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Preclinical toxicology and tissue platinum distribution of novel oral antitumour platinum complexes: ammine/amine platinum(IV) dicarboxylates

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Abstract. The preclinical toxicology and tissue platinum distribution of a series of six orally given antitumour platinum complexes [ammine/amine platinum(IV) dicarboxylates] with structural variations of their alicyclic amine (c-C_5, c-C_6 or c-C_7), axial dicarboxylate (CH_3, C_3H_7 or NHC_2H_5) or leaving substituents (Cl_2 or OCOOCO) was studied in the mouse. Platinum tissue levels measured at 48 h after a single oral dose at 0.5 of the MTD were highest in the liver (6.0–19 μg/g) and second highest in the kidney (2.8–12 μg/g), and these levels were up to 5 times higher than those reported with equi-toxic doses of i.v. cisplatin and i.v. carboplatin. Platinum levels in the lung, heart, spleen, skin, skeletal muscle and brain were all ≤3.1 μg/g at this dose level. Liver platinum levels measured at 2 h, 2 days, 6 days and 10 days after a single oral dose at the MTD ranged widely (from 15 to 109 μg platinum/g), were related to the number of carbon atoms in the axial dicarboxylate and alicyclic amine groups (r = 0.9389) and showed a diversity of time-course profiles. Elevations of plasma ALT activity were recorded with single oral doses of JM225 and JM256 at the MTD. Accumulation of platinum in the liver with repeated oral dosing weekly for 4 consecutive weeks at 0.5 of the MTD occurred with JM269 (3.3-fold increase, $P < 0.05$) and JM225 (2.4-fold increase, $P < 0.05$), and elevated plasma ALT activity (44 ± 33 IU/l) was recorded with repeated oral doses of JM269. JM216 was selected from this series of analogues for further study on the basis of the elevated plasma ALT activity (JM225, JM256 and JM269), liver platinum accumulation (JM269 and JM225), poor activity against human ovarian carcinoma xenografts (JM291) or severe emetogenesis (JM221) of other examples. Following a single oral dose of JM216 at the MTD, transient reductions in the WBC (nadir, 1.6×10⁹/l, 2 days, 74% reduction), platelet count (nadir, 613×10⁹/l, 10 days, 33% reduction) and bone marrow cellularity (nadir, 0.5×10⁷ nucleated cells/femur, 4 days, 75% reduction) were found, and these had recovered by 21 days after treatment. Jejunal mucosal disaccharidase activity following single MTDs indicated that small-intestinal mucosal damage was less severe for oral JM216 (nadir maltase activity, 68% ± 16% of control, NS) than for i.v. cisplatin (nadir maltase activity, 35% ± 6.0% of control, $P < 0.01$) and i.v. carboplatin (nadir maltase activity, 38% ± 6.4% of control, $P < 0.01$). In conclusion, the liver is the major tissue platinum depot for orally delivered ammine/amine platinum(IV) dicarboxylates and is a site of toxicity for examples of this class. Oral JM216 causes dose-limiting leucopenia but produces less gastrointestinal toxicity than either i.v. cisplatin or i.v. carboplatin at the MTD in the mouse.

Introduction

Cisplatin and carboplatin are the existing antitumour platinum drugs, but they have the disadvantages of exhibiting cross-resistance, requiring i.v. administration and producing severe acute and chronic toxicity. The requirement for measures to circumvent acute nephrotoxicity and emesis frequently necessitates hospitalisation. An oral platinum drug causing less toxicity but having activity similar to that of the existing agents, which would be free of the requirement for inpatient supportive measures, could be a significant advance. The ammine/amine platinum(IV) dicarboxylate class of platinum complexes have been shown to have oral antitumour activity and bioavailability in mice [3] and to lack cross-resistance with cisplatin in vitro against human ovarian carcinoma cell lines [4]. Moreover, these oral platinum complexes have a wider therapeutic

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Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; carboplatin, *cis*-diamminecyclobutanedicarboxylatoplatinum(II); cisplatin, *cis*-diamminedichloroplatinum(II); EDTA, ethylenediaminetetraacetic acid; MTD, maximally tolerable dose; NS, not significant

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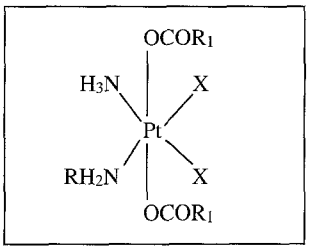
Table 1. Chemical structures of ammine/amine platinum(IV) dicarboxylate complexes



	R	R_1	X
JM225	c-C_5H_9	CH_3	Cl
JM216	c-C_6H_{11}	CH_3	Cl
JM291	c-C_6H_{11}	CH_3	ocooco[a]
JM221	c-C_6H_{11}	C_3H_7	Cl
JM256	c-C_6H_{11}	NHC_2H_5	Cl
JM269	c-C_7H_{13}	CH_3	Cl

[a] Bidentate

index than parenteral cisplatin and carboplatin against a murine tumour model [5]. Such an oral platinum preparation could potentially lessen the morbidity of chemotherapy, improve patients' quality of life, and further rationalise the use of health-service resources.

