Differential effect of *cis*-platinum (*cis*-diamminedichloroplatinum) on regulation of liver and kidney haem and haemoprotein metabolism

Possible involvement of γ -glutamyl-cycle enzymes

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The treatment of rats with *cis*-platinum (*cis*-diamminedichloroplatinum) for 1, 3 or 7 days elicited vastly different responses in the liver and the kidney in activities of enzymes of haem-metabolism pathway and γ -glutamyl-cycle enzymes. The differences resided in the magnitude, direction and the time course of responses. In general, the liver was by far less severely affected, and when a response was elicited, it displayed an earlier onset (1–3 days), with a return to normal at 7 days. In the kidney, however, the effects were notable after 3 days of treatment, and became more pronounced at 7 days. Specifically, the activity of 5-aminolaevulinic acid (ALA) synthetase and contents of cytochrome *P*-450 and the microsomal haem were decreased in the liver. In contrast, in the kidney, cytochrome *P*-450 and haem concentrations were significantly increased, with no change in ALA synthetase activity. The increase in the kidney haem content appeared to reflect an increased formation of haem, as suggested by the elevated activity of ferrochelatase and the concomitant decrease in tissue porphyrin levels. In the kidney, a time-dependent and pronounced inhibition of activities of γ -glutamylcysteine synthetase, the rate-limiting enzyme in glutathione production, and γ -glutamyl transpeptidase, the first enzyme in glutathione breakdown, were observed. The enzyme activities, 7 days after treatment, were only 40 and 60% of the control values respectively. In contrast, these enzyme activities were not affected in the liver. Complexing *cis*-platinum with cysteine considerably intensified the entire spectrum of effects of *cis*-platinum in the kidney. Notably, cytochrome *P*-450 concentration and haem oxygenase activity were increased to about 3.5 and 6 times the control values, respectively. γ -Glutamylcysteine synthetase activity was decreased to less than 20% of the control. It is suggested that the differential effectiveness of *cis*-platinum in the liver and the kidney in alternating haem metabolism is related to the vast differences which exist between these organs in the activities of γ -glutamyl-cycle enzymes. It is further suggested that this may promote the formation in the kidney, but not in the liver, of a *cis*-platinum–cysteine complex that is more stable, and thus biologically more effective, than the parent compound.

INTRODUCTION

Alterations in the activities of haem-metabolism pathway enzymes frequently correspond closely with the cellular toxicity of chemicals [see review articles by Maines (1984), De Matteis (1978) and Elder (1978)]. Nearly all toxic chemical agents studied to date have been shown to affect the pathway to a certain extent (Maines, 1984; De Matteis, 1978; Elder, 1978). Moreover, toxic chemicals often cause alterations in cellular GSH levels and the activities of the enzymes of γ -glutamyl cycle (Maines, 1984). In the present study the effect of *cis*-platinum (*cis*-diamminedichloroplatinum) on haem- and GSH-metabolism pathways was examined in order to gain some understanding of the well-known tissue specific toxicity of this compound to the kidney.

Cis-Platinum is considered as the single most effective chemotherapeutic agent against a broad spectrum of malignancies (Rosenberg *et al.*, 1969; Thomson, 1974; Burchenal, 1978). Renal toxicity, however, constitutes a major limiting factor in the clinical use of this co-ordinated platinum complex (Blachley & Hill, 1981). Moreover, in

the kidney, *cis*-platinum has been shown to cause a pronounced increase in concentration of the microsomal haem and cytochrome *P*-450 (Litterst *et al.*, 1983; Jollie & Maines, 1985). Indeed, the magnitude of increase in concentration of the cytochrome in the kidney surpasses that elicited by most other agents (Jollie & Maines, 1985; Litterst *et al.*, 1975). On the other hand, the concentration of the haemoprotein in the liver is refractory to *cis*-platinum treatment (Litterst *et al.*, 1975, 1983). In the kidney, *cis*-platinum also causes perturbations in activities of various enzymes of haem-metabolism pathway (Jollie & Maines, 1985) and has been reported to cause alterations in GSH concentration (Litterst *et al.*, 1979). The effect of *cis*-platinum on hepatic haem and GSH metabolism is not known.

In the kidney, regulation of haem metabolism appears to differ from that in the liver (Maines, 1982). In the former organ, the activity of the mitochondrial ALA synthetase, which catalyses the first, and the rate-limiting, step in biosynthesis of haem (Granick & Urata, 1963), does not appear to be regulated through a feedback-repressor mechanism. Haem is believed to regulate the

Abbreviations used: *cis*-platinum, *cis*-diamminedichloroplatinum: ALA, 5-aminolaevulinic acid; uro, uroporphyrinogen.

activity of this enzyme in the liver by such a mechanism (Granick & Urata, 1963). Moreover, in the kidney, unlike the liver, the activities of ALA synthetase and haem oxygenase are altered (Maines, 1982) by thiol agents known for their ability to disrupt GSH metabolism (Boyland & Chasseaud, 1970). Haem oxygenase constitutes the initial and the rate-limiting step in the haem-degradation pathway (Tenhunen *et al.*, 1968; Maines & Kappas, 1974). In turn, the activity of various metal ions, including platinum, known for their ability to regulate haem metabolism (Maines & Kappas, 1977a), is vastly modified by thiols such as cysteine and GSH (Maines & Kappas, 1977b).

