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Energy-dispersive X-ray fluorescence determination of platinum in plasma, urine, and cerebrospinal fluid of patients administered *cis*-dichlorodiammineplatinum(II)

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Summary. *A method involving the use of an energy-dispersive X-ray fluorescence spectrometer was developed for assaying total platinum concentrations in body fluids of patients treated with the antitumor drug cis-dichlorodiammineplatinum(II). Sample preparation by this procedure is simple, consisting in adding an internal standard (Zr) to 1 ml of biological fluid or tissue homogenate, pipetting 20 µl of the sample onto a Mylar sample holder, and drying. This produces a thin-film sample, which effectively eliminates absorption enhancement effects due to other elements in the specimen. Standard addition studies were found to be linear in the concentration range of interest (0.1–10.0 µg/ml), with correlation coefficients exceeding 0.99. Minimum detection limits range from 0.10 to 0.25 µg Pt per ml, depending on the body fluid, which is adequate for routine patient monitoring after normal chemotherapeutic doses of cis-dichlorodiammineplatinum(II). In preliminary studies with mammalian liver, standard addition experiments were found to be linear and the minimum detection limit was found to be 1.4 µg/g dry weight.*

Introduction

Since the initial discovery by Rosenberg et al. [18, 19] that *cis*-dichlorodiammineplatinum(II) (DDP, NSC 119875) exhibits a broad spectrum of antitumor activity and inhibits replication of *E. coli* cells, DDP has undergone extensive clinical study [17, 20]. The drug has been found to be extremely effective in cases of testicular cancer. Efficacy has also been shown in a number of other malignancies, including carcinoma of the head and neck, bladder, or ovary [20]. In addition to its high activity against several malignancies, DDP is known to be a radiosensitizer [7, 14] and has shown to be synergistic with cranial irradiation in a rat intracerebral tumor model [6].

Clinical and pharmacological studies have shown that plasma platinum concentrations decline biphasically after a single IV injection of DDP, the initial alpha phase having a half-life of about 30 min and the slow beta phase, a half-life of 2 or 3 days [5, 9]. The average cumulative urinary excretion of Pt is about 23% of the total dose during the first 24 h, about 17% being excreted during the first 4 h [9].

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Platinum concentrations in biological fluids have been determined by flameless atomic absorption spectroscopy [10, 13, 15, 16], neutron activation analyses [13], and gamma counting using DDP radiolabeled with ^{193m}Pt [5], and also by X-ray fluorescence spectroscopy [1] and high-performance liquid chromatography [2]. The atomic absorption and neutron activation methods usually require significant sample manipulation and treatment with strong oxidizing acids to wet-ash the sample, although Priesner et al. [16] have recently devised a method for flameless atomic absorption spectroscopy in which sample preparation is reduced to homogenization and dilution with a detergent solution. The use of radiolabeled DDP for routine monitoring of plasma Pt concentrations in patients is generally not recommended because of the obvious radiation hazards. Sample preparation is also an involved procedure for determination of DDP by liquid chromatography, requiring derivatization and extraction steps before the actual analysis.

This report describes a rapid, simple procedure for measuring total platinum concentrations in urine, plasma, cerebrospinal fluid, and tissue by use of energy-dispersive X-ray fluorescence spectroscopy. In addition to standard addition data for Pt in these biological samples, which demonstrate the precision and lower limits of detection and quantitation of the method, results obtained for samples from patients administered DDP are also given. Use of this method has the advantage of having sample pretreatment minimized to the addition of an internal standard and having high precision at the Pt concentrations found in the plasma of patients who are undergoing chemotherapy with DDP.

Materials and methods

Materials. Crystalline DDP was obtained from the National Cancer Institute, Bethesda, MD, USA. Standard solutions of platinum and zirconium (certified AAS grade) were obtained from Alfa Products, Danvers, MA, USA. Mylar film (2.5 µm thick) used for sample support was obtained from Chemplex Industries, Inc., Eastchester, NY, USA.

