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Short communication

Improved high-performance liquid chromatographic detection of paclitaxel in patient's plasma using solid-phase extraction, and semi-micro-bore C_{18} separation and UV detection

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Abstract

Although a number of analytical methods for taxanes have been published, none of them are sufficiently suitable for use in a medical setting. In this study, we established an improved analytical HPLC/UV detection method using a Sep-Pak C_{18} cartridge for extraction and a semi-micro-bore column for separation. This method employed here reduced chromatographic background signals, and allowed a more sensitive analysis of taxanes in human blood sample. The recovery of taxanes after the solid-phase extraction procedure was over 90%. Chromatographic separation of paclitaxel and docetaxel was achieved within 30 min with no interference peak by a semi-micro-bore column, packed either with C_{18} (Wakosil 5C18 RS) or pentafluorophenyl (Curosil/Taxol) materials. The method was reproducible with coefficients of variation less than 6%. This analytical procedure was simple and sensitive with lower quantification limit of 3 ng/ml. The improved sensitivity achieved by the popular HPLC/UV apparatus, which is available in hospitals, would vouch safer and more efficient therapy with taxane.

Keywords: Paclitaxel; Docetaxel; HPLC/UV; Semi-micro-bore column; Sep-Pak C_{18}; Therapeutic drug monitoring

1. Introduction

During the past decade, several newer chemotherapeutic agents, including taxanes, topotecan, irinotecan, and gemcitabine, have demonstrated substantial single-agent activity in the treatment of advanced carcinoma [1]. Recently, a number of trials combining these new drugs either with a platinum agent or with each other as doublets have shown substantial activity [2]. Among these new drugs, taxanes, originally isolated from the bark of the tree *Taxus brevifolia*, is potent inhibitors of cell replication and have strong antitumor activities. The antimitotic activity of taxane results from its ability to block the late G2 and M phases of the cell cycle. Taxanes, paclitaxel and docetaxel, have significant antitumor activities in several human tumors, including advanced ovarian, lung and breast cancers [3]. Paclitaxel-induced cell death is concentration-dependent [4,5]. On the other hand, the therapy with taxanes also causes sever side effects, such as leucopenia, neutrophil depletion, disorder of peripheral nerve nausea and vomiting [6]. A pharmacokinetic study of paclitaxel has clearly demonstrated the relationship between dose-limiting toxicity and serum its concentration [7]. In patients receiving taxane therapy, the individual plasma drug concentration appears to be up to threefold variability [8]. Thus, the therapeutic drug monitoring (TDM) during the treatment of taxane drug should be substantially beneficial for patients.

Numerous analytical methods for determination of taxanes in biological fluids have been proposed [9–14]. Most of them rely on HPLC separation with UV [9–12] or mass detection [13,14]. The HPLC method with a UV detector is convenient for TDM in a medical setting. In recent years, the dose-dense chemotherapy, a weekly regimen with a lower dose of taxane, has been introduced to prevent myelosuppression [15]. With this regimen,

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an assay method with a lower quantification limit approximate 10 nM (8 ng/ml) should be required for the TDM of paclitaxel [16] and previously published HPLC/UV methods have insufficient sensitivity [17]. On the other hand, the majority extraction methods of taxanes from plasma are based on the solid-phase extraction [9], which is packed with CN packing materials, or the organic solvent extraction [10,11] method. These extraction methods do not always give good results, especially when taxane levels are very low in the blood. In this study, we improved the HPLC/UV method for taxane detection using a semi-micro-bore analytical column combined with a C_{18} solid-phase extraction step. We also discussed TDM in patients receiving dose-dense weekly chemotherapy of paclitaxel.

2. Material and methods

2.1. Chemicals

Standard paclitaxel and docetaxel were prepared from commercial available products from Bristol-Myers Squibb (Tokyo, Japan) and Sanofi Aventis (Tokyo, Japan), respectively. Sep-Pak C_{18} cartridge was purchased from Waters (360 mg type, Milford, MA, USA). Phosphoric acid and *n*-hexane were obtained from Nacalai (Kyoto, Japan). All the other chemicals were products of HPLC or reagent grade from Wako (Osaka, Japan). Re-distilled water was used throughout the study.

