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Intracellular metabolites of cisplatin in the rat kidney

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Summary. The appearance of low-molecular-weight metabolites of cisplatin in the cytosol of cells from the cortex and outer medulla of the rat kidney has been examined using HPLC up to 24 h following cisplatin administration. Comparison was made between these metabolites and those present in plasma, urine and liver. The effect of sodium chloride (NaCl) pretreatment, which is known to reduce cisplatin-induced nephrotoxicity, on these metabolites was also investigated. Platinum levels in the kidney cortex and medulla and the cytosol reached maximal levels within 1 h of i.p. injection of 5 mg/kg cisplatin. At least six platinum species, including cisplatin, were present 1 h post-dosing, with the principal species being the parent drug; all of these species were either neutral or negatively charged. Although the concentration of most of the platinum species fell with time, that of one species eluting before cisplatin rose, and by 24 h it was the major metabolite. Cisplatin and two other major cytosolic platinum species were also present in urine and plasma, both of which also contained a number of charged species that were absent from the cytosol. The liver cytosol contained at least five metabolites 1 h post-dosing, but, in contrast to the kidney cytosol at the same time, the predominant species was that eluting before cisplatin and not cisplatin itself. One of the metabolites in the cytosol and urine had the same retention time as an adduct of cisplatin with glutathione and with cysteine. Urinary samples also contained a metabolite coeluting with aquated cisplatin. Pretreatment of animals with NaCl significantly reduced the platinum concentration in the kidney, with a corresponding decrease in the cytosolic metabolites; this may have contributed significantly to the reduction in cisplatin-induced nephrotoxicity after NaCl pretreatment. NaCl also significantly reduced a possible aquated species present in the urine, which may also have contributed to the reduction in nephrotoxicity. The data suggest that cisplatin itself may be the nephrotoxic species, since it is the intracellular platinum compound present in highest concentration during the early critical period after its administration.

Introduction

The major dose-limiting side effect of cisplatin is nephrotoxicity. Kinetic and toxicological studies [1, 4] suggest that the critical effects of this drug on the kidney occur within 0–4 h after its administration, well before the maximal functional disturbance at 96–120 h. Previous studies in this laboratory [3, 4] have demonstrated the presence of platinum-containing transformation products of cisplatin in plasma 15 min following a bolus dose. Although these compounds probably arise from cisplatin principally by simple ligand-exchange reactions not involving metabolism [4, 8] it is convenient to refer to them as metabolites. It has been demonstrated that the parent drug and several of the metabolites are actively secreted by the kidney, whereas other metabolites are reabsorbed [3, 5, 7]. Furthermore, a mixture of metabolites generated in vitro in plasma has been shown to be more nephrotoxic than cisplatin alone; consequently, one or more of the metabolites might play a role in the development of the nephrotoxic lesion [4]. It is not known how cisplatin or its metabolites enter renal cells, but accumulation of platinum in the kidney occurs rapidly, reaching maximal concentrations within 6 h following a single dose [2, 10]. A large proportion of the intracellular platinum is found in the cytosol (60%–70% 24 h after dosing), the remainder being distributed amongst the other sub-cellular fractions [2]. Of the cytosolic platinum, 30%–50% is ultrafilterable (i.e. not bound to protein) 24 h after dosing, but little is known of the chemical nature of this fraction.

Mason and co-workers [11, 12] investigated intracellular metabolites of cisplatin using gel filtration and ion exchange chromatography. They demonstrated the presence of both neutral and positively charged low-molecular-weight species within 15 min of cisplatin administration, the concentration of these species subsequently falling as the concentration of protein-bound platinum rose. Safirstein et al. [15] reported that the ultrafilterable platinum species present in the kidney 24 h after dosing with cisplatin are non-toxic and non-mutagenic.

In the present study we used HPLC to separate the low-molecular-weight metabolites of cisplatin found in the cytosol of cells from the cortex and outer medulla of kidneys from rats up to 24 h following cisplatin treatment. We compared the chromatographic behaviour of these metabolites with that of metabolites found in plasma, urine and cells from the liver, an organ on which cisplatin has no

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toxic side effects at therapeutic dose levels [18]. We also investigated changes in the relative amounts of these metabolites in animals protected from nephrotoxicity by the administration of NaCl prior to cisplatin.

Materials and methods

Chemicals. Cisplatin (*cis*-dichlorodiammine platinum II) was a gift from Johnson-Matthey Research, Reading. HPLC-grade methanol was obtained from Koch-Light Ltd., Suffolk, and 1-heptanesulphonic acid was supplied by Sigma Chemical Co. Ltd., Poole. Other chemicals were standard laboratory grade from BDH, Poole. Water for HPLC was glass double-distilled.