Instrumentation. For X-ray fluorescence analyses we used a Model 0600 ultra-trace X-ray tube excitation subsystem equipped with a 2-kW (50 KV max., 50 mA max.) X-ray generator, a Mark AAA Si(Li) detector/cryostat with 30 mm^2 active area, a Model 7000 micro-X universal spectrometer, and a Model 6100 data processor (all from Kevex Corp., Foster

City, CA, USA). For excitation we used an Ag-anode X-ray tube operated at 35 kV and 15–50 mA. Before exciting the sample, the X-ray beam was filtered with 200 μm Ag. Characteristic X-rays fluoresced from the excited samples were counted by the Si(Li) detector for 600 s (live time). After acquisition, X-ray fluorescence spectra were stored on Scotch 3M Model 740/2-0 diskettes.

Sample preparation. Sample holders were prepared by cementing X-ray Mylar film onto non-pigmented 35-mm slide mounts (Pako Corp., Minneapolis, MN, USA) with rubber cement. For biological fluids (plasma, urine, CSF), 5 μl of a 1,000 mg/ml solution of zirconium (Zr) was added to 1.0 ml of the fluid, and a thin film of the mixture was then prepared by pipetting 20 μl onto the center of the Mylar sample holder and drying at 37° C.

Tissue samples were prepared by first lyophilizing the sample to dryness; the dried sample was then weighed and reconstituted with a solution containing Zr at a concentration of 5.0 μg/ml (1 ml solution/100 mg dried tissue). The sample was then homogenized and 10 μl of the homogenate was pipetted onto the Mylar sample holder and dried at 37° C. Standard curves for Pt were prepared from data on serial dilutions of AAS-grade Pt standard in pooled control samples of each biological sample.

Data analysis. The contribution of the Mylar sample support to the sample spectrum was removed by subtracting a spectrum of the Mylar sample holder obtained under the same conditions as the biological sample. Pt was quantitatively measured by using the Pt Lα emission (9.43 keV, weighted average) after appropriate background modeling and subtraction. To correct for the interfering Zn Kβ peak (9.57 keV, weighted average) the spectrum of pure Zn was normalized to the Zn Kα peak (8.63 keV, weighted average) of the sample spectrum, and the normalized pure Zn spectrum was subtracted from the sample spectrum. The area of the Pt Lα peak (Pt window = 9.22–9.66 keV) was then ratioed to the area of the Zr Kα peak (Zr window = 15.36–16.08 keV), which acts as the internal standard. All of these operations were performed by use of programs included in the Kevex Model 6100 data processor.

Results

Because the samples were prepared as thin films (5–10 mg/cm^2), absorption-enhancement effects of the sample matrix were negligible [8] and were not included in data analysis. Standard curves were generated for Pt in each of the biological samples by the method of standard addition. A separate standard curve was required for each type of specimen because the wet-to-dry weight ratios are different for each sample. The ratio of the Pt Lα peak to the internal standard Zr Kα peak was linear in the concentration range of interest (0.1–10.0 μg/ml). Linear regression analysis resulted in the equations $y = 4.34 \times 10^{-2} x + 1.71 \times 10^{-3}$ for the plasma standard curve, $y = 3.99 \times 10^{-2} x + 7.67 \times 10^{-3}$ for urine, $y = 4.50 \times 10^{-2} x + 5.03 \times 10^{-3}$ for cerebrospinal fluid, and $4.00 \times 10^{-3} x - 2.15 \times 10^{-3}$ for rat liver, where y is the ratio of the areas of the Pt Lα peak to the Zr Kα peak and x is the concentration of Pt added to the sample (μg/ml fluid or μg/g dry weight tissue). Correlation coefficients in all cases were greater than 0.99.

