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Determination of platinum in tumour tissues after cisplatin therapy by electrothermal atomic absorption spectrometry

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Abstract

A simple procedure is described for the determination of platinum in tumours after cisplatin therapy. Tumours were digested in 65% nitric acid by incubation at 37°C for 2 days and platinum analysed under optimum conditions by electrothermal atomic absorption spectrometry with Zeeman background correction. The reproducibility of measurements in general was better than $\pm 2\%$. The calibration graph was linear from 30.0 up to 1000 μg l^{-1} of platinum, while the limit of detection (3σ) was found to be 3.0 μg l^{-1} (sample volume 20 μl). Aqueous standard solutions and the standard addition method were applied in the calibration procedure. Under the recommended analytical conditions, the sample matrix did not influence the determination of platinum significantly. In 72% of samples analysed the differences between results obtained by the two calibration procedures did not exceed $\pm 5\%$.

Keywords: Platinum determination; Electrothermal atomic absorption spectrometry with Zeeman background correction; Aqueous standards and standard addition calibration; Tumour tissues

1. Introduction

Cisplatin (*cis*-diaminedichloroplatinum II) is one of the most effective and widely used chemotherapeutic drugs in the treatment of metastatic testicular and ovarian tumours, advanced bladder cancer, head and neck cancers and others [1]. Treatment modalities combining cisplatin with irradiation [2], hyperthermia [3], biological response modifiers [4], electric pulses (electrochemotherapy) [5,6] and other chemotherapeutic drugs [7] have also been studied with the aim of increasing antitumour effectiveness. The critical intracellular target for cisplatin is DNA and its cytotoxicity is thought to be mediated by inhibition of DNA synthesis subsequent to formation of cisplatin-DNA cross-links [8]. Therefore, cisplatin uptake and accumulation in the cells is an important determinant for tumour cell sensitivity and consequently for the response of tumours to cisplatin treatment alone or in combined therapy schedules. For this reason, it is important to determine cisplatin accumulation in tumour and

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normal tissues for selecting the optimal dose and schedule of cisplatin administration [9].

There are several analytical techniques for determination of platinum in biological materials. The most sensitive is inductively coupled plasma-mass spectrometry (ICP-MS) with a limit of detection (LOD) of 1 ng Pt l^{-1} [10] or sector field ICP-MS with an even lower LOD of 0.24 ng Pt l^{-1} [11]. Both techniques enable determination of physiological platinum levels in the urine of non-exposed persons [10,11]. In exposed persons, treated with platinum-containing drugs, platinum in biological fluids (urine, serum, blood) may be determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) [12–14], which offers an LOD between 20 and 50 μg Pt l^{-1}. In many cases this sensitivity is not satisfactory, so the most frequently used technique is electrothermal atomic absorption spectrometry (ETAAS) [15–25]. This technique is much cheaper than ICP-MS and sensitive enough (LOD about 5 μg Pt l^{-1}) for the determination of platinum in such biological materials. Various sample preparation and various calibration procedures have been reported. LeRoy et al. [15] analysed platinum in urine and tissues by ETAAS. Urine samples were determined directly without any pretreatment with simple calibration using aqueous standard solutions. Tissue samples were digested by heating in 60% nitric acid, followed by addition of 70% perchloric acid, evaporation, dissolution in a known volume of hydrochloric acid and platinum determination by the standard addition method. Hopfer et al. [16] demonstrated that in diluted samples of urine and plasma, standard additions are unnecessary when platinum is determined by ETAAS with Zeeman correction. Smeyers-Verbeke et al. [17] proved that platinum in blood, plasma, serum and urine can be determined by ETAAS without any special pretreatment of the samples and that measurements can be made against aqueous standard solutions. Their proposed method was therefore very suitable for routine drug monitoring. Andrews et al. [18] determined platinum in carcinoma cell lysates by ETAAS with simple calibration against aqueous standard solutions. Siddik et al. [19] compared a digestion procedure for plasma, blood and tissues in concentrated nitric acid on a hot plate at 230°C, and a procedure in which these samples were solubilizated in hyamine hydroxide. Platinum was determined by ETAAS by the standard addition method. Excellent agreement of results was obtained between hyamine hydroxide solubilization and nitric acid digestion. Among various biological samples platinum was also determined in tumours. Deurloo et al. [20] digested tumours in 65% nitric acid at 170°C in a Parr teflon digestion bomb for 2 h and determined platinum by ETAAS with the standard addition method. Vadiei et al. [21] digested tissue samples in hyamine hydroxide by incubation at 55°C for 5 h and addition of 0.3 M hydrochloric acid. Platinum in the samples was determined by ETAAS using the standard addition method. Yang et al. [23] reported a simple procedure for decomposition of carcinoma cells. To the pellet of cells, 30 μl of concentrated nitric acid was added and the samples incubated overnight at 37°C in a water bath. The next day, 170 μl of distilled water was added and platinum determined by ETAAS with the standard addition method.