The side effects of platinum-based drugs are severe and variously include damage to the kidneys, gastrointestinal system, nervous system and bone marrow [6]. An oral platinum drug developed for outpatient use would require a lack of major toxicity. Work is described herein on the toxicology and tissue distribution of a series of six antitumour platinum(IV) complexes of the ammine/amine platinum(IV) dicarboxylate class, short-listed as potential candidates for clinical testing on the basis of their oral antitumour activity and bioavailability in mice [3].

Previous data on the tissue platinum distribution of i.v. cisplatin and i.v. carboplatin in the mouse were generally indicative of the known toxic effects of these clinical agents, e.g. nephrotoxicity and myelosuppression, and were obtained using single doses at the MTD or a dose at 0.5 of the MTD and time points of up to 10 days after treatment [1]. Peak plasma platinum levels after oral administration of ammine/amine platinum(IV) dicarboxylates in mice occur approximately 2 h after drug ingestion (Sarah Morgan, personal communication). For comparative purposes, the tissue distribution and toxicology studies of oral ammine/amine platinum(IV) dicarboxylates used doses and time points similar to those applied in previous published work for i.v. cisplatin and i.v. carboplatin and incorporated a 2-h time point. The haematological toxicities of cisplatin and carboplatin in rodents are generally reflective of their respective clinical effects [7], and small-intestinal mucosal disaccharidase activity in mice has previously been established as a sensitive method for the evaulation of cisplatin-induced gut damage [8]. The effects on peripheral blood counts and small-intestinal mucosal disaccharidase activity of the lead oral compound (JM216) were also studied in the mouse.

Materials and methods

Drug administration. Cisplatin, carboplatin and ammine/amine platinum(IV) dicarboxylate complexes (Table 1) were synthesised and supplied by the Johnson Matthey Technology Centre (Blount's Court, Sonning Common, Reading, Berkshire, UK). Female BALB/c mice weighing 20–25 g were used in all experiments. Cisplatin was dissolved in sterile 0.9% sodium chloride and carboplatin, in sterile 5% dextrose by sonication (MSE 150-W Ultrasonic Disintegrator, 15 μm for 15 s) and both were given i.v. via the lateral tail vein. Transient whole-body hyperthermia (≤40° C for ≤3 min) was used to facilitate the i.v. injections. Ammine/amine platinum(IV) dicarboxylate complexes were suspended in arachis oil by sonication (MSE 150-W Ultrasonic Disintegrator, 15 μm for 15 s) immediately before being given by oral gavage. Control mice were treated with the respective i.v. or p.o. drug vehicle. Mice were fasted for 18 h prior to drug administration while access to drinking water was maintained at all times. All treatments were given between 0930 and 1200 hours. Platinum complexes were given as single oral or i.v. doses at the MTD or at 0.5 of the MTD.

Dose-finding studies. The MTD was defined as the dose that caused reversible (>5%) body weight loss that was ≤25% lower than the dose causing moribundity. The MTDs for i.v. cisplatin (7 mg/kg) and i.v. carboplatin (120 mg/kg) were as previously described [9]. The MTDs for oral ammine/amine platinum(IV) dicarboxylates were determined by studying four or more dose levels at dose increments of approximately 20% in groups of four to six mice. A daily record of body weight and signs of drug-induced toxicity was made for a total of 28 days. Mice were immediately killed at the onset of moribundity by cervical dislocation. The MTDs and body weight loss at the MTD (percentage of fall in body weight, day of nadir) were as follows: JM225, 180 mg/kd (18%, day 8); JM216, 200 mg/kg (10%, day 5); JM291, 320 mg/kg (8.1%, day 8); JM221, 150 mg/kg (6.1%, day 6); JM256, 150 mg/kg (8.6%, day 4); and JM269, 1000 mg/kg (15%, day 8). Moribundity was encountered for all examples at doses higher than these.

Sample collection. After single oral doses at the MTD or 0.5 of the MTD, blood and tissues were collected at time points ranging from 2 h to 10 days. Blood was collected from an axillary incision during terminal halothane anaesthesia into tubes containing EDTA (1 mg) for peripheral blood counts or into tubes containing heparin (10 units) for determinations of plasma ALT and ALP activity and platinum concentration. Tissue samples were collected, weighed and set aside for preparation for histological examination and platinum analysis. Bone marrow was flushed from the femoral medullary cavity with 5 ml of sterile phosphate-buffered saline, then disbursed by gently syringing with a 20-gauge needle, centrifuged (2000 *g*, 5 min), re-suspended in 1 ml of phosphate-buffered saline and counted immediately. Jejunal mucosa was collected by removing a 3-cm section of the jejunum 10 cm distal to the pyloric sphincter, flushing the luminal contents away with sterile sodium chloride solution (0.9%, w/v) and then gently scraping off the mucosal surface with a scalpel blade. The scrapings were weighed and frozen until analysis for disaccharidase activity.

