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Determination of metabolites of a novel platinum anticancer drug JM216 in human plasma ultrafiltrates

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Abstract

The present study describes the application of on-line liquid chromatography-electrospray ionisation in conjunction with a high resolution magnetic sector mass spectrometer to identify metabolites of a platinum(IV) anticancer drug JM216 [bis(acetato)amminedichloro(cyclohexylamine)platinum(IV)] in human plasma. Four metabolites were identified following incubation of JM216 in human plasma: JM118 [amminedichlorocyclohexylamineplatinum(II)], a platinum(II) complex; JM383 [bis(acetato)amminedihydroxo(cyclohexylamine)platinum(IV)]; JM518 [bis(acetato)amminechloro(cyclohexylamine)hydroxoplatinum(IV)] and its isomer JM559. The platinum complexes mass spectra were dominated by the natriated $[M + Na]^+$ ion. Elemental compositions of these natriated ions were confirmed by accurate mass measurement on a magnetic sector mass spectrometer in the course of LC/MS analysis. This study demonstrates the capability of direct LC-ESI/MS with accurate mass measurement for analysis of platinum complexes in biological samples. Our results suggest that LC-ESI/MS is a powerful technique for structure elucidation of novel metabolites, and could make valuable contributions to drug metabolism research.

Keywords: Platinum anticancer drug; Metabolites; Human plasma ultrafiltrate; Electrospray ionisation/mass spectrometry

1. Introduction

Cisplatin [*cis*-diamminedichloro-platinum-(II)] [1, 2] and carboplatin [*cis*-diammine cyclobutane-1,1-dicarboxylato-platinum(II)] [3] (Fig. 1) are widely used for the treatment of a range of human malignancies, particularly ovarian and testicular cancers. However, patients treated with cisplatin develop severe side effects including neurotoxicity and nephrotoxicity. Moreover, many cancers are intrinsically resistant or acquire resistance to both agents, and their clinical spectrum of activity is similar. Resistance to these agents is believed to be multifactorial [4]. Therefore, it is necessary to develop more effective analogues which overcome these drawbacks. A platinum(IV) complex bis(acetato)amminedichloro(cyclohexylamine)platinum(IV) (JM216) (Fig. 1) is currently undergoing clinical evaluation, as an orally administrable anticancer drug. Preclinical studies with this complex have shown that it can overcome resistance to cisplatin and carboplatin [5]. In vitro and in vivo metabolism studies have shown that the complex undergoes extensive metabolism [6]. A better understanding of the pharmokinetics and metabolic fate

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of this compound may give improved insight into its mode of action. In addition, confirmation of the presence of metabolites in patients, and subsequent evaluation of their biological activities, is a prerequisite for the clinical development of this compound.

The lack of a suitable, sensitive analytical technique has often hindered the quantitation and identification of platinum drugs and their metabolites in biological samples. For example, analytical methods such as liquid chromatography (LC) in conjunction with UV detection, flameless atomic absorption or inductively coupled plasma-atomic emission spectrometry [7–10] usually indicate the presence of platinum complexes, but do not provide structural information. ^{195}Pt NMR spectroscopy requires larger quantities of pure sample, and is not suitable for analysing platinum complexes which are unstable in solution [11]. However, the recent introduction of liquid chromatography/mass spectrometry with electrospray ionisation (LC-ESI/MS) has simplified the analysis of platinum complexes in biological fluids [12–14]. ESI, being a soft ionisation technique [15], has advantages for examining involatile and labile compounds such as platinum complexes by MS over other ionisation techniques including electron impact, thermospray ionisation, or fast atom bombardment [16–18]. Moreover, ESI provides better sensitivity than other ionisation techniques. Off-line LC-laser desorption/time-of-flight mass spectrometry has been applied to the study of platinum–glutathione adducts, but the resolution is insufficient for reliable interpretation of the mass spectra of unknown metabolites [19].

With the recent introduction of a new atmospheric pressure ionisation (API) source operated in the electrospray ionisation (ESI) mode, installed with a heated capillary, the performance of LC-ESI/MS has been enhanced [20,21]. The most noticeable improvement is the acceptance of a higher flow rate ($\approx$600 μl min^{-1}) into the magnetic sector mass spectrometer, which allows introduction of the LC effluents directly into the mass spectrometer, and is ideal and convenient for LC/MS analysis. A sector instrument can provide accurate mass information as first demonstrated by Cody et al. [22] and Starrett and DiDonato [23]. The present study describes the application of ESI/MS to the analysis of JM216 and its metabolites produced during incubation with human plasma. The reference compound, polyethylene glycol, was infused into the magnetic sector mass spectrometer, followed by infusion of solutions containing the analytes.

