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Review

Simple and rapid determination of carboplatin in plasma by high-performance liquid chromatography. Error pattern and application to clinical pharmacokinetic studies

L. Zufía, A. Aldaz*, C. Castellanos, J. Giráldez

Pharmacy Department, University Hospital of Navarra, c/Pío XII s/n, 31008 Pamplona, Spain

Abstract

Carboplatin is an antitumor agent widely employed in cancer chemotherapy. A specific and selective method for the determination of carboplatin in human plasma and its applications to pharmacokinetic investigations is described. One ultrafiltration step, through a Centrifree micropartition system (Amicon) at 2000 *g* for 10 min, is the only requirement as sample treatment. The resulting solution is injected into an Inertsil ODS-2 (5 μm, 25 cm×4.6 mm I.D.) analytical column. The mobile phase consisted of 0.1 *M* potassium dihydrogenphosphate with 1 m*M* dipotassium edetate adjusted to a pH between 3 and 3.5. The limit of quantitation was 1 mg/l. The method showed good recovery (100.68±5.49%) and precision: the within-day relative standard deviation (RSD) for carboplatin (3–350 mg/l) was 2.07% and the between-day RSD for carboplatin, in the previously described range, was 1.31%. We determined the assay error pattern for proper weighting of serum level data in pharmacokinetic models. The selectivity (discrimination between the parent drug and platinum-containing species such as carboplatin metabolites), simplicity and speed of this assay for free carboplatin quantitation should facilitate pharmacokinetic investigations and therapeutic drug monitoring.

Keywords: Review; Error pattern; Carboplatin

Contents

*Corresponding author.

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1. Introduction

Platinum complexes are now a well-established class of antitumor agents and play an important role in cancer chemotherapy. Pharmacokinetics/pharmacodynamics have been well characterized during the last decade. Carboplatin [*cis*-diammine(1,1-cyclobutanedicarboxylato) platinum (II)] was introduced into clinical trials in 1981 to help circumvent some of the toxicities of cisplatin. Its greater chemical stability in comparison with cisplatin accounts for its lower reactivity with nucleophilic sites of DNA, and may therefore be related to the higher dose necessary to obtain an antitumor effect similar to that of cisplatin [1,2].

Carboplatin has been tested clinically in tumors responsive to cisplatin, in those not previously considered for treatment by the latter drug and in patients whose treatment was limited by cisplatin-induced nephrotoxicity or neurotoxicity [5–8]. Although carboplatin has replaced cisplatin in chemotherapy regimens of some diseases, such as ovarian or lung carcinoma, it is still unclear whether carboplatin has equivalent efficacy to cisplatin across all disease types.

In oncological practice, doses of anticancer agents are often adjusted to body surface area, but for many drugs this strategy fails to control drug concentration in individual patients. Since cancer chemotherapy is often associated with high toxic risks, adaptive dosing and therapeutic drug monitoring based on pharmacokinetic parameters may reduce toxicity and even enhance efficacy. For carboplatin, individual dosage strategies, such as the Calvert [3] or Chatelut [4] formulas based on the target systemic exposure to carboplatin (AUC), have already been introduced into clinical practice and might help to maximize efficacy and minimize toxicity.

The methods used for the pharmaceutical and biomedical analysis of carboplatin can be divided into two groups: (a) nonspecific methods determining only the total or free quantity of the element platinum, in plasma or plasma ultrafiltrate, and (b) selective methods analyzing carboplatin itself. Among the nonspecific methods the most commonly used is atomic absorption spectrophotometry (AAS). The procedures developed for cisplatin [9–11] can be applied to determine the concentration of platinum in plasma ultrafiltrate, (ultrafiltration is necessary because protein bound platinum was found to be pharmacologically and toxicologically inactive [12]), and urine without pretreatment, and in blood and tissues after destruction [13–15]. These methods typically give a relative standard deviation (RSD) of the assay of less than 10% and can measure free platinum concentrations down to the order of 50 to 100 μg/l [16–18].