Platinum analysis. Tissues weighing approximately 200 mg were blotted dry, weighed, solubilised with hyamine hydroxide and diluted with 0.1 *M* HCl [10]. Plasma samples were diluted with dialysed water and analysed without further preparation. Platinum analysis was undertaken by flameless atomic absorption spectrometry using either an Instrument Laboratory Atomic Absorption Spectrometer (Instrument Laboratory Atomic Absorption Spectrometer models 457 655 and model 254, Wilmington, Mass., USA) or a Perkin Elmer Spectrophotometer (Perkin Elmer models 1100B and HGA700, Ueberlingen, Germany). Absorption was measured at 265.9 nm. Platinum concentrations were calculated by an external standard curve for plasma and the standard addition method for tissue.

Pathology. Plasma ALT and ALP activities were analysed using an automated clinical machine (Roche Cobas FARA). Tissues for histo-

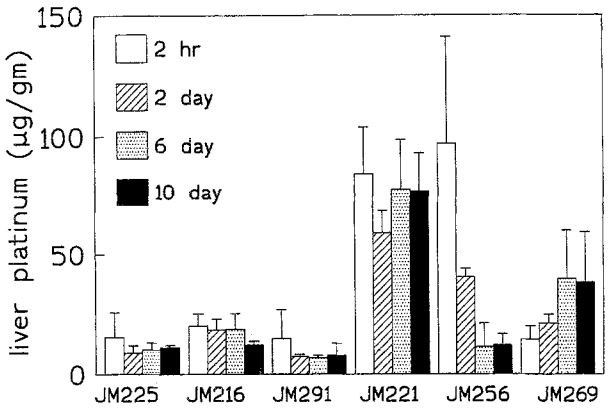


Fig. 1. Time course of the platinum content of the liver following single oral doses of ammine/amine platinum(IV) dicarboxylates at the MTD (mean values ± SD, $n = 3$)

logical examination were prepared by fixation in modified methacarn (methanol, 600 ml; inhibisol, 300 ml; and acetic acid, 100 ml) for at least 24 h, dehydration with ethanol, embedding in paraffin wax and staining with haematoxylin and eosin. Peripheral blood counts were measured using a Coulter 660 cell analyser. Nucleated bone-marrow cell counts were made using a haematocytometer and cell viability was assessed by trypan blue exclusion. Jejunal mucosal disaccharidase activity was measured according to the method of Dahlqvist [11] and normalised to total protein concentration as determined by the Lowry assay [12]. Disaccharidase activity was expressed as a percentage of the activity of control mice treated with the respective i.v. or p.o. drug vehicle. The significance of differences was tested by Student's *t*-test.

Results

Tissue platinum levels were measured at 48 h following a single oral dose of ammine/amine platinum(IV) dicarboxylates at 0.5 of the MTD (Table 2). Tissue platinum content was highest in the liver (range, 6.0–19 µg platinum/g) and next highest in the kidney (range, 2.8–12 µg platinum/g). Thereafter, all tissue platinum levels were ≤3.1 µg platinum/g, and were ranked in the order of spleen (range, 0.7–3.1 µg platinum/g), lung (range, 0.8–2.4 µg platinum/g), skin (range, 0.4–1.6 µg platinum/g), heart (range, 0.6–1.6 µg platinum/g), skeletal muscle (range, 0.2–1.0 µg platinum/g) and brain (range, 0.1–0.2 µg platinum/g). The more lipophilic complexes (JM221, JM256 and JM269) produced higher liver platinum levels (range, 13–19 µg platinum/g) than did those with smaller axial or amine groups (JM225, JM216 and JM291; range, 6.0–9.1 µg platinum/g), but the platinum content measured in other tissues at this dose did not relate to the chemical structure of the platinum complex given.

Liver platinum content was measured at 2 h, 2 days, 6 days and 10 days following single oral doses of ammine/amine platinum(IV) dicarboxylates at the MTD (Fig. 1). The liver platinum time-course profiles were very variable within the series, with some complexes displaying a gradual decline in platinum levels over the 10-day time course (e.g. JM256), whereas others showed static liver platinum levels throughout this time course (e.g. JM221), and further examples demonstrated a tendency for a steady increase in liver platinum content after a single dose (e.g. JM269). Moreover, liver platinum levels ranged widely for this series of platinum complexes (range of highest liver platinum level, 15–109 µg platinum/g), and were related to the number of carbon atoms in the axial and amine groups ($r = 0.9389$; Fig. 2A). A similar relationship was found between the size of these substituents and both the kidney platinum content ($r = 0.8455$) and the plasma platinum concentration ($r = 0.9379$) after single oral doses at the MTD (Fig. 2B).

