Cancer Chemother Pharmacol (1996) 38: 155–162

ORIGINAL ARTICLE

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Biotransformation of the platinum drug JM216 following oral administration to cancer patients

Received: 8 May 1995/Accepted: 6 October 1995

Abstract This study evaluates the metabolic profile of JM216 [bis(acetato)ammine-dichloro(cyclohexylamine) platinum(IV)], the first orally administrable platinum complex, in plasma ultrafiltrates of 12 patients ($n = 2$–4 time points per patient) following different doses of drug (120, 200, 340, 420, 560 mg/m^2). The biotransformation profile was evaluated by high-performance liquid chromatography (HPLC) followed by atomic absorption spectrophotometry (AA). The AA profiles were compared with those previously identified by HPLC on line with mass spectrometry (HPLC-MS) in plasma incubated with JM216. A total of six platinum peaks (Rt = 5.5, 7.2, 10.6, 12.4, 15.6, and 21.6 min, respectively) were observed in patients' plasma ultrafiltrate samples, of which only four appeared during the first 6 h post-treatment. Four of these coeluted with those observed and identified previously in plasma incubation medium. No parent JM216 was detected. The major metabolite seen in patients was the Pt II complex JM118 [*cis*-amminedichloro-(cyclohexylamine)platinum(II)] and was observed in all the patients. Interestingly, the second metabolite was shown to coelute with the Pt IV species JM383 [bis-acetatoammine(cyclohexylamine)dihydroxoplatinum (IV)]. Both JM118 and JM383 were identified by HPLC-MS in a clinical sample. Peak C, which was a minor product (less than 5% of the free platinum), coeluted with JM559 [bis-acetatoammine-chloro(cyclohexylalamine)hydroxoplatinum(IV)]. The cytotoxicity profile of all three metabolites in a panel of cisplatin-sensitive and -resistant human ovarian carcinoma cell lines was very close to that of the parent drug. In addition, the concentrations of JM118 reached in patients' plasma ultrafiltrate were comparable with the cytotoxic levels of the compound determined in the ovarian carcinoma panel of cell lines. Two metabolites were seen in patients but not in the in vitro incubation medium, suggesting the involvement of a possible enzyatic reaction. Thus, the biotransformation profile following oral administration of JM216 shows a variety of Pt(IV) and Pt(II) metabolites in plasma that differ significantly from other systemically applied platinum drugs.

Key words JM216 · Platinum complexes · Metabolism · Human plasma ultrafiltrates

Introduction

Platinum anticancer agents are widely used in the treatment of ovarian, testicular, head and neck, and small-cell lung carcinoma [1, 2, 9, 10, 21, 22, 27]. The major toxicities of these compounds are nephrotoxicity, myelosuppression and peripheral neuropathy [2]. Previous studies have established that biotransformation products of platinum complexes can be responsible for their antitumor efficacy and side effects. As an example, the hydrolysis products of cisplatin are known to be 1,000-fold more reactive than the parent drug itself [3, 4]. It has also been demonstrated that the nephrotoxicity of methionine-cisplatin complexes is higher than that of cisplatin [5], although cisplatin itself seems to be responsible for the nephrotoxic effects [19]. Platinum(IV) complexes such as iproplatin (CHIP) and tetraplatin (Ormaplatin) are reduced intra- and extracellularly to their platinum(II) counterparts, which can lead to more reactive species [8, 25]. Although ligand-exchange reactions are mainly responsible for the

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biotransformation of platinum complexes, the resulting compounds are often conveniently called metabolites [13].

JM216, bis(acetato)amminedichloro(cyclohexylamine) platinum(IV) [7], is the first orally administrable platinum drug and is currently undergoing phase II clinical trials under the auspices of the United Kingdom Cancer Research Campaign [16, 18]. This drug shows activity in human tumor cell lines exhibiting resistance to cisplatin [10, 12]. In preclinical rodent models, JM216 has shown no nephrotoxicity or peripheral neuropathy [15, 17]. In the initial single-dose phase I study, 31 patients received doses ranging from 60 to 700 mg/m^2 JM216. No limiting toxicity could be established and emesis was found to be easily controlled with prophylactic antiemetic therapy [18]. The pharmacokinetics of total and ultrafilterable platinum following JM216 administration in patients, samples has been investigated and has shown saturation in absorption at doses higher than 300 mg/m^2 [18]. Plasma platinum was highly protein-bound. Studies of the pharmacokinetics of the drug have focused on measuring the total and ultrafilterable platinum (non-protein-bound), but the extent of metabolism and the time course of the metabolism profile have yet to be determined. Incubation of JM216 in plasma revealed the formation of several platinum complexes, including JM118, JM383, JM518 and JM559 (26).

