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CISPLATIN METABOLITES IN PLASMA, A STUDY OF THEIR PHARMACOKINETICS AND IMPORTANCE IN THE NEPHROTOXIC AND ANTITUMOUR ACTIVITY OF CISPLATIN

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Abstract—For rats dosed with cisplatin the rate of appearance in plasma of ultrafilterable metabolites containing platinum has been investigated using HPLC. At least seven species containing platinum in addition to cisplatin are present 15 min following injection i.p. of 15 mg kg^{-1}. Unchanged cisplatin has been almost completely eliminated from the plasma within 3 hr of dosing; however, metabolite species are still present. The same metabolite species form when cisplatin is incubated *in vitro* with plasma although in different proportions. After incubation for 24 hr at 37° a mixture of metabolites is produced which contains less than 4% cisplatin. This mixture, when injected i.p. into rats, is nephrotoxic at doses of platinum at which cisplatin is not. The mixture of metabolites has considerably less antitumour activity than cisplatin when tested against the mouse L1210 leukemia assay. Although no metabolite species has been unequivocally identified we present evidence which suggests that amongst the principle metabolite species are an hydrolysis product and methionine substitution products of cisplatin. A mixture of cisplatin methionine substitution complexes showed neither antitumour nor nephrotoxic properties. However, an hydrolysis product was shown to be nephrotoxic at a dose of platinum at which cisplatin is not.

The work reported here is the first direct experimental demonstration that cisplatin metabolites are more nephrotoxic but less effective antitumour agents than the parent compound.

Cisplatin (*cis*-dichlorodiammine platinum II) is a potent antitumour agent with severe dose-limiting nephrotoxic side-effects [1]. When introduced into animals or patients it is known that some of the parent drug is converted into other compounds [2]. This conversion probably involves simple chemical reactions and possibly some enzymic reactions. The details of these mechanisms and the extent of their contribution to the overall changes have not been elucidated; we refer to the mixed products of these processes as metabolites. Until recently [2] investigations into the mechanism, rate and extent of metabolism of cisplatin were limited by the lack of a suitable method for separation of the metabolites. Nevertheless, a role for cisplatin metabolites in the development of the nephrotoxic lesion has been suggested by several workers [3–5]. Others have suggested that the production of metabolites is a necessary prerequisite for the antitumour activity of cisplatin [6–8]. The metabolites of cisplatin which form in plasma include both protein bound high molecular weight species and low molecular weight, non-protein bound ultrafilterable species. It has been shown [9] that protein bound platinum does not cause nephrotoxicity in rats. In this paper we report an investigation into the pharmacokinetics of the low molecular weight metabolites of cisplatin. Using a method for the separation of cisplatin metabolites developed by us [2] we have investigated the rates of production of different metabolites *in vivo* and *in vitro* in rat plasma and the biological activity (nephrotoxicity and antitumour activity) of a mixture of metabolites.

MATERIALS AND METHODS

Animals. Male Wistar rats, 200–250 g (Charles River, Margate), were housed individually in plastic cages. Male DBA2 mice, 20–23 g (Bantin and Kingman, Hull), were housed in groups of 6–10 in plastic cages. All animals were allowed *ad lib.* access to food and water.

Chemicals. Cisplatin (*cis*-dichlorodiammine platinum II), chromatographically pure, was a gift from Johnson Matthey Research, Reading. Chromatographic solvents and other chemicals were of HPLC grade (BDH Chemicals, Poole). Water used in HPLC was glass double distilled, deionized and Millipore filtered.

Separation of cisplatin metabolites. Metabolite species and unchanged cisplatin present in samples of protein-free plasma (pfp) were separated by HPLC using a method previously described [2]. In principle the method involved loading samples (10–200 μl) onto Spherisorb octadecyl silica (5 μm) columns (0.5 × 25 cm or 50 × 1 cm) which had been pre-equilibrated with 5 mM sodium dodecyl sulphate (an ion-pairing agent). The samples were then eluted with a gradient of increasing concentration of 90% v/v aqueous acetonitrile and 0.5 cm^3 fractions of the column effluent collected for analysis. Fractions were analysed for platinum using flameless atomic absorp-

tion spectroscopy (NFAA) with a detection limit of approximately 10 ng/cm³ Pt. Samples of pfp containing low concentrations of platinum were concentrated 20-fold by lyophilization prior to separation. (Increasing the concentration of samples by more than 20-fold results in a loss of resolution when the samples are subsequently separated by HPLC.)

