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A STUDY OF THE PROTECTIVE EFFECT OF CHLORIDE SALTS ON CISPLATIN NEPHROTOXICITY

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Abstract—In rats NaCl and NH_4Cl (25 mmoles/kg, p.o.) were found to be equally effective at preventing nephrotoxicity when administered to rats 90 min before cisplatin (5 mg/kg, i.p.) but $(NH_4)_2SO_4$ did not protect. The severity of nephrotoxicity, taken as the maximum elevation in blood urea concentration, showed a high degree of correlation with urinary chloride concentration, but not with urinary pH or volume. Sodium chloride did not protect against nephrotoxicity when administered 3 or 24 hr after cisplatin. Sodium chloride showed protection against nephrotoxicity caused by cisplatin metabolites only at low doses of platinum. For animals pretreated with NaCl (25 mmoles/kg) or water p.o. the urinary excretion of total platinum, cisplatin and six of the seven metabolites separated by hplc was not significantly different between the two treatments during the 0–5-hr period post dosing. However, one metabolite, possibly a nephrotoxic hydrolysis product, was excreted in significantly smaller amounts in the urine of animals pretreated with NaCl ($P < 0.05$). Furthermore, in all cisplatin treated animals the amount of this species excreted correlated with the severity of nephrotoxicity. Whilst this suggests that chloride ions may protect against the nephrotoxicity of cisplatin by inhibiting its rate of metabolism this metabolite accounts for only 2.5% of the platinum excreted. Furthermore, the data do not exclude the possibility that NaCl prevents cisplatin-induced nephrotoxicity by preventing renal ischaemia, which may normally follow cisplatin treatment, or that the renal uptake or transport of platinum may be inhibited by NaCl.

Cisplatin (*cis*-dichlorodiammine PtII) is an anti-tumour agent which is active against a variety of human tumours [1] but nephrotoxicity is the dose limiting side-effect [2]. Various treatment regimens have been devised to reduce the severity of this side-effect and thereby to increase the therapeutic index of the drug. Litterst [3] reported that the inclusion of hypertonic sodium chloride in the injection vehicle reduced the nephrotoxicity of cisplatin in rats. Recently Ozols *et al.* [4] have shown that the administration of cisplatin to patients in hypertonic saline can reduce the nephrotoxicity of the drug and allow higher doses to be tolerated. However, the mechanism by which sodium chloride is able to prevent cisplatin nephrotoxicity has not been established. Bertolero and Litterst [5] examined the effects of ammonium chloride on cisplatin induced nephrotoxicity but did not observe substantial differences in toxicity when compared to animals treated with cisplatin alone. One possible effect of increased plasma and urinary chloride concentration could be suppression of replacement of the chloride ligands of cisplatin. We have shown that when cisplatin is added to normal rat plasma it gives rise by ligand replacement, both *in vivo* and *in vitro*, to a variety of transformation products, or metabolites, which *in vivo* are also subsequently found in the urine [7, 8]. We have also shown [7] that the metabolites are more potent nephrotoxic agents than cisplatin itself. We here report an investigation into the biochemical mechanisms by which salts influence the nephrotoxicity of cisplatin and its metabolites.

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MATERIALS AND METHODS

Animals. All the animals used in this study were male Wistar rats (Charles River, Margate) of 200–250 g. For experimental purposes all animals were housed individually in metabolism cages and allowed *ad libitum* access to food and water.

In one experiment animals were prepared by surgical cannulation of the ureters. The cannulae were led subcutaneously from the abdomen to the nape of the neck enabling the collection of urine into plastic saddles directly from the conscious animal. The time taken for passage of urine through the cannula from kidney to saddle was not more than 60 sec for any animal.

