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Zwitterionic hydrophilic interaction liquid chromatography-tandem mass spectrometry with HybridSPE-precipitation for the determination of intact cisplatin in human plasma

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ABSTRACT

Cisplatin is a first-line chemotherapeutic for the treatment of a wide variety of cancers since its discovery in the 1960s. Although various techniques have been reported for the measurement of total platinum in biological matrices, such as inductively coupled plasma-mass spectrometry and derivatization procedures, a specific, sensitive and robust assay for the quantification of intact cisplatin is still lacking. Therefore, we present a rapid, selective, sensitive, and reliable UHPLC-MS/MS based method for the determination of intact cisplatin in human plasma in support of a Phase II clinical trial. The optimal chromatographic behavior of cisplatin was achieved on a Syncronis HILIC column (50 × 2.1 mm, 1.7 µm, zwitterionic stationary phase). The retention behavior of cisplatin on this zwitterion-based stationary phase was well described by an adsorptive interaction model. A simple sample preparation based on protein precipitation combined with the removal of phospholipids by HybridSPE-precipitation was developed. The method was proven to be free of a relative matrix effect. The assay was validated within a range of 20 – 10,000 ng/mL using 100 µL of plasma sample. The intra and inter-day precisions were all less than 7.6%, and none of the bias was greater than 13.1%, thus corroborating that the developed method is precise and accurate. As a proof of concept, the assay has been successfully applied to plasma samples obtained from different patients who were enrolled in the Phase II trial and were treated with cisplatin.

1. Introduction

Platinum (Pt) drugs are coordination complexes of platinum with multiple inorganic or organic ligands with cisplatin being the simplest platinum drug in which the Pt atom is associated with two amine and two chlorine leaving ligands [1]. As the first chemotherapeutic agent that was used clinically since 1970 [2,3], cisplatin still holds a leading position for the treatment of various types of solid tumors (head and neck, ovarian, gastric, lung, bladder, and testicular, etc.) [4–6]. It is well known that cisplatin is partly hydrolyzed to a monohydrated complex (MHC) upon administration [7,8], and that both cisplatin and MHC undergo irreversible ligand exchange reactions with biological nucleophiles such as nucleotides, methionine, glutathione, and albumin [9,10]. Both cisplatin and MHC are responsible for the antitumor effects as well as the toxic side effects (e.g. nephrotoxicity) [7,11]. After ligand exchange, the biotransformed platinum products are no longer biologically active [10,12].

Numerous analytical methodologies have been developed in the last decades for the determination of cisplatin [13,14]. However, quantitative determination of MHC is scarcely reported due to the lack of a pure standard of MHC. Some studies reported the measurement of MHC using the hydrolysis equilibrium solution of cisplatin in distilled water [15,16]. However, as found in an exhaustive mass spectroscopic hydrolysis study [17], the hydrolysis equilibrium approach presented in literatures does not provide a reliable way of obtaining a standard with a traceable exact amount of MHC useful for calibration purposes. This essentially prohibits a quantitative MHC measurement despite the analytical potential of the sample treatment, chromatographic and detection.

Bioanalytical determination of cisplatin is most often based on non-selective techniques, targeting the Pt, which cannot distinguish between different Pt-containing complexes. These non-selective techniques include phosphorescence [18], X-ray fluorescence [19], atomic absorption spectroscopy (AAS) [20–22] and inductively coupled plas-

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ma-mass spectrometry (ICP-MS) [23–25]. ICP-MS is the most frequently used tool for the measurement of total platinum for the drug cisplatin [14,26]. However, circulating total platinum represents a mixture of platinum species, with differing biological activity and toxicity profiles. Thus, the amount of total platinum does not reflect the authentic concentration of intact cisplatin and/or MHC and could lead to erroneous interpretations of the clinical pharmacokinetics and pharmacodynamics of cisplatin.

Selective methods for the determination of cisplatin itself in biological matrices are based on HPLC followed by different kinds of on-line or off-line detectors such as radioactivity detection [27], UV detection [10,16,28–30], flameless atomic absorption spectrometry (FAAS) [15], ICP-MS [31–34] and MS/MS [35]. UV-based methods usually require pre-column or post-column derivatization due to the low molar absorptivity of cisplatin in the UV spectrum [10,28–30,36]. Pre-column derivatization is a sensitive, yet unselective approach because of the formation of the same derivatization product for cisplatin, MHC as well as other Pt-based complexes (e.g. Pt-methionine and Pt-glutathione). This leads to an overestimation of the concentration of intact cisplatin in biological samples [37]. Post-column derivatization requires a microwave-assisted heater system and enormous efforts to maintain the sensitivity and robustness of the method [10,16]. HPLC with off-line detection such as FAAS requires tedious collection of different fractions and is therefore of limited use in medium to high-throughput applications [15]. HPLC-ICP-MS is a sensitive technique for the measurement of cisplatin in biological matrices, such as plasma ultrafiltrate, by detecting the specific mass of platinum [31–33]. However, chromatographic separation is still a requirement to provide satisfactory specificity and the particular hyphenated technique is not readily available in regular bioanalytical labs.

