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Sensitive liquid chromatography–mass spectrometry assay for quantitation of docetaxel and paclitaxel in human plasma

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Abstract

We have developed a high-performance liquid chromatography–electrospray ionization mass spectrometry (LC–MS) method for quantifying docetaxel and paclitaxel in human plasma. The assay fulfills the need for defining the lower plasma concentrations of these antineoplastic agents that result from a number of changes in how these agents are used clinically. The assay uses paclitaxel as the internal standard for docetaxel, and vice versa; solid-phase extraction; a Phenomenex Hypersil ODS (5 μm, 100×2 mm) reversed-phase analytical column; an isocratic mobile phase of 0.1% formic acid in methanol–water (70:30, v/v); and mass spectrometric detection using electrospray positive mode electron ionization. The assay has a lower limit of quantitation (LLOQ) of 0.3 n*M* and is linear between 0.3 n*M* and 1 μ*M* for docetaxel. For paclitaxel, the LLOQ was 1 n*M*, and the assay is linear between 1 n*M* and 1 μ*M*. We demonstrated the suitability of this assay for docetaxel by using it to quantify the docetaxel concentrations in plasma of a patient given 40 mg/m^2 of docetaxel and comparing those results to results produced when the same samples were assayed with an HPLC assay using absorbance detection. In a similar manner, the suitability of the assay for paclitaxel was demonstrated by using it to quantify the concentrations of paclitaxel in the plasma of a patient given 15 mg/m^2 of paclitaxel and comparing those results to results produced when the same samples were assayed with an HPLC assay using absorbance detection. The LC–MS assay, which proved superior because of its greater sensitivity and relatively short (7 min) run time, should be an important tool for future pharmacokinetic analyses of docetaxel and paclitaxel.

Keywords: Docetaxel; Paclitaxel

1. Introduction

The taxanes, docetaxel and paclitaxel, represent important antineoplastic agents with broad spectra of antitumor activity [1–7]. Quantitation of docetaxel and paclitaxel concentrations in the plasma of patients is important because previous studies have

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demonstrated a correlation between drug exposure and the myelosuppression resulting from taxane therapy [8–12]. Although previous HPLC assays for docetaxel and paclitaxel have been described, most have relied on UV absorbance detection [13–24], and each of these suffers from a relative lack of sensitivity. The need for more sensitive assays for docetaxel and paclitaxel has become more important as a result of several developments that result in lower plasma concentrations of these drugs. The first is that the doses of taxanes used in combination with other cytotoxic agents are often less than the recommended phase II doses defined when taxanes were used as single agents. The second is that clinicians have begun to investigate weekly taxane therapy as an alternative to the every-three-week administration schedule that is currently the standard of care [25–28], and weekly taxane therapy often utilizes doses lower than those administered on an every-three-week basis. In addition, there is continued interest in evaluating prolonged infusions of docetaxel and paclitaxel, which are associated with low plasma drug concentrations [29–33]. Furthermore, the interest in the claimed antiangiogenic properties of low, non-cytotoxic concentrations of docetaxel and paclitaxel has prompted clinical evaluation of dosing strategies that produce such concentrations [34–38]. Finally, recognition of the very high plasma binding of docetaxel and paclitaxel has spurred interest in evaluating the low, unbound concentrations of these drugs rather than the total drug in plasma [38–40]. Quantitation of these lower concentrations is important for establishing pharmacokinetic/pharmacodynamic relationships, such as have been defined for single-agent, every-three-week therapy, and also for exploring potential drug–drug interactions. Recent reports have described paclitaxel assays based on LC–MS–MS instrumentation [41–44]. While these have addressed the need for increased sensitivity, the availability of the required instrumentation is more limited than is desirable for broad application of the assays described. Recognizing the need for sensitive and broadly applicable HPLC assays for docetaxel and paclitaxel, we have developed a liquid chromatography–electrospray ionization mass spectrometry method that is approximately 50 times more sensitive than current HPLC–UV methods and is suitable for application to biological matrices, such as patient plasma.

2. Experimental

2.1. Materials

Docetaxel was supplied by Aventis Pharmaceuticals (Parsippany, NJ, USA). Paclitaxel was supplied by the Toxicology and Pharmacology Branch of the National Cancer Institute (Bethesda, MD, USA). Methanol (Optima grade), acetonitrile (Optima grade), hexane (HPLC grade), and ammonium acetate (enzyme grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (minimum 95%) was purchased from Sigma (St. Louis, MO, USA). Control human plasma was prepared by centrifugation of outdated, citrate-anticoagulated blood obtained from the Central Blood Blank (Pittsburgh, PA, USA). Medical-grade nitrogen and liquid nitrogen were purchased from Praxair (Pittsburgh, PA, USA).

