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Clinical report

Effect of cyclosporin A on protein binding of teniposide in cancer patients

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We investigated the effect of cyclosporin A (CSA) on protein binding of teniposide (VM26) in 16 patients with metastatic renal cell carcinoma receiving i.v. VM26 alone over 24 h (total dose, 200 mg/m^2) and in association with CSA (5 mg/kg/2 h followed by 30 mg/kg/48 h i.v.). CSA was used in an attempt to overcome multidrug resistance. The unbound fraction (%fu) of VM26 was significantly ($p = 0.04$) higher in the cycles with CSA (median 0.8; range 0.4–1.9) than in the cycles with VM26 alone (median 0.5; range 0.1–1.6). Both total VM26 area under curve concentration ($AUC_{0-\infty}$) and free VM26 $AUC_{0-\infty}$ increased after treatment with CSA, but the median increase in free $AUC_{0-\infty}$ was higher (2.7-fold) than total $AUC_{0-\infty}$ (1.5-fold) ($p = 0.04$). Bilirubin was significantly ($p < 0.01$) increased after CSA but no association was observed between bilirubin level and %fu of VM26. Albumin was in the normal range after both VM26 alone and VM26 plus CSA. The nadir of absolute neutrophil count (ANC) after VM26 plus CSA (median 700/μl, range <100–2860/μl) was lower than after VM26 alone (median 1900/μl, range 200–6000/μl) ($p = 0.0007$). The median percentage of ANC compared to the pretreatment value (ANC nadir/ANC pretreatment × 100) was 39.0% (range 3.1–98.8%) in the cycles with VM26 alone and 16.9% (range 1.4–97.9%) ($p = 0.007$) after VM26 plus CSA. Percentage change of neutrophils significantly correlated with free $AUC_{0-\infty}$ VM26 in the cycles with VM26 alone and VM26 plus CSA ($p = 0.04$, $r = -0.53$ and $p = 0.04$, $r = -0.52$, respectively). Only a trend which failed to reach significance was observed between total $AUC_{0-\infty}$ VM26 and percentage change of neutrophils in the cycles with VM26 alone and in association with CSA (p = NS, $r = -0.33$ and $p = 0.055$, $r = -0.49$, respectively). In conclusion, patients treated with CSA had higher systemic exposure to unbound VM26. [© 1999 Lippincott Williams & Wilkins.]

Key words: Cyclosporin, protein binding, teniposide.

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Introduction

Teniposide (VM26) is a derivative of epipodophyllotoxin with a broad spectrum of anticancer activity.[1] Its target is the nuclear enzyme topoisomerase II, which catalyzes the passage of DNA across adjacent strands during cell division. VM26 is readily taken up by the cells and high levels of VM26 accumulate within the cells.

Pharmacokinetics and protein binding are other important variables that may influence the pharmacodynamic effect of VM26 in patients.[2] The major toxicity of VM26 at conventional doses is myelosuppression, particularly neutropenia. Rodman *et al.*[3] reported that the concentrations of VM26 in the plasma of patients with acute leukemia were significantly higher when toxicity was encountered. An even more significant correlation emerged when the steady-state concentration of responders was compared with that of non-responders.

VM26 is a highly protein-bound drug. Over 99% of VM26 is protein-bound over a wide range of drug concentrations.[4,5] The potential influence of plasma protein binding on the disposition and effect of drugs is well recognized, and has been reviewed extensively.[6] It is generally presumed that the protein-unbound fraction of drug in plasma is the primary drug moiety producing a pharmacologic effect. The protein binding of VM26 is of such an order that its alteration is likely to influence not only its pharmacokinetics *in vivo* but also its cytotoxic effect. A small decrease in the bound fraction (99 to 97%) would yield a relatively large increase in unbound (active) drug concentration (a 3-fold increase, i.e. 1 to 3%), which could alter the pharmacodynamics of VM26.[7,8]