In addition, there are major differences between the liver and the kidney in the activities of enzymes of γ -glutamyl cycle and GSH metabolism (Meister, 1981). Although the concentration of GSH in the kidney is lower than in the liver, the turnover of GSH, and the activities of the cycle enzymes, by far exceed those of the liver. Notably, the activity of γ -glutamyl transpeptidase exceeds that of the liver by two to three orders of magnitude (Sekura & Meister, 1974). In the kidney, this membrane-bound enzyme is involved in amino acid transport and the transport of GSH from the plasma into the organ (Meister *et al.*, 1980). The transpeptidase is not only involved in uptake of GSH by the kidney, but is also directly involved in the degradation of GSH; indeed, the first step in the breakdown of GSH is catalysed by this enzyme (Meister, 1981). Also, in the kidney the activity of the cytosolic enzyme γ -glutamylcysteine synthetase, the first enzyme in the biosynthesis of GSH, surpasses that of the liver by approximately an order of magnitude (Meister, 1981).

The present study was undertaken to explore whether the tissue-specific toxicity of *cis*-platinum to the kidney extends to its effects on the liver and the kidney haem metabolism and, if so, to examine the possible involvement of γ -glutamyl-cycle enzymes in the toxicity of *cis*-platinum in the kidney.

MATERIALS AND METHODS

Materials

Porphyrin compounds were obtained from Porphyrin Products, Logan, UT, U.S.A. *cis*-Platinum and other biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All chemicals were of analytical grade. The cysteine complex with *cis*-platinum was prepared as described by Daley-Yates & McBrien (1982) by incubating cysteine with *cis*-platinum in a 2:1 molar ratio in distilled water for 0.5 h at 37 °C.

Animals and tissue preparations

Male Sprague-Dawley rats (180–220 g) were purchased from Harlan Industries, Madison, WI, U.S.A., and maintained on Purina rat chow and water *ad libitum*. *cis*-Platinum was injected as a single dose of 9 mg/kg in a vehicle of 0.9% NaCl. *cis*-Platinum, its cysteine complex and cysteine were administered intraperitoneally. Control animals received saline or cysteine, depending on the experimental protocol. All injections were made between 09:30 and 10:00 h. The regimen of treatments is given in the legends to the appropriate Tables and Figures. The animals were decapitated and the liver and the kidneys were perfused *in situ* with saline. The organs were homogenized in 4 vol. of 0.01 M-Tris/HCl buffer,

pH 7.4, containing 0.25 M-sucrose. The mitochondrial, the microsomal and the cytosol fractions were prepared by differential centrifugation as detailed previously (Kutty & Maines, 1982). The tissue homogenate was utilized for determinations of total porphyrin and GSH contents, and the activity of γ -glutamyl transpeptidase. The activities of ALA synthetase and ferrochelatase were measured in the mitochondrial fraction. The microsomal fraction was used for measurements of haem oxygenase activity and contents of cytochromes *P*-450 and *b*₅. The activities of ALA dehydratase, uroporphyrinogen (uro)-I synthetase, biliverdin reductase, γ -glutamylcysteine synthetase and GSSG reductase were assessed in the cytosolic fraction (105000 *g* supernatant). Biliverdin reductase was purified from the rat liver as described previously (Kutty & Maines, 1981) and used in haem oxygenase assay system.

Assay procedures

The activity of ALA synthetase was measured by the procedure of Marver *et al.* (1966) as described before (Maines, 1980). The activity of ALA dehydratase was determined by using the method of Mauzerall & Granick (1956). The procedure described by Granick *et al.* (1975) was employed for the spectrofluorometric assay of uro-I synthetase activity. Coproporphyrin-I was utilized as the standard. Ferrochelatase activity was determined by the kinetic method described by Jones & Jones (1970); deuteroporphyrin and Co²⁺ were used as substrates. The rate of enzyme activity was assessed by measuring deuterocobalt-haem formation. The activity of haem oxygenase was measured as detailed before (Maines & Kappas, 1975) using Fe-protoporphyrin (haem *b*) as the substrate. The activity of biliverdin reductase was determined as described previously (Kutty & Maines, 1981), at pH 8.6, with NADPH as the cofactor. GSSG reductase activity was assayed by the method of Massey & Williams (1965). The assay medium (1.0 ml) contained enzyme sources (80–100 μ g of protein), EDTA (3 mM), bovine serum albumin (2 mg), NADPH (0.1 mM), GSSG (3 mM) and potassium phosphate buffer (50 mM, pH 7.6). The reaction was started by the addition of GSSG, and the rate of activity was assessed from the disappearance of NADPH. The blank assay system did not contain GSSG. GSSG reductase assay was carried out at 25 °C by use of a dual-beam spectrophotometer, a unit of activity being defined as that amount of enzyme catalysing the oxidation of 1 nmol of NADPH/min per mg of protein.

