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ROLE OF MITOCHONDRIA IN CISPLATIN-INDUCED OXIDATIVE DAMAGE EXHIBITED BY RAT RENAL CORTICAL SLICES

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Abstract—The role of mitochondria with regard to cisplatin-induced renal toxicity has been examined *in vitro*. The mitochondria were prepared from rat renal cortical slices which had been exposed to cisplatin. Incubation of the kidney slices with 2 mM cisplatin for various periods depleted glutathione (GSH) and increased thiobarbituric acid reactive substances (TBARS) in a time-dependent manner, a change which indicates lipid peroxidation in the mitochondria. The content of GSH was significantly depleted within 15 min of incubation, while TBARS formation was increased after 60 min of incubation. Marked depletion (30%) of GSH and increased TBARS formation (2-fold) were observed after 60 min and 120 min of incubation, respectively. Furthermore, cisplatin also depleted GSH and induced TBARS formation in the mitochondria in a concentration-dependent fashion. Cisplatin (0.5 mM) depleted GSH, but did not increase the production of TBARS. In addition, the fluorescence intensity of 8-anilino-1-naphthalenesulphonic acid (ANS)-bound to mitochondrial membranes was decreased after 120 min of incubation with 2 mM cisplatin. Several parameters were measured as indicators of damage to mitochondria and cellular integrity and they showed that cytotoxicity occurred subsequent to both GSH depletion and TBARS formation. Cisplatin-induced depletion of GSH is an early event and a determinant step in oxidative stress to mitochondria in the kidney cortex and may lead to irreversible cell injury.

Cisplatin, a widely used antineoplastic agent, is a well-known nephrotoxicant in humans and animals [1–3]. Although the exact biochemical mechanisms responsible for the nephrotoxicity of cisplatin have not yet been defined, the involvement of oxidative stress is indicated by several lines of evidence. Lipid peroxidation in the kidney tissue was increased after either one dose *in vivo* or incubation of renal cortical slices with cisplatin [4–8], cisplatin depleted glutathione (GSH†) *in vitro* [8, 9] and protein thiols *in vivo* [10, 11], the activities of antioxidative enzymes, such as Cu, Zn-superoxide dismutase, GSH peroxidase, GSH *S*-transferase, catalase and GSH reductase, were decreased [12, 13] and furthermore radical scavengers and antioxidants have been used to protect against cisplatin-induced nephrotoxicity [5–8, 14, 15]. Nevertheless, the role played by lipid peroxidation in the pathogenesis of kidney damage by oxidative stress is still controversial. Vermeulen and Baldew [16] have recently demonstrated that cisplatin did not induce lipid peroxidation in rat kidney microsomes containing a NADPH-generating system and also did not reduce the activity of a cytosolic GSH-dependent protective factor against Fe^{2+}-ascorbate-induced lipid peroxidation. Thus, it appears that lipid peroxidation is not a major cause of cisplatin-induced nephrotoxicity.

Cisplatin has a site-specific nephrotoxic effect and pathological studies have shown that the major site of renal injury by cisplatin in rats is the S_3 segment of the proximal tubule located in the outer stripe of the outer medulla [17, 18]. Cells of the proximal convoluted tubule are generally rich in mitochondria and the cells of the S_3 segment are particularly active in relation to the metabolism of GSH [19]. Studies of the subcellular distribution of platinum have shown that mitochondria and cytosol contained the highest concentration of platinum [10]. The mitochondrion may therefore be a critical target for the toxic effects of cisplatin and there is growing evidence for this possibility. Earlier investigations showed that cisplatin reduced state 3 renal mitochondrial respiration and increased mitochondrial calcium accumulation after cisplatin administration to the rat [20]. Inhibition of oxygen consumption and net K^+ transport were observed when renal proximal tubules were exposed to cisplatin [21] and cisplatin also caused damage to mitochondrial DNA [22, 23]. Daley-Yates and McBrien [24] suggested that pathological effects of cisplatin on kidney mitochondria are an early manifestation of its nephrotoxicity. The swelling and vacuolation of mitochondria may be the result of osmotic forces initiated by ionic imbalance in mitochondria with impaired ATPase activity. Only recently, Kameyama and Gemba [25] reported that cisplatin decreased GSH and calcium uptake in mitochondria and diethylmaleate, a GSH depletor,