2.2. Blood samples

Blood was withdrawn into a heparinized tube from healthy volunteers and three patients after obtaining their informed consents. The plasma sample was obtained after centrifugation at 1200 g for 5 min and stored at −30 °C until analysis.

2.3. Sample preparation for quantification of paclitaxel

An aliquot of 5 μl of 100 ng docetaxel (internal standard) in methanol was added to 0.5 ml of plasma sample. The sample was diluted with 4.5 ml of 20 mM ammonium acetate buffer (pH 5.0). The sample solution was passed through a Sep-Pak C_{18} cartridge pre-washed with 10 ml methanol and 10 ml of 20 mM ammonium acetate. After rinsing with 5 ml of 10 mM ammonium acetate, 5 ml of 20% methanol in 10 mM ammonium acetate and 2 ml of *n*-hexane, the cartridge was vacuum dried for 5 min. The taxanes were then eluted with 5 ml of acetonitrile. The eluant was dried under a stream of nitrogen at 50 °C. The dried residue was re-resolved in 100 μl of the mobile-phase solution. An aliquot of 50 μl of the sample was injected into a HPLC apparatus (Jasco 900 series, Tokyo, Japan) equipped with a UV detector (Jasco model UV 970, Tokyo, Japan).

2.4. Calibration curve

The known amount of paclitaxel, ranging from 3 to 1200 ng/ml, was spiked into 0.5 ml of drug-free human plasma from volunteers and carried through the same workup procedure as described above in triplicate. The calibration curve of paclitaxel was constructed by plotting peak-area ratios of authentic compounds to internal standard.

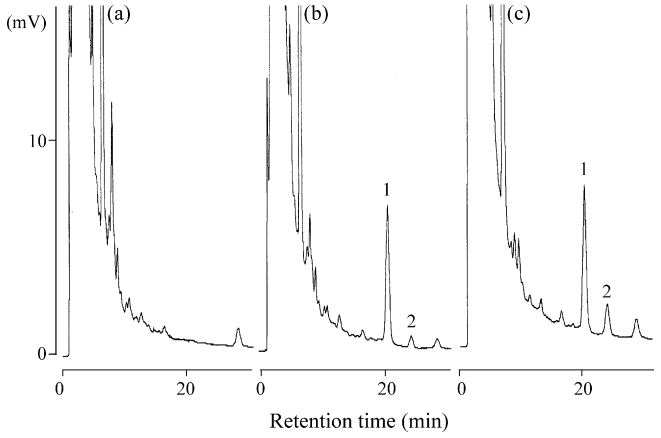

Fig. 1. Typical chromatographic separations of taxanes on Wakosil column. (a) Blank human plasma, (b) plasma spiked with docetaxel (100 ng/ml) and paclitaxel (12 ng/ml), and (c) Patient plasma under paclitaxel therapy. Peak 1, docetaxel (internal standard); 2, paclitaxel.

2.5. HPLC conditions

Taxanes were measured by HPLC/UV at wavelength of 230 nm. The separation of each compound was achieved on a Wakosil 5C18 RS column (250 mm × 2.0 mm i.d., Wako, Osaka, Japan) or a Curosil/Taxol (250 mm × 2.0 mm i.d., Phenomenex, Torrance, CA, USA), which is packed with pentafluorophenyl materials and is commercially recommended for taxane analysis. The mobile phase of 20 mM potassium phosphate buffer (pH 3.0) and acetonitrile (55:45 v/v) was delivered at a flow rate of 0.25 ml/min at 45 °C.

3. Results

3.1. Chromatographic separation

Chromatographic separation of paclitaxel and docetaxel was achieved within 30 min without any interference peak from either Wakosil 5C18 RS or Curosil/Taxol (Figs. 1 and 2). However, the chromatographic background signals derived from blank plasma using the Wakosil column (Fig. 1a) were slightly lower than those of the Curosil/Taxol column (Fig. 2a). The retention times of paclitaxel and docetaxel on the Wakosil column were 24.5 and 20.7 min, respectively. Those on the Curosil/Taxol column were 21.6 and 18.7 min for paclitaxel and docetaxel, respectively.

