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Metabolic studies of an orally active platinum anticancer drug by liquid chromatography–electrospray ionization mass spectrometry

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Abstract

Bis(acetato)amminedichloro(cyclohexylamine) platinum(IV) (JM216) is a new orally administered platinum complex with antitumor properties, and is currently undergoing phase II clinical trials. When JM216 was incubated with human plasma ultrafiltrate, 93% of the platinum species were protein-bound and 7% were unbound. The unbound platinum complexes in the ultrafiltrates of human plasma were analysed using a liquid chromatography–electrospray ionization–mass spectrometry (LC–ESI-MS) method. Apart from the parent drug, four metabolites were identified and characterised. These include JM118 [amminedichloro(cyclohexylamine) platinum(II)], JM383 [bis(acetato)ammine(cyclohexylamine)dihydroxo platinum(IV)] and the two isomers JM559 and JM518 [bis(acetato)amminechloro(cyclohexylamine) hydroxo platinum(IV)]. Their elemental compositions were determined by accurate mass measurement during the LC analysis, to confirm their identities. Quantitation of these metabolites by off-line LC atomic absorption spectroscopy demonstrated that JM118 is the major metabolite in plasma from patients receiving JM216 treatment.

1. Introduction

Bis (acetato) amminedichloro (cyclohexylamine) platinum(IV) (JM216) [1] is a mixed amine platinum(IV) dicarboxylate complex and an analogue of cisplatin [*cis*-diamminedichloro platinum(II)] [2] designed for oral administration. In preclinical studies the complex exhibited cytotoxic activity against a panel of human ovarian and testicular carcinoma cell lines including those with acquired resistance to cisplatin [3,4]. In addition, in vivo antitumor activity after oral dosing was reported to be far superior to that observed for cisplatin, carboplatin or tetraplatin in the cisplatin-sensitive murine ADJ/PC6 plasmacytoma tumor model [3]. JM216 is currently undergoing phase II clinical trials for the treatment of a variety of tumors, particularly ovarian and lung. The present study investigated the in vitro biotransformation of JM216 by incubating the drug with human plasma. Identification of metabolites was carried out by comparing chromatograms and mass spectra of metabolites with those of synthesized authentic standards using LC–ESI-MS. The high-resolution magnetic sector mass spectrometer used was fitted with an atmospheric pressure ionization (API) source which could accommodate a high LC flow-rate

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(600 μl/min). Simultaneous on-line LC–ESI-MS and accurate mass measurements on the platinum complexes provided the elemental compositions of these compounds, as required for verification of the structures of the metabolites. Quantitative analysis of JM216 and its metabolites was conducted by off-line LC–flameless atomic absorption spectroscopy (LC–AAS), the most commonly used analytical technique for quantifying platinum complexes [5].

2. Experimental

2.1. Reagents

JM216, JM118, JM383, JM518, JM559 were synthesized and provided by Johnson Matthey Technology Centre. HPLC grade solvents were purchased from Merck (Darmstadt, Germany) and Romil Chemicals (Cambridgeshire, UK).

2.2. Preparation of human plasma ultrafiltrate

Pre-treatment, 3-h post-treatment plasma obtained from patients receiving JM216 (an oral single dose of 700 mg/m^2) or control human plasma (4 ml) was incubated with JM216 (120 μM) in darkness for 1 h at 37°C and was ultrafiltered at 1900 g at 4°C using an Amicon Centrifree Micropartition System, molecular mass cut-off 10 000 (Silverstone, Gloucester, UK). Aliquots of the samples [50 μl for the human plasma ultrafiltrate (HPUF) and 100 μl for the patient plasma ultrafiltrate] were analysed by LC–MS. An incubation containing JM216 and saline at pH 7 was used as control.

2.3. On-line LC–MS analysis

The HPLC system consisted of a LDC MS 4100 pump (Thermo Separation, Riviera Beach, FL, USA) and a 250 × 4.6 mm PLRP-S polymer column (Polymer Laboratories, Shropshire, UK) operated at 600 μl/min. The mobile phase was water (A) and methanol (B) and the sample was eluted using a linear gradient of 30–95% B in 30 min.