VM26 is a chemotherapeutic agent involved in multidrug resistance (MDR)[9] and *in vitro* studies demonstrated that inhibition of P-glycoprotein (P-gp) activity restores sensitivity to VM26. As reported for

other chemotherapeutic agents,[10-13] we previously demonstrated that the use of cyclosporin A (CSA) as a MDR modulator in patients is associated with an increased pharmacodynamic effect of VM26.[14] However, a chemosensitizing effect was observed only in P-gp-expressing cells with a rapid growth kinetics, such as hemopoietic precursor cells, but not in MDR cells with longer doubling times, such as renal carcinoma cells. This results in an increased toxicity in normal tissues, without antitumor effects. It has been suggested that CSA could also interfere with the clearance of cytotoxic agents through competition for efflux pumps involved in hepatic drug clearance and biliary or renal excretion, and thus alter drug pharmacokinetics. The consequence is a significant increase of the area under the curve concentration versus time (total $AUC_{0-\infty}$) of VM26[14] and thereby contributes to toxicity in normal tissues.[15,16]

At present no data have been reported on the effect of CSA on the protein binding of VM26 and its pharmacodynamic consequences. In a previous study,[14] we used CSA to overcome resistance of renal cell carcinomas to VM26; this gave us the unique opportunity to investigate the modulation effect of CSA on protein binding of VM26.

Materials and methods

Patient selection and treatment protocol

Details of the clinical protocol are reported elsewhere.[14] Briefly, all patients had a diagnosis of metastatic renal cell carcinoma in progression after standard treatment, WHO performance status ≤ 2, renal and hepatic tests ≤ 1.5 normal value, absolute neutrophil count (ANC) $> 2000/\mu l$, platelets $> 100\,000/\mu l$, and absence of symptomatic CNS metastases and/or other diseases requiring drugs known to be nephrotoxic or affecting CSA metabolism. Informed consent was obtained in accordance with institutional guidelines. The percentage change in hematological parameters during pharmacological treatments was calculated as nadir value/pretreatment value $\times$ 100.

Patients were treated with VM26 alone over 24 h (total dose, 200 mg/m^2) and with the combination of VM26 (Vumon; Bristol-Myers, Rome, Italy) and CSA (Sandimmun; Sandoz, Milan, Italy). CSA (5 mg/kg) was administered as an i.v. loading dose during a 2 h period (−2 to 0 h) of the protocol, followed by i.v. continuous infusion for 48 h (15 mg/kg/24 h) (0 and 48 h of the protocol) together with VM26 (0-24 h). The patients were clinically evaluated according to the usual phase II trial criteria (WHO) and blood tests were performed weekly. Evaluations for toxicity were performed after the first or second course of VM26 alone and after the second course of VM26 plus CSA in order to compare pharmacokinetic data obtained during the same time. However, evaluations performed after the second cycle of VM26 plus CSA indicated that toxicity was not cumulative.

No patients received drugs known to interfere with protein binding of epipodophyllotoxins when they entered the study.

Sample collection, drug assays and pharmacokinetic analyses

To determine VM26 concentration, blood samples were obtained just before the onset of i.v. infusion of VM26 (0 h), and at 1, 6, 12, 18, 24, 24.5, 25, 26, 28, 32, 36, 48 and 60 h after it. Plasma was stored at −20°C until analysis. To determine whole-blood CSA concentration, blood samples were obtained at −2, 0, 6, 12, 36, 48, 60 and 72 h, and blood was stored at −70°C until analysis. These samples were drawn during the first cycle of VM26 alone and during the first cycle of VM26 plus CSA.

VM26 and CSA levels were determined as previously reported.[14] Briefly, VM26 plasma concentrations were measured by a reversed-phase HPLC method with UV absorbance, as described by Evans *et al.*[7] The limit of quantitation was 0.25 μg/ml: both intraday and interday imprecision and inaccuracy of the method were within 15%. CSA blood concentrations were measured by a FPIA method using the CSA monoclonal TDx (Abbott Laboratories, North Chicago, IL) with a sensitivity of 40 ng/ml and an interassay CV less than 10%.