The aim of this study, conducted in parallel with the phase I trial, was to evaluate the number and nature of the metabolites detected in patients' plasma ultrafiltrates following treatment with JM216 and to compare the metabolism profile with that identified by high-performance liquid chromatography on line with mass spectrometry (HPLC-MS) in plasma incubated with JM216. The retention time of metabolites observed in patients' ultrafiltrate samples were also compared with those of exogenously synthesised metabolites. Mass spectrometry with selected ion monitoring of a patient sample is presented. The possible role of the major metabolites in determining the antitumor properties of JM216 was evaluated through determination of cytotoxicity against a panel of cisplatin-sensitive and -resistant human ovarian carcinoma cell lines.

Patients and methods

Drugs

JM216, JM118, JM383 and JM518 were obtained from Johnson Matthey Technology Centre (UK). The structures are shown in Fig. 1. The complexes JM118 and JM216 were prepared as previously described [6, 7].

Patients

The metabolic profiles were examined in plasma ultrafiltrates from 12 patients in the single-dose study after treatment with 120 ($n = 1$), 200 ($n = 2$), 300 ($n = 2$) , 420 ($n = 4$), 540 mg/m^2 ($n = 3$). For each patient, two to four different sampling time points were analysed according to material remaining after pharmacokinetic analysis. Samples containing less than 75 ng/ml were not run due to the limit of detection of the method. The patients' ultrafiltrates were obtained by centrifugation at 1900 g and 4°C for 45 min on an Amicon Centrifree Micropartition System (10,000 mol. wt. cut-off) and were then flash-frozen and stored in liquid nitrogen until analysed. The plasma ultrafiltrate of a patient treated with 700 mg/m^2 JM216 was analysed on the HPLC-MS.

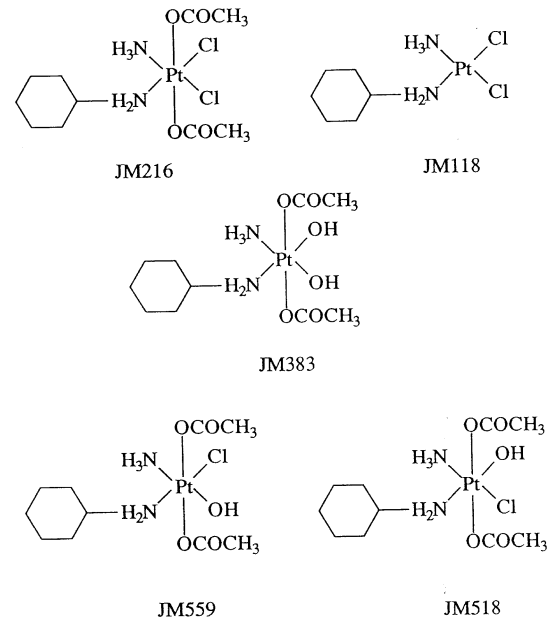

Fig. 1 Structure of JM216 and its biotransformation products identified by HPLC-MS

High-performance liquid chromatography

Two 510 Waters pumps connected to a gradient controller and a manual U6 K injection loop of 500 µl were used in this study. Patients' plasma ultrafiltrates (100–200 µl) were separated on a reverse-phase polymeric column (PLRP-S, 5 µM, 4.6 mm × 25 cm; Polymer Laboratories, UK) with an acetonitrile/water gradient from 15/85 to 90/10 over 30 min at a 0.6-ml/min flow rate.

The metabolism profile was determined by collecting 0.2-min fractions with an LKB fraction collector and subsequently analysing them for platinum content on an atomic absorption spectrophotometer. Identification of the metabolites was achieved by coelution with authentic material. The HPLC column was washed every three runs with 100 µl of 200 mM diethyl dithiocarbamate so as to eliminate platinum contamination.

Furnace atomic absorption spectrophotometry

A Perkin Elmer (model 1100) atomic absorption spectrophotometer with a graphite furnace (model 700) was used for this study. A five-stage temperature program was used. Samples (50 µl) of the HPLC effluent were introduced into the furnace at 60°C. The absorption of platinum was recorded at 265.9 nm. Platinum was quantified in the samples using an external-standard calibration method with platinum standards ranging between 0 and 100 ng/ml. Quality controls

were included in duplicate at the beginning and end of each run at the level of 20 and 50 ng/ml.