Preparation of blood and plasma samples for hplc separation. Blood samples were taken from the caudal veins of rats. The samples were immediately centrifuged to remove cells and the plasma was then filtered through Amicon centriflow ultrafiltration membranes (CF25) to remove proteins. These operations were performed as quickly as possible, in any event in less than 5 min after collection. Pfp thus produced was examined by HPLC to determine the composition of cisplatin metabolites or used for *in vitro* incubations (see below). Pfp samples not required for immediate HPLC separation were stored at −20° and analysed immediately after thawing. Samples of whole plasma used in *in vitro* incubations with cisplatin were filtered through Amicon ultrafilters prior to separation by HPLC.

Assessment of nephrotoxicity. Nephrotoxicity was assessed in rats by the measurement of blood urea (BUN) and by histopathological examination of the kidneys. Blood was withdrawn from the caudal vein and urea determined using a Boehringer, Mannheim kit based on urease. BUN levels were determined daily for seven days following injection of potential nephrotoxic agents. Kidneys for histopathological examination were exposed in animals anaesthetized with 50 mg/kg sodium pentobarbital (i.p.) and perfused *in situ* with 5% formaldehyde in phosphate buffered saline (pH 7.4). After excising and slicing into two the kidney halves were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Sections (5 μm) were stained with periodic acid Schiff reagent and haemotoxylin.

Antitumour effects of platinum compounds. Antitumour effects of platinum compounds were assessed in mice carrying L1210 leukemia. Mice were inoculated i.p. with 10^5 L1210 cells on day 0. After 24 hr animals were dosed i.p. with the test solutions. Six to ten animals were used in each test group. The animals were weighed daily and their life spans measured. The median length of life for controls was 9 days, animals surviving more than 30 days were counted as cured.

RESULTS

Cisplatin metabolism in vivo

Rats were injected with a fresh solution of cisplatin in saline (15 mg/kg i.p.). Blood samples were collected at various times and the composition of the metabolites in pfp determined by HPLC. The recovery of platinum from the columns was complete. Figure 1 shows HPLC elution profiles for pfp 2 hr and 3 hr after injection. Figure 1(I) shows the presence of nine discrete platinum species in rat pfp 2 hr after injection of cisplatin. Species B is unchanged cisplatin. The other species, assigned letters A to G have been quantified in animals between 15 min and 3 hr post injection. These data are shown in Table 1. The data indicate that the proportions of the principal

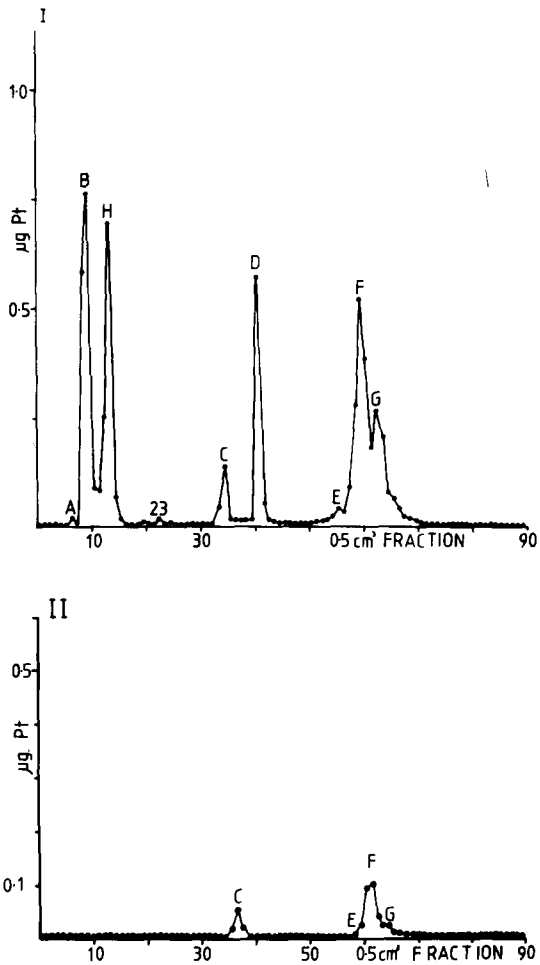

Fig. 1. HPLC elution profiles of platinum species in protein free rat plasma obtained 2 hr and 3 hr after injection of 15 mg/kg cisplatin. Samples: 100 μl of 20-fold concentrated pfp. Vertical axis shows μg Pt per 0.5 cm³ fraction. B is unchanged cisplatin (column 25 × 5 cm Spherisorb ODS).