3.2. Calibration curve

The standard curves were expressed as the regression equation of a straight-line, $y = ax + b$, where y is the concentration, a the slope, x the peak area ratio against internal standard and b is the y-axis intercept, by the weighted liner regression analysis. The equation describing the calibration curves for paclitaxel using the Wakosil and Curosil/Taxol columns were

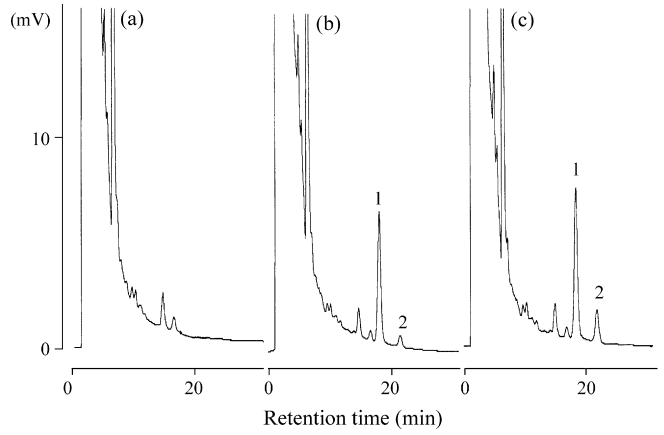

Fig. 2. Typical chromatographic separations of taxanes on Curosil/Taxol column. (a) Blank human plasma, (b) plasma spiked with docetaxel (100 ng/ml) and paclitaxel (12 ng/ml), and (c) Patient plasma under paclitaxel therapy. Peak 1, docetaxel (internal standard); 2, paclitaxel.

Table 1
Recovery of taxanes from human plasma

Spiked concentration (ng/ml)	Recovery (%)	CV (%)
Paclitaxel		
12	91.6	3.8
150	91.8	3.6
300	92.6	3.4
Docetaxel		
100	92.0	3.2

The data are expressed as the mean of six analyses.

$y = 121.38x + 2.22$ and $y = 125.68x + 3.72$ with correlation coefficient of 0.999 and 0.999, respectively. The lower quantification limits of paclitaxel and docetaxel were 3 ng/ml (signal to noise; S/N > 3) spiked in control plasma.

3.3. Recovery and precision studies

The recovery of paclitaxel after the solid-phase extraction procedure employed here was evaluated at three concentrations, namely, 12, 150 and 300 ng/ml ($n = 6$). The efficiency of extraction from plasma was 91.6, 91.8 and 92.6% at 12, 150 and 300 ng/ml concentrations, respectively, using the Wakosil column (Table 1). The recovery of internal standard (docetaxel) was 92.0% (Table 1).

The accuracy and precision study of the assay method was conducted at five different concentrations of paclitaxel (Table 2). The method was reproducible with coefficients of variation (CV) less than 6%.

Table 3
Paclitaxel concentrations in the blood of patients who were receiving the dose-dense chemotherapy of paclitaxel with a daily dose of 30–70 mg/m^2

Patient	Cancer type	Dose (mg/m^2)	Time after infusion (h)	Blood level (ng/ml)
1	NSCLC	70	24	35.6
			48	17.8
2	NSCLC	70	24	38.2
			48	18.8
3	MOC	60	126	15.6
		30	24	35.8

NSCLC, non-small cell lung cancer; MOC, metastatic ovarian cancer.

3.4. Application of the method

Plasma paclitaxel concentrations were determined in a metastatic ovarian cancer and two non-small cell lung cancer patients, who were receiving the dose-dense weekly chemotherapy of paclitaxel with a daily dose of 30–70 mg/m^2. Blood was taken 24–126 h after paclitaxel treatment. Paclitaxel was clearly detected from the patient plasma samples (Fig. 1c and 2c), and the levels were determined as 15.6–38.2 ng/ml (Table 3).

