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ROLE OF ENDOGENOUS SULFUR-CONTAINING NUCLEOPHILES IN AN *IN VITRO* MODEL OF *cis*-DIAMMINEDICHLOROPLATINUM(II)-INDUCED NEPHROTOXICITY

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Abstract—Quiescent LLC-PK$_1$ cells have been shown to be a good model of *cis*-diamminedichloroplatinum(II) (DDP)-induced nephrotoxicity. In these nonproliferating porcine kidney epithelial cells, DDP inhibition of protein synthesis rate is the major correlate of cytotoxicity. We report here the use of this cell line to investigate the role of endogenous sulfur-containing nucleophiles in DDP-induced nephrotoxicity. Reaction of DDP with glutathione (GSH), cysteine, or methionine for up to 24 hr led to concentration- and time-dependent loss of its toxic effects, whereas dissolution of DDP alone did not alter its reduction of viability or protein synthesis rate in LLC-PK$_1$ cells. Treatment of these cells with differing cytotoxic concentration of DDP produced an identical transient increase in intracellular GSH, whereas compounds known to bind GSH, *trans*-diamminedichloroplatinum(II) (t-DDP) and diethyl maleate (DEM), rapidly depleted LLC-PK$_1$ intracellular GSH levels. Buthionine sulfoximine (BSO) treatment decreased intracellular GSH to 10% of control without altering cell viability or protein synthesis rate. However, BSO-pretreated LLC-PK$_1$ cells exhibited enhanced DDP-induced toxicity. $CdCl_2$ treatment produced a 30-fold induction of metallothionein-like, cadmium-binding proteins and a 10-fold increase in metallothionein isoform I (MT-I) mRNA, but this induction had no effect on DDP-induced reduction of viability or protein synthesis rate. Protracted DDP exposure did not induce MT-I mRNA levels in LLC-PK$_1$ cells.

The selective and severe nephrotoxicity associated with *cis*-diamminedichloroplatinum(II) (DDP†) administration has led to the formulation of many hypotheses concerning its mechanism of toxicity. DDP binding to extracellular sulfur-containing nucleophiles, glutathione (GSH), or metallothioneins is central to many of these hypotheses. Equivocal data have been reported concerning the role of these compounds in DDP-induced nephrotoxicity. For example, platinum-containing species from rat plasma that coeluted on HPLC with the reaction product of DDP and distilled water were demonstrated to be more nephrotoxic, whereas species that coeluted with a DDP-methionine reaction product were less nephrotoxic than parent DDP in rats [1]. In contrast, other investigators reported that methionine injection following DDP administration enhances nephrotoxicity in rats [2]. Similarly, while the majority of reports do not support a direct intracellular reaction between GSH and DDP in renal tissue [3, 4], an indirect interaction between the nephrotoxic mechanisms of DDP and GSH-dependent cellular processes has been suggested based upon increased lethality and nephrotoxicity of DDP in rodents following depletion of GSH with diethyl maleate (DEM) or buthionine sulfoximine (BSO) [3, 5].

Protection from the lethal effects of DDP treatment in mice has been achieved by preadministration of different metals known to induce hepatic and renal metal-binding proteins, including metallothioneins [6, 7]. However, only bismuth-containing compounds demonstrated a positive correlation between survival and ^{203}Hg-binding capacity in kidney homogenates. Litterst *et al.* [3] have also shown protection from lethality of DDP by pretreatment of rats with $CdCl_2$, but inconsistent protection from DDP-induced nephrotoxicity and no significant changes in subcellular platinum distribution led them to suggest that the mechanism of death may be different in metal-pretreated animals. Mason *et al.* [8] demonstrated binding of platinum to high and low molecular weight fractions of rat renal tissue using gel filtration. Upon further resolution, however, neither platinum-binding fraction proved to be authentic metallothionein. Neither these platinum-binding proteins nor metallothioneins were induced by DDP treatment.

