Platinum levels in various tissues of a patient died 181 days after cisplatin treatment determined by electrospray ionization mass spectrometry

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INTRODUCTION

An important problem in relation to clinical drug schedule avoiding side effects is to know in what form and in what concentration the drug retained in tissues of patients. Cisplatin, *cis*-diamminedichloro platinum (II), is reported to bind to DNA and protein displaying anti-tumor activities [1-6], but it is easily hydrolyzed when the concentration of Cl^- in the surroundings is lowered [3,7]. Although free cisplatin is far more toxic than protein-bound cisplatin causing cell death, little is known concerning to the effect of high concentrations of protein-bound cisplatin in tissues. The tissue distribution of platinum (Pt) appears to be dependent on its dose [8], intervals [1-6] and the kind of organs [1-6]. The effect of intervals was studied until 90 days on human plasma and urine [5], and it was found that the decay of Pt was represented by multi-exponential functions expressed by at least 4 half-life periods at 6 min, 34 min, 22 h and 143 h in human plasma [2]. However, studies on Pt levels in tissues were limited mostly to a short test period such as 12 days in animal tissues [1-4, 6]. Recently, we have reported Pt levels in human tissues from a patient died 44 days after cisplatin overdose [9]. Soon after the case, we have encountered to another case where a patient died 181 days after cisplatin overdose, although the patient's death was due to the progress of recurrent malignant lymphoma.

MATERIALS AND METHODS

HNO_3, Pt^{4+} and Ag^+ solutions of atomic absorption grade, cisplatin and other chemicals of analytical grade were obtained from Wako Pure Chemical Ltd., Japan. IAA suitable for nucleic acid purification was obtained from Sigma-Aldrich Co., USA. Pt^{4+} and Ag^+ standard solutions at 1 μg/μl, respectively, were used as stock solutions. Cisplatin was dissolved in 1 M HCl at 0.5 μg Pt^{2+}/μl and was used as a stock solution. Pure water, having a specific resistance of 18 MΩcm, was used. All glassware or plastics were soaked in conc. or 0.3 M HNO_3, respectively, overnight and rinsed at least 10 times with pure water.
Ethical approval was obtained for the removal of tissue from one patient and two reference subjects. A 45-year-old female with malignant lymphoma received an

accidental overdose of 160 mg cisplatin/24 h, administered as an intravenous infusion for almost 4 days. The administration was stopped on day 5 since her hearing disturbance appeared on day 3. The total cisplatin dose received was 600 mg and the patient's body weight was 59.2 kg. Intravenous hydration and administration of diuretics were initiated on day 7. The patient received 14 sessions of the transfusion and 6 sessions of dialysis until day 41, when she recovered to some extent. However, remaining tumor continued to grow and spread throughout the body, in spite of radiation therapy. She died on day 181 after the beginning of cisplatin administration. A forensic autopsy was performed 2 days after her death and tissues, blood and urine were collected, and Pt levels of them were examined. Corresponding specimens from two healthy females (32 years and 64 years old) subjected to forensic autopsy, were obtained and subsequently used as the reference material.

Tissues from reference subjects were spiked with cisplatin at either 0, 6, 20, 100, 200 or 2000 ng Pt^{2+}/g wet weight and Ag^{+} at either 0 or 1000 ng/g wet weight. Patient tissues were spiked with Ag^{+} at 1000 ng/g wet weight. One g of wet tissue was mixed with 1 ml of conc. HNO_3 and wet-ashed at 85℃ for 8 h [9]. A spherical cancer in liver with 1.5 cm-diameter was cut into two halves and the half (1 g) was wet-ashed similarly as other tissues. The final volume of the wet-ashed solution was adjusted to 2 ml.

Analytical procedure

The pH of the wet-ashed solution (10 μl) was adjusted to between 3 and 7 with either 10 M NaOH or 7 M HNO_3. Small differences of aqueous volumes due to pH adjustment did not influence greatly on the final volume of IAA added in the subsequent step, since the solubility of IAA in the pH adjusted tissue solution was quite low. A 1-μl aliquot of 1 M DDC was then added to the solution. After 3 min, 10 μl of IAA was added and mixed for 30 sec, and separated by centrifugation at 13,000 rpm for 30 s. The IAA layer was mixed with 10 μl of 1 M oxalic acid for 10 sec and centrifuged. A 1-μl aliquot of the IAA layer was subjected to ESI-MS. The peaks of both $Pt(DDC)_3^{+}$ and $Ag(DDC)_2^{+}$ appeared 1 min after the injection.

