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High-performance liquid chromatographic bio-analysis and preliminary pharmacokinetics of the experimental antitumour drug vintriptol

O. VAN TELLINGEN* and H.R. VAN DER WOUDE

*Department of Clinical Chemistry, Netherlands Cancer Institute,
Antoni van Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX Amsterdam (The Netherlands)*

J.H. BEIJNEN

Slotervaart Hospital, Amsterdam (The Netherlands)

K.S.P. BHUSHANA RAO

Medgenix, Brussels (Belgium)

W.W. TEN BOKKEL HUININK

*Clinical Research Unit, Antoni van Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX Amsterdam
(The Netherlands)*

and

W.J. NOOYEN

*Department of Clinical Chemistry, Netherlands Cancer Institute, Antoni van
Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX Amsterdam (The Netherlands)*

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ABSTRACT

A high-performance liquid chromatographic procedure, including sample pretreatment, is presented for the analysis of the experimental antitumour drug vintriptol in plasma. The sample pretreatment involved liquid–liquid extraction of the buffered (pH 3) sample with chloroform. Vinblastine was used as internal standard. Separation was achieved on a Hypersil ODS (5 μm) column with a mobile phase of acetonitrile–phosphate buffer. Electrochemical detection (at $+0.70$ V) was used, giving a detection limit of 2 μg/l. The applicability of the assay was demonstrated in a pharmacokinetic study with eight cancer patients who received 45 or 50 mg/m^2 vintriptol in

a phase I study. A three-compartment model was used to fit the plasma concentration-time curves. Pharmacokinetic parameters are presented.

INTRODUCTION

Vincristine (VCR) and vinblastine (VBL) are naturally occurring vinca alkaloids (Fig. 1) derived from the plant *Catharanthus roseus* G. Don. These drugs are used for the treatment of a variety of neoplastic disorders. Their minor structural differences lead to different antitumour spectra, potencies and toxicities. In order to improve the therapeutic efficacy and/or to change to antitumour spectrum, analogues have been developed. Vindesine (VDS) was the first of these semi-synthetic derivatives shown to be clinically useful. Other analogues are currently under investigation and some have already been
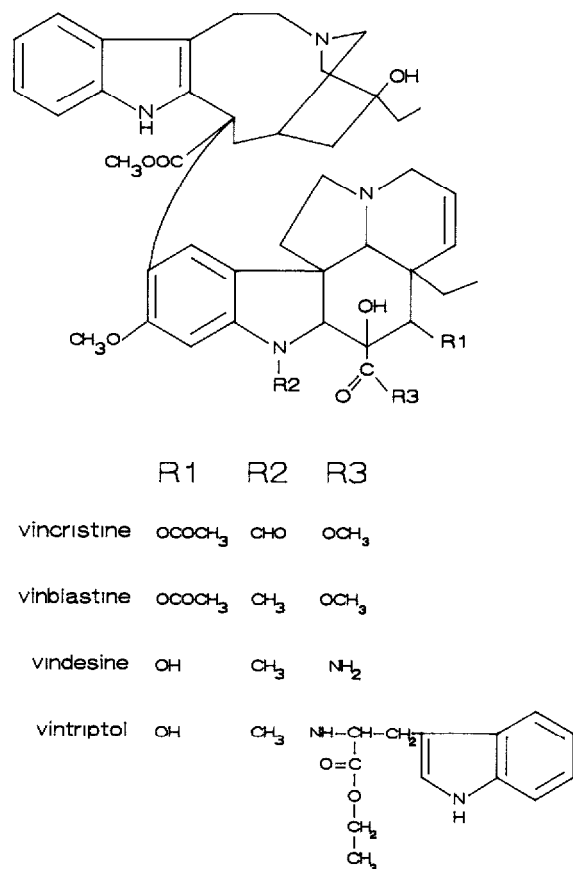

Fig. 1. Molecular structures of vinblastine, vincristine, vindesine and vintriptol.

tested in clinical trials [1,2]. In a series of 21 vinblastine-23-oyl amino acid derivatives that were tested for antitumour activity in murine leukemia P388 and L1210 and in 6C3HED lymphosarcoma test models, 4-deacetyl-3-L-O-ethyltryptophan-vinblastine [vintriptol (VtrpE)] was shown to be an effective compound [3,4]. These and other animal data justified the clinical evaluation of VtrpE, and the compound is now being tested in phase I trials in our institute.

