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Short Communication

Bio-analysis of *N*-(deacetyl-*O*-4-vinblastoyl-23)-L-ethyl isoleucinate, methane sulphonate (VileE) by high-performance liquid chromatography with fluorescence detection*

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Introduction

Vincristine and vinblastine (VBL) are naturally occurring vinca alkaloids used for the treatment of a variety of neoplastic disorders. To improve the therapeutic efficacy and/or to change the antitumour spectrum of this class of compounds, analogues have been developed of which vindesine was the first to be clinically useful. In a series of 21 vinblastine-23-oyl amino acid derivatives tested for antitumour activity, vintriptol (VtrpE), the L-tryptophan ethyl ester and VileE, the L-isoleucine ethyl ester of deacetylvinblastine (Fig. 1) showed to be the most effective compounds [1, 2]. VtrpE has already passed phase I clinical evaluation and is now being investigated in a phase II clinical study. A high-performance liquid chromatographic (HPLC) assay for the analysis of VtrpE has been designed and preliminary pharmacokinetics of the drug have been reported [3, 4]. VileE is now being evaluated in preclinical studies and planned for a phase I study soon. This paper is the first report on the quantification of this experimental cytotoxic compound in plasma. Pharmacokinetic evaluation of a new antitumour drug constitutes an important part of a phase I study and as the first doses are very low the assay must possess a high sensitivity. The presented assay fulfills these requirements and will be used in the phase I study of VileE.

Experimental

Materials and reagents

Vinblastine (VBL) was obtained from Eli Lilly (Utrecht, The Netherlands) and *N*-(deacetyl-*O*-4-vinblastoyl-23)-L-ethyl isoleucinate, methane sulphonate (VileE) originated from Medgenix (Brussels, Belgium). All other reagents were purchased from E. Merck (Darmstadt, FRG) and were of analytical quality, except for chloroform and acetonitrile which were of HPLC grade. Water purified with the Millipore-Q system (Waters, Bedford, MA, USA) was used throughout.

Sample preparation

The vinca alkaloids were extracted from plasma in glass tubes fitted with Teflon covered screw caps. To 500 μl plasma, 10 μl internal standard solution [10 mg l^{-1} VBL in acetonitrile–water (90:10, v/v)], 2.5 ml 0.5 M phos-

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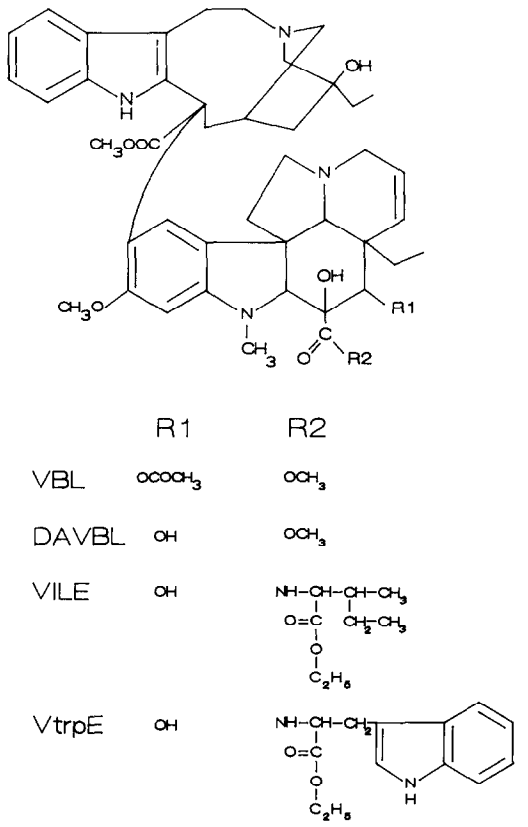

Figure 1
Structures of vinblastine (VBL), deacetylvinblastine (DAVBL), *N*-(deacetyl-*O*-4-vinblastoyl-23)-L-ethyl isoleucinate, methane sulphonate (VileE) and vintriptol (VtrpE).

phate buffer pH 3.0 and 5 ml chloroform was added. The tubes were shaken vigorously for 10 min. After centrifugation for 10 min at 2500*g* (4°C), the aqueous layer was discarded. The organic phase was transferred into a clean tube and evaporated to dryness under nitrogen (37°C). The residue was dissolved in 100 µl acetonitrile by sonification for 5 min. An aliquot of 50 µl was subjected to chromatography.

Liquid chromatography

Chromatographic analyses were performed using a HPLC system consisting of a Spectroflow SF400 pump, a Spectroflow 980 fluorimetric detector (Kratos, Ramsey, NJ, USA), a MSI 660 autosampler (Kontron, Basel, Switzerland) and a model CR-3A integrator (Shimadzu, Kyoto, Japan). Samples were chromatographed on a glass column (200 × 3 mm) packed with 5 µm Spherisorb Si material, preceded by a guard column (10 × 3 mm) packed with straight-phase material (Chrompack, Middelburg, The Netherlands). The mobile phase comprised a mixture of acetonitrile–10 mM citrate buffer pH 3.0 850:150 (v/v) containing 10 mM tetrabutylammonium bromide (TBABr). The excitation monochromator was set at 270 nm, while emission was monitored using a 320 nm longpass filter. Peak height ratios of VileE or VBL were used for quantitative calculations.

Stability of VileE was tested in spiked plasma samples yielding final concentrations of 20 or 1000 µg l^{-1}. The samples were stored at −20°C. Their concentrations were measured relative to a set of fresh diluted standards over a storage period of 4 months.