2. Experimental

2.1. Chemicals

JM118, JM383, JM518, ^{14}N-JM216 and ^{15}N-JM216 were synthesised and supplied by the Johnson Matthey Technology Centre [24,25]. All HPLC grade solvents used were supplied by Merck (Darmstadt, Germany).

2.2. Incubation of JM216 with human plasma

^{14}N-JM216 or ^{15}N-JM216 (120 μM) was incubated with human plasma (4 ml) for 1 h at 37°C. The plasma was ultrafiltered using an Amicon Centrifree micropartition system, 10 000 MW cut off (Silverstone, Gloucester, UK) centrifuged at 1900g at 4°C. The ultrafiltrate were stored at −70°C until analysis. Aliquots of the samples (50 μl) were analysed by LC/MS. Incubation containing JM216 and saline at pH 7 was used as control.

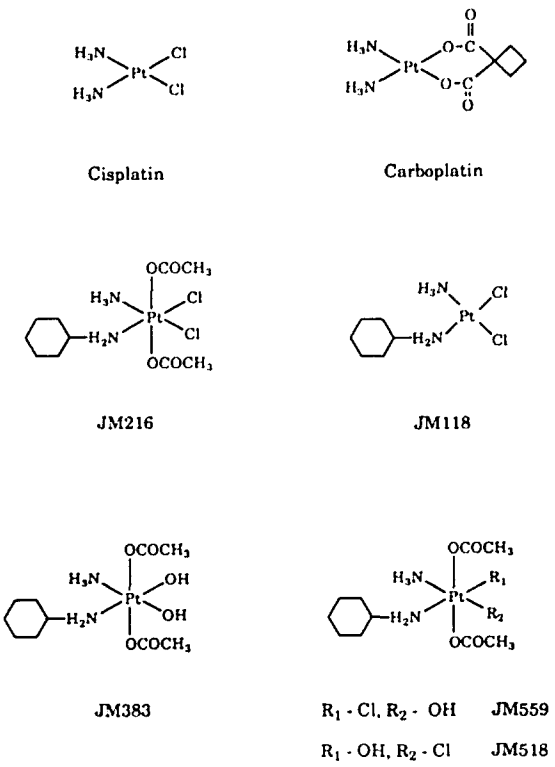

Fig. 1. Structures of the platinum complexes referred to in this study.

Table 1
Theoretically calculated relative abundancies (in parentheses) of molecular ions containing $PtCl_n$ when $n = 0$, 1 and 2

	A	(A + 1)	(A + 2)	(A + 3)	(A + 4)	(A + 5)
Pt	194(97%)	195(100%)	196(75%)	0	0	0
PtCl	229(92%)	230(94%)	231(100%)	232(30%)	233(43%)	0
$PtCl_2$	264(72%)	265(73%)	266(100%)	267(47%)	268(58%)	269(7%)
JM216	498(65%)	499(74%)	500(100%)	501(55%)	502(59%)	503(14%)
JM118	380(68%)	381(74%)	382(100%)	383(52%)	384(58%)	385(11%)
JM383	462(87%)	463(100%)	464(78%)	465(10%)	466(20%)	467(2.5%)
JM518	480(81%)	481(93%)	482(100%)	483(39%)	484(43%)	485(5%)

2.3. LC/MS conditions

LC/MS was performed on a Finnigan MAT 900 magnetic sector mass spectrometer (EB type, Bremen, Germany) equipped with a Finnigan API source operated in the positive ESI mode. The mobile phase (methanol/water) was delivered at 600 μl min^{-1} with a LDC MS 4100 pump (Thermo Separation Ltd., Riviera Beach, FL). Samples were analysed on a 25 cm × 4.6 mm PLRP-S reverse-phase column (Polymer Laboratories, Shropshire, UK). The sample was eluted using a linear gradient from 15 to 95% methanol in water (w/w) between 0 and 30 min, followed by an isocratic elution at 95% methanol/5% water for 10 min.