Plasma ALT and ALP activity was measured, and samples of liver were taken for histopathology at 2 h, 2 days, 6 days and 10 days following single oral doses at the MTD (Fig. 3). Plasma ALT activity was elevated by up to 2 times the control levels following oral dosing with JM225 at 2 h, 6 days and 10 days and following oral administration of JM256 at 2 h and 6 days. Plasma ALT and ALP activities were otherwise within the control range. Plasma ALT activity was not related to either the liver platinum content ($r = 0.3780$) or the time-course profile of liver platinum levels. All livers, including those from mice receiving the oral drug vehicle alone (arachis oil), showed a transient histological change comprising hepatocellular rarefaction and vacuolar change for which periodic acid-Schiff (PAS) staining was indicative of glycogen accumulation. All treatment groups showed a transient increase above baseline in the frequency of pyknotic cells in the basal crypts of the small-intestinal mucosa. Samples of kidney, lung and heart taken at the same time points showed no histological abnormality.

Repeated-dose oral treatment was studied for a series of diacetato ammine/amine platinum(IV) complexes (JM216,

Table 2. Tissue platinum distribution of oral ammine/amine platinum(IV) dicarboxylates in mice 48 h after a single oral dose at 0.5 of the MTD

	Platinum concentration (µg/g tissue)					
Tissue	JM225	JM216	JM291	JM221	JM256	JM269
Liver	8.6±3.1	9.1±5.0	6.0±3.1	19 ±1.6	13 ±2.9	16 ±2.2
Kidney	8.8±2.4	4.5±1.1	2.8±0.8	3.5±1.2	3.0±0.6	12 ±1.1
Spleen	3.1±1.7	2.1±0.4	1.8±1.3	0.7±0.3	1.5±0.3	2.2±1.2
Lung	2.4±1.2	1.3±0.2	0.8±0.4	1.3±0.4	1.4±0.3	1.8±0.2
Skin	1.6±0.6	1.0±0.6	1.0±0.3	0.1±0.1	0.4±0.1	1.1±0.4
Heart	1.6±0.5	0.6±0.3	0.6±0.4	1.5±0.4	1.1±0.4	1.1±0.7
Skeletal muscle	0.7±0.2	0.2±0.2	0.5±0.1	0.5±0.1	1.0±0.3	0.2±0.2
Brain	ND	0.1±0.1	ND	0.2±0.1	ND	0.1±0.1

Data represent mean values ± SD ($n = 3$). ND, Not determined

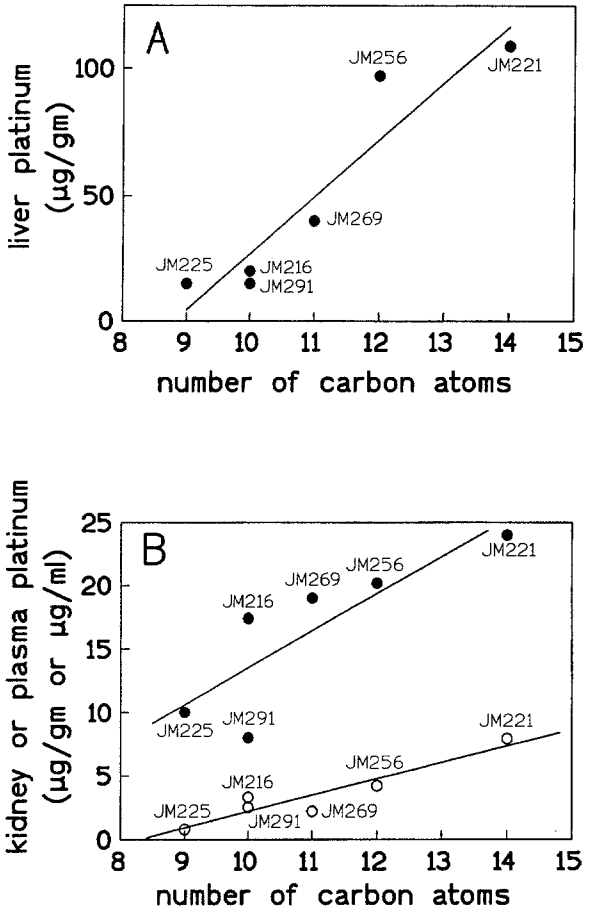


Fig. 2A, B. Highest platinum level measured in the liver (**A**), kidney (**B**, *filled circles*) and plasma (**B**, *open circles*) following single MTD doses of p.o. ammine/amine Pt(IV) dicarboxylates versus the number of carbon atoms in their alicyclic amine and axial dicarboxylate substituents (liver, $r = 0.9389$; kidney, $r = 0.8455$; plasma, $r = 0.9379$; each data point represents the mean value for 3 determinations)