The assay of γ -glutamylcysteine synthetase was carried out as described by Sekura & Meister (1977). The reaction mixtures (1.0 ml) contained ATP (5 mM), MgCl₂ (20 mM), L-glutamate (10 mM), α -L-aminobutyrate (10 mM), EDTA (0.1 mM), bovine serum albumin (0.05 mg) and Tris/HCl buffer (10 mM, pH 8.2). The reaction was initiated by the addition of the enzyme source (1–2 mg of protein). α -L-Aminobutyrate was not added to the blank assay mixture. Duration of incubation was 30 min at 37 °C. The reaction was terminated by the addition of 1 ml of 10% (w/v) trichloroacetic acid and the protein was precipitated by centrifugation (5000 *g*, 10 min). The liberated phosphate (P_i) in the supernatant fraction was determined colorimetrically (720 nm) by the method of Taussky & Shorr (1953). A unit of activity was defined as that amount of enzyme that catalysed the release of 1 μ mol of P_i/h per mg of protein.

The activity of γ -glutamyl transpeptidase was measured as described by Tate & Meister (1974). The assay system (1.0 ml) consisted of enzyme source (20–50 μ g of protein), glycylglycine (2 mM), γ -L-glutamyl *p*-nitroanilide (2.5 mM), Triton X-100 (1.0%), NaCl (7.5 mM) and Tris/HCl buffer (0.05 M, pH 8.0). Sodium deoxycholate (1.0%) was used in the assay system for measurement of the liver transpeptidase activity. The reaction was initiated by the addition of γ -L-glutamyl *p*-nitroanilide. The release of *p*-nitroaniline was monitored by an increase in A_{405} . A millimolar absorption coefficient of 9.9 $\text{mM}^{-1}\cdot\text{cm}^{-1}$ was used for the measurement of the enzymic activity. The activity is expressed as μ mol or nmol of product formed/min per mg of kidney or liver protein, respectively. GSH was measured by the method of Cohn & Lyle (1966), with authentic GSH as the standard.

Spectral studies

Kidney cytochrome *P*-450 content was measured by using the procedure described by Jones *et al.* (1980), which corrects for cytochrome oxidase contamination. Liver cytochrome *P*-450 concentration was determined by the method of Omura & Sato (1964). The pyridine haemochromogen method of Paul *et al.* (1953) was used for measurement of microsomal haem content. The total porphyrin concentration was assessed spectrofluorometrically (Granick *et al.*, 1975); the excitation wavelength was 400 nm with a slit width of 20 nm, and the emission wavelength was 654 nm with a 20 nm slit. Coproporphyrin-I was utilized as the standard. Protein was

measured by the method of Lowry *et al.* (1951). The GSH values were detected fluorometrically, using excitation and emission wavelengths of 328 nm and 430 nm respectively.

The spectral studies were carried out with a SLM amino DW-2C spectrophotometer. Fluorescence measurements were conducted on an Aminco-Bowman fluorimeter. The results are expressed as means $\pm$ s.d. for four determinations; one rat was used for each determination. Results were analysed by the Student's *t* test and a *P* value of ≤ 0.05 denoted significance.

RESULTS

Comparative effects of *cis*-platinum treatment on liver and kidney haem metabolism and microsomal haemoproteins

The comparative time-dependent effects of *cis*-platinum treatment on the activity of ALA synthetase and on the microsomal contents of cytochrome *P*-450 and haem in the kidney and liver are shown in Fig. 1. As is shown, in these organs the pattern of responses elicited by the *cis*-platinum complex was reciprocal in nature, and the time courses of effects were dissimilar. In general, the onset of effects in the liver preceded those in the kidney, and whereas in the liver the effects were depreciative in nature, in the kidney they were augmentative. In the liver, 3 days after *cis*-platinum treatment, significant decreases in activity of ALA synthetase and contents of the cytochrome and haem were observed. In the case of cytochrome *P*-450, a significant decrease of nearly 30%

Fig. 1. Time-dependent effects of *cis*-platinum on the microsomal contents of cytochrome *P*-450 and haem and the activity of the mitochondrial ALA synthetase in the liver (■) and the kidney (□)

Sprague-Dawley rats (180–220 g) were treated with *cis*-platinum (9 mg/kg intraperitoneally) and killed at the indicated intervals. The microsomal and the mitochondrial fractions were prepared and used for determination of the values of indicated parameters. The methods used for preparation of tissue fractions and assay procedures are described in detail in the Materials and methods section. Results are means ($\pm$ s.d.) for four determinations. Control values: cytochrome *P*-450, liver and kidney, 1.01 ± 0.10 and 0.1 ± 0.01 nmol/mg of protein respectively. Microsomal haem, liver and kidney, 1.82 ± 0.14 and 0.19 ± 0.01 nmol/mg of protein respectively. ALA synthetase, liver and kidney, 159.5 ± 23.5 and 260.1 ± 16.9 pmol of ALA/h per mg of protein respectively.

*P ≤ 0.05 when compared with the control group.

in its content was noted as early as 1 day after treatment. When measured 1 and 3 days after treatment, significant effects on the above parameters in the kidney could not be detected. On the other hand, in the kidney 7 days after *cis*-platinum treatment, a nearly 2.5-fold increase in the concentration of cytochrome *P*-450 was observed. The increase in the concentration of the cytochrome was accompanied by a significant increase in the microsomal haem content. At this time, in the liver, the microsomal contents of cytochrome *P*-450 approximated the control values.

The response elicited by *cis*-platinum treatment in the activity of ALA synthetase in the liver and the kidney deviated from the expected pattern. In the kidney, the activity of the enzyme was not altered in the course of *cis*-platinum treatment, and increases in the microsomal haem and cytochrome *P*-450 content appeared independent of the activity of this enzyme. The observed responses are not consistent with the concept of feedback mechanism for the regulation of ALA synthetase activity (Granick & Urata, 1963).

