Cancer Chemother Pharmacol (1991) 28: 277–282
034457049100102D

Cancer Chemotherapy and Pharmacology

The effects of cisplatin on the concentration of protein thiols and glutathione in the rat kidney

P. Mistry[1], Y. Merazga, D. J. Spargo[2], P. A. Riley[2], and D. C. H. McBrien

Biology and Biochemistry Department, Brunel University, Uxbridge, Middlesex UB8 3PH, UK
[1] Present address Department of Biochemical Pharmacology, Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, UK
[2] Department of Chemical Pathology, University College and Middlesex School of Medicine, London W1P 6DB, UK

Received 5 October 1990/Accepted 20 February 1991

Summary. The effect of a nephrotoxic dose of cisplatin (5 mg/kg) on the concentrations in the rat kidney of both glutathione and protein-bound thiols was investigated. Total glutathione and oxidised glutathione were measured in the cortex and outer medulla using specific enzyme-based assays. The high-molecular-weight thiols were quantified in cells of the proximal tubule using a cytochemical technique. The concentration of total glutathione (oxidised and reduced) in the kidney cortex and outer medulla was significantly higher than that of controls at 1 h following cisplatin administration. The amount by which the concentration in treated animals exceeded that in controls increased to 50% at 72 h and remained significantly elevated for 120 h following treatment. This increase was mainly attributable to an increase in the concentration of reduced glutathione. In contrast, the concentration of protein thiols in the proximal tubules decreased significantly at 8 h after dosing, reaching a nadir 29% below that of controls at 120 h, thus coinciding with the maximal functional disturbance in the kidney as reflected by the concentration of blood urea. The decrease in protein thiols could not be correlated stoichiometrically with the platinum concentration in the cortex and outer medulla, which reached a peak of 16.3 ± 0.3 μg/g wet tissue at 72 h after treatment. Evidently cisplatin perturbs the equilibrium that is said to exist between the concentration of reduced glutathione and that of protein thiols. This perturbation occurs well before the onset of overt functional disturbance of the kidney and is evident before the point at which the damage to the kidney caused by cisplatin becomes irreversible.

Offprint requests to: P. Mistry

Introduction

Cisplatin [*cis*-dichlorodiammineplatinum(II)] is a potent antitumour agent with severe dose-limiting nephrotoxic side effects. The biochemical mechanism of this toxicity is unknown. The avidity of platinum for sulphur-containing ligands has often been invoked in hypothetical mechanisms explaining the nephrotoxicity of cisplatin [6, 12]. Both high- and low-molecular-weight molecules containing thiol functional groups may undergo ligand-exchange reactions with platinum. The principal high-molecular-weight thiol-containing molecules found in kidney cells are proteins and the principal low-molecular-weight thiol is glutathione. Protein-bound thiol groups are thought to be in equilibrium with low-molecular-weight thiol species such as glutathione, and the maintenance of appropriate concentrations of these species in the reduced state is essential for numerous cellular functions, including inactivation of peroxides and free radicals, detoxification of xenobiotics and membrane transport, and for the activity of many enzymes [2, 11]. The nephrotoxicity of mercury (and some other heavy metals) has been suggested to be a consequence of perturbations in the concentration of cellular thiols [4, 18]. Variations in the concentration in the rat kidney of low-molecular-weight thiols such as glutathione [12–14] and changes in the total thiol concentration (protein and non-protein) [12] have been reported to follow treatment with cisplatin. Levi et al. [12] report that at 72 h after cisplatin administration, a reduction in total renal thiol content coincided with an increase in the concentration of non-protein thiols.

In the present study we applied two different approaches to the investigation of cisplatin-induced changes in renal thiol concentrations. We used specific enzyme-based assays to measure both reduced and oxidised glutathione concentrations in the cortex and outer medulla. We also measured protein-thiol concentrations in the cells of the proximal tubule, the principal site of the cisplatin-induced nephrotoxic lesion [7], using a cytochemical technique in conjunction with a video microdensitometer.

Materials and methods

Animals and chemicals

Male Wistar rats weighing 300–350 g were obtained from Charles River, Margate. Cisplatin (DDP) was a gift from Johnson-Matthey Research, Reading. DDD (2,2′-dihydroxy-6,6′-dinaphthyldisulphide) and glutathione reductase type III from baker's yeast were purchased from Sigma Chemical UK Ltd. Fast Blue B was purchased from BDH Chemicals Ltd. Poole.