Dosing solutions. Cisplatin, chromatographically homogeneous when analysed by hplc (see below) was a gift from Johnson–Matthey Research (Reading, U.K.). Solutions in 0.9% w/v NaCl were prepared immediately before use and injected i.p. Mixed metabolites of cisplatin were prepared *in vitro* as previously described [7] by incubating cisplatin (200 $\mu g\ cm^{-3}$) at 37° for 24 hr in sterile rat plasma ultrafiltrate prepared by filtering fresh rat plasma through Amicon CF25 membrane ultrafilters (removing proteins of mol. wt > 25,000). After addition of cisplatin the solution was sterilised by passage through Millipore 0.2 μ filters. After incubation the platinum content of the filtrate was determined by flameless atomic absorption spectrophotometry (see below) and the amount of residual unchanged cisplatin by hplc (see below). Solutions used in experiments contained not more than 4% of platinum as unchanged cisplatin. Solutions were injected i.p.

Salt solutions NH_4Cl, NaCl and $(NH_4)_2SO_4$ (BDH, Poole, Analar grade) were prepared as 2.5 M aqueous solutions and administered to animals (25 mmoles/kg, approx. 1.5–2.0 cm^3 per animal) by stomach intubation.

Assessment of nephrotoxicity. Nephrotoxicity was assessed by measurement of blood urea nitrogen (BUN). Blood samples were withdrawn from treated rats via the caudal vein and urea measured in plasma samples using a urease based assay kit (Boehringer, Mannheim).

Chloride determinations. The chloride concentrations of urine and plasma was determined by titration using a Corning Model 925 Chloride Analyser.

Platinum determination. The platinum content of solutions (urine, hplc fractions, plasma etc.) was determined by flameless atomic absorption spectrophotometry using a Perkin Elmer 2380 spectrophotometer with HGA400 graphite furnace and temperature controller and AG40 autosampler using argon as the inert gas. Sample volumes were 20–50 μl. A four-stage temperature programme was used, viz. drying for 20–50 sec at 110°; charring for 20–60 sec at 1400°; atomization for 5 sec at 2500° using max. power; cleaning at 2700° for 3 sec. Standardisation was using platinum chloride solution up to 1 $\mu g\,cm^{-3}$ and the detection limit was 10 $ng\,cm^{-3} \pm 0.1\%$. Urine samples were diluted, as appropriate up to 1:100 with distilled water before analysis.

Chromatography of cisplatin metabolites. Cisplatin metabolites in urine were separated by hplc using a method previously described [8]. When frozen urine samples were analysed the samples were thawed immediately before use and insoluble residues (such as urates) removed by centrifugation in an Eppendorf 5412 mini-centrifuge for 1 min.

The modification of cisplatin nephrotoxicity by salts. Four groups of rats were taken. Each animal in the first three groups was dosed with a salt solution [either NH_4Cl, NaCl or $(NH_4)_2SO_4$, 25 mmoles/kg] p.o. Each animal in the fourth group was dosed with an equivalent volume of distilled water. Ninety minutes after this pretreatment every animal was dosed with cisplatin (5 mg/kg) i.p. Following cisplatin treatment urine was collected in two batches over the first 24 hr (0–3 hr and 3–24 hr). The urinary volume, pH, platinum content and chloride content of each batch for each animal was determined. For 7 days following cisplatin treatment daily blood samples were collected from each animal and the BUN concentration determined.

Sodium chloride and the nephrotoxicity of cisplatin metabolites. Five groups of rats were taken. Animals in two of the groups were pretreated by dosing with NaCl 25 mmoles/kg p.o. Ninety minutes after pretreatment animals in one of the groups was injected i.p. with cisplatin metabolites at a dose of platinum equivalent to 2 mg/kg cisplatin. The same dose was given to a group of animals not pretreated with saline. The other pretreated group was dosed, 90 min after pretreatment, with metabolites at a dose equivalent to 5 mg/kg cisplatin and the same dose was given to another of the groups not pretreated with saline. The fifth group of animals were untreated controls. Urine from each animal was collected for 24 hr following platinum treatment and analysed for platinum. Blood samples were collected on day 5 following platinum treatment and assayed for BUN.

