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SHORT COMMUNICATION

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The tissue distribution in BALB/c mice of C-14-labeled JM216, an orally active platinum antitumour compound

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Abstract The ammine/amine platinum(IV) dicarboxylates have been developed as orally active platinum antitumor agents, and one of these, $[PtCl_2(NH_3)(C_6H_{11}NH_2)(OCOCH_3)_2]$ (JM216), is undergoing clinical trials at present. A synthesis method was developed to radiolabel JM216 with carbon 14 at the carboxylate carbon. The labeling efficiency was 92%, and the purity as shown by high-performance liquid chromathography (HPLC) was 96% after recrystallisation. The radiolabeled JM216 was given orally to BALB/c mice and detailed tissue-distribution data were obtained (blood plasma, kidney, liver, spleen, brain, lung, muscle and skin) for time points of 2 h and 2, 6 and 10 days. Comparison of these data with previously reported data for distribution of platinum obtained by atomic absorption spectroscopy has shown distinct differences, especially for the liver and the kidney. This clearly indicates a difference in behaviour between the labeled ligand and the platinum centre, suggesting detachment of the ligand in vivo.

Key words JM216 · Platinum tissue distribution · BALB/c mice · Carbon 14

Introduction

Since the introduction of cisplatin [*cis*-diamminedichloroplatinum(II), cis-DDP] therapy there have been great improvements in the treatment of bladder, ovarian and testicular cancers [1, 2]. There has been much further research to develop new platinum drugs to moderate the serious side effects (such as nephrotoxicity and nausea, amongst others) and to extend the range of action to cisplatin-resistant tumours. However, only one such drug, CBDCA [*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II), carboplatin], has proceeded beyond clinical trials to be licensed for clinical use.

The development of an oral platinum drug is advantageous in terms of both ease of administration and also the possibility of treatment of outpatients with the resultant reduction of hospitalisation costs. Both cisplatin and CBDCA are given by i.v. injection and have been found to be poorly absorbed by the gastrointestinal tract when given orally (bioavailability in the mouse 32% for cis-DDP, 10% for CBDCA) [3]. Comparison of the biodistribution of Pt-195m-labeled CBDCA by i.v., i.p. and oral administration routes [4] has shown a similar distribution in all administration regimes, but the platinum concentrations measured after oral administration were 4–5 times lower than those detected after administration via the other routes.

A new class of platinum antitumour compounds, the platinum(IV) ammine/amine dicarboxylates, has been developed by Johnson Matthey, the Institute of Cancer Research and Bristol-Myers Squibb. These compounds can be given orally and display excellent antitumour activity in vitro and in animal models [5, 6]. The compounds are suitable for oral administration as they are neutral, kinetically inert, acid-stable and lipophilic; the compounds are well absorbed from the gastrointestinal tract, unlike their bis-hydroxy platinum(IV) or platinum(II) analogues [3]. There is evidence that the compounds are less emetogenic than cis-DDP in an animal model [7] and that they display activity against certain cis-DDP-resistant tumour lines in vitro. The platinum(IV) mixed-amine compounds have the general formula $[PtCl_2(OCOR_1)_2NH_3(RNH_2)]$, whereby R_1 and R can be aliphatic, alicyclic or aromatic. The compounds display solubility in both water and organic solvents, and this property may contribute to the antitumour activity displayed by the compounds by influencing the path of transition of cell membranes [8].

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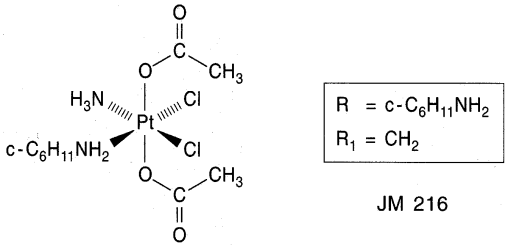