MS was performed on a Finnigan MAT 900 magnetic sector mass spectrometer equipped with an ESI source (Bremen, Germany). The spray voltage was set to 3 kV and the heated capillary temperature was maintained at 250°C. The instrument was optimized in the positive ion mode by constantly infusing (via a syringe pump) a 4 pmol/μl solution of gramicidin S. Ion detection was in the centroid mode with an array-type focal plane detector (PATRIC, position and time resolved ion counter). Data was acquired over the mass range m/z 200–700 at a resolving power of 1200 (full width at half height) during normal acquisition. During the accurate mass measurement, the resolving power of the instrument was adjusted to approximately 4000. Polypropylene glycol (PPG 425, 10 pmol/μl) dissolved in water–methanol (1:1, v/v) was chosen as the reference compound. The solution was infused into the mass spectrometer via the sheath liquid inlet of the ESI source at 5 μl/min during the LC analysis. The scan range was m/z 410–530 in 2.5 s. Tandem mass spectrometry was performed on a Finnigan MAT TSQ 700 triple quadrupole mass spectrometer operated with an ESI source (San Jose, CA, USA). Argon was used as the collision gas, at a pressure of 40 kPa and the collision energy was maintained at −30 eV. All the data acquisition analysis was controlled by a DEC data system with Finnigan ICIS and ICL software.

2.4. Off-line LC–AA analysis

LC analyses were performed using a Waters Model 510 pump (Millipore, Herts., UK) and a PLRP-S column at 600 μl/min flow-rate. Plasma ultrafiltrates (50 μl) were analysed and the eluent was collected by a LKB fraction collector at 0.2-min intervals. The platinum absolute levels were determined on a Perkin-Elmer Model 1100B atomic absorption spectrophotometer (Ueberlingen, Germany) equipped with a graphite furnace Model 700. Samples (50 μl) were injected at 60°C and the absorbance of the atomized platinum was measured at 266 nm. Calibration was achieved by injecting standards at concentrations of 100, 50, 20, 10 and 5 ng/ml.

Quality controls were added in each run of 30 unknown samples at the level of 50 and 20 ng/ml, and 15% deviation from nominal concentration was allowed. The lower limit of quantification is 3 ng/ml (20% C.V.).

3. Results and discussion

Once in circulation, platinum complexes are taken up by the tissue. They then either bind irreversibly to the plasma protein, or undergo metabolism and excretion. Most platinum complexes, including cisplatin, are heavily bound to protein in the plasma, subsequently losing their cytotoxicity and therapeutic values [6–8]. On the other hand, the ultrafiltrable fraction contains the intact drug and metabolites. These non-protein-bound species react with DNA or intracellular enzymes, and are responsible for the antitumor and toxic properties of the drug [9]. When the ultrafiltrates were analysed by AAS, 93% of the platinum species (including JM216) was bound and only 7% was present in the ultrafiltrate as "free" components.

When the human plasma ultrafiltrate was analysed by LC–ESI-MS, the reconstructed ion chromatograms indicated that the sample contained four metabolites (Fig. 1). A typical ESI mass spectrum of platinum complex displays a characteristic cluster pattern [10] due to the platinum (^{194}Pt, ^{195}Pt, ^{196}Pt) and chloride ion (^{35}Cl and ^{37}Cl) isotopes, and is usually dominated by a sodiated molecule as the base peak with minimal fragmentation. The parent drug which eluted at 29 min showed a pseudo-molecular ion at m/z 521 ($[M + Na]^+$). Two less intense signals at m/z 499 and 537 were observed, which represented the $[M + H]^+$ and $[M + K]^+$ ions, respectively (Fig. 2a). Accurate mass measurement indicated that the sodiated adduct of JM216 had a molecular mass of 521.0473 (calculated molecular mass of $^{194}Pt^{35}Cl_2C_{10}H_{22}N_2O_4Na$ is 521.0482). The component at 27:12 min was an endogenous constituent and did not contain platinum.