For both pharmacokinetic investigations and in vitro experiments, it is desirable to have a selective method for the determination of free carboplatin (the active species) because treatment efficacy and toxicity are both related to the concentration of free carboplatin and not to the concentration of other platinum-containing species which may arise from the degradation or metabolism of carboplatin and could interfere in the quantitation of free carboplatin and the evaluation of the efficacy/toxicity. Normal-phase and reversed-phase high-performance liquid chromatography (HPLC) systems have been used with a number of detection techniques: UV detection [19–24], post-column derivatization plus UV detection [25,26], quenched phosphorescence [27] and electrochemical detection [28]. These methods also require an ultrafiltration stage and typically give assay RSDs of less than 10%, but have a limit of detection of about 500 μg/l or more. These techniques distinguish between carboplatin and free ultrafilterable Pt, the decarboxylated product of carboplatin.

In order to study in detail the pharmacokinetics of an antitumor platinum complex, it is necessary first to develop a method for determining the quantity of

that complex in biological samples. We describe a quick, simple but highly selective reversed-phase method with a UV detection system to measure carboplatin concentrations in human plasma ultrafiltrate. The chromatographic peak homogeneity was confirmed by the use of a photodiode array detector to obtain multi-wavelength chromatograms. We determined the error pattern for this analytical methodology as a part of the validation procedure. We have used the method to determine successfully plasma concentrations of carboplatin in cancer patients receiving infusion of high doses of carboplatin.

2. Experimental

2.1. Reagents

A Carboplatin reference standard was kindly supplied by Bristol-Myers Squibb (Evansville, USA), HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany) and analytical-grade potassium dihydrogenphosphate, dipotassium edetate and phosphoric acid were obtained from Panreac (Barcelona, Spain). For stock solutions, all compounds were dissolved in Milli-Q water on the day of assay.

2.2. Sample preparation

Blood samples were collected in heparinized tubes and immediately centrifuged at 5000 *g* for 5 min. Plasma ultrafiltrate was obtained by ultrafiltration through a Centrifree micropartition system (Amicon) at 2000 *g* for 10 min. The resulting solution, 100 μl, was transferred to vials and injected into the HPLC system. Ultrafiltrate for each sample was stored at −25°C until analysis. Biological samples should be stored frozen and analyzed within a week of collection to obtain valid results [20].

Standards were prepared from normal human plasma spiked with different amounts of carboplatin and analyzed as patient samples.

2.3. High-performance liquid chromatography

The HPLC equipment consisted of a HP 1100 Model with a diode array detector. Separation of compounds was achieved using an Inertsil ODS-2 (25 cm×4.6 mm I.D.) analytical column protected by a LiChospher 100RP-18 pre-column (5 μm, 4.0×4.0 mm). The mobile phase was 0.1 *M* potassium dihydrogenphosphate with 1 m*M* dipotassium edetate adjusted to pH between 3 and 3.5 with 85% orthophosphoric acid.

Flow-rate was monitored at 1 ml/min and the column temperature was held at 40°C until the end of the chromatogram. The detector wavelength was set at 229 nm.

2.4. Determination of carboplatin in plasma

The concentrations of carboplatin were determined by reference to the calibration graph obtained in the concentration range 3–350 mg/l using a least-squares analysis of standard concentrations.

The limit of quantification in plasma was calculated as three times the standard deviation of the lowest concentration included in the calibration graph (3 mg/l).

2.5. Determination of recovery, precision and accuracy

The recovery from plasma was evaluated by comparing preparations of appropriate standards in water and in plasma. The standards in water were analyzed directly, while the standards in plasma were ultrafiltered prior to analysis. By comparing the areas of pure standards with those of extracted plasma samples containing the same amount of standards, we determined the recovery coefficient.

Standards in water were also analyzed after being ultrafiltered, to determine whether the Centrifree membrane adsorbed any of the drug.

Four replicates with four different concentrations ranging from 3 to 350 mg/l of carboplatin in human plasma were processed as described above to determine the within-day and between-day reproducibility.

The precision of the method at each concentration was calculated as the RSD of the mean using the following equation: RSD=(SD/mean)×100.

The accuracy of the procedure was determined by

expressing the mean calculated concentration as a percentage of the spiked concentration.

2.6. Practical determination of assay error pattern for the analytical method and application to clinical pharmacokinetic studies

Estimation of the standard deviations (SDs) of each serum drug concentration are a fundamental part of the objective function in the Bayesian fitting of the individual pharmacokinetic parameters. The weighting method employed here was the Fisher information (the reciprocal of the variance by which each serum concentration is measured) [29].

