Journal of Pharmaceutical and Biomedical Analysis 138 (2017) 373–377

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Quantification and clinical application of carboplatin in plasma ultrafiltrate

Kim Downing[a,*], Berit Packert Jensen[a], Sue Grant[a], Matthew Strother[b], Peter George[a]

[a] Canterbury Health Laboratories, Christchurch, New Zealand
[b] Department of Oncology, Christchurch Hospital, Christchurch, New Zealand

ARTICLE INFO

Article history:
Received 19 October 2016
Received in revised form 18 January 2017
Accepted 21 January 2017
Available online 27 January 2017

Keywords:
Carboplatin
Platinum
ICP-MS
Ultrafiltrate
Stability
Therapeutic drug monitoring

ABSTRACT

Carboplatin is a chemotherapy drug used in a variety of cancers with the primary toxicity being exposure-dependant myelosuppression. We present the development and validation of a simple, robust inductively coupled plasma mass spectrometry (ICP-MS) method to measure carboplatin in plasma ultrafiltrate. Plasma ultrafiltrates samples were prepared using Amicon Ultra 30,000 da cut-off filters and then diluted with ammonia EDTA before ICP-MS analysis. The assay was validated in the range 0.19–47.5 mg/L carboplatin in ultrafiltrate. The assay was linear ($r^2 > 0.9999$), accurate (<6% bias, 12% bias at LLOQ) and precise (intra- and inter-day precision of <3% coefficient of variation). No matrix effects were observed between plasma ultrafiltrate and aqueous platinum calibrators and recovery was complete. The assay was applied to 10 clinical samples from patients receiving carboplatin. Incurred sample reanalysis showed reproducible values over 3 analysis days (<6% CV). As plasma stability prior to ultrafiltration has been a major concern in previous clinical studies this was studied extensively at room temperature (22 °C) over 24 h. Carboplatin was found to be stable in both spiked plasma (n = 3) and real patient samples (n = 10) at room temperature for up to 8 h before ultrafiltration. This makes routine measurement of carboplatin concentrations in clinical settings feasible.

1. Introduction

Carboplatin (cisdiammine 1-1 cyclobutanedicarboxylate platinum) is an antineoplastic drug used to treat various malignancies such as lung cancer, neuroblastoma, germ-cell tumors, osteosarcoma, retinoblastoma, hepatoblastoma, brain tumors, lymphoma and ovarian carcinoma [1,2]. Carboplatin forms adducts between platinum and DNA leading to single strand breaks [3–5]. Toxicities to carboplatin include myelosuppression, oto- and nephrotoxicity [1,6]. Toxicity and efficacy are dependent on drug exposure [7,8]. Carboplatin dosed to an area under the curve (AUC) of 7–8 mg min/mL has been shown to have an almost 50% risk of grade 3 thrombocytopenia, and an AUC 9–10 has a 50% probability of grade 3 leucopenia [8]. The Calvert formula (Carboplatin dose (mg) = target AUC (mg/mL min) × [GFR (mL/min) + 25]) has been developed to rationalize carboplatin exposure, with dosing based on a target AUC range [8–11]. However, patients dosed using the Calvert formula still Exhibit 15–23% mean error in dose prediction [12–14]. There has been progress towards overcoming this variability in exposure with adaptive modelling to predict subsequent carboplatin doses and achieve the target AUC [15]. A few strategies which require a limited amount of sampling (limited sampling models) have also been developed which require only one measurement (which correlates to the AUC) and can be used to predict the AUC [16]. In both scenarios, measurement of the drug concentration is required for more accurate intra- and inter-patient dosing and a robust and validated analytical method is needed [15,16].

Methods based on HPLC–MS/HPLC-UV, AAS and ICP-MS for measurement of carboplatin have been previously described, however ICP-MS methods have the advantage of increased sensitivity. HPLC-based methods have additional limitations in terms of sample stability as the intact carboplatin molecules are measured rather than the elemental platinum measured by AAS and ICP-MS [4,6,8,17,18]. ICP-MS methods for platinum analysis (carboplatin, cisplatin, oxaliplatin etc) have been published with many different aims, especially with biological or environmental measurement as previously reviewed [6,8]. These methods are largely similar at the ICP-MS level, with adaptations to their sample processing to reflect their purpose (i.e. laser ablation with solid samples, HPLC for protein analyses, or dilution for chemotherapy).

In clinical studies, one of the major concerns raised in measurement of carboplatin has been the impact of storage of plasma or

* Corresponding author.
E-mail address: kim.downing@cdhb.health.nz (K. Downing).

http://dx.doi.org/10.1016/j.jpba.2017.01.045
0731-7085/

whole blood samples and time-to-ultrafiltration on the reliability of assay results [7,17,19,20]. In the literature, there is some data on carboplatin stability such as Gaver and Erkmen [4,19,21]. Stability for carboplatin may change with method used to detect it however, as the ICP-MS measures direct platinum in the ultrafiltate, rather than the carboplatin molecule such as HPLC. Ultrafiltrate platinum has been shown to correlate to carboplatin AUC, making this a useful measurement [8]. Gaver [4,19] reports values based on HPLC, suggesting that carboplatin (37 °C, spiked plasma) degrades to 63–77% of the original concentration within 2 h. However, Erkmen [21] provides data obtained by FAAS showing a loss of 2.4% ultrafilterable platinum per day if plasma is stored at −20 °C before ultrafiltration, with no change at −70 °C. Using FAAS, no loss of ultrafilterable platinum from spiked carboplatin was observed after 4 h at 37 °C, but a loss of ~30% was seen at 24 h [8,25]. As FAAS is similar to ICP-MS in its direct measurement of platinum, it is likely closer to the stability as determined by ICP-MS. These papers are the best data we currently have for carboplatin stability in plasma, before ultrafiltration and this data is clearly incomplete.