Animals. Male Wistar rats weighing 300–400 g (Charles River, Margate) were used in all studies. They were housed either in groups of 2–4 in plastic cages or individually in metabolic cages. All animals were allowed access to food and water ad lib.

Cisplatin metabolites in plasma. Two rats were dosed with 5 mg/kg cisplatin; 30 min later blood was taken from each by heart puncture with ether anaesthesia and collected into heparinised syringes. The blood was immediately centrifuged and protein-free plasma samples were prepared by ultrafiltration using Amicon Centrifree filters (mol. wt. cut-off, 30,000 daltons). Aliquots were used immediately for HPLC analysis (see below).

Cisplatin metabolites in urine. Three rats were dosed i.p. with 5 mg/kg cisplatin (2 mg/ml in 0.9% w/v NaCl) and housed individually in plastic metabolic cages. Urine was collected in ice-cooled containers for 1 h post-dosing and centrifuged in an Eppendorf microfuge; aliquots were immediately used for HPLC analysis (see below).

Cisplatin metabolites in kidney. Three groups of two rats were dosed i.p. with 5 mg/kg cisplatin (2 mg/ml in 0.9% w/v NaCl) and both animals in each group were killed at 1, 6 and 24 h post-dosing, respectively, by cervical dislocation. The kidneys were immediately removed onto ice and all subsequent procedures carried out at 4° C. The cortex and outer medulla of each kidney was dissected and homogenised (3:2 w/v) in 10 m*M* phosphate buffer (pH 7.4) containing 0.25 *M* sucrose. The cytosol fraction was prepared by centrifuging the homogenate at 100,000 *g* for 50 min. Cytosolic ultrafiltrate was prepared as for plasma (see above) and immediately used for HPLC analysis. In a preliminary experiment, cytosolic ultrafiltrate was prepared from the kidneys of two rats dosed similarly with cisplatin and killed 1 h later. In one animal, the preparation exactly followed the protocol described above. In the other, prior to dissection the kidney was perfused with cold homogenising buffer through the renal artery to remove all blood and displace the glomerular filtrate, tubular fluid and formative urine from the kidney. Samples of kidney homogenate, cytosol and cytosolic ultrafiltrate were stored at −20° C prior to platinum analysis. The platinum content of kidney homogenates was determined after solubilisation in Hyamine hydroxide using the method of Siddik et al. [16].

Cisplatin metabolites in liver. Two rats were dosed i.p. with 5 mg/kg cisplatin (2 mg/ml in 0.9% w/v NaCl) and killed by cervical dislocation 1 h post-dosing. The livers were removed and cytosolic ultrafiltrate was prepared and analysed as described above for the kidney.

NaCl pretreatment and cisplatin metabolism. Two rats were dosed p.o. with 25 mmol/kg NaCl (3.125 mol/l NaCl) 90 min prior to i.p. administration of 5 mg/kg cisplatin (2 mg/ml in 0.9% w/v NaCl). The rats were placed in metabolic cages and urine was collected into ice-cooled containers for 1 h following cisplatin treatment. At that time the animals were killed by cervical dislocation, the kidneys were removed and cytosolic ultrafiltrate prepared and analysed as previously described. The chloride concentration of the ultrafiltrate was determined using a Corning model 925 chloride analyser.

Separation of cisplatin metabolites by HPLC. Platinum species in samples of plasma ultrafiltrate, urine and cytosolic ultrafiltrates were separated by HPLC using the method of Mauldin et al. [13]. Samples (200 µl) were loaded onto a Whatman Partisil ODS-3 column (4.5 × 250 mm) with a guard column (4.5 × 50 mm) of the same material. The column had been pre-equilibrated with 5 m*M* 1-heptanesulphonic acid (pH 3.4) (solvent A) for a minimum of 60 min. Samples were initially eluted at a flow rate of 1.0 ml/min with solvent A, followed at 10 min by a linear gradient of increasing concentrations of solvent B (90% aqueous methanol), reaching 100% solvent B concentration at 40 min. Fractions (0.5 ml) of the effluent were collected and analysed for platinum using flameless atomic absorption spectrometry as previously described [3].