The SD usually has a nonlinear relationship to the serum concentration. One way to compute the probable SD with which a single determination of a serum drug concentration is measured, is to do replicate measurements of several representative samples and determine the mean and SD of each sample. This was done by measuring four concentrations levels included in the calibration fit. Each concentration was determined four times a day for 4 consecutive days and the mean and SD for each day were plotted and adjusted to the best fit. The assay error pattern enables us to calculate the probable SD of an individual serum concentration measured with the analytical method validated in our laboratory and to evaluate the credibility of each patient pharmacokinetic parameter values.

3. Results and discussion

3.1. Assay validation

The analytical methodology was validated in terms of selectivity, recovery, linearity, limit of quantitation, precision, accuracy and finally the error pattern was determined.

3.1.1. Selectivity

Possible interference from endogenous constituents of human plasma ultrafiltrates was evaluated by analyzing plasma samples obtained from different donors. Most of the material absorbing at 229 nm eluted before the carboplatin peak. No interfering peaks were observed and no significant peaks were found at the retention time of carboplatin. Fig. 1 shows the chromatogram obtained from a patient who had not received carboplatin and Fig. 2 shows a representative chromatogram of a human plasma extract after administration of carboplatin. As we can see, potential degradation products of carboplatin, cyclobutane mono- and dicarboxylic acids were not interfering in the chromatogram and carboplatin was perfectly resolved. This system, therefore, complete-

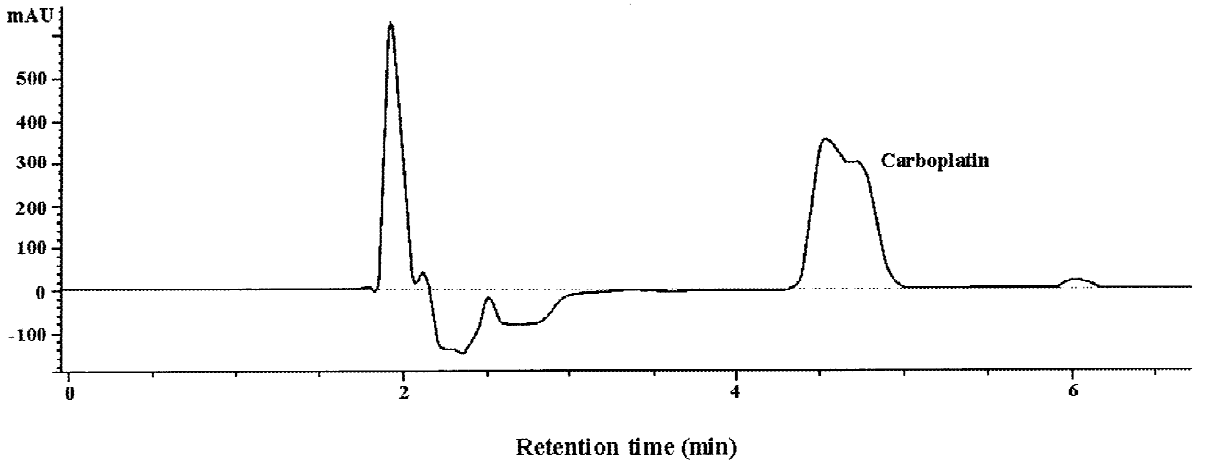

Fig. 1. HPLC of a plasma extract without carboplatin.

