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# Pharmacokinetics of Teniposide (VM26) and Etoposide (VP16-213) in Children with Cancer*

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***Summary.*** *The clinical pharmacokinetics of VM26 and VP16-213 were assessed in 15 children (median age 10 years) with acute leukemia, using a new high-performance liquid chromatography–electrochemical assay. Pharmacokinetic parameters were calculated by both model-dependent and compartment model-independent methods. These studies demonstrated substantial differences in the central volumes of distribution ($VD_c$), steady-state volumes of distribution ($VD_{ss}$) and systemic clearances ($Cl_s$) of VM26 and VP16-213; with the $VD_c$, $VD_{ss}$, and $Cl_s$ all being smaller for VM26. Systemic clearances determined by model-independent methods were 5.2 ± 1.0 ml/min/m² (mean ± SD) for VM26 and 17.8 ± 11.2 ml/min/m² for VP16-213. The major metabolites detected in serum and urine were the hydroxy acids. Low levels of the picro-lactone isomers were detected in some patients while the aglycones were not detected in the serum or urine of any patients.*

## Introduction

The pharmacokinetics of two relatively new epipodophyllotoxin derivatives (VM26, teniposide; VP16-213, etoposide) have not previously been evaluated in children. Previous comparative pharmacokinetic studies have only been conducted in adults, used radioactive compounds, and lacked specificity in identification of metabolic products. Because of the established activity of these two drugs in acute leukemias, neuroblastoma, and other pediatric malignancies [7, 11], and the absence of comparative pharmacokinetic studies in pediatric patients, our previously reported reverse phase high-performance liquid chromatography (HPLC)-electrochemical detection assay [12] was used to quantitate VP16-213, VM26 and their metabolites in plasma and urine, and to determine pharmacokinetic parameters of the two parent drugs in children with leukemia.

## Methods

From the known conversions of podophyllotoxin [8], we have developed the potential metabolic scheme illustrated in Fig. 1. In this scheme, the 2 : 3 trans-lactone undergoes isomerization to a 2 : 3 cis- (or picro-) lactone. The hydroxy acids of VM26 and VP16-213 are included since they have previously been reported to be the principal metabolites eliminated in the urine of adult patients [2, 13]. Possible metabolic production of 4′-demethyl-epipodophyllotoxin aglycones and conjugates of the parent drugs is included in our proposed scheme, although these metabolites have not been detected in human specimens and the metabolic pathways for their production are undefined. Based on this potential metabolic scheme, the cis-hydroxy acids, picro-lactone isomers and aglycone of VM26 and VP16-213 were synthesized, purified, confirmed by IR, NMR and high-resolution mass spectrometry, and used as metabolite standards for development of an HPLC assay. Resolution of all potential metabolites of VP16-213 was achieved isocratically using a μBondapak Phenyl column (Waters, Assoc., Milford, MA, USA), a mobile phase of water/acetonitrile/acetic acid (74/25/1) and a flow rate of 1 ml/min (see Fig. 2). Chromatographic conditions were the same for analysis of VM26 with the exception of water/acetonitrile/acetic acid (64/35/1) as the mobile phase. A commercially available electrochemical detector (Bioanalytical Systems, Inc., West Lafayette, Ind., USA) was used at an applied oxidative potential of 0.7 V. Urine was quantitated by direct injection of 20 μl sample after the appropriate internal standard (IS) was added. Serial plasma samples were either directly injected or extracted with chloroform and ethyl acetate after addition of $(NH_4)_2SO_4$ and internal standard. VP16-213 was used as internal standard for the VM26 assay and VM26 hydroxy acid was used as internal standard for VP16-213 quantitation. The peak height ratios of parent drug or metabolite to internal standard were plotted against known concentrations (0.1–50 μg/ml) and this linear calibration curve was used to determine drug concentration in patient samples.

The pharmacokinetics of VM26 and VP16-213 were assessed in 15 children with acute leukemia (ages 3 months to 18 years).

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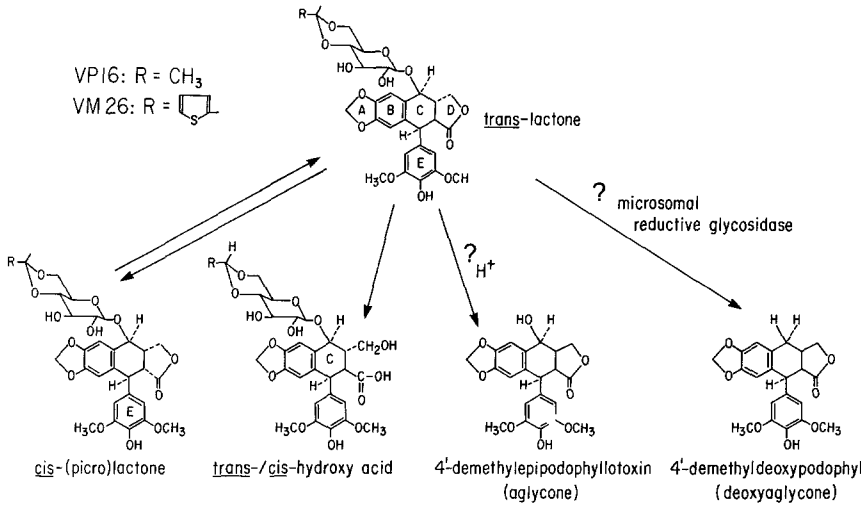