Results

Intracellular metabolites in the kidney and liver cytosol

Preliminary experiment. In a preliminary experiment, when cytosol was prepared from perfused and unperfused kidneys there was very little difference at 1 h after cisplatin treatment in the amounts of platinum species eluted from the HPLC column prior to the start of the methanol gradient at fraction 20 (data not shown). However, the unperfused kidney yielded a larger amount than the perfused kidney of species eluting after fraction 20 (cationic species). These species are also found in urine (see later); their presence in the ultrafiltrate from the unperfused kidney, albeit in trace amounts, probably reflects the contamination of the cytosol with formative urine from the kidney tubules. This contamination was not substantial 1 h post-dosing and, at later times, would have made a decreasing contribution to total platinum in the cytosol due to the fall in urinary platinum concentration and, in particular, to the decreased amounts of cationic species. Because of the need to keep the duration of tissue preparation to a minimum, cytosolic ultrafiltrates were prepared in all subsequent experiments without prior perfusion of the kidneys.

Distribution of platinum in the kidney. Within 1 h of the i.p. injection of 5 mg/kg cisplatin, the concentration of platinum in the kidney cortex and outer medulla reached its maximal level, fell by a small but significant amount at 6 h, then rose again to the 1-h level by 24 h (Table 1). The concentration of platinum in the cytosol also peaked at 1 h but fell slightly by 24 h. Over the 24-h period post-dosing, the proportion of the total platinum in the kidney found in

Table 1. Distribution of platinum in the kidney at various times following cisplatin administration

	Time		
	1 h	6 h	24 h
Animals (*n*)	3	3	3
μg Pt/g tissue:			
Kidney (total)	13.34±0.27	12.48±0.07**	13.56±0.77 (NS)
Cytosol	6.08±0.28	5.53±0.32 (NS)	5.25±0.82 (NS)
Cytosolic Pt (as % of total kidney Pt)	46±3	45±3	39±8
Cytosolic Pt (μg/ml):			
Total	6.74±0.39	6.13±0.22*	5.55±0.86 (NS)
Ultrafilterable	3.09±0.09	2.23±0.19**	2.25±0.41*
Protein-bound	3.65±0.37	3.90±0.37 (NS)	3.31±0.46 (NS)
Ultrafilterable Pt (as % of total cytosolic Pt)	46±3	36±4**	41±2**

Difference from distribution at 1 h: * $P < 0.1$; ** $P < 0.05$; NS, not significant

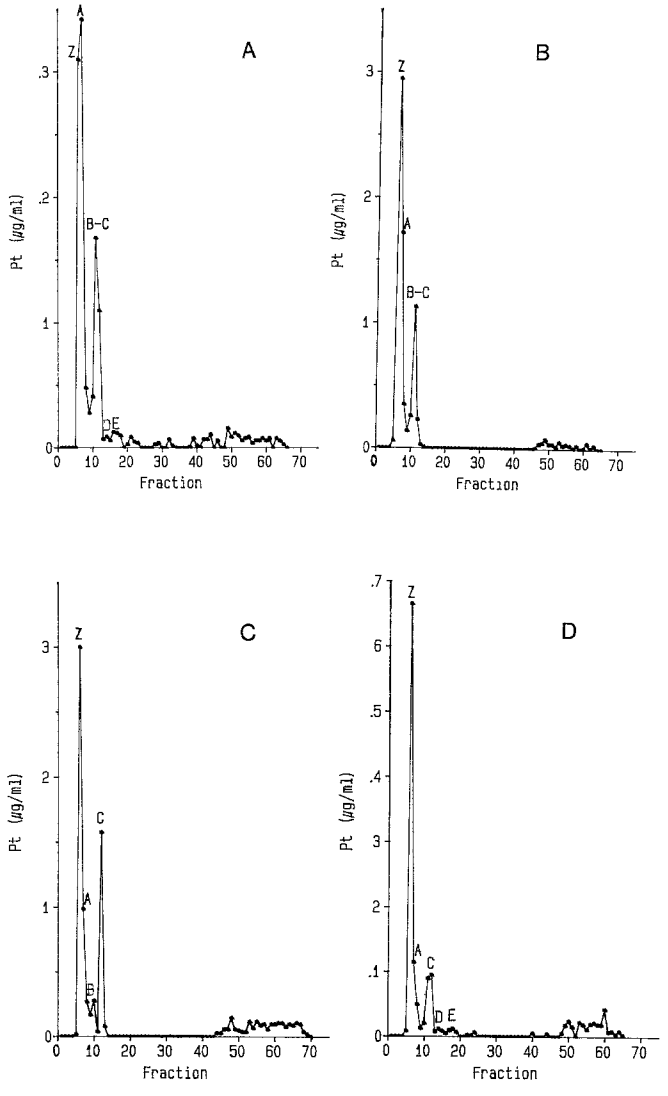

Fig. 1. HPLC elution profiles of platinum species in rat-kidney cytosolic ultrafiltrate (*A*) 1 h, (*B*) 6 h and (*C*) 24 h following treatment with cisplatin (5 mg/kg) and (*D*) in rat-liver cytosolic ultrafiltrate 1 h post-treatment. Peak *A* is unchanged cisplatin. Sample sizes, 200 μl