Results and Discussion

HPLC provides a sensitive and selective tool for the quantification of vinca alkaloids in biological material. Either reversed-phase HPLC with electrochemical detection [4, 5] or ion exchange straight-phase chromatography (IESPC) with fluorescence detection [6] was used. Since problems were reported using reversed-phase HPLC [4], we have optimized the IESPC for the quantification of VileE. The capacity factors of VileE and several other vinca alkaloids are given in Table 1. Sample pretreatment of biological fluids containing the vinca alkaloids vincristine, vinblastine and vindesine have been described using solid-phase extraction on Bond Elut CN columns [5, 6] but these vinca alkaloids can be effectively extracted from plasma with chloroform if samples are diluted with an aqueous buffer solution [4]. This study demonstrates that *N*-(deacetyl-*O*-4-vinblastoyl-23)-L-ethyl isoleucinate, methane sulphonate (VileE) could also be extracted from plasma into chloroform. As shown in Fig. 2 the optimal recovery of VileE was achieved with a buffer of pH 3. Because VBL was well separated from VileE and exhibited a similar recovery profile during sample pretreatment, it was very suitable as an internal standard.

Table 1
Capacity factors (k') of several vinca alkaloids

VBL	1.75
DAVBL	2.33
VileE	1.33
VtrpE	1.33

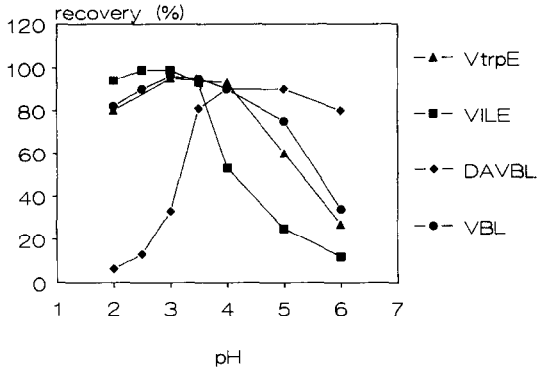

Figure 2
Recovery of VBL, DAVBL, VtrpE and VileE extracted from plasma. 0.5 ml plasma (containing 1 mg l^{-1} of each vinca alkaloid) was mixed with 2.5 ml phosphate buffer (pH) varied from 2 to 6) and extracted with 5 ml chloroform. Recovery was calculated relative to a standard sample diluted in acetonitrile.

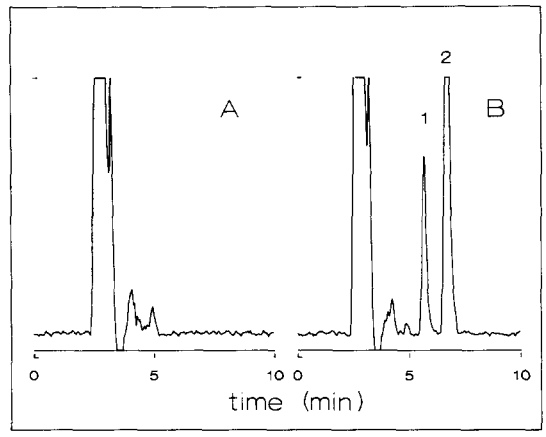

Figure 3
Chromatograms of plasma samples. (A) Represents a chromatogram of a blank plasma sample. (B) The same sample spiked with 20 μg l^{-1} VileE (1. VileE, 2. VBL).

Table 2
Reproducibility of the assay

Within run reproducibility				
Concentration (μg l^{-1}):	2	5	10	>10
RSD (%):	14.0	6.7	3.0	2.6
Run to run reproducibility				
Concentration (μg l^{-1}):	24.6		718	
RSD (%):	8.3		2.3	

The within run relative standard deviations (RSD) were calculated from the variations found between triplicates. Run to run RSDs were calculated from the concentrations found in the control samples analysed with each series.

Figure 3 depicts typical chromatograms of a blank and a spiked plasma. No interfering peaks were present allowing selective determination. The characterization of the assay was established over six separate days and the results on the reproducibility are presented in Table 2. All calibration curves were linear and pass through the origin. Slope and intercept data from a typical calibration curve ($Y = a^*X + b$) obtained in one series were for the concentration range 0–1000 μg l^{-1} (blank plus nine concentrations); a = 0.002422 ± 0.000014, b = 0.00500 ± 0.016711 and r = 0.9991 and for the concentration range 0–20 μg l^{-1} (blank plus four concentrations); a = 0.002610 ± 0.000040, b = −0.00079 ± 0.001067 and r = 0.9971. The detection limit of the assay was 2 ng ml^{-1} using a 500 μl sample. In plasma stored at −20°C VileE was stable for at least 4 months. In conclusion, a simple, accurate and sensitive method is presented for the analysis of VileE in plasma which is intended to be used for phase I pharmacokinetic analysis of the drug.

References

[1] K.S.P. Bhushana Rao, M.P. Collard, J.P.C. Dejonghe, G. Atassi, J.A. Hannart and A. Trouet, *J. Med. Chem.* **28**, 1079–1088 (1985).
[2] K.S.P. Bhushano Rao, M.P. Collard and A. Trouet, *Anticancer Res.* **5**, 379–386 (1985).
[3] O. van Tellingen, H.R. van der Woude, J.H. Beijnen and W.J. Nooyen, *Pharm. Weekbl. Sci. Ed.* **11** (suppl.), N10 (1989).
[4] O. van Tellingen, H.R. van der Woude, J.H. Beijnen, K.S.P. Bhushana Rao, W.W. ten Bokkel Huinink and W.J. Nooyen, *J. Chromatogr.* In press.
[5] D.E.M.M. Vendrig, J. Teeuwsen and J.J.M. Holthuis, *J. Chromatogr.* **424**, 83–94 (1988).
[6] D.E.M.M. Vendrig, J. Teeuwsen and J.J.M. Holthuis, *J. Chromatogr.* **434**, 145–155 (1988).

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