The effect upon cisplatin nephrotoxicity of administration of sodium chloride after cisplatin treatment. Three groups of rats were dosed with cisplatin 5 mg/kg i.p. After 3 hr the animals in one of the groups were dosed with NaCl 25 mmoles/kg p.o. Animals in another group were dosed with the same amount of NaCl 24 hr following cisplatin treatment. Animals in the third group were used as cisplatin treated controls. A fourth group of animals was not treated with cisplatin but each animal received the standard dose of NaCl at 3 hr. Blood was collected on day 5 following cisplatin treatment and assayed for BUN.

The effect of sodium chloride on the urinary excretion of cisplatin and its metabolites. Two groups of rats were taken. One group of rats was pretreated by dosing with NaCl 25 mmoles/kg p.o., the other group was dosed with the same volume of distilled water. After 90 min the animals in both groups received cisplatin 5 mg/kg i.p. The urine collection vessels of the metabolic cages were surrounded by a solid CO_2/ethanol mixture in order to freeze the urine immediately it had been voided by the animals. Urine was thus collected for a 5-hr period following cisplatin treatment. Each urine sample was then quickly thawed, mixed, divided into several batches and rapidly refrozen. The samples were stored at −20° prior to analysis. Urine samples were analysed for chloride, for total platinum, and, following hplc separation, for cisplatin and its metabolites. Five days following cisplatin treatment blood samples were collected and analysed for BUN.

The stability of cisplatin and its metabolites in rat urine. Two rats prepared by surgical cannulation of the ureters, as described above, were used. One animal was pretreated with NaCl solution and the other animal pretreated with water as in the previous experiment and 90 min later both animals were treated with cisplatin 5 mg/kg i.p. The urine collecting in the plastic saddles was removed continuously, using a Pasteur pipette, and immediately frozen. Urine was collected for 5 hr following cisplatin treatment. For hplc separation each urine sample was thawed, mixed and an aliquot immediately applied to the column.

RESULTS

The modification of cisplatin nephrotoxicity by salts

In a preliminary experiment rats were dosed p.o. with 25 mmoles/kg NH_4Cl and the plasma chloride concentration determined at various times after dosing. The mean plasma chloride concentration in untreated control animals was 109.3 mM. In the treated animals the mean peak plasma chloride concentration was 121.2 mM and this occurred 90 min after dosing. For the experiment in which salts were used in an attempt to modify cisplatin nephrotoxicity the values for BUN on day 5 and the other results are shown in Table 1. In all animals where BUN was elevated the peak value was found 5 days after cisplatin treatment.

Table 1. The effect of salts on the nephrotoxicity of cisplatin (5 mg/kg) in the rat

	NH_4Cl	$(NH_4)_2SO_4$	NaCl	Cisplatin alone	Control
(N)	4	4	8	6	9
BUN day 5 (mg/dl)	28 ± 6‡	83 ± 27	27 ± 6‡	96 ± 47†	20 ± 6
§Urine vol. day 1 (cm^3)	15 ± 1	30 ± 5†	29 ± 4†	14 ± 5	13 ± 2
Total urinary Pt, day 1 (μg)	699 ± 218	362 ± 22‡	584 ± 127	742 ± 65	—
Urinary pH, day 1	5.7 ± 0.2†‡	7.6 ± 1.3	6.9 ± 0.3	7.2 ± 0.1	7.5 ± 0.6
Urinary chloride (mM)					
0–3 hr	346 ± 85†‡	90 ± 15†	314 ± 21†‡	85 ± 24†	179 ± 24*
3–24 hr	285 ± 29	56 ± 29†	191 ± 31	73 ± 11†	—

Values ± S.D.
* 0–24 hr value.
† Significantly different from control ($P < 0.01$).
‡ Significantly different from cisplatin alone ($P < 0.01$).
§ 0–3 hr urine volume for all three salt treatments not significantly different.

For the animals pretreated with NaCl and NH_4Cl the urinary chloride concentration 0–3 hr after cisplatin treatment was higher than in the period 3–24 hr after cisplatin. Irrespective of the nature of the pretreatment the 0–3 hr urine contained 70–90% of the total platinum excreted in the first 24 hr.