The above mentioned papers in the literature reported various simple to more complex digestion procedures for a range of biological samples and calibration by aqueous standards or the standard addition method in the determination of platinum by ETAAS. Tissues were in general digested in acids by heating and evaporation or in heated teflon bombs and platinum determined by ETAAS with the standard addition method. However, in studies of combined treatment modalities with cisplatin aiming to optimise dose and timing of cisplatin administration, a large series of tumours needs to be analysed. In such cases a simple digestion and measurement procedure for determining cisplatin accumulation in tumour tissues is required. Therefore, the aim of our work was to investigate the possibilities of simple digestion of tumour tissue in 65% nitric acid combined with determination of platinum by ETAAS with Zeeman background correction. For this purpose calibration procedures based on aqueous standard solutions and the standard addition method were compared and critically appraised.

Table 1
Measurement parameters for determination of platinum in tumour tissues by ETAAS with Zeeman background correction

Electrothermal atomization programme						
Stage No.	Stage	Temp.(°C)		Time (s)		Gas flow (ml min^{-1})
		Start	End	Ramp	Hold	
1	Dry	60	80	15	10	200
2	Dry	80	100	10	10	200
3	Dry	100	150	10	5	200
4	Ash	150	1200	10	20	100
5	Atomization	2700	2700	0	4	0
6	Clean	2800	2800	0	5	200
7	Cool	0	0	0	5	200

Wavelength: 265.9 nm.
Slit width: 0.40 nm.
Lamp current: 12.5 mA.
Sample volume: 20 μl.

2. Materials and methods

2.1. Instrumentation

Platinum was measured by electrothermal atomic absorption spectroscopy on a Hitachi Z-8270 Polarized Zeeman Atomic Absorption Spectrophotometer equipped with an autosampler. Integrated absorbances (A·s) were used in the calculation of platinum concentrations. Pyrolytically coated graphite tubes were applied throughout the measurements. The measurement parameters for the determination of platinum in tumour tissues by ETAAS with Zeeman background correction are presented in Table 1.

2.2. Reagents

Merck Suprapur acids and water doubly distilled in quartz were used for the preparation of samples and standard solutions. All other chemicals were of analytical reagent grade.

A standard platinum stock solution Titrisol (1.000 ± 0.002 g Pt l^{-1} as $H_2(PtCl_6)\cdot 6H_2O$ in water) was obtained from Merck. Cisplatin (0.5 g l^{-1} of *cis*-diamminedichloroplatinum-(II)) ready-to-use Platinex solution was purchased from Bristol Myers Squibb.

2.3. Sample preparation

Subcutaneous tumours were induced dorsolaterally in mice by injection of viable tumour cells. After 6–8 days when the tumour reached ≈ 45 mm^3 mice were treated with cisplatin at a dose of 4 mg kg^{-1} injected intravenously [5]. To determine the platinum concentration in the tumours, mice were sacrificed at various time intervals after therapy. Tumours were excised and removed from the overlying skin. The weight of the tumours was approximately 0.1000 g.

2.4. Digestion procedure

Each tumour was weighed into a 15 ml graduated polyethylene tube (Costar) and 1 ml of 65% nitric acid added. Samples were incubated at 37°C for at least 2 days. The endpoint of digestion (quantitative digestion) was reached when the digested samples were transformed into completely clear solutions. Since the particular tumour weighted only about 0.1000 g and Pt in tumour is not distributed proportionately in the whole tissue, it was not possible to divide tumour in two samples for comparison analysis with other digestion techniques. The present digestion procedure of tumour tissue is very similar to that reported by Yang et al. [23], who digested a pellet of

carcinoma cells. After decomposition samples were left at room temperature and were diluted to 10 ml with water before analysis. The advantage of the proposed digestion procedure lies in its simplicity. A small volume of acid was used and during incubation, samples did not need any attention. Time-consuming handling of samples (evaporation of acids at elevated temperatures [15,19], digestion in Parr bombs [20]) was avoided and only dilution with water was necessary before measurement. Hence, the procedure is very convenient for analysis of large series of samples.

3. Results and discussion

3.1. Calibration graph, limit of detection and reproducibility of measurements

Standard solutions were prepared in 100 ml calibration flasks. An appropriate amount of stock standard solution and 0.5 ml of 65% nitric acid were added and diluted to the mark with water. Aqueous standard solutions which were prepared from Merck Titrisol stock platinum standard and those prepared from Bristol Myers Sqiubb cisplatin gave the same sensitivity of measurement. Therefore, Merck Titrisol stock standard solution was used in the calibration procedure.