the cytosol ranged between 46% and 39% (Table 1). The slight fall in the platinum concentration of the cytosol over the 24-h period is accounted for by a small but significant reduction in the ultrafilterable fraction over the first 6 h. The concentration of protein-bound platinum in the cytosol remained at the same level between 1 and 24 h post-dosing (Table 1).

Cisplatin metabolites in the cytosol. Typical patterns of metabolites found in the kidney cytosol at 1, 6 and 24 h post-dosing can be seen in Fig. 1A–C. The species eluting from the HPLC column before commencement of the gradient at fraction 20 were either uncharged or negatively charged. The pattern of peaks in this region indicates the presence of at least six species. Species A and Z were never fully resolved, and the resolution of species B and C was variable. However, in replicate runs for each time point the combined amounts of B and C did not vary. Metabolites D and E were present at very low concentration and were not always detected in replicate runs for the same time point. At 1 h post-dosing, the principal species present was A (unchanged cisplatin); at this time species B and C were also present in larger amounts than in later samples. Over the period 1–24 h post-dosing, the amounts of species A, B, D and E fell; however, the amount of species Z rose as the amount of A fell, and the former became the predominant metabolite species by 6 h post-dosing. Although separated by only a single fraction, the chromatographic behaviour of A and Z were completely reproducible in all samples; we are certain that they are separate species and not a single species variably overlapping into two fractions. Although virtually 100% of the platinum in the 1-h cytosol (and in all plasma and urinary samples) applied to the HPLC column was recovered in the eluted fractions, the recoveries from the 6- and 24-h cytosolic ultrafiltrates amounted to only 67%±7% and 86%±12%, respectively. The loss of platinum from these samples may arise from the demonstration by Mason et al. [11, 12] that as time progresses an increasing amount of platinum becomes bound to low-molecular-weight proteins (<30,000 daltons), which may be ultrafiltered but would not then elute from the HPLC column. The liver cytosol 1 h post-treatment also contained species A, C, D, E and Z (Fig. 1D), but,

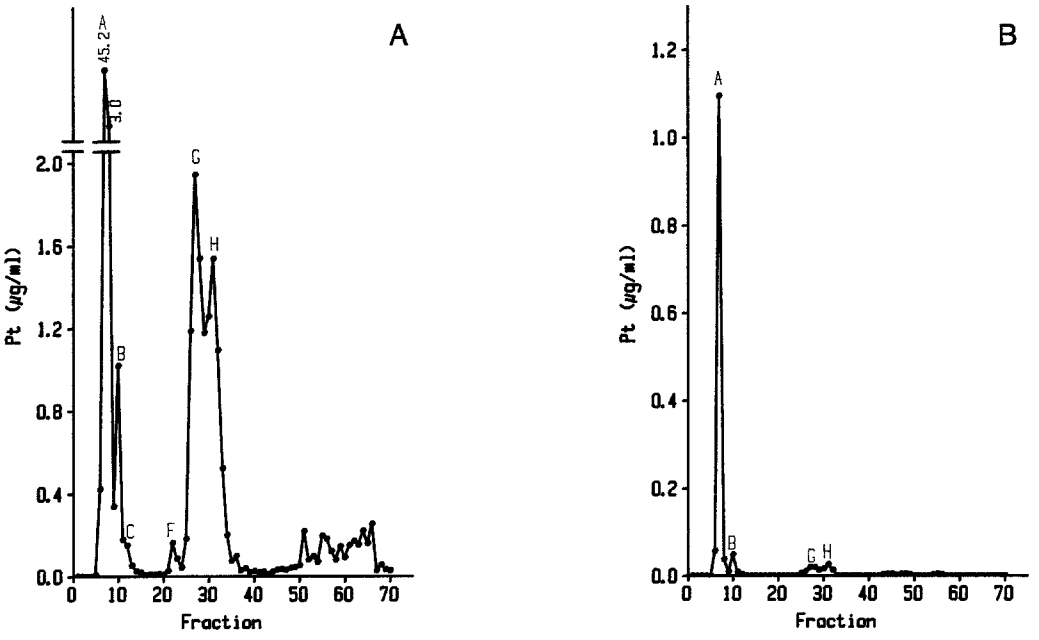

Fig. 2. HPLC elution profiles of platinum species (*A*) in urine collected 0–1 h following treatment with cisplatin (5 mg/kg) and (*B*) in plasma ultrafiltrate collected 30 min post-treatment. Peak *A* is unchanged cisplatin. Sample size, 200 μl

in contrast with kidney cytosol at the same time, species Z was predominant, with only very little of species A occuring.

