determinations of the WBC count or absolute neutrophil counts around the anticipated time of the nadir. Unfortunately, absolute neutrophil counts, which would have been a better indicator of infectious risk than WBC counts, were not obtained in this study.

Our findings suggest that a small degree of the enhanced leukopenia observed with high-dose CsA when

Table 3 Sigmoid Emax model nonlinear regression parameter estimates and model performance using the unbound etoposide area under the curve (AUC) as the pharmacodynamic response factor (*WBC* white blood cell)

$$\%\ \text{decrease in WBC count} = E_{max} \times PDRV^{H+Z\times\delta} / (PDRV_{50} + Z \times \beta) + PDRV^{H+Z\times\delta}$$

Parameter	Estimate	95% CI (lower, upper)
Regression parameter estimates		
H	0.897	0.829, 0.966
δ	0.228	0.219, 0.236
AUC_{50}	4.44	3.65, 5.23
β	0.039	−1.23, 1.31
Data	Mean or value	95% CI Lower, upper
Model performance		
Observed % decrease WBC	79.3	74.6, 83.9
Calculated % decrease WBC	79.2	75.4, 82.9
Error	−0.17	−3.0, 2.7
Absolute error	6.4	4.9, 7.8
Correlation (r)	0.790	–
R-squared	0.625	–

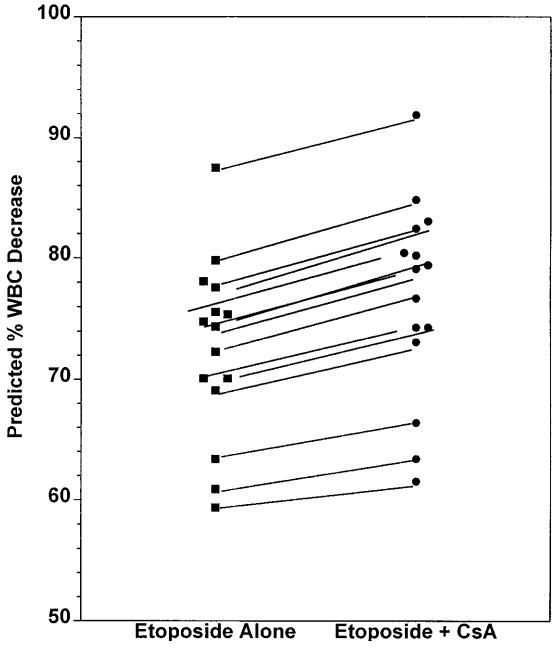

Fig. 3 Observed versus predicted % decrease in WBC count for the sigmoid Emax model fit of the AUC (0 to infinity) of unbound etoposide and % decrease in WBC count

combined with etoposide may be attributable to a mechanism such as inhibition of P-gp in bone marrow precursor cells. This would be consistent with the in vitro observations of Chao et al. [3], where CsA was shown to enhance the toxicity of etoposide to normal hematopoietic precursor cells in bone marrow purging experiments. However, the majority of the observed effect, with the use of CsA as an inhibitor of MDR, appears to be pharmacokinetic in origin, where high concentrations of CsA result in (1) a decrease in etoposide clearance, attributable to the modulator's effects on P-gp mediated hepatic and renal excretion and (2) an increase in unbound etoposide, as a consequence of CsA-induced hyperbilirubinemia. Future trials of agents used to modulate P-gp mediated MDR should include pharmacokinetic and pharmacodynamic assessments to determine the contribution of the modulating agent to antineoplastic-induced response and toxicity.

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