Minimum detection limits (L_D) and determination limits (L_Q) for Pt were calculated by the method of Currie [4]. These represent the concentrations of Pt at which a detectable signal for Pt will be present 95% of the time (L_D) and at which a quantitative precision as small as 10% (relative standard deviation) will be attained (L_Q). For concentrations between those determined by L_D and L_Q a quantitative estimate of the concentration of Pt can be made, but at decreased precision. The following working relations for L_D and L_Q were used:

$$L_D = 3.29\,\sigma_B$$
$$L_Q = 10\,\sigma_B$$

where σ_B is the standard deviation of the background counts. The values for L_D for human plasma, urine, and cerebrospinal fluid were found to be 0.240, 0.250, and 0.100 μg/ml, respectively, while values for L_Q were found to be 0.730, 0.750, and 0.300 μg/ml, respectively. For rat liver, L_D was determined to be 1.4 μg/g dry weight and L_Q was 4.2 μg/g dry weight.

Variability of the analysis was examined by analyzing plasma samples that had been spiked with Pt at concentrations near the minimum detection limit and above the determination limit (Table 1). Within-sample variability, as determined by the standard error on analyzing the same sample 10 times, was 2%–3% when the Pt concentration in plasma was near the L_D (0.5 μg Pt/ml) and about 1% when the Pt level was above L_Q (2.5 μg/ml). Within-specimen variability, as determined by the standard error on analyzing 10 different samples of the same biological specimen, was 2%–6% near L_D and 1%–2% above

Table 1: Precision of analysis of Pt in biological fluids (area Pt Lα/area Zr Kα[a])

Experiment	Plasma		Urine		Cerebrospinal fluid	
	A[e]	B[f]	A	B	A	B
Within-sample variability[b]	0.0258 ± 0.0008	0.1139 ± 0.0014	0.0220 ± 0.0005	0.1012 ± 0.0007	0.0316 ± 0.0005	0.1348 ± 0.0007
Within-specimen variability[c]	0.0258 ± 0.0005	0.1116 ± 0.0019	0.0328 ± 0.0021	0.1050 ± 0.0012	0.0332 ± 0.0007	0.1349 ± 0.0012
Between-specimen variability[d]	0.0281 ± 0.0010	0.1194 ± 0.0030	0.0231 ± 0.0008	0.1112 ± 0.0035	–	–

[a] Ratios are expressed as the mean of 10 analyses ± SEM
[b] The same sample and Mylar sample holder were analyzed 10 times
[c] Ten samples were prepared from the same specimen of biological fluid and analyzed
[d] Ten samples from different patient/volunteer sources were analyzed
[e] Experiment A: Biological fluids were supplemented with Pt to give a concentration of 0.50 μg/ml
[f] Experiment B: Biological fluids were supplemented with Pt to give a concentration of 2.50 μg/ml

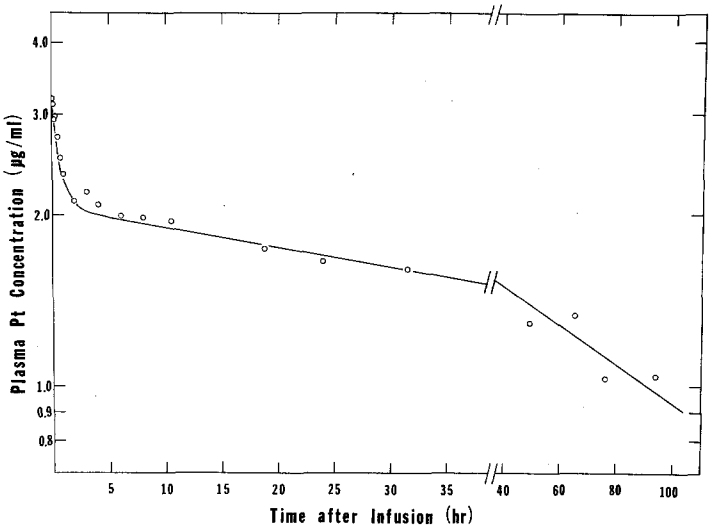

Fig. 1. Patient plasma Pt concentrations following IV administration of DDP (120 mg/m^2)

L_Q. Between-specimen variability, as determined by analyzing fluids from 10 different patients, was 2.5%–3.6% for either concentration.