Urine and plasma cisplatin metabolites

The HPLC elution pattern of platinum species present in the 0- to 1-h urinary sample is shown in Fig. 2A. Species A, B and C, which were the principal species found in the 1-h cytosol, were also present in the 0- to 1-h urinary sample. However, the urine also contained a number of cationic species (which elute in fractions 20 and above) that were not found in the cytosol. The principal cationic species were G and H. Plasma ultrafiltrate samples collected 30 min after dosing also contained species A, B, G and H, species A being predominant (Fig. 2B).

Table 2. The effect of NaCl pretreatment on the distribution of platinum in the kidney

	Treatment	
	Cisplatin	NaCl + cisplatin
Animals (*n*)	3	3
μg Pt/g tissue:		
Kidney	13.34 ± 0.27	8.21 ± 3.57*
Cytosol	6.08 ± 0.28	3.20 ± 1.09**
Total kidney Pt in cytosol (%)	46 ± 3	40 ± 5
Cytosolic Pt (μg/ml):		
Total	6.74 ± 0.39	3.55 ± 1.22**
Ultrafilterable	3.09 ± 0.09	1.85 ± 0.84*
Protein-bound	3.65 ± 0.37	1.70 ± 0.39***
Ultrafilterable Pt (as % of total)	46 ± 3	50 ± 8

Significant difference between treatments: * $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$

The effect of NaCl pretreatment on cisplatin metabolism

The administration of NaCl prior to cisplatin treatment significantly reduced both total ($P < 0.1$) and cytosolic ($P < 0.05$) platinum concentrations 1 h post-dosing compared with cisplatin alone (Table 2). Furthermore, NaCl pretreatment significantly reduced the fraction of cytosolic platinum bound to protein (Table 2). Ultrafilterable cytosolic platinum was also reduced in concentration following NaCl pretreatment, but the difference is not statistically significant. Corresponding decreases in the amounts of platinum in the peaks of species A, Z and B, C were seen after HPLC analysis (Fig. 3A), although the proportion of total cytosolic platinum contained in these peaks was similar to that in the control (Table 3). The chloride concentration in the cytosolic ultrafiltrates was 22 m*M* in animals pretreated with NaCl compared with 20 m*M* in control animals. Figure 3B shows the effect of pretreating cisplatin-dosed animals with NaCl on the cisplatin metabolites present in the 0- to 1-h urinary samples. The amounts of platinum eluting in the region of metabolites G and H (fractions 14–34) were reduced by 66% (Table 4), whereas, the amount of species A was increased by 17% in NaCl-pretreated animals compared with those treated with cisplatin alone.

Production of cisplatin metabolites in vitro

To try to identify some of the metabolite peaks, cisplatin was incubated in vitro with solutions of single compounds carrying potential substituting ligands and the products were separated by HPLC. Figure 4A shows the pattern obtained after cisplatin was incubated in distilled water for 24 h at 37° C. There is only one distinct peak other than that of unchanged cisplatin. Also shown in Fig. 4 are the results of incubating cisplatin with glutathione, cysteine and methionine in 10 m*M* phosphate buffer (pH 7.4) containing 150 m*M* NaCl for 5 h. To prevent oxidation of the