Quiescent LLC-PK$_1$ cells have been shown to be a good model of DDP-induced nephrotoxicity and its modulation by thiol-containing chemoprotectors [9]. DDP-induced reduction of protein synthesis rate is the major correlate of cytotoxicity in these porcine renal epithelial cells. We report here the role of

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† Abbreviations used: DDP, *cis*-diamminedichloroplatinum(II); t-DDP, *trans*-diamminedichloroplatinum(II); DEM, diethyl maleate; BSO, buthionine sulfoximine; GSH, reduced glutathione; MT-I and MT-II, metallothionein isoform I and II, respectively; medium, Dubecco's modified Eagle's medium/Ham's Nutrient Mixture F-12 with 15 mM (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) and L-glutamine supplemented with 1% (v/v) antibiotic/antimycotic solution; BSS, balanced salt solution; and PBS, phosphate-buffered saline.

endogenous sulfur-containing nucleophiles in DDP-induced cytotoxicity and reduction of protein synthesis in this porcine kidney cell line.

METHODS

Materials. Antibiotic/antimycotic solution and trypsin were obtained from Gibco (Grand Island, NY). Fetal calf serum was purchased from Flow Laboratories (McLean, VA). Scintiverse II and all HPLC grade organic solvents were purchased from Fisher (Rochester, NY). Monobromobimane was purchased from Calbiochem (La Jolla, CA). HAWP filters were obtained from the Millipore Corp. (Bedford, MA). Sephadex G-75 was purchased from Pharmacia (Piscataway, NJ). Agarose was bought from Bio-Rad Laboratories (Richmond CA), and Nu-Sieve low melting agarose was obtained from FMC Bioproducts (Rockland, ME). All radioactive compounds were purchased from Amersham (Arlington Heights, IL). All other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO).

Cell culture. LLC-PK_1 cells (porcine kidney epithelial cells) were purchased from the American Type Culture Collection at passage number 196 and used between passages 215 and 220. The cell culture procedure has been described in detail elsewhere [9]. After growing to confluence, monolayers were maintained in serum-free medium for 1–2 days before drug treatment. All platinum solutions were freshly prepared in sterile balanced salt solution (BSS): 114 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.8 mM Na_2HPO_4, 0.2 mM NaH_2PO_4, 16 mM $NaHCO_3$, 5.5 mM glucose, and saturated with 5% CO_2, 95% air (pH 7.4) at 37°. All other drugs were prepared in medium. Less than 10 min elapsed between solution preparation and application to monolayers. Drug incubations were 60 min unless stated otherwise. In some experiments, either GSH, cysteine, or methionine was reacted with DDP in BSS at 37° for up to 24 hr before application of the mixture to cells. All monolayers were washed twice with phosphate-buffered saline (PBS, 37°, pH 7.4) before and after all drug incubations.

Viability assay and protein synthesis rate. Viability was assessed by measuring adherent cell protein. This method has been shown to correlate with lactate dehydrogenase (LDH) release and trypan blue exclusion in this system [9] and has been employed by others as an index of toxicity to adherent monolayers [10]. Monolayers were washed twice with PBS, digested in 0.5 M NaOH for 5 min, and then protein content was quantified using Bradford's assay [11]. Protein synthesis rate was determined by measuring the rate of L-[4,5-^{3}H]leucine ([^{3}H]leucine) incorporation into trichloroacetic acid-insoluble material [9].

Intracellular GSH. LLC-PK_1 cultures (9.6 cm^2/well) were removed from the incubator and immediately placed on ice. Medium was aspirated and monolayers were washed twice with ice-cold PBS. GSH concentrations were determined as described elsewhere [9]. Briefly, cell digests were incubated with a solution of monobromobimane and then analyzed by HPLC with fluorometric detection. An isocratic mobile phase of 16.4% methanol and 0.25% acetic acid in water (pH 3.9) flowing at 1.5 mL/min was used with an Econosphere C-18 (250 × 4.6 mm, 5 μm particle) column. The retention time for GSH was 9.5 min. Fluorescence of the GSH-bimane derivative was linearly related to concentrations over the range of 0 to 5 mM.