ESI-MS operating conditions

ESI-MS was performed on a TSQ 7000 LC/MS/MS quadrupole mass spectrometer (ThermoQuest, Tokyo, Japan) in the positive ion mode. A 1-μl aliquot of the IAA layer was injected manually in the direct flow injection mode. The mobile phase consisted of methanol at a flow rate of 200 μl/min. The spray voltage was set at +4.5 kV, and the fused silica capillary temperature was set at 280℃ since the peaks of both $Pt(DDC)_3^{+}$ and $Ag(DDC)_2^{+}$ increased following an increase in temperature from170 to 280 ℃. Nitrogen was used as sheath gas (68 p.s.i.) and also auxiliary gas (8 units) to assist the nebulization. The electron multiplier was set at 1.3 kV, and the scan time, at 1.8 s between *m/z* 100 and 1000.The quantitation of Pt was conducted by detecting simultaneously two molecular ions at *m/z* 639 for $Pt(DDC)_3^{+}$ and *m/z* 403 for $Ag(DDC)_2^{+}$ as internal standard (IS) by mass chromatography.

RESULTS AND DISCUSSION

Figure 1 shows an ESI-MS of 1 μl of IAA extracted from patient liver (a) and its tumor mass (b) containing 500 pg Ag as IS. A cluster of peaks around *m/z* 403 were the signal of $Ag(DDC)_2^+$, and that around *m/z* 639, the signal of $Pt(DDC)_3^+$. Pt and Ag ions showed clusters of peaks with their isotopes, and the shapes of the clusters proved useful for identification purposes. Our previous study indicated that the peaks of $Ag(DDC)_2^+$ and $Pt(DDC)_3^+$ were not interfered with by the impurities extracted from tissues [9].

Table 1: Pt levels in tissues, blood and urine obtained from the patients overdosed with cisplatin

	Present patient	Previous patient [9]:
	600 mg cisplatin/58.2 kg b.w.	426 mg cisplatin/56.3 kg b.w.
	Survival time: 181 days	Survival time: 44 days
	Pt level[a]	Pt level[a]
Sample	(ng/g or ml)	(ng/g or ml)
cerebrum	38	48
thymus	190	60
cerebellum	66	36
heart	640	267
adrenal	1,240	365
testis	–[b]	653
ovary	180	–[b]
pancreas	670	413
lung	710	487
spleen	160	290
kidney	1,030	1,280
cancer in liver	2,750	–[b]
liver	2,050	1,680
mamma	440	–[b]
small intestine	480	–[b]
head skin	810	–[b]
blood	25	14[c]
urine	28	58[c]