metabolite (F) and cisplatin (B) do not vary substantially between different animals. However, considerably more variation is seen in the amounts of the metabolites present at lower concentrations. It can be seen in Table 1 that the concentration of ultrafilterable platinum in plasma falls rapidly and exponentially after injection ($t_{1/2}$ = 33 min), reflecting both an increase in the binding of platinum to plasma proteins and the removal of soluble platinum species by the kidney. By 3 hr following dosing the concentration of unchanged cisplatin remaining in the plasma was very low and was undetectable in one of the animals used. The principal metabolite species are those which elute in fractions 35 (C) and 60–62 (F). Fifteen minutes after injection C is the most abundant metabolite and accounts for approximately 10% of the total ultrafilterable plasma platinum. At 3 hr post injection, when almost no unchanged cisplatin remains in the plasma and the total platinum content of the plasma is much lower, species F is the most abundant metabolite and C and F are approximately 15% and 50% of the ultrafilterable plasma platinum respectively. Figure 2 shows

Table 1. Cisplatin metabolites formed in rat plasma *in vivo*

Time following injection (hr)	Total ultra-filterable platinum (μg/cm³)	Plasma protein binding (%)	Platinum species as a % of total ultrafilterable platinum							
			A	B (cisplatin)	C	D	E	F	G	H
0.25	12.39 ± 3.5	37.5 ± 8.5	ND	54.7 ± 1.7	9.7 ± 7.2*	3.4 ± 2.5	2.1 ± 0.3	6.2 ± 2.8	5.3 ± 4.5	7.2 ± 8.8*
1.0	1.69 ± 0.27	54.5 ± 7.7	1.2 ± 0.9*	49.5 ± 13.2	5.5 ± 4.1	4.0 ± 3.3	3.2 ± 1.1	13.7 ± 4.3	4.9 ± 2.8	4.2 ± 3.4*
2.0	0.39 ± 0.16	89.8 ± 2.7	0.22 ± 0.21*	23.5 ± 3.5	6.1 ± 2.5	12.6 ± 0.6	7.0 ± 4.9	40.0 ± 11.6	8.2 ± 4.8	5.4 ± 0.1
3.0	0.14 ± 0.04	95.6 ± 0.8	1.2 ± 1.1*	0.35 ± 7.3*	13.5 ± 7.3	2.3 ± 2.2*	13.4 ± 6.3	47.1 ± 8.5	11.4 ± 1.9	0.5 ± 0.1*

ND, not detectable in any animal (values are means ± S.D., N = 3).
* Not detected in one animal.

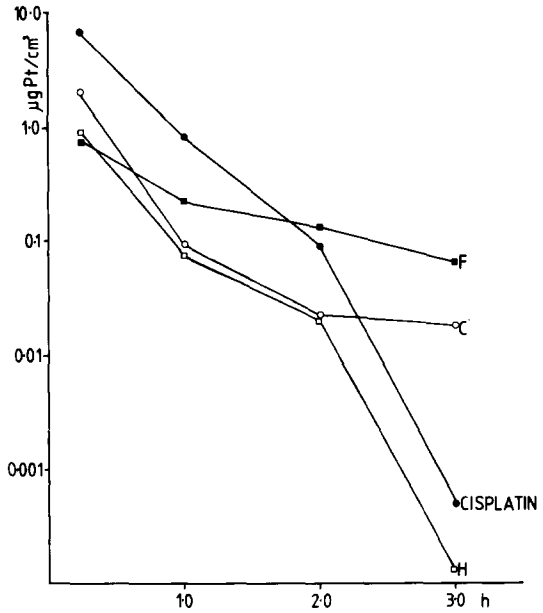

Fig. 2. The change in concentration *in vivo* of cisplatin and three metabolite species; C, F and H, between 15 min and 3 hr after injection of cisplatin at 15 mg/kg i.p. Concentration is a log scale.

the changes in the relative concentrations of unchanged cisplatin and the principal metabolites in the plasma in the 0–3 hr period.

Formation of metabolites in vitro

Cisplatin was incubated at 1 mg/cm³ in whole rat plasma at 37° in the dark. Prior to incubation plasma was sterilized by passage through 0.2 μ Millipore filters. Samples were removed at various times, protein removed by ultrafiltration and the platinum species in the pfp examined by HPLC as before. Table 2 shows the results and Fig. 3 shows typical HPLC elution profiles. It can be seen (Table 2) that the increase in binding of platinum to plasma proteins during the incubation causes a fall in the concentration of total platinum in the ultrafiltrate. This decline is rapid and exponential with a $t_{1/2}$ of 57 min. After 24 hr approximately 10% of the initial concentration of platinum is still in solution. The HPLC elution profiles (Fig. 3) show peaks for platinum species with the same retention volumes as those observed *in vivo*. If the species formed *in vitro* are chemically identical to the species formed *in vivo* with the same retention volumes then species F is the most abundant metabolite formed *in vitro* eventually forming more than 50% of the total soluble platinum. After 24 hr incubation unchanged cisplatin is present only in trace amounts (Fig. 3). When pfp is used directly for incubation with cisplatin (200 μg/cm³) the loss of soluble platinum from the plasma due to protein binding does not occur and the metabolite species form in higher concentrations than when whole plasma is used. By incubating pfp and cisplatin under these conditions for 24 hr (Fig. 4) it is possible to produce a solution containing a mixture of metabolites which does not contain more than 4% of platinum as unchanged cisplatin. Solutions of metabolites

