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Analytical and clinical validation of a dried blood spot assay for the determination of paclitaxel using high-performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

Background: Paclitaxel (PCT) is a chemotherapeutic drug widely used for the treatment of several types of tumors, and its use is associated with severe adverse events, mainly neurologic and hematopoietic toxicities. The relation between systemic exposure and clinical response to PCT was previously described, making paclitaxel a potential candidate for therapeutic drug monitoring (TDM). The use of dried blood spot (DBS) sampling could allow complex sampling schedules required for TDM of PCT. The aim of this study was to develop and validate an LC-MS/MS assay for the quantification of PCT in DBS.

Methods: PCT was extracted from one 8 mm DBS punch with a mixture of methanol and acetonitrile, followed by chromatographic separation in a Kinetex C18 (50 × 4.6 mm, 2.6 µm) column. Detection was performed in a 5500-QTRAP® mass spectrometer, with a run time of 2.3 min.

Results: The assay was linear in the range of 2.5 to 400 ng mL^{-1}. Precision (CV%) and accuracy at the concentration levels of 7.5, 40 and 150 ng mL^{-1} were 1.69–4.9% and 106.25 to 109.92%, respectively. PCT was stable for 21 days at 25 and 45 °C. The method was applied to DBS samples obtained from 34 patients under PCT chemotherapy. The use of a simple correction factor, derived from the correlation between PCT concentrations in plasma and DBS in this set of patients, allowed unbiased estimation of PCT plasma concentrations from DBS measurements, with similar clinical decisions using either plasma or DBS measurements.

Conclusions: DBS testing of PCT concentrations represents a promising alternative for the dissemination of PCT dose individualization.

1. Introduction

Paclitaxel (PCT) is a chemotherapeutic drug used for the treatment of many tumors, including breast [1], head and neck [2], ovarian [3] and non-small cell lung cancer [4]. PCT was isolated from a Pacific Yew tree in 1971 [5] and was approved for clinical use by the US FDA in 1992. PCT mechanism of action is based on selective tubulin polymerization, preventing the cellular microtubule depolymerization, which in turn inhibits cell division [6].

PCT administration is usually associated with several adverse events, mainly neurologic and hematopoietic toxicities [7]. The inter-individual tolerability to these effects is partially related to pharmacokinetics and pharmacogenetics differences among patients, particularly in PCT clearance [8]. PCT pharmacokinetics is nonlinear due to saturable elimination and distribution of the drug [9]. PCT metabolic clearance can be affected by genetic polymorphisms [10], demographic, physiologic and pathologic factors and by drug interactions [11].

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The pharmacokinetic parameter that better represents the relation between exposure to PCT and clinical response is the time that plasma concentration remains above a threshold of 0.05 μM (Tc > 0.05 μM) [11–13]. Considering this target, algorithms had been proposed to individualize treatment with PCT based on the individual exposure to the drug [14]. The proposed therapeutic range for Tc > 0.05 μM in three weekly chemotherapy regimens is 26–31 h, and for weekly regimens is 10–14 h [12,13]. The determination of Tc > 0.05 μM can be performed with only one plasma concentration, obtained 24 h after the beginning of the drug infusion, using a proper pharmacokinetic model [12]. In this context, a simple Excel®-based tool to calculate Tc > 0.05 μM was recently described, based on a single PCT concentration [13]. Recent studies showed that PCT dose adjustment based on Tc > 0.05 μM lead to a significant reduction in the occurrence of adverse effects, without a reduction in clinical efficacy [4].

Therapeutic drug monitoring (TDM) of PCT is a promising tool to optimize chemotherapy with this drug, but is highly dependent on the availability of reliable and clinically implementable assays. The current sampling strategy to estimate pharmacokinetics parameters of PCT requires plasma separation from venous blood, after phlebotomy. This conventional sampling approach requires specialized professionals and infrastructure [12,13,15]. Alternatively, the use of dried blood spots (DBS) samples could allow remote or self-sampling, also being an alternative for sample transportation from distant sites to reference laboratories due to higher analyte stability, increased biosafety, and simplified logistics, usually not requiring refrigeration [15–17]. These DBS advantages are particularly relevant for TDM of PCT in limited-resources settings, where outpatients have significant difficulties to return to a specialized center to collect blood samples at the required time of 24 h post-infusion.

The clinical application of TDM using DBS as the sampling strategy demands analytical methods with high sensitivity and specificity, such as liquid chromatography-mass spectrometry (LC-MS/MS), particularly considering the small amount of sample available for testing [15,18]. Additionally, before clinical application, DBS drug measurement assays require extensive validation, including tests to evaluate the impact of blood hematocrit in its accuracy [15–17]. Moreover, a clinical application study is mandatory to adequately translate DBS concentrations to plasma levels [15]. Despite the previous description of PCT assays in DBS [19,20], there is no report of a comprehensive, DBS-specific, method validation, as well as no data on the clinical performance of the methods.