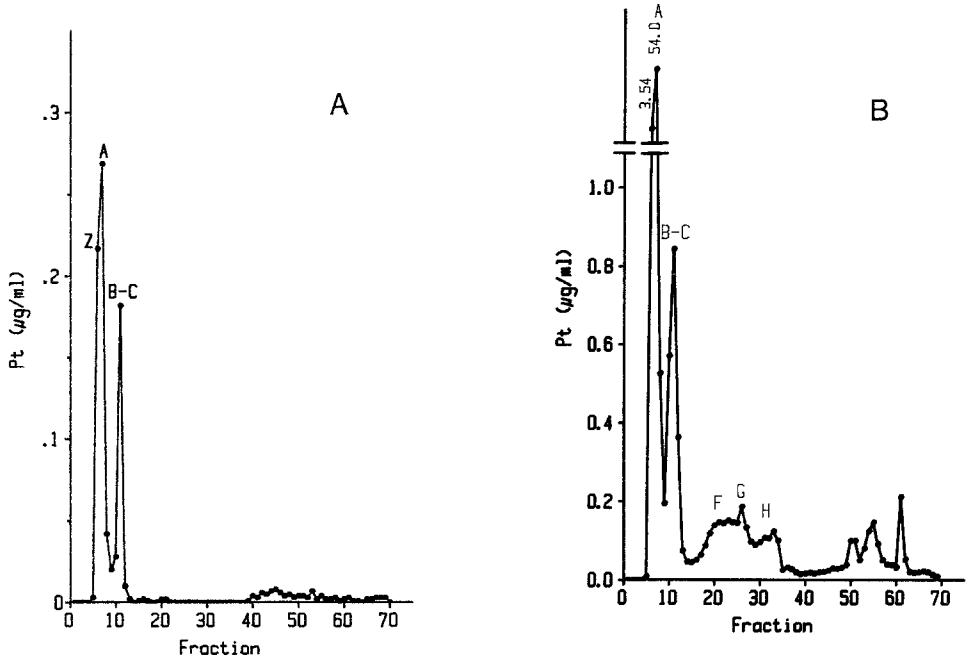

Fig. 3. HPLC elution profile of platinum species (*A*) in kidney cytosolic ultrafiltrate 1 h following treatment with cisplatin (5 mg/kg) in rats pretreated with NaCl (25 mmol/kg) and (*B*) in urine collected 0–1 h following treatment with cisplatin (5 mg/kg) in rats pretreated with NaCl (25 mmol/kg). Peak *A* is unchanged cisplatin. Sample size, 200 μl

Table 3. The effect of NaCl pretreatment on intracellular cisplatin metabolites in the kidney

	Treatment	
	Cisplatin	NaCl + cisplatin
Pt in cytosolic ultrafiltrate (μg/ml)	3.09 ± 0.09	1.85 ± 0.84*
Pt in fractions 6–9:		
μg	0.33 ± 0.03	0.20 ± 0.07*
Pt recovered from HPLC (% of total)	54.60 ± 5.10	59.00 ± 6.50
Pt in fractions 10–13:		
μg	0.16 ± 0.01	0.09 ± 0.03*
Pt recovered from HPLC (% of total)	26.50 ± 2.80	26.90 ± 5.30

* Significant difference between treatments ($P < 0.1$)

free thiols, all solutions and incubation mixtures were thoroughly gassed with N_2 prior to use and incubations were carried out in the presence of N_2.

Discussion

Our data confirm that the accumulation of platinum in the kidney following cisplatin treatment is a rapid process. By 1 h post-dosing, the total kidney platinum concentration is maximal (Table 1). Mason et al. [11] report that a peak in total kidney platinum occurs as early as 15 min post-dosing, thereafter falling for 2 h before slowly rising to the initial level 24 h later. However, these authors did not perfuse the kidneys to remove blood and formative urine before analysis. Moreover, they used different cisplatin doses (1 and 10 mg/kg in addition to 5 mg/kg) and whole kidney, whereas we used only cortex and outer medulla and they gave the drug s.c. Although by 1 h after dosing urinary platinum levels had fallen sufficiently that contamination

Table 4. The effect of NaCl pretreatment on urinary metabolites of cisplatin 0–1 h after dosing

Treatment	Urinary Pt (μg/ml)	Pt in peak (as % of Pt in sample)			
		6–9[a]	Fraction 10–13	14–34[b]	Total recovered from HPLC
Cisplatin	198.3 ± 71.7	76.1 ± 4.6	2.0 ± 0.3	16.8 ± 4.0	97 ± 4
NaCl + cisplatin	196.2 ± 110.5 (NS)	89.3 ± 2.1**	2.5 ± 0.5 (NS)	5.7 ± 1.8**	98 ± 5

[a] Principally unchanged cisplatin
[b] Aquated plus other unidentified species
* Significant difference between treatments ($P < 0.05$); NS, not significant