The pharmacokinetic analysis of VM26 was performed by a one-compartment model in which drug infusion and elimination were considered as zero-order and first-order processes, respectively. Pharmacokinetic parameters [peak concentration (C_{max}), terminal elimination rate constant (K), apparent volume of distribution (V), systemic clearance (CL), elimination half-life ($t_{1/2\beta}$) and area under the curve ($AUC_{0-\infty}$)] were determined by PCNONLIN 4.0, a non-linear regression program. The terminal elimination rate constant (K) was estimated by unweighted least-squares linear regression analysis of the elimination phase of the VM26 plasma concentration-time curve.

VM26 protein binding

The method used to determine unbound VM26 was adapted from a technique described by Robieux *et*

al.[17] for unbound VP16 measurement. In short, 1 ml of plasma was ultrafiltered using the disposable Centrifree Micropartition device (cut-off 30 000 Da) from Amicon (Beverly, MA) for separation of free VM26. After centrifugation (2000 *g* for 30 min at 25°C) about 0.5 ml of ultrafiltrate was obtained from the starting plasma sample. The 0.5 ml ultrafiltrate sample was spiked with the internal standard etoposide (0.5 μg) and extracted with 1 ml of chloroform. After 20 min shaking, samples were centrifuged at 1000 *g* for 5 min; the organic phase was dried under vacuum and rediluted in methanol (50 μl). Then 25 μl of this solution was injected into a Waters (Milford, MA) high-performance liquid chromatography (HPLC) system equipped with a fluorimetric detector (λ_{ex} 288 nm, λ_{em} 328 nm). Separation was achieved eluting a mobile phase made up of water:acetonitrile:glacial acetic acid (66:33:1) at a flow of 1 ml/min through a Waters 300 × 3.9 mm μBondapak phenyl column (125 A, 10 μm). Under these conditions the retention times were 4.8 min for VP16 and 8.6 min for VM26, respectively. Concentrations were quantitated with the Millennium 2010 chromatography manager software from Waters by adopting the internal standard method. The sensitivity of the method was 0.025 μg/ml; in this range of concentrations (0.025–0.5 μg/ml), intraday and interday variabilities were within 20%. The unbound fraction (%fu) was calculated from total and protein unbound VM26 concentrations at peak (C_{max}) as follows:

$$\%fu = (\text{free } C_{max}/\text{total } C_{max}) \times 100$$

Our and other *in vitro* studies[8] demonstrated that teniposide protein binding is not concentration dependent over the range we obtained in the plasma of the patients (< 25 μg/ml). Moreover, no significant differences were observed in the %fu between samples obtained during time to C_{max} (t_{max}) (range 12–26 h) or during other times of a single cycle with teniposide.

Area under the curve ($AUC_{0-\infty}$) of free VM26 was obtained from $AUC_{0-\infty}$ and unbound fraction (%fu) as:

$$\text{free } AUC_{0-\infty} = \text{total } AUC_{0-\infty} \times \%fu$$

Statistics

A complete sampling schedule was performed during the first course of VM26 alone and plus CSA for each patient. Thus, paired concentration-time courses and pharmacokinetic parameters were available.

All statistical analyses were performed by non-parametric tests because several parameters did not follow a normal distribution, as determined by the Shapiro-Wilk *W*-test. The Wilcoxon test was used for statistical evaluation when the two groups of parameters were compared.

A search for correlation between kinetic and dynamic parameters was performed by the Spearman test. The significance of the coefficients of the correlation found was determined by the table reported elsewhere.[18]

Results

A total of 16 patients [nine males, seven females; median age 59 years (range 44–75)] were treated with VM26 alone and VM26 plus CSA. Nine patients received two courses of VM26 alone and then crossed over to CSA. Among these, six had progressive disease and three stable disease. Since no response was observed after two courses of VM26 alone, the remaining seven patients crossed over to VM26 plus CSA after only one course of VM26. The median number of courses of VM26 plus CSA was 2 (range 2–5). Addition of CSA did not improve the response rate in the patients (seven patients had progressive disease, eight stable disease and one patient was not evaluable for response).