Fig. 1 Structure of JM216

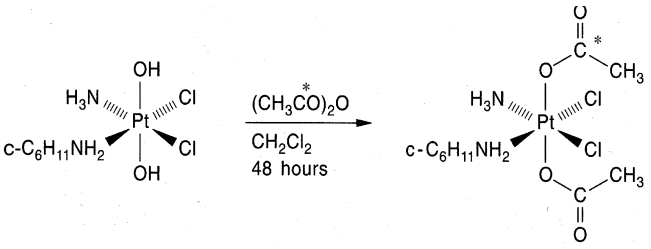


Fig. 2 Synthesis of C-14-labeled JM216

Platinum(IV) compounds undergo ligand-substitution reactions much more slowly than their platinum(II) analogues [9], and it is therefore most likely that this class of drug is reduced in vivo to the more reactive derivatives $[PtCl_2(NH_3)(RNH_2)]$. DNA-binding studies using the compound $[PtCl_2(NH_3)(C_6H_{11}NH_2)]$, the expected metabolite of $[PtCl_2(OCOC_3H_7)_2NH_3(C_6H_{11}NH_2)]$, have shown [10] that the major adduct formed by the platinum centre is an intrastrand cross-link between adjacent guanosine residues; this behaviour is analogous to that of cisplatin and supports the theory that the platinum(IV) ammine/amine compounds act as pro-drugs to the active platinum(II) species.

However, there is also evidence that the mode of action of the platinum(IV) complexes is not as straightforward as this. Studies on the DNA interaction of oxoplatin [*cis*-diamminedichloro-*trans*-dihydroxyplatinum(IV)] have shown that oxoplatin will coordinate directly to DNA, forming adducts in which the platinum(IV) state is maintained. The reaction is slower than that seen with the platinum(II) analogue cis-DDP, although hydrolysis of chloride ions before binding to DNA is necessary (as with cis-DDP) and a similarly strong preference for guanosine residues is displayed [11].

After administration of a platinum(IV) complex, one would expect that a significant amount of the complex would be reduced before the cellular target is reached. It has been shown that incubation of one of the platinum(IV) mixed-amine complexes, JM216 [*bis*-acetatodichloroammineclohexylamineplatinum(IV)], in human plasma gives rise to *cis*-amminedichlorocyclohexylamineplatinum(II) (JM118), but also to the complexes *bis*-acetatoammineclohexanedihydroxoplatinum(IV) (JM383) and *bis*-acetatoammineclorocyclohexanehydroxoplatinum(IV) (JM518). JM118 and JM383 have also been identified in clinical samples and both have been shown to have strong antitumour activity [12, 13]. It is thus quite conceivable that the action of JM216 arises due to one or more metabolitic pathways.

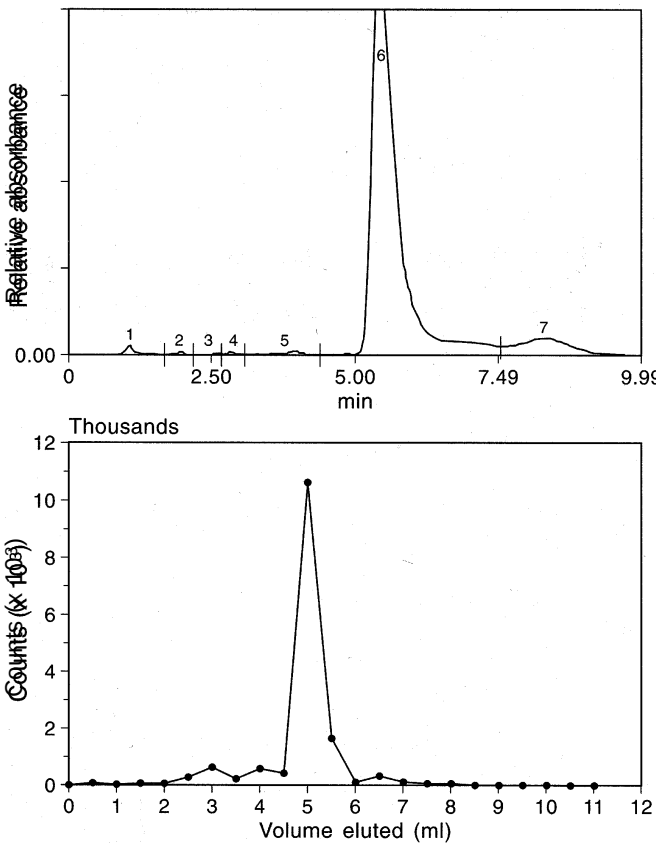


Fig. 3 UV-Vis (210-nm) chromatogram of C-14-labeled JM216 (*top*) and radiochromatogram of C-14-labeled JM216 (*bottom*)

In the present study a radiosynthesis of JM216 was completed (the structure of the complex is shown in Fig. 1) and the compound was labeled by C-14 at the carboxylate carbon. A detailed tissue-distribution study was carried out in BALB/c mice with this radiolabeled compound using an oral dose of 200 mg/kg as a suspension in arachis oil, with tissue levels of the label being measured at time points of 2 h and 2, 6 and 10 days. Similar animal organ-distribution studies have been reported [14] in an identical model in which tissue concentrations of platinum were measured by atomic absorption spectroscopy (AAS). Our experiments were designed to compare the distribution of platinum (found in the previous study by the ICR [14]) and of the C-14 ligand.