The retention time (19:18 min) and the mass spectrum of metabolite I (at m/z 403, $[M + Na]^+$), resembled the results obtained from the authentic reference material [amminedichloro(cyclohexylamine) platinum(II)] (JM118) (Fig. 2b). The less intense cluster at m/z 419 corresponds to the $[M + K]^+$ ion. Further confirmation was achieved by comparing the accurate mass data of the $[M + K]^+$ ion at m/z 418.9962 with the calculated accurate mass data ($^{194}Pt^{35}Cl_2C_6H_{16}N_2K$ is 418.9956). JM118 is a Pt(II) complex, it reflects the reduction of JM216 via loss of the two axial carboxylate groups. Metabolic conversion of Pt(IV) complexes to Pt(II) complexes is well documented (i.e. iproplatin and tetraplatin) [11–14]. Pt(II) complexes such as cisplatin readily react with DNA, causing a variety of intra-strand and a small proportion

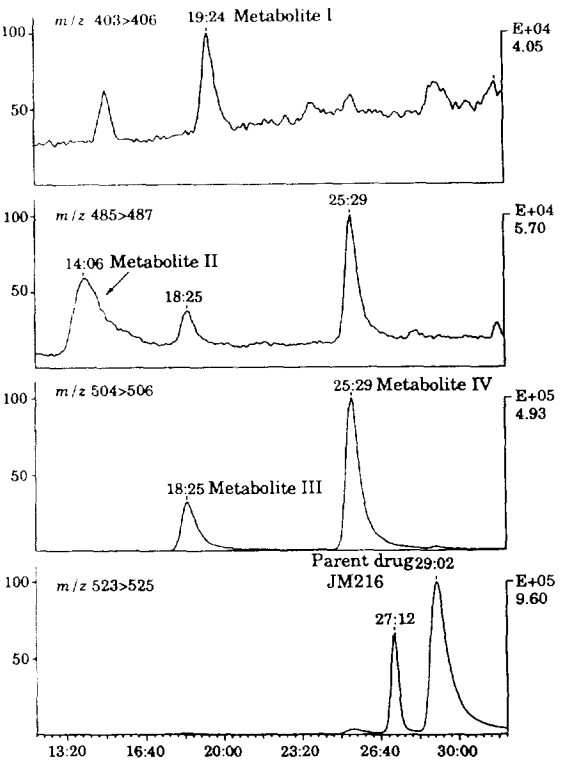

Fig. 1. Reconstructed ion chromatograms of HPUF after incubation of plasma with JM216 and analysed by LC–ESI-MS. Metabolite I: JM118; metabolite II: JM383; metabolites III and IV: two isomers of JM518; and the parent drug JM216. Each peak is labelled with the scan time. The two peaks at 18:25 and 25:29 min with m/z 485 > 487 were derived from metabolites III and IV.