The enzymic basis for an increase in the microsomal haem and cytochrome *P*-450 contents in the kidney and a decrease in these entities in the liver, was investigated by measuring activities of several enzymes in pathways of haem biosynthesis and degradation. Also, the microsomal concentration of cytochrome *b*₅ was measured. As shown in Table 1, notable differences existed between the liver and the kidney in these pathways in the pattern and/or the magnitude of responses elicited by *cis*-platinum. In the liver, with the exception of the effect exerted on the activity of ALA dehydratase, *cis*-platinum was rather ineffective. In this organ, the activity of ALA dehydratase was moderately depressed at 3 and 7 days after *cis*-platinum treatment. However, since the activity of this enzyme exceeds that of ALA synthetase by more than an order of magnitude, the noted decrease in the activity of the dehydratase could not have had a significant effect on cellular haem levels. The total porphyrin content and the activities of uro-I synthetase and ferrochelatase, two key

enzymes of haem-biosynthesis pathway, and biliverdin reductase, the last enzyme in haem-degradation pathway, were not altered 1, 3 or 7 days after treatment. Moreover, an increase in activity of haem oxygenase was noted at 1 day, but not at 3 or 7 days, after treatment.

From the results in Table 1, *cis*-platinum appeared to have selectively affected cytochrome *P*-450 in the liver, inasmuch that a decrease in the level of cytochrome *b*₅ in this organ was not observed in the course of *cis*-platinum treatment. This finding suggests that the above-described decrease in the liver microsomal haem concentration (Fig. 1) mainly reflected the decrease in the cellular content of cytochrome *P*-450. Furthermore, data shown in Fig. 1 and Table 1 may be interpreted as suggesting that, in the liver, an increase in the activity of haem oxygenase at 1 day after *cis*-platinum treatment contributed to the observed decrease in the microsomal cytochrome *P*-450 content, whereas the decrease in ALA synthetase activity was possibly responsible for decreases in the microsomal haem and haemoprotein contents at 3 days after treatment.

In the kidney, the pattern of response of the haem-metabolism pathway to *cis*-platinum treatment vastly differed from that observed in the liver. As shown in Table 1, an increase in haem oxygenase activity was observed throughout the experimental period. Similarly, the activity of ferrochelatase was substantially increased at 3 and 7 days after *cis*-platinum treatment. On the other hand, the activities of uro-I synthetase and biliverdin reductase were significantly decreased. Also, notable and progressive decreases in the total porphyrin level and the activity of ALA dehydratase were observed during the course of treatment. Inhibition of the activity of ALA dehydratase and uro-I synthetase, however, does not appear to contribute to the observed decrease in the porphyrin concentration in a major way. The activity of ALA dehydratase, when measured 7 days after *cis*-platinum treatment, exceeded that of ALA synthetase by more than 2-fold, and that of uro-I synthetase was only marginally inhibited at this time. Therefore, in the face

Table 1. Effect *in vivo* of *cis*-platinum treatment on activities of the enzymes of haem-metabolism pathway, and porphyrin and cytochrome *b*₅ contents in rat liver and kidney

The liver and the kidneys were obtained from rats treated with *cis*-platinum as described in the legend to Fig. 1. Various cell fractions were prepared as described in the Materials and methods section. The tissue homogenate was used for measurement of total porphyrin content. The mitochondrial fraction was used for measurement of ALA dehydratase activity; the microsomal fraction was used for assay of haem oxygenase activity and cytochrome *b*₅ content. The activities of ALA dehydratase, uro-I synthetase and biliverdin reductase were measured in the cytosol fraction (105000 *g* supernatant). Measurements were made as described in the text. Results shown are means $\pm$ S.D. for four determinations. *P $\leq$ 0.05 when compared with the control group.

Organ	Duration of treatment (days)	ALA dehydratase (nmol/h per mg)	Uro-I synthetase (pmol/h per mg)	Ferrochelatase (nmol/h per mg)	Porphyrins (pmol/mg)	Cytochrome <i>b</i> ₅ (nmol/mg)	Haem oxygenase (nmol/h per mg)	Biliverdin reductase (nmol/min per mg)
Liver	Control	4.56 $\pm$ 0.50	187 $\pm$ 9	9.57 $\pm$ 0.76	4.82 $\pm$ 0.24	0.43 $\pm$ 0.24	2.86 $\pm$ 0.37	1.00 $\pm$ 0.14
	1	4.90 $\pm$ 0.94	218 $\pm$ 9	8.04 $\pm$ 2.10	4.60 $\pm$ 0.41	0.43 $\pm$ 0.05	4.35 $\pm$ 0.75*	0.85 $\pm$ 0.06
	3	3.38 $\pm$ 0.32*	195 $\pm$ 16	9.01 $\pm$ 1.81	4.02 $\pm$ 0.26	0.56 $\pm$ 0.17	2.38 $\pm$ 0.27	0.75 $\pm$ 0.21
	7	3.00 $\pm$ 0.44*	190 $\pm$ 8	9.52 $\pm$ 3.12	5.45 $\pm$ 0.45	0.56 $\pm$ 0.06	2.96 $\pm$ 0.41	1.04 $\pm$ 0.11
Kidney	Control	1.43 $\pm$ 0.80	291 $\pm$ 21	6.3 $\pm$ 0.6	35.9 $\pm$ 5.4	0.08 $\pm$ 0.01	1.11 $\pm$ 0.13	5.39 $\pm$ 0.54
	1	0.95 $\pm$ 0.06*	295 $\pm$ 15	6.4 $\pm$ 0.2	27.9 $\pm$ 3.6	0.08 $\pm$ 0.02	2.23 $\pm$ 0.17*	5.21 $\pm$ 0.53
	3	1.10 $\pm$ 0.12*	241 $\pm$ 29*	9.7 $\pm$ 0.7*	27.9 $\pm$ 1.5*	0.10 $\pm$ 0.01*	2.38 $\pm$ 0.17*	4.03 $\pm$ 0.81*
	7	0.68 $\pm$ 0.41*	245 $\pm$ 22*	14.0 $\pm$ 1.1*	20.9 $\pm$ 1.9*	0.14 $\pm$ 0.01	3.20 $\pm$ 0.49*	2.68 $\pm$ 0.37*