Reactive protein-thiol concentration in proximal tubules

Cisplatin treatment and preparation of kidney sections. Experimental animals were injected i.p. with a freshly prepared solution of DDP (2 mg/ml) in 0.9% NaCl at a dose of 5 mg/kg DDP. Control rats were injected with an equivalent volume of 0.9% NaCl. Animals were allowed access to food and water ad libitum. At 1, 3, 8, 24, 48, 72, 96 and 120 h following treatment, animals were anaesthetised with sodium pentobarbital (50 mg/kg i.p.) and the kidneys were excised, cut into 3-mm-thick transverse sections and frozen in *n*-hexane maintained at −70° C using the method of Smith et al. [20]. Frozen sections (5 μm) were cut using a Slee cryostat, air-dried for 15 s and fixed in 95% aqueous ethanol for at least 15 min before staining for protein thiols.

Staining for reactive protein thiols. The sections were stained using the method of Nöhammer [15]. This method involves the incubation of sections in a solution of DDD for 7 h followed by a 3-min wash in acetone and a 3-min incubation in acetate buffer (pH 4). The sections are then washed three times in acetone (5 min) and once in distilled water (5 min) before azo-coupling with Fast Blue B (FBB) for 15 min. Next, they are washed in tap water for 5 min and then in distilled water before finally being mounted in immersion glycerin.

Cytodensitometry. The absorbance of the DDD-FBB protein-thiol complex in the sections was measured at 560 nm using a Zeiss photomicroscope fitted with a Hitachi KP4 video camera (Hitachi-Denshi Ltd. UK), which served as the detector for an Intellect 200 image analyser (Quantel Ltd. UK) as previously described [21]. An in-house programme written in FORTRAN enabled the operator to delineate the proximal tubules on the video image of sections in which the measurements were to be made. A composite reference image was built up by recursive video processor using ten images of unoccupied areas of the slide immediately adjacent to the section. Absorbance was calculated using a pixel-by-pixel comparison of the same areas of the object and of reference images, thereby obviating the influence of shading and differential sensitivity of the detector without recourse to lengthy computation or image processing [21]. The extinction coefficient for the dye was 1.9×10^4 at 560 nm and the path length was assumed to be equivalent to the section thickness (5 μm). For each kidney section, approximately 20 cross sections of a proximal tubule located adjacent to a glomerulus were individually measured; between 3 and 5 sections/animal were measured, and 2–8 animals were used for each time point. The results were analysed using Student's *t*-test.

Glutathione

Total glutathione. Total glutathione [reduced glutathione (GSH) + oxidised glutathione (GSSG)] was determined in homogenates of the kidney cortex and outer medulla using the dithionitrobenzene (DTNB) recycling assay originally described by Owens and Belcher [16] and later modified by Tietze [23]. Tissue for analysis was homogenised in ice-cold metaphosphoric acid (3%, w/v) and centrifuged at 100,000 *g* for 40 min at 4° C. Supernatant was diluted with an equal volume of distilled water; 200-μl samples were added to 2.5 ml 0.05 mM phosphate buffer (pH 7), 0.8 ml 1 mM Na-ethylenediamine-tetraacetyl acid (Na-EDTA) (pH 7), 30 μl 0.1 M DTNB and 100 μl GSSG reductase (2.2 units/ml); and the reaction was started by the additon of 100 μl 5 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH). The rate of change in absorbance at 412 nm was monitored for 5 min and compared with a standard curve generated using different concentrations of GSH.

GSSG and total glutathione. GSSG and total glutathione concentrations were measured separately using the method of Griffith [8]. Tissue for analysis was homogenised in 10% (w/v) sulfosalycilic acid (5 vol/g tissue) and centrifuged at 100,000 *g* for 20 min. For measurement of GSSG, the free GSH was masked by adding 2 μl 2-vinylpyridine and 6 μl triethanolamine to 100 μl supernatant. The sample was incubated for 1 h at 25° C. To 25 μl sample after derivitisation, we added 100 μl 6 mM DTNB, 700 μl 0.3 mM NADPH in 6.3 mM EDTA, 143 mM sodium phosphate buffer (pH 7.5) and water to a final volume of 1 ml and the mixture was incubated for 12–15 min at 30° C. The reaction was started by the addition of 10 μl GSSG reductase (50 units/ml) and the rate of change in absorbance was recorded at 412 nm. Total glutathione concentration was measured by analysis of 25 μl aliquots of the original supernatant, whereby the derivatisation step with the masking agent was omitted.