**Fig. 1.** Potential metabolic scheme for VM26 and VP16-213. See text for basis

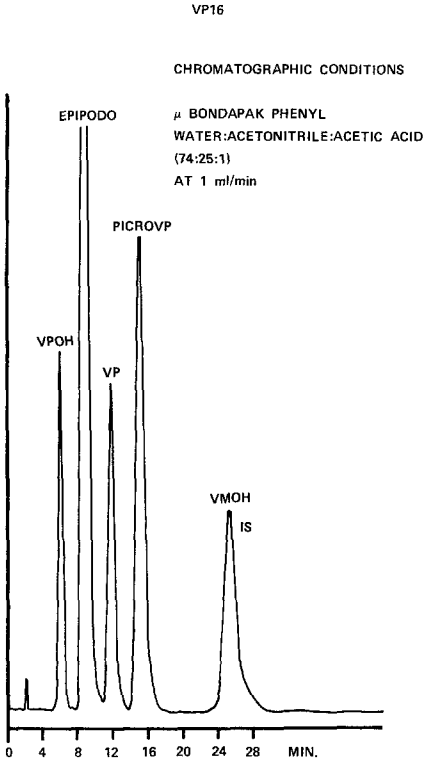


**Fig. 2.** Reverse-phase HPLC chromatogram of VP16-213 and metabolites with electrochemical detection at an oxidative applied potential of 0.7 v

Plasma samples were obtained over 48 h from six patients administered VM26 (165 mg/m$^2$) and nine patients given VP16-213 (200–250 mg/m$^2$) as a 30–145 min intravenous infusion. All evaluated patients had normal renal and hepatic function based on serum creatinine and total bilirubin concentrations, respectively.

Pharmacokinetic parameters describing VM26 and VP16-213 disposition were calculated by both model-dependent and model-independent (compartmental-independent) methods. For model-dependent parameters, serum concentration-time data were fit to the appropriate multiexponential equation using the NONLIN [10] computer program. A weighting function of 1 was used for all curve-fitting procedures and the method of Loo-Riegelman [9] was used to adjust intercept values for the length of intravenous infusions. The slopes and intercepts were used to calculate Ke, $T_{1/2}\alpha$, $T_{1/2}\beta$, $VD_c$ and systemic clearance ($Cl_s$) using standard two-compartment first-order pharmacokinetic equations [6].

Model-independent (compartmental-independent) values of systemic clearance ($Cl_s$) and steady-state volume of distribution ($VD_{ss}$) were calculated by the following equations:

$$VD_{ss} = \frac{\text{Dose (mg/m}^2)}{AUC_0^\infty} \cdot \left[ \frac{AUMC_0^\infty}{AUC_0^\infty} - \left( \frac{K_0 \cdot t}{\text{Dose}} \right) \left( \frac{t}{2} \right) \right],$$

where $K_0$ is the drug infusion rate (mg/min) and t is the length of the drug infusion (min). The area under the concentration-time curve (AUC) and area under the moment curve (AUMC) were calculated by the log-trapezoidal method [14].

## Results

VP16-213 and VM26 were both adequately described by a two-compartment first order pharmacokinetic model with respective model parameters as summarized in Table 1. Using model-dependent equations, the systemic clearance of VP16-213 was 19.5 ± 11.7 ml/min/m$^2$ versus 5.4 ± 1.4 ml/min/m$^2$ for VM26. Using model-independent methods, the mean (± SD) systemic clearance of VP16-213 was 17.8 ± 11.2 ml/min/m$^2$ versus 5.2 ± 1.0 ml/min/m$^2$ for VM26. These clearances are similar to those reported in adults [14] (ml/min/m$^2$) with an almost three times faster clearance of VP16 compared to VM26. The hydroxy acids were detectable in the serum of some patients and were the principal metabolites detected in the urine. Detectable concentrations of the picro-isomers were present in some patients while the aglycones and conjugates remain unidentified in the plasma or urine of any patient.