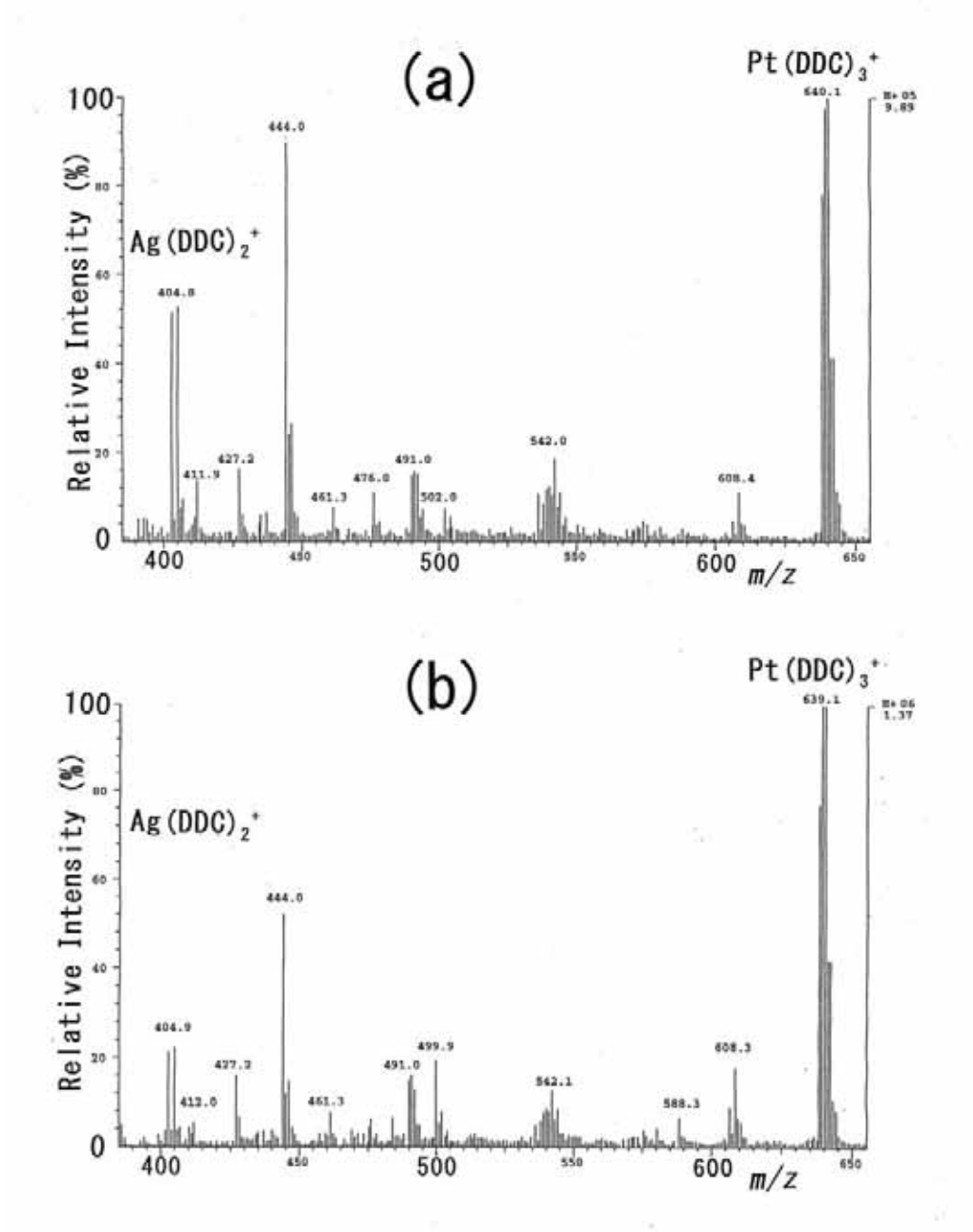

Fig. 1: Mass spectra obtained from IAA extracts of patients' liver (a) and a tumor mass inside the liver (b), containing 500 pg Ag as IS respectively.

