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Specific determination of intact cisplatin and monohydrated cisplatin in human plasma and culture medium ultrafiltrates using HPLC on-line with inductively coupled plasma mass spectrometry

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Abstract

We have developed a specific assay for cisplatin in human plasma ultrafiltrate (PUF) and cell culture medium ultrafiltrate (MUF) using HPLC on-line with inductively coupled plasma mass spectrometry (ICP-MS). Separation of cisplatin (6 min) and monohydrated cisplatin (12 min) was achieved using a μBondapak C_{18} column (Waters) and a mobile phase (0.075 mM sodium dodecyl sulfate and 3% methanol, adjusted to pH 2.5 with triflic acid) pumped at a flow rate of 0.5 mL/min. The analytes were detected with little background interference by ICP-MS monitoring of platinum masses (*m/z* 194/195). Calibration curves were linear over three orders of magnitude (0.05–8 μM) and the limit of quantitation was 0.1 μM. Intra- and inter-assay accuracy (range 91.6–113%) and precision (range 1.00–12.3%) were acceptable for PUF and MUF. The method was applied to determining cisplatin during ex vivo incubation of the drug in whole human blood at 37 °C. In conclusion, a specific, sensitive and reliable HPLC–ICP-MS assay has been established for determining intact cisplatin in PUF and MUF.

Keywords: Cisplatin; Inductively coupled plasma mass spectrometry; Monohydrated cisplatin

1. Introduction

Cisplatin is a platinum based drug that is widely used in the clinical treatment of various cancers, including testicular, ovarian, lung, and head and neck cancer [1]. Cisplatin reacts indirectly with nitrogen atoms on DNA to form cross-links which inhibit DNA replication, cell division and induce apoptosis [2]. Preceding these DNA reactions, cisplatin undergoes ligand displacement reactions where water replaces one of its chloride atoms, resulting in the formation of monohydrated cisplatin, which is more reactive with nitrogen than the parent drug [3]. In addition, cisplatin and monohydrated cisplatin react with nitrogen, sulfur and oxygen residues on other biomolecules such as plasma proteins. As a result of these reactions, a variety of platinum species may be present in the body after treatment with cisplatin. There has been considerable interest in determining the concentration of cisplatin and monohydrated cisplatin in the body and under experimental conditions in matrices such as plasma ultrafiltrate (PUF) and cell culture medium ultrafiltrate (MUF) because of these important clinical applications. Given the reaction constants of cisplatin hydration [3,4] and the chloride concentration (110 mM) and pH (7) of PUF and MUF, the major biologically-active platinum species detected after cisplatin treatment in PUF and MUF are expected to be unchanged cisplatin and monohydrated cisplatin. Dihydrated cisplatin would form very slowly, and also would be removed rapidly by reacting with biological nucleophiles, in PUF or MUF.

A common approach for determining the concentration of cisplatin has been to measure the total platinum content of blood fractions and other biological fluids by flame and graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) [5]. To avoid detection of platinum that may have become deactivated by reactions with plasma proteins, plasma is often

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deproteinized by solvent protein precipitation or ultrafiltration before analysis. However, ultrafiltrates and solvent extractions of plasma may still contain different platinum species including the intact cisplatin, hydrated cisplatin and inactive forms of platinum. To further distinguish the species present in the systemic circulation after cisplatin treatment, there has been increasing interest in the use of species-specific analytical techniques [6,7].

HPLC–ICP-MS is a technique that has gained popularity for detecting different chemical species of trace elements in environmental, biological and clinical samples [8]. By directly coupling the HPLC column to an ICP-MS, which is set to detect the specific mass of interest (*m/z* 194/195 for platinum), specific and sensitive detection can be achieved with little background interference from complex biological matrices. HPLC–ICP-MS has been already utilized for determining platinum-based drugs such as satraplatin [9], ZD0473 [10] and BBR3464 [11]. Previous studies have shown promise for the specific detection of cisplatin and its metabolites [12,13], but there is still little information about the application of HPLC–ICP-MS to the quantitative analysis of cisplatin in plasma or medium ultrafiltrate. In this paper we report, for the first time, the development and validation of a specific HPLC–ICP-MS assay for detection of cisplatin and monohydrated cisplatin in PUF or MUF.