Materials and methods

Radiosynthesis of C-14-labeled JM216

The method used in the radiosynthesis follows that used by Johnson Matthey in larger-scale synthesis of the same compound and has been used with success in our laboratory to acetylate *cis-trans*-dichlorodihydroxybis(isopropylamine)platinum(IV) (CHIP) [8]. The reaction scheme is shown in Fig. 2.

bis-Hydroxodichloroammineclohexylamineplatinum(IV)(JM149) was obtained from Johnson Matthey Technology Centre, Sonning

Table 1 Tissue distribution in female BALB/c mice of carbon-labeled JM216, expressed as (tissue counts/g)/(dose counts)×100%

Tissue	2 h	2 days	6 days	9.5 days
Liver	1.279 ±0.364	0.609 ±0.055	0.359 ±0.027	0.145 ±0.010
Left kidney	1.234 ±0.379	0.480 ±0.046	0.442 ±0.035	0.211 ±0.016
Right kidney	1.161 ±0.335	0.500 ±0.056	0.433 ±0.029	0.202 ±0.018
Plasma	0.296 ±0.049	0.177 ±0.040	0.061 ±0.025	0.037 ±0.001
Spleen	0.422 ±0.072	0.298 ±0.169	0.422 ±0.098	0.071 ±0.019
Skin	0.279 ±0.050	0.129 ±0.065	0.228 ±0.026	0.073 ±0.025
Muscle	0.201 ±0.056	0.120 ±0.029	0.131 ±0.033	0.112 ±0.059
Lung	0.338 ±0.090	0.281 ±0.063	0.381 ±0.073	0.131 ±0.047
Brain	0.175 ±0.029	0.111 ±0.020	0.102 ±0.044	0.114 ±0.014

Table 2 Tissue distribution in female BALB/c mice of carbon-labeled JM216, expressed as (tissue counts/whole organ)/(dose counts)×100%

Time point	Organ	Uptake	±	SD
2 h	Brain	0.038	±	0.007
	Left kidney	0.157	±	0.059
	Right kidney	0.149	±	0.051
	Liver	1.830	±	0.522
	Spleen	0.033	±	0.007
2 days	Brain	0.024	±	0.005
	Left kidney	0.059	±	0.006
	Right kidney	0.067	±	0.009
	Liver	0.872	±	0.080
	Spleen	0.018	±	0.017
6 days	Brain	0.022	±	0.010
	Left kidney	0.062	±	0.005
	Right kidney	0.057	±	0.004
	Liver	0.516	±	0.039
	Spleen	0.027	±	0.007
9.5 days	Brain	0.024	±	0.003
	Left kidney	0.031	±	0.003
	Right kidney	0.032	±	0.003
	Liver	0.207	±	0.015
	Spleen	0.006	±	0.002

Common, Reading. Acetic anhydride; *N,N*-dimethylacetamide; and dichloromethane were obtained from Sigma Chemical Co., Poole, Dorset. C-14-labelled acetic anhydride was obtained from ICN Biomedicals, Thame, Oxfordshire.

Stirring of JM149 (166 mg) in dichloromethane (2 ml) together with acetic anhydride (68 µl) and C-14-labeled acetic anhydride (5 µl, 400 µCi) for 36 h affords JM216 as a white solid. The product was washed with acetone (1 ml) and then recrystallised from hot DMA (0.5 ml) and water (1 ml). The mixture was stirred in an ice bath for 2 h to ensure maximal crystallisation. The supernatant was removed and the dried product was kept in darkness until needed for use. (JM216 is stable in solid form and made up for injection only immediately before its administration.)

Yield and purity testing

All waste solutions were kept, and samples of these and the product were counted using a scintillation counter. The total activities were as follows:

Product activity (157.9 mg JM216) = 178.5 µCi
All combined residues (washing and recrystallisation) = 208.54 µCi.

