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Short communication

Determination of desoxyepothilone B in nude mice plasma by liquid–liquid extraction and reversed-phase high-performance liquid chromatography

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Abstract

A novel reversed-phase high-performance liquid chromatographic (HPLC) method has been established for the determination of a newly developed anti-cancer agent desoxyepothilone B (dEpo B) in nude mice plasma. The sample preparation involved deproteination of 200 μl of plasma sample first, followed by liquid–liquid extraction of the resultant supernatant with chloroform. The compound taxol was used as the internal standard. Chromatographic separations were carried out on a 250 mm × 4.6 mm Zorbax SB-phenyl column with acetonitrile–0.25% orthophosphoric acid (50/50, v/v) as mobile phase and UV detection at 250 nm. For dEpo B and taxol at the concentration level of 10 μg/ml in nude mice plasma, the absolute extraction recoveries were 85.3 and 87.2%, respectively. The linear quantification range of the method was 0.1–100 μg/ml in nude mice plasma with linear correlation coefficients greater than 0.999. The within-day and between-day relative standard deviations (R.S.D.s) for dEpo B at 0.5, 2.5 and 10 μg/ml levels in nude mice plasma fell in the range of 2.8–4.8 and 1.5–4.6%, and the within-day and between-day recoveries were in the range of 96.5–101.7 and 97.7–101.2%, respectively.

Keywords: Desoxyepothilone B; High-performance liquid chromatography; Nude mice plasma

1. Introduction

The epothilones (Epos) are a new class of 16-membered macrolides, which are currently under evaluation for antitumor potential both in vitro and in vivo [1–5]. They share a similar cellular mechanism of action to taxol, which is currently used as the front-line therapeutic agent in a variety of solid forms of cancer, including ovarian, breast, colon, lung and liver neoplasmas. Both the epothilones and taxol exert their biological effects by stabilizing microtubule assemblies, thus leading to the arrest of cell division and eventual cell death. By far the most intriguing property of the epothilones at the in vivo level is their lack of cross-resistance to multidrug-resistant (MDR) cell lines when compared with major antitumor agents, including taxol, vinblastine, adriamycin, camptothecin and etoposide, all of which are currently used in clinical settings. In addition, the epothilons are also more water-soluble and more readily available through chemical synthesis than taxol. Therefore, the epothilones are a class of compounds of current interest in the pharmaceutical research field. Recently, a newly synthesized epothilone analogue Z-12,13-desoxyepothilone B (dEpo B, Fig. 1) has shown to be an efficacious microtubule-targeted antitumor agent in vivo, particularly against MDR models of human cancer [4,5]. For its further study, it is necessary to develop an analytical method for the determination of such a compound, especially in biological fluids. However, to our knowledge, there has been no report so far in any literature on the determination of this drug in biological fluids. The in vitro and in vivo study in our lab for the development of dEpo B urged

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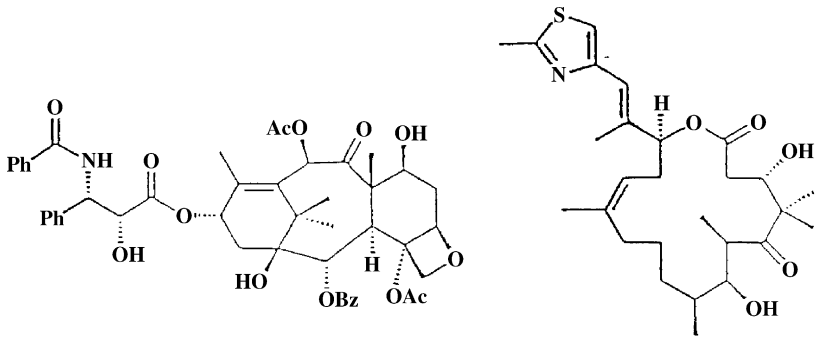

Fig. 1. Chemical structures of taxol and dEpo B.

us to establish an analytical method for this promising drug. Literature shows that sample pretreatment with liquid–liquid extraction [6–8] or solid-phase extraction [9–12], followed by HPLC separation with ultraviolet (UV) detection, has been a commonly used means for the analysis of UV-absorbing drugs in biological matrices. Here, we report a simple and useful determination method for dEpo B in nude mice plasma by liquid–liquid extraction and high-performance liquid chromatography, which has been successfully employed in its pharmaceutical studies in our lab.