Table 1. Effect of CSA on pharmacokinetics of VM26 in 16 patients with renal cell carcinoma

	VM26	VM26 plus CSA	Fold increase	p^a
Elimination half-life (h)	6.8 (2.8–14.8)[b]	9.3 (3.7–22.3)[b]	1.4 (0.5–5.2)[b]	0.007
Total $AUC_{0-\infty}$ (μg/ml·h)	290.3 (168.4–791.0)	432.9 (194.2–1055.1)	1.5 (0.8–2.2)	0.001
Clearance (ml/min/m^2)	0.62 (0.3–1.2)	0.4963 (0.2–1.0)	0.7 (0.4–1.3)	0.03
Volume of distribution (l/m^2)	5.5 (4.4–9.0)	5.9 (4.8–11.6)	1.1 (0.5–2.4)	NS
Fraction unbound (%)	0.50 (0.1–1.6)	0.75 (0.4–1.9)	1.5 (0.4–6.0)	0.04
Free $AUC_{0-\infty}$ (μg/ml·h)	1.4 (0.4–5.1)	3.8 (1.0–10.4)	2.7 (0.8–10.2)	0.0008

[a]Comparison was made between median values by the Wilcoxon rank test.
[b]Median (range).

Plasma pharmacokinetic parameters were determined in all the patients during the first cycle of VM26 alone and VM26 plus CSA (see Table 1). In the cycles with VM26 plus CSA, at the end of the 48 h CSA infusion, whole-blood CSA levels ranged from 1830 to 4501 ng/ml (median 2415 ng/ml). CSA significantly ($p < 0.01$) increased both total and free VM26 $AUC_{0-\infty}$. Median total VM26 $AUC_{0-\infty}$ was 290.3 μg/ml·h (range 168.4–791.0) and 432.9 μg/ml·h (range 194.2–1055.1) in the cycles with VM26 alone and in association with CSA, respectively. In the cycles with CSA, median increments of total VM26 $AUC_{0-\infty}$ were 1.5 (range 0.8–2.2, p=0.001). The fold increase in free VM26 $AUC_{0-\infty}$ in the cycles with VM26 plus CSA compared to the cycles with VM26 alone was significantly greater (p=0.04) than that observed for total VM26 $AUC_{0-\infty}$. Median free VM26 $AUC_{0-\infty}$ was 1.4 μg/ml·h (range 0.4–5.1) and 3.8 μg/ml·h (range 1.0–10.4) in the cycles with VM26 alone and in association with CSA, respectively. The median increase in free VM26 $AUC_{0-\infty}$ after addition of CSA was 2.7-fold (range 0.8–10.2) (p=0.0008). Overall, we did not observe any significant association between CSA levels and fold increases in free VM26 $AUC_{0-\infty}$. However, it is to note that the sole patient where free VM26 $AUC_{0-\infty}$ was decreased (from 1.24–0.97 μg/ml·h, respectively, in the cycles with VM26 alone and VM26 plus CSA) had the lowest whole-blood CSA levels.

CSA affected the %fu of VM26: after VM26 plus CSA the median %fu of VM26 was 0.8 (range 0.4–1.9), whereas the median %fu of VM26 in the cycles with VM26 alone was 0.5 (range 0.1–1.6). By comparing %fu of VM26 within each patient the median fold increase was 1.4-fold (range 0.7–6.0) (p=0.04). Increases in %fu did not appear to be a cumulative effect. Determinations of %fu performed in subsequent cycles of VM26 plus CSA for five patients did not show any significant variation in %fu compared to those observed in the first cycle of VM26 plus CSA (data not shown). Other pharmacokinetic parameters of VM26 administered alone or together with CSA are compared in Table 1.