This equates to a yield of 89% since only half of the C-14 is available for labeling during the reaction with acetic anhydride. For study of the quality control of JM216, reverse-phase high-performance liquid chromatography (HPLC) with both UV-Vis detection and beta-scintillation counting was used. The liquid chromatograph used consisted of two pumps (Shimadzu LC-6A) linked by a mixing chamber, with the flow rate and/or composition being determined by a pump control system (Shimadzu). All assays were performed at room temperature. A Kontron Instruments HPLC Detector 432 set at 210 nm with attenuation sensitivity 0.5 was used for UV-Vis analysis; this was connected to a PC with a Softron PC integration package.

Acquired UV-Vis data were stored and manipulated after each assay was complete. The eluent was collected using a fraction collector (Pharmacia UK) in fractions of 0.5 ml, and these fractions were counted using a beta-scintillation counter (Hewlett Packard). Comparison of UV-Vis and gamma-counter data was thus possible; the retention times of known standards and of radioactive complexes are almost identical. Identification of the radioactive fractions was thus possible using the retention time; hence, calculation of the radioactive purity of the desired complex was possible.

The column used was an 18-cm, 4.6-mm C_{18} Nucleosil column with a precolumn; isocratic elution conditions were used, the solvent being 3:1 (v/v) water:acetonitrile and the flow rate, 1.25 ml/min. Figure 3 shows the chromatograms obtained by UV-Vis and beta-counting; the overall purity of the final product was found to be 96%. The main impurity would seem to be $[PtCl_2(OCO\text{-}CH_3)(OH)(NH_3)(C_6H_{11}NH_2)]$ due to incomplete reaction, the majority of this impurity being removed during recrystallisation.

Tissue distribution of C-14 label following oral administration of JM216

BALB/c mice (female, approximately 20 g) were obtained from the Biological Services Unit of the University of Manchester. Arachis oil was obtained from Sigma Chemical Co. Five animals were used for each time point of 2 h and 2, 6 and 9.5 days post-administration. Each animal received the dose resulting in 10% lethality (LD_{10}) [11] of 200 mg/kg body weight, 3.8 mg (3-µCi activity) per animal. The dose was given by gavage as a suspension in arachis oil (ca. 0.4 ml, 10 mg/ml) using a 4-cm oral needle. Food was withheld overnight before administration. The animals were killed by exsanguination under fluothane anaesthesia. They were then weighed and a blood sample (approx. 1 ml) was removed by cardiac puncture. Tissue samples were taken of the liver, left and right kidney, spleen, brain, muscle and fat and were weighed in vials. Weights of the whole organs were recorded before a smaller (ca. 50 mg) was taken for analysis. The blood sample was centrifuged (2000 rpm, 10 min) and a plasma sample (50 µl) was taken. All the samples were dissolved in 1 ml digestion medium (Optisolv) and then made up to 10 ml with scintillation cocktail (Optiphase). The samples were counted with appropriate internal standards on a scintillation counter.

Results and discussion

Carbon-14 label distribution

Table 1 shows the numerical data obtained for the distribution of the carbon 14 label in the blood plasma and tissue samples, expressed as a percentage of the dose per gram of tissue (or milliliter of plasma) following oral administration

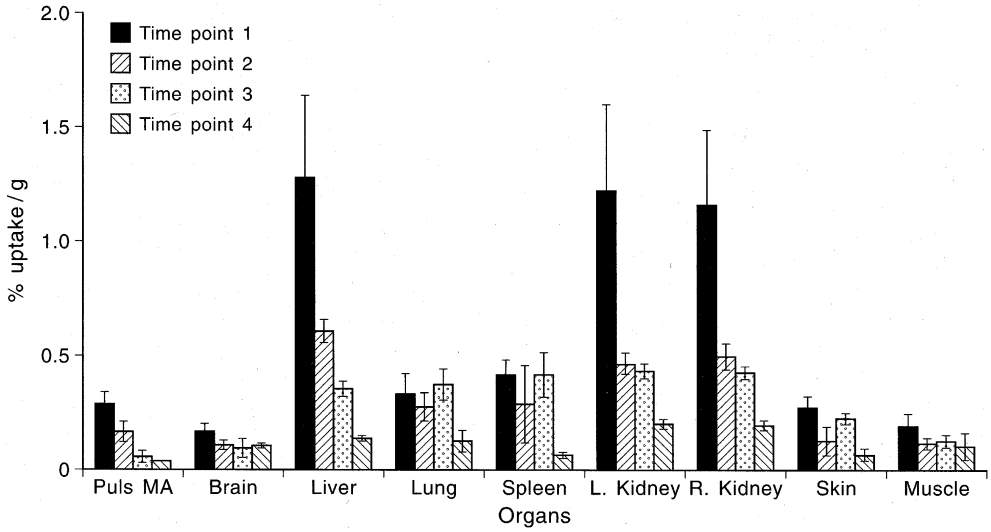


Fig. 4 Tissue label concentrations, expressed as (tissue counts/g)/(dose)×100%, detected at time points of 2 h and 2, 4 and 10 days