2. Experimental

2.1. Chemicals and reagents

dEpo B was obtained from McKesson Bioservices (1592 Rockville Pike, Suite E, Rockvill, MD 20852, USA). Taxol (purity 99%) was purchased from Sigma (St. Louis, MO 63178, USA). All other reagents were of analytical grade or higher, and deionized water was used throughout the study. Mobile phase used in HPLC was filtered and degassed using a 0.2-μm membrane filter (Gelman Sciences, Ann Arbor, MI, USA) with a filtration system (Millipore, MA, USA).

2.2. HPLC instrumentation and conditions

The HPLC system was composed of a Beckman Gold module 406, with computer-controlled 126 solvent delivery system and its 168 UV detector. The analytical column was a Zorbax SB-phenyl 250 mm × 4.6 mm i.d. (Agilent Technologies, USA), coupled with an inline guard column (RP-18, 5 μm, Lichrospher 100, EM Sciences, Gibbstown, NJ, USA). The mobile phase consisted of acetonitrile–0.25% orthophosphoric acid (50/50, v/v) and was pumped at 1.5 ml/min. The eluent was monitored at 250 nm. The ratios of peak area of dEpo B to the internal standard taxol versus concentrations of the standard were used for quantitative computations.

2.3. Preparation of standards

Stock solutions of dEpo B (2 mg/ml) were prepared in methanol. Serial dilutions in methanol were made to the follow-

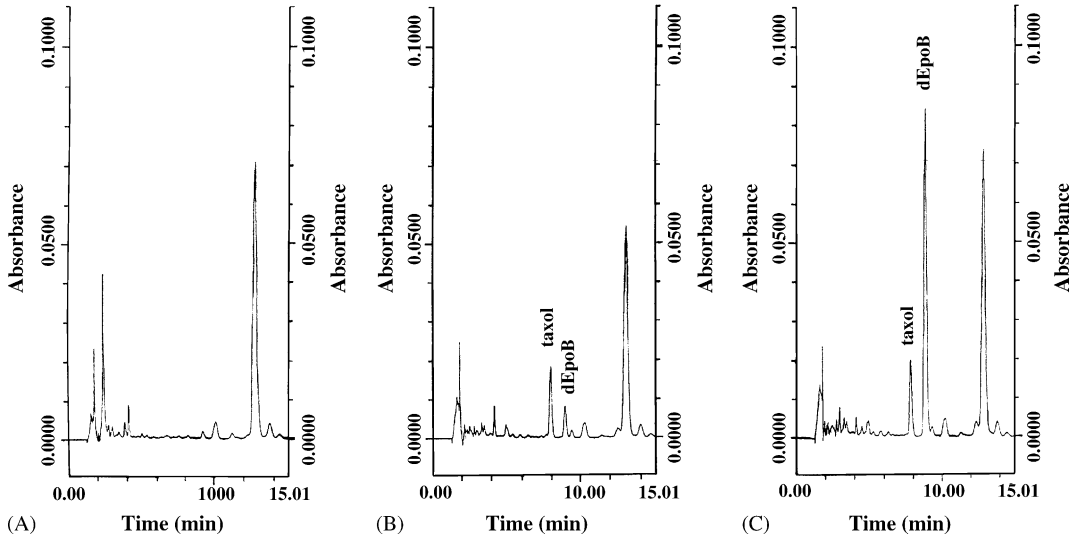

Fig. 2. Chromatograms: (A) nude mice plasma blank; (B) 1 μg/ml dEpo B and 10 μg/ml of taxol in nude mice plasma; (C) 10 μg/ml dEpo B and 10 μg/ml of taxol in nude mice plasma. HPLC conditions: column, Agilent Zorbax SB-phenyl (250 mm × 4.6 mm i.d., 5 μm); mobile phase, acetonitrile–0.25% orthophosphoric acid (50/50, v/v) at 1.5 ml/min; UV detection at 250 nm.

Table 1
Within-day and between-day variations and recoveries of HPLC analysis of dEpo B in nude mice plasma

Spiked dEpo B in plasma (μg/ml)	Within-day ($n=6$)		Between-day ($n=5$)	
	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)
0.5	2.8	100.3	4.1	97.7
2.5	4.8	96.5	4.6	97.8
10.0	2.9	101.7	1.5	101.2

ing final concentrations: 1, 2, 5, 25, 100, 200 and 1000 μg/ml for use as dEpo B working solutions. Similarly, a solution of taxol at 100 μg/ml was prepared in methanol for use as internal standard. All the solutions were stored at −20 °C. Calibration curve samples were prepared by adding 20 μl of the final dEpo B working solutions to 180 μl of plasma (final volume to 0.2 ml) on ice, resulting in final concentration of dEpo B being at 0.1, 0.2, 0.5, 2.5, 10, 20 and 100 μg/ml in nude mice plasma. The samples were then extracted immediately with the following procedure.