Recovery of glutathione. Kidney homogenates with added known amounts of GSH and GSSG were analysed by both analytical methods.

The effect of cisplatin treatment on renal glutathione concentration. Groups of eight rats were dosed with cisplatin (5 mg/kg i.p.) and control groups of four animals were treated with the same volume of saline (0.9%, w/v). At each time point, a group of cisplatin-treated rats and a group of control animals were taken. Each animal was anaesthetised with sodium pentabarbital (50 mg/kg) prior to the removal of both kidneys. The renal vein and artery of the first kidney of each animal was clamped before removal to permit free circulation of blood in the second kidney until it was also removed. Portions of the cortex and outer medulla were taken from each kidney and either immediately homogenised for analysis for total glutathione using the method of Owens and Belcher [16] (see above) or stored at −20° C for platinum analysis. In a similar experiment, kidneys removed at 3 and 72 h following cisplatin treatment were analysed for GSSG and total glutathione using the method of Griffith [8] (see above).

Renal platinum concentration

The portions of the cortex and outer medulla of kidneys removed from animals at various times following treatment with cisplatin were analysed for platinum using flameless atomic absorption spectrometry after solubilisation according to the method of Siddik et al. [19].

Blood urea nitrogen determination

Blood urea nitrogen (BUN) was measured in controls and cisplatin-treated animals (dose, 5 mg/kg) on day 5 following treatment as previously described [5].

Results

Protein-thiol concentrations in kidney proximal tubules

The intensity of stain for protein thiols in the proximal tubules from control rats was greater than that in those from the 5-day cisplatin-treated group (Fig. 1). Quantitation of protein thiols using the video microdensitometer at various times after cisplatin treatment (5 mg/kg i.p.) showed a significant ($P < 0.01$) reduction in the concentration of protein thiols within 8 h of dosing (Fig. 2). The