of JM216, i.e. T/D % = (Tissue counts/g)/(Dose)×100%. Table 2 shows the numerical data recorded for the label, expressed as a percentage of the dose per organ, i.e., *(Tissue counts/organ)/(Dose)*×100%.

The tissue-label concentrations are expressed as bar charts in Figs. 4 and 5 (counts per gram and counts per organ, respectively). The first point to note is that there are counts in all tissues at the first time point. This demonstrates that by 2 h post-administration the drug has been absorbed into the organs and systemic circulation, i.e. C-14-labeled JM216 is absorbed after oral administration.

Secondly, similar changes in concentration in liver and kidney samples after oral administration of JM216 are displayed. These tissues have the maximal concentration of label at the 2-h time point, with removal occurring over the duration of the experiment. This removal appears to be biphasic, the major fall in concentration of the label (53% in liver, 60% in kidney) being removed at between 2 h and 2 days and the rest, over the time scale of the experiment. It is noteworthy that this tailing off is due to two reasons: firstly, the normal biological elimination of the drug as a course of the normal metabolism, and secondly, the expected removal of carbon-14 label prior to or during in vivo reduction of platinum(IV) to the platinum(II) species, the active metabolite. These results suggest that the kidney and liver play a part in this reduction (or removal of acetate). The other tissues (lung, spleen, muscle) show steadier concentrations, especially over the first 6 days; however measurable concentrations of carbon-14 label are present in all the tissues. This suggests the following:

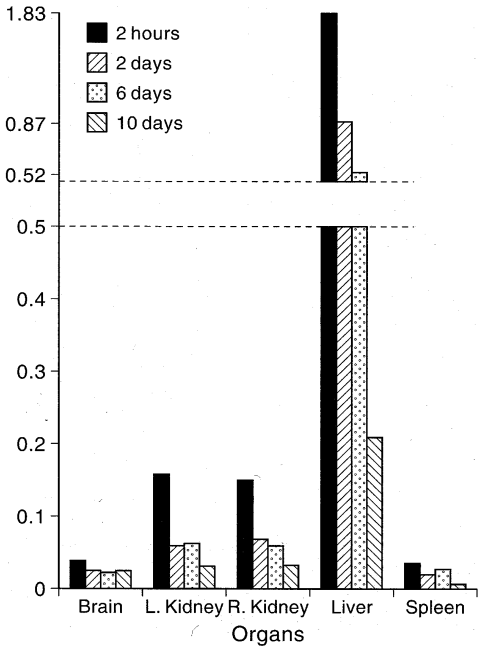
1. Either metabolism to the active form takes place over a long period (JM216 is present unchanged) – plasma metabolite studies appear to discount this.
2. The presence of another C-14-containing metabolite – liquid chromatography with on-line mass spectrometry (LC-MS) of biological fluids has shown the presence of the acetate-containing complexes JM383 and the two isomers of JM518 [$\{PtCl(OCOCH_3)_2(OH)(NH_3)(C_6H_{11}NH_2)\}$], among other metabolites such as JM118 [12, 13]. The possibility of binding of such platinum(IV) complexes to DNA exists, although JM518 is very short-lived [12]. There is also the possibility of other species such as glutathione adducts that contain platinum bound to C-14.
3. Metabolism of C-14-containing acetate groups.

The increased standard error of the earlier time points is probably a factor of the differing stomach contents of individual animals; overnight fasting of the animals before the experiment was performed to minimise this error.

Comparison of the tissue distribution of the label and of platinum

The animal model was chosen such that comparison of tissue-distribution data found using C-14-labeled JM216 and FAAS of platinum (published ICR data [14]) was valid.