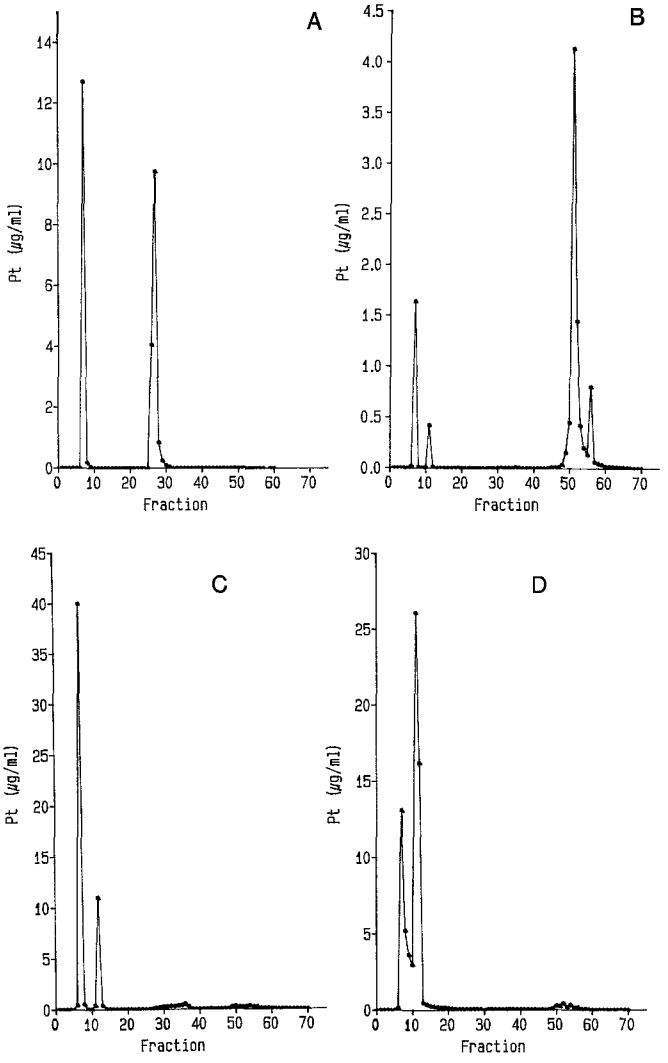

Fig. 4. HPLC elution profiles of platinum species produced in vitro by incubation of (*A*) 3.33 m*M* cisplatin in water at 37° C for 24 h (sample size, 20 μl), (*B*) 0.1 m*M* cisplatin with 2.0 m*M* methionine (sample size, 200 μl), (*C*) 2.0 m*M* cisplatin with 2.0 m*M* reduced glutathione (GSH) (sample size, 200 μl), and (*D*) 2.0 m*M* cisplatin with 10.0 m*M* cysteine (sample size, 200 μl). Incubations *B*, *C* and *D* were carried out for 5 h at 37° C in 10 m*M* phosphate buffer (pH 7.4) containing 150 m*M* NaCl and gassed with N_2. All incubations were done under aseptic conditions in a dark environment

of whole-kidney homogenate with formative urine did not contribute substantially to either the total kidney platinum or the species detectable in the cytosol (for example, compare Fig. 1A with Fig. 2A), this contamination may be more significant at earlier time points, when the urinary platinum concentration is much higher. The early rise and fall in total kidney platinum reported by Mason et al. [12] may reflect this diminishing contribution of urinary platinum to the total.

Reviewing earlier observations, Safirstein et al. [15] state that the process of uptake of platinum in the kidney is "essentially complete" by 1–6 h post-dosing. However, such a statement does not take account of reports that the kidney tubule not only filters but secretes and reabsorbs platinum species [3]; thus at least part of the platinum content of the kidney cells is in a state of dynamic flux. Such a state may explain why, even 24 h after dosing, 40% of the total kidney platinum is in the cytosol and 41% of that is ultrafilterable (Table 1), whereas at this time the platinum detectable in plasma is virtually all protein-bound [4, 17]. The concentration of platinum in the kidney cytosol peaked at 1 h, then fell to a slightly lower level, which was maintained up to 24 h post-dosing (Table 1). This fall is accounted for by a reduction in the concentration of ultrafilterable platinum; the concentration of platinum bound to protein does not change over the period 1–24 h (Table 1). These results are similar to those of Mason et al. [11]. The cytosolic platinum accounted for between 39% and 46% of the total kidney platinum; of this, 32%–49% was non-protein-bound. These values are lower than those reported by Choie et al. [2], probably because of differences in the preparation of the cytosol.

