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A dried blood spot assay for paclitaxel and its metabolites

Feifan Xie[a,*], Elien De Thaye[a], An Vermeulen[a], Jan Van Bocxlaer[a], Pieter Colin[a,b]

[a] Laboratory of Medical Biochemistry and Clinical Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium

[b] Department of Anesthesiology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

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ABSTRACT

After being used for decades in clinical screening, dried blood spots (DBS) have recently received considerable attention for their application in pharmacokinetic and toxicokinetic studies in rodents. The goal of this study was to develop and apply a DBS-based assay for a pharmacokinetic study of paclitaxel (PTX) and its metabolites in SCID/Beige mice. A fast and sensitive UHPLC–MS/MS method has been developed for the simultaneous determination of PTX, its three metabolites (6α-hydroxy-paclitaxel, 3′-*p*-hydroxy-paclitaxel, and 6α,3′-*p*-dihydroxy-paclitaxel) and its stereoisomer 7-*epi*-paclitaxel. The 10 μL DBS sample was extracted with methanol for 20 min at 37 °C. After dilution of the extracts with water in a ratio of 1:1, the analytes were separated on a reversed-phase 2.1 mm I.D. column using gradient elution. The total run time was 2.5 min. The analytes were detected by use of multiple reaction monitoring mass spectrometry. The extraction recoveries of the compounds were all greater than 60%, resulting in a quantification limit of 1 ng/ml. The calibration curves ranged from 1 to 1000 ng/ml. The intra-day and inter-day imprecision (%CV) across three validation runs over four quality control levels were less than or equal to 14.6%. The accuracy was within ±11.9% in terms of relative error. The described method is advantageous in terms of its ease-of-use and speed compared to other published PTX assays. The method's usefulness was demonstrated by applying it to a preclinical pharmacokinetic investigation of PTX and its metabolites in SCID/Beige mice with an intraperitoneal administration of 50 mg/kg Abraxane®.

1. Introduction

Paclitaxel (PTX, Fig. 1) is a natural product discovered in 1968 by isolation from the bark of the pacific yew tree Taxus brevifola [1]. It is a widely used antineoplastic drug for the treatment of multiple types of tumors including ovarian, stomach, endometrial, colon, breast cancer and so on [2–4]. Despite its clinical efficacy, current PTX-based therapies are characterized by non-linear pharmacokinetics, a narrow therapeutic window, and severe dose-limiting toxicities (DLTs) [5,6].

PTX is predominantly metabolized in the liver by cytochrome P450 (CYP) enzymes. The primary metabolite 6α-hydroxy-paclitaxel (6α-OH-PTX, Fig. 1) is formed by CYP2C8, whereas the secondary metabolite 3′-*p*-hydroxy-paclitaxel (3′-*p*-OH-PTX, Fig. 1) is generated by CYP3A4 [7,8]. These two metabolites can be further metabolized to a minor metabolite 6α,3′-*p*-dihydroxy-paclitaxel (6α,3′-*p*-di-OH-PTX, Fig. 1). In addition, the C-7 chiral center in the taxane ring of PTX is notably labile and can readily undergo epimerization in an aqueous environment, resulting in 7-*epi*-paclitaxel (7-*epi*-PTX, Fig. 1), a biologically active [9] and thermodynamically more stable stereoisomer under physiological conditions [6,10].

Intraperitoneal chemotherapy (IPC) with PTX has a strong pharmacokinetic rationale in the treatment of peritoneal carcinomatosis. Hereto, PTX is directly administered into the abdominal cavity [11]. In this way, by lowering the systemic exposure, DLTs should be reduced [12]. However, controversy remains regarding the optimal treatment strategies that will optimize efficacy whilst minimizing systemic exposure. Quantification of PTX together with its metabolites is of major importance to gain insights into the absorption process and the extent of first-pass metabolism during IPC treatment. Current attempts to gain fundamental insights in the disposition of PTX following IPC treatment have focused on rodent models [13,14]. In order to exploit the full potential of these animal models, an efficient sampling procedure coupled to a rapid and sensitive analytical method is highly desirable.

* Corresponding author.
E-mail addresses: Feifan.Xie@UGent.be (F. Xie), Elien.DeThaye@UGent.be (E. De Thaye), AnMC.Vermeulen@UGent.be (A. Vermeulen), Jan.VanBocxlaer@UGent.be (J. Van Bocxlaer), Pieter.Colin@UGent.be (P. Colin).

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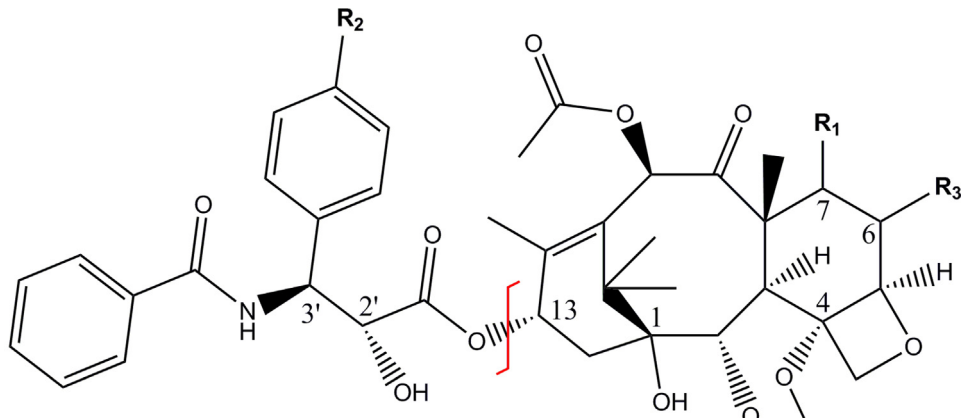