GSH depletion. GSH was depleted by a 1-hr treatment with DEM (0.3 to 3 mM) in BSS or 22–24 hr of treatment with BSO (0.1 to 1 mM) in medium [12]. Monolayers were exposed to DDP immediately upon completion of GSH depletor pretreatment. The GSH depletors were not included in the DDP treatment.

Metallothionein induction. LLC-PK_1 cells were exposed to $CdCl_2$ in medium for 22–24 hr to induce metallothionein production before proceeding withe drug treatments. Viability experiments showed that concentrations of 1 and 5 μM $CdCl_2$ were not significantly toxic to these cells.

Induction of "metallothionein-like" proteins was quantified using the standard Cd-hemoglobin assay adapted to cultured cells [10]. LLC-PK_1 cells (1.8×10^7) were trypsinized, concentrated to a pellet, and then resuspended in 100 μL of 5 mM Tris–HCl buffer (pH 8.6). Cells were lysed by three cycles of freezing and thawing and then centrifuged at 12,000 *g* for 25 min at 0°. The supernatant fraction (100 μL) was removed and combined with 100 μL of Tris buffer containing 2.2 μM $CdCl_2$ and 2 μCi $^{109}CdCl_2$. This solution was incubated in a microfuge tube at 37° for 1 hr. Bovine hemoglobin [2% (w/v); 100 μL] was added , and the solution was placed in a boiling water bath for 90 sec, cooled on ice, and then centrifuged. This procedure was repeated three times. The final supernatant fraction (200 μL) was loaded onto a Sephadex G-75 column (0.7 × 20 cm) and eluted with Tris–HCl buffer at 6 mL/hr. Fractions (100 μL) were collected every minute for 1 hr and analyzed directly with a Beckman Gamma 4000 counter. A solution of blue dextran was eluted to calibrate the column. Authentic metallothionein eluted with $V_e/V_o = 1.8$ to 2.2.

Mouse metallothionein-I cDNA probe. Escherichia coli transformed with pBX_Δ (a pBR322 derivative) plasmid containing two copies each of mouse MT-I c-DNA 355 basepair insert (isolated and characterized by Durnam *et al.* [13]) was provided by Professor G. Diamond, University of Rochester. Plasmid purification exactly followed the published method [14]. pBX_Δ was digested with Bam H1, and the MT-I inserts were isolated by gel purification using Nu-Sieve low-melting agarose. Isolated MT-I cDNA insert was labeled with [α-^{32}P]dCTP by random priming [15] to a specific activity of 10^8 cpm/μg DNA.

RNA isolation and Northern blot analysis. Total cellular RNA was isolated from LLC-PK_1 cells by the method of Chomczynski and Sacchi [16]. LLC-PK_1 cells (150 cm^2 flasks) were treated with $CdCl_2$ (1 or 5 μM) or DDP (12 or 25 μM) in medium for 5 or 24 hr, trypsinized, pelleted, and then frozen before RNA isolation. Yields averaged 6.4 μg total RNA/mg protein in control cells. Viability experiments demonstrated that these concentrations of $CdCl_2$ or