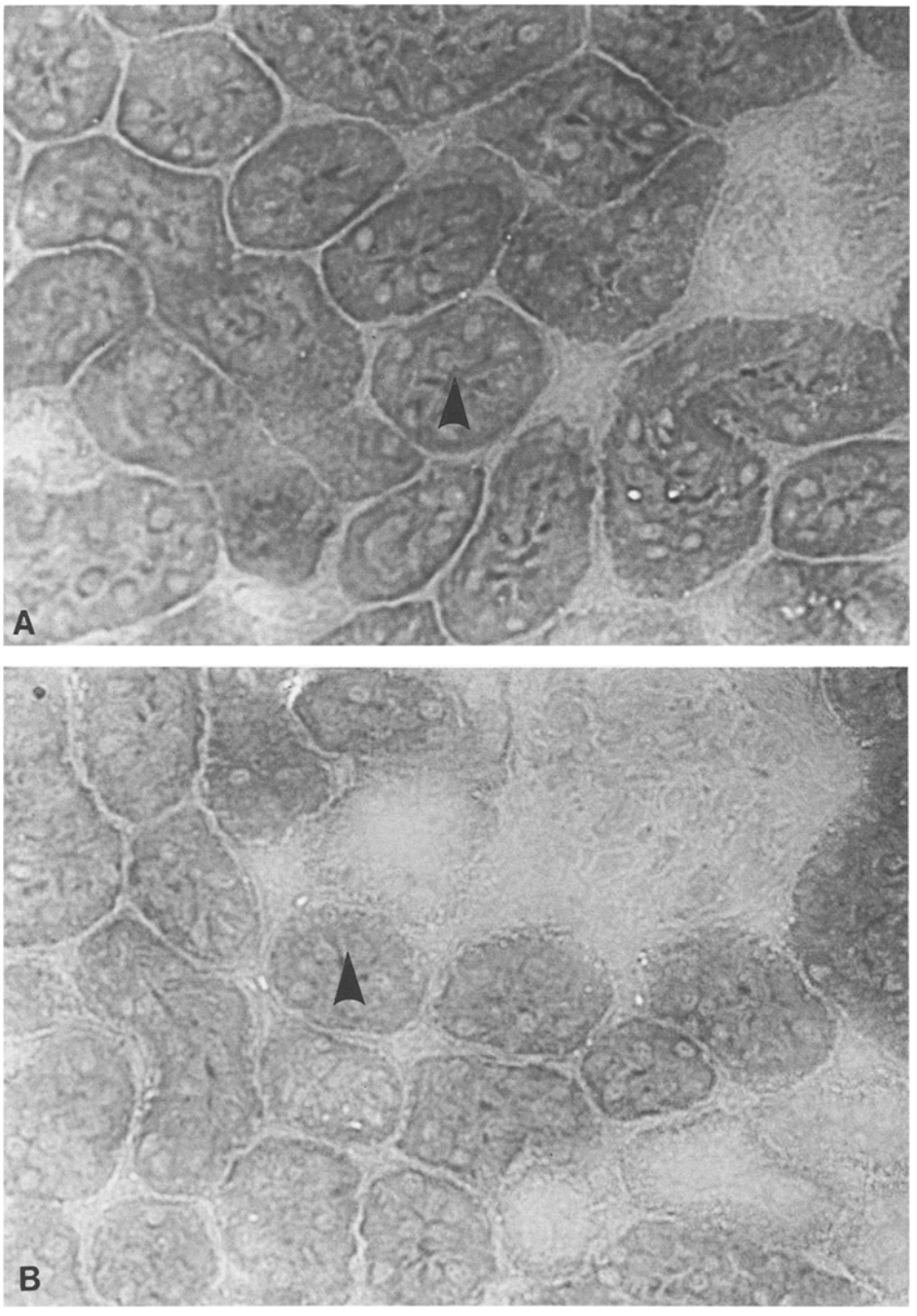

Fig. 1A,B. Kidney sections from male Wistar rats treated with **A** saline and **B** 5 mg/kg i. p. cisplatin stained for reactive protein thiols. Sample proximal tubule cross-sections used for measurement are indicated by *arrowheads*

concentration of protein thiols in the tubules continued to decline reaching a nadir 29% below the control levels at 120 h following cisplatin treatment. At this time the mean BUN concentration in cisplatin-treated animals (86.9 ± 18.6 mg/dl) was significantly higher than that in controls (29.2 ± 6.2 mg/dl). These values are similar to those previously reported by Daley-Yates and McBrien [5], which were maximal on day 5 following cisplatin treatment.

The effect of cisplatin on renal glutathione concentration

Cisplatin treatment provoked an increase in the concentration of total glutathione in the cortex and outer medulla (Fig. 3, Table 1). By 1 h following cisplatin treatment, total glutathione levels in treated rats had significantly elevated in comparison with those in controls ($P < 0.0005$), reaching 50% above the control values at 72 h post-treatment ($P < 0.0005$) and remaining high for up to 120 h post-treatment.

Analysis of the kidney cortex and outer medulla of rats for GSSG and for total glutathione using the method of Griffith [8] (Table 2) revealed that the increase in total glutathione following cisplatin treatment was caused by an

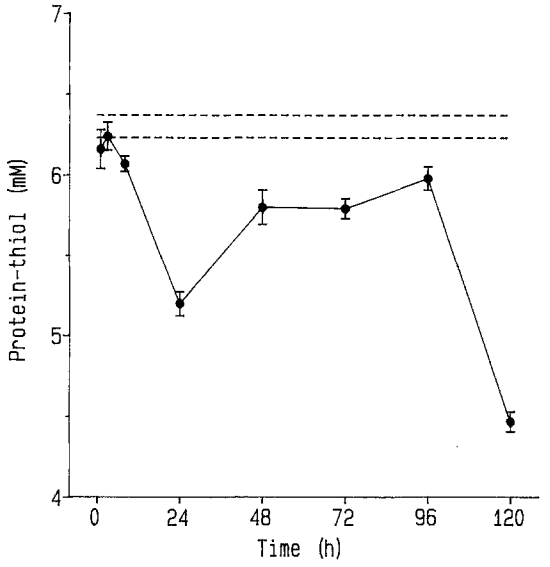

Fig. 2. The effect of cisplatin on the concentration of reactive protein thiols in the proximal tubule. Values represent means ± SEM. The number of animals examined at each time point was 2–4; 8 control animals were treated with 0.9% NaCl at 3 h prior to removal of the kidneys. For 8 h, $P < 0.01$. For 24, 48, 96 and 120 h, $P < 0.001$