Considering its potential clinical relevance and the lack of a comprehensively validated bioanalytical method for the determination of PCT in DBS, in the context of TDM, this study aimed to develop and validate a clinically applicable LC-MS/MS method for the quantification of PCT.

2. Experimental

2.1. Standards, solvents and materials

PCT and deuterated PCT (PCT-D5) were acquired from Toronto Research Chemicals (NorthYork, Canada). Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany) and ammonium formate, ammonium acetate, formic acid and 1-chlorobutane were acquired from Sigma Aldrich (Saint Louis, USA). Whatman 903® paper was obtained from GE Healthcare (Westborough, USA). Ultra-pure deionized water was supplied by a Milli-Q RG unit from Millipore (Billerica, MA, USA).

2.2. Solutions

Stock (1000 μg mL^{-1}), intermediate (100 μg mL^{-1}) and working (0.05, 0.10, 0.20, 0.50, 1.00, 2.00, 4.00, 8.00 μg mL^{-1}) solutions of PCT were prepared by dissolution in methanol. The internal standard (IS) working solution, at 1 μg mL^{-1}, was prepared by dilution of intermediate solution of PCT-D5 with methanol. Working solutions of PCT were prepared at concentrations 20 times higher than calibration and control levels by dilution with methanol. DBS extraction solution was a mixture of methanol and acetonitrile (90:10, v/v), containing PCT-D5 at 4 ng mL^{-1}. The IS solution for plasma analysis was PCT-D5 at 1 μg mL^{-1}, also in methanol.

2.3. Chromatographic and mass spectrometric conditions

DBS and plasma samples were analyzed using a 1260 Infinity liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA), coupled to a 5500-QTRAP® hybrid triple quadrupole mass spectrometer (ABSciex, Concord, Canada). The chromatographic separation was performed with a Kinetex C18 (50 × 4.6 mm, 2.6 μm) column, from Phenomenex (Torrance, CA, USA), maintained at 30 °C. The mobile phase consisted of ultra-pure water (A, 25%) and methanol (B, 75%), both containing formic acid (0.1%, v/v) and 2 mmol L^{-1} of ammonium formate, eluted at isocratic mode. The mobile phase flow rate was 0.5 mL min^{-1}. Chromatographic run time was 2.3 min. The 5500-QTRAP® mass spectrometer was equipped with a TurboIonSpray™ interface using electrospray ionization in the positive ionization mode. Nitrogen was used as curtain, collision and nebulizer gas. The source parameters were: ion source temperature, 650 °C; ion spray voltage, 4.5 kV; entrance potential (EP), 10 V; nebulizer gas (GS1) pressure, 60 psi; auxiliary gas (GS2) pressure, 60 psi; and curtain gas pressure, 15 psi. The analyses were performed in multiple reaction monitoring (MRM) mode. For each compound, three MRM transitions were chosen for quantification and confirmation of PCT and two for PCT-D5, and optimized by constant infusion of working solutions of each analyte (20 ng mL^{-1} in water/methanol, 1:1 v/v). Table 1 shows the optimized conditions of declustering potential (DP), collision energy (CE), collision cell exit potential (CXP) and the retention time for PTC and PTC-D5. Analyst 1.6.2 software was used for data collection and MultiQuant 3.0.1 for data processing.

2.4. Preparation of DBS

Calibration and quality control DBS samples were prepared by pipetting 50 μL of blood on Whatman 903® paper, followed for a minimum drying time of 3 h before extraction. Calibrators and quality control samples were prepared by diluting working solution of PCT with venous blood in the proportion 1:20 (v/v). Blood used for the preparation of validation DBS samples had a Hct% of 40 unless otherwise stated. DBS samples from finger-pricks were obtained by application of one drop of blood to the paper, directly from the patient finger, without touching the surface of the collection area. DBS samples obtained from patients were allowed to dry at room temperature for 3 h after collection and then stored in plastic bags with desiccants at room temperature.

Table 1
Optimized parameters for analysis of paclitaxel and paclitaxel-D5 by liquid chromatography-tandem mass spectrometry.

Analyte	MRM transitions (*m/z*)[a]	DP (V)[b]	CE (V)[c]	CXP (V)[d]	Retention time (min)
Paclitaxel	854.3 → 509.2	186	17	11	2.0
	854.3 → 569.2	186	13	27	
	854.3 → 551.2	186	15	13	
Paclitaxel-D5	859.4 → 569.2	181	13	13	2.0
	859.4 → 509.2	181	19	25	

[a] Quantifier transitions were underlined, *MRM* multiple reaction monitoring.
[b] *DP* declustering potential.
[c] *CE* collision energy.
[d] *CXP* collision cell exit potential.