The animals pretreated with NH_4Cl or NaCl had BUN levels on day 5 which were not significantly different from control animals not treated with cisplatin (Table 1). Both pretreatments elevated urinary chloride concentrations to values which peaked at approximately twice those found in untreated animals. Animals pretreated with NH_4Cl had a urinary pH significantly lower than the controls ($P < 0.01$). Ammonium sulphate was included as a pretreatment in an attempt to lower urinary pH in the absence of an elevation in urinary chloride. However, $(NH_4)_2SO_4$ did not lower urinary pH. Animals pretreated with $(NH_4)_2SO_4$ had elevated BUN levels on day 5 which were not significantly different from those of animals treated with cisplatin alone ($P < 0.01$).

Regression analysis was performed on different paired sets of data from Table 1 to investigate possible correlations between the different parameters. The correlation coefficients (r) between pairs selected from BUN, urinary chloride concentration, urinary volume, urinary pH and urinary platinum excretion are shown in Table 2. Only urinary chloride concentration and BUN show a high degree of correlation ($r = 0.82$).

Sodium chloride and the nephrotoxicity of cisplatin metabolites

The results of the experiment in which an attempt was made to modify the nephrotoxicity caused by cisplatin metabolites by the administration of sodium chloride are shown in Table 3. Sodium chloride provided full protection against nephrotoxicity, as measured by BUN, at the lower dose of metabolites but gave no protection at the higher dose. The higher dose of metabolites (Table 3) induced a more severe uraemia than the equivalent dose of cisplatin (Table 1).

The effect upon cisplatin nephrotoxicity of administration of sodium chloride after cisplatin treatment

The administration of NaCl after cisplatin treatment did not affect the elevation of BUN on day 5 after treatment (Table 4). The general condition of the animals which received the NaCl 24 hr after cisplatin treatment appeared to be considerably worse than the other cisplatin treated animals. One animal in this group died before day 5, an abnormal occurrence at this dose. Weight loss and diarrhoea were also more severe in this group.

The effect of sodium chloride on the urinary excretion of cisplatin and its metabolites

The results of these analyses are shown in Table 5. Figure 1 shows a typical hplc separation of platinum

Table 2. Correlation coefficients for various pairs of parameters

Parameters	Correlation coefficient (r)
BUN day 5; urinary chloride day 1 (0–3 hr)	0.82
BUN day 5; urine volume day 1	0.58
BUN day 5; urinary pH day 1	0.11
BUN day 5; urinary platinum day 1	0.39
Urinary pH day 1; urinary platinum day 1	0.53
Urine volume day 1; urinary platinum day 1	0.53

Table 3. The effect of sodium chloride on the nephrotoxicity of cisplatin metabolites

Treatment	Platinum dose* (mg/kg)	BUN day 5 (mg/dl)	Total urinary platinum day 1 (μg)
Mixed metabolites	5	147 ± 31.8‡	513 ± 210.9
Mixed metabolites	2	81.8 ± 33.7†	360.2 ± 148.2
NaCl + metabolites	5	146.9 ± 79.9‡	618.9 ± 109.9
NaCl + metabolites	2	27.9 ± 1.6†§	251.6 ± 36.3
Control	—	20.3 ± 6.4	—

Values ± S.D. For all treatments N = 6.
* Expressed as equivalent dose of cisplatin.
† Significant difference between treatments ($P < 0.01$).
‡ Difference between treatments not significant ($P < 0.01$).
§ Not significantly different from control ($P < 0.01$).

Table 4. The effect upon cisplatin nephrotoxicity of administering NaCl after the drug

Treatment	N	BUN day 5 (mg/dl)
Cisplatin alone	8	87.5 ± 33.4*
Cisplatin + NaCl after 3 hr	5	119.8 ± 45.2†
Cisplatin + NaCl after 24 hr	5	117.3 ± 19.0†
Control	15	20.2 ± 6.4

Values ± S.D.
* Significantly different from control ($P < 0.01$).
† Not significantly different from cisplatin treatment ($P < 0.01$).

Table 5. Components of the blood and urine of rats dosed with cisplatin (5 mg/kg i.p.) with or without NaCl pretreatment (25 mmoles/kg p.o.)

