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Alterations in Hepatic and Renal Levels of Glutathione and Activities of Glutathione S-Transferases from Rats Treated with Cis-dichlorodiammineplatinum-II

Charles L. Litterst, Samuel Tong*, Yoichiro Hirokata**, and Zahid H. Siddik***

Laboratory of Medicinal Chemistry and Biology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Building 37, Room 6D28, Bethesda, MD 20205, USA

Summary. *Adult female rats were treated intraperitoneally with 8 mg/kg of cis-dichlorodiammineplatinum (II). At various times after treatment 1, 3, 5, 8, 12 days replicate animals were killed and liver and kidney cytosols examined for activity of glutathione-dependent transferase activities and levels of glutathione. Hepatic levels of glutathione were depressed by 13–28% at 1, 3, 5 days after dosing. Renal levels of glutathione were increased by 3–5 fold at 8 and 12 days after drug administration. Renal levels of glutathione were decreased at nearly all times studied with a nadir at 5 days. Activity of glutathione s-acryl transferase was increased and S-epoxidetransferase was decreased at 5, 8, 12 days after dosing. When cisplatinum was added to incubation mixtures in vitro, no changes in enzyme activities were observed. When cisplatin and reduced glutathione were determined chromatographically in tissue cytosols from treated rats, 30% of the recovered platinum was associated with glutathione. In tissue cytosols, greater than 95% of the total platinum content was retained in the supernatant when protein was precipitated with trichloroacetic acid, while only 3–5% of the protein was retained.*

Introduction

Cis-Dichlorodiammineplatinum-II (cisPt) is an antineoplastic drug with activity toward numerous animal and human tumors. The pharmacodynamics, distribution, and toxicity of the drug are well established [17, 19, 31]. The drug decomposes spontaneously by replacement of labile chlorine atoms with water or hydroxyl groups in a process termed aquation. It has been proposed that the electrophilic aquation site of the cisPt molecule should interact avidly with various nucleophiles, most specifically nitrogen- or sulfur-containing molecules. For instance, the binding of cisPt to DNA is thought to occur at a nitrogen atom of guanine [21]. Similarly, several thiol-containing compounds have been shown to reduce the in vivo toxicity of cisPt, possibly by such binding [4, 5, 9, 33]. Furthermore, cisPt has been shown to bind to the sulfur group of phosphorothioates [29] and to the sulfur of methionine [23], and to interact with the sulfhydryl groups of thymidylate synthetase in vitro [2]. It thus seemed reasonable to expect an interaction in vivo between cisPt and endogenous sulfur-containing molecules, such as the ubiquitous glutathione. Preliminary data [19] suggested that cisPt may alter glutathione-dependent cytosolic drug metabolism, but a thorough study of this important area has not been conducted. We report here studies of the effect of cisPt treatment on a variety of glutathione-dependent enzymatic pathways, endogenous levels of glutathione, and a possible in vivo association between platinum and glutathione.

Methods and Materials

Female Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing approximately 200 g were treated IP with 8 mg cisPt/kg in 0.9% NaCl (1.0 mg/ml). At various times after treatment, control and treated animals were killed by cervical dislocation. Livers and kidneys were removed and a post-microsomal supernatant (cytosol) was prepared by differential ultracentrifugation as described in detail previously [18]. Protein content was determined according to the method of Lowry et al. [20]. Levels of reduced glutathione (GSH) and total glutathione (GSH + GSSG) were determined by the methods of Ellman [7] and Tietze [30], respectively. The

Reprint requests should be addressed to C. L. Litterst

* *Present address:* Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA

** *Present address:* Dept. Anesthesiology, Kyushu University Medical School Fukuoka, Japan

*** *Present address:* Dept. of Biochemical Pharmacology, Institute for Cancer Research, Sutton, Surrey, England

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method of Mize and Langdon was used to assay glutathione reductase activity [25]. Activities of three glutathione S-transferases were determined under conditions that have been detailed previously [28]. Briefly, incubations contained reduced glutathione (10 m*M*), diluted cytosol (0.6–3.0 mg protein), and an appropriate substrate and buffer. Substrates for glutathione S-aryl-, S-aralkyl-, and S-epoxidetransferases and their respective final concentrations were: 3,4-dichloronitrobenzene (DCNB; 1 m*M*), 4-nitrobenzyl chloride (NBC; 1.0 m*M*), and 1,2-epoxy-3-(4-nitrophenoxy)propane (ENPP; 0.5 m*M*). The in vitro effect of cisPt on enzyme activities was investigated with cytosol from untreated rats. Prior to incubation cisPt was added to the cytosol at final concentrations of 400 and 800 μ*M*. In vitro enzyme activity was determined immediately, and after standing at room temperature for 1 or 2 h. The incubation medium contained 150 m*M* KCl-tris buffer [18].