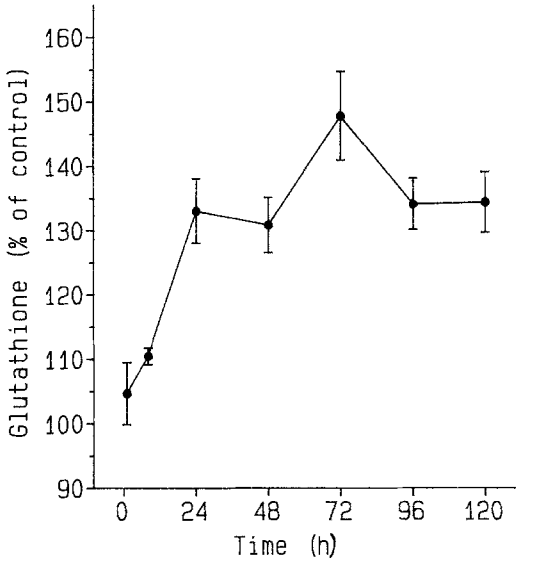

Fig. 3. The effect of cisplatin on the concentration of total glutatione in the cortex and outer medulla of the rat kidney as measured by the method of Owens and Belcher [16]. Values are expressed as a percentage of the control values (mean ± SEM). For the numbers of animals examined at each time point, see Table 1

increase in the concentration of reduced glutathione, since the concentration of GSSG in treated rats remained essentially unchanged in comparison with that in control animals.

Studies on the recovery of known amounts of GSH and GSSG that had been added to kidney homogenates gave values of approx. 110% as analysed by the method of Owens and Belcher [16] and 95% according to the method of Griffith [8]. The full data will be published elsewhere (Merazga et al., manuscript in preparation). In our hands, the commonly used method of analysis for GSH and GSSG

Table 1. Total glutathione concentration in the kidney cortex and outer medulla of rats as determined by the method of Owens and Belcher [16]

Time (h)	Total glutathione (μg/g tissue)	
	Saline-treated controls	Cisplatin-treated rats
0	798 ± 61.5	–
1	988 ± 2.5	1,050 ± 48.2
8	1,050 ± 3.9	1,160 ± 13.1
24	786 ± 21.5	1,050 ± 39.5
48	893 ± 55.4	1,170 ± 38.2
72	943 ± 28.9	1,390 ± 64.8
96	930 ± 1.6	1,250 ± 31.1
120	923 ± 22.5	1,240 ± 43

Data represent mean values ± SD. In all, 4 animals per cisplatin-treated group and 2 animals per saline-treated control group were examined at each time point; the two kidneys from each animal were analysed separately

Table 2. Total glutathione and GSSG in the kidney cortex and outer medulla of rats as determined by the method of Griffith [8]

Time (h)	Total glutathione (μg/g tissue)		GSSG (μg/g tissue)	
	Saline--treated controls	Cisplatin--treated rats	Saline--treated controls	Cisplatin--treated rats
3	818 ± 25.1	818 ± 25.3	62.5 ± 1.6	65.6 ± 3.1
72	855 ± 25.5	1,240 ± 25.1	64.9 ± 2.4	72.9 ± 2.3

Data represent mean vallues ± SEM. In all, 2 animals per saline-treated group and 6 animals per cisplatin-treated group were examined at each time point; the two kidneys from each animal were analysed separately

[9] gave recoveries of only 55% in similar experiments and gave values for total renal glutathione that consistently amounted to only 60–65% of those obtained in the same samples using the other two methods.

Renal platinum concentrations

Following cisplatin treatment (5 mg/kg i.p.), the total platinum concentration in the cortex and outer medulla reached a level of 16.3 ± 0.3 μg/g wet tissue at 72 h following treatment, declining thereafter to 9.1 ± 0.3 μg/g at 5 days (Fig. 4). Thus, the peak platinum concentrations coincided with the peak glutathione levels and occurred earlier than the nadir in protein-thiol concentration.