2.2. Procedure

2.2.1. Sample preparation

Standard curves were prepared by placing triplicate, 1-ml samples of human plasma, containing 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, or 1 μM docetaxel or paclitaxel, into 1.5-ml microcentrifuge tubes. Ten microliters of a 1 μM solution of internal standard (paclitaxel for docetaxel assays and docetaxel for paclitaxel assays) in methanol were added to each tube, and the tubes were vortexed briefly. The samples were centrifuged at 12 000 *g* for 5 min and then applied to Sep-Pak CN cartridges (1-ml capacity, Waters, Milford, MA, USA) that had been conditioned with two 1-ml aliquots of methanol followed by two 1-ml aliquots of 0.01 *M* ammonium acetate, pH 5.0. After samples had been applied, the cartridges were washed sequentially with two 1-ml aliquots of 0.01 *M* ammonium acetate, pH 5.0; two 1-ml washes of 0.01 *M* ammonium acetate, pH 5.0–methanol (80:20, v/v); and 1 ml of hexane. After the cartridges had been dried by applying vacuum for 1 min, 1 ml of acetonitrile was applied to each cartridge. The resulting eluates were collected in 12×75 mm borosilicate glass tubes and evaporated to dryness under a stream of nitrogen at 27 °C [35]. Each dried residue was redissolved in 100 μl of methanol–water (70:30, v/v), vortexed briefly, trans-

ferred to HPLC autosampler vials, and 10 μl were injected into the LC–MS system.

2.2.2. HPLC–MS system

The HPLC system consisted of an Agilent Model 1100 autosampler with a 100 μl sample loop (Agilent Technologies, Palo Alto, CA, USA), an Agilent 1100 Quaternary pump, and a Hypersil C_{18} (ODS) (5 μm, 100×2 mm) analytical column (Phenomenex, Torrance, CA, USA). The isocratic mobile phase, consisting of 0.1% formic acid in methanol–water (70:30, v/v), was pumped at 0.2 ml/min, and the run time was 7 min. Column eluate was analyzed with a ThermoFinnigan aQa mass spectrometer (ThermoQuest, San Jose, CA, USA) operating in electrospray, positive-single-ion mode to monitor 808.1 m/z for docetaxel and 854.0 m/z for paclitaxel. The insert probe temperature was set at 250 °C with 5000 V applied as the ion spray voltage and 10 V as the orifice voltage. Nitrogen gas flow was fixed by the tank head unit set at 75 p.s.i. (520 kPa). The system was operated with ThermoFinnigan Excalibur Software installed on a Gateway 2000 (N. Sioux City, SD, USA) Pentium II 300 MHz computer. The I.S. ratio was calculated for each standard by dividing the analyte peak area by the peak area of the internal standard. Standard curves of docetaxel and paclitaxel were constructed by plotting the I.S. ratio versus the known concentration of analyte in each sample. Standard curves were fit by linear regression with weighting by $1/y^2$, followed by back calculation of concentrations.

2.3. Demonstration of applicability to biological samples

To demonstrate the applicability of this LC–MS method to pharmacokinetic samples, it was used to quantify concentrations of docetaxel in the plasma of a patient who received a 40 mg/m^2 dose as a 30-min infusion and concentrations of paclitaxel in the plasma of a patient who received a 15 mg/m^2 dose as a 1-h infusion. Prior to treatment, each patient gave written, informed consent as approved by the Institutional Review Board. Blood samples were collected into heparinized tubes before taxane delivery. In the patient given docetaxel, samples were also obtained at 15, 25, 45, 60, 90 min, and 3.5, 5.5, 7.5, and 24.5 h after initiation of the docetaxel infusion. In the patient given paclitaxel, samples were also obtained at 10, 15, 60, 70, 75, 90, 105 min and 2, 2.5, 4, 5, 8, 16, 24, and 30 h after initiation of the paclitaxel infusion. Each sample was centrifuged at approximately 1000 *g* for 10 min, and the resulting plasma layer was stored at −70 °C until analyzed with the procedure described above. Docetaxel and paclitaxel concentrations were calculated by comparing the I.S. ratio measured for each sample to the weighted, linear function derived from the standard curve that related the I.S. ratio to docetaxel or paclitaxel concentration. In order to evaluate the potential superiority of the LC–MS assay over available HPLC methods, the results of LC–MS assays were compared to results of the same plasma samples assayed by HPLC with absorbance detection. Docetaxel assays used the same extraction and column packing as described for LC–MS studies, but the column was of 4.6 mm inner diameter, the mobile phase consisted of acetonitrile–water (45:55, v/v) and the column eluate was monitored at 227 nm with a Waters 2487 variable-wavelength detector. Paclitaxel concentrations were determined with a previously described modification of the method of Jamis-Dow et al. [24,45]. Specific modifications to the method included use of 1 ml of plasma sample, 10 μl of 1 μ*M* cephalomannine internal standard, and Spe-ed Cartridges (Applied Separations, Allentown, PA, USA).