This paper describes a method for the analysis of VtrpE in plasma of cancer patients. Preliminary pharmacokinetic data from patients who received dosages at or near the maximal tolerated dose (MTD) are also presented.

EXPERIMENTAL

Solvents and reagents

VDS, VBL and VCR were obtained from Eli Lilly (Utrecht, The Netherlands) and VtrpE from Medgenix (Brussels, Belgium). All other reagents were purchased from E. Merck (Darmstadt, F.R.G.) and were of analytical quality except for chloroform and acetonitrile, which were of HPLC grade. Water was purified by the Millipore-Q system (Water Assoc., Bedford, MA, U.S.A.).

Instrumentation and chromatography

Chromatographic analyses were performed using an HPLC system consisting of a Spectroflow 400 pump (Kratos, Ramsey, NJ, U.S.A.), an automatic sampling device MSI 660 (Kontron, Basle, Switzerland), the AMOR electrochemical detector provided with a standard glassy-carbon electrode (Spark, Emmen, The Netherlands) and a Model CR-3A integrator (Shimadzu, Kyoto, Japan). For some experiments UV detection at 270 nm using a Spectroflow 757 detector (Kratos) was used. Samples were chromatographed on a glass column (100 mm × 3 mm I.D.) packed with 5-μm Hypersil ODS material and preceded by a guard column (10 mm × 3 mm I.D.) packed with reversed-phase material (Chrompack, Middelburg, The Netherlands). The buffer used for the preparation of the mobile phase consisted of 50 mM KH_2PO_4, 0.5 g/l NaCl and 20 mg/l K_2EDTA, adjusted to a pH of 6.0 with a 5 M NaOH solution. The buffer was filtered through 0.45-μm cellulose acetate filters (HAWP) (Waters Assoc.). The mobile phase was prepared by mixing 620 ml of acetonitrile and 380 ml of buffer, and was degassed by vacuum. The flow-rate was 0.4 ml/min and the detection potential was maintained at +0.70 V.

Analytical procedure

The buffer used during the extraction procedure consisted of a 0.5 M KH_2PO_4 solution in water adjusted to pH 3.0 with 5 M hydrochloric acid. Extraction was carried out in glass tubes equipped with a PTFE-covered screw cap. A 500-μl volume of plasma and 50 μl of internal standard [20 mg/l VBL in acetoni-

trile–water (80:20)] were mixed with 2.5 ml of buffer. A 5-ml volume of chloroform was added, and 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 layer was transferred to a clean tube and evaporated under nitrogen (37°C). The residue was dissolved by sonication for 5 min in 62 μl of acetonitrile. Next, 38 μl of the phosphate buffer used for the preparation of the mobile phase were added, and 50 μl of the mixture were injected into the chromatographic system.

Calibration and quality control

Standard samples were prepared by making the appropriate dilutions of VtrpE in blank plasma. These samples were analysed with each run for construction of a calibration curve. Control samples were prepared by pooling patient plasma and were analysed with each series of samples to determine the day-to-day accuracy. Patient and control samples were analysed in duplicate. Standard and control samples were stored at $-20°$C. Peak-height ratios of VtrpE/VBL were used for the calculation of concentrations.

Patients

Eight patients received 45 ($n=6$) or 50 ($n=2$) mg/m^2 VtrpE as a short intravenous infusion (ca. 2 min) every week. During the first course venous blood samples were drawn from the opposite arm and collected in heparinized tubes before administration and 1, 5, 10, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 12, 18, 24 and 48 h after infusion. Plasma was separated immediately by centrifugation (10 min, 2000 g) and kept at $-20°$C until analysis within two months.