Currently, there is thus little practical data to show how long a sample can be left before ultrafiltration and still be viable for measurement with the best data relying on *in vitro* spiking experiments which may differ from the actual stability of carboplatin in patients [8,21]. We therefore aim to describe a simple, fast and reliable ICP-MS method for quantitating carboplatin in ultrafiltrate, and to clarify the data in the literature surrounding pre-analytical carboplatin stability.

2. Materials and methods

2.1. Materials

Amicon Ultra 0.5 mL, 30 kDa cut-off filters used for ultrafiltration, Centrifree 30 kDa cut-off filters, Vivaspin 30 kDa cut-off filters, 1000 mg/L iridium chloride certipur stock solution in 7% HCl and chromatography grade 2 *iso*-propanol were all purchased from Merck (Darmstadt, Germany). Platinum stock solution 1000 mg/L in 5% HCl used for calibration was bought from O2SI Smart Solutions (Charleston SC, USA). Carboplatin used for spiking experiments and quality controls were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Powdered EDTA, TritonX100 and 69% nitric acid was purchased from BDH Lab Supplies (Poole, England). Trace metals grade ammonia hydroxide (22%) and the water purification system used to produce water I (Easypure II system) were purchased from ThermoFisher (Pittsburgh, PA, USA). 99.9995% argon and 99.9995% helium were purchased from BOC (Surrey, England). All standards and samples were prepared in trace element free plastic tubes (Thermo Fisher Scientific New Zealand, North Shore City, NZ). Whole blood patient samples were collected in non-gel lithium heparin tubes (Becton, Dickinson and Company, Franklin Lakes, NK, USA) and plasma separated off into polypropylene tubes.

2.2. Reagent preparation

For dilution of standards and samples, an ammonia EDTA diluent was made. This consisted of 0.004% w/v EDTA, 0.008% w/v TritonX100, 0.4% v/v isopropanol and 0.56%v/v ammonium hydroxide in water I. This diluent is generically used in the laboratory for ICP-MS assays. The internal standard solution of iridium chloride was prepared to 1 g/L in water I.

2.3. Standard preparation

Calibration standards were made by diluting a platinum stock solution (1000 mg/L in 5% HCl) with water I to concentrations of 1.0 μg/L, 2.5 μg/L, 25 μg/L and 250 μg/L of platinum (corresponding to 0.19, 0.475, 4.75 and 47.5 mg/L carboplatin in the samples). Standards were prepared fresh each batch.

2.4. Sample preparation

Whole blood samples were centrifuged at 4500 × g for 5 min, 4 °C using an Eppendorf 5810R centrifuge. Once centrifuged, 350–500 μL of plasma was pipetted into the Amicon Ultra 0.5 mL, 30,000 da cut-off filter, centrifuged at 12,300 × *g* for 10 min using a Gyrozen Mini microcentrifuge. The ultrafiltrate obtained was stored at −20 °C until analysis.

Ultrafiltrate samples for analysis were thawed to room temperature and vortexed. Specimens were then diluted 1:100 with the ammonia EDTA diluent solution using 50 μL of sample with an autodilutor (Hamilton Microlab 600) followed by addition of 100 μL internal standard (final concentration of 20 μg/L iridium).

2.5. Instrumentation

The ICP-MS used was a 7700 series analyser from Agilent (Agilent Technologies, California, USA). The ICP-MS was equipped with a Mira Mist nebulizer and a quartz torch (2.5 mm). Specimens were sampled by a ASX-520 series autosampler encased within a plastic housing to prevent evaporation. Pt^{194} and Pt^{195} isotopes were selected for measurement as these are the most abundant isotopes, with a known ratio between them to discount any unknown interferences. Pt^{194} was used in any calculations and reporting. ICP settings used were: an RF power of 1.35 kW forward, RF matching of 1.84 V; gas flow settings of 0.9 L/min carrier argon and 0.2 L/min dilution argon with 4 mL/min helium used as a collision gas as this lowered the background signal. Nickel cones were used for both the sampling and skimming cones. A sampling depth of 8 mm was used. A spectrum acquisition mode was used with a dwell time of 200 ms, utilising 2 replicates per sampling, 40 sweeps per replicate, a total acquisition time of 0.4 min and a 15 s stabilization time. A 40 s post-wash time was also employed. All data was collected with Mass Hunter version B.01.03 SP1 (Agilent Technologies, California, USA).

2.6. Ethics

The clinical component of this study was approved by the Health and Disability Ethics Committee of New Zealand

2.7. Evaluation of ultrafiltration device

In a pilot study, Centrifree 30 kDa, Vivaspin 30 kDa and Amicon 30 kDa filters were all tested for recovery. Platinum-free plasma was spiked to 5 mg/L carboplatin, vortexed thoroughly and left at room temperature. Ultrafiltration was done at 0, 1 and 2 h post spike, in each of the three ultrafiltration devices. Ultrafiltration was performed for each device according to manufacturer specifications in order to attain the largest possible plasma ultrafiltrate (pUF) volume (22 °C, highest centrifugation speed, maximum sample volume, longest stated centrifugation time). The Pt concentrations for the ultrafiltrated samples were compared with aqueous standards representing 100% recovery.

2.8. Method validation

Method validation was carried out according to the following criteria.