Concerning the toxic pharmacodynamic effects, significant differences were observed between treatments with VM26 alone and in association with CSA. After addition of CSA, five and three patients had G4 and G3 neutropenia, respectively, whereas only three cases of G3 neutropenia were observed in the cycles with VM26 alone. The individual values of ANC nadir were lower after VM26 plus CSA (median 700/μl, range <100–2860/μl) than after VM26 alone (median 1900/μl, range 200–6000/μl) (p=0.0007). The median percentage in ANC compared to the pretreatment value was 39.0% (range 3.1–98.8%) in the cycles with VM26 alone and 16.9% (range 1.4–97.9%) after VM26 plus CSA (p=0.007). Thrombocytopenia with platelet counts less than 50 000/μl occurred in one patient treated with VM26 plus CSA. Other side effects observed after addition of CSA were G2 mucositis (one patient), 3-fold AST increase (one patient) and epigastric pain (three patients). In the patients treated with VM26 plus CSA, the median value of bilirubin (median 2.5 mg/100 ml; range 1.2–6.5 mg/100 ml) was significantly ($p < 0.01$) higher than in the patients treated with VM26 alone. In these latter, bilirubin was in the normal range (<1.5 mg/100 ml).

Other parameters of liver function (i.e. total protein, albumin, triglycerides and cholesterol) were near the normal range without significant differences in the cycles with VM26 alone and in association with CSA (see Table 2). Creatinine was also close to the normal range (≤1.5), and creatinine clearance estimated by the method of Jelliffe[19] ranged between 44 and 105 ml/min (median 60 ml/min), without significant differences in the cycles with VM26 alone and in association with CSA.

Significant correlations by the Spearman test were observed between ANC nadir and total VM26 $AUC_{0-\infty}$ in the cycles with VM26 alone (p=0.04, $r = -0.53$) and in association with CSA (p=0.004, $r = -0.68$) and between ANC nadir and free VM26 $AUC_{0-\infty}$ in the cycles with VM26 plus CSA (p=0.02, $r = -0.59$) (Figure 1). When comparisons were made with percentage change in ANC at nadir with respect to the pretreatment value, significant correlations were found only with free VM26 $AUC_{0-\infty}$ both in the cycles with VM26

Table 2. Liver function assessment in the cycles with VM26 alone and in association with CSA

	Normal range	VM26	VM26 plus CSA	p
Total protein (g/dl)	6.0–8.5	7.1 (5.2–8.3)[a]	7.1 (5.2–11.1)[a]	NS
Albumin (g/dl)	3.2–5.5	4.2 (3.2–4.7)	4.2 (3.2–4.8)	NS
Triglycerides (mg/dl)	36–170	95 (34–168)	105 (29–217)	NS
Cholesterol (mg/dl)	<200	134 (56–185)	107 (43–225)	NS

[a]Median (range).