JM291, JM225 and JM269) with structural variations of the alicyclic amine (c-C_5, c-C_6 or C-C_7) and leaving group (Cl_2 or OCOOCO) positions, which were given at 0.5 of the MTD weekly for a total of four doses. Liver platinum content was measured at 48 h after the first and fourth doses, and plasma and liver samples were taken for histology and determination of plasma ALT activity at 48 h after the fourth dose (Table 3). There was significant accumulation of platinum in the liver with repeated oral dosing of JM225 (2.4-fold, $P < 0.05$) and JM269 (3.3-fold,

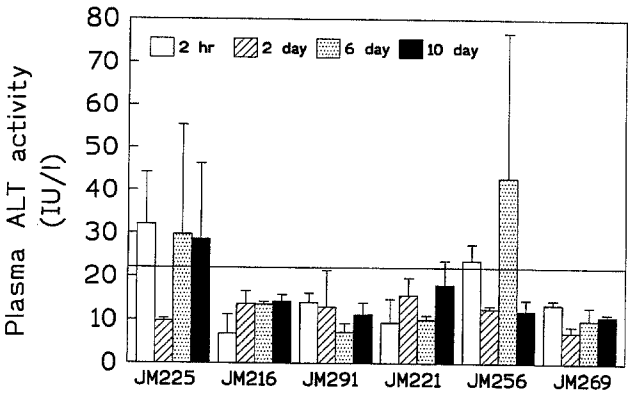


Fig. 3. Time course of plasma ALT activity following single oral doses of ammine/amine Pt(IV) dicarboxylates at the MTD (mean values ± SD, $n = 3$). ———, Upper 95% probability limit of plasma ALT activity (22 IU/l) in control mice

$P < 0.05$). Plasma ALT activity was elevated 48 h after four consecutive oral doses of JM269 (47 ± 22 IU/l). The magnitude of increase in liver platinum content with repeated dosing was related to the plasma ALT activity measured 48 h after the last oral dose ($r = 0.9030$). No histological liver abnormality occurred with repeated oral dosing other than those attributable to the oral drug vehicle (arachis oil).

The time course of haematological toxicity following a single oral dose of JM216 at the MTD was studied from day 1 to day 28 (Fig. 4). Leucopenia occurred with a nadir at 2 days (1.6×10^9/l, 74% reduction) and leucocyte counts recovered between days 14 and 21. Thrombocytopenia was mild by comparison with the nadir occurring at day 10 (613×10^9/l, 33% reduction) and recovery, by days 14–21. Nucleated bone-marrow cell counts fell to the nadir at day 4 (0.5×10^7/femur, 75% reduction), with recovery occurring at day 10. There was a minor change in haemoglobin concentration at day 2 (13.7 ± 0.8 g/l, 5% reduction).

The comparative time course of small-intestinal toxicity was studied by measurement of disaccharidase activity in scrapings of the jejunal mucosa from 2 to 14 days following single i.v. or oral doses of cisplatin, carboplatin or JM216 at the MTD (Fig. 5). Maltase activity in scrapings of jejunal mucosa fell following i.v. dosing with cisplatin and carboplatin between days 2 and 10 [nadir maltase activity (% of control): i.v. cisplatin, 35% ± 6.0% (day 10); i.v. carboplatin, 38% ± 6.4% (day 4); both, $P < 0.01$ in comparison with controls] and had recovered by day 14. Mucosal sucrase activity also fell following i.v. administration

Table 3. Liver platinum content and plasma ALT activity during repeated-dose oral treatment with diacetato ammine/amine platinum(IV) complexes at 0.5 of the MTD weekly for a total of four doses

		Liver platinum content (µg platinum/g)[a]				ALT (IU/l)[b]
		1st dose	4th dose	X-fold increase	*P*	
JM216	(c-C_6, Cl_2)	9.1 ± 5.0	14 ± 2.3	1.5	NS	14 ± 0.1
JM291	(c-C_6, OCOOCO)	6.0 ± 3.1	9.1 ± 2.1	1.5	NS	13 ± 2.6
JM225	(c-C_5, Cl_2)	8.6 ± 3.8	20 ± 2.7	2.4	< 0.05	18 ± 5.6
JM269	(c-C_7, Cl_2)	16 ± 2.2	53 ± 20	3.3	< 0.05	47 ± 33

Liver platinum content was measured 48 h after the first and fourth doses, and plasma ALT activity was measured 48 h after the fourth dose [a] Mean values ± SD [b] Mean values ± SD (upper limit of control values, 22 IU/l)