To determine the nonspecific binding of platinum to tissue proteins, each tissue fraction (homogenate, 9,000 *g* supernatant, cytosol) was analyzed for protein content and for platinum content before and after precipitation of proteins with ice-cold 4% (w : v) sulfosalicylic acid. Glutathione present in protein-free supernatants was isolated by thin layer chromatography (TLC) with *n*-butanol : acetic acid : water (4 : 1 : 2) [10]. Plates were air-dried and then exposed to iodine vapors to allow visualization of the glutathione-containing area. Each sample on the plate was then arbitrarily divided into the glutathione-containing area ($R_f = 0.35–0.65$), and those areas between the origin and glutathione and between glutathione and the solvent front. Silica gel was scraped from these areas and eluted with 2–3 ml 0.1 *N* HCl. After vigorous mixing the sample was centrifuged and the HCl supernatant was then analyzed for platinum content by means of atomic absorption spectrophotometry [13]. The behavior of freshly prepared cisPt standards was also determined in the same TLC system. When known amounts of cisPt were added to a TLC plate and the plate then analyzed as described, recovery was approximately 70%. All data on Pt content of TLC plates have been corrected for this recovery.

Results

Table 1 shows the effect of cisPt treatment on the activity of glutathione-dependent transferases and amounts of glutathione in post-microsomal supernatants of liver and kidney. In the liver, the only consistent and noteworthy change was a decrease in glutathione. In the kidney, however, the effects of cisPt treatment were more evident. Activity of glutathione-S-aryltransferase (DCNB) was increased 2–4 times at 5, 8, and 12 days post-treatment. Effects at earlier times were variable and time-dependent. At the same times that S-aryltransferase was significantly increased, the activity of glutathione S-epoxidetransferase was significantly decreased. Total glutathione was increased by 3–5 times normal at 8 and 12 days post-treatment, but by lesser amounts at earlier times. Interestingly, reduced glutathione was increased only on the first day after treatment. Glutathione reductase was decreased at all times except day 3.

Table 1. Alterations of in vitro parameters of glutathione-dependent drug metabolism in cytosol from liver and kidney of rats treated with cisPt (8 mg/kg). Data are expressed as percentage of control[a] and represent means of at least three animals per time

Parameter	Days post-treatment				
	1	3	5	8	12
Liver					
S-Aryltransferase	104	110	112	102	110
S-Epoxidetransferase	96	95	103	98	120[b]
S-Aralkyltransferase	95	89	107	91	82[b]
Reduced glutathione	87[b]	88	77[b]	98	104
Total glutathione	78[b]	82[b]	72[b]	98	110
GSH reductase	109[b]	98	101	92	97
Kidney					
S-Aryltransferase	76[b]	138	174[b]	371[b]	201[b]
S-Epoxidetransferase	100	102	69[b]	85[b]	87[b]
S-Aralkyltransferase	100	124[b]	92	108	94
Reduced glutathione	134[b]	93	102	100	93[b]
Total glutathione	169[b]	102	141[b]	330[b]	535[b]
GSH reductase	88[b]	102	77[b]	85[b]	87[b]

[a] Control values for hepatic and renal enzyme activities (expressed as nmol product formed/min per mg protein) and glutathione (expressed in m*M*) are, respectively: S-Aryltransferase 43.3 ± 5.8, 3.00 ± 0.40; S-Epoxidetransferase 131 ± 16, 146 ± 16; S-Aralkyltransferase 361 ± 59, 266 ± 35; GSH-Reduced glutathione 5.9 ± 1.4; 4.3 ± 0.4; total glutathione 7.1 ± 0.4, 6.4 ± 0.8; GSSG reductase 105 ± 11, 115 ± 7

[b] treated value significantly different from control ($p < 0.05$)

When various concentrations of cisPt were added in vitro to cytosol from control rats and the tissue allowed to stand for up to 2 h few consistent, dose-related changes in enzyme activity were observed (Table 2). One explanation for this may be that the chloride ion concentration in the in vitro incubation mixture was sufficiently high to prevent the rapid activation by aquation of the cisPt molecule, and thus preclude an early binding or effects [16].