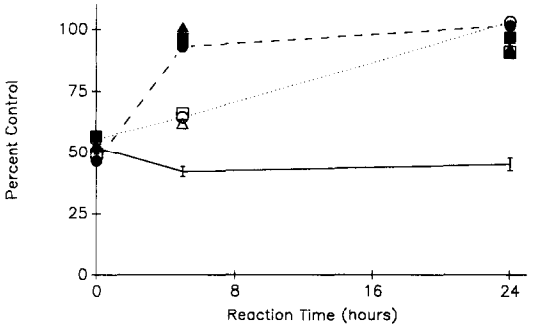

Fig. 1. Inhibition of protein synthesis rate in LLC-PK$_1$ cells by DDP preicubated with reduced glutathione, cysteine, or methionine. DDP (300 μM in BSS) and reduced glutathione, cysteine, or methionine (0.5 or 3 mM) were incubated for up to 24 hr at 37°. At the times indicated, aliquots of DDP/sulfur-containing nucleophile reaction mixtures were applied to monolayers for 60 min. Protein synthesis was determined immediately after drug exposure. Data are presented as percent precipitable dpm incorporated/mg protein/hr incubation relative to untreated control. The average protein synthesis rate in control cells was 3.5×10^5 dpm incorporated/mg protein/hr incubation. Each point is the average of three triplicate experiments. Key: (I) 300 μM DDP in BSS, (▲) 300 μM DDP and 3 mM cysteine, (●) 300 μM DDP and 3 mM methionine, (■) 300 μM DDP and 3 mM glutathione, (○) 300 μM DDP and 0.5 mM cysteine, (△) 300 μM DDP and 0.5 mM methionine, and (□) 300 μM DDP and 0.5 mM glutathione.

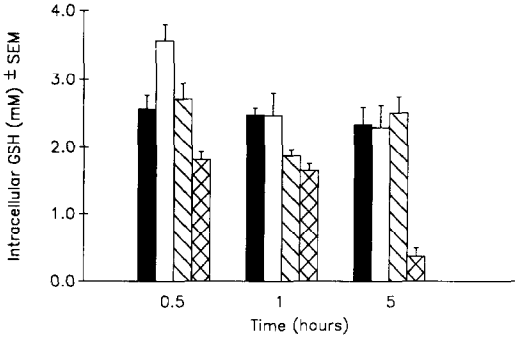

Fig. 2. Time course of LLC-PK$_1$ intracellular GSH levels after treatment with DDP or t-DDP. Monolayers were treated with drug for 1 hr and then assayed for intracellular GSH levels at the times indicated after the end of drug exposure. Data are presented as intracellular concentration of GSH assuming 13 μL of intracellular water volume per 10^7 cells [18]; means ± SE, N = 4–20. Key: (■) BSS-treated control, (□) 400 μM DDP, (▨) 400 μM t-DDP, and (▩) 1 mM t-DDP.

DDP were slightly less than those that produced significant loss of protein from the culture plate after a 24-hr exposure (data not shown).

The relative amount of total cellular RNA specific for MT-I was determined by Northern and dot blotting analysis [17]. Autoradiographs of dot blots were scanned with an LKB Ultrascan densitometer.

RESULTS

Toxicity of putative extracellular platinum metabolites. The hypothesis that extracellular metabolites of DDP are more nephrotoxic than parent drug was examined by reacting cytotoxic concentrations of DDP with GSH, cysteine, or methionine for up to 24 hr before a 1-hr treatment of LLC-PK$_1$ cells with each mixture (Fig. 1). DDP-induced reduction of protein synthesis was unchanged by incubation in BSS. Neither GSH, cysteine, nor methionine significantly altered DDP-induced toxicity when the mixture was immediately applied to cells. However, DDP incubation with each sulfur-containing nucleophile greatly decreased its toxic effect. Exposure of LLC-PK$_1$ cells to 24-hr reaction mixtures of DDP (300 μM) alone or DDP with GSH, cysteine, or methionine (3 mM) yielded 36 ± 4, 98 ± 5, 108 ± 5, and 97 ± 4% control viability at 72 hr respectively.