4. Discussion

Traditionally, agent development has involved evaluation of dose–response relationships and clinically objective endpoints (e.g., survival, cancer incidence). In recent years, several innovative strategies have been advanced for improving

Table 2
Accuracy and precision study on the paclitaxel assay

Prepared (ng/ml)	Run-to-run			Day-to-day		
	Detected (ng/ml)[a]	Accuracy (%)	CV (%)	Detected (ng/ml)[a]	Accuracy (%)	CV (%)
Wakosil column						
6	5.88 ± 0.39	−3.8	5.28	5.82 ± 0.46	−4.4	5.66
12	12.17 ± 0.56	4.3	5.25	11.20 ± 0.66	−2.2	5.52
30	29.11 ± 1.38	−1.6	4.27	28.91 ± 1.57	−3.2	4.57
150	145.66 ± 6.78	5.2	3.75	149.90 ± 7.76	6.5	5.87
300	288.54 ± 18.5	−5.7	4.25	301.02 ± 18.9	5.5	5.66
Curosil/Taxol column						
6	5.78 ± 0.32	4.6	5.25	5.88 ± 0.31	5.0	5.01
12	11.17 ± 0.56	−5.5	4.25	11.00 ± 0.86	−5.5	4.41
30	27.92 ± 1.25	−6.3	3.80	28.99 ± 1.57	4.3	4.05
150	140.53 ± 5.38	−6.0	3.55	141.86 ± 6.54	−5.8	5.07
300	290.22 ± 20.5	−4.5	3.89	301.02 ± 17.9	8.0	4.23

[a]Data are expressed as means ± S.D. of six analyses.

the efficiency of agent evaluation, including pharmacokinetic/pharmacodynamic (PK/PD) and intermediate endpoint biomarker-guided development. The PK/PD approach, which includes TDM, is well suited for those agents with a narrow therapeutic index and substantial PK variability. From this point of view, simple and sensitive analytical methods are required for such drugs. Although a number of analytical methods for taxanes have been published, none of them are sufficiently suitable for use in a medical setting. Most methods published for both paclitaxel and docetaxel are based on the principles for extraction and separation published by Willey et al. in 1993 [10], relying on sample preparation by CN material packed solid-phase extraction followed by separation by HPLC and detection by absorbance at 225–230 nm. The lower limit of HPLC/UV detection has been reported as 10 ng/ml paclitaxel in blood [10]. Under the current dose-dense chemotherapy of taxane, however, the lower quantification limit of assay method has been recommended approximate 10 nM (8 ng/ml) [16]. Mass spectrometry based methods, which have quantification limits of sub-nanomolar range [13,14], are superior in taxane analysis. However, these methods are not always applicative in a medical setting, since the mass spectrometry is expensive in equipment and running cost. The method reported in this paper was simple and relatively sensitive having a lower quantification limit of 3 ng/ml. The extraction method employed here reduced chromatographic background signals, and allowed a more sensitive analysis of taxanes in a medical setting. In this extraction procedure, the vacuum step after rinsing the cartridge was essential to reduce background signals. In addition, quantification of docetaxel was successfully achieved when paclitaxel was used as an internal standard (data not shown). The improved sensitivity achieved by the popular HPLC/UV apparatus, which is available in hospitals, would provide a safer therapy with taxane.

Pharmacokinetic studies have shown that the major toxicity of paclitaxel, neutropenia, appears to be related to the duration of time that plasma paclitaxel concentrations are at or above a threshold value [18]. Neutropenia is not directly related to either peak plasma concentration or area under the concentration curve of paclitaxel, although the relationship between therapeutic efficacy and paclitaxel disposition is, as yet, undefined [15,19]. A significant relationship between neutropenia and the time of paclitaxel plasma levels of greater than 50 ng/ml has been observed, with a predicted time of 10.4 h to decrease cell count by 50% [20]. The important point in taxane therapy is that there is significant interpatient variability of plsama drug concentration [8]. One of the patient studied here initially received paclitaxel at a dose 60 mg/m^2, however, she suffered severe neutropenia (patient #3 in Table 3). Plasma paclitaxel concentration was very high even 126 h after administration. The dose of paclitaxel was then reduced to 30 mg/m^2, resulting in a tolerable blood level of this agent. This indicates that TDM during the early stage of the treatment with paclitaxel is essential.

In conclusion, we improved the HPLC method for the determination of taxane level using the Sep-Pak C_{18} cartridge and semi-micro-analytical column. This method would vouch safer and more efficient therapy with taxane.

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