In rats treated for 1 h with cisPt, the liver and kidney contained 94%–98% acid-insoluble proteins (Table 3). Platinum loss after acid addition was variable, however, and depended on tissue and tissue fractions studied (Table 3). In both liver and kidney, the amount of platinum retained after acid addition was lowest for crude homogenate (48%–54%), while the cytosol retained larger amounts of platinum (94%–111%). When protein and platinum concentrations were studied 30 h after animal treatment or 1 h after in vitro addition to control homogenates, qualitatively similar results were obtained (data not presented).

In the TLC system described, standards of cisPt ran more slowly ($R_f = 0.18–0.26$) and had a different

Table 2. Effect of in vitro addition of cisPt on glutathione-dependent S-aryl and S-epoxidetransferase activities (expressed as nmol/min per mg protein) in liver and kidney

Pathway	Pt^{+2} conc (μM)	Hours post-treatment		
		0	1	2
Liver				
S-Aryltransferase	0	73.0 ± 12.4	70.2 ± 18.1	66.6 ± 14.7
	400	73.0 ± 10.1	60.0 ± 14.2	59.0 ± 17.0
	800	61.0 ± 8.8	60.0 ± 14.9	53.8 ± 9.4
S-Epoxidetransferase	0	123 ± 8	101 ± 12	102 ± 10
	400	115 ± 11	131 ± 14[a]	119 ± 14
	800	115 ± 10	79 ± 8[a]	91 ± 11
Kidney				
S-Aryltransferase	0	3.2 ± 0.9	3.7 ± 0.9	5.4 ± 1.0
	400	3.2 ± 1.2	3.7 ± 0.6	5.4 ± 0.8
	800	3.1 ± 0.7	3.6 ± 0.7	4.2 ± 1.2
S-Epoxidetransferase	0	107 ± 12	113 ± 12	67 ± 8
	400	85 ± 11[a]	94 ± 10	66 ± 14
	800	75 ± 11[a]	94 ± 13	66 ± 12

[a] $p < 0.05$

Table 3. Effect of acid precipitation on tissue Pt and protein concentrations in liver and kidney fractions 1 h after animal treatment with cisPt. Parenthetical number is the percent remaining in the acid supernatant

Tissue	Fraction		Protein (mg/ml)	Pt (μg/ml)
Liver[a]	Homogenate	Total[a]	57.8	0.54
		Retained[a]	1.12 (1.9%)	0.29 (54%)
	Cytosol	Total	30.2	0.28
		Retained	1.05 (3.5%)	0.31 (111%)
Kidney[b]	Homogenate	Total	36.6	0.95
		Retained	1.12 (3.1%)	0.46 (48%)
	Cytosol	Total	20.6	0.54
		Retained	1.15 (5.6%)	0.51 (94%)

[a] GSH content of liver was 0.54 ± 0.01 mg/ml for all three fractions
[b] GSH content of kidney was 0.26 ± 0.01 mg/ml for all three fractions
[c] Concentration before addition of acid
[d] Concentration in the acid supernatant after sediment of precipitate by centrifugation

color (grey-brown) than did standards of glutathione ($R_f = 0.41$; yellow). When protein-free tissue fractions from platinum-treated rats were spotted and run, the results were the same as when standards of cisPt and GSH were co-chromatographed. Approximately 30% of the recovered platinum was present at the R_f of, and with the color of, GSH. The remaining 60%–70% of the recovered platinum was present at the R_f of, and with the color of, a cisPt standard.

Discussion

Much recent work has established the ability of platinum-containing compounds to inhibit the activity of various enzymes [1–3, 8, 23], and the inhibition has often been related to direct interaction between platinum and sulfur atoms on the enzyme molecule [2, 3, 8, 23]. Thus the interaction of cisPt with sulfhydryl-containing glutathione seemed likely, particularly in tissues with high concentrations of glutathione and glutathione-dependent enzymes (liver and kidney) and where the predominant platinum-induced toxicity was known to occur (kidney).

Decreases in hepatic glutathione have been previously reported for several drugs [24]. The cisPt-induced decrease in hepatic glutathione (Table 1) was probably not due entirely to a direct reaction between cisPt and GSH, because the decrease in the latter was about 100-fold greater than the hepatic platinum content previously determined [18]. This is consistent with the work of Levi et al., who found that cisPt produced a decrease in total cellular thiols but that cisPt did not interact directly with thiol-containing molecules in vitro [14]. Thus an indirect mechanism may be responsible for the decrease in hepatic GSH content.