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† Abbreviations: GSH, glutathione; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); TBA, 2-thiobarbituric acid; ANS, 8-anilino-1-naphthalenesulphonic acid, ammonium salt; Tris, tris(hydroxymethyl) aminomethane; TBARS, thiobarbituric acid reactive substances; SH, sulphydryl; GDH, glutamate dehydrogenase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; NAG, *N*-acetyl-β-glucosaminidase.

enhanced these toxic effects. Another piece of evidence showed that cisplatin-induced nephrotoxicity was closely related to mitochondrial dysfunction through the impairment of GSH peroxidase [26].

Altogether, these observations suggest that oxidative damage to mitochondria may be involved in cisplatin-induced nephrotoxicity and so the role of lipid peroxidation and its relationship to GSH depletion in mitochondria, aspects of cisplatin nephrotoxicity which have not previously been studied in detail, have now been investigated with rat renal cortical slices *in vitro*. Cisplatin was observed to deplete GSH, increase the production of thiobarbituric acid reactive substances (TBARS), affect membrane integrity and cause leakage of the mitochondrial marker enzyme, aspartate aminotransferase (AST).

MATERIALS AND METHODS

Chemicals. Cisplatin was a generous gift from the Johnson Matthey Research Centre (Berks, U.K.). L-Cysteine, 1,1,3,3-tetraethoxypropane, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent), 8-anilino-1-naphthalenesulphonic acid, ammonium salt (ANS), tris(hydroxymethyl) aminomethane (Tris), *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide and bovine serum albumin were obtained from the Sigma Chemical Co. (Poole, U.K.). L-Glutamic acid, sodium salt and glycine were purchased from BDH Chemicals Ltd (Poole, U.K.). 2-Thiobarbituric acid and 4-nitrophenol were from the Aldrich Chemical Co. Ltd (Dorset, U.K.) and all other chemicals were of reagent grade.

Preparation of renal cortical slices. Adult male Wistar albino rats (210–260 g) were obtained from the Department of Pharmacology and Therapeutics animal house. Rats were killed by cervical dislocation. The kidneys were quickly removed, decapsulated and placed in ice-cold rinse medium, gassed with pure oxygen. The rinse medium was composed of 97 mM NaCl, 40 mM KCl, 0.74 mM $CaCl_2.H_2O$ and 7.5 mM sodium phosphate buffer, pH 7.4. The renal cortical slices (about 0.5 mm in thickness) were prepared freehand with Rocket skin graft Blades (Rocket of London Ltd, Watford, U.K.) as previously described [27]. The kidney was placed on filter paper fixed to a rectangular glass base (5 × 25 cm). A ground-glass slide (2.5 × 7.5 cm) was then pressed against the kidney and the blade was drawn between slide and kidney. Three cortical slices were cut from each surface of kidney. The slicing apparatus and kidney were kept wet at all times with rinse medium. After preparation, the slices were blotted with filter paper and 150–200 mg of the slices were added to glass conical flasks (25 mL Erlenmeyer flasks, Duran, Astell Scientific, Kent, U.K.) each of which contained oxygenated incubation medium (5 mL) with or without cisplatin. The incubation medium contained 1 mM glycine, 1 mM glutamate and 0.2 mM L-cysteine in rinse medium. This combination of amino acids was used to sustain GSH concentration in the slices [28]. Each flask was thoroughly gassed with pure oxygen for 1 min after addition of the slices and medium and then stoppered promptly with a rubber bung. The flasks were incubated in a shaking waterbath at 100 cycles/min at 37° for various times. Cisplatin was dissolved in incubation medium prior to use.