The API source consisted of a heated metal capillary maintained at 250°C. The spray voltage was set to 3 kV. Positive ions were detected with an array-type focal plane detector (PATRIC™). Polypropylene glycol (PPG 425) dissolved in methanol/water (50:50 v/v) was chosen as the reference compound. The solution was infused into the mass spectrometer (Harvard infusion pump, MA, USA) via the sheath liquid inlet of the API source at 5 μl min^{-1} during the LC analysis. The scan range was m/z 420 to 530 u in 2.5 s for accurate mass measurement. This was chosen to cover all masses of interest including two reference masses (PPG and $[PPG + Na]^+$). Prior to the ESCAN, an electrical calibration was performed with these two reference masses. The resolving power of the instrument (full width at half height) was adjusted to approximately 4000 during the accurate mass measurement. For low resolution measurements, the instrument was scanned from m/z 200 to 700 u at a resolving power of 1200. MS/MS spectra were obtained using argon as the collision gas at a pressure of 0.3 mtorr on a Finnigan MAT TSQ 700 mass spectrometer. Data acquisition analysis was controlled by a DEC data system with Finnigan ICIS and ICL software.

3. Results and discussion

The identification of metabolite was achieved firstly by comparing their retention times on the LC column with that of the authentic reference compounds, and secondly by comparing their mass spectra. The isotopic pattern contributed by platinum (^{194}Pt:^{195}Pt:^{196}Pt) and chloride ion (^{35}Cl:^{37}Cl) produces a characteristic ion cluster for $PtCl_n$ complexes (Table 1), which is useful for interpreting the mass spectra of this class of compounds, and distinguishing them from non-platinum containing molecules. In addition, the mass spectra of the metabolites obtained following incubation of human plasma with ^{15}N-JM216 (^{15}N on the NH_3 functional group) and ^{14}N-JM216 were compared. All the metabolites examined showed one mass unit increment following incubation with ^{15}N-JM216 compared to ^{14}N-JM216. This information provides further evidence that the metabolites observed all retained the NH_3 group in the molecule.

The LC separation of the human plasma ultrafiltrate (HPUF) after incubation with JM216 with plasma is shown in Fig. 2. The profile was very similar to that observed with plasma ultrafiltrate samples obtained following oral administration of JM216 in preclinical models and in patients [26]. From the ion chromatograms, four metabolites are observed and identified. The parent drug has a retention time of 30 min. The mass spectrum of this molecule is dominated by the natriated adduct at m/z 521 ($[M + Na]^+$) (Fig. 3(a)). In addition, two less intense signals were observed in the spectrum at m/z 499 and 537, consistent with the protonated molecule ($[M + H]^+$) and the kalidated molecule ($[M + K]^+$) of JM216. Accurate mass data indicated the measured molecular weight of 521.0473 u (calculated molecular weight 521.0482).

Metabolite I has a retention time of 21 min 52 s, which is identical to that of authentic JM118. The mass spectrum displayed two clusters at m/z 403 and 419, both containing two chloride ions, and representing the natriated and kalidated molecules of this metabolite (Fig. 3(b)). The presence of JM118 was verified by the accurate mass data of the kalidated adduct (MW 418.9962), which was one of the major adducts obtained from this metabolite. Conversion of the quadrivalent JM216 to the divalent JM118 is an expected metabolic pathway for a Pt(IV) complex. Pendyala et al. [27] and Gibbons et al. [28] reported that Pt(IV) compounds are readily reduced to their Pt(II) analogues in biological media. Subsequently, the Pt(II) products are thought to be responsible for the antitumour activity of Pt(IV) complexes by reacting with DNA and other biological nucleophiles [29]. JM118 is four to six times more cytotoxic than JM216 against a panel of cell lines [30]. In addition, JM118 is more cytotoxic than cisplatin and appears to be non-cross-resistant in some cell lines [30].