Table 2. Cisplatin metabolites formed in rat plasma *in vitro*

Incubation time (hr)	Total ultra-filterable platinum ($\mu g/cm^3$)	Plasma protein binding (%)	Platinum species as a % of total ultrafiliterable platinum							
			A	B (cisplatin)	C	D	E	F	G	H
0.25	597 ± 138	10.5 ± 2.4	0.02 ± 0.02*	87.5 ± 6.7	1.8 ± 0.9	0.3 ± 0.2*	2.3 ± 1.8	7.0 ± 3.8	0.3 ± 0.2*	0.9 ± 0.7
1.0	465 ± 81	30.2 ± 5.2	0.07 ± 0.07*	49.9 ± 4.4	3.0 ± 1.1	1.0 ± 0.7	4.3 ± 2.9	18.6 ± 10.2	2.1 ± 0.4	1.8 ± 1.0
5.0	269 ± 3	59.6 ± 0.6	0.8 ± 0.7*	41.5 ± 1.7	3.0 ± 0.4	0.01 ± 0.01*	5.4 ± 1.7	42.7 ± 6.1	7.1 ± 4.2	3.4 ± 1.9
24.0	100 ± 7	85.0 ± 5.9	2.3 ± 2.2	3.2 ± 3.2*	5.6 ± 0.9	2.0 ± 2.0*	17.4 ± 0.6	44.0 ± 9.9	6.0 ± 0.7	10.0 ± 5.0

* Not detected in one sample (values are means ± S.D., N = 2).

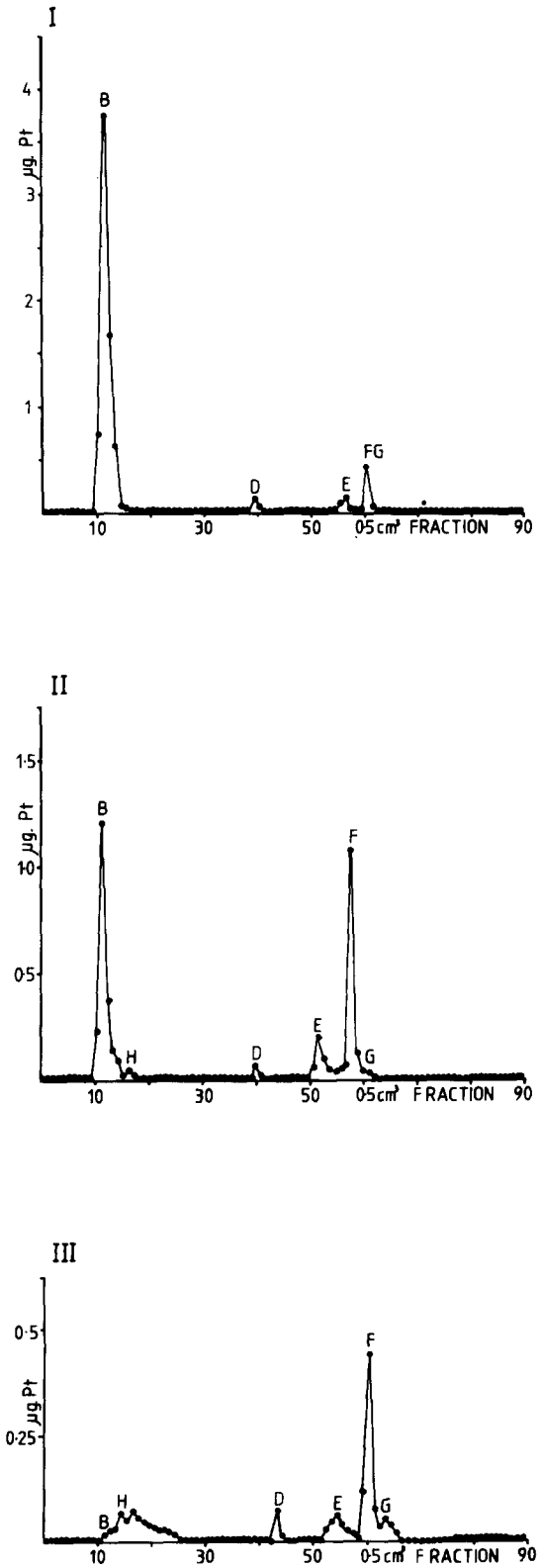

Fig. 3. HPLC elution profiles of platinum species in rat protein free plasma after incubation *in vitro* of 1 mg/cm³ cisplatin with whole rat plasma for 15 min (I); 5 hr (II); 24 hr (III) at 37° under sterile conditions. Vertical axis is μg Pt per 0.5 cm³ fraction. B is unchanged cisplatin (column 25 × 0.5 cm Spherisorb ODS).

can be readily produced in this way in concentrations high enough for an investigation into the toxicity and antitumour properties of the mixed metabolites.