Component	Treatment (N = 5) Cisplatin	Cisplatin + NaCl
Urinary chloride, mM, 0–5 hr*	82.3 ± 21.4	328.5 ± 40.9‡
BUN day 5 (mg/dl**)	113.5 ± 30.4	23.7 ± 6.6‡
Urine volume 0–5 hr (cm^3)	4.8 ± 1.6	6.4 ± 2.3§
Urinary platinum 0–5 hr		
(μg/cm^3)	96.0 ± 33.7	69.2 ± 36.3§
(μg total)	462.5 ± 239.0	416.8 ± 183.7§
μg Pt in individual species excreted in urine 0–5 hr		
A	73.4 ± 27.1	43.9 ± 29.5
B (cisplatin)	215.2 ± 116.9	253.5 ± 122.3
C	20.5 ± 13.2	19.5 ± 11.7
D	22.8 ± 19.5	13.2 ± 6.2
E	16.1 ± 8.8	13.4 ± 7.2
F	11.7 ± 2.1	6.5 ± 2.3†
G	6.7 ± 4.9	3.7 ± 3.9
H	18.1 ± 16.5	32.5 ± 14.6
U	80.1 ± 55.6	30.9 ± 16.3

Values ± S.D.
* Control value 181.4 ± 25.6 mM chloride.
** Control value 20.3 ± 6.4 mg/dl BUN.
† Difference between treatments significant ($P < 0.05$).
‡ Difference between treatments significant ($P < 0.01$).
§ Difference between treatments not significant ($P < 0.01$).

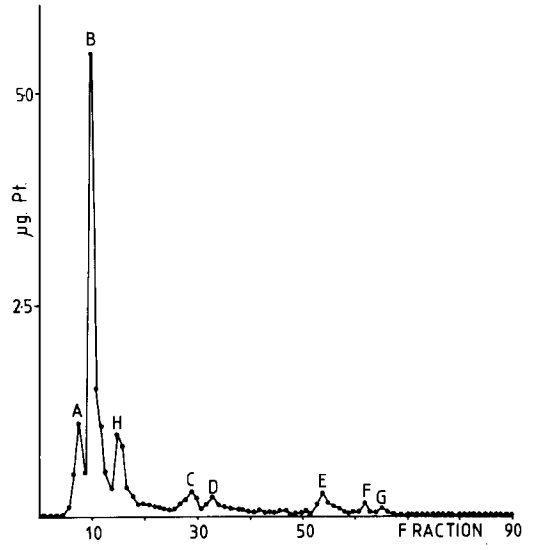

Fig. 1. Separation by hplc of platinum species in rat urine collected 0–5 hr after treatment with NaCl and cisplatin. Fractions of column eluate were collected for platinum analysis. Fraction B is unchanged cisplatin.

species in the urine of a rat treated with sodium chloride and cisplatin. It can be seen that eight discrete platinum species are present in the urine, labelled A to H on the figure. Species B is the unchanged parent compound, cisplatin. In estimating the amount of platinum in each peak the platinum in each fraction was assigned to a particular peak, was divided between pairs of peaks or was unassigned to any peak. The unassigned platinum has been called U and is shown as a separate species in Table 5. Using the hplc method all the platinum applied to the column is recovered in the eluate. The total amount of platinum excreted in the urine by animals in both treatment groups is not significantly different. Nor are there any significant differences between treatments for the amounts of cisplatin or any individual metabolite excreted in the urine in the first 5 hr after treatment except for species F ($P < 0.05$). Furthermore, for all animals treated only with cisplatin and for one animal treated with both cisplatin and NaCl in which BUN was elevated by an amount greater than one standard deviation above the mean of the untreated control animals there was a high degree of correlation, $r = 0.96$ (all values falling within the 95% confidence limits) between BUN on day 5 and the amount of species F excreted in the first 5 hr following cisplatin treatment (Table 5).