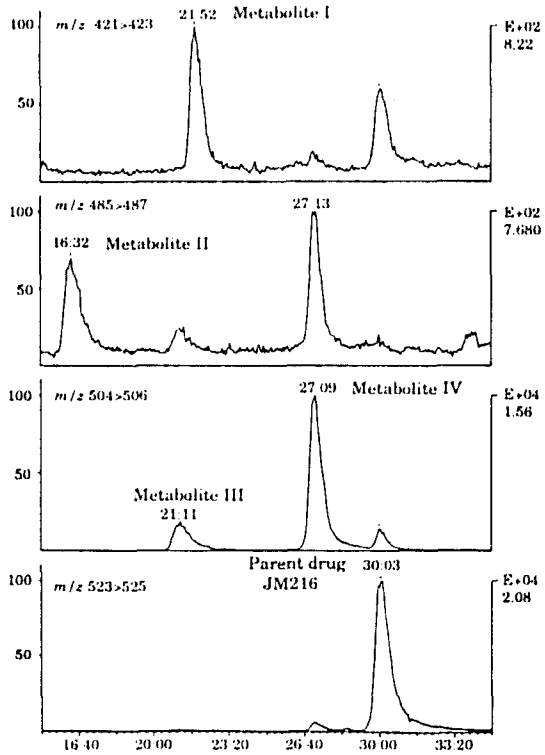

Fig. 2. JM216 was incubated with human plasma and the ultrafiltrate was analysed by LC/MS. The reconstructed ion chromatograms showing the presence of the parent drug and the four metabolites: Metabolite I = JM118; Metabolite II = JM383; Metabolite III = JM559 and Metabolite IV = JM518. Each peak is labelled with the scan time.

The ESI mass spectrum of Metabolite II is shown in Fig. 3(c) and is identified as JM383 (t_R = 16 min 22 s). The m/z 485 ion corresponds to the natriated molecule $[M + Na]^+$ of dihydroxo-JM216. The isotopic pattern displayed in the mass spectrum differs from that of JM216 and JM118, which indicates clearly that the two chloride ions of JM216 have been displaced. The proposed structure was confirmed by the accurate mass data; the calculated molecular weight of the natriated adduct of dihydroxo-JM216 is 485.1160 and the measured value obtained from the mass spectrometer is 485.1142.

From the reconstructed ion chromatogram, two metabolites have been observed at t_R = 21 min 11 s and 27 min 9 s. Metabolites III and IV are identified as the two isomers of monohydroxo-monochloro-JM216 (MW 480, Fig. 1). Their mass spectra are presented in Figs. 3(d) and 3(e). The natriated molecule at m/z 503 ($[M + Na]^+$) predominates, and the diagnostic fragment ion at m/z 463 is attributed to $[M + H - H_2O]^+$. Metabolite IV had an identical LC retention time to an authentic sample of JM518. The product-ion mass spectra of metabolites III and IV were consistent with the product-ion mass spectrum of the synthetic JM518. The MS/MS spectrum of metabolite IV is shown in Fig. 3(f). It contains a series of cluster ions at: m/z 486 ($[M + Na - H_2O]^+$); m/z 468 ($[M + Na - HCl]^+$); m/z 426 ($[M + Na - H_2O - CH_3COOH]^+$); m/z 409 ($[M + Na - CH_3COOH - Cl]^+$); m/z 349 ($[M + Na - 2CH_3COOH - Cl]^+$). Based on these data, Metabolite IV was identified as JM518, monohydroxy-monochloro-JM216 and metabolite III was the corresponding isomer JM559 (Fig. 1). These metabolites were produced in different amounts in the HPUF, with metabolite IV being present in insufficient quantity for accurate mass measurement. The measured mass value of 503.0825 was in good agreement with the calculated value of 503.0821 for the natriated molecule. The biological significance and cytotoxicity of these metabolites are under investigation.

A large proportion of platinum in plasma following administration of cisplatin and other platinum complexes is irreversibly protein bound [31], and only the ultrafiltrable (non-protein bound) fraction is believed to contain the biologically active species with antitumour