Preparation of mitochondria. After incubation, the slices were gently removed from the flasks with forceps and blotted on filter paper and a mitochondrial fraction was prepared by differential centrifugation according to Schenkman and Cinti [29]. Slice mitochondria were isolated in ice-cold 150 mM KCl which was used instead of sucrose in view of its interference with the measurement of malondialdehyde [30]. The homogenates (10% w/v) were prepared in a glass homogenizer tube (10 mL capacity) with a teflon pestle (FSA Laboratory Supplies, Loughborough, U.K.) for 10 strokes (Black & Decker H501 Homogenizer). The homogenates were centrifuged at 600 *g* for 5 min with an MSE Prespin 65 ultracentrifuge. The supernatant obtained was centrifuged at 12,000 *g* for 10 min. The pellets representing the mitochondrial fraction were resuspended in 150 mM KCl, except for the mitochondria used to assess GSH. All these processes were carried out at 0–4°. Protein content was determined by the method of Lowry *et al.* [31] with bovine serum albumin as standard.

Determination of GSH in mitochondria. GSH in the mitochondria was determined by the method of Sedlak and Lindsay [32] with little modification. The mitochondrial pellets were suspended in 20 mM EDTA, 2 mL mitochondrial suspension (1–2 mg protein/mL), was added to a tube with 0.1 mL of trichloroacetic acid (1 g/mL). After shaking on the whirlmixer for 1 min, the tube was centrifuged at 3000 *g* for 10 min. An aliquot (1 mL) of the resulting supernatant was added to a tube containing 2 mL of 400 mM Tris–HCl–20 mM EDTA buffer (pH 8.9) and 0.1 mL of 10 mM DTNB in methanol and then mixed. The absorbance was measured at 412 nm within 5 min and the GSH concentration was calculated with L-cysteine as the standard.

Lipid peroxidation assay. The extent of lipid peroxidation was expressed as TBARS. Mitochondrial suspension (1 mL; 1–2 mg protein/mL) was added to 2 mL of a solution (0.375% w/v TBA, 15% w/v trichloracetic acid, and 0.25 M HCl) and the reaction mixture was heated in a boiling-water bath and then cooled and centrifuged at 3000 *g* for 15 min to remove particulate matter. TBARS in the supernatant were determined at 532 nm by the method of Buege and Aust [33] with 1,1,3,3-tetraethoxypropane as standard.

ANS fluorescence intensity. The fluorescent ligand ANS was used as a probe to monitor the physical state of the mitochondrial membrane. Mitochondrial suspension (3 mL; 0.05 mg/mL in 50 mM phosphate buffer, pH 7.4) was labelled by addition of 1.5 mM ANS (0.1 mL) to give a final ANS concentration of 0.05 mM in the reaction medium. After the membranes had reacted with ANS at 25° for 15 min, the relative fluorescence intensity was measured with a Perkin–Elmer 203 fluorescence spectrophotometer and a Perkin–Elmer 150 xenon power supply. The excitation wavelength for ANS was 380 nm and the emission was measured at 485 nm. The data are presented as relative fluorescence intensity.

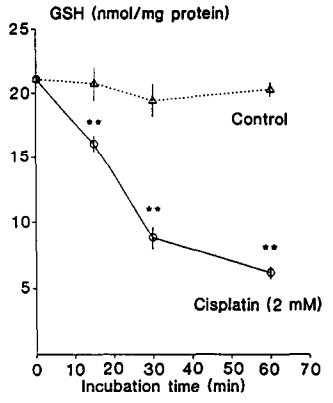

Fig. 1. Effect of duration of incubation on GSH in the mitochondria isolated from rat renal cortical slices. The slices were incubated in the medium with or without cisplatin at 37° for different periods. Each value represents the mean ± SE of five observations. Significant differences from control are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$.