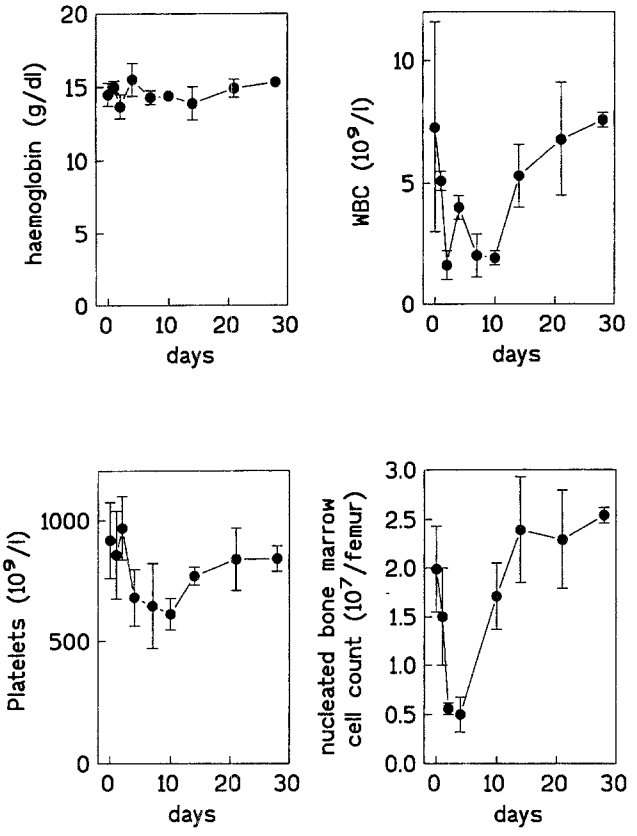


Fig. 4. Time course of peripheral blood haemoglobin concentration, WBC count, platelet count and bone-marrow nucleated cell count following a single oral dose of JM216 at the MTD (mean values ± SD, $n = 3$)

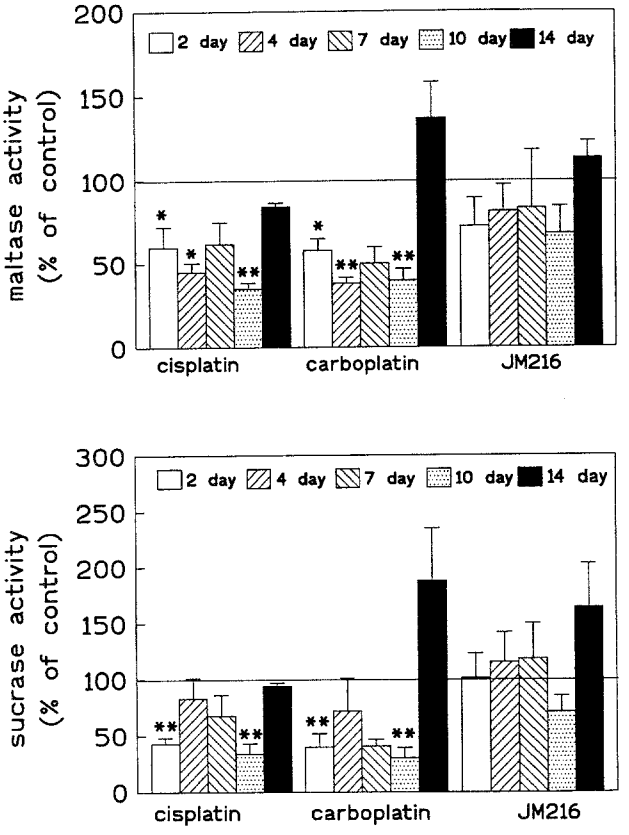


Fig. 5. Time course of jejunal mucosal maltase and sucrase activity following single doses of i. v. cisplatin, i. v. carboplatin and p. o. JM216 at the MTD (mean values ± SE, $n = 3$). * $P < 0.05$, ** $P < 0.01$

of cisplatin and carboplatin between days 2 and 10 [nadir sucrase activity (% of control): i. v. cisplatin, 34% ± 16% (day 10); i. v. carboplatin, 30% ± 1.6% (day 10); both, ($P < 0.01$ in comparison with controls] and had recovered by day 14. In contrast, there was no significant reduction in jejunal mucosal maltase or sucrase activity after oral JM216 treatment.

Discussion

The comparative toxicology and tissue distribution of a series of six novel oral platinum complexes were investigated. These six ammine/amine platinum(IV) dicarboxylates had structural variations of the alicyclic amine (c-C_5, c-C_6 or c-C_7), axial dicarboxylate (CH_3, C_3H_7 or NHC_2H_5) or leaving substituents (Cl_2 or OCOOCO), and the studies were undertaken in the mouse following single doses at the MTD and at 0.5 of the MTD. Tissue platinum levels were highest in the liver and second highest in the kidney, and these levels were up to 5 times higher than those reported for i. v. cisplatin and i. v. carboplatin in the mouse at equi-toxic doses [1]. The tissue platinum content of the spleen, lung, skin, heart and brain was ≤3.1 μg platinum/g, and these levels were within the range previously reported with i. v. cisplatin and i. v. carboplatin.