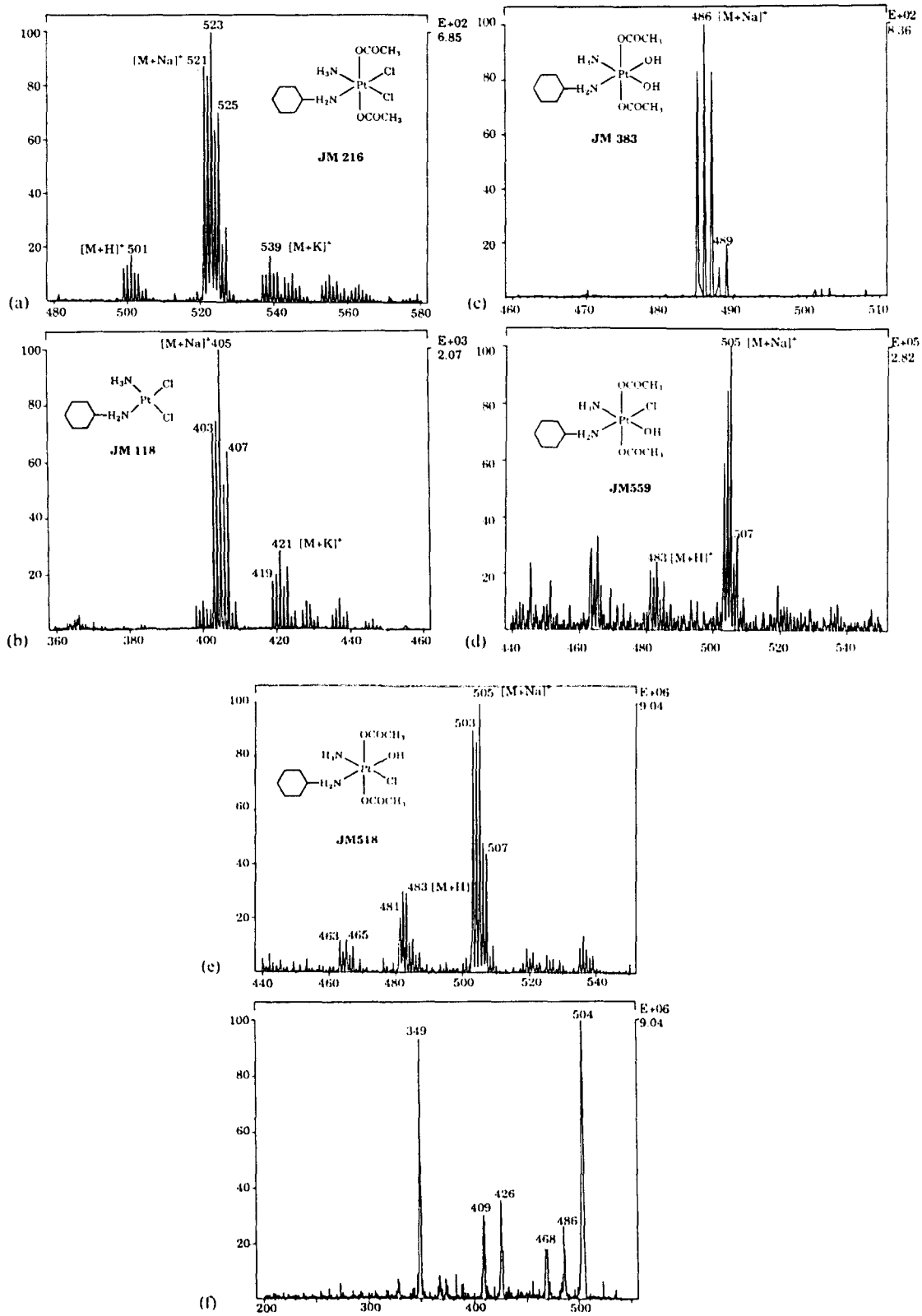

Fig. 3. ESI mass spectra of (a) Parent drug JM216; (b) Metabolite JM118; (c) Metabolite JM383; (d) Metabolite JM559 and (e) Metabolite JM518; (f) product-ion mass spectrum of Metabolite JM518.

and toxic properties. The level of JM216 metabolites present in the HPUF was estimated to be about 1 μg ml^{-1} for the most abundant metabolite JM118 [32] based on off-

line LC separation followed by atomic absorption spectrophotometric detection (LC-AAS). This value compares favourably with a level of 200 ng ml^{-1} of JM118 in the plasma of several patients as determined using the same off-line LC-AAS technique [27]. Hence, LC-ESI MS would have the sensitivity to determine the metabolites of JM216 in clinical samples. It is also indicated by LC-AAS that approximately 10% of the platinum complexes were eluted near the solvent as the glutathione or methionine adducts. Incubation of JM216 with saline at pH 7.0 showed only trace amounts of JM118 and JM383 (data not shown).

4. Conclusion

The ability to detect and characterise involatile and unstable metabolites of platinum complexes in biological fluid using on-line liquid chromatography/high resolution mass spectrometry has been demonstrated for the first time. In addition, our data indicate that although the analysis can be performed either on a quadrupole or a magnetic sector mass spectrometer, the magnetic sector mass spectrometer is more sensitive (by a factor of 25), and the accurate mass information obtained from the latter instrument is extremely useful in verifying the structures of unknown metabolites. This technique will undoubtedly have wide application in the pharmaceutical industry.

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