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Sulfur-containing amino acids decrease cisplatin cytotoxicity and uptake in renal tubule epithelial cell lines

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Abstract *Purpose*: Nephrotoxicity is one of the major dose-limiting side-effects of cisplatin (DDP). The disproportionate accumulation of cisplatin in kidney tissue may play an important role; however, therapeutic measures to prevent this prime cause of nephrotoxicity are not available. Because certain amino acids (AAs) have been reported to modulate DDP nephrotoxicity in vivo, we explored the potential of all 20 protein AAs, *N*-acetylcysteine and DL-homocysteine to reduce DDP cytotoxicity and uptake in S_1, S_3 (proximal tubule), and DCT (distal convoluted tubule) cell lines. *Methods*: Immortalized but non-transformed renal tubule epithelial cell lines, derived from specific portions of the nephron of an SV40 transgenic mouse, were grown to confluency and exposed to various concentrations of DDP for 1 h with or without concurrent exposure to AAs in an otherwise AA-free Krebs-Ringer buffer (KRB). After 1 h, cell layers were washed and replenished with medium for cytotoxicity assays, or processed immediately for the determination of DDP accumulation. Cytotoxicity was assessed 48 h later by an MTT assay, and DDP uptake after 1 h was determined by atomic absorption spectroscopy. *Results*: In an initial screening where the cells were concurrently incubated with 0.25 m*M* DDP and 1 m*M* AA for 1 h in KRB, only cysteine (Cys), methionine (Met), *N*-acetylcysteine and DL-homocysteine reduced DDP toxicity. This effect was enhanced at 5 m*M* AA and most potent for Cys, which reduced DDP cytotoxicity by 79 ± 3% in S_3 cells, by 78 ± 12.2% in DCT cells, and by 19 ± 3.6% in S_1 cells ($P < 0.05$). Reduction of cytotoxicity was less for Met, DL-homocysteine, and *N*-acetylcysteine, in decreasing order. All four AAs also inhibited DDP uptake in renal cells, with Cys as the strongest inhibitor. Inhibition of DDP accumulation by 1 m*M* Cys after 1 h was 39% in S_3 cells, 38% in DCT cells, and 28% in S_1 cells. Again, reduction of uptake was less for the three other AAs. Pre-complexing of DDP with Cys for 16 h increased its uptake by 8- to 30-fold compared with native DDP, but markedly inhibited its toxicity. Thus, pre-complexing of DDP with Cys could not explain the reduced uptake of DDP, but could partly account for the reduction in cytotoxicity. Double-reciprocal Lineweaver-Burk plots of DDP concentration-versus-uptake rates at a constant concentration of Cys suggested that Cys competitively inhibited DDP uptake in S_1 and DCT cells, and in a more complex fashion in S_3 cells. *Conclusions*: We conclude that Cys, Met, *N*-acetylcysteine, and DL-homocysteine differentially inhibit DDP toxicity and uptake in cultured S_1, S_3, and DCT cells, and that the inhibition of uptake, as well as the complexation of DDP with Cys within the cell, may prevent toxicity. The structural element R-CH(NH_2)-[CH_2]$_{1-2}$-S-R, which is common to all four molecules, may play a crucial role in blocking the transport of DDP, and could have future clinical applications.

Key words Cisplatin · Nephrotoxicity · Amino acids · Cytotoxicity · Uptake · Transport

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Introduction

Cisplatin (DDP) is one of the most effective chemotherapeutic agents for the treatment of a variety of human solid tumors, including testicular, ovarian, bladder,

lung, and head and neck cancers [13]. Its major dose-limiting side-effect is nephrotoxicity [4]. Although we have demonstrated recently that the accumulation rate of DDP within a specific renal cell type is not the sole determinant for individual cell sensitivity [10], the disproportionate accumulation of DDP within the kidney compared with other organs, and its retention for days after a single dose, may be the most critical aspect of its nephrotoxicity [2]. The highly efficient reabsorption of various molecules from the luminal fluid on its way through the nephron may play a critical role in DDP accumulation. However, the transport mechanism whereby DDP enters cells has not been identified for the past 25 years, and therapeutic measures to prevent renal DDP accumulation are not available.

In the past certain AAs have been shown to prevent DDP nephrotoxicity in vivo: L-cysteine [3], L-methionine [5], *N*-acetylcysteine [1], glycine [12], L-arginine [11], and *N*-benzoyl-β-alanine [19]. The mechanism of protection, however, is not well understood. Modulation of DDP uptake in renal tissues by AAs has not been documented, except for *N*-acetylcysteine that seemed to reduce DDP accumulation in the kidney [1]. Here we investigated the effects of all 20 protein AAs, *N*-acetylcysteine, and DL-homocysteine on DDP cytotoxicity and uptake in immortalized but non-transformed renal tubule epithelial cell lines (RTE cells). The cell lines were isolated previously from an SV40 large T antigen transgenic mouse from specific portions of the nephron; S_1 cells from an early portion of the proximal tubule, S_3 cells from a late portion of the proximal tubule, and DCT cells from the distal convoluted tubule [8]. In this study we show that sulfur-containing AAs inhibit DDP cytotoxicity and uptake with differing potencies, depending on the molecule and on the cell type. We also demonstrate that pre-complexation of DDP with cysteine (Cys) may not be the mechanism of uptake inhibition, since cisplatin-cysteinyl conjugates are taken up at higher rates. Our data suggest instead, that cysteine may competitively inhibit DDP uptake in S_1 and DCT cells, and in a more complex fashion in S_3 cells. The structural element R-CH(NH_2)-[CH_2]$_{1-2}$-S-R, which is common to sulfur-containing AAs, may play a crucial role in blocking the transport of DDP, and could have future clinical applications.

Materials and methods

Cell culture

S1, S3, and DCT cell lines were established as previously described [8]. Our approach was based upon studies by Brinster and colleagues, which suggested the feasibility of creating immortalized cell culture lines from transgenic mice expