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Short communication

Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection

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Abstract

An isocratic reversed-phase high-performance liquid chromatographic method with ultraviolet detection at 230 nm has been developed for the determination of paclitaxel in human plasma. Plasma samples were prepared by a selective one-step liquid–liquid extraction involving a mixture of acetonitrile–n-butyl chloride (1:4, v/v). Paclitaxel and the internal standard docetaxel were separated using a column packed with ODS-80A material, and a mobile phase consisting of water–methanol–tetrahydrofuran–ammonium hydroxide (37.5:60:2.5:0.1, v/v). The calibration graph for paclitaxel was linear in the range 10–500 ng/ml, with a lower limit of quantitation of 10 ng/ml, using 1 ml plasma samples. The extraction recoveries of spiked paclitaxel and docetaxel to drug-free human plasma were 89.6±8.52 and 93.7±5.0%, respectively. Validation data showed that the assay for paclitaxel is sensitive, selective, accurate and reproducible. The assay has been used in a single pharmacokinetic experiment in a patient to investigate the applicability of the method in vivo. © 1998 Elsevier Science B.V.

Keywords: Paclitaxel

1. Introduction

Paclitaxel (Taxol) is a highly functionalized diterpene amide from the bark of the Pacific yew tree, Taxus brevifolia [1], having a bulky-fused taxane ring system with a rare four-membered oxetane ring and an ester side chain at C13 (Fig. 1). The compound is a potent inhibitor of cell replication in malignant tissues, a property attributed to its ability to stabilize microtubules and block the transit of cycling cells from the G_2-phase to the M-phase [2,3]. In clinical studies, paclitaxel has demonstrated considerable activity against a variety of malignancies, including platinum-refractory ovarian cancer, breast cancer and non-small cell lung cancer (reviewed in [4]).

Over the last few decades, numerous methods have been developed for the determination of paclitaxel in human plasma samples, including biochemical procedures based on tubulin-formation [5,6], competitive-inhibition enzyme immunoassays

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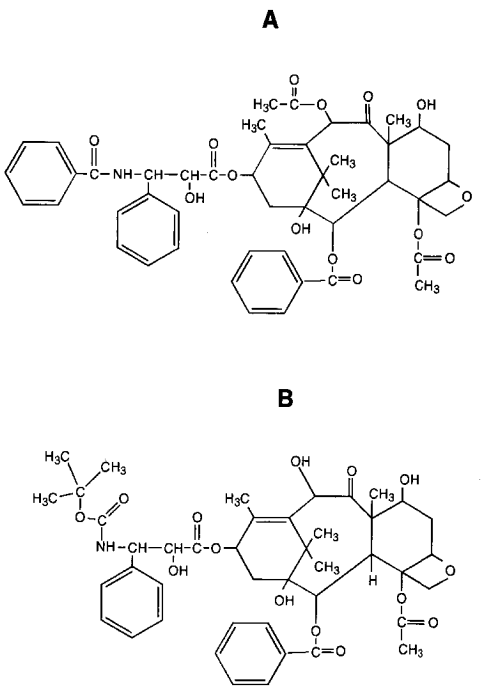

Fig. 1. Chemical structures of paclitaxel (A) and the internal standard docetaxel (B).

[7,8] and capillary electrophoresis [9]. Lately, however, high-performance liquid chromatography (HPLC) has emerged as the technique of choice for accurate pharmacokinetic monitoring of paclitaxel during clinical trials because of its rapidity, selectivity and sensitivity. The available HPLC methods have generally employed ultraviolet (UV) absorption detection techniques and a variety of sample clean-up procedures (Table 1) [10–20]. To date, all analytical procedures with sufficient sensitivity to justify application in pharmacologic studies involving serial blood sampling have employed tedious and expensive solid-phase extraction (SPE) techniques, either alone [15–20] or in combination with protein precipitation [11] or liquid–liquid extraction (LLE) [13,14]. In addition, recent experiments have shown that the paclitaxel-formulation vehicle (Cremophor EL), which is present in clinical samples at concentrations up to 1.0% (v/v), can have a profound effect on assay performance by affecting the SPE-extraction efficiency for paclitaxel [21].

In this report, we describe an alternative HPLC method with UV detection for the determination of paclitaxel in human plasma with a lower limit of quantitation (LLQ) of 10 ng/ml, which avoids the use of SPE for sample clean-up. The method is a modification of our procedure routinely applied for the analysis of the related compound docetaxel (Taxotere) [22] and involves a rapid and highly selective one-step LLE. A pilot pharmacokinetic study in a cancer patient receiving the drug was included to investigate the suitability of the method for clinical use.

Table 1
High-performance liquid chromatographic methods available for the analysis of paclitaxel in human plasma

Sample pretreatment	Internal standard	Detection wavelength (nm)	LLQ (ng/ml)	Reference
PP (acetonitrile)	–	227	Not reported	Wiernik et al. [10]
PP (acetonitrile)+SPE (C_{18})	–	227	25	Grem et al. [11]
LLE (ethylacetate)+gradient elution	n-Cyclohexylbenzamide	227	25	Longnecker et al. [12]
LLE (ethylacetate)+column-switching	Cephalomannine	229	10	Song and Au [13]
LLE (tert.-butylmethylether)+SPE (C_{18})	–	227	85	Rizzo et al. [14]
SPE (nitrile)	–	227	10	Willey et al. [15]
SPE (nitrile)	2′-Methylpaclitaxel	227	10	Huizing et al. [16]
SPE (C_{18})	Cephalomannine	230	11	Jamis-Dow et al. [17]
SPE (C_{18})	Cephalomannine	230	20	Mase et al. [18]
SPE (C_{18})	n-Hexyl-p-hydroxybenzoate	227	15	Ohtsu et al. [19]
SPE (C_{18})	n-Nitrosodiphenylamine	227	10	El-Yazigi et al. [20]

Abbreviations: LLQ=lower limit of quantitation; PP=plasma protein precipitation; LLE=liquid–liquid extraction; SPE=solid-phase extraction.

2. Experimental

2.1. Materials

Paclitaxel powder (batch 484034; purity, 98.3% by reversed-phase HPLC) and commercially available paclitaxel formulated in Cremophor EL–dehydrated ethanol USP (1:1, v/v; Taxol) were kindly donated by Bristol-Myers Squibb (Woerden, Netherlands). The internal standard, docetaxel (batch 14PROC92320; purity, 98.0% by reversed-phase HPLC), was obtained from Rhône-Poulenc Rorer (Vitry-sur-Seine Cedex, France). Reference standards of the paclitaxel metabolites 6α,3′-p-dihydroxypaclitaxel, 3′-p-hydroxypaclitaxel and 6α-hydroxypaclitaxel were isolated and purified from feces samples from a patient, as described in detail previously [23]. The structures of the metabolites were confirmed by on-line photodiode array detection and fast-atom bombardment mass spectrometry. Stock solutions of paclitaxel, docetaxel and the metabolites were prepared in dimethylsulfoxide at 1 mg/ml and were stored in glass at −20°C. Cremophor EL was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of the highest grade available and were from Rathburn (Walkerburn, UK). Water was purified and deionized by the Milli-Q-UF system (Millipore, Milford, MA, USA) and was used throughout. Human plasma was obtained from healthy volunteers.

2.2. Sample preparation

A 100-μl volume of the internal standard [10 μg/ml in methanol–water (1:1, v/v)] and 5 ml of acetonitrile–n-butylchloride (1:4, v/v) were added to 1 ml of human plasma in a 12-ml glass tube and closed with a PTFE-faced cap. The tube was rocked on a multi-tube vortex-mixer for 5 min, and centrifuged at 4000 g for 5 min. Next, the organic layer was transferred to a clean glass tube with a pasteur pipette and evaporated to dryness under a stream of nitrogen at 60°C, taking care to discontinue evaporation immediately after a residue appeared in the bottom (approximately 45 min). A 125-μl volume of methanol–water (1:1, v/v) was added to the residue and reconstituted by ultrasonication for 1 min. The contents of the tube was transferred to a low-volume insert of glass and a 100-μl aliquot was subjected to chromatography.

2.3. Chromatography

The chromatographic system consisted of a constaMetric 3200 pump (LDC Analytical, Rivera Beach, FL, USA), a Waters 717plus autosampler (Milford, MA, USA), a model SpH99 column oven (Spark Holland, Meppel, Netherlands) and a Spectra Physics UV-2000 detector (San Jose, CA, USA). The stationary phase was 5 μm Inertsil ODS-80A material packed in a 150×4.6 mm I.D. stainless-steel column (GL Science, Tokyo, Japan) protected by a Lichrospher 100 RP-18 guard column (4.0×4.0 mm; 5 μm particles). The mobile phase consisted of water–methanol–tetrahydrofuran–ammonium hydroxide (37.5:60:2.5:0.1, v/v), with the pH adjusted to 6.0 (formic acid). The flow-rate of the mobile phase was set at 1.0 ml/min, and the eluent was monitored at ambient room temperature at an absorption wavelength of 230 nm. The column temperature was 60°C.

With each chromatographic run, duplicate calibration standards were prepared in blank human plasma by serial dilution at concentrations of 10, 25, 50, 100, 250 and 500 ng/ml. Sets of quality control (QC) samples were prepared in batch in the same manner at 10, 40, 200, 400 and 15 000 ng/ml. The QC sample containing the highest concentration was used to investigate the effect of sample dilution and/or limited volume injection. Acquisition and integration of data were performed with the ChromCard data analysis system connected to an ICW chromatographic work station (Fisons, Milan, Italy). Calibration graphs were calculated by weighted ($1/x^2$) least-squares linear regression analysis of the peak area ratio of paclitaxel and the internal standard (ordinate) versus the drug concentration of the nominal standard (abscissa).

2.4. Validation

Method validation was performed according to the guidelines recorded in the conference report on ‘Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies’ [24], with minor modifications as described previously [22]. All

validation runs were performed on four consecutive days, and included a calibration curve processed in duplicate and a set of QC samples in quintuplicate analyzed with repeated cycles of freezing and thawing.

2.5. Pharmacokinetic study

Blood samples (5 ml) were obtained in heparinized tubes from a patient who gave informed written consent according to institutional guidelines before treatment. Samples were collected prior to dosing and at 1.50, 3.00, 3.08, 3.25, 3.50, 3.75, 4.00, 5.00, 7.00, 9.00, 11.00, 15.00 and 27.00 h after administration of 225 mg/m^2 of paclitaxel by a 3-h intravenous infusion. Following centrifugation at 4000 g for 5 min, the plasma fraction was separated and stored frozen at −20°C until analysis within two weeks. The pharmacokinetic profile was fitted to a three-compartment open model using the MW\Pharm KinFit computer program (MediWare, Groningen, Netherlands) [25].

3. Results and discussion

3.1. Specificity

Fig. 2 displays typical chromatograms resulting from HPLC analysis of 1 ml human plasma extracts from a blank sample (A) and a sample spiked to contain 50 ng/ml of paclitaxel (B). As shown, paclitaxel (t_R=7.5 min) and the internal standard, docetaxel (t_R=8.5 min), were well resolved and adequately separated from endogenous plasma components under the optimum analytical conditions used. The overall chromatographic run time was established at 30 min.

Certain drugs that are commonly used clinically in the pre- or postchemotherapy period were selected to check for potential interference in the assay for paclitaxel. These included acetaminophen, alizapride, codeine, dexamethasone, domperidon, lorazepam, metoclopramide, morphine, paroxetine and ranitidine. After injection into the chromatograph post-extraction, only paroxetine was found to give a significant interfering peak during the analysis, around the retention time of paclitaxel (approximately 7.0 min). The selectivity and resolution of the HPLC system was also confirmed by co-injection of paclitaxel with three known cytochrome P450-mediated metabolites, i.e., 6α,3′-p-dihydroxypaclitaxel (t_R=3.4 min), 3′-p-hydroxypaclitaxel (t_R=4.0 min) and 6α-hydroxypaclitaxel (t_R=5.9 min) [23].

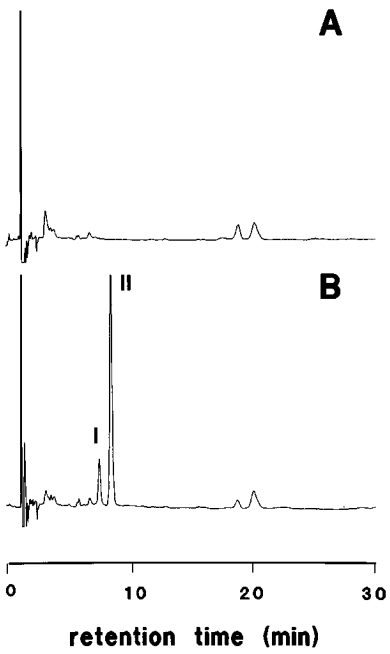

Fig. 2. Typical isocratic reversed-phase HPLC chromatograms of a blank human plasma sample (A) and a sample spiked at a paclitaxel concentration of 50 ng/ml (B). Peaks corresponding to paclitaxel (t_R=7.5 min) and the internal standard (docetaxel; t_R=8.5 min) are indicated by I and II, respectively.

3.2. Validation characteristics

The assay was found to be linear over the range 10–500 ng/ml of paclitaxel in plasma, with a mean regression correlation coefficient of 0.9998 (n=4). The percentage deviation of the predicted concentration in each calibration sample, calculated from the nominal concentration, was ≤10%, using the reciprocal of the concentration as the weighting factor.

In blank human plasma, ten out of twenty samples spiked at 5 ng/ml were outside the acceptable ±20% deviation limits, whereas at 10 ng/ml, all were

within 4% of the nominal concentration. Based on these results, the LLQ was established at 10 ng/ml. At the upper limit of quantitation, i.e. 500 ng/ml, both the mean percentage deviation and the between-run precision were also less than 5%. The within-run and between-run variability, expressed as the percentage relative standard deviation calculated by one-way analysis of variance, were less than 3% at the four QC concentrations analyzed (Table 2). Similar results were obtained from the analysis of QC samples spiked to contain 1% (v/v) Cremophor EL, demonstrating a lack of interference in assay performance in the presence of this vehicle. Therefore, accuracy and precision of the assay were found to be acceptable for the analysis of plasma samples, in support of pharmacokinetic studies. The mean overall extraction efficiencies were 89.6±8.5% for paclitaxel (n=47) and 93.7±5.0% for docetaxel (n=47), which was used as internal standard, and were independent of the spiked concentration.

The described analytical method was applied in our institute to a phase I and pharmacokinetic study of paclitaxel in combination with cisplatin in patients with advanced solid cancer. A representative plasma concentration–time profile of paclitaxel after an intravenous dose of 225 mg/m^2 is depicted in Fig. 3. The fitting of these concentrations to a three-compartment model is in agreement with previous data [26].

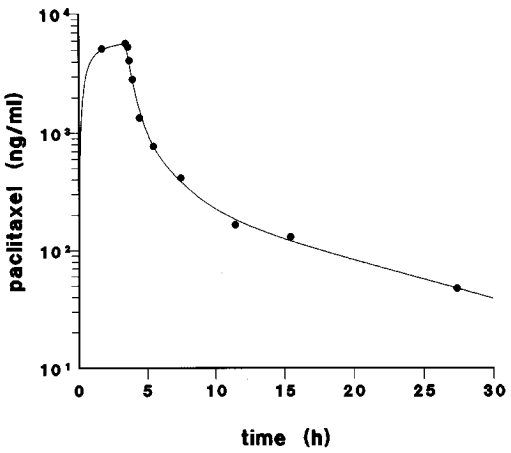

Fig. 3. Plasma concentration–time profile in a human subject following administration of a 225 mg/m^2 dose of paclitaxel.

4. Conclusions

In conclusion, the method described for the determination of paclitaxel in human plasma is specific, accurate and precise, and is selective and sensitive (LLQ, 10 ng/ml) enough to be used in clinical trials. As sample pretreatment consists of a rapid, one-step solvent extraction, it avoids the use of the complicated and expensive SPE techniques that were used in previously reported procedures. In addition, it

Table 2
Accuracy, within-run and between-run precision for the analysis of paclitaxel in spiked quality control samples in the absence and presence of 1.0% (v/v) Cremophor EL

Added (ng/ml)	Recovered (ng/ml)	DEV (%)	WRP (%)	BRP (%)	n
QC samples without Cremophor EL					
10	10.14±0.47	+1.40	2.22	2.76	20
40	40.36±0.49	+0.91	1.50	1.02	20
200	199.8±1.84	−0.12	2.06	0.67	20
400	411.1±0.86	+2.77	0.47	0.58	20
15 000	15 080±201.6	+0.54	2.99	1.91	20
QC samples containing *1.0% (v/v)* Cremophor EL					
40	43.04±3.77	+7.06	–	–	3
200	201.8±6.71	+0.91	–	–	3
400	416.5±8.98	+4.13	–	–	3
15 000	15 630±351.0	+4.20	–	–	2

Abbreviations: %DEV=percent deviation (accuracy); WRP=within-run precision; BRP=between-run precision; n=number of replicate observations within each validation.

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