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Short communication

Improved high-performance liquid chromatographic analysis of teniposide in human plasma

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Abstract

A simple and practical high-performance liquid chromatographic analysis has been developed for measuring teniposide (VM26) in human plasma. The present analytical method has improved extraction efficiency from human plasma, therefore allowing determination of VM26 in a clinical setting using ultraviolet detection alone. Furthermore, sample preparation was simplified and shortened through use of a one-step extraction procedure. VM26 and internal standard (ibuprofen) were extracted from human plasma (0.5 ml) with ethyl acetate. A phenyl μBondapak column eluted with a mobile phase, consisting of acetonitrile–distilled water–acetic acid (30:68:2, v/v/v) was used for separation, and quantitation was achieved with a UV monitor set at 240 nm. Average extraction efficiency was 96.8±6.6% for VM26 between 1 and 25 μg/ml, and 91.4±4.3% for internal standard, with both intra- and inter-day coefficients of variation being less than 10%. The detection limit with a 100-μl injection was estimated at 0.2 μg/ml with a signal-to-noise ratio of 3 for VM26 in human plasma. The stability data of VM26 in plasma, standard and stock solutions were also obtained. The present method was found to be an alternative to the previously reported method with an electrochemical detection, and can be easily applied to routine clinical pharmacokinetic studies of VM26.

Keywords: Teniposide

1. Introduction

The glycosidic podophyllotoxin derivative, teniposide (VM26), is effective in combination with other approved anticancer agents for treatment of acute lymphocytic leukemia, lymphomas, and neuroblastoma in children and ovarian carcinoma [1–3]. In a phase I trial, inter-patient pharmacokinetic variability yielded a four- to six-fold difference in systemic exposure with the same dose, which could be linked to clinical response [4,5]. Therefore, it is essential to monitor plasma VM26 levels and achieve an effective systemic exposure for optimizing individual pharmacokinetics.

Although several HPLC analytical methods for podophyllotoxin derivatives have been reported [6–10], VM26 was generally used as an internal standard (I.S.) for etoposide assay. Therefore, data on analytical efficiency for VM26 have not been sufficient

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for the clinical application of those assay methods. Sinkule and Evans [9] and Canal et al. [10] reported both extraction efficiencies and detection limits using electrochemical detection.

The present method has improved extraction efficiency from human plasma with a simple extraction procedure and has been easily applied to routine clinical study of VM26 using UV detection alone.

2. Experimental

2.1. Chemicals and reagents

VM26 was a gift from the manufacturer (Bristol Laboratories, Syracuse, NY, USA). The stock solution (1 mg/ml) was made in methanol and stored at −38°C for no longer than 1 week. The standard solutions (1, 2, 5, 10 and 25 μg/ml VM26 and 100 μg/ml ibuprofen) were prepared by dilution of the stock solution with the mobile phase, consisting of acetonitrile–distilled water–acetic acid (30:68:2, v/v/v). All other chemicals and reagents were of analytical and reagent grade.

2.2. HPLC apparatus and analytical conditions

The HPLC system (Jasco Gulliver Series, Jasco Corporation, Tokyo, Japan) equipped with an isocratic pump (PU-980), an injector (AS-950) maintained at 4°C, a column oven (CO-960) at 50°C, a UV absorbance detector (UV-970) set to a wavelength of 240 nm and an integrator (HP Laserjet 4PJ, Yokogawa Hewlett-Packard, Tokyo, Japan), which was operated by a system station (LCSS-905). An analytical column (30 cm×4 mm, 10-μm, μBondapak phenyl, Waters, Milford, MA, USA) and a precolumn (Guard-Pak module with phenyl insert, Waters) were used for separation of VM26 from other metabolites and plasma components. The mobile phase was eluted at a constant flow-rate of 1.5 ml/min.