In the kidney, substantial increases in total glutathione occurred without significant changes in reduced glutathione. Other divalent metal ions than Pt-II also have been shown to increase GSH [26], but concomitant measurements of total glutathione were not made, so direct comparison with the present results is difficult. Extremely high GSSG/GSH ratios are uncommon [32] because of a feedback inhibition of protein synthesis [11]. Therefore, it is questionable whether a prolonged elevation of the magnitude observed in Table 1 could be maintained for several days without drastic consequences. It may be possible, therefore, that the large increase in total glutathione may be due to the presence of an unidentified acid-soluble substance released from the damaged kidneys that interferes with the analytical method for glutathione. The concomitant occurrence of elevated total glutathione levels and decreased glutathione reductase activity is probably coincidental because the reductase, even though it is involved in maintaining glutathione in the reduced form, is probably not rate-limiting in this reaction [27].

Hepatic GSH S-transferase activities were generally unaffected by the cisPt treatment. In contrast, the renal S-aryltransferase (DCNB) and S-epoxidetransferase (ENPP) activities appeared to be more sensitive to the drug. The preferential increase in only the renal S-aryltransferase activity is an effect which is similar to that seen after treatment with pesticides [12].

The most dramatic changes occurred in kidney at times (5 and 8 days) closely corresponding to the most severe renal toxicity [31]. It is tempting to postulate a cause-effect relation between a platinum-glutathione interaction and the severity of renal injury, particu larly since the extraction of glutathione by the kidney is proposed to occur in the proximal tubules [22], the site of the tubular necrosis observed following cisPt administration [31]. It is just as likely, however, that glutathione and the S-transferases are affected in a nonspecific manner secondary to the severe renal damage. This latter explanation gains credence in light of the results of the in vitro study (Table 2), where cisPt had no apparent effect. However, it should be kept in mind that the 2-h incubation time for the in vitro study may have been too short to allow for aquation of the cisPt molecule and subsequent binding of the aquated molecule to GSH or other cellular constituents [8].

The acid-precipitation studies suggest that there may not be a generalized, random binding of platinum to tissue proteins because the addition of acid to tissue fractions resulted in 95%–98% loss of protein but only a 0%–60% loss of platinum. Other studies of acid-precipitable platinum have led to similar conclusions [6]. Unfortunately, the design of this study does not provide any evidence for the nature of the acid-soluble platinum species or whether it is in solution in the cytosol or is bound to an acid-soluble polypeptide or other substance. The presence of large amounts of glutathione in the acid-soluble tissue fractions suggest one possible source of binding for platinum. Histones from nuclear chromatin are also acid-soluble and have been implicated in platinum binding [15], so they represent another potential acid-soluble binding site for platinum.

Because 60%–70% of the platinum from protein-free, acidic supernatants was recovered from TLC plates at an R_f lower than that of GSH, it appears that a majority of the platinum was unassociated with GSH. Whether the remaining 30% of the platinum was bound to glutathione or merely exhibited an altered migration when in the presence of glutathione cannot be determined from this study.

References

1. Allen RC, Gale GR, Oulla PM, Gale AO (1978) Effect of certain antitumor platinum compounds on kidney esterases. Bioinorg Chem 8:83–89
2. Aull JL, Rice AC, Tebbetts LA (1977) Platinum complex interactions with sulfhydryl groups of thymidylate synthetase. Biochemistry 16:672–677
3. Aull JL, Allen RL, Bapat AR, Daron HH, Friedman ME, Wilson JF (1979) Effects of platinum complexes on seven enzymes. Biochim Biophys Acta 571:352–358
4. Borsch RF, Pleasants ME (1979) Inhibition of cisplatinum nephrotoxicity by diethyldithiocarbamate. Proc Natl Acad Sci USA 76:6611–6614
5. Burchenal JH, Kalaher K, Dew K, Lokys L, Gale G (1978) Studies of cross-resistance, synergistic combinations and blocking of activity of platinum derivatives. Biochimie 60:961–965
6. Choie D, del Campo A, Guarino A (1980) Subcellular localization of *cis*-dichlorodiammineplatinum(II) in rat liver and kidney. Toxicol Appl Pharmacol 55:245–252
7. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77
8. Friedman ME, Melius P, Teggins JE, McAuliffe CA (1978) Interactions of *cis* and *trans*-platinum(II) complexes with dehydrogenase enzymes in the presence of different mono- and polynucleotides: Evidence for a ternary complex. Bioinorg Chem 8:341–353
9. Howell SB, Taetle R (1980) Effect of sodium thiosulfate on *cis*-dichlorodiammineplatinum(II) toxicity and antitumor activity in L1210 leukemia. Cancer Treat Rep 64:611–616
10. Javitt NB (1976) Biochemical probes for the study of binding and conjugation of glutathione S-transferases. In: Arias I, Jakoby W (eds) Glutathione: Metabolism and function. Raven Press, New York, p 309
11. Kosower NS, Kosower EM (1976) Functional aspects of glutathione disulfide and hidden forms of glutathione. In: Arias I, Jakoby W (eds) Glutathione: Metabolism and function. Raven Press, New York, p 159