Compound	R_1	R_2	R_3
PTX	◀OH	H	H
7-epi-PTX	····OH	H	H
3'-p-OH-PTX	◀OH	OH	H
6α-OH-PTX	◀OH	H	····OH
6α,3'-p-di-OH-PTX	◀OH	OH	····OH

Fig. 1. Chemical structures of paclitaxel, 7-*epi*-paclitaxel, 3′-*p*-hydroxypaclitaxel, 6α-hydroxy paclitaxel, and 6α,3′-*p*-dihydroxypaclitaxel, and their fragmentation pattern.

Dried blood spot (DBS) sampling is a form of microsampling where small volumes of blood samples are applied on absorbent paper [15]. The most notable benefits of DBS sampling are the low required volume thus resulting decrease in the number of animals to be included in pharmacokinetic (PK)/toxicokinetic (TK) studies [16–18], and the improved stability in the dried blood spot matrix as compared to whole blood/plasma samples [19]. However, some challenges need to be overcome for the implementation of DBS analysis. The most common disadvantages are hematocrit, complex matrix (whole blood plus paper), required high sensitivity, and the comparability of DBS and plasma derived concentrations [20].

Different bioanalytical techniques have been described for the determination of PTX, including immunoassays [21], capillary electrophoresis [22], HPLC with UV detection [23] and liquid chromatography-tandem mass spectrometry (LC–MS/MS) [24–26]. Of these, the LC–MS/MS technique has been the most frequently applied method due to its sensitivity and selectivity [27]. There have been several LC–MS/MS assays published for the quantification of PTX and its metabolites [3,28–30] in various biological matrices such as plasma, serum, urine and tissue. As far as we know, there is only one publication reported for the determination of PTX in human DBS [31]. This method has a relatively narrow calibration range (0.2–20 ng/ml) and its clinical utility has not been demonstrated. Our report here is describing the quantification of PTX including its metabolites in dried blood spots obtained from animal experiments.

The goal of the present study was to develop a rapid and sensitive LC–MS/MS method for the simultaneous quantification of PTX and its metabolites (3′-*p*-OH-PTX, 6α-OH-PTX, and 6α,3′-*p*-di-OH-PTX) as well as the stereoisomer 7-*epi*-PTX, in mice DBS samples based on a simple extraction procedure. The use of DBS, a short gradient elution, and satisfactory sensitivity demonstrate that this LC–MS/MS method is superior to previous methods. This method to quantify PTX and its metabolites was successfully applied to a pharmacokinetic study where 10 SCID/Beige mice received an intraperitoneal dose of 50 mg/kg Abraxane®.

2. Materials and methods

2.1. Reagents and chemicals

Paclitaxel, $^{13}C_6$-Paclitaxel (internal standard (IS)), 3′-*p*-hydroxy-paclitaxel, 6α-hydroxy-paclitaxel, 6α-hydroxy-paclitaxel-d5 (IS), 6α,3′-*p*-dihydroxy-paclitaxel, and 7-*epi*-paclitaxel were all purchased from Toronto Research Chemicals (Toronto, Canada). ULC–MS grade water and methanol were obtained from Biosolve (Valkenswaard, The Netherlands). The PKI Bioanalysis Card (GR2261004) was supplied from PerkinElmer Health Sciences (Greenville, South Carolina, USA). All other solvents or chemicals were analytical grade or better.

2.2. Instrumentation and set-up

2.2.1. UHPLC–MS/MS

An Acquity BEH C18 column (50 × 2.1 mm, 1.7 μm) with an Acquity BEH C18 VanGuard pre-column (5 × 2.1 mm, 1.7 μm) installed in an Acquity UPLC H-class system (Waters, Milford, MA, USA) was used for chromatographic separation. Mobile phases, delivered at 0.45 mL/min, consisted of 0.2% formic acid either in 90/10 (*v*/*v*) water/methanol (eluent A) or in methanol (eluent B). The total run time for each separation was 2.5 min, and the linear gradient elution program was as follows: 0–1.25 min, 50% B to 80% B; 1.25–1.5 min, 80% B to 100% B; 1.5–2.0 min, 100% B to 50% B; 2.0–2.5 min, 50% B. A divert valve program switching alternatively between detector and waste was incorporated as follows: 0–0.75 min: to waste; 0.75–2.0 min: to detector; 2.0–2.5 min: to waste. The column temperature was kept at 50 °C, and the injection volume was 10 μL.

The LC eluate was led directly into a Waters Xevo TQS tandem mass spectrometer (Waters, Milford, MA, USA) with electrospray ionization (ESI) in the positive-ion, multiple reaction monitoring (MRM) mode. The MS/MS instrument was operated with a capillary voltage of 3.0 kV and source temperature of 150 °C. The desolvation gas (nitrogen) flow rate was set to 1000 L/h at a tem-