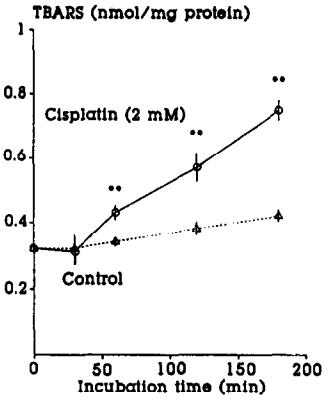

Fig. 2. Effect of duration of incubation on lipid peroxidation, assessed by production of TBARS in the mitochondria isolated from rat renal cortical slices. The other conditions were as in Fig. 1.

Assay of enzyme activity. The mitochondrial damage and cellular integrity were estimated by measurement of enzyme activity in either the mitochondria and/or the incubation medium. Glutamate dehydrogenase (GDH; EC 1.4.1.3) activity in mitochondria was measured with the Boehringer test-combination kit (Boehringer-Mannheim, Germany). AST (EC 2.6.1.1) in mitochondria and medium and lactate dehydrogenase (LDH; EC 1.1.1.27) in the medium were assessed with Sigma kits (Poole, U.K.). *N*-Acetyl-β-glucosaminidase (NAG; EC 3.2.1.30) activity in the medium was determined by the conversion of *p*-nitrophenyl *N*-acetyl-β-glucosaminide to *p*-nitrophenol by the method of Maruhn [34] with some modifications. The reaction mixture containing 0.05 mL incubation medium and 1 mL of 10 mM *p*-nitrophenyl *N*-β-glucosaminide in 50 mM citrate–100 mM phosphate buffer (pH 4.2) was incubated in a water bath at 37° for 30 min. The reaction was terminated by 4 mL of 50 mM sodium tetraborate decahydrate–NaOH buffer (pH 9.8) and the concentration of *p*-nitrophenol formed was determined from the absorbance at 410 nm. All enzyme activities are expressed as international units per litre.

Statistics. The renal cortical slices for each experiment were prepared from four to five rats and the results are expressed as means ± SE of five observations. The data were analysed with an unpaired two-tailed Student's *t*-test. A probability level of $P < 0.05$ was considered as statistically significant.

RESULTS

Depletion of GSH in the mitochondria

Incubation of slices with 2 mM cisplatin caused a dramatic depletion of GSH in the mitochondria in a time-dependent fashion (Fig. 1). GSH levels were significantly decreased as early as 15 min of incubation (77% of control value) and a marked depletion (30%) occurred after 60 min of incubation (Fig. 1). Furthermore, 0.5 mM cisplatin significantly decreased GSH levels and 2 mM of cisplatin was found to deplete GSH to the greatest extent (27%) after 120 min of incubation (Table 1).

Lipid peroxidation in the mitochondria

Lipid peroxidation in the mitochondria was assessed by the estimation of TBARS formation and

Table 1. Effect of cisplatin concentration on GSH, TBARS, relative fluorescence intensity of ANS and protein content of the mitochondria isolated from rat renal cortical slices

	Cisplatin concentration (mM)			
	0	0.5	1	2
GSH (nmol/mg protein)	14.4 ± 2.4	7.9 ± 0.8†	5.2 ± 0.7†	4.0 ± 0.9†
TBARS (nmol/mg protein)	0.33 ± 0.01	0.36 ± 0.03	0.39 ± 0.02*	0.54 ± 0.02†
Fluorescence intensity	53 ± 1	54 ± 2	54 ± 1	45 ± 2†
Protein (mg/g wet wt)	16.6 ± 1.1	14.1 ± 1.5	14.4 ± 0.8	11.4 ± 0.4†

The slices were incubated in the medium either with or without different concentrations of cisplatin at 37° for 120 min. Each value represents the mean ± SE of five observations.
Significant differences from control are indicated by $^{*}P < 0.05$, †$P < 0.01$.

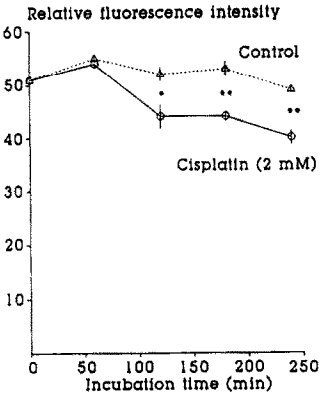

Fig. 3. Effect of duration of incubation on the fluorescence intensity of ANS bound to mitochondrial membranes isolated from rat renal cortical slices. The other conditions were as in Fig. 1.