Data analysis

Pharmacokinetic parameters were calculated by non-linear regression analysis. The post-infusion plasma data were best fitted in a three-compartment open model:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\tau t}$$

The half-lives of the elimination constants were calculated as:

$$T_{1/2}(\alpha) = \frac{\ln(2)}{\alpha}$$

$$T_{1/2}(\beta) = \frac{\ln(2)}{\beta}$$

$$T_{1/2}(\tau) = \frac{\ln(2)}{\tau}$$

The area under the curve (AUC) from the first sample point to infinity was calculated as:

$$\text{AUC} = \frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\tau}$$

The AUC during the early missing period (start of infusion until the first sample drawn) was calculated using the linear trapezoidal rule as recommended by Chiou [5]. Both AUC values were added to obtain the area under the complete curve.

The plasma clearance

$$Cl = \frac{\text{dose}}{\text{AUC}}$$

and the volume of distribution

$$V_D = \frac{\text{dose}}{\text{AUC} \cdot \tau}$$

were calculated using classical pharmacokinetics.

RESULTS AND DISCUSSION

Solubility and stability of VtrpE

VtrpE is supplied as the sulphate salt and is readily soluble in water and acetonitrile, but virtually insoluble in 0.05 M phosphate buffer solutions. In high concentrations in water it is very stable, but in low concentrations (less than 20 mg/l) considerable amounts are lost, probably due to adsorption. A linear response curve can be obtained from 5 to 20 000 μg/l, however, when it is diluted in mobile phase. Problems of non-linearity occurring from analytical columns are discussed under *Chromatography*.

Plasma samples containing VtrpE were stable for at least 2 months if stored at $-20°C$.

Sample preparation

Extraction of VtrpE and other vinca alkaloids with chloroform from water yielded high recoveries (ca. 100%), but the recovery obtained by direct extraction from plasma was low (30%). Extraction from plasma with high recoveries is possible, however, if samples are prediluted with an aqueous buffer solution. The recovery obtained for the various tested vinca alkaloids was dependent on pH (Fig. 2). An optimum for VtrpE was found at pH 3. The extraction mixtures should be shaken vigorously for at least 10 min. The residue must first be dissolved in pure acetonitrile by sonication before the addition of mobile phase buffer. The virtually identical profiles for VtrpE and VBL make the
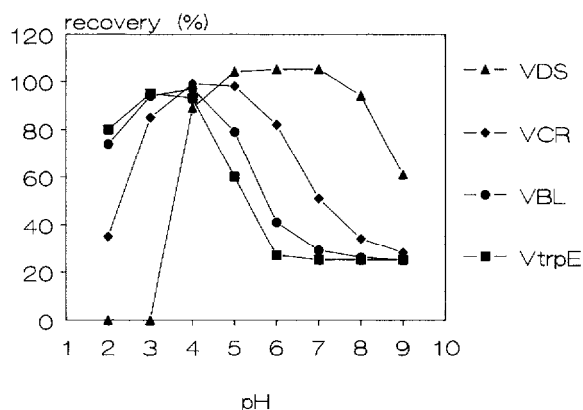
recovery (%)

Fig. 2. Extraction of vinca alkaloids from plasma. A 500-μl volume of plasma (containing 0.5 μg each of VBL, VCR, VDS and VtrpE) was mixed with 2.5 ml of 0.5 M phosphate buffer and extracted with 5 ml of chloroform. The pH of the buffer ranged from 2 to 9. The recovery was recorded relative to a mixture of the compounds diluted in mobile phase.

TABLE I

RECOVERY OF THE EXTRACTION PROCEDURE

Spiked plasma samples were extracted in quadruplicate. The recovery is recorded relative a standard solution dissolved in mobile phase.

Concentration (μg/l)	Recovery (%)	Coefficient of variation (%)
5	87.2	15.8
50	91.5	1.0
500	90.4	1.1
5000	93.4	6.0
20000	92.4	2.3

latter a good internal standard. The recovery of the extraction procedure at various concentrations is given in Table I. No evidence for any adsorption during the sample preparation procedure was noticed.