In order to determine the relative activity of each potential metabolite, in vitro cytokinetic studies (flow cytometry) and cytotoxicity studies (clonogenic

**Table 1.** Summary of VM26 and VP16-213 pharmacokinetic parameters (mean ± SD)

| | Compartmental model-independent[a] | | Model-dependent[a] | | | | |
|---|---|---|---|---|---|---|---|
| | Systemic clearance[a] (ml/min/m$^2$) | $VD_{ss}$[a] (l/m$^2$) | Systemic clearance (ml/min/m$^2$) | $VD_c$ (l/m$^2$) | Ke ($H^{-1}$) | $T_{1/2\alpha}$ (H) | $T_{1/2\beta}$ (H) |
| VM26: | 5.2 ± 1.0 | 3.4 ± 0.7 | 5.4 ± 1.4 | 1.8 ± 1.1 | 0.23 ± 0.9 | 0.9 0.2 | 9.6 ± 2.7 |
| VP16-213: | 17.8 ± 11.2 | 4.8 ± 2.8 | 19.5 ± 11.7 | 3.1 ± 2.0 | 0.42 ± 0.17 | 1.2 ± 1.7 | 5.8 ± 3.2 |

[a] Model-independent: Systemic clearance ($Cl_s$) = Dose/$AUC_0^\infty$

[b] Model-dependent parameters derived from NONLIN best-fit to two-compartment model; systemic clearance ($Cl_s$) = Ke · $VD_c$; $VD_c$ = Dose/(A + B)

assay) were performed after 18H incubations of human leukemia cells (CCRF-CEM) according to previously described methods [4]. These studies demonstrated both qualitative and quantitative differences between the parent drugs and their potential metabolites. Concentrations of the picro-lactone isomers required to produce 50% inhibition of cloning efficiency ($IC_{50}$) or an increase in the percent of cells in $G_2$ + M (with a marked reduction in mitotic figures) were about 100-fold higher than the parent trans-lactone. The aglycones produced metaphase arrest and the $IC_{50}$ was at least 10-fold less than VP16 and equitoxic with VM26. The hydroxy acids produced no measurable cytotoxicity or cytokinetic effects when tested at concentrations of 0.1–10 μg/ml.

## Discussion

This study is the initial report of VM26 and VP16-213 pharmacokinetics in children, using a sensitive and specific HPLC-electrochemical assay. The disposition of VM26 is clearly different from VP16, as previously reported in adults [7]. The systemic clearance of VP16-213 was approximately three-fold greater than VM26 and the central volume of distribution ($VD_c$) was about two-fold larger for VP16-213. In our pediatric study population, the mean values for systemic clearance and $VD_c$ (when normalized to body surface area) and the relative differences between VM26 and VP16-213 were similar to the previously reported values for adults [7]. Although there was close agreement between the $VD_c$ for each drug in these two studies (VM26, 1.8 vs. 1.5; VP16-213; 3.1 vs. 3.3 l/m$^2$, mean $VD_c$), the $VD_{ss}$ in our study was considerably lower for both drugs and was different for VM26 and VP16-213. The low $VD_{ss}$ for each drug is consistent with the high degree of protein binding of each, with the smaller $VD_c$ and $VD_{ss}$ for VM26 consistent with the greater extent of VM26 plasma protein binding. The adequate discription of VM26 by a two-compartment model is in contrast to a previous report in adults [7], which may be due to the length of the intravenous infusions, differences in sampling times, number of samples, or differences in analytical methodology. Close agreement between systemic clearances calculated by both model-independent and model-dependent methods support the adequacy of the two-compartment model for VM26 in our study.

The hydroxy acids of VM26 and VP16-213 appear to be the major metabolic products detectable in plasma and urine, based on chromatographic peaks eluting in the region of the cis-hydroxy acids. Relatively low (< 5 μg/ml) concentrations of the picro-lactone isomers were detected in the plasma of some patients. The low cytotoxicity of the picro-isomers when assessed by clonogenic assays using CCRF-CEM cells ($IC_{50} \geq$ 100-fold higher than parent drugs) and the minimal serum concentration of the picro-isomers, suggest that these compounds are "metabolites" of minor clinical importance. However, this observation and the previous report that picro-podophyllotoxin is about 100-fold less active at inhibiting microtubular assembly than the trans-lactone podophyllotoxin [3, 5] indicate the importance of the stereochemistry of these compounds. The absence of detectable plasma concentrations of the aglycones in the present study does not rule out their potential production or tissue localization, necessitating future investigation of these potential metabolites or their conjugates.

The present study and future investigations further defining the individual components of systemic clearance (i.e., metabolic clearances, renal clearance) and evaluating potential differences in the extent of metabolism of VM26 and VP16-213, should provide a more complete basis for the clinical use of these two drugs.

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