Discussion

The binding of platinum to sulphydryl groups has been suggested as being involved in the mechanism of nephrotoxicity of cisplatin [6, 12]. Using a cytochemical technique, we measured the effects of a nephrotoxic dose of cisplatin on the concentration of protein-thiols in cells of the proximal tubule, the main site of the lesion [7]. In processing of the tissue during the staining procedure, all low-molecular-weight thiol species are removed [15]. We

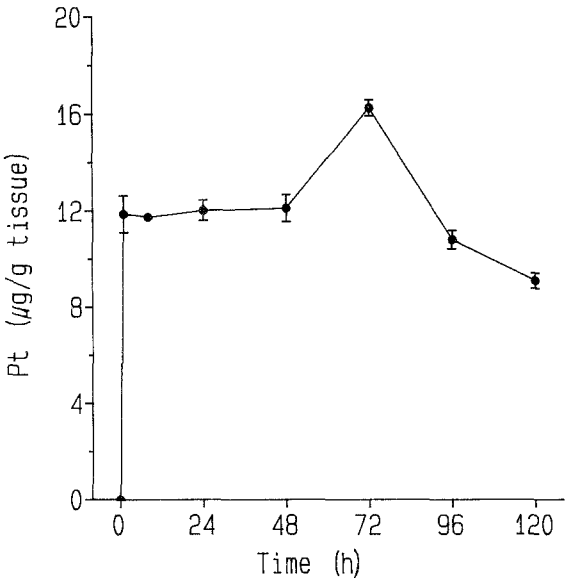

Fig. 4. Platinum concentration in the cortex and outer medulla of the rat kidney following a single dose of cisplatin (5 mg/kg i. p.)

also measured the overall glutathione concentration in the cortex and outer medulla following cisplatin treatment using specific enzyme-based assays that enable us to dinstinguish between reduced and oxidised glutathione. If there were a rapidly established equilibirum between reduced glutathione and protein thiols, any changes in concentration following the administration of cisplatin should occur in concert in the pools of both species. The concentration of protein thiols did not change for up to 3 h following the administration of cisplatin; thereafter, a significant reduction was observed at 8 h. In contrast, the total renal glutathione concentration rose following cisplatin administration reaching a peak at 72 h post-dosing and remaining significantly elevated at day 5. It is therefore evident that there is no rapid equilibrium between GSH and protein-bound thiols unless the GSH concentration in cells of the proximal tubule falls whilst the average concentration in the cortex and outer medulla rises. Although we cannot exclude that possibility, it seems very unlikely. Since changes in the concentration of GSH and of protein thiols following cisplatin administration are not co-ordinated, we will consider the phenomena separately.

Our observations of a reduction in the concentration of protein thiols following cisplatin dosing confirm the findings of Levi et al. [12], who used a biochemical assay and reported a reduction in the concentration of total renal thiols (protein plus non-protein) of 14% on day 5 following dosing with cisplatin. These authors suggested that the reduction in total thiol concentration was caused by a fall in protein-thiol concentration because they had observed an increase in renal non-protein-thiol concentration at 72 h post-dosing. Our observation that the fall in protein-thiol concentration can be detected as early as 8 h post-dosing indicates that this biochemical change occurs long before the onset of functional disturbance as detected by an elevation in BUN levels. Borch and Pleasants [3] have reported that the administration of diethyldithiocarbamate DDTC to rats treated with cisplatin protects maximally against nephrotoxicity, provided that it is given within 4 h after the initial dose, and that the protective effect is much diminished if it is given at $\geqslant 6$ h after cisplatin. Clearly, some of the biochemical changes that occur in the kidney within a few hours of cisplatin administration are of crucial importance in determining the extent of the subsequent nephrotoxic lesion.

The further fall in the protein-thiol concentration in the proximal tubules, which occurs at between 96 and 120 h after dosing with cisplatin (Fig. 2), coincides with the development of necrosis within the tubules. The brush-border and cellular contents are shed into the tubular lumen and, at this stage, the fall in protein thiols is associated with a loss of total protein from the cells.