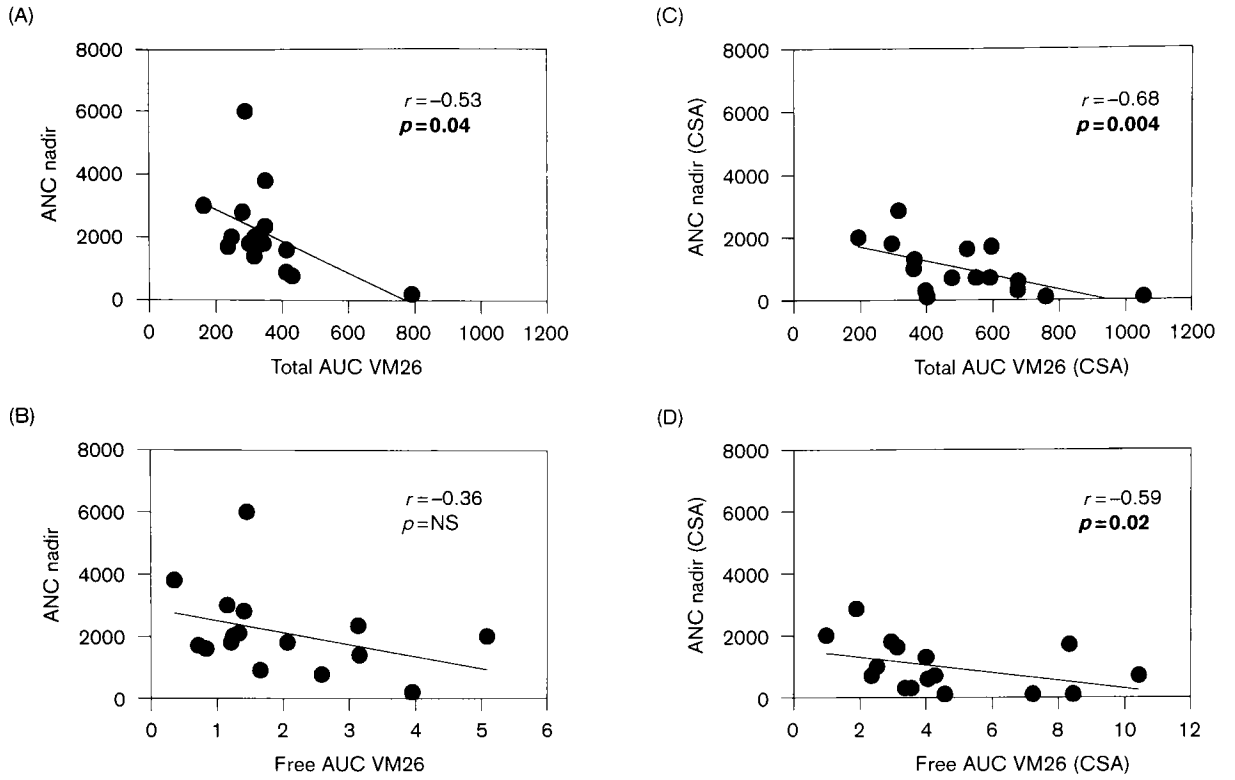

Figure 1. Scatter diagram of total $AUC_{0-\infty}$ VM26/free $AUC_{0-\infty}$ VM26 and ANC nadir in patients treated with VM26 alone (A/B, respectively) or VM26 and CSA (C/D, respectively); *p* and *r* (coefficient of correlation) values were determined according to the Spearman test.

alone (p=0.04, r=−0.53) and in those with VM26 plus CSA (p=0.04, r=−0.52) (Figure 2). Conversely only a trend was observed between percentage change in ANC at nadir and total VM26 $AUC_{0-\infty}$ (p=NS, r=−0.33 and p=0.055, r=−0.49 in the cycles with VM26 alone and in association with CSA, respectively).

Finally, despite the increase in bilirubin level after CSA administration, no significant correlations were observed between %fu of VM26 and bilirubin plasma level (r=0.20, p=NS) or between %fu of VM26 and total proteins, albumin, triglycerides, cholesterol, creatinine and creatinine clearance, respectively (data not shown).

Discussion

CSA has been extensively used to overcome drug resistance both in the preclinical and clinical setting. CSA treatments are generally associated with an increased total $AUC_{0-\infty}$ of antineoplastic drugs[20] and it has been argued that these pharmacokinetic variations could contribute to explain the increased toxicity of antineoplastic drugs, which has been generally observed after CSA treatment. However, it is widely accepted that only unbound drug in the plasma (i.e. drug not bound to plasma proteins) is available for membrane transport and receptor interaction, and thus is the pharmacologically active drug.[6] On these grounds, free $AUC_{0-\infty}$, rather than total $AUC_{0-\infty}$, could play a crucial role for pharmacodynamic effects, especially for antineoplastic drugs with higher protein binding, such as VM26.[7,21,22]

We have previously published[14] that the dosage of CSA we used in the patients reported in this study allowed us to obtain CSA plasma levels effective in inhibiting P-gp activity in *in vitro* experimental models. This resulted in an increased pharmacodynamic effect of VM26, as evidenced by the greater neutrophil toxicity in the cycles with VM26 plus CSA compared to the cycles with VM26 alone. In accordance with other studies,[15,16] we concluded that increased hematologic toxicity (pharmacodynamic effect) could be due to an inhibition of P-gp activity in hemopoietic precursor cells overexpressing P-