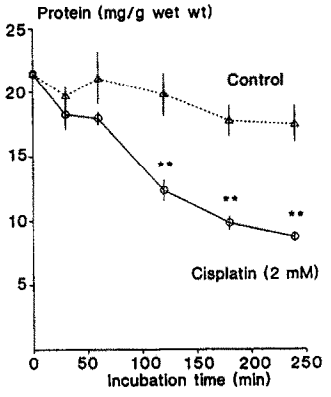

Fig. 4. Effect of duration of incubation on mitochondrial protein concentration in rat renal cortical slices. The other conditions were as in Fig. 1.

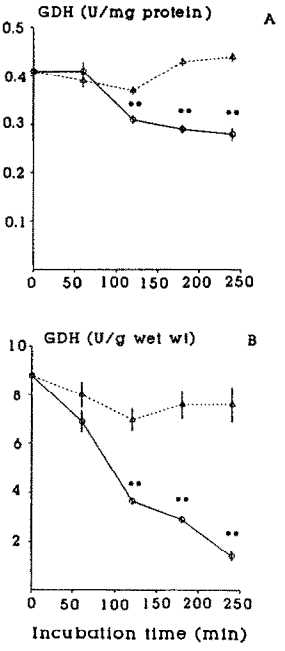

Fig. 5. Effect of duration of incubation on GDH activity in mitochondria isolated from rat renal cortical slices. (- -△- -) Control; (—○—) 2 mM cisplatin. The other conditions were as in Fig. 1.

cisplatin caused a time-dependent increase in the formation of TBARS. The production of TBARS was increased after 60 min of incubation and TBARS was maximal (1.8-fold higher than that in control) after 180 min of incubation (Fig. 2). TBARS formation was also increased by exposure to cisplatin at 1 mM, but not 0.5 mM, after 120 min of incubation (Table 1).

ANS fluorescence intensity

The addition of 2 mM cisplatin to the incubation medium decreased the fluorescence intensity of ANS bound to mitochondrial membranes after 120 min of incubation (Fig. 3). Lower concentrations of cisplatin (less than 2 mM) had no effect, however, on the fluorescence intensity (Table 1).

Mitochondrial protein content of the slices and enzyme activity

The mitochondrial content of the slices, expressed as the ratio between mg of mitochondrial protein and g wet weight slices (mg protein/g wet wt) decreased in a linear manner with increasing incubation time (Fig.4). After 120 min of incubation, the mitochondrial protein content was 20 mg protein/g wet wt in the control, while that in the presence of cisplatin was 12 mg protein/g wet wt. The mitochondrial protein concentrations were decreased to 50% of the controls after exposure to cisplatin for 240 min (Fig. 4). Lower concentrations of cisplatin had little effect on the mitochondrial protein content (Table 1).

The effects of cisplatin on enzyme activities in the mitochondria are shown in Figs 5 and 6. GDH activity, expressed per mg protein, in the mitochondrial fraction was directly inhibited at 120 min by exposure to 2 mM cisplatin (Fig. 5A) and when the GDH activity is expressed per g wet wt of slices, the decrease in activity was even greater (Fig. 5B). The activity of GDH was similar in the groups exposed to less than 2 mM cisplatin, whether the activity was expressed per mg protein or per g wet wt (Table 2). The activity of AST per mg protein in the mitochondria was unaffected by various periods of incubation with cisplatin (Fig. 6A), but, when AST activity was expressed per g wet wt of slices, a reduction in activity was observed in the slices treated with 2 mM cisplatin for longer than 120 min (Fig. 6B). There was no reduction of the activity in slices when treated with lower concentrations of cisplatin for 120 min (Table 2).