2.3. Extraction procedure

A 0.5-ml volume of human plasma in a 10-ml glass tube was extracted with ethyl acetate (4 ml) after the addition of 0.5 ml of internal standard (I.S., 100 μg/ml ibuprofen in mobile phase), 0.2 *M* acetate buffer (pH 3) and 0.5 ml of saturated ammonium sulfate. The extraction was performed by mechanical shaking for 15 min. After centrifugation (1000 *g*) for 10 min, 3 ml of the upper organic layer was transferred to another glass tube. The organic layer was gently evaporated at 40°C using a rotary evaporator (Shibata, Tokyo, Japan). The residue was reconstituted with 500 μl of the mobile phase, and an aliquot (100 μl) was injected into the HPLC system.

2.4. Analytical efficiency

VM26 standard solution (1, 2, 5, 10 or 25 μg/ml) was spiked in blank plasma donated from a healthy female or pooled plasma from four healthy volunteers (one female and three male). This was extracted according to the procedure described above, and the residues were reconstituted in 500 μl of the mobile phase. Equivalent concentrations of VM26 (1, 2, 5, 10 or 25 μg/ml) in the mobile phase were added to the residue of 0.5-ml blank plasma extracts. Extraction efficiency was calculated by comparing the peak area of VM26 extracted with that of the same concentration added to the plasma residue to give a final volume of 0.5 ml after extraction of blank plasma. For intra-day reproducibility, five replicates of plasma sample (0.5 ml) each containing VM26 standard solution were tested on the same day. For inter-day variability, the extraction procedure was performed on each of 5 separate days. The intra- and inter-day coefficients of variation (as percentage) were calculated (see Table 1).

2.5. Stability

Degradation of VM26 was determined in order to ensure the stability of VM26 in methanol (stock solution), in mobile phase (standard solution) and in human plasma. The stock solution (1 mg/ml in methanol), stored at −38°C, was evaluated every week. VM26 standard solution (5 μg/ml in the mobile phase), stored at 4°C, was sampled (0.5 ml) on days 0, 1, 2, 3 and 7. VM26 (5 μg/ml spiked in plasma) was evaluated on days 0, 1, 3, 7 (4°C) or on day 0 and after 1 month (−70°C). The concentration of VM26 was quantitated by HPLC as described

Table 1
Analytical efficiency of VM26 in human plasma

Concentration added (μg/ml)	Concentration found (μg/ml)	C.V. (%)	Recovery (%)	C.V. (%)
Intra-day				
1	1.06±0.03	3.0	91.8±5.2	5.7
2	2.05±0.08	4.1	104.1±4.6	4.4
5	4.97±0.18	3.6	95.2±3.4	3.6
10	10.95±0.52	4.8	104.7±6.1	5.8
25	24.65±0.38	1.5	93.8±2.0	2.2
I.S.			91.6±4.0	4.4
Inter-day				
1	1.12±0.09	8.2	94.0±7.6	8.1
2	2.06±0.12	6.0	97.8±8.2	8.4
5	4.93±0.14	2.9	96.9±2.7	2.7
10	10.79±1.00	9.2	95.5±8.5	8.9
25	25.01±0.02	0.1	93.6±2.2	2.2
I.S.			91.2±4.6	5.1

C.V.=coefficient of variation.

above. The stability data were obtained as the remaining percentage against the area on day 0.

2.6. Preliminary clinical application

To determine the usefulness of the present procedure, ten plasma samples were obtained from a male cancer patient with non-small cell lung carcinoma after infusion of 60 mg/m^2 VM26 over 1 h. A 5-ml volume of blood was drawn into heparinized tubes prior to drug administration, at the 30th min of infusion and at 0 (completion of infusion), 10 min, 1, 2, 4, 6, 12 and 23 h postinfusion, then centrifuged for 10 min at 4°C. The plasma samples were stored frozen at −70°C until analyzed.