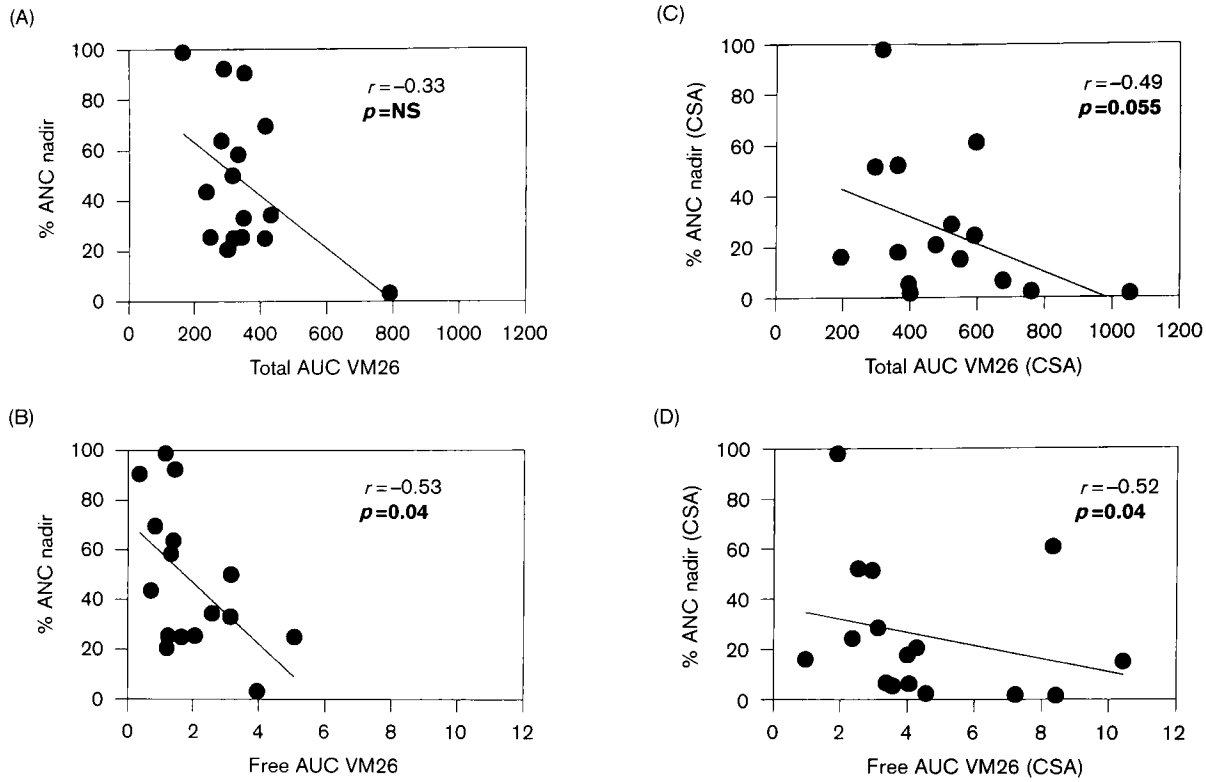

Figure 2. Scatter diagram of total $AUC_{0-\infty}$ VM26/free $AUC_{0-\infty}$ VM26 and percentage change of ANC nadir (ANC nadir/ANC pretreatment × 100) in patients treated with VM26 alone (A/B, respectively) or VM26 plus CSA (C/D, respectively); *p* and *r* (coefficient of correlation) values were determined according to the Spearman test.

gp[23,24] and/or to a pharmacokinetic effect, as demonstrated by the increase in total $AUC_{0-\infty}$ of VM26.