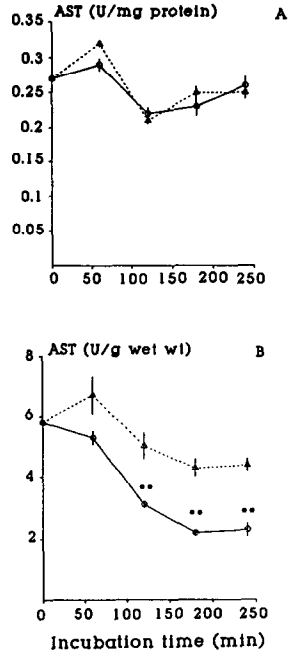

Fig. 6. Effect of duration of incubation on AST activity in mitochondria isolated from rat renal cortical slices. (- -△- -) Control; (—○—) 2 mM cisplatin. The other conditions were as in Fig. 1.

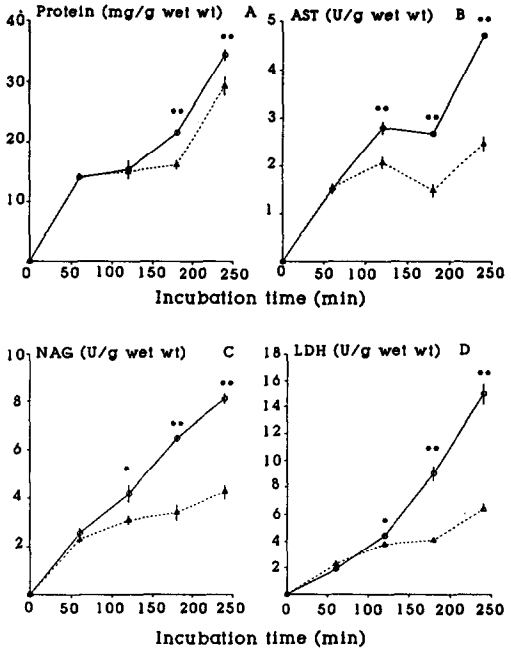

Fig. 7. Effect of duration of incubation on the release of protein and enzyme (AST, NAG and LDH) activities released from rat renal cortical slices. (- -△- -) Control; (—○—) 2 mM cisplatin. The other conditions were as in Fig. 1.

Table 2. Effect of cisplatin concentration on GDH and AST of the mitochondria isolated from rat renal cortical slices

Enzyme	Cisplatin concentration (mM) 0	0.5	1	2
GDH				
(U/mg protein)	0.38 ± 0.01	0.39 ± 0.01	0.36 ± 0.01	0.27 ± 0.03*
(U/g wet wt)	6.2 ± 0.3	5.4 ± 0.4	5.2 ± 0.4	3.4 ± 0.4*
AST				
(U/mg protein)	0.28 ± 0.02	0.31 ± 0.02	0.28 ± 0.01	0.30 ± 0.01
(U/g wet wt)	4.7 ± 0.5	4.1 ± 0.2	4.0 ± 0.3	3.4 ± 0.1*

The slices were incubated in the medium either with or without different concentrations of cisplatin at 37° for 120 min.

Significant differences from control are indicated by *$P < 0.01$. The other conditions were as in Table 1.

Protein content and enzyme leakage released from the slices into the incubation medium

The leakage of protein from the slices was significantly increased after 180 min of incubation (Fig. 7A) and furthermore, AST, NAG and LDH activities in the incubation medium were enhanced after 120 min of incubation. The AST, NAG and LDH activities released by the slices were all about 2-fold higher than the corresponding controls at 240 min (Fig. 7B, C and D). All the enzyme activities were similar to those of the controls when the concentration of cisplatin was less than 2 mM (Fig. 8).