To illustrate the utility of the technique, Fig. 1 shows data on the analysis of plasma sampled after the IV administration of DDP (120 mg/m^2) to a patient. An apparent biphasic decrease (as previously observed) is seen in this patient, with half-lives of 0.53 h and 87.7 h for the α and β phases, respectively. Urine samples collected after administration of DDP to this patient indicated that 25.7% of the administered Pt had been excreted by 24 h and a total of 26.3% by 48 h.

Cerebrospinal fluid samples obtained by lumbar puncture from four patients receiving 100–120 mg DDP/m^2 body surface area were analyzed. Pt was detectable at concentrations of 0.37 and 0.12 μg/ml in two of the patients. The precision of the analysis at the L_D (100 ng/ml) for CSF is about 35% (SEM). Pt was not detected in CSF from the other two patients receiving DDP in this dosage, nor in patients who had received DDP 15 mg/m^2 per day for 4 days [21].

Discussion

In initial studies in our laboratory we used energy-dispersive X-ray fluorescence spectroscopy to determine Pt in biological specimens without an added internal standard. The method was based on the assumption that the inelastic scatter peak of the exciting Ag Kα emission was proportional to the mass of the sample [3]. By determining the ratio of the area of the Pt Lα peak to this scatter peak, the area is mass-normalized and is a measure of the concentration of Pt in the sample. However, the wet-to-dry ratios of the various biological samples are not consistent and require the measurement of dissolved solids to correct for this variation. An easier and more precise method is to add an internal standard in the preparation of the sample. We chose zirconium as the internal standard because it is not normally present in biological samples, at least in concentrations measurable by X-ray fluorescence spectrometry.

Our method is rapid and simple. It also is sensitive and specific, with minimum detection limits in the range of 0.10–0.25 μg/Pt ml for fluid samples (1.4 μg/g dry weight for tissue samples), or about 2–5 ng Pt per analyzed sample. If increased sensitivity is required, additional concentration procedures could be devised to enable quantitation of more dilute specimens. As shown in the results presented in this report, the method also has the advantage of high precision in the concentration ranges that have been observed in patient plasma samples.

With flameless atomic absorption spectrophotometry the reported minimum detectable concentrations in plasma and urine are 0.03 [13] and 0.003 [11] μg Pt/g fluid. However, these procedures usually require much more sample manipulation: wet-ashing and concentrating steps. The 2-ng lower limit for detecting total platinum in biological samples reported by LeRoy et al. [13] is about the sensitivity limit of the X-ray fluorescence spectrometer used in these studies, but with our procedure 20–50 samples can be analyzed per day, and other trace elements in the samples can be monitored, should this be necessary. However, it should be noted that our method does suffer from the same disadvantage as atomic absorption spectroscopy and radiolabeled analysis of platinum, in that only total Pt concentrations are measured. However, unbound Pt may be determined after ultrafiltration of the fluid or homogenized tissue sample through a membrane filter to remove protein and protein-bound Pt [10].

In these studies, we have demonstrated that tissue levels of Pt can also be determined easily by energy-dispersive X-ray fluorescence spectroscopy. Sample preparation consists of lyophilization, reconstitution with an internal standard solution, and homogenation before analysis. Minimum detection limits are comparable to those in plasma or urine.

The utility of these procedures lies in their ready application for routine monitoring of patients given DDP. As with most antineoplastic agents at chemotherapeutic doses, DDP is quite toxic and may cause symptoms of hematological, renal, and neural toxicity [12]. Our simple technique permits the routine monitoring of plasma Pt, and potentially the correlation of data on concentrations in plasma with therapeutic response and toxicity.

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