Table 6. Platinum species in the urine of rats with surgically cannulated ureters after cisplatin treatment with or without NaCl pretreatment

Component	μg platinum in urine 0–5 hr	
	Cisplatin	NaCl + Cisplatin
Total platinum	344.4	303.5
A	24.6	23.5
B (cisplatin)	203.5	211.7
C	7.3	13.4
D	18.7	13.9
E	6.4	5.8
F	9.9	5.1
G	2.2	0.7
H	2.9	3.1

The stability of cisplatin and its metabolites in rat urine

In the previous experiment because the animals did not have cannulated ureters the urine produced spent varying times in the ureters and bladders of the animals before being voided and frozen in the urine collection vessels of the metabolic cages. It is, therefore, possible that some of the platinum species excreted in the urine would have had time, before the urine was frozen, to undergo ligand exchange reactions and if that had occurred the pattern of species seen in the urine after hplc separation would not be representative of the pattern in the urine as it was originally produced. This possibility was investigated using rats prepared by surgical cannulation of the ureters. Neither urine sample thus obtained had stood unfrozen for more than 2 min after leaving the kidney before the start of the hplc separation. The results can be seen in Table 6. The results closely parallel those obtained from intact animals (Table 5).

Following this experiment a sample of urine from the rat which had been treated with cisplatin without NaCl pretreatment was incubated at 37° for 1 hr then examined by hplc and the proportions of cisplatin and its metabolites in the urine were compared with those found in the sample analysed immediately after collection. Unchanged cisplatin fell from 59.1% of the total urinary platinum to 55.1% and species F increased from 2.9 to 4.0%.

In a further experiment cisplatin 200 $\mu g\ cm^{-3}$ was added to fresh urine from an untreated rat and incubated at 37°. Samples were withdrawn after 5 min and 1 hr for analysis. Analysis by hplc revealed that species A–G were all present in the urine. After 5 min incubation 93.1% of the total urinary platinum was unchanged cisplatin and 1.2% was species F. After 1 hr percentages were 84.5 and 1.3 respectively.

DISCUSSION

Cisplatin nephrotoxicity and salts

Pretreatment of rats with NaCl or NH_4Cl prevents the onset of nephrotoxicity when animals are subsequently treated with cisplatin. Our observation that NH_4Cl can protect against cisplatin induced nephrotoxicity contrasts with that of Bertolero and Litterst [5]. They found that NH_4Cl made little difference to the severity of cisplatin induced nephrotoxicity, however, they employed a different experimental protocol from ours. Ammonium sulphate did not provide protection (Table 1). The treatment of rats with salts (NaCl, NH_4Cl and $(NH_4)_2SO_4$) modifies the urinary composition by elevating the chloride concentration, by reducing the pH or by inducing diuresis (Table 1). However, of these parameters only the urinary chloride concentration showed a high degree of correlation with nephrotoxicity as measured by BUN (Table 2). This

result is consistent with that of Litterst [3] who showed that mice could be protected from the lethality of cisplatin when the drug is administered i.p. in hypertonic saline (4.5%). The rationale for his approach was based on the assumption that cisplatin is converted into hydrolysis products (which are more toxic than cisplatin) at low chloride concentrations and that this process is inhibited at higher chloride concentrations. However he did not measure urinary or plasma chloride levels, or demonstrate that the conversion of cisplatin to hydrolysis products had been arrested.

Chloride ions and cisplatin metabolism

Earhart *et al.* [6] compared the excretion of cisplatin and total platinum in rats with pharmacologically induced chloruresis and in chloride deprived rats. In the first 24 hr after treatment animals on the high chloride regimen excreted more platinum as the parent drug (98.5%) than those animals on the low chloride regimen (91.2%). The high chloride group were also protected against nephrotoxicity. However, our data, which show that in 1 hr 15.5% of cisplatin added to urine may be converted to other platinum species, suggests that the small differences Earhart *et al.* [6] observed in the conversion of cisplatin in their samples may have arisen during the urine collection period since freezing was delayed for 1 hr. Our data (Tables 5, 6) do not show any significant differences in the excretion of seven out of eight platinum species, including cisplatin, found in the urine when animals were treated with cisplatin with or without pretreatment with NaCl. However, species F does occur in significantly lower concentration in the urine of animals treated with NaCl. This could have arisen either by a reduction in the rate of formation of species F in the urine or by a decrease in the renal permeability to species F. The fact that the concentration of species F in the urine correlates with the severity of the nephrotoxicity ($r = 0.96$) is strong evidence for a causal relationship.