2.4. Extraction of plasma

The internal standard solution (20 μl) was added to plasma (0.2 ml) in Eppendorf tubes on ice. Following the addition of 0.8 ml of acetonitrile/water (70/30, v/v), each tube was vortex-mixed and then centrifuged at 14,000 rpm for 10 min to deproteinate. The resultant supernatant was transferred to a 120 mm × 7.5 mm glass tube, to which 3 ml of chloroform was added. The tube was capped, vortex-mixed for 10 s and then centrifuged at 2500 rpm for 5 min. The organic phase was transferred to a clean 120 mm × 7.5 mm glass tube and dried under a gentle stream of compressed air. The dried extract was reconstituted with 200 μl of methanol–0.25% orthophosphoric acid (70/30, v/v) prior to HPLC analysis.

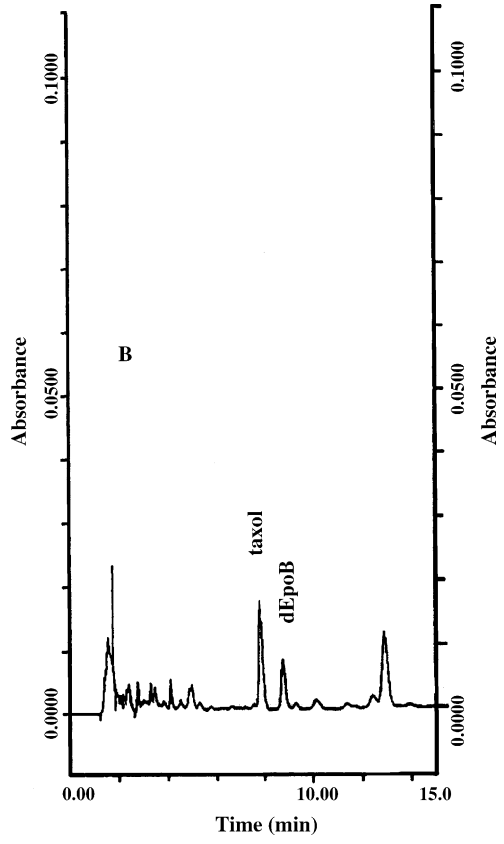

Fig. 3. Typical analytical chromatogram from nude mice plasma after administration of dEpo B. Other conditions are the same as in Fig. 2.

2.5. Linearity, reproducibility and recoveries

The absolute extraction recoveries of dEpo B and internal standard taxol from plasma were calculated by comparing peak areas obtained by direct injection of the standard solution, with those obtained in plasma samples which were spiked with the same amount of the standard solution, and subjected to a complete extraction procedure. The within-day precision and recoveries of the present method were determined by the analysis of dEpo B plasma samples spiked in nude mice blank plasma at three different concentration levels (0.5, 2.5 and 10 μg/ml of dEpo B in nude mice plasma). Six parallel determinations were made at each level. The between-day precision and recoveries were determined in the same way, but on five different days in a 3-week period. The concentrations of dEpo B in nude mice plasma samples were found based on a seven-point internal standard calibration curve established according to Sections 2.3 and 2.4. The precision of the method at each concentration was expressed as the relative standard deviation (R.S.D.) for the determined values. The recoveries of the method were determined by expressing the mean concentrations found as a percentage of the spiked concentrations.

2.6. Initial pharmacokinetic study in nude mice

Nude mice weighing about 20 g were administered dEpo B at a dose of 30 mg/kg either by i.v. via tail vein or by i.p. administration. At 5, 15, 30, 60, 120 min, and 6 and 24 h after drug dosing, the nude mice were killed and the blood was collected in heparinized Eppendorf tubes. The blood was immediately centrifuged for one min at maximum speed in Eppendorf centrifuge. Two hundred microliters of plasma was analyzed immediately. For each time point, three parallel experiments were made.