Table 2. Effects *in vivo* of *cis*-platinum treatment on activities of GSSG reductase, γ -glutamyl transpeptidase and γ -glutamylcysteine synthetase and content of GSH in the liver and the kidney

Male Sprague-Dawley rats (180–220 g) were treated with *cis*-platinum (9 mg/kg intraperitoneally) or saline. At the indicated times the animals were killed, the liver and the kidneys were perfused and the homogenate and the cytosol fractions were prepared as described in the Materials and methods section. The concentration of GSH and the activity of γ -glutamyl transpeptidase were measured in the homogenate. The activities of GSSG reductase and γ -glutamylcysteine synthetase were measured in the cytosol fraction. The indicated parameters were measured as described in the text. Results shown are means $\pm$ S.D. of four determinations; one rat was used for each determination. * $P \leq 0.05$ when compared with the control animals.

Organ	Duration of treatment (days)	[GSH] (mM)	GSSG reductase (units†)	γ -Glutamyl transpeptidase (units‡)	γ -Glutamylcysteine synthetase (units§)
Liver	Control	4.0 $\pm$ 0.3	173 $\pm$ 12	0.23 $\pm$ 0.06	1.34 $\pm$ 0.13
	1	4.7 $\pm$ 0.3	173 $\pm$ 16	0.22 $\pm$ 0.05	1.49 $\pm$ 0.16
	3	4.0 $\pm$ 0.7	157 $\pm$ 24	0.14 $\pm$ 0.09	1.11 $\pm$ 0.20
	7	4.5 $\pm$ 0.6	177 $\pm$ 18	0.28 $\pm$ 0.09	1.21 $\pm$ 0.21
Kidney	Control	2.2 $\pm$ 0.4	210 $\pm$ 58	0.15 $\pm$ 0.02	92 $\pm$ 9
	1	2.1 $\pm$ 0.3	265 $\pm$ 34	0.15 $\pm$ 0.01	96 $\pm$ 7
	3	2.2 $\pm$ 0.4	250 $\pm$ 39	0.11 $\pm$ 0.02*	68 $\pm$ 12*
	7	2.5 $\pm$ 0.6	263 $\pm$ 12	0.09 $\pm$ 0.02*	37 $\pm$ 14*

† A unit is defined as 1 nmol of NADPH oxidized/min per mg of protein.

‡ A unit is defined as 1 nmol or 1 μ mol of *p*-nitroanilide produced/min per mg of protein for the liver and the kidney, respectively.

§ A unit is defined as 1 μ mol of P_I/h per mg of protein.

of an unchanged ALA synthetase activity and an increase in ferrochelatase activity, concomitant with a decrease in total porphyrin content, the above-observed elevation in the cellular haem level in the kidney (Fig. 1) could reflect an enhanced rate of conversion of porphyrin to haem. This suggestion is consistent with a previous report on this observation (Jollie & Maines, 1985). Alternatively, alterations by *cis*-platinum of the composition of constituents of the endoplasmic-reticulum membranes could lead to an increase in apocytochrome *P*-450 and/or holoenzyme in the membranes.

The effect *in vitro* of *cis*-platinum on activities of the enzymes of haem biosynthesis and degradation listed in Table 1 was also investigated. Addition *in vitro* of up to 500 mM-*cis*-platinum to assay systems did not affect enzyme activities (results not shown). This finding suggests that the inhibition of the enzyme activities *in vivo* was not a direct action resulting from binding of *cis*-platinum to enzyme proteins.