DISCUSSION

Numerous studies have suggested that oxidative stress is one of the mechanisms of cisplatin-induced nephrotoxicity [4–9, 13–15]. The present experiments have provided evidence that this effect involved cisplatin-dependent depletion of GSH and an increase in lipid peroxidation, as monitored by TBARS formation, in the mitochondria (Figs 1 and 2). The GSH levels in the mitochondria were significantly decreased at as early as 15 min of incubation and this decrease preceded the increases in lipid peroxidation caused by cisplatin after 60 min of incubation (Fig. 2). The results are similar to

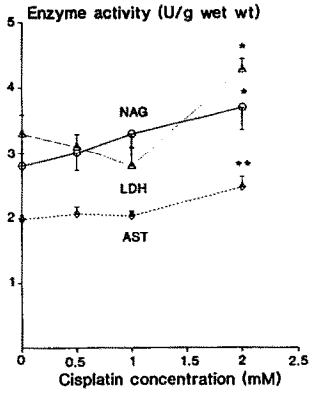

Fig. 8. Effect of cisplatin concentration on AST, NAG and LDH activities released from rat renal cortical slices after 120 min of incubation.

those of a previous study [8] where cisplatin (2 mM) caused a marked depletion of GSH in rat cortical slices after 30 min exposure, which suggested that the mitochondrion is an early target for cisplatin-induced depletion of GSH in the tissues. Schnellmann and Mandel [35] have suggested that GSH is distributed between two pools within the proximal tubules: the mitochondrial pool is larger, containing 72% of the cellular GSH while the cytoplasmic pool contains the remaining 28%.

Unlike the mitochondrion, it seems less likely that the endoplasmic reticulum is a major target for oxidative stress induced by cisplatin, because, it did not induce lipid peroxidation in rat kidney microsomes nor did it reduce the activity of a microsomal GSH-dependent protective factor against lipid peroxidation induced by Fe^{2+}-ascorbate [16]. Furthermore, cisplatin administered *in vivo* decreased the sulphydryl (SH) groups in the mitochondrial fraction but not the microsomal one [10], so it is likely that the mitochondria are a more important target for oxidative stress by cisplatin.

The profound importance of GSH in relation to hepatotoxicity has also been documented for chemicals that deplete GSH, and they include carbon tetrachloride, 1,1-dichloroethylene, paracetamol (acetaminophen), bromobenzene and thioacetamide [36]. In the case of paracetamol it has been shown that its toxic metabolite *N*-acetyl-*p*-benzoquinone imine stimulated the oxidation of both NADH and NADPH, with subsequent loss of the nucleotides by hydrolysis [37]. Oxidation of the pyridine nucleotides was necessary but not sufficient to cause the release of Ca^{2+} from mitochondria. Disturbance of Ca^{2+}-homeostasis and depletion of pyridine nucleotides may be early events in cisplatin toxicity which precedes the onset of lipid peroxidation. The depletion of GSH by diethylmaleate accelerates lipid peroxidation in kidney cortical slices [9], whereas dithiothreitol, a SH-reducing agent, protects against the lipid peroxidation and cytotoxicity caused by cisplatin [14]. Similarly, administration of GSH to rats ameliorated cisplatin-induced kidney damage [15].

Cisplatin also reduced the mitochondrial activity of GSH peroxidase, an enzyme for which the concentration of reduced GSH is important [26]. Mitochondria play an important role in the regulation of free cytosolic calcium and Monte *et al.* [38] have proposed that perturbation of intracellular calcium homeostasis was critically associated with depletion of protein thiols which occurred more rapidly in cells with decreased levels of GSH. This conclusion was supported by the observation that the simultaneous treatment of renal cortical slices with cisplatin and diethylmaleate decreased even further the uptake of calcium by mitochondria [25]. Thus the ability of cisplatin to deplete GSH at an early stage may be a determinant step in the subsequent induction of lipid peroxidative processes, disturbance of calcium homeostasis and subsequent nephrotoxicity. It is difficult, however, to be certain as yet which changes are a cause and which are a consequence of toxicity.