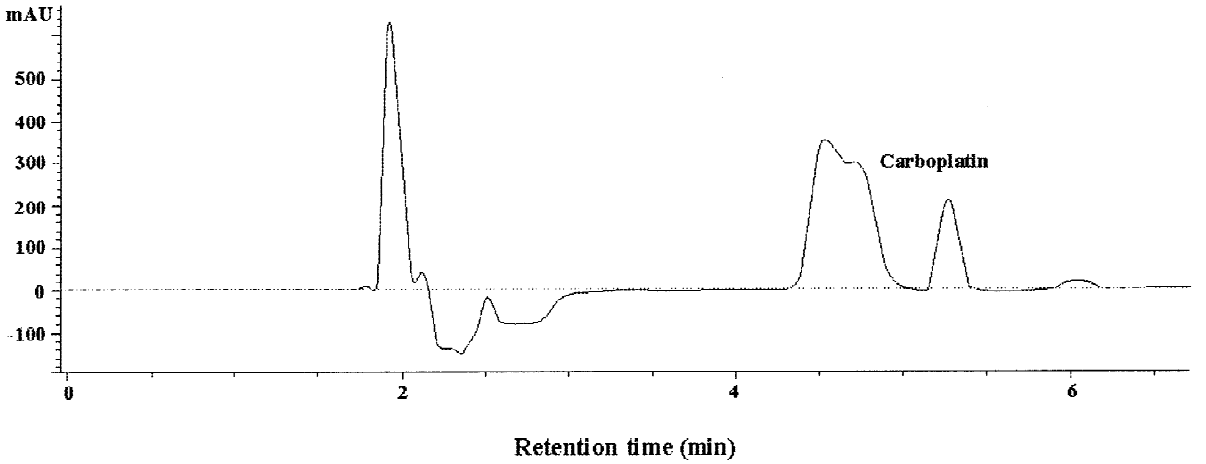

Fig. 2. HPLC of a human plasma extract 3 h after administration of 1200 mg/m^2 of carboplatin.

ly separated carboplatin from endogenous constituents and degradation products.

3.1.2. Recovery

Recovery was determined according to the ratio of the areas of standards prepared in water and in plasma. The overall mean recovery of carboplatin was (mean±SD) 100.68±5.49 (n=5). As shown in Table 1, recoveries for carboplatin were similar for every concentration studied.

In the procedure, plasma samples were ultrafiltered through a membrane (that excluded proteins with a molecular mass greater than 30 000), and so the carboplatin recovered in the ultrafiltrate is the carboplatin which was present in the plasma sample as free, nonprotein bound drug.

Carboplatin was shown not to be bound to the Centrifree membrane: the mean recovery of carboplatin in the ultrafiltrate was 98% relative to unfiltered aqueous standards.

Table 1
Recovery of carboplatin from human plasma (n=5), results are shown as mean±SD (standard deviation)

Carboplatin concentration (mg/l)	Recovery (%)
325	100.92±4.03
145	97.75±4.23
64	96.12±5.24
32	104.94±3.27
12	110.20±5.63
3	94.16±3.86

3.1.3. Linearity and the limit of quantification

The range of reliable response was established on the basis of five triplicate standards in plasma covering the concentration range of 1 to 500 mg carboplatin/l. The calibration line $y=12.74x+77.35$ was estimated for a range of 3–350 mg carboplatin/l and represents the mean of the three graphs corresponding to the triplicate standards. The correlation coefficient (r) for each calibration graph was greater than 0.999 and the RSDs of the response factors (RFs) for each concentration assayed were below 10%. Peak area was linear with concentration up to at least 500 mg/l. The lower limit of detection was 0.5 mg/l since the peak area for this concentration was distinguishable from the responses given by the carboplatin free control plasma. The limit of quantification was 1 mg/l, the signal-to-noise ratio for this concentration was approximately 3:1.

We developed our method to measure carboplatin plasma concentrations in patients under the specific protocol used and developed in our hospital, the University Hospital of Navarra (Spain). This protocol involves high doses (1200–1500 mg/m^2), a 1-h infusion and sampling between 1 and 6 h after administration,

Table 2
Within-day and between-day precision and accuracy of the HPLC determination of carboplatin in human plasma ($n=4$)

Carboplatin concentration (mg/l)	Within-day		Between-day	
	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
325	2.1	4.7	2.17	5.9
145	2.16	3.84	1.59	5.32
97	2.12	1.02	0.69	1.65
32	1.60	5.99	1.05	6.85
6	2.38	4.35	1.51	5.87
3	1.96	2.21	2.64	3.10

3.1.4. Precision and accuracy

The precision was good. The mean within-day RSD was of 2.07%, yielding a mean accuracy of 3.8%. The mean between-day RSD was 1.31%, with a mean accuracy of 4.92%. Table 2 gives precision and accuracy of this method for the concentrations assayed.

3.1.5. Practical determination of the assay error pattern and applications of the analytical method in clinical pharmacokinetic studies

We determined a calibration curve which included concentration levels similar to those found in patients with high risk breast cancer treated at high doses of carboplatin under the above mentioned protocol developed in the University Hospital of Navarra.