The mechanism by which cisplatin decreases the concentration of protein thiols in the proximal tubule prior to the development of necrosis is unknown, but it cannot be a simple one-to-one interaction between platinum and thiol groups since the maximal concentration of platinum in the kidney (83.4 ± 1.7 nmol/g) is much smaller than the reduction in protein-thiol concentration (1.83 mM). Levi et al. [12] have suggested that the reduction in the concentration of protein thiols could be due to an inhibition of disulphide reductase similar to that brought about by mercurial compounds [18]. More recently, Sugihara et al. [22] have suggested that the generation of free radicals may play a part in the development of cisplatin nephrotoxicity, since these authors could achieve protection against toxicity using free-radical scavengers. Free radicals could interact with thiols, including protein-bound thiols, reducing their concentration and causing an increase in intracellular Ca^{2+} ion concentration and the development of toxicity [2]. However, if this were the mechanism of cisplatin nephrotoxicity, it would be expected that the concentration of reduced glutathione would fall before the reduction in the concentration of protein thiols became apparent [2]. Our data show that the GSH concentration in the cells rises as the protein-thiol concentration falls and that there is no concomitant increase in the intracellular concentration of GSSG.

Mercury causes a decrease in the renal concentration of protein thiols and it has been suggested that this is the cause of mercury-induced nephrotoxicity [4, 18]. The results of Johnson [10], however, suggest that protein thiols may not be involved, since Sprague-Dawley rats treated with diethyl maleate and mercury together showed a reduction in renal cortical protein-thiol concentration similar to that in animals treated with mercury alone, even though the diethyl maleate protected the first group of animals from mercury-induced alterations in renal function. However, mercury is reported to cause a reduction in the concentration of non-protein as well as protein thiols in the kidney [4, 18]. This reduction occurs within 3 h of dosing and, unlike that caused by cisplatin, mercury-induced renal failure has a rapid onset that is coincident with the fall in thiol concentration.

The increase in the cortical and medullary glutathione concentration, which we observed to peak at 72 h following cisplatin treatment, was similar in extent (50%) to those in previous reports [12, 13]. However, Leyland-Jones et al. [13] observed that the peak occurred within

20 min of cisplatin administration. There is no obvious reason for this discrepancy in the observations, although there are differences in the experimental protocols. Leyland-Jones et al. used a higher dose of cisplatin (6 mg/kg) and a different route of administration (i.v.) than we used in our experiments. Moreover, although these authors also measured total glutathione by the method of Owens and Belcher [16], their kidney samples were frozen prior to analysis. The concentration of total renal glutatione reported by Leyland-Jones et al. [13] in their untreated control animals was only 661 ± 169 μg/g wet tissue weight as compared with 932 ± 20.8 μg/g wet tissue weight observed in the present study. Kidney is rich in γ-glutamyl transpeptidase, and this enzyme is known to reduce the glutathione concentration in tissues frozen prior to analysis [1].

The reason for cisplatin treatment bringing about the observed changes in protein thiol and glutathione concentration in the kidney requires further investigation. The mechanism by which cisplatin administration brings about an increase in renal glutathione concentration is not known. The increase could arise from an increase in the rate of glutathione synthesis, since kidney cells have a high rate of glutathione turnover and can synthesize glutathione in response to cellular injury [17]. Clearly, if the increase in glutathione synthesis is a response to a toxic insult arising from cisplatin treatment, it is an insufficient response since it does not prevent ultimate kidney damage. We have embarked on an investigation into the effect of cisplatin treatment on the enzymes of glutathione synthesis in the kidney. The significance of the reduction in the concentration of protein thiols in kidney tubules in cisplatin-induced nephrotoxicity may become clearer when we investigate the effects of protective treatments such as the administration of DDTC or sodium chloride on these alterations.

Acknowledgements. We thank Dr. P. Principe for her advice in connection with the protein-thiol determination. We gratefully acknowledge the support of the Cancer Research Campaign.