We have recently reported [7] that the metabolites of cisplatin are nephrotoxic at doses of platinum at which cisplatin is not. We also reported that species F is one of the major metabolites of cisplatin in rat plasma and that there is some evidence which suggests species F could be the hydrolysis product—diaquodiammine platinum. This species has been shown to be nephrotoxic in rats [7].

Sodium chloride and the renal transport of platinum

We have previously reported the renal fractional clearance, relative to inulin, of cisplatin and its principal metabolites in the rat [8]. Amongst the metabolites species F is secreted with a fractional clearance of 2.1. This value means that not more than half the amount of this species in the urine has arrived by glomerular filtration and at least half by transtubular transport. If NaCl prevented the renal tubular secretion of species F then its only route into the urine would be by glomerular filtration. The fractional clearance would then be 1.0 and a 50% reduction in the urinary concentration of species F would be observed—as reported in Tables 5 and 6. Renal tubular transport is a mechanism by which platinum species can enter kidney cells and is therefore a prerequisite for nephrotoxicity provided nephrotoxicity arises by an intracellular mechanism. We propose to investigate the effects of NaCl on the fractional clearance of platinum metabolites.

Sodium chloride, renal homeostasis and ischaemia

Since the formation of only one cisplatin metabolite was significantly reduced by saline treatment (Tables 5 and 6) and since this reduction in concentration was by only 50% it is difficult to envisage how this could account for the complete abolition of nephrotoxicity and the mechanism suggested in the preceding section requires experimental verification. An alternative explanation is that NaCl could act indirectly by influencing the response of the kidney to the platinum (for example by modification of renal haemodynamics). We examined this hypothesis in two ways. First we investigated the effect of administering NaCl 3 or 24 hr after cisplatin. Despite the fact that nephrotoxicity takes several days to manifest itself, peaking on the fifth day after treatment, no protection was conferred by this retrospective treatment (Table 4). Therefore, it is possible that the event against which NaCl protects occurs less than 3 hr after cisplatin dosing.

Our second approach was to attempt to reduce the nephrotoxicity of preformed cisplatin metabolites by pretreatment with NaCl. The metabolites of cisplatin are nephrotoxic agents and they may be responsible for nephrotoxicity when cisplatin is administered [7]. If chloride ions act by preventing the formation of cisplatin metabolites then NaCl should not be able to protect animals from the nephrotoxicity induced by the metabolites, unless the chloride ions can convert the metabolites back to cisplatin. Our results (Table 3) were not conclusive since protection by NaCl was obtained but only at the lower dose of metabolites. It is possible that the protective mechanism is overloaded by the higher dose of metabolites and we propose to investigate this sharp dose response further.

Cisplatin-induced nephrotoxicity could possibly arise via a mechanism involving the induction of renal ischaemia. Safirstein *et al.* [9] demonstrated a fall in glomerular filtration rate following cisplatin treatment. Sodium chloride can protect against the nephrotoxicity of several agents which are thought to act via ischaemic mechanisms [10] possibly by increasing renal blood flow [11]. Treatment of rats with cisplatin alone results in the excretion of urine with a lower than normal chloride concentration in the absence of diuresis (Tables 1 and 5). This disturbance of renal salt homeostasis could be a key event in the development of the renal lesion since it could lead, via tubuloglomerular feed-back, to ischaemia. Hypochloruria (Tables 1, 5) is the earliest renal disturbance so far to have been observed *in vivo* following cisplatin treatment in the rat.

On the basis of our results it is possible to propose several roles for NaCl in modifying cisplatin nephrotoxicity. It may act via the inhibition of the formation, uptake or transport of a single nephrotoxic metabolite or via a mechanism in which NaCl

circumvents a pathological process which results when platinum causes renal ischaemia.

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