Chromatography

HPLC provides a selective tool for the quantification of vinca alkaloids in biological material. The combination of reversed-phase HPLC and electrochemical detection allows accurate quantification down to the μg/l level [6,7]. As with many other basic drugs, vinca alkaloids show peak broadening on reversed-phase columns. We tested various materials, e.g. Chromspher C_{18}, Spherisorb-ODS2, Hypersil-ODS and μBondapak C_{18}. Only Hypersil-ODS in combination with a mobile phase of pH 6–7 did not give excessive peak tailing.
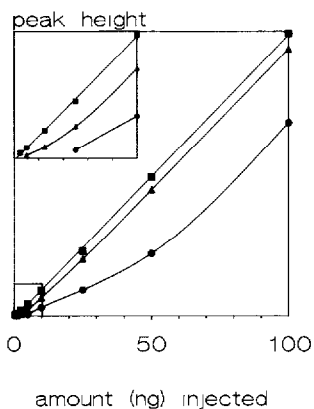
Fig. 3. Non-linear response curves. VtrpE diluted in mobile phase (range 10–2000 μg/l) was injected into the chromatographic system. UV detection at 270 nm was used. A non-linear response was obtained if a new column was used. The linearity was restored if a small amount of vindesine was added to the mobile phase. (●) Mobile phase; (▲) mobile phase plus 20 μg/l VDS; (■) mobile phase plus 200 μg/l VDS.
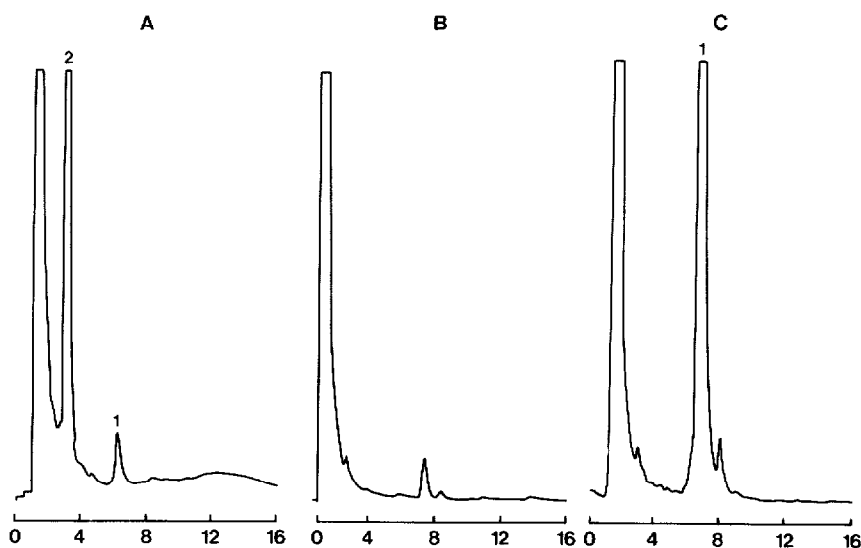

Fig. 4. Chromatograms of plasma samples. (A) Plasma sample spiked with 20 μg/l VtrpE. Peaks: 1 = VtrpE; 2 = VBL. (B) Plasma sample from a patient prior to the administration of VtrpE. No internal standard is added. No interference was present for VtrpE, and interference for VBL amounted less than 2% of the amount added as internal standard. (C) Plasma sample from a patient 30 min after administration of VtrpE (1). No internal standard is added. No other peak except that of unchanged VtrpE has appeared.

TABLE II

QUALITY CONTROL

Run-to-run coefficients of variation were calculated from the concentration found in control samples analysed with each series. The within-day accuracy was calculated from the variation found between duplicates.