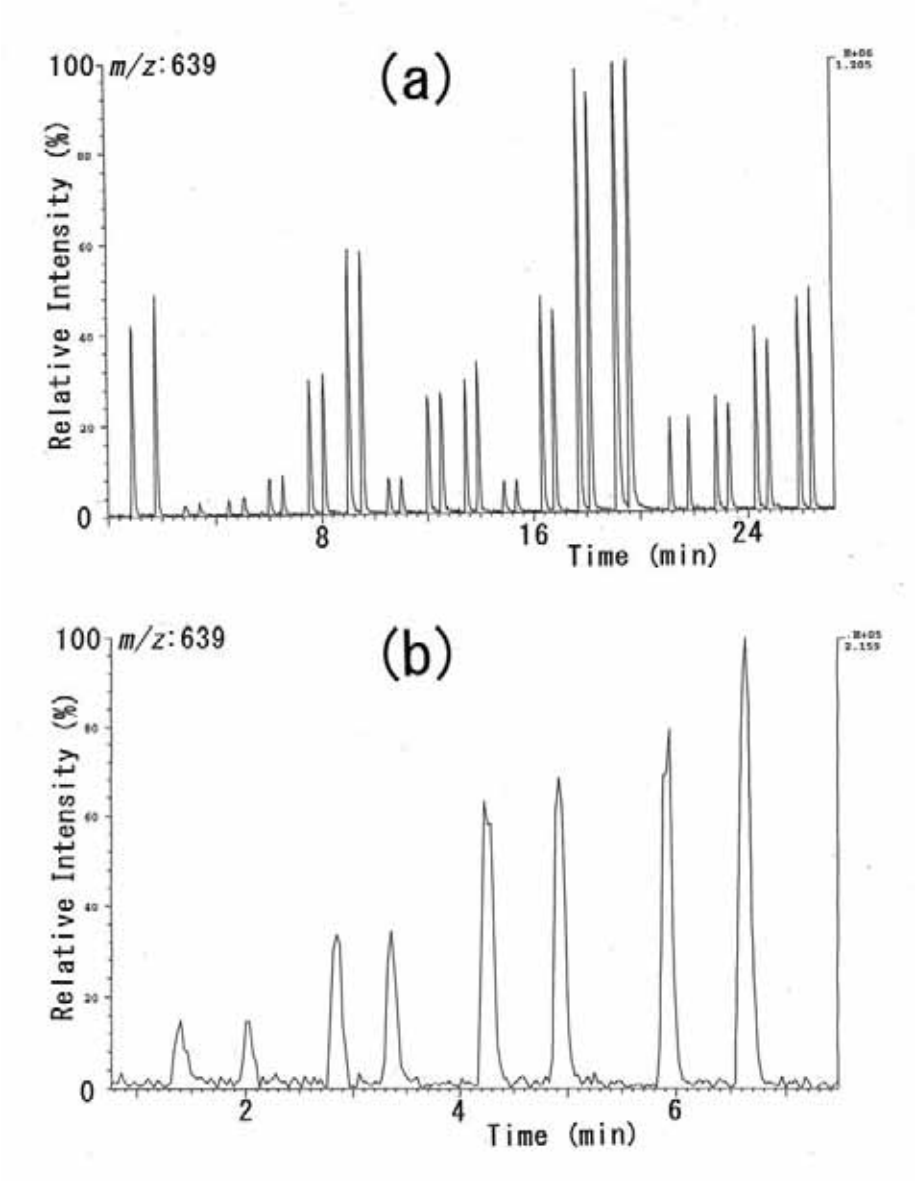

Fig. 2 Mass chromatograms obtained by direct flow injections being monitored at *m/z* 639. (a): One μl each of IAA extracts was injected in duplicate; the samples injected were: the kidney standard spiked with 1 μg Pt/g, patient samples such as the cerebrum, cerebellum, thymus, heart, adrenal gland, ovary, pancreas, lung, spleen, kidney, liver, tumor in the liver, mamma, small intestine and head skin, and the kidney standard spiked with 1 μg Pt/g, being arranged from left to right. (b): One μl each of IAA extracts of urine samples; urine standards spiked with Pt at 5, 10 and 20 ng/ml and patient urine, being arranged from left to right in duplicate.

Figure 2(a) shows the mass chromatograms obtained from flow injections monitored at *m/*z 639 for $Pt(DDC)_3^+$. A 1-μl aliquot each of IAA extracted from the reference samples and patient samples was injected in duplicate, respectively; the samples injected were the kidney standard spiked with 1 μg Pt/g, patient samples such as the cerebrum, cerebellum, thymus, heart, adrenal gland, ovary, pancreas, lung, spleen, kidney, liver, tumor mass in the liver, mamma, small intestine and head skin, and the kidney standard spiked with 1 μg Pt/g, respectively, from left to right. Figure 2(b) shows mass chromatograms for urine standards spiked with Pt at 5, 10 and 20 ng/ml and patient urine, respectively, being injected in duplicate from left to right.
Pt levels in samples obtained from the patient were summarized in table 1 and were compared with those in the previous patient died 44 days after cisplatin overdose [9]. The distribution of Pt was organ specific. Kidney showed the highest Pt level among all organs in short periods up to 12 days [1-6]. The tendency that Pt level in kidney was lower than that in liver noticed in the previous patient, was much more pronounced in the present patient died 181 days after cisplatin overdose.The present patient received 600 mg cisplatin and the detoxifying treatment started on day 7 whereas previous patient received 462 mg of cisplatin and the treatments started on day 3 [9]. This may be one of the reasons why the present patient showed higher Pt levels than the previous patient, although the duration of time was much longer in the present patient than that in the previous patient. Results in table 1 also demonstrated that Pt levels in tissues were remained rather high whereas Pt levels in blood and urine were quite low. These results indicate that cisplatin may be bound to tissue proteins when the concentration in blood was high, was excreted quite slowly into urine via blood circulation.

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