The concentrations determined were 325, 145, 97, 32, 6 and 3 mg/l (the same as those used in the precision and accuracy study). Blank samples were not included because the quantification software was not able to integrate when the peak area was zero. Jelliffe et al. [29] emphasized that measuring blanks and reporting concentrations below detectable limits is very important to optimal Bayesian therapeutic drug monitoring, as is the need for an accurate assay error pattern for proper weighting of serum level data in both individually fitted patient pharmacokinetic models and in population pharmacokinetic models.

We used the Assfit6 module in USC-Pack (University of Southern California) to find the best polynomic equation and Statistica, Edition‘99 (StatSoft) to obtain the graphic design (Fig. 3) to

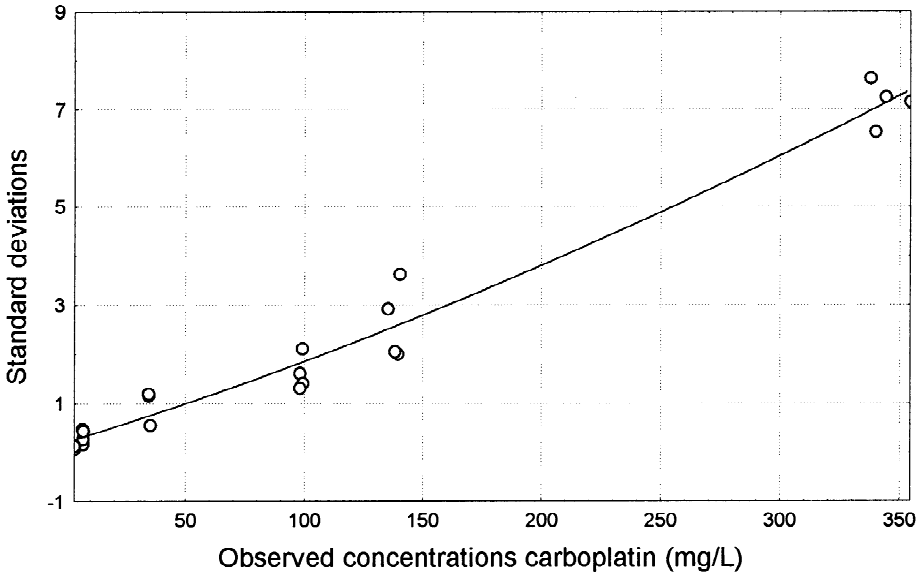

Fig. 3. Plot for the best estimated polynomic fit for the assay error pattern (Statistica Edition‘99, StatSoft).

Table 3
Experimental means and SDs for each concentration plotted in the assay error pattern

Carboplatin concentration (mg/l)	Mean (mg/l)	SD
323.75	339.75	6.54
	354.53	7.15
	337.8	2.26
	344.46	7.25
145.67	140.28	3.62
	135.25	2.92
	139.43	2.00
	138.32	2.05
97.13	98.13	1.61
	99.26	2.11
	99.44	1.41
	98.22	1.31
32.38	34.44	1.2
	35.17	1.6
	34.97	1.16
	34.48	1.2
6.48	6.78	0.48
	6.93	0.17
	6.83	0.27
	7.0	0.44
3.24	3.46	0.14
	3.25	0.098
	3.31	0.065
	3.34	0.13

accommodate our data (Table 3). The best fit corresponded to a second-order polynomial, $SD = 0.2172 + 0.01491x + 0.00001511x^2$ where SD is the assay standard deviation and x is the measured serum concentration. The R^2 coefficient value was good: 0.972.

4. Conclusions

The method we describe is very simple, quick, specific and selective. The only sample treatment required is one ultrafiltration step and only one C_{18} reversed-phase column is used (others authors describe methods requiring three columns in series [30]). The whole chromatographic process takes only 10 min and resolves carboplatin from its potential degradation products (cyclobutane mono- and dicarboxylic acids) and other metabolites. Results are reproducible; Gaver and Deeb [20] obtained variable results when carboplatin (which is highly polar) was applied to typical silica columns.

The simplicity and speed of this assay should facilitate pharmacokinetic research and therapeutic drug monitoring in cancer patients. The method has been successfully applied in our hospital laboratory.

Finally, we emphasize the importance of the assay error pattern to the validation of analytical methods in general, especially those assays to be used in pharmacokinetic studies.

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