3. Results

With the chromatography conditions described, paclitaxel eluted at approximately 3.2 min, and docetaxel eluted at approximately 3.7 min (Fig. 1). There was baseline separation of docetaxel and paclitaxel, and no endogenous materials interfered with measurement of either taxane. The sample preparation described resulted in 103±7% recovery of docetaxel and 108±3% recovery of paclitaxel when compared to direct injection of an equivalent amount of either compound in mobile phase. The assay also proved suitable for use with an autosampler as there was no decay in peak shape or area of docetaxel or paclitaxel solutions that were prepared in mobile phase and monitored during a 24-h incubation at room temperature. The assay was linear

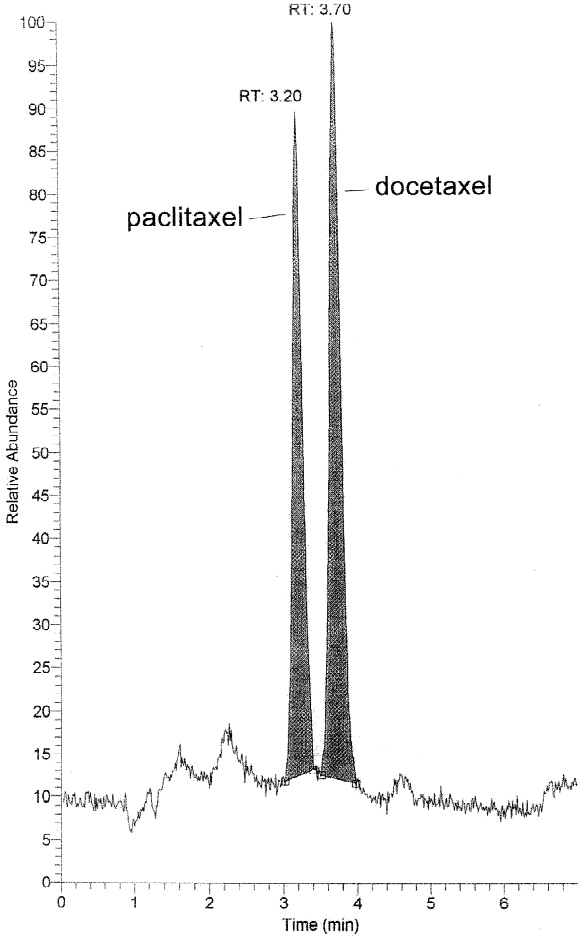

Fig. 1. Chromatogram of 0.01 μ*M* docetaxel and 0.01 μ*M* paclitaxel extracted from control, human plasma.

over the concentration range of 0.0003–1 μ*M* for docetaxel and 0.001–1 μ*M* for paclitaxel.

The correlation coefficients for three successive docetaxel triplicate standard curves were 0.9931, 0.9935, and 0.9912, respectively. Back-calculated values for docetaxel varied by 2–15 and 2–14% from the theoretical values of 0.0003 and 1 μ*M*, respectively. When expressed as percentage coefficient of variation, the within-day variation in docetaxel triplicate standards was always $<5.6\%$ at all concentrations. Between-day variation in three, successive, triplicate docetaxel standard curves was also minimal, as the coefficient of variation of the slopes associated with these repeated standard curves was 7.8%. Also, the coefficients of variation were 4.6 and 3.5% at 0.003 and 0.3 μ*M*, respectively, when these concentrations of docetaxel were added to 10 ml of plasma, and 10 replicate 1-ml aliquots were analyzed.

The correlation coefficients for three successive paclitaxel triplicate standard curves were 0.9903, 0.9923, and 0.9914, respectively. Back-calculated values for paclitaxel varied by 1–15 and 6–14% from the theoretical values of 0.001 and 1 μ*M*, respectively. When expressed as percentage coefficient of variation, the within-day variation in triplicate paclitaxel standards was always $<11\%$ at all concentrations. Between-day variation in three, successive, triplicate paclitaxel standard curves was also minimal, as the coefficient of variation of the slopes associated with these repeated standard curves was 11.1%. Also, the coefficients of variation were 4.3 and 5.7% at 0.03 and 0.3 μ*M*, respectively, when these concentrations of paclitaxel were added to 10 ml of plasma, and 10 replicate 1-ml aliquots were analyzed