Intracellular reduced glutathione and DDP-induced toxicity. DDP significantly ($P < 0.05$) elevated LLC-PK$_1$ intracellular GSH levels 30 min after exposure, but these returned to normal within the next 30 min and remained normal for at least 5 hr (Fig. 2). The transiently increased GSH levels did not correlate with DDP-induced toxicity in LLC-PK$_1$ cells [9]. *trans*-Diamminedichloroplatinum(II) (t-DDP) reacts with GSH at rates several hundred fold faster than DDP [19]. Toxic concentrations of t-DDP (1 mM) produced a rapid and persistent reduction in GSH concentration, while non-toxic concentrations (400 μM) reversibly depleted GSH content. Although these results were not supportive of direct reaction between GSH and DDP, depletion of cellular GSH prior to DDP treatment should permit an assessment of an indirect interaction. Both DEM and BSO were potent depletors of GSH levels in LLC-PK$_1$ cells (Table 1), and the degree of depletion agreed with results obtained with other cell lines [20]. In contrast to DEM, concentrations of BSO that significantly reduced GSH levels did not modify LLC-PK$_1$ cell viability or protein synthesis rate. Therefore, BSO pretreatment was deemed superior to DEM as a means of depleting intracellular GSH levels prior to DDP exposure. LLC-PK$_1$ intracellular GSH concentrations were not significantly different immediately and 1 hr after BSO treatment, thus ensuring that GSH levels did not rebound during the period of DDP exposure. DDP-induced reduction in cell viability and protein synthesis rate were similarly and significantly ($P < 0.05$) enhanced by BSO pretreatment (Fig. 3A).

Metallothionein. The following experiments were designed to evaluate the correlation between DDP-induced cytotoxicity and LLC-PK$_1$ intracellular levels of metallothioneins. Cadmium chloride treatment induced production of high, intermediate, and low molecular weight Cd-binding proteins with V_e/V_o = 1.0 to 1.3, 1.8 to 2.2, and 2.5 to 2.7 respectively (Fig. 4). These values and the low basal levels of metallothioneins are consistent with other investigations of kidney homogenates from $CdCl_2$-treated rodents [3]. The degree of induction was approximately 3-, 6-, and 10-fold at 1 μM and 16-, 28-, and 52-fold at 5 μM $CdCl_2$ for the low, intermediate, and

Table 1. Depletion of LLC-PK$_1$ intracellular GSH

Drug treatment (mM)	Intracellular GSH* (nmol/mg protein) t = 0 hr†	t = 1 hr‡	Cell viability§ (% of control)	Protein synthesis‖ (% of control)
Control	32.6 ± 2.5	31.8 ± 1.7	100 ± 4	100 ± 3
DEM for 1 hr				
0.3	26.1 ± 0.5	23.8 ± 1.4	89 ± 4	94 ± 2
3	3.3 ± 0.1	2.6 ± 0.9	4 ± 1	16 ± 2
BSO for 24 hr				
0.1	9.7 ± 0.2	10.8 ± 0.7	102 ± 3	97 ± 5
0.5	4.2 ± 0.2	4.8 ± 0.8	99 ± 4	97 ± 1
1.0	3.1 ± 0.1	3.4 ± 0.7	¶	¶

LLC-PK$_1$ cells were treated with DEM in BSS or BSO in medium for the times indicated and then assayed as described. GSH depletion by 0.5 mM BSO treatment was not different from 1 mM BSO exposure but was significantly different from 0.1 mM BSO treatment ($P < 0.05$). Intracellular GSH levels were not significantly different when a 1-hr BSS incubation was inserted between BSO exposure and GSH determination.

* Values are means ± SE; N = 3.

† GSH was assayed immediately after BSO treatment.

‡ Cells were treated with BSO, incubated in BSS for 1 hr, and then assayed for GSH content.

§ Protein remaining attached to culture plate 72 hr after the end drug treatment expressed as a percent of control; controls cells had 41.9 μg protein/cm^2. Values are means ± SE, N = 6.

‖ Disintegrations per minute of L-[4,5-^{3}H]leucine incorporated/mg protein/hr expressed as a percent of untreated control; control cells had an average protein synthesis rate of 3.5×10^6 dpm incorporated/mg protein/hr incubation. Values are means ± SE, N = triplicate experiments.