LC-MS/MS is a highly selective, reliable and sensitive methodology for the quantitation of analytes in biological samples by using specific precursor-to-product ion transitions. Previous studies have shown promising applications of LC-MS/MS for the determination of Pt-based drugs such as carboplatin [38] and oxaliplatin [39]. However, only poorly detectable ions were found for cisplatin regardless of the types of acid modifiers and solvents employed [1,2,8,40], reflecting the analytical challenges for direct quantitation of cisplatin using LC-MS/MS. Several studies have reported the use of pre-column derivatization of cisplatin for LC-MS/MS quantitation [41–43]. Although this approach can promote the ionization of cisplatin, it results in a measurement of cisplatin together with other Pt-based complexes aforementioned. To our knowledge, only one LC-MS/MS method has been described for the quantification of intact cisplatin in rat kidney and liver [35].

Reliable determination of intact cisplatin in biological samples is not easy since cisplatin can rapidly react with nucleophilic species in the matrices [44] and undergoes hydrolysis in aqueous medium [8]. Most of the bioanalytical work on cisplatin was done in plasma [16,31] or blood ultrafiltrate [10]. The aim here is to remove, and consequently reduce cisplatin interaction with, proteins. However, this does not alleviate the instability of cisplatin in the aqueous environment of the ultrafiltrate: cisplatin is also highly susceptible to hydrolysis. The above mentioned LC-MS/MS assay uses a conventional liquid-liquid extraction followed by a tedious evaporation procedure [35]. As such, it does not meet the requirements for a high throughput determination of cisplatin in biological fluids such as plasma.

Intraperitoneal chemoperfusion (IPEC) is a type of normothermia or hyperthermia therapy used in combination with surgery in the treatment of advanced abdominal cancers [45]. In this procedure, (warmed) anti-cancer drugs are infused and circulated in the peritoneal cavity for a short period of time. In support of the assessment of the efficacy of cisplatin-based IPEC in cancer patients, a simple, reliable, and high throughput assay for the determination of intact cisplatin in human plasma is appreciated. Therefore, the aim of this study was to develop and systematically validate a selective and sensitive UHPLC-MS/MS assay for the measurement of intact cisplatin in human plasma. As a proof of concept, this method was subsequently used to quantify cisplatin in plasma samples from an IPEC study in patients with peritoneal carcinoma receiving intraperitoneal chemoperfusion of cisplatin. We think that the method developed presents a new strategy for the direct determination of Pt-based drugs in complex biological matrices, such as plasma.

2. Experimental

2.1. Reagents and chemicals

Cisplatin and dichloro(ethylenediamine)platinum(II) (internal standard, IS) standards were purchased from European Directorate for the Quality of Medicines Council of Europe and Sigma-Aldrich (Belgium), respectively. Ultra liquid chromatography-MS (ULC-MS) grade water and acetonitrile were obtained from Biosolve (Valkenswaard, The Netherlands). Formic acid, ammonium formate and HybridSPE®-Plus 96-Well Plates (50 mg/well, volume 2 mL) were supplied from Sigma-Aldrich (Brussels, Belgium). All other chemicals were of analytical grade. Li-heparinized human plasma was purchased from Seralab (West Sussex, United Kingdom).

2.2. Instrumentation and set-up

Chromatographic separation of cisplatin was evaluated on several HILIC columns including an Acquity BEH HILIC column (50 × 2.1 mm, 1.7 µm) from Waters (Milford, MA, USA), a SeQuant ZIC-HILIC column (100 × 2.1 mm, 3.5 µm, zwitterionic stationary phase) from Millipore (Umeå, Sweden), and a Syncronis HILIC column (50 × 2.1 mm, 1.7 µm, zwitterionic stationary phase) from Fisher Scientific (Aalst, Belgium). Reversed-phase columns including an Acquity BEH C18 column (50 × 2.1 mm, 1.7 µm) and an Acquity HSS T3 column (50 × 2.1 mm, 1.7 µm) from Waters (Milford, MA, USA) for reversed-phase chromatography were also tested during method development. For method validation and sample analysis, the chromatographic analysis was performed on the Syncronis HILIC column installed in an Acquity UHPLC H-class system (Waters, Milford, MA). The mobile phase (MP) consisted of MP A (10/85/5 acetonitrile/water/50 mM ammonium formate buffer, pH 3.75) and MP B (95/5 acetonitrile/50 mM ammonium formate buffer, pH 3.75). Compounds were eluted using the conditions shown in Table 1 with a divert valve switched alternatively between detector and waste as follows: 0–0.6 min: to waste; 0.6–1.25 min: to detector; 1.25–5.0 min: to waste. The column temperature was maintained at 45 °C, and the injection volume was 2 µL.

Eluted components were detected using a Waters Xevo TQS tandem mass spectrometer (Waters, Milford, MA) operated in positive electrospray ionization (ESI^+) mode. The optimized instrument conditions were as follows: electrospray capillary voltage 4.0 kV, cone voltage 25 V, and source temperature of 125 °C. The desolvation gas (nitrogen) flow rate was set to 1000 L/h at a temperature of 500 °C, and collision gas (argon) flow was maintained at 0.15 mL/min. The collision energy was set at 14 eV for both cisplatin and dichloro(ethylenediamine)

Table 1
Elution conditions for cisplatin and internal standard.

Time (min)	Flow rate (mL/min)	% MP A	% MP B
Initial	0.40	5.0	95.0
1.10	0.40	5.0	95.0
1.20	1.20	50.0	50.0
2.50	1.20	50.0	50.0
2.60	1.20	5.0	95.0
4.80	1.20	5.0	95.0
5.00	0.40	5.0	95.0