When plasma samples from patients receiving low doses of docetaxel (Fig. 2) and paclitaxel (Fig. 3) were analyzed by LC–MS and HPLC methods relying on absorbance detection, the data were similar for samples obtained during drug infusion and the first 4–8 h after the end of drug administration. However, the LC–MS method allowed plasma concentrations of docetaxel (Fig. 2) and paclitaxel

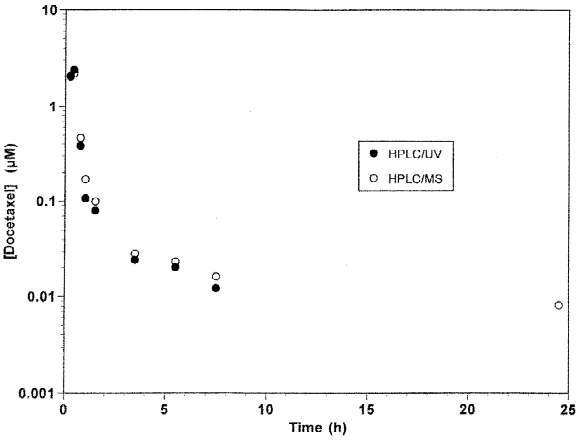

Fig. 2. Time course of docetaxel in plasma of a patient after a 30-min i.v. infusion of a 40 mg/m^2 dose. (●) Values determined by HPLC with absorbance detection monitoring at 227 nm. (○) Values determined by LC–MS assay.

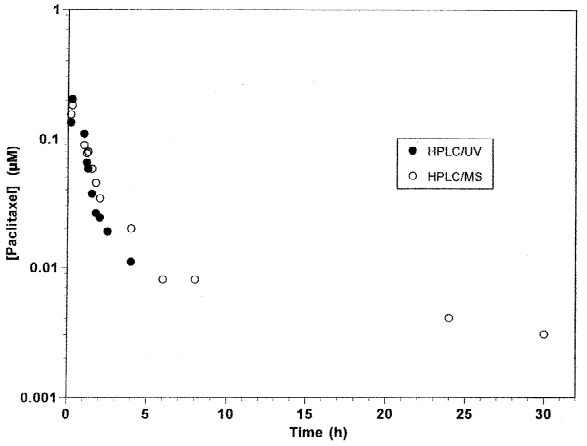

Fig. 3. Time course of paclitaxel in plasma of a patient after a 1-h i.v. infusion of a 15 mg/m^2 dose. (●) Values determined by HPLC with absorbance detection monitoring at 227 nm. (○) Values determined by LC–MS assay.

(Fig. 3) to be followed for much longer periods of time after drug delivery than did HPLC methods relying on absorbance detection. In both patients studied, data from HPLC with absorbance detection would have indicated a much shorter half-life, smaller area under the plasma concentration versus time curve, and larger total body clearance than actually existed (Figs. 2 and 3).

4. Discussion

Although HPLC assays for docetaxel and paclitaxel have been published previously [1–7], their suitability for clinical pharmacology studies of these agents has been reduced by shifts in standard of practice that involve lower doses of each taxane [25–28]. These lower doses of drug result in lower plasma drug concentrations that rapidly decrease to concentrations below those quantifiable with absorbance detection [13–24]. The LC–MS method described in the current manuscript overcomes this problem of assay sensitivity yet remains applicable to biological matrices and is capable of being implemented in laboratories with standard LC–MS instrumentation. The incentive for performing additional clinical pharmacology studies with docetaxel and paclitaxel is multifactorial. Studies in patients receiving every-three-week treatment with taxanes have established pharmacokinetic/pharmacodynamic relationships between drug exposure and the neutropenia associated with taxane treatment [8–12]. Additionally, docetaxel and paclitaxel have each demonstrated a broad range of antitumor activity [1–7], and each drug is being evaluated further in combination with a variety of other cytotoxic and non-cytotoxic antineoplastic agents. With each of these combinations, the question of pharmacokinetic drug–drug interactions is considered; therefore, there is an ongoing need to characterize plasma concentrations of docetaxel and paclitaxel in clinical studies. The LC–MS method described in the current manuscript is suitable for this next generation of taxane clinical pharmacology studies because of its sensitivity and the lack of interference from endogenous or co-administered materials. The 1-ml sample volume required for the assay is reasonable, the LLOQ of the assay is approximately 50 times lower than those of previously described HPLC–absorbance detection assays of docetaxel and paclitaxel, and the 7-min run time is suitable for reasonable throughput of samples. The overall applicability of this method is evidenced by its currently being employed in a variety of clinical trials at our institution.

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