In the present study we used an HPLC technique to investigate the speciation of platinum complexes in rat-kidney cytosolic ultrafiltrate over a 24-h period following cisplatin administration. The HPLC technique was that described by Mauldin et al. [13] and is similar to the method that we have previously used [3] which, however, does not require such a long period for pre-equilibration of the column with the first eluant (cf 1 and 16 h). We showed that within 1 h of treatment with cisplatin, at least six different neutral or negatively charged platinum species appear in the kidney cytosolic ultrafiltrate. No distinct species carrying a net positive charge, such as the mono- or di-aquo metabolites (which would have been expected to elute in fractions 20 and above) were detected by us in the kidney cytosol. Safirstein et al. [15] reported finding mainly neutral platinum species in the cytosol 24 h post-dosing. In contrast, Mason et al. [11, 12] concluded that 15 min after cisplatin administration, kidney cytosol contains some positively charged low-molecular-weight platinum species, since these were retained by a cation-exchange column. The presence of such species may have been due to contamination of the cytosol with formative urine, which contains a high concentration of platinum at this early time point. Of the six platinum species we detected in the cytosol, species A is unchanged cisplatin. Species Z elutes very closely to cisplatin but nearer to the void volume and thus may be a negatively charged species. Species C elutes with the same retention volume as adducts of cisplatin with glutathione and with cysteine (Fig. 4). Urine (0–1 h) and plasma (30 min) samples also contained species G, which elutes with the same retention volume as an aquated cisplatin sample. This species corresponds with the species F reported by Daley-Yates and McBrien [3, 4], who used a slightly different chromatographic procedure. Generated in vivo, this species, thus coelutes with aquated cisplatin in two different chromatographic procedures.

The liver seems to convert cisplatin rapidly, principally to species Z and C – species Z being the major metabolite 1 h post-dosing. In contrast with the kidney, there is very little intracellular unchanged cisplatin at this time. The fact that species Z and C are major products of cisplatin biotransformation in the liver and that cisplatin causes no hepatotoxicity suggests that species Z and C are not cytotoxic. Further evidence for this is that we showed that 24-h post-dosing, species Z and C are also the principal soluble platinum species in the kidney, and Safirstein et al. [15] have demonstrated that kidney cytosolic ultrafiltrate prepared 24 h post-dosing is non-toxic. If production of these

metabolites is a detoxification mechanism, it would clearly be valuable to identify the substituting ligands unambiguously; we intend to undertake further work to investigate this. If cisplatin metabolites are involved in the mechanism of cisplatin nephrotoxicity, as has been suggested [4], then metabolites present in the first 4 h of exposure to cisplatin but absent (or present in diminished concentration) at later times are the most likely candidates, since the events leading to irreversible damage occur during the first 4 h [1, 4]. On this basis, unchanged cisplatin could be a candidate for the toxic species, as it fulfils the kinetic criteria.

Pretreatment of animals with NaCl significantly reduces cisplatin-induced nephrotoxicity [6, 9, 14]. One mechanism for this effect that has been postulated is an alteration in cisplatin metabolism, and changes in urinary metabolites of cisplatin have been reportedly induced by NaCl pretreatment [14]. In the present study, pretreatment with NaCl significantly reduced both total kidney and cytosolic platinum concentrations at 1 h post-dosing. In the cytosol both protein-bound and ultrafilterable platinum concentrations were reduced, with a corresponding decrease in the various cytosolic platinum species. These data suggest that the early reduction in the total uptake of platinum by the kidney (particularly the reduction in cisplatin uptake) following NaCl pretreatment may play a role in reducing cisplatin-induced nephrotoxicity.

The proportions of different metabolites in the urine were also altered by NaCl pretreatment, with a significant reduction in the amount of species G (which elutes with the same retention volume as aquated species) and species H and a corresponding increase in the concentration of unaltered cisplatin. Daley-Yates and McBrien [6] also reported a 50% reduction in the concentration of the same species in the urine of rats pretreated with NaCl. If this metabolite is really a reactive, aquated species, the reduction in its urinary concentration may be the significant event in the reduction of cisplatin nephrotoxicity by NaCl pretreatment. However, despite the chromatographic evidence, no direct, unequivocal evidence has yet been obtained to identify any of the substituting ligands found on platinum in vivo following cisplatin administration. The devising of suitable, sufficiently sensitive analytical techniques to investigate the chemical nature of these metabolites remains one of the most urgent tasks remaining to be tackled by those interested in the metabolism of platinum drugs.

In summary, cisplatin itself appears to be the most likely toxic species to occur within the cells of the cortex and outer medulla of the kidney during the period following cisplatin administration, when nephrotoxicity is initiated. However, a contribution to the overall cytotoxicity of cisplatin by a metabolite, possibly an aquated species, in the lumen of the kidney tubules cannot be ruled out on present evidence. The liver can apparently rapidly convert cisplatin to non-toxic metabolites.

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