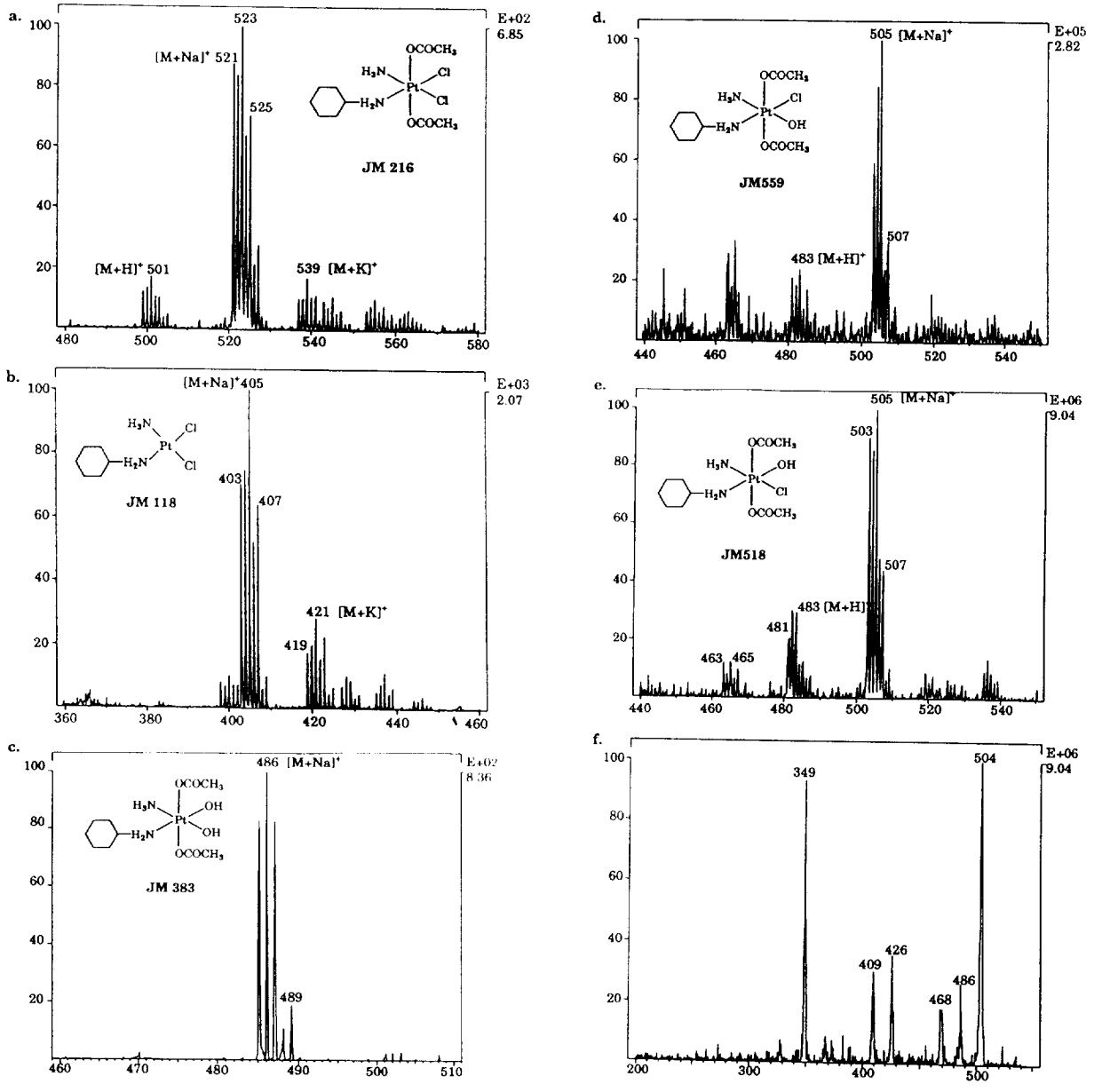

Fig. 2. ESI mass spectra of the metabolites: (a) parent JM216; (b) JM118; (c) JM383; (d) and (e) the two isomers of JM518; (f) product-ion mass spectrum of the metabolite JM518.

of inter-strand crosslinks and cytotoxicity [15]. When JM118 was examined against six ovarian carcinoma cell lines, it was approximately 4.8-fold more potent than cisplatin [16]. Moreover, JM118 has been shown to circumvent acquired resistance in both a human ovarian and a human cervical carcinoma cell line [17,18].

The mass spectrum of metabolite II (Fig. 2c) is consistent with the sodiated pattern of bis(acetato)ammine(cyclohexylamine)dihydroxo

platinum(IV), JM383. The mass spectrum showed a different isotopic cluster ion to that of JM118 or JM216, which strongly suggested that the two chloride ions of JM216 had been displaced. The LC elution time (14:06 min) and the accurate mass measurement of the metabolite were identical to those obtained from an authentic sample of JM 383 (calculated for $^{194}PtC_{10}H_{24}N_2O_6Na$ is 485.1160; measured: 485.1142). The two components observed at 18:25 min and 25:29 min with m/z 485–487 were derived from metabolites III and IV.