Fig. 5 Organ label concentrations, expressed as (tissue counts/organ)/(dose)×100%, detected at time points of 2 h and 2, 4 and 10 days (*L.* Left, *R.* right)



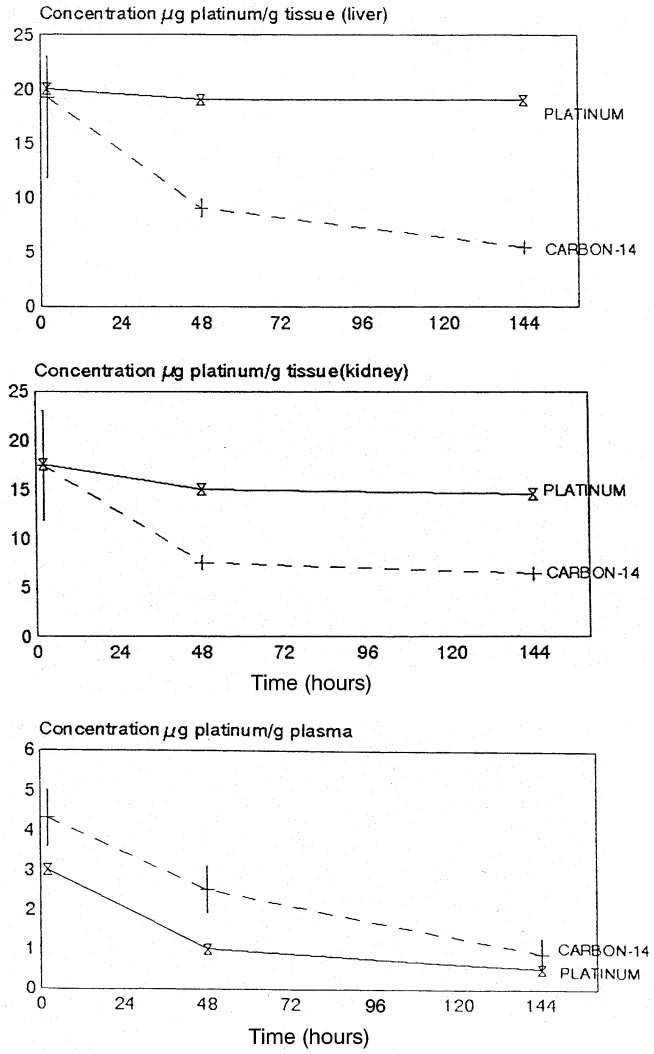


Fig. 6 Percentage of uptake/g in liver, kidney and plasma of platinum (ICR data [11]) and platinum bound to carbon label (beta scintillation, this work) as detected at time points of 2 h and 2, 6 and 10 days after a single oral LD_{10} dose of JM216

Figure 6 shows a comparison of the tissue levels of the C-14 label and of platinum detected in the liver, kidney and plasma, respectively.

For all the tissues the level of platinum and carbon label found at the initial time point is similar; there appears to be no loss of C-14 label (and, hence, acetate ligand) from platinum at this time. This points to the finding not only that the JM216 molecule is absorbed from the gut intact but also that platinum is absorbed only in this form, any reactions (e.g. formation of JM118) taking place after absorption.

Finally, the differing levels of platinum and C-14 detected in the tissues with time appear to point to removal of the C-14 from the platinum centre. The levels of C-14 fall rapidly (60% of the maximum in the kidney and 53% in the liver in just 2 days as compared with 14% and 5% for platinum, respectively; Fig. 6). This clearly points to in vivo dissociation of the acetate, with the platinum and acetate following a different metabolic pathway in the kidney and liver. This is consistent with the theory that the acetate groups are removed from JM216 and the platinum(IV) species is thus reduced to the active platinum(II) species $[PtCl_2(NH_3)(C_6H_{11}NH_2)]$ (JM118). An LC-MS study has confirmed that JM216 is short-lived in blood plasma; this metabolism takes place quickly [13].

These results appear to suggest that any acetate containing the metabolites JM383 and JM518 must be short-lived in the kidney and liver. The presence of steady levels of C-14 in other tissues (skin, muscle, lung and spleen) at later time points, coupled with the observation that JM216 itself is short-lived in vivo, would appear to suggest that some platinum- and C-14-containing complex, perhaps a metabolite of JM383 or JM518, is bound in these other tissues.

The comparison of blood plasma levels of platinum and C-14 label shown in Fig. 6 gives a higher level of label than expected, even allowing for experimental error. It is suggested that this is due to removal of the C-14 acetate from the JM216 and its elimination from the tissues via the systemic circulation.

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