Comparative effects of *cis*-platinum treatment of γ -glutamyl-cycle enzymes in liver and kidney

The cellular basis for the differential effect of *cis*-platinum on the haem-metabolism pathway in liver and kidney was investigated by examining the responses of the enzymes of the γ -glutamyl cycle and GSH-metabolism pathway to *cis*-platinum treatment. Results presented in Table 2 show the comparative effects of *cis*-platinum treatment for 1, 3 or 7 days on cellular content of GSH and activities of GSSG reductase, γ -glutamyl transpeptidase and γ -glutamylcysteine synthetase in the liver and the kidney. As shown, except for a modest increase in GSH content in the liver 1 day after *cis*-platinum treatment, the GSH levels in both organs were unchanged throughout the course of the experiment. A modest increase (30%) in kidney GSH content 1 day after *cis*-platinum has been previously noted by other investigators (Litterst *et al.*, 1979). However, we did not note such an increase in the present study. The

experimental basis for this difference is not clear. Similarly, in both organs, the activity of GSSG reductase did not differ from that of the control at any experimental points subsequent to *cis*-platinum treatment. On the other hand, responses elicited by *cis*-platinum treatment on activities of γ -glutamylcysteine synthetase and γ -glutamyl transpeptidase in the two organs were vastly different. As shown, in the kidney the transpeptidase activity was significantly decreased at 3 and 7 days after treatment; at the latter time point, the activity was decreased to about 60% of that of the control value. In contrast, in the liver this activity was unchanged. Similarly, in the liver the activity of γ -glutamylcysteine synthetase was not affected by *cis*-platinum treatment. In the kidney, however, a marked decrease in the activity of the synthetase was noted in the treated animals. As shown, the enzyme activity was reduced by nearly 25% 3 days after treatment, and further declined to only about 40% of the control value at 7 days. The present finding that a significant reduction in the activity of γ -glutamylcysteine synthetase in the kidney was not associated with a decrease in the cellular level of GSH may, in part, reflect a decreased rate of GSH turnover subsequent to the inhibition of γ -glutamyl transpeptidase activity in this organ.

Since the activity of γ -glutamylcysteine synthetase is feedback-regulated by GSH, and is limited by the supply of cysteine (Meister, 1984), the effect of the *cis*-platinum-cysteine complex on the activity of the synthetase was investigated. As shown in Fig. 2, in the kidney of rats treated with the complex, the inhibitory effect of *cis*-platinum on the activity of the synthetase was further augmented; in this case, the activity was reduced to a mere fraction of the control value (less than 20%). The cysteine complex of *cis*-platinum did not produce a significant effect on the synthetase activity in the liver. The inability to detect a significant effect on the activity of this enzyme could reflect the limit of sensitivity of the assay system for detecting low levels of enzyme activity

Fig. 2. Comparative effects of *cis*-platinum and *cis*-platinum-cysteine complex on the activity of γ -glutamylcysteine synthetase in the liver and the kidney

Male Sprague-Dawley rats (180–220 g) were treated (intraperitoneally) with *cis*-platinum (▤) or its cysteine complex (▨) as detailed in the Materials and methods section and killed 7 days later. The cytosol fraction was prepared from perfused organs and utilized for determination of γ -glutamylcysteine synthetase activity. Experimental details are provided in the Materials and methods section. Results are means $\pm$ S.D. for four determinations. * $P \leq 0.05$ when compared with the control (□) group; ** $P \leq 0.05$ when compared with *cis*-platinum-treated group.

in the liver. Also, treatment with cysteine alone had no effect on the activity of the synthetase in either organs (results not shown).

Comparative effects of *cis*-platinum and *cis*-platinum-cysteine complex on haem-metabolism enzymes

In subsequent studies the possibility of potentiation of the effect of *cis*-platinum, by complexing it with cysteine, on haem-metabolism enzymes and cytochrome *P*-450 in the liver and the kidney was examined (Figs. 3 and 4). In this series of experiments, rats were treated once with *cis*-platinum, *cis*-platinum-cysteine complex or cysteine and killed 7 days later. The data pertaining to haem oxygenase activity and cytochrome *P*-450 content are shown in Fig. 3. As this Figure shows, in comparison with the *cis*-platinum-treated rats, in animals treated with the cysteine complex the magnitude of increase in the activity of haem oxygenase and in the content of cytochrome *P*-450 was further exaggerated. The activity of haem oxygenase was nearly 6 times the control value and the microsomal content of cytochrome *P*-450 was increased to about 3–5 times that of the control. In the liver, however, the cysteine complex was as ineffectual as *cis*-platinum in altering the activity of haem oxygenase and the concentration of cytochrome *P*-450; values similar to those shown in Table 1 (7 days) were obtained. Cysteine treatment alone exerted no effect on the activity of the oxygenase and content of cytochrome *P*-450 in either organ (results not shown).

The effect of treatment of rats with *cis*-platinum-cysteine complex (7 days) on the activities of ALA

Fig. 3. Comparative influence of treatments with *cis*-platinum and *cis*-platinum-cysteine complex on the microsomal concentration of cytochrome *P*-450 and activity of haem oxygenase in the kidney

Rats were treated with *cis*-platinum (▤) or *cis*-platinum-cysteine complex (▨) as detailed in the Materials and methods section and killed after 7 days. The microsomal fraction was prepared and used for measurements of haem oxygenase activity and cytochrome *P*-450 concentration. Experimental procedures are described in detail in the Materials and methods section. Results are means $\pm$ S.D. for four determinations. * $P \leq 0.05$ when compared with the control (□) group; ** $P \leq 0.05$ when compared with the *cis*-platinum-treated group.