Mass spectrometry

The LC system used was a Waters 600 MS on line with a Finnigan Mat TSQ 700 triple quadrupole mass spectrometer fitted with an electrospray ionisation source. A 100-µl plasma ultrafiltrate sample was analysed on a 4.6×250 mm PLRP-S column with a methanol/water gradient from 30/70 to 95/5 over a 30-min period. Selective ion monitoring was performed using the previously identified masses (405–406 and 422–423 for the sodium and potassium adducts of JM118; 486–487, 506–507, 524–525 for the sodium adducts of JM383, JM518 and JM216, respectively). The limit of detection for the different metabolites was evaluated in plasma ultrafiltrate.

Plasma incubations

Fresh human plasma was incubated with 120 µ*M* JM216 at 37°C for 2 h and subsequently ultrafiltered as previously described and analysed by HPLC followed by atomic absorption spectrophotometry (AA) and HPLC-MS.

Cell culture

Established human ovarian carcinoma cell lines were used in this study, namely, HX 62, SKOV-3, CH1, CH1 CISR, 41M, 41M CISR, A2780 and A2780 CISR, and were selected as previously described to represent examples of intrinsic and acquired cisplatin resistance [11]. Their biological properties and sensitivity to the clinically used platinum compounds have been described elsewhere [11].

Cells grew as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Imperial Laboratories, Andover, UK), 2 m*M* glutamine, 10 µg insulin/ml, 0.5 µg hydrocortisone/ml and 2.5 µg amphotericin B/ml in an atmosphere containing 10% CO_2 and 90% air. Cells were checked routinely for the presence of mycoplasma and were found to be negative throughout the course of these experiments. The lines used were checked for consistency in morphology and tumor-doubling time during the period of the study. The growth-inhibitory properties of JM216 and the metabolites JM118, JM383 and JM518 were determined using a 96-h period of drug exposure and assayed using a sulforhodamine B assay as described previously [12].

Results

Metabolic profile in plasma incubation and patients' plasma ultrafiltrate

Figure 2A shows the metabolic profile in the ultrafiltrate of plasma incubated with JM216 and the retention time of the different metabolites identified by mass spectrometry. The metabolic profile in a patient's plasma ultrafiltrate sample at 3 h post-treatment with 420 mg/m^2 JM216 is shown in Fig. 2B. Six platinum peaks were observed in this patient with retention times (Rt) of 5.5, 7.2, 10.6, 12.4, 15.6 and 21.6 min, respectively, and no JM216 was detectable.

The first eluting peak (A) was observed in both samples. The second metabolite coeluted with JM383, and spiking of a dilution of plasma incubated with JM216 with clinical material increased this peak (Fig. 2C, D). The third peak coeluted with JM559, and the JM559 peak observed in the plasma incubated with JM216 increased when clinical material was added. The fourth peak (D) was not present in the incubation medium. JM518 was not present in patients' samples