Hydrolysis and methionine substitution products of cisplatin

When investigating the toxicity and antitumour activity of the mixed metabolites we included in the tests, for comparison, solutions containing transformation products of cisplatin produced in simpler *in vitro* systems than pfp. A solution of cisplatin in distilled water (1 mg/cm^3) was incubated for 24 hr at 37° in the dark. An aliquot of this solution was analysed by HPLC and Fig. 4 shows the elution profile. There is one distinct peak other than that of unchanged cisplatin. This new peak elutes in fractions 60–62 where species F elutes when pfp is examined. This solution of hydrolysis products was tested for antitumour and nephrotoxicity *in vivo* (see below).

Amino acids form substitution complexes with cisplatin. Methionine substitution complexes show

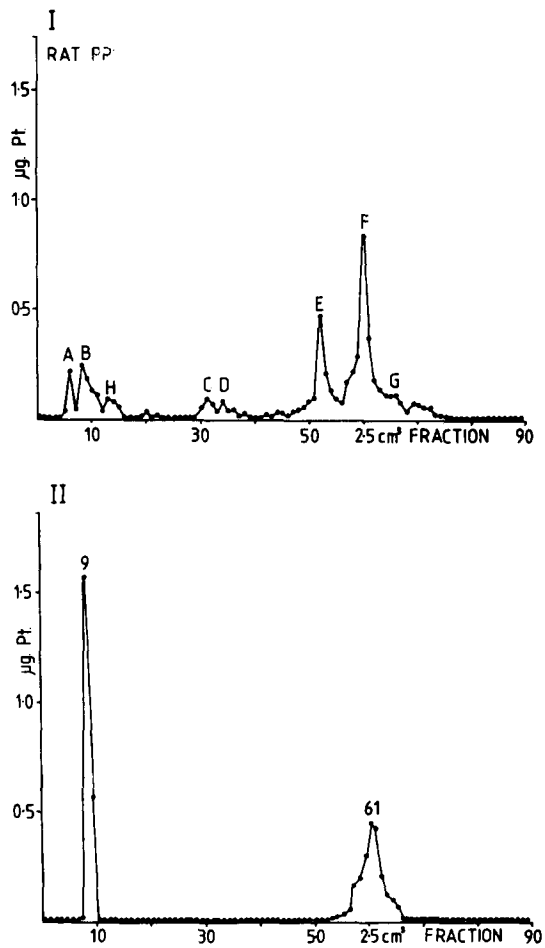

Fig. 4. HPLC elution profiles of platinum species produced following incubation of (I) rat protein free plasma with 200 µg/cm^3 cisplatin *in vitro* for 24 hr at 37° under sterile conditions (200 µl sample); (II) 1 mg/cm^3 cisplatin in distilled water for 24 hr at 37° (20 µl sample). Vertical axis is µg Pt per cm^3. Unchanged cisplatin, B, elutes in fraction 9 (column 50 × 1.0 cm Spherisorb ODS).