3. Results and discussion

We have modified previously published methods which extracted VM26 twice with chloroform or ethyl acetate, separated through reversed-phase column and monitored using ECD [9,10], to develop a simple and practical procedure for routine monitoring of VM26 in human plasma using UV detection alone. Typical chromatograms are shown in Fig. 1. The retention times were about 16 min (VM26) and 21 min (I.S.), respectively. Although there were several chromatographic peaks observed in blank plasma within 14 min retention, no peak interfered with VM26 or the I.S. VM26 is almost completely metabolized, which can account for nonrenal clearance [3]. The separation of VM26 from metabolites, including the picro lactone form, could be obtained only using μBondapak phenyl or cyano reversed-phase columns [7–10]. Although we had no authentic samples of VM26 metabolites, the main metabolites could be detected in the present analytical method because the same type of analytical column, mobile phase and ethyl acetate extraction as in the method of Sinkule and Evans [9] were utilized.

The extraction efficiency was a mean of 96.8±6.6% for VM26 (all validation data between 1 and 25 μg/ml) and 91.4±4.3% for I.S. (all validation data). Quantitative precision evaluated by inter- and intra-day variability was less than 10% for VM26 and I.S.. A better extraction efficiency for VM26 was obtained, compared to the 66% [9] and about 90% [10] reported previously. Although extracted plasma sample in methanol (50 μl) had been injected to HPLC in the previous study [9], we found predominant shoulders on both peaks of VM26 and I.S. in methanol after 100-μl injection (Fig. 1B). Data on extraction efficiency in their study [9] was obtained by comparing methanol reconstitution (extracted VM26) and the equivalent amount in the mobile phase, which might be related to lower extraction efficiency. The same study [9] monitored

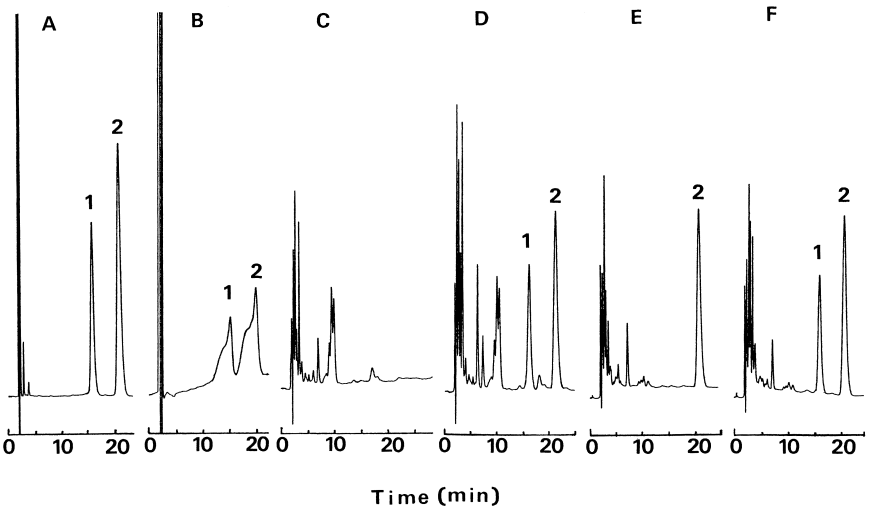

Fig. 1. Typical chromatograms of VM26 (5 μg/ml, 1) and internal standard (ibuprofen 100 μg/ml, 2) in mobile phase (A), in methanol (B) and a blank plasma sample (C), and chromatograms of a blank plasma spiked with 5 μg/ml of VM26 (D), predose (E) and 2 h postinfusion (F) plasma samples from a male cancer patient receiving 60 mg/m^2 of VM26.

VM26 at UV 280 nm, which showed about one-tenth fold less sensitivity of that by electrochemical detection (detection limit of 0.05 μg/ml). We used the more effective wavelength of UV 240 nm in the mobile phase and extracted VM26 by longer shaking (for 15 min), therefore, improved extraction efficiencies for both VM26 and I.S. and a satisfactory detection limit for clinical application were obtained in spite of the simple extraction procedure and use of UV detection alone.