Pharmacokinetic data reported in the present work clearly demonstrated that after the reversal treatment with CSA, free $AUC_{0-\infty}$ of VM26 was significantly increased (p=0.0008) compared to the cycles with VM26 alone. Increases in free $AUC_{0-\infty}$ were greater than those expected from the increase in total $AUC_{0-\infty}$ of VM26. The increase of total $AUC_{0-\infty}$ was 1.5-fold, whereas the increase of free $AUC_{0-\infty}$ was 2.2-fold (p=0.04, by comparing increases of total and free $AUC_{0-\infty}$ of VM26). Free $AUC_{0-\infty}$ is determined by total $AUC_{0-\infty}$ and %fu (free $AUC_{0-\infty}$=total $AUC_{0-\infty}$ × %fu), therefore variations in %fu are also expected to explain the variation of free $AUC_{0-\infty}$ after CSA treatment. According to this suggestion, we observed that CSA significantly increased (p=0.04) the %fu of VM26 compared to the treatment without CSA. Median %fu of VM26 was 0.5 (range 0.1-1.6) in the cycles with VM26 alone, whereas it was 0.8 (range 0.4-1.9) in the cycles with VM26 plus CSA.

At present, the increase in %fu of VM26 after CSA is not clearly understood. CSA and VM26 has a different plasma carrier: VM26 mainly binds to albumin[25] and CSA binds mostly to lypoproteins.[26] Therefore, we can reasonably suppose that the increase in %fu may not be due to a competitive effect between CSA and VM26 in the protein binding. Several factors[27] could affect the protein binding of epipodophyllotoxins. Moreover, other compounds in the CSA formulation (i.e. Cremophore EL) are known as MDR modifiers and may affect VM26 protein binding or other VM26 pharmacokinetic parameters.

Petros *et al.*[8] have reported that the lower plasma protein binding (higher %fu) of VM26 could be related to the lower serum albumin concentrations. Stewart *et al.*[21] have focused on the effect of hyperbilirubinemia in reducing the protein binding of etoposide as a consequence of bilirubin displacement from albumin, which is a common plasma carrier for bilirubin and epipodophyllotoxins.[25] It must be considered that in our patients reversal treatment with CSA significantly increased the bilirubin plasma level. This latter is a substrate for P-gp and inhibition of the activity of P-gp expressed in biliary canaliculi increases the bilirubin plasma level.[28] However, no significant correlation between total bilirubin and %fu of VM26 was found in

our patients, thus indicating that other factors than total bilirubin (if it did) affected %fu of VM26.

Impairments in renal or liver function are reported to be other factors affecting %fu of epipodophyillotoxins,[22] mainly by reducing the plasma levels of albumin or by altering its interaction with the antineoplastic agents.[29] However, in our patients, albumin plasma levels and transaminases were close to the normal range (except for one patient) without variations in the cycles with VM26 alone and VM26 plus CSA, and so were the levels of creatinine and the estimated creatinine clearance.[19] No significant association was observed between %fu and these parameters. Other more sensitive investigations than those used in this study may have defined impaired renal or liver functions more clearly. Nevertheless, these data seem to exclude displacement of VM26 from the binding site of albumin due to compounds accumulating as a result of renal or hepatic dysfunction or to a decrease in albumin plasma level.

Finally, pharmacodynamic analysis was performed in order to investigate the effect of total and free VM26 $AUC_{0-\infty}$ on ANC in the cycles with VM26 alone and in association with CSA. For this purpose, comparisons were made both with ANC at nadir and with the percentage change in ANC at nadir with respect to the basal value. Overall, our data indicate that both total and free $AUC_{0-\infty}$ play a role in the pharmacodynamic effect of VM26. A significant association was observed between free $AUC_{0-\infty}$ and percentage change in ANC (p=0.04, $r=-0.53$ and p=0.04, $r=-0.52$ in the cycles with VM26 alone and VM26 plus CSA, respectively). Conversely when comparisons were made with ANC at nadir, more strict associations were observed with total $AUC_{0-\infty}$ (p=0.04, $r=-0.53$ and p=0.004, $r=-0.68$ in the cycles with VM26 alone and VM26 plus CSA, respectively). Even if further studies on a greater number of patients are needed to establish whether unbound or total VM26 is the main determinant for pharmacodynamic effects, our data clearly demonstrate that the reversal treatment with CSA significantly increases the %fu and free $AUC_{0-\infty}$ of VM26. This suggests a further and new insight for pharmacodynamic and pharmacokinetic interactions between CSA and epipodophillotoxins.

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