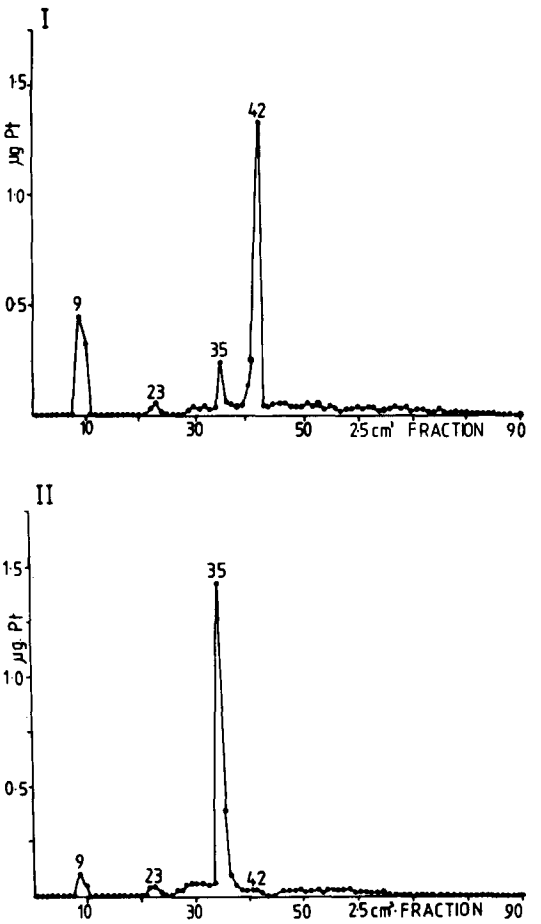

Fig. 5. HPLC elution profiles of platinum species produced after incubation of 1 mg/cm^3 cisplatin with methionine in: (I) 1:1 and (II) 1:2 mole ratios for 4 hr at 37° in 150 mM NaCl (20 µl samples). Vertical axis is µg Pt per cm^3. Unchanged cisplatin elutes in fraction 9 (column 50 × 1.0 cm Spherisorb ODS).

powerful inhibitory activity towards renal ATPases *in vitro* [5]. Cisplatin (1 mg/cm^3) in 150 mM NaCl was incubated with methionine in mole ratios of 1:1 and 1:2 for 4 hr at 37° in the dark. Figure 5 shows the HPLC elution profiles for these solutions. Several peaks other than unchanged cisplatin can be seen in each solution. Two of these peaks elute in fractions 35 and 41–42, where peaks C and D elute when pfp is examined (Fig. 1,I). The 1:1 mixture was tested *in vivo* for antitumour and nephrotoxic properties (see below).

Nephrotoxicity of cisplatin metabolites

Samples of pfp incubated for 24 hr at 37° with 200 µg/cm^3 cisplatin in the dark contain only trace amounts of unchanged cisplatin and were used to investigate the nephrotoxicity of the mixed metabolites. All solutions used in preparing incubation mixtures for subsequent injection into animals were sterilized prior to mixing and incubation by passage through 0.2 µm Millipore filters and then incubated under aseptic conditions. Rats were divided into

groups of at least 6 and after treatment were examined for the development of nephrotoxicity as described in Materials and Methods. Control groups of animals were injected on day 0 with pfp in amounts equivalent to those given to metabolite treated animals. Two groups were injected i.p. with pfp preincubated with cisplatin, as above, to produce solutions of mixed metabolites, to give doses of platinum equivalent to 2 mg/kg and 5 mg/kg cisplatin. Two groups were injected i.p. with cisplatin solution to give doses of 2 and 5 mg/kg. One group was treated with a solution of cisplatin hydrolysis products prepared as described above, to give a dose of platinum equivalent to 2 mg/kg cisplatin. A final group was treated with a solution of methionine substitution complexes of cisplatin prepared as described above, with equimolar cisplatin and methionine, to give a dose of platinum equivalent to 5 mg/kg cisplatin. The results of this investigation are shown in Table 3. In all groups where BUN levels were elevated, maximum elevation occurred on day 5 after treatment. Histopathological damage to the kidney was also assessed on day 5. The extent of proximal tubular necrosis, which was confined principally to the pars recta, was used as an indicator of tissue damage. This was manifest chiefly by loss of the microvilli of the brush border and cellular disorganization and the extent of this damage was assessed by eye (Table 3).

Antitumour activity of cisplatin metabolites

The antitumour activity of the mixed cisplatin metabolites produced by incubation of cisplatin (200 $\mu g/cm^3$) in pfp at 37° in the dark under aseptic conditions was assessed in the mouse L1210 leukemia assay as described in Materials and Methods. Two groups of mice were treated with the metabolite solution i.p. at doses equivalent to 7.5 or 5.0 mg/kg cisplatin. Two groups were treated with cisplatin at the same dosing rates. Two groups were dosed with the solution of methionine substitution complexes of cisplatin prepared as described above with equimolar cisplatin and methionine in amounts sufficient to give doses equivalent to 7.5 and 15.0 mg/kg cisplatin. One control group of animals was dosed with pfp containing no platinum at the same volume as the plasma in the higher dose of metabolites. A second control group was given sterile saline i.p. The results are shown in Table 4.

DISCUSSION

Cisplatin metabolism

Metabolites of cisplatin are detectable in plasma within 15 min of cisplatin being injected into rats (Table 1). In the period following injection cisplatin is removed from the plasma by a number of processes including binding to plasma proteins, dissemination into the tissues and removal by the kidney. As this happens the metabolites form an increasingly high proportion of the diminishing pool of filterable platinum compounds in the plasma (Fig. 2). Although no metabolite achieves a plasma concentration as high as that of cisplatin, the fact that cisplatin is rapidly removed from the plasma whilst metabolite species persist for longer, means that although they are present in lower concentration the metabolite species have more time to bring about an effect. We have only been able to follow the pharmacokinetics for up to 3 hr, after which the plasma platinum concentrations fall below the level where the individual metabolites can be detected by NFAA following HPLC. During that period 70% of the initial dose, and almost all the unchanged cisplatin, is excreted in the urine.