References

1. Anderson ME (1985) Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 113: 548
2. Bellomo G, Orrenius S (1985) Altered thiol and calcium homeostasis in oxidative hepatocellular injury. Hepatology 5: 876
3. Borch RF, Pleasants ME (1979) Inhibition of cis-platinum nephrotoxicity by diethyldithiocarbamate rescue in a rat model. Proc Natl Acad Sci USA 76: 6611
4. Clarkson TW (1972) The pharmacology of mercury compounds 6545. Annu Rev Pharmacol 12: 375
5. Daley-Yates PT, McBrien DCH (1984) Cisplatin metabolites in plasma, a study of their pharmacokinetics and importance in the nephrotoxic and antitumour activity of cisplatin. Biochem Pharmacol 33: 3063
6. Dedon PC, Qazi R, Borch RF (1986) Potential mechanisms of cisplatin toxicity and diethyldithiocarbamate rescue. In: McBrien DCH, Slater TF (eds) Biochemical mechanisms of platinum antitumour drugs. IRL Press, Oxford, p 255
7. Dobyan DC, Levi J, Jacobs C, Kosek J, Weiner MW (1980) Mechanism of cis-platinum nephrotoxicity: II. Morphologic observations. J Pharmacol Exp Ther 213: 551
8. Griffith OW (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinyl pyridine. Anal Biochem 106: 207
9. Hissin PJ, Hilf R (1976) A fluorometric method for determination of oxidised and reduced glutathione in tissues. Anal Biochem 74: 213
10. Johnson DR (1982) Role of renal cortical sulphydryl groups in development of mercury induced renal toxicity. J Toxicol Environ Health 9: 119
11. Kosower NS, Kosower EM (1983) Glutathione and cell membrane thiol status. In: Larsson A, Orrenius S, Holmgren A, Mannervik B (eds) Functions of glutathione. Raven Press, New York, P 307
12. Levi J, Jacobs C, Kalman SM, McTique M, Weiner MW (1980) Mechanism of cisplatinum nephrotoxicity: 1. Effect of sulfhydryl groups in rat kidneys. J Pharmacol Exp Ther 213: 545
13. Leyland-Jones B, Morrow C, Tate S, Urmacher C, Gordon C, Young CW (1983) *cis*-Diamminedichloroplatinum(II) nephrotoxicity and its relationship to renal γ-glutamyl transpeptidase and glutathione. Cancer Res 54: 5072
14. Litterst CL, Tong S, Hirokata Y, Siddik ZH (1982) Alterations in hepatic and renal levels of glutathione and activities of glutathione-*S*-transferase from rats treated with *cis*-dichlorodiammineplatinum(II). Cancer Chemother Pharmacol 8: 67
15. Nöhammer G (1982) Quantitative microspectrophotometrical determination of protein thiols and disulfide with 2,2′-dihydroxy-6,6′-dinaphthyldisulfide (DDD). The variety of DDD-staining methods demonstrated on Erlich ascites tumour cells. Histochemistry 75: 219
16. Owens CW, Belcher RV (1965) A colorimetric micro-method for the determination of glutathione. Biochem J 94: 705
17. Reed DJ, Beatty PW (1980) Biosynthesis and regulation of glutathione: toxicological implications. In: Hodgson E, Bend JR, Philpot RM (eds) Rev Biochem Toxicol, Vol 2. Elsevier, New York, p 213
18. Shore V, Shore B (1962) Effects of mercurials on renal sulfhydryl and disulfide groups. Am J Physiol 203: 15
19. Siddik ZH, Boxall FE, Harrap KR (1987) Flameless atomic absorption spectrophotometric determination of platinum in tissues solubilised in hyamine hydroxide. Anal Biochem 163: 21
20. Smith MT, Loveridge N, Willis ED, Chayen J (1979) The distribution of glutathione in the rat liver lobule. Biochem J 182: 103
21. Spargo DJ, Riley PA (1988) Computerized digital video microdensitometry applied to the quantitation of intracellular macromolecules or their functional groups. In: Nigam SK, McBrien DCH, Slater TF (eds) Eicosanoids, lipid peroxidation and cancer. Springer, Berlin Heidelberg New York, p 315
22. Sugihara K, Nakano S, Koda M, Tanaka K, Fukuishi N, Gemba M (1987) Stimulatory effect of cisplatin on production of lipid peroxidation in renal tissues. Jpn J Pharmacol 43: 247
23. Tietze F (1969) Enzymatic method for quantitative determination of nanogram amounts of total and oxidised glutathione. Anal Biochem 27: 502