¶ Experiment was not performed.

high molecular weight species respectively. Some investigators have questioned the accuracy of assigning the intermediate molecular weight species as metallothioneins [21]. A substantial body of evidence exists to support transcriptional initiation as the primary, if not exclusive, mechanism of heavy metal induction of metallothioneins [22]. Therefore, a more conclusive demonstration of metallothionein induction in LLC-PK$_1$ cells was sought by measuring relative mRNA levels of MT-I after induction with $CdCl_2$. Northern analysis detected only a single band (approximately 500 bases) when total cellular RNA from $CdCl_2$-treated cells was hybridized with the MT-I probe. Poly-adenylated MT-I mRNA of this size has been reported by several laboratories [23, 24]. Total cellular RNA from control and DDP-treated cells did not hybridize to the MT-I probe to a detectable extent (data not shown). Dot blot analyses of untreated LLC-PK$_1$ cells showed MT-I mRNA levels just above the limit of detection (Table 2). This same result has been reported for mouse renal MT-I mRNA [25]. MT-I mRNA levels from DDP-treated cells were not significantly different from untreated cells at 5 and 24 hr after drug exposure (data not shown). However, $CdCl_2$ treatment markedly elevated MT-I mRNA levels in LLC-PK$_1$ cells in a dose-dependent manner. Cadmium chloride (5 μM for 24 hr) treatment of LLC-PK$_1$ cells also increased the rate of protein synthesis to 116% of untreated controls ($P < 0.05$), while 1 μM $CdCl_2$ was without significant effect. This parallels *in vivo* data showing a 40% increase in translatable mRNA from kidneys of rats administered maximally inducing doses of $CdCl_2$ [26]. In spite of this evidence for metallothionein induction, pretreatment of LLC-PK$_1$ cells with 1 or 5 μM $CdCl_2$ for 24 hr failed to significantly alter DDP-induced cytotoxicity or reduction in protein synthesis rate (Fig. 3B).

DISCUSSION

LLC-PK$_1$ cells have been demonstrated to be a good *in vitro* model of DDP-induced nephrotoxicity; reduction of protein synthesis rate is a major determinant of cytotoxicity in this system [9]. We have exploited this model to examine the roles of endogenous sulfur-containing nucleophiles in DDP-induced cytotoxicity. The hypothesis that extracellular metabolites of DDP, including aquated DDP and DDP–methionine complexes, contribute to *in vivo* nephrotoxicity has been proposed by some investigators [1, 2]. In LLC-PK$_1$ cells, however, the unvaried toxicity of DDP indicates that it is either stable in BSS for 24 hr or that the aquated species formed are equitoxic with DDP in LLC-PK$_1$ cells. Given the very short half-time of aquated DDP in 100 mM chloride ion solution (<5 min at 37°), the former is considered more probable. Therefore, it seems unlikely that extracellularly generated aquated metabolites of DDP significantly contribute to its cytotoxicity in LLC-PK$_1$ cells. Furthermore, these data corroborate the slow reaction of GSH, cysteine, and methionine with DDP at extracellular chloride concentrations [19] and indicate that the products of these reactions are of reduced nephrotoxic potential when presented extracellularly.

The role of intracellular GSH in DDP-induced nephrotoxicity could involve direct reaction of DDP and GSH, with protection of critical cellular sites or indirect maintenance of cell viability after DDP exposure through GSH-dependent processes, e.g. DNA repair mechanisms. If a drug were to bind