Although the free radicals involved in cisplatin-induced lipid peroxidation have not yet been identified, the free active oxygen species such as superoxide anion, hydrogen peroxide or hydroxyl radicals, may be involved since the activities of antioxidative enzymes were decreased *in vivo* [12, 13]. The fact that these enzymes were decreased does not necessarily indicate that radicals are involved because the enzymes may be inhibited by covalent binding and/or complexation with a transformation product of cisplatin. Further studies will be required to determine the interrelationships between lipid peroxidation, depletion of GSH, disturbance of calcium homeostasis, formation of active oxygen species and antioxidative enzymes with this experimental model.

Interestingly, the depletion of GSH in the mitochondria by either 2 mM cisplatin at 15 min (77% of control) or 0.5 mM cisplatin at 120 min (55%) was not accompanied by lipid peroxidation. Cisplatin caused lipid peroxidation only when the mitochondrial GSH depletion reached a critical value (about 30% of control) in both time-course and concentration-effect studies. Therefore, under our experimental conditions, it appears that GSH must fall to this critical threshold concentration before lipid peroxidation will occur.

The negatively charged probe ANS binds to the region of the polar head group of the lipid bilayer in the membrane [39] and this binding, rapidly enhances fluorescence and gives a blue-shift in the emission maximum, compared with ANS in aqueous bufer. The results here showed that cisplatin decreased the fluorescence intensity of ANS bound to mitochondrial membranes after incubation with cisplatin (2 mM) for 120 min. The decrease in fluorescence could arise in two ways. One possibility is a decrease of the quantum yield of the bound ANS in the mitochondria, which suggests that the naphthalene ring of the ANS molecule penetrates less deeply into the hydrophobic region of membrane and hence is more accessible to the aqueous environment. This implies a change in the packing of the ANS molecules between phospholipid polar head groups and also, possibly, a change in the orientation of the phospholipid as the lipid interior

of the membrane becomes less fluid because of lipid peroxidation [40]. On the other hand, the number of ANS molecules bound to the membrane is strongly influenced by the surface charge potential and is inversely proportional to the value of the negative membrane surface potential [41]. So if cisplatin increases the negative charge density of the membrane, this will decrease the binding sites of ANS in the membrane and lower the fluorescence.

Damage to mitochondria by cisplatin was examined by measurement of the mitochondrial protein concentration and also several enzyme markers. The mitochondrial protein in the slices was dramatically decreased after 120-min exposure to 2 mM cisplatin (Fig. 4) and there was a subsequent increase in protein concentration in the incubation medium after 180 min (Fig. 7). In addition, the effects of cisplatin on GDH and AST in the mitochondrial matrix were investigated. After 120 min of incubation, 2 mM cisplatin directly inhibited the activity of GDH, but had no effect on the activity of AST, when expressed per mg protein. Although the exact reasons for these differences are not known, one possibility is that GDH contains an active SH group(s) [42], to which cisplatin may bind and thus inhibit the enzyme so that it could not be measured in the medium. GSH protects against chemical attack on critical SH groups in proteins, and modification of SH groups of enzymes often leads to inhibition of enzyme function. It is therefore possible that cisplatin inhibits ATPase [24] and gluconeogenesis [7, 8] through such a mechanism. Both GDH and AST activities in the slices were decreased when expressed on the basis of g wet wt as a result of a loss of mitochondria in the slices and this is consistent with leakage of AST activity from the slices in the presence of cisplatin.

The leakage of the cytosolic enzyme LDH and lysosomal NAG have been used to monitor the cytotoxicity of cisplatin [8] and the present results show that 2 mM cisplatin increased the leakage of these enzymes (Fig. 7C and D) after 120 min, which suggests that severe damage to cell integrity occurred. It is generally thought that lipid peroxidation destroys the biological membranes in which it occurs and so such damage to mitochondrial membranes by cisplatin will lead to disturbance of mitochondrial function, loss of their ultrastructural architecture and changes in membrane fluidity and permeability.

In summary, the results point to depletion of the mitochondrial GSH pool by cisplatin as an early and primary event and it is postulated that peroxidative damage to the mitochondria and subsequent changes in the physical state of mitochondrial membranes are related to the cytotoxicity caused by cisplatin.

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