12. Kulkarni AP, Fabacher DL, Hodgson E (1980) Pesticides as inducers of hepatic drug metabolizing enzymes. II. Glutathione S-transferases. Gen Pharmacol 11: 437–441
13. LeRoy A, Wehling M, Sponseller H, Litterst C, Gram T, Guarino A, Becker D (1977) Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem Med 18: 184–191
14. Levi J, Jacobs C, Kalman S, McTigue M, Weiner M (1980) Mechanism of *cis*-platinum nephrotoxicity. I. Effects of sulfhydryl groups in rat kidney. J Pharmacol Exp Ther 213: 545–550
15. Lippard SJ, Hoeschele JD (1979) Binding of *cis*- and *trans*-dichlorodiammineplatinum(II) to the nucleosome core. Proc Natl Acad Sci USA 76: 6091–6095
16. Litterst CL (1981) Alterations in the toxicity of *cis*-dichlorodiammineplatinum-II and in tissue localization of platinum as a function of NaCl concentration in the vehicle of administration. Toxicol Appl Pharmacol 61: 99–108
17. Litterst CL, Torres IJ, Guarino AM (1977) Plasma levels and organ distribution of platinum in the rat, dog and dogfish shark following single intravenous administration of *cis*-dichlorodiammineplatinum(II). J Clin Hematol Oncol 7: 169–179
18. Litterst CL, Sikic BI, Mimnaugh EG, Guarino AM, Gram TE (1978) In vitro drug metabolism in male and female athymic nude mice. Life Sci 22: 1723–1730
19. Litterst CL, LeRoy AF, Guarino AM (1979) Disposition and distribution of platinum following parenteral administration of *cis*-dichlorodiammineplatinum(II) to animals. Cancer Treat Rep 63: 1485–1492
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275
21. Macquet JP, Butour JL (1978) Modification of DNA secondary structure upon platinum binding: a proposed model. Biochimie 60: 901–914
22. McIntyre TM, Curthoys NP (1980) The interorgan metabolism of glutathione. Int J Biochem 12: 545–551
23. Melius P, Friedman ME (1977) Complexes of platinum with polypeptides and proteins. Inorganic Perspectives in Biology and Medicine 1: 1
24. Mitchell JR, Hinson JA, Nelson SD (1976) Glutathione and drug-induced tissue lesions. In: Arias I, Jakoby W (eds) Glutathione: Metabolism and function. Raven Press, New York, p 357
25. Mize CE, Langdon RG (1962) Hepatic glutathione reductase. I. Purification and general kinetic properties. J Biol Chem 237: 1589–1595
26. Sasame HA, Boyd MR (1978) Paradoxical effects of cobaltous chloride and salts of other divalent metals on tissue levels of induced glutathione and microsomal mixed function oxidase components. J Pharmacol Exp Ther 205: 718–724
27. Siddik ZH, Drew R, Gram T (1980a) Metabolism and biliary excretion of sulfobromophthalein in vitamin A deficiency. Biochem Pharmacol 29: 2583–2588
28. Siddik ZH, Mimnaugh E, Trush M, Gram T (1980b) Effect of vitamin A deficiency on hepatic renal and pulmonary glutathione S-transferase activities in the rat. Biochem J 188: 889–893
29. Strothcamp KG, Lippard SJ (1976) Platinum binds selectively to phosphorothioate groups in mono- and polynucleotides: A general method for heavy metal staining of specific nucleotides. Proc Natl Acad Sci USA 73: 2536–2540
30. Tietze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathion: Applications to mammalian blood and other tissues. Anal Biochem 27: 502–522
31. Ward J, Fauvie K (1976) Nephrotoxic effects of *cis*-diamminedichloroplatinum(II) in male F344 rats. Toxicol Appl Pharmacol 38: 535–547
32. Whikehart DR (1975) Total and oxidized glutathione in bovine corneal epithelium and endothelium. Exp Eye Res 21: 89–92
33. Yuhas JM, Culo F (1980) Selective inhibition of the nephrotoxicity of *cis*-dichlorodiammineplatinum(II) by WR-2721 without altering its antitumor properties. Cancer Treat Rep 64: 57–64

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