The mass spectra of metabolites III and IV (t_R = 18:25 and 25:30 min) primarily gave pseudomolecular ion at m/z 503, coinciding with the sodiated molecule of bis(acetato)amminechloro(cyclohexylamine)hydroxo platinum(IV) (Fig. 2d,e). The elution time of metabolite IV was identical to that of JM518, and metabolite III is thus tentatively identified as its isomer JM559. Additional evidence confirming the structural assignment of these two isomers was elicited from tandem mass spectrometry. A collision-induced dissociation mass spectrum of m/z 505 gave fragment ions at m/z 486 ($[M + Na - H_2O]^+$), m/z 468 ($[M + Na - HCl]^+$) and m/z 426 ($[M + Na - H_2O - CH_3COOH]^+$). Elimination of the entire ($[M + Na - CH_3COOH - Cl]^+$) moiety gave rise to the abundant peaks at m/z 409; and the base peak at m/z 349 was attributed to loss of ($[M + Na - 2CH_3COOH - Cl]^+$) (Fig. 2f). The measured molecular mass of the JM518 sodiated adduct is 503.0825, in good agreement with the calculated value ($^{194}Pt^{35}ClC_{10}H_{23}N_2O_5Na$: 503.0821). However, the signal intensity of metabolite III was insufficient for accurate mass measurement.

Displacement of the chloride ligand by a hydroxy-group is an established metabolic route of platinum complexes, and the resulting hydroxo group forms a stable bond with platinum with variable antitumor activities [19]. The cytotoxicity of JM518 is similar to that of JM216 against the ovarian cell lines, while JM383 is less cytotoxic (F. Raynaud, pers. commun.).

Off-line LC–AAS was used to determine the level of platinum complexes in the ultrafiltrates. JM518 and JM383 were the major metabolites observed and represented approximately 1.15 and 1.18 μg/ml of plasma, respectively, whereas JM118, JM216 and the isomer of JM518 were present at 0.66, 1.15 and 0.238 μg/ml of plasma, respectively. Some unidentified components eluted in the solvent front were probably glutathione or methionine adducts and accounted for $< 10\%$ of the unbound platinum complexes. Only trace amounts of JM118 and JM383 were detected in the control incubation.

Fig. 3 represents the selected-ion monitoring chromatograms of the patient plasma ultrafiltrate when examined by LC–ESI–MS on a quadrupole mass spectrometer. Two metabolites were detected, i.e. JM383 and JM118. As indicated by off-line LC–AAS, JM118 was the major metabolite in the patient plasma ultrafiltrate, and the sample contained only trace amounts of unchanged parent drug ($< 2\%$). Neither JM518 nor its isomer were detected in the post-treat-

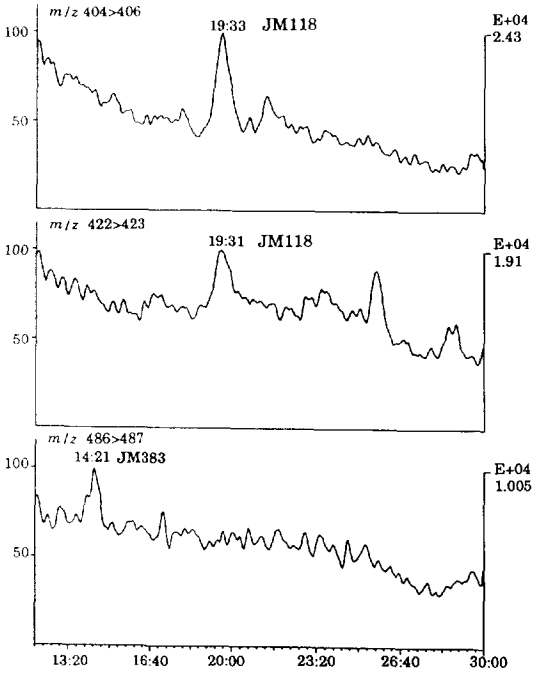

Fig. 3. Selected-ion chromatograms of the plasma ultrafiltrate obtained from patients treated with JM216 and analysed by LC–ESI-MS showing the presence of metabolite I, JM118, and metabolite II, JM383.

ment plasma sample. No platinum complexes were observed in the patient pre-treatment plasma ultrafiltrate sample.

4. Conclusions

Information on the biotransformation products from patients treated with platinum complexes has previously been limited by the lack of analytical techniques capable of determining these species at a sufficiently low level. In the present study LC–ESI–MS with accurate mass measurement and tandem mass spectrometry has been successfully applied to the study of platinum complexes, affording unambiguous identification of four metabolites of JM216 in human plasma ultrafiltrate, JM118 and JM383 in patient plasma ultrafiltrate.

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