When cisplatin is incubated *in vitro* with whole plasma (Table 2) or pfp (Fig. 4) species can be recognized which have the same retention volumes as those formed *in vivo* although they are present in different proportions. The difference in the proportions of the metabolites can be explained as follows. *In vitro* the relative proportions of the metabolites are not affected by differential excretion [2, 11]. Moreover, *in vitro*, in order to produce sufficient material for subsequent testing, we used a much higher initial concentration of cisplatin than *in vivo*. This could have had the effect of depleting the plasma of some substituting ligands. Thus the change in the relative proportions of C and F *in vitro* compared with *in vivo* conditions might be due to the substituting ligand(s) which give rise to metabolite F being in much greater concentration in plasma than

Table 3. Nephrotoxicity of cisplatin and its metabolites

Treatment	N	Dose (mg/kg)	Blood urea day 5 (mg/dl)	Renal damage (histopathological)	Maximum loss body wt. day 5 (%)
Control	15	—	20.0 ± 6.4	−	
Mixed metabolites	6	2	81.8 ± 33.7*†	+	7.5
Mixed metabolites	6	5	147.1 ± 31.9*‡	+++	20.8
Cisplatin	6	2	19.0 ± 4.5†	−	3.4
Cisplatin	9	5	95.6 ± 46.8*‡	++	8.5
Hydrolysis products	6	2	40.7 ± 6.4*	+	12.8
Methionine : cisplatin (1 : 1)	6	5	23.0 ± 2.5	−	nm

nm, not measured. Doses expressed as equivalent dose of cisplatin based on platinum content.
BUN values are mean ± S.D.
* Significantly different from control ($P < 0.01$).
† Treatments significantly different from each other ($P < 0.01$).
‡ Treatment significantly different from each other ($P < 0.05$).

Table 4. Activity of cisplatin and its metabolites in the L1210 antitumour assay

Treatment	N	Dose* (mg/kg)	Median day of death (range in parenthesis)	Number alive on day 30	Increase in lifespan (%)
Cisplatin	10	7.5	20	9	>233
Cisplatin	10	5.0	13 (12–14)	0	44
Metabolites	10	7.5	12 (5–23)	0	33
Metabolites	10	5.0	12 (11–14)	0	33
Methionine : cisplatin (1 : 1)	7	7.5	11 (10–12)	0	22
Methionine : cisplatin (1 : 1)	7	15.0	12 (12–14)	0	33
Control (pfp)	10	—	9 (8–11)	0	0
Control (saline)	6	—	9 (8–10)	0	0

* Doses expressed as equivalent dose of cisplatin based on platinum content.

those which give rise to metabolite C. We have some evidence, discussed below, that the respective ligands could be water and methionine, and therefore these inferences about their concentrations would be logical.

Possible identity of some metabolites

We have not been able to produce metabolite species sufficiently pure and in the quantities required for unambiguous analysis. However, Fig. 4(II) indicates that a hydrolysis product of cisplatin produces a peak in the HPLC elution profile with the same retention volume as species F. Many hydrolysis products of cisplatin are reactive and they would not be expected to accumulate in plasma and other biological environments where ligands of much higher affinity are available. When cisplatin is reacted in water with a 2 molar excess of silver nitrate and then allowed to come to equilibrium the diaquodiammine platinum species forms [7]. When a solution prepared in this way was examined by HPLC it contained a single species which eluted with the same retention volume (fractions 60–62) as species F (unpublished results). If species F is an hydrolysis product it is surprising that it is produced in such relatively large amounts in plasma which has a chloride concentration of 100 mM; 60% in 24 hr. Under identical conditions, an incubation of cisplatin in NaCl (100 mM) results in only 15% conversion of cisplatin to species F (data not shown). It is possible that in plasma an agent is present which can catalyse this hydrolysis reaction.

Figure 5 shows that methionine substituted species of cisplatin elute with the same retention volumes as species C and D. Since methionine can form a variety of different substitution complexes with cisplatin [12] it is not possible to specify the precise structure of the complexes which form in cisplatin–methionine incubation mixtures. However, the changes in proportions of the species as the ratio of cisplatin to methionine is varied (Fig. 5) suggest that the species eluting in fraction 42 could be a 1 : 1 complex, and the species eluting in fraction 35 may be a 1 : 2 cisplatin to methionine complex. We cannot suggest a composition for the species eluting in fraction 23.