dehydratase, uro-I synthetase and biliverdin reductase in the kidney are shown in Fig. 4. As with other parameters discussed above, the effects of *cis*-platinum were intensified as the result of complexing with cysteine. As noted, in comparison with *cis*-platinum-treated rats, in animals treated with the cysteine complex the rate of activity of the noted enzymes was significantly decreased. Indeed, ALA dehydratase and biliverdin reductase activities were reduced to a fraction of those of the control. Again, cysteine treatment alone did not affect these enzyme activities when compared with the control animals (results not shown). Although modest differences were detected between *cis*-platinum-treated animals and *cis*-platinum-cysteine-treated rats in activity of ferrochelatase and porphyrin concentration, these differences were not statistically significant. In these animals, ferrochelatase activity measured 13.7 ± 2.3 as against 11.56 ± 1.2 nmol of cobalt-deuteroporphyrin/min per mg of protein, respectively, and the corresponding values for total porphyrin concentrations were 21.0 ± 0.99 as against 18.6 ± 2.9 pmol/mg of protein. Furthermore, the mitochondrial ALA synthetase activity did not significantly differ in the two groups of treated animals. The enzyme activity in the *cis*-platinum-treated and *cis*-platinum-cysteine-complex-treated animals measured 325 ± 34 as against 257 ± 46 pmol of ALA/h per mg of protein, respectively. In the liver, at this time no significant difference between *cis*-platinum and *cis*-platinum-cysteine complex in eliciting a response in the above mentioned variables could be detected.

Fig. 4. Comparative influence of *cis*-platinum and *cis*-platinum-cysteine complex on activities of ALA dehydratase, uro-I synthetase and biliverdin reductase in the kidney

Male Sprague-Dawley rats (180–220 g) were treated with *cis*-platinum (▨) or *cis*-platinum-cysteine complex (▩) as described in the text. The indicated enzyme activities were measured in the cytosol fraction. Experimental details regarding tissue preparations and assay procedures are provided in the Materials and methods section. * $P \leq 0.05$ when compared with the control (□) group; ** $P \leq 0.05$ when compared with the *cis*-platinum-treated rats.

DISCUSSION

The tissue-specific toxicity of *cis*-platinum to the kidney is well documented (Blachley & Hill, 1981; Litterst *et al.*, 1983). The present results show that *cis*-platinum treatment also elicits vastly different responses in the liver and the kidney haem-metabolism activities and in the activities of the γ -glutamyl-cycle enzymes. In general, tissue responses differed in magnitude, direction and the time course. In the kidney, a larger number of parameters were affected than in the liver, and when a response was elicited, its magnitude was more pronounced, and the response was expressed at a later time point, than in the liver. In the latter organ, for the most part, significant changes were observed at 1–3 days after treatment, and at 7 days the values returned to normal. In the kidney, a delayed response was noted and, in general, the greatest deviations from the control values were detected 7 days after *cis*-platinum treatment.

It would be tempting to interpret the differential action of *cis*-platinum on haem-metabolism pathway in the liver and the kidney as a reflection of a rapid clearance of the compound from the liver with its gradual accumulation in the kidney. This, however, may not be an accurate representation of the molecular basis for the differential effects; rather, the differential effects may be related to a change in the molecular form of the compound. The expressed belief that the gradual accumulation of *cis*-platinum in the kidney may not be responsible for the differential biological potency of the compound in this organ is consistent with the reported time course of tissue distribution of *cis*-platinum in the liver and the kidney (Litterst *et al.*, 1975). Litterst *et al.* (1975) measured the

tissue concentration and distribution of *cis*-platinum in the liver and the kidney by measuring the platinum content of the tissues. These investigators did not find major differences in the concentration of platinum in the liver between 10 min and 6 days after injection. Similarly, major differences in the concentration of platinum in the kidney were not detected when measured at 30 min and 6 days after *cis*-platinum treatment. These investigators, however, noted that throughout the experiment the concentration of platinum in the kidney was in 5-fold excess of that of the liver. Therefore it appears that other factors contribute to the selective effect of *cis*-platinum on the kidney, such as the above stated transformation of *cis*-platinum to a biologically more effective form. The ability of the tissue, in this instance the kidney, to promote such molecular biotransformation could be involved in the presently observed differential responses of the liver and the kidney to *cis*-platinum.

One plausible factor responsible for this biotransformation is the vast differences in the activity of γ -glutamyl cycle enzymes that exist between the liver and the kidney (Sekura & Meister, 1974). As noted in the present study, in these organs a close similarity existed between the activity of *cis*-platinum to affect enzymes of the haem-metabolism pathway and those of the γ -glutamyl cycle. Moreover, complexing *cis*-platinum with cysteine potentiated the effects of *cis*-platinum on several haem-metabolism parameters, as well as the activity of γ -glutamyl cysteine synthetase in the kidney, but not in the liver. The known mechanism of GSH breakdown involves conversion into its constituent amino acids (γ -glutamic acid, cysteine and glycine) through the sequential activities of γ -glutamyl transpep-

tidase and dipeptidase. As noted above (Fig. 2 and Table 2; Meister, 1981) the activity of γ -glutamyl transpeptidase in the kidney exceeds that of the liver by two to three orders of the magnitude; therefore by far a greater rate of GSH degradation activity occurs in this organ than in the liver (Meister, 1981). It may be postulated that the differential activity of γ -glutamyl-cycle enzymes in the liver and kidney may lead to the preferential formation of the cysteine complex of *cis*-platinum in the kidney which, in turn, causes the differential effect of *cis*-platinum in this organ. Specifically, the possibility exists that, at the cellular level, binding of *cis*-platinum to cysteine, produced as the result of the degradation of GSH, as well as the fraction taken up from the plasma, results in the formation of *cis*-platinum-cysteine complex. It may be further postulated that, in the kidney, the severely inhibited γ -glutamylcysteine synthetase activity (Table 2) would result in a decreased utilization of cysteine for GSH production and hence an increased availability of the amino acid for binding with *cis*-platinum.