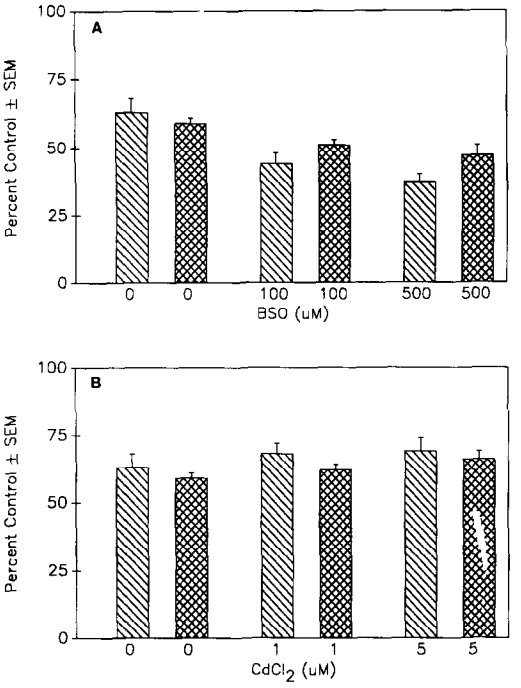

Fig. 3. DDP-induced reduction in LLC-PK$_1$ cell viability and protein synthesis rate after pretreatment with BSO or $CdCl_2$. LLC-PK$_1$ cells were pretreated with BSO or $CdCl_2$ at the concentrations indicated for 22–24 hr before exposure to 300 μM DDP for 1 hr. Cells were either immediately assayed for protein synthesis rate or re-incubated for 48 hr before being assayed for viability. Control cells had 41.9 μg protein/cm^2 and an average protein synthesis rate of 3.5×10^5 dpm incorporated/mg protein/hr incubation. Data are presented as a percent of control values; means ± SE, N = 3 triplicate experiments. Paired *t*-tests indicated significant differences between control and 100 or 500 μM BSO-pretreated cells for both viability and protein synthesis rate data ($P < 0.05$). There was no significant difference between control and any $CdCl_2$-pretreated cells for either viability or protein synthesis data. (A) BSO pretreatment: (▨) viability, and (■) protein synthesis rate. (B) $CdCl_2$ pretreatment: (▨) viability, and (■) protein synthesis rate.

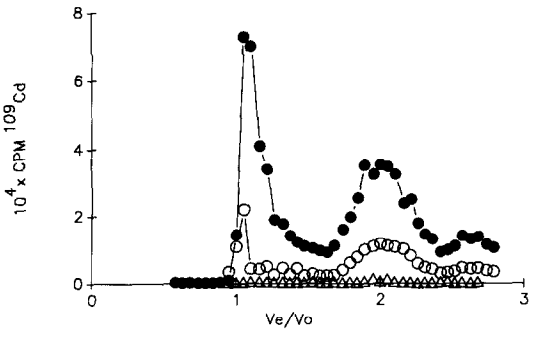

Fig. 4. Gel filtration elution profile of $CdCl_2$-pretreated and untreated LLC-PK$_1$ cells. Derivatized homogenates from LLC/PK$_1$ cells were loaded onto a Sephadex G-75 column and eluted with buffer as described in Methods. Separate fractions were collected every minute; blue dextran eluted in fraction 19. Data are presented as cpm ^{109}Cd versus elution volume normalized to void volume (V_e/V_o). Authentic metallothionein co-eluted with the intermediate molecular weight species. Key: (△) untreated control, (○) 1 μM $CdCl_2$ for 24 hr, and (●) 5 μM $CdCl_2$ for 24 hr.

Table 2. Induction of MT-I mRNA in LLC-PK$_1$ cells

Drug	Exposure time (hr)	MT-I mRNA induction*
$CdCl_2$, 1 μM	5	1.8 ± 0.1
	24	1.6 ± 0.2
$CdCl_2$, 5 μM	5	9.1 ± 1.2
	24	10.3 ± 0.3

LLC-PK$_1$ cells were exposed to drug at the concentrations and times given. MT-I mRNA was assayed immediately after drug exposure as described in the Methods.