The calibration graph for aqueous standard solutions was linear from 30.0 up to 1000 μg Pt l^{-1}. The limit of detection (LOD), calculated on a 3σ basis (a value of three times the standard deviation of the blank) was found to be 3.0 μg Pt l^{-1}. The wide linear concentration range and low LOD were appropriate for determination of platinum in tumour tissue after cisplatin therapy.

The reproducibility of measurements was tested in six parallel determinations of two digested tumour tissues. The results were calibrated by aqueous standard solutions. The average standard concentration of Pt in the first digest was 157 μg Pt l^{-1} with the relative standard deviation of measurements (R.S.D.) $\pm$ 0.9%, and in the second digest 239 μg Pt l^{-1} with R.S.D. $\pm$ 1.3%, respectively.

3.2. Comparison of two calibration procedures

In a variety of biological samples (plasma, urine, [16,17], carcinoma cells [18]) measurements of platinum by ETAAS can be made by simple calibration with aqueous standards. To estimate whether analysis of platinum in tumour tissue could also be performed against aqueous standards, aqueous standard calibration and the standard addition method were compared. For this purpose, 18 tumours were digested by the recommended analytical procedure. Analysis of platinum in tumour tissues was performed in the linear concentration range with a correlation coefficient of the calibration graph of 1.0000. The results of these measurements and the correlation between the standard addition method against aqueous standard calibration are presented in Table 2. It is evident from these data that with the recommended analytical procedure the sample matrix does not influence the determination of platinum significantly. In 72% of samples analysed the differences between results obtained by the two calibration procedures (Δ%) do not exceed $\pm$ 5%. Similar agreement was reported by Hopfer et al. [16] for plasma and urine samples. The differences between results obtained by calibration against aqueous standards and the standard addition method did not exceed $\pm$ 8 and $\pm$ 6%, respectively. Smeyers-Verbeke et al. [17] also obtained excellent agreement of results between the two calibration procedures. The differences in urine samples did not exceed $\pm$ 1% and in blood samples $\pm$ 4%.

On the basis of our observations (Table 2) it can be concluded that determination of platinum in tumour tissue by ETAAS can be performed against aqueous standards. The agreement of results between aqueous standards and the standard addition method for tumour tissue is similar to that reported for other biological samples like plasma, urine and blood [16,17].

The proposed analytical procedure for determination of platinum in tumour tissue offers a simple digestion procedure and calibration against aqueous standards, which is much more rapid than the time-consuming standard addition method. This is particularly important in the in-

Table 2
Concentrations of platinum in tumour tissues determined by ETAAS

Sample	Aqueous standard calibration		Standard addition calibration		Standard addition/aqueous standard (Δ%)
	Pt (μg l^{-1})	RSD (%)	Pt (μg l^{-1})	RSD (%)	
1	66.0	±3.4	71.9	±3.7	+8.9
2	59.7	±1.8	67.0	±3.5	+12.2
3	58.4	±0.8	58.0	±1.0	−0.7
4	76.6	±1.3	78.6	±2.8	+2.6
5	37.7	±2.4	40.0	±1.6	+6.1
6	60.6	±3.5	62.6	±1.7	+3.3
7	73.9	±1.2	78.5	±0.6	+6.2
8	55.6	±4.9	53.2	±7.9	−4.3
9	48.2	±3.5	46.5	±1.9	−3.5
10	62.4	±2.1	62.8	±5.4	+0.6
11	39.1	±2.3	38.7	±0.5	−1.0
12	53.4	±3.2	52.2	±4.4	−2.2
13	136	±0.6	147	±0.8	+8.1
14	159	±0.6	163	±0.7	+2.5
15	103	±1.1	107	±1.8	+3.8
16	96.3	±0.2	101	±1.3	+4.8
17	118	±0.7	115	±7.0	−2.5
18	87.6	±0.5	86.2	±4.2	−1.6

Comparison of two calibration procedures: aqueous standard solutions and standard addition method.
The results represent the average of three parallel determinations

vestigations of combined treatment modalities where a large series of tumour samples needs to be analysed for cisplatin accumulation.

4. Conclusion

A method is presented for analysis of platinum in tumours after cisplatin therapy based on digestion in 65% nitric acid and determination of platinum by electrothermal atomic absorption spectrometry with Zeeman background correction. The method is sensitive and accurate and allows calibration with aqueous standard solutions. Due to the simplicity of the digestion and measurement procedure, it is very suitable for application to investigations of platinum accumulation in tumour tissue, particularly when many tumour samples have to be digested and analysed.

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