The time course of liver platinum content following single oral doses of ammine/amine platinum(IV) dicarboxylates at the MTD showed a diversity of profiles with examples displaying either a gradual decay (JM256; c-C_6, NHC_2H_5), a static (JM221; c-C_6, C_3H_7) or an increasing liver platinum content (JM269; c-C_7, CH_3) at time points ranging from 2 h to 10 days. This finding contrasted with the consistent decline in liver platinum content reported for i. v. cisplatin and i. v. carboplatin treatment after a single bolus dose in the mouse [1]. Moreover, there was a 7-fold (from 15 to 109 μg platinum/g) range of the highest liver platinum levels determined for these platinum(IV) complexes following single oral doses at the MTD, which was related to the size of their axial aliphatic and alicyclic amine functions. A similar correlation between kidney and plasma platinum concentrations and the size of these substituents was also found.

These results show that the tissue platinum distribution of oral ammine/amine platinum(IV) dicarboxylates contrasts with that of i. v. cisplatin and i. v. carboplatin, in that the liver (and not the kidney) is the major tissue platinum depot, and that the platinum levels achieved in this tissue were higher (by several factors) than those obtained with the conventional agents, highlighting the potential for this organ as a site of drug toxicity. Moreover, the variability in liver platinum content and time course appeared to be related to both the structure of the aliphatic axial and alicyclic

amine substituents and the systemic oral bioavailability of these octahedral platinum(IV) complexes.

Elevations in plasma ALT activity, indicative of enzyme release from damaged hepatocytes, were recorded following maximally tolerated single oral doses of JM225 and JM256. These increases were not related to either the level or the time-course profile of liver platinum content. They were not associated with histological abnormalities at the MTD, with the exception of a transient fatty change attributable to the arachis-oil drug vehicle and glycogen accumulation. Liver platinum content and plasma ALT activity was further studied with repeated-dose administration schedules. Significant accumulation of platinum in the liver was recorded with repeated oral dosing of JM269 (c-C_7) and JM225 (c-C_5), and elevated plasma ALT activity was recorded following four consecutive weekly oral doses of JM269. Moreover, plasma ALT activity was related to the magnitude of increase in liver platinum content with repeated oral dosing. These results suggest that oral ammine/amine platinum(IV) dicarboxylates are capable of inducing liver damage and that this toxicity may be related to the size of the alicyclic amine function and to the extent to which platinum is laid down in the liver with repeated oral dosing.

JM216 was selected from this series of six platinum(IV) complexes for further study. This complex produced neither significant hepatic platinum accumulation with repeated oral dosing nor elevations in plasma ALT activity in mice. Furthermore, oral JM216 has a superior therapeutic index by comparison with parenteral cisplatin and carboplatin in mice bearing the ADJ PC6 plasmacytoma [3], and its activity against human ovarian carcinoma cell lines and xenografts is at least similar to that of the existing platinum drugs [5]. In the ferret, oral JM216 has displayed an emetogenic potential similar to that of i.v. carboplatin [3], whereas this complex has shown a lack of nephrotoxicity at the MTD in rodents [13]. By comparison, the oral analogues JM225 and JM269 produced significant hepatic platinum accumulation, oral JM221 induced severe emesis in the ferret [3], oral JM291 displayed poor activity against human ovarian carcinoma xenografts [2] and oral JM256, JM225 and JM269 caused significant liver enzyme release in mice.

The haematological and gastrointestinal toxicities of JM216 were studied at time points ranging from 1 to 28 days following a single oral dose at the MTD. Transient severe reductions in the peripheral WBC and the bone-marrow nucleated cell content (both by approximately 75%) were recorded 2 days after treatment. Less severe reductions were seen in the peripheral blood platelet count (by 33% at day 10) and haemoglobin concentration (by 5% at day 2). The leucopenia induced by oral JM216 was of a severity similar to that reported for i.v. cisplatin and i.v. carboplatin in rodents at comparable doses, whereas the anaemia and thrombocytopenia were less severe than the abnormalities induced by the clinical agents [7]. The comparative gastrointestinal toxicity of i.v. cisplatin, i.v. carboplatin and oral JM216 given as single doses at the MTD was investigated by assaying disaccharidase activity in scrapings of the small-intestinal mucosa. There was a 70% reduction in jejunal mucosal disaccharidase activity for the reference i.v. platinum agents, which had recovered by 14 days after treatment. However, there was no significant change in disaccharidase activity, indicating a lack of small-intestinal mucosal damage with oral JM216. These results suggest that the dose-limiting effects of oral JM216 in mice are myelosuppression and leucopenia and that small-intestinal mucosal damage is less severe for oral JM216 than for i.v. cisplatin or i.v. carboplatin at the MTD in mice.