2. Experimental

2.1. Materials

Cisplatin (CDDP) and trifluoromethanesulfonic acid (triflic acid) were purchased from Sigma, St Louis, MO, USA. Thallium and platinum solutions were from Spex CertiPrep, Metuchen, NJ, USA. Methanol of chromatography grade was from Lab Scan Analytical Sciences, Dublin, Ireland. Sodium dodecyl sulphate (SDS) (Scientific Supplies, Auckland, NZ), 70% nitric acid, sodium hydroxide (Scharlau, Barcelona, Spain) and other chemicals were of analytical grade, unless otherwise indicated. Sterile 0.9% saline was purchased from Baxter Healthcare, Old Toongabbie, NSW, Australia. Alpha Modified Eagles Medium (αMEM) and fetal calf serum (FCS) were obtained from Invitrogen, Grand Island, NY, USA. Milli Q water (Millipore, Bedford, MA, USA) was used throughout the study. Argon of instrument grade (99.99%) and carbon dioxide of food grade (95%) were supplied by BOC Gases (Auckland, NZ). Drug free human plasma was supplied by the Auckland Regional Blood Services (Newmarket, Auckland, New Zealand). Human whole blood was donated by healthy volunteers and was collected from the left brachial vein into heparinized vacuum tubes (Vacutainer Systems, Rutherford, NJ, USA). All procedures were approved by The University of Auckland Human Ethics Committee.

2.2. Instrumentation

To detect cisplatin and monohydrated cisplatin, samples were analyzed using a method combining HPLC on-line with ICP-MS. A HPLC system consisted of a HP1100 binary pump, a rheodyne injector fitted with a 50 μL sample loop (Hewlett-Packard, Wilmington, DE, USA) and a μbondapak C_{18} (5 μ, 300 mm × 3.9 mm) column (Waters, Milford, MA, USA). The aqueous mobile phase was filtered through a membrane filter (0.45 μm; Millipore, Molsheim, France), degassed by sonication bath and spiked with 0.5 ng mL^{-1} thallium as the internal standard and pumped isocratically at a flow rate of 0.5 mL/min. At least 12 h were required for equilibrating the column prior to each analytical run. HPLC eluate flowed directly into the nebulizer through a 10 cm piece of 0.25 mm diameter PEEK tubing. A HP 4500 ICP-MS system (Hewlett Packard, Yokogawa, Japan) principally composed of a V groove nebulizer, a quartz torch, nickel sample/skimmer cones, a Scott double pass spray chamber maintained at −1 °C, a quadrupole mass analyzer, an electron multiplier detector (ETP Pty Ltd, Ermington, NSW, Australia), a CFT series recirculating chiller (Neslab, Portsmouth, NH, USA) and an ASX-500 auto sampler (CETAC Technologies, Omaha, NE, USA). Optimization of the platinum signal was performed for each analytical run using 10 ng mL^{-1} platinum in 1% nitric acid by adjusting Extract/Einzel/Omega ion lenses, AMU/Axis gain/offset and Plate/Pole bias. The platinum and thallium signals were monitored continuously throughout each run by single ion monitoring of *m/z* 194/195 and 205 with a dwell time of 100 ms and a replicate time of 6000 ms. Platinum chromatograms were integrated by RTE Integrator using HP4500 ICP-MS Chromatographic Software C.01.00 and ChemStation A.02.00 (Agilent Technologies, Avondale, PA, USA). Platinum peak areas were divided by thallium counts measured at the time of elution of each platinum peak for internal standardisation.

2.3. Stock solutions, calibration standards and quality control samples

On each occasion, a stock solution of 1 mM cisplatin was prepared in 0.9% saline with water bath sonication from which working solutions of 100, 10 and 1 μM were prepared by further dilution. A stock solution of 1000 μg mL^{-1} thallium was diluted with 1% nitric acid to make up a working solution of 100 ng mL^{-1}. PUF and MUF were prepared by spinning an aliquot of blank plasma or blank FCS-supplemented αMEM in Centrisart I filter (20 kDa cut-off, Sartorius AG, Goettingen, Germany) at 3000 rpm for 30 min (Centrifuge model J-6M, Beckman Instruments, Palo Alto, CA, USA). The ultrafiltrate was collected and stored at −20 °C. Calibration standards, at 0.05, 0.1, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 μM and quality control samples, at 0.1, 2.0 and 8.0 μM, were prepared in duplicate with PUF or MUF, respectively. Calibration curves were generated by plotting internal standard-corrected platinum peak areas versus nominal concentrations of calibration standards. Calibration curves were analyzed by linear regression analysis using Prism 3.0 (GraphPad Software, San Diego, USA).

2.4. Method validation

2.4.1. Accuracy and precision

Intra- or inter-assay accuracy and precision of the assay was determined by analyzing PUF or MUF samples containing