Insofar that the supply of cysteine is usually considered limiting in the production of GSH (Meister, 1984), the occurrence of the above-postulated molecular interactions could be expected to lead to an excess cellular levels of glycine and glutamic acid in the kidney and their subsequent excretion in the urine. Indeed, the reported findings by Leyland-Jones *et al.* (1983) provide credence to this suggestion. These investigators have noted that the inhibition of γ -glutamyl transpeptidase activity in rats by *cis*-platinum causes a profuse and prolonged (up to 8 days) amino aciduria. In *cis*-platinum-treated rats, greater than 20-fold increases in urinary levels of glutamic acid and glycine were detected. Interestingly, however, cysteine was not detectable in the urine.

The present findings clearly show that *cis*-platinum-cysteine complex is the more effective form of *cis*-platinum in altering haem metabolism and γ -glutamyl-cycle enzymes (Figs. 2-4). The molecular basis for the greater efficacy of the complex may be related to the greater biological stability of the cysteine complex. The concept that *cis*-platinum-cysteine complex is the more stable and potent form of *cis*-platinum is supported by findings reported by Daley-Yates & McBrien (1982) on the strong binding affinity of *cis*-platinum to the -SH group of cysteine; these investigators have shown that this binding renders *cis*-platinum sluggish for ligand-exchange reactions. It is known that free amino acids are particularly accessible to ligand exchange reactions with *cis*-platinum (Daley-Yates & McBrien, 1982; Howe-Grant & Lippard, 1980). Therefore, the formation of the cysteine complex may effectively prolong tissue retention, and thus the biological half-life, of *cis*-platinum. Moreover, according to Daley-Yates & McBrien (1982), *cis*-platinum-amino acid complexes could be degraded in the kidney. It follows that such an occurrence could cause the gradual release of *cis*-platinum from cysteine to maintain elevated levels of *cis*-platinum in the tissue for biological interactions. Thus the involvement of the following processes may be considered in explaining the differential time course of action of *cis*-platinum in the liver and the kidney, and the differential potency of the compound to alter haem metabolism in these organs. The exceedingly low levels of transpeptidase activity in the liver preclude the formation and/or retention of *cis*-platinum-cysteine complex in the liver. Therefore, the effects on haem metabolism are less pronounced and are of short

duration. In the kidney, however, the gradual conversion of *cis*-platinum to *cis*-platinum-cysteine complex, concomitant with the inhibition of the transpeptidase and the synthetase activities, would lead to the retention, and thus the prolonged biological activity, of *cis*-platinum. This postulate is consistent with the reported marked differences in platinum content of the liver and the kidney of *cis*-platinum-treated rats (Litterst *et al.*, 1975).

The mechanism by which *cis*-platinum causes decreased γ -glutamyl transpeptidase and γ -glutamylcysteine synthetase activities is not clear, although loss of enzyme protein from the kidney is a likely mechanism for this observation. However, the inhibitory action of *cis*-platinum on γ -glutamylcysteine synthetase activity may not solely reflect a loss of a fraction of the enzyme protein from the organ. This suggestion is consistent with the finding (Table 2) that activity of GSSG reductase, which is, as is the synthetase, a cytosolic enzyme, was not inhibited in *cis*-platinum-treated rats. Therefore, unless a selective loss of γ -glutamylcysteine synthetase from the kidney had occurred, it would appear that this factor did not solely contribute to the decreased activity of the synthetase. Furthermore, γ -glutamylcysteine synthetase is not a -SH dependent enzyme. Therefore, it would appear unlikely that *cis*-platinum-mediated inhibition of its activity involved a direct inhibition of the enzyme protein. Accordingly, the inhibition of the activity may involve additional processes such as an altered turnover of the enzyme and/or inhibition of enzyme protein synthesis. Moreover, since the enzyme activity is regulated by feedback inhibition by GSH (Meister, 1984), the involvement of GSH in the presently observed inhibited enzyme activity could be considered. However, in light of the finding that in the kidney the level of GSH in course of the study was not changed, renders this possibility rather unlikely.

On the other hand, decrease in the transpeptidase activity, which is bound to the renal tubules, and is a -SH-dependent enzyme, could reflect the direct inhibition of the enzyme protein, a suggestion which is in agreement with the reported findings by Borch & Pleasants (1979). Alternatively, the enzyme activity could be decreased, in part, as the result of loss of enzyme protein due to the nephrotoxicity of *cis*-platinum, and/or inhibited production of the enzyme protein. Similarly, observed decreases in activities of ALA dehydratase, uro-I synthetase and biliverdin reductase, all of which are SH-dependent cytosolic proteins, could reflect the direct inhibition of the enzymes *in vivo*; however, this suggestion is not consistent with results of studies *in vitro*. Again, a likely explanation for the noted decrease is loss of enzyme protein due to the nephrotoxicity of *cis*-platinum and/or inhibited synthesis of enzyme proteins.

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