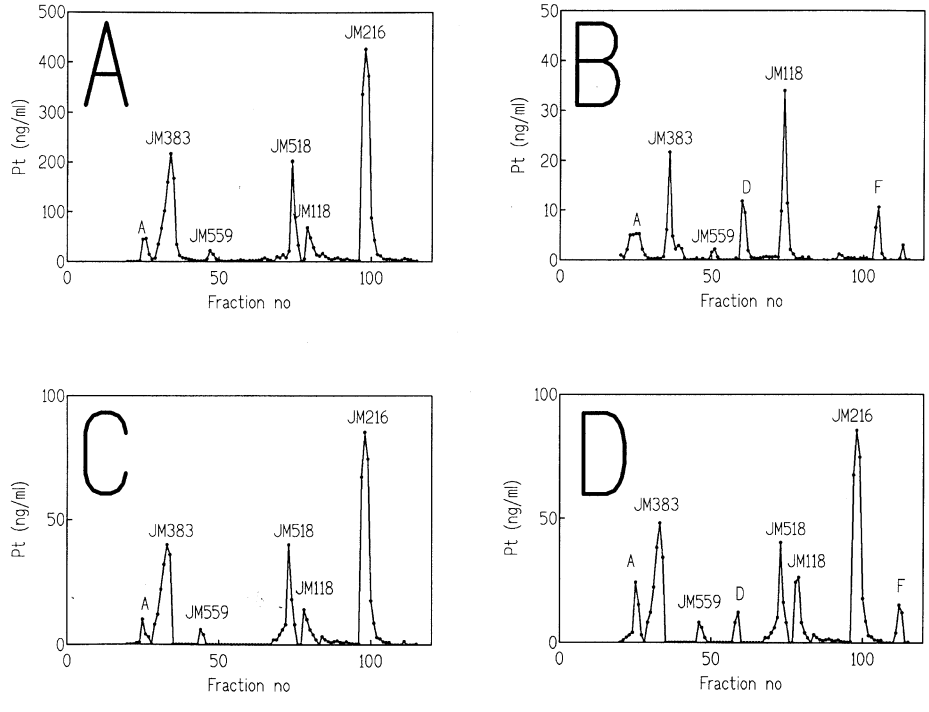

Fig. 2A–D Metabolic profile (**B**) in a patient's plasma ultrafiltrate at 3 h post-treatment with 420 mg/m^2 JM216 and (**A**) in plasma ultrafiltrates obtained by incubating 120 µ*M* JM216 in human plasma for 2 h . Metabolic profile in plasma ultrafiltrate obtained by incubation of JM216 at 5 µ*M* in plasma diluted with blank plasma (**C**) and spiked with a patient's plasma ultrafiltrate at 4 h post-treatment with 700 mg/m^2 JM216. The biotransformation products were separated by HPLC on a PLRPS 25-cm column with an acetonitrile/water gradient (15–90% acetonitrile over 30 min) and absolute platinum levels were measured in 0.2-min fractions by atomic absorption spectrophotometry

and its quantity did not increase when clinical sample was added to plasma incubation medium. A late-eluting metabolite was observed in patients' samples but not in incubation medium (F).

Table 1 shows the percentage of each metabolite detected in different patients' plasma ultrafiltrates following various doses of JM216. Only two patients' samples showed traces of the parent drug ($< 2\%$, data not shown). Peaks A and JM383 appeared in all patients throughout the sampling time (24 h). In different gradient conditions, it appeared that peak A actually constituted three different hydrophilic species (data not shown), amongst which one may have been glutathione adduct. Metabolites D and JM118 were observed in all patients at the earlier time points (before 6 h) but were not always seen at the later time points (6–24 h). JM118 was observed in eight of ten patients and metabolite F, in four of ten patients.

The total platinum eluted from the HPLC column accounted for 93–103% of the free platinum measured in the pharmacokinetics studies (data not shown) [16], indicating that the metabolites had eluted from the column. JM118 was the major metabolite in 22 of 27 samples collected at 1–6 h post-treatment (the peak concentration of free platinum occurring at 3–4 h post-treatment) and represented on average 31% of the total free platinum. JM383 was the major platinum species in 5 of the 22 samples taken at 1–6 h post-treatment and was the major metabolite in 5 of 7 samples taken at 6–24 h post-treatment. The levels of this metabolite were very low, representing $< 5\%$ of the total ultrafilterable platinum. The chemical transformations leading to the formation of these metabolites are illustrated in Fig. 3.

HPLC-MS findings

Figure 4 shows the HPLC-MS ion chromatograms monitoring the mass of the metabolites. JM383 (Rt = 14.21 min, m/z 486–487) and JM118 (Rt = 19.31 min, m/z 405–406 and 422–423) were the two metabolites

Table 1 Pt levels expressed in percent as measured in plasma ultrafiltrates following HPLC separation. Peaks separated as A (fraction 19–25), JM383 (fraction 32–39), JM559 (fraction 49–53), D (fraction 59–63), JM118 (fraction 72–79) and F (fraction 105–110). JM216 eluted at fraction 98–101