3. Results and discussion

3.1. Separation and extraction conditions

The chemical structures of dEpo B and the internal standard taxol are shown in Fig. 1. In this study, a Hypersil C18 (Jones Chromatography, USA) column with different relative composi-

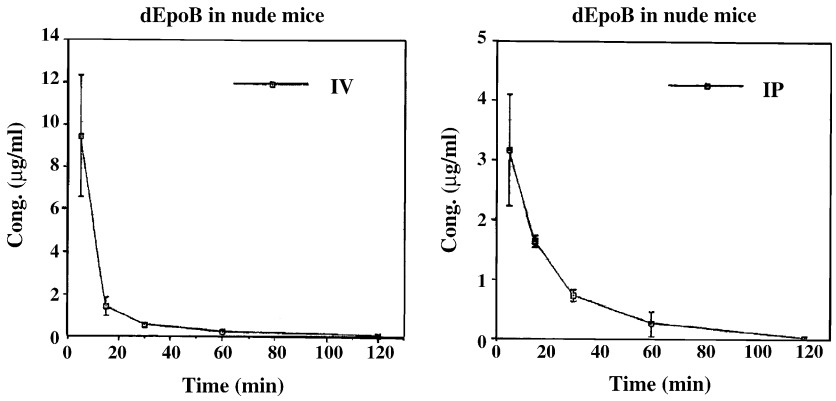

Fig. 4. Plasma concentration–time curve profiles in nude mice following administration of 30 mg/kg of dEpo B by i.v. (A) and i.p.(B) injection.

tions of acetonitrile or methanol to 0.25% orthophosphoric acid as mobile phase were first investigated for the separation of dEpo B and taxol. However, the column could not give a symmetrical and sharp peak for dEpo B. Next, Zorbax SB-C18 and Zorbax SB-phenyl columns were chosen for further investigation. As a result, symmetrical and sharp peak for both dEpo B and taxol could be obtained on both columns. After optimization, separation was chosen on a 250 mm × 4.6 mm Zorbax SB-phenyl column with acetonitrile–0.25% orthophosphoric acid (50/50, v/v) as mobile phase at a flow rate of 1.5 ml/min. Under this condition, dEpo B and taxol were well separated with retention times at about eight and nine min, and no significant endogenous peaks from plasma appeared at their retention times. For the extraction of plasma, ethyl acetate was also investigated as an extractant. However, compared with chloroform, ethyl acetate gave more endogenous peaks under the selected separation conditions. Therefore, chloroform was chosen as the extractant in this study. The dEpo B has two absorption peaks at about 210 and 250 nm and taxol has maximum absorbance at about 225 nm. Considering that the sensitivity of dEpo B was of primary importance in this study, and UV detection at higher wavelengths can often decrease baseline noise, the detection was chosen at 250 nm. This was in favor of the detection of dEpo B, but at the cost of sensitivity of the internal standard of taxol. The representative chromatograms for nude mice plasma samples with spiked dEpo B under optimized separation and extraction conditions are shown in Fig. 2.

3.2. Linearity, reproducibility and recoveries

Chromatograms obtained from different batches of nude mice blank plasmas contained no significant interfering endogenous peaks co-eluting with dEpo B and taxol.

For dEpo B and taxol at the concentration level of 10 μg/ml in nude mice plasma, the absolute overall extraction recovery was found to be 85.3 ± 10.9% ($n = 80$) and 87.2 ± 8.6% ($n = 80$), respectively, indicating a fairly good extraction efficiency.

Using the ratio of the peak area of dEpo B to the internal standard taxol as a quantification signal, the seven-point calibration curves (0.1, 0.2, 0.5, 2.5, 10, 20 and 100 μg/ml of dEpo B in nude mice plasma) were linear with mean correlation coefficients (r) for daily calibration curves being greater than 0.999. By using 200-μl of the plasma sample volume, the limit of detection for dEpo B was found to be 0.04 μg/ml at a signal-to-noise ratio of three and the limit of quantitation was determined at 0.1 μg/ml in plasma. The within-day and between-day variations and recoveries at three different concentration levels of dEpo B in nude mice plasma (0.5, 2.5 and 10 μg/ml) are illustrated in Table 1. The within-day and between-day R.S.D.s fell in the range of 2.8–4.8 and 1.5–4.6%, and the within-day and between-day recoveries were in the range of 96.5–101.7 and 97.7–101.2%, respectively.

3.3. Application in pharmacokinetic study in nude mice

The usefulness of the developed method for clinical application was also demonstrated by conducting a pharmacokinetic study in nude mice. A typical chromatogram from nude mice plasma after administration of dEpo B is shown in Fig. 3. Fig. 4 shows the plasma concentration-time curve profiles in nude mice after being administered 30 mg/kg of dEpo B by i.v. and i.p. injection. Rapid clearance of dEpo B in nude mice plasma was observed.

In conclusion, a simple and useful HPLC method for the quantitative determination of dEpo B in plasma samples has been established. The method has been successfully used for the in vivo stability studies for the development of dEpo B.

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