Nephrotoxicity of cisplatin metabolites

The data in Table 3 show that the solution of mixed metabolites possesses increased ability to induce nephrotoxicity in rats compared to cisplatin at equivalent doses. At the lower dose tested (2 mg/kg) cisplatin is not nephrotoxic, whereas the mixed metabolites induce a four-fold increase in blood urea concentration and induce histopathological changes in the kidney. The observation that the metabolites induce nephrotoxicity suggests that these compounds could be involved in the aetiology of the nephrotoxicity of cisplatin itself. The hydrolysis products of cisplatin are more nephrotoxic than cisplatin, but less nephrotoxic than the mixed metabolites (Table 3). This result is consistent with species F being a nephrotoxic agent since species F is present in the hydrolysis products at a lower concentration than in the mixed metabolites.

The cisplatin–methionine (1 : 1) complex was not nephrotoxic (Table 3). This result conflicts with some previous observations. Firstly, we have shown, *in vitro*, that renal ATPases are more sensitive to inhibition by cisplatin–methionine (1 : 1) than by cisplatin alone [5]. Other workers [13] have evidence that the administration of methionine to rats 2 hr after cisplatin results in enhanced nephrotoxicity. However, we have data (unpublished results) demonstrating that rats injected with cisplatin–methionine (1 : 1) complex (Fig. 5,I) excrete in their urine different platinum species from those injected. Thus the failure of cisplatin–methionine mixtures to induce nephrotoxicity when injected into rats i.p. could be due to the transformation of an active species before it reaches the kidney.

Anti-tumour effects of cisplatin metabolites

From the data presented in Table 4 it can be seen that although the mixed metabolites do have some antitumour effect in the L1210 assay system they are less effective at the higher dose rate than an equivalent dose of cisplatin. At the lower dose rate the difference was small and not significant. At the higher dose 9 out of 10 animals treated with cisplatin were cured of the tumour. However, at the same dose none of the metabolite treated animals were cured and 40% of them died before the controls, presumably of drug toxicity. This was inferred from the loss in weight of the animals (data not shown) which was at its maximum on day 6 following injection (−27%) whereas cisplatin treated animals had

lost weight on this day (−15%) compared to the controls (+11%). Because this made comparison difficult the test was repeated at a lower dose. At the lower dose no treatment produced any cures and no deaths occurred before the controls in the group of animals treated with metabolites. When rats are dosed with cisplatin at 15 mg/kg the majority of the dose is excreted unchanged and it is known that only a small proportion ends up as filterable metabolites [2], and if the drug behaves similarly in mice, it follows that in mice given cisplatin at 7.5 mg/kg considerably less than 5.0 mg/kg would end up as filterable metabolites. We have shown (Table 4) that even at a dose rate of 5.0 mg/kg the metabolites have only residual antitumour activity. In the case of animals dosed with cisplatin at 7.5 mg/kg (Table 4) the dose of metabolites, arising from the production of metabolites *in vivo*, would be very much less than a dose equivalent to 5 mg/kg. Therefore the metabolites of cisplatin investigated in this study could not be responsible for the antitumour effect of cisplatin.

The methionine–cisplatin mixture was also effective at prolonging life but there was a significant proportion of unreacted cisplatin in the mixture such that 15 mg/kg platinum contained 2.4 mg/kg cisplatin.

Clinical implications

From the foregoing it can be concluded that cisplatin metabolites are more nephrotoxic but less effective antitumour agents than cisplatin alone. This observation provides a rationale for two empirical treatments which have been devised for minimizing the nephrotoxicity of cisplatin whilst retaining the antitumour effects.

Borch and co-workers [14, 15] have shown that the nephrotoxic effects of cisplatin can be attenuated without affecting the antitumour effects if the metal chelating agent diethyldithiocarbamate is administered to animals up to 4 hr after cisplatin treatment. We have demonstrated that by this time virtually all the unchanged cisplatin has been eliminated from the body. Therefore, the most likely explanation would appear to be that it is not cisplatin but a metabolite which is responsible for the nephrotoxicity. Moreover the abolition of nephrotoxicity without effect upon the antitumour activity suggests that the two effects are brought about by different chemical entities.

In a clinical investigation the administration of cisplatin in hypertonic saline to patients has been reported not only as reducing the nephrotoxicity but as permitting a doubling of the maximum tolerated dose, eliciting higher levels of response from tumours which, with normal therapeutic doses of cisplatin, only partially respond [16]. The success of this treatment probably depends on the ability of the high chloride ion concentration to suppress the replacement of the chloride ligands of cisplatin and the formation of metabolites. Our demonstration that cisplatin metabolites are more nephrotoxic than cisplatin but are not responsible for the antitumour activity of the drug provides support for this hypothesis and we propose to investigate further the effects of chloride ion concentration on the extent of metabolite formation *in vivo*.

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