* Induction of MT-I mRNA relative to control; mean ± range, N = 2 separate experiments.

significantly to intracellular GSH, then drug exposure would be expected to deplete GSH levels. Experiments with compounds that rapidly form stable products with GSH, t-DDP [19] and DEM, confirm this relationship. However, treatment of LLC-PK$_1$ cells with highly cytotoxic concentrations of DDP did not deplete, but rather, transiently elevated GSH levels. Although this argues against a direct reaction between DDP and GSH, GSH-dependent processes other than scavenging did appear critical to the maintenance of cell viability after DDP treatment. This conclusion is based on the observed enhancement of DDP-induced cytotoxicity and inhibition of protein synthesis after BSO treatment. These findings in LLC-PK$_1$ cells concur with what other investigators have demonstrated *in vivo* [3–5].

Most of the controversy surrounding the role of metallothioneins in DDP-induced nephrotoxicity probably stems from the different methods used to induce and to assay metallothioneins [3, 6–8]. Since cadmium induction of metallothioneins operates at the level of transcriptional initiation [22], quantitation of MT-I mRNA appeared the most definitive method for assaying induction of metallothioneins in LLC-PK$_1$ cells. Two points were addressed: (i) whether $CdCl_2$ induction of metallothioneins could modify DDP-induced cytoxicity, and (ii) whether DDP could induce metallothionein production. Neither $CdCl_2$ induction of metallothioneins nor MT-I mRNA levels correlated with DDP-induced cytoxicity in LLC-PK$_1$ cells. Cadmium induction of MT-I and MT-II mRNA has been shown to be quantitatively similar in several murine tissues, including kidney [27]. These data indicate that $CdCl_2$-induced levels of cellular metallothioneins do not modulate DDP-induced cytotoxicity in quiescent LLC-PK$_1$ cells. Furthermore, under maximally inducing conditions for other metals [22], DDP exposure failed to induce MT-I mRNA production,

whereas $CdCl_2$ treatment significantly elevated MT-I mRNA levels. This suggests that metallothioneins do not detoxify DDP significantly in LLC-PK_1 cells by a mechanism similar to cadmium detoxification. Other investigators also have been unable to detect renal metallothionein induction by DDP *in vivo* [8].

Elevation of metallothioneins in tumors made resistant to the antiproliferative actions of DDP has been established. Bakka *et al.* [28] have reported significant platinum binding to metallothioneins in tumor cells continuously cultured in medium containing $CdCl_2$ and then exposed to DDP. These investigators concluded that $CdCl_2$-induced metallothioneins could reduce the antiproliferative effect of DDP on human skin epithelial and murine fibroblast cell lines. Kelly *et al.* [29] have shown cross-resistance to $CdCl_2$ in a human head and neck carcinoma cell line made resistant to DDP exposure. These investigators demonstrated good correlation between metallothioneins and MT-II mRNA overproduction and DDP resistance in several tumor cell lines. The data reported here, however, are based on the effect of $CdCl_2$-induced metallothioneins on DDP-induced cytotoxicity in a nonproliferating renal epithelial cell line. These results may reflect different roles for metallothioneins in the antitumor and nephrotoxic actions of DDP.

It has been suggested that $CdCl_2$ treatment may induce metallothioneins saturated with cadmium that are unreactive toward platinum complexes. However, displacement of cadmium from Cd_7-metallothionein by platinum(II) has been reported, and the reaction rates of metallothioneins with platinum complexes are exceedingly slow [30]. In the reaction of native metallothionein with a 100-fold molar excess of K_2PtCl_4, a platinum complex that is more reactive than DDP, platinum represented less than half of the metal ions bound to metallothionein after 24 hr [30]. Both zinc and cadmium in the native protein were replaced by platinum. However, the substantial formation of oligomeric products reflects the extensive disruption of the metal thiolate clusters by tetrachloroplatinate in this reaction. Thus, it is unlikely that the direct reaction of native metallothionein with DDP occurs rapidly enough to have a significant effect on the acute toxicity of this drug.

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