For this work, the MTD was defined as the dose causing significant (>5%) but reversible loss of body weight that was ≤25% below the dose known to cause moribundity. The conclusions drawn regarding the comparative tissue distribution and toxicology of these oral ammine/amine platinum(IV) dicarboxylates, i.v. cisplatin and i.v. carboplatin were based on the assumption that these studies were undertaken at equi-toxic doses, and variability of the MTD determinations were a potential source of bias. However, at the MTD, body weight loss ranged from 6.1% to 18%, and all MTD estimates were a median of only 20% lower than the dose at which moribundity was encountered at an incidence of 18%–33%, consistent with only a modest degree of variability in these MTD determinations. An alternative may have been to use the dose lethal to 10% of the study population (LD_{10}) or LD_{50}, but there is increasing concern regarding the use of lethality as an experimental endpoint [14, 15], and the day-to-day reproducibility of the lethality testing of antineoplastic agents in mice can be poor [16].

In conclusion, the liver is the major tissue depot for oral ammine/amine platinum(IV) dicarboxylates and is a site of toxicity for examples of this class. The structure of the aliphatic axial and alicyclic amine substituents was related to the content, time-course profile and accumulation of platinum in the liver, and hepatic enzyme release with single and repeated oral dosing. Oral JM216 was selected for further study on the basis of its antitumour activity and toxicity profile and caused dose-limiting leucopenia but less gastrointestinal toxicity than i.v. cisplatin or i.v. carboplatin at the MTD in the mouse.

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References

1. Siddik ZH, Jones MR, Boxall FE, Harrap KR (1988) Comparative distribution and excretion of carboplatin and cisplatin in mice. Cancer Chemother Pharmacol 21: 19–24
2. Kelland LR, Jones M, Gwynne JJ, Valenti M, Murrer BA, Barnard CFJ, Vollano JF, Giandomenico CM, Abrams MJ, Harrap KR (1993) Antitumour activity of orally administered ammine/amine platinum(IV) dicarboxylate complexes against a panel of human ovarian carcinoma xenografts. Int J Oncol 2: 1043–1048
3. Harrap KR, Murrer BA, Giandomenico C, Morgan SE, Kelland LR, Jones M, Goddard PM, Schurig J (1991) Ammine/amine platinum IV platinum dicarboxylates: a novel class of complexes

which circumvent intrinsic cisplatin resistance. In: Howell SB (ed) Platinum and other metal coordination compounds in cancer chemotherapy. Plenum, New York, pp 391–399
4. Kelland LR, Murrer BA, Abel G, Giandmenico CM, Mistry P, Harrap KR (1992) Ammine/amine platinum(IV) dicarboxylate complexes: a novel class of platinum complex exhibiting selective cytotoxicity to intrinsically resistant human ovarian carcinoma cells. Cancer Res 52: 822–828
5. Kelland LR, Abel G, McKeage MJ, Jones M, Goddard PM, Valenti M, Murrer BA, Harrap KR (1993) Preclinical antitumour evaluation of bis-acetato-ammine-dichloro-cyclohexylamine platinum(IV): an orally active platinum drug. Cancer Res 53: 2581–2586
6. Reed E, Kohn KW (1990) Platinum analogues. In: Chabner BA, Collins JM (eds) Cancer chemotherapy: principles and practice. J. B. Lippincott, Philadelphia, pp 465–490
7. Siddik ZH, Boxall FE, Harrap KR (1987) Haematological toxicity of carboplatin in rats. Br J Cancer 55: 375–379
8. Allan S, Smyth JF, Hay FG, Leonard RCF, Wolf CR (1986) Protective effect of sodium-2-mercaptoethanesulfonate on the gastrointestinal toxicity and lethality of *cis*-diamminedichloroplatinum. Cancer Res 46: 3569–3573
9. Siddik ZH, Dible SE, Boxall FE, Harrap KR (1986) Renal pharmacokinetics and toxicity of cisplatin and carboplatin in animals. In: McBrien DCH, Slater TF (eds) Biochemical mechanisms of platinum antitumour drugs. IRL Press, Oxford, pp 171–198
10. Siddik ZH, Boxall FE, Harrap KR (1987) Flameless atomic absorption spectrophotometric determination of platinum in tissues solubilized in hyamine hydroxide. Anal Biochem 163: 21–26
11. Dahlqvist A (1968) Assay of intestinal disaccharidases. Anal Biochem 22: 99–107
12. Lowry OH, Rosenbrough NH, Farr AL, Randall RJ (1951) Protein measurement with the Folin reagent. J Biol Chem 193: 265
13. McKeage MJ, Morgan SE, Boxall FE, Murrer BA, Hard GC, Harrap KR (1993) Lack of nephrotoxicity of oral ammine/amine platinum(IV) dicarboxylate complexes in rodents. Br J Cancer 67: 996–1000
14. Aldhous P (1991) Tide turns against LD50. Nature 353: 489
15. Balls M (1991) Why modification of the LD50 test will not be enough. Lab Anim 25: 198–206
16. Guarino AM, Rozencweig M, Kline I, Penta JS, Venditti JM, Lloyd HH, Holzworth DA, Muggia FM (1979) Adequacies and inadequacies of assessing murine toxicity data with antineoplastic agents. Cancer Res 39: 2204–2210