Dose (mg/m^2)	Time (h)	Metab.A (%)	383 (B) (%)	559 (%)	Metab.D (%)	118 (%)	Metab.F (%)	Pt (ng/ml)
120	3	20	23.3	[a]	18.7	43	[a]	144
120	4.1	23.7	13.2	[a]	13.8	28.2	[a]	114
120	6.15	58.9	18.1	[a]	14.5	8.5	[a]	
200	3	50	14.8	[a]	9.8	25	[a]	221
200	4.1	25.8	24.9	[a]	13.6	35.6	[a]	102
200	6.2	30	22.4	[a]	11.6	36	[a]	35
200	1	10.2	19.5	2.5	19	35.2	13.1	103
200	1.5	13.7	21.8	4.9	15.7	30.8	14	17
200	2	18.9	22.5	5.9	16.3	25.1	14.4	17
300	1	8.7	30	[b]	42.2	19.1	[b]	71
300	1.45	14	29.3	[b]	20	36.6	[b]	153
300	2.1	13.2	12.5	[b]	26.6	54.8	[b]	122
300	3	15.2	7.5	[b]	20.4	57.8	[b]	41
300	4	9.4	26.4	[b]	22.4	43.7	[b]	61
300	6	30	35.1	[b]	12.8	22.1	[b]	45
300	8.15	76.2	35.8	[b]	[b]	[b]	[b]	28
420	2	8.2	41.6	3.7	19	27	[b]	106
420	3.2	18.1	35	[b]	15	31.9	[b]	91
420	12.3	21.9	43.6	[b]	15.6	18.9	[b]	84
420	3.1	20.5	34.3	18.7	3.4	23	[b]	132
420	4	15.9	35	3.8	18	27.2	[b]	140
420	24.3	64.3	32.2	[b]	[b]	[b]	3.5	78
420	4	13	16.2	[b]	18.2	40.3	12.3	90
420	5.9	27.8	11.8	3.1	15.4	29	12.8	97
420	22.4	85.9	14.1	[b]	[b]	[b]	[b]	21
420	3	25.1	17.4	2.3	16.9	3.9	[b]	82
420	3.85	16.1	23.2	2.6	14.5	32.4	[b]	113
420	6.7	29.2	29.6	[b]	11.5	22.2	[b]	72
540	3.3	14.1	28.9	3.7	13.3	40	[b]	63
540	4.15	10.8	34.9	4.7	16.5	33	[b]	65
540	7.8	44.4	55.6	[b]	[b]	[b]	[b]	34
540	3	14.7	25.6	7.4	21.8	33.7	[b]	73
540	4	17.7	15.5	[b]	11.1	45.1	[b]	51
540	2	5.1	21.9	41.2	4.5	24.1	[b]	178
540	4	14.4	28	4.4	15	19.1	18.3	102

[a] Not measured
[b] Not detectable

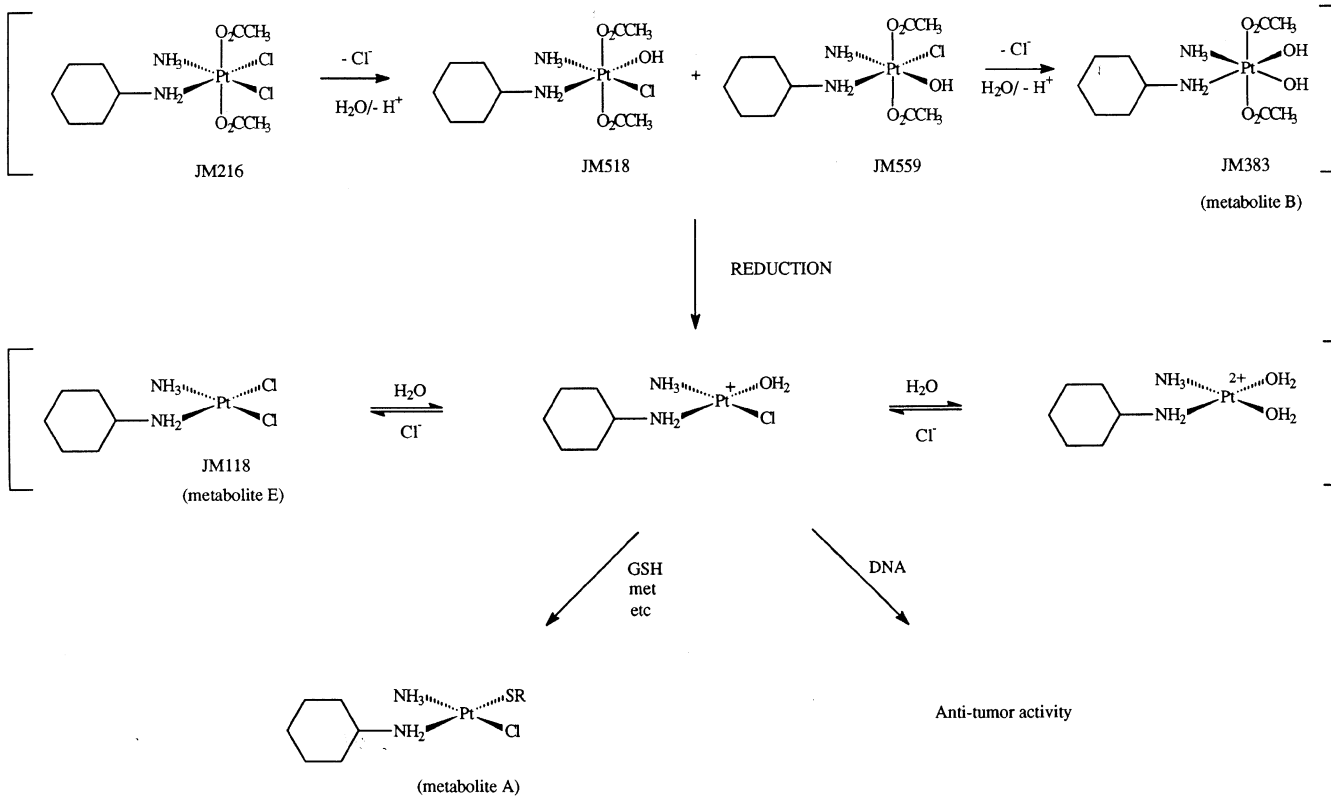

Fig. 3 Chemical transformations leading to the formation of JM216 metabolites

detected in this patient. No signal was detected for JM518 or JM559 (m/z = 504–507, Rt = 18.1 and 24.43 min) or JM216 (m/z = 524–527, Rt = 28.03 min). The limit of detection by HPLC-MS for each metabolite was 25 ng/ml for JM216, 10 ng/ml for JM383 and JM518, and 50 ng/ml for JM118 (see Fig. 5 for a chromatogram of plasma ultrafiltrate spiked with 100-ng/ml concentrations of JM118, JM383, JM518 and JM216). None of these peaks were observed in a blank plasma (data not shown).

In vitro cytotoxicity

Table 2 shows the cytotoxicity data for the different platinum complexes previously identified in plasma incubation medium. All compounds tested showed an activity profile and potency similar to that of the parent drug. Of the three identified metabolites, the major metabolite JM118 is the most potent inhibitor of tumor cell growth in vitro, the mean 50% growth-inhibitory concentration (IC_{50}) across the eight cell lines being 0.7 μM as compared with 1.1 μM for JM216 itself and 2.8 μM for cisplatin itself. Moreover, JM118 showed non-cross-resistance with cisplatin in the 41M pair of cell lines, where resistance has been shown to be the result of reduced drug transport [14], and partial cross-resistance in the two other pairs of lines. It was significantly more potent than the other platinum(IV) complexes in the A2780 pair. JM518 showed a potency and pattern of activity similar to that of JM216, with the mean IC_{50} value being 0.8 μM, whereas the Pt(IV) compound JM383 was generally about 10-fold less potent (mean IC_{50} value of 9.8 μM) but exhibited a low level of cross-resistance across all three cell lines with acquired cisplatin resistance.

Discussion

This study clearly establishes that no JM216 can be detected in patients' plasma ultrafiltrates as early as 20 min post-administration. This is contrary to what has been observed with cisplatin [2, 27], carboplatin [20] and iproplatin [24]. However, oxaliplatin was rapidly transformed in plasma incubation medium [23], and tetraplatin was shown to be extremely rapidly transformed into platinum(II) complexes, even in tissue-culture medium [8].

The major metabolite of JM216 in patients' samples appears to be a platinum(II) species, namely, JM118. This finding was also confirmed by HPLC-MS. The rapid conversion of platinum(IV) species into platinum(II) species is a well-known phenomenon and has previously been described [8, 23, 25]. Our study provides

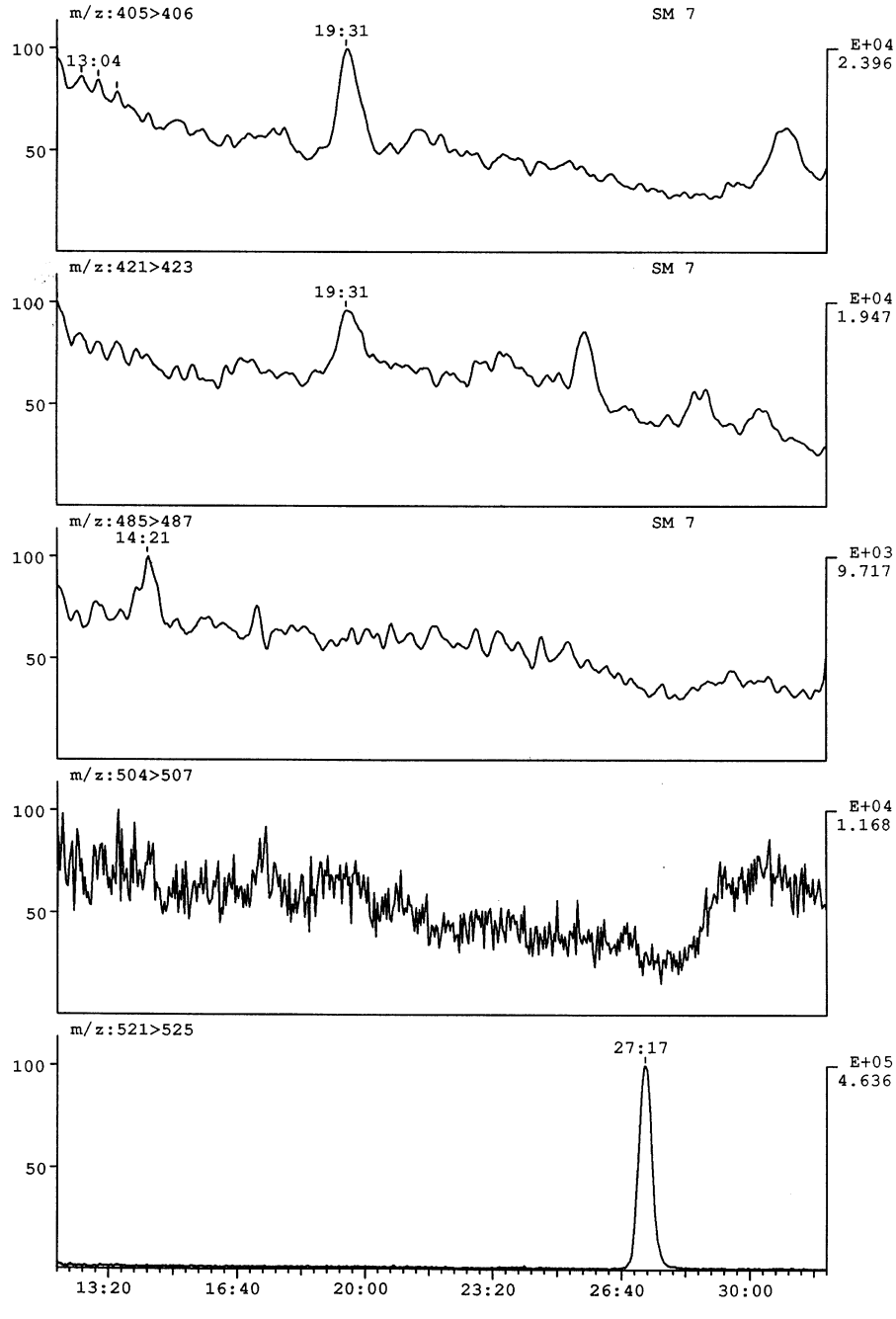

Fig. 4 Selected ion chromatograms of a patient's plasma ultrafiltrate at 3 h following treatment with JM216 at 700 mg/m^2

absolute evidence for the conversion of JM216 into JM118 in a patient's sample. The concentrations of JM118 achieved in the plasma ultrafiltrates were in the cytototoxic range as established by the IC_{50} value of the compound in our ovarian panel of cell lines. JM118 was detectable at 6 h after JM216 administration, which is a good indicator in terms of therapeutic efficacy. It was the major metabolite when the levels of free platinum were maximal (Cmax). JM383 has also been observed in patients' samples by HPLC-MS. Interestingly, in the case of JM383 the acetato axial ligands remained, whereas hydrolysis occurred at the chloride atoms. In our chromatographic conditions, JM383 eluted close to the solvent front, and it is thus not certain whether all of peak B measured by AA is JM383. Two metabolites (D and F) were detected in patients' samples but not in the human plasma incubation medium, which suggests that they could be formed by enzymatic reaction in the liver or by cellular metabolism. They have yet to be identified. JM518 was observed in incubation medium but not in patients' samples. This could have been due to a rapid clearance of JM518 or to a high reactivity of the compound. Very low levels of a platinum-containing metabolite ($< 5\%$ of total platinum) were found to coelute with JM559.

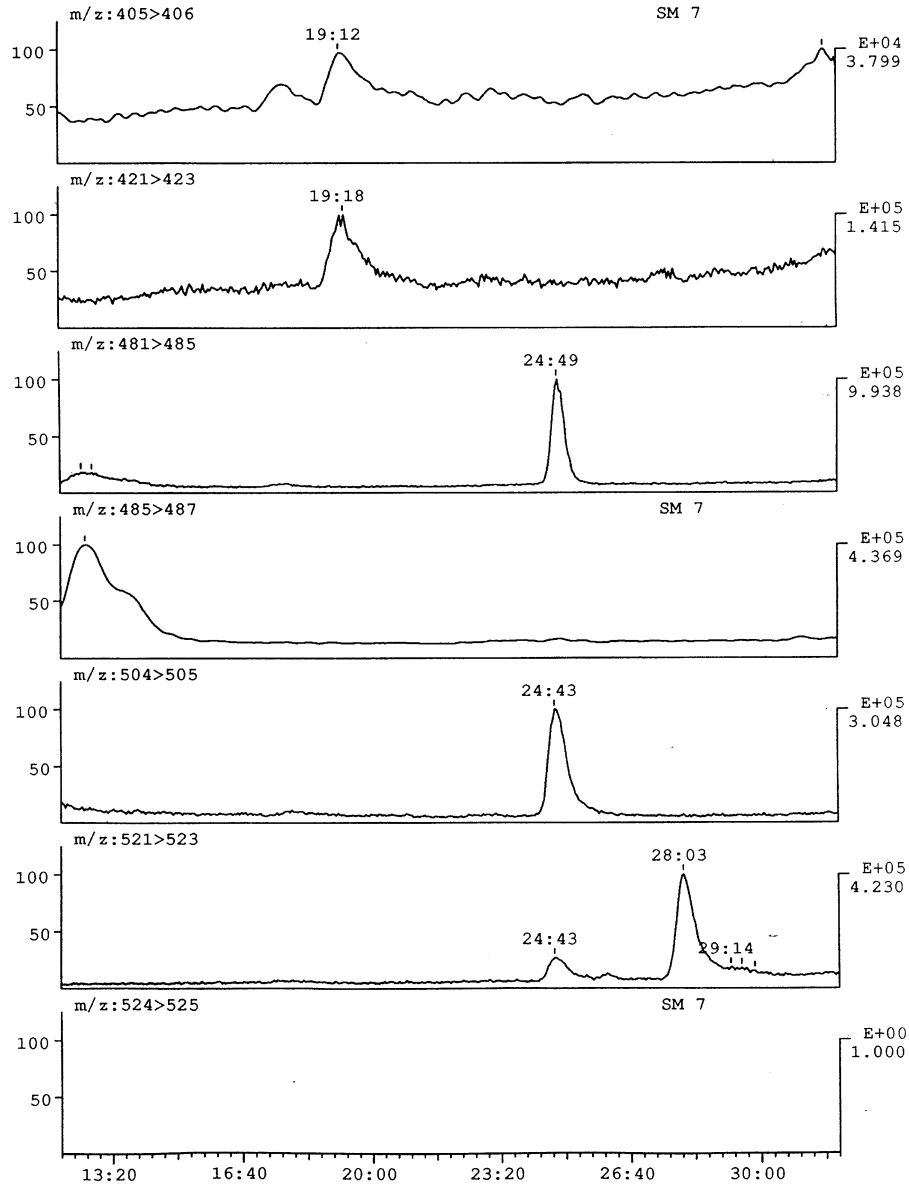

Fig. 5 Selected ion chromatograms of a plasma ultrafiltrate spiked with 100-ng/ml concentrations of JM216, JM118, JM383 and JM518

Table 2 Cytotoxicity of JM216, its identified metabolites and cisplatin in our panel of ovarian carcinoma cell lines, expressed as the concentration inhibiting cell growth by 50% (IC_{50}, in μM) after 96 h of cell culture. Data represent mean values ($n = 2$–3 experiments). Resistance factors (IC_{50} resistant line/IC_{50} parent line) are given in parentheses (*cis Pt* Cisplatin)

	HX62	SKOV-3	CH1	CH1 CisR	41M	41M CisR	A2780	A2780 CisR
JM216	3.7	1.6	0.084	0.33 (4)	0.48	0.51 (1.1)	0.34	1.53 (4.5)
JM118	1.9	2.1	0.066	0.38 (5.7)	0.33	0.32 (1)	0.083	0.4 (4.8)
JM383	37	21	0.74	1.35 (1.8)	6.5	4.35 (0.9)	4.2	3.7 (0.9)
JM518	3.1	1.1	0.02	0.082 (4.1)	0.24	0.44 (1.8)	0.31	0.93 (3)
cisPt	12.6	4.4	0.1	0.7 (7)	0.25	1.3 (5.2)	0.3	3.2 (10.7)

The cytotoxicity of platinum complexes is believed to result from the interaction of these complexes with DNA. The metabolism profiles observed in the present study do not take into consideration reactions with proteins or DNA. However, the characterisation of JM216 metabolites should allow a better understanding of its mechanism of action. The metabolism pattern observed following JM216 administration was shown not to depend upon the dose of treatment.

In conclusion, this study has established that orally applied JM216 is completely converted into at least six different compounds, amongst which are JM118 and JM383. Furthermore the metabolic profiles are consistent with the cytotoxicity of the compound. That no parent drug could be detected in patient plasma together with the observed mixture of platinum(II) and platinum(IV) complexes in patients' plasma ultrafiltrates indicates that JM216 differs from the other platinum compounds studied to date.

Acknowledgements This work was supported by the Cancer Research Campaign, Bristol Myers Squibb Ltd, and Johnson Matthey PLC.

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