Most previous studies utilized etoposide as an I.S. for VM26. Etoposide could not be applied in the present study because interference from plasma was detected on the chromatogram at 240 nm. We used ibuprofen as an I.S., and also examined the effectiveness of indomethacin as an I.S. which eluted at 28 min on the chromatogram. Therefore, if ibuprofen is coadministered in a clinical setting, indomethacin can be applied as an I.S..

VM26 was inactivated by acid treatment, which may be attributed to the increased lability of the O-4,6 acetyl bond to acid hydrolysis because of the adjacent 2-thienyl group in VM26 [11]. Therefore, the stability of VM26 in the stock solution, standard solution or plasma was studied in the present study. The peak area of VM26 (5 μg/ml in the mobile phase) decreased according to apparent first-order kinetics, and the remaining percentage at day 7 was a mean of 25% of the day 0 value. The degradation half-life in the mobile phase was a mean of 3.55±0.4 day (n=3), suggesting that the remaining VM26 might be less than 95% at 6.3 h postreconstitution. Therefore, it is recommended to inject the HPLC sample as soon as possible after reconstitution with the mobile phase, and use the cooling unit, if possible. VM26 in methanol was stable at −38°C after 1 week (mean 99.8% of day 0 value), but the area after 2 weeks was slightly smaller (mean 96.9%). The stock solution should be prepared every other week. In plasma, VM26 was stable at 4°C for 1 week (mean 103.2% of day 0 value), and stable at −70°C for at least 1 month (mean 98.4% of day 0 value)

The plasma concentration–time profile of VM26 in a cancer patient receiving 60 mg/m^2 is shown in Fig. 2. VM26 in plasma declines biexponentially. VM26 is most often administered as an infusion over 30–60 min at daily to weekly intervals, and peak plasma concentration was about 30 μg/ml in a patient with ovarian cancer receiving 100 mg/m^2 over 1 h [12] and about 50 μg/ml at a dose of 165 mg/m^2 over 40 min [9]. The peak plasma con-

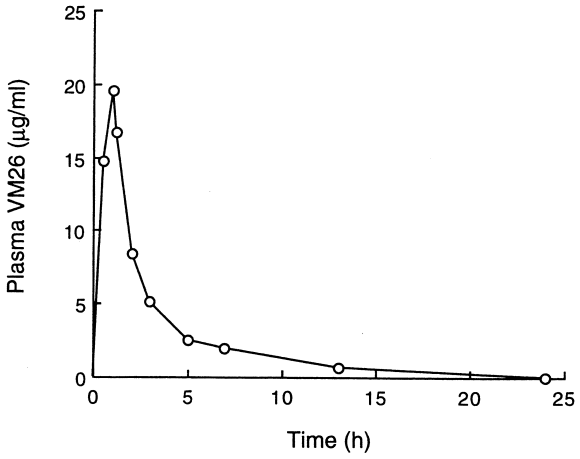

Fig. 2. Plasma concentration–time curve of VM26 for a male cancer patient receiving 60 mg/m^2 of VM26 infused over 1 h.

centration after 60 mg/m^2 over 1 h in the present study, was 19.5 μg/ml, almost corresponding to reported values.

VM26 competitively inhibited 4-hydroxylation of *S*-mephenytoin with human liver microsome at a concentration corresponding to the plasma level easily achieved with a clinically used dose [13]. It has been reported that the O-demethylation pathway of VM26 is primarily mediated by CYP3A4 [14]. These in vitro results may account for the wide inter-patient pharmacokinetic variability and drug interactions of VM26 with other drugs metabolized by CYP2C and 3A in a clinical setting. It may, therefore, be important to evaluate the pharmacokinetics of VM26 in vivo. The present method was found to be easily applicable for routine monitoring of plasma VM26 level.

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