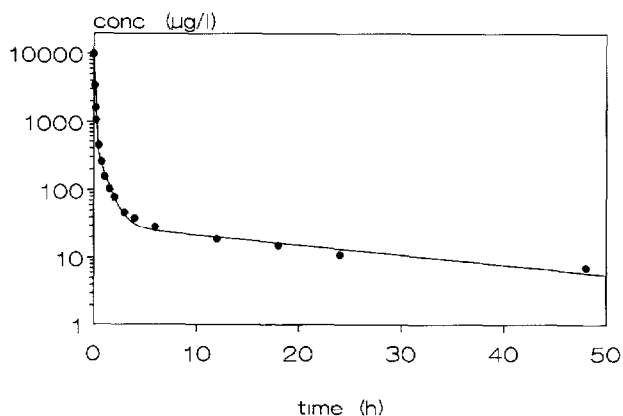| Concentration (μg/l)	Coefficient of variation (%)	n
Within-run		
< 10	8.0	10
> 10 and < 100	2.8	52
> 100	2.0	78
Run-to-run		
44	5.1	12
2560	4.0	12

Fig. 5. Plasma concentration decay curve. A patient received 45 mg/m^2 VtrpE as a short intravenous infusion. The concentration in plasma decreased in accordance with a three-compartment open model.

However, we observed a non-linear concentration response curve of VtrpE diluted in mobile phase, when we installed a new column (Fig. 3). This non-linearity, which appears to be caused by a reversible binding of the compound to the analytical column, diminished if the column was used for some time (1–2 weeks and ca. 100–200 pretreated plasma samples injected). Treating a new column overnight with a mobile phase containing 5 mg/l VtrpE in an attempt to shield the adsorption sites, followed by reequilibration with mobile phase, did not lead to a more linear curve. Linear curves on new column were obtained if sufficient (200 μg/l) VDS was added to the mobile phase during the analyses,

TABLE III

PHARMACOKINETIC PARAMETERS FOR VtrpE

Patient	Dose (mg)	Body weight (kg)	Plasma half-lives (h)			AUC (mg h/l)	V_d (l/kg)	Cl (l/h/kg)
			α	β	τ			
De	80[a]	66	0.0570	0.651	15.0	2.37	11.0	0.511
Ve	70[a]	50	0.0656	0.619	20.6	2.22	18.7	0.630
Ka	75[a]	65	0.0982	1.394	24.1	3.05	13.1	0.378
St	95[a]	88	0.0582	1.055	18.3	2.17	13.1	0.497
Ho	90[a]	84	0.0611	1.055	21.5	2.80	11.8	0.382
Sc	85[a]	81	0.0740	1.010	18.3	3.23	8.6	0.325
Mean[a]			0.0690	0.964	19.6	2.64	12.7	0.454
S.D.[a]			0.0155	0.290	3.1	0.45	3.4	0.113
Ja	85[b]	65	0.0621	1.072	21.2	2.75	14.5	0.476
Pe	100[b]	76	0.0338	0.727	24.2	2.32	19.8	0.567

[a] 45 mg/m^2.
[b] 50 mg/m^2.

probably because VDS competes with VtrpE for the adsorption sites. Further research will be needed to elucidate this phenomenon.

Although the detection limit of VtrpE in mobile phase using the more practical UV detection at 270 nm is 8 μg/l (signal-to-noise ratio 5:1), the limit of determination in plasma samples was higher than 20 μg/l owing to the presence of endogenous compounds. Therefore electrochemical detection operating at a potential of +0.70 V was used to combine sufficient sensitivity with optimal selectivity. No interfering compounds were found in blank plasma samples (Fig. 4). Although some interference was present for VBL, it was minor compared with the amount of VBL added as internal standard. If VDS was added to the mobile phase, background current increased and pulsation became a severe problem. In order to maintain sufficient sensitivity, the installation of a high-efficiency pulse damper is required.

Quality control

A summary of the day-to-day and within-day accuracy is given in Table II. Excellent reproducibility was obtained throughout the entire concentration range.

Pharmacokinetics results

A typical concentration–time curve is depicted in Fig. 5. The results of the pharmacokinetic study are summarized in Table III. The ranges of the pharmacokinetic parameters are narrow, indicating little inter-patient variability. This is in contrast with the results obtained for other vinca alkaloids [8,9]. The half-lives were comparable with those reported for VBL. Plasma AUC

levels of VtrpE are approximately ten times higher than those reported for VBL.

CONCLUSION

A method is presented for the analysis of VtrpE in plasma. Liquid–liquid extraction with chloroform is a suitable sample pretreatment method. The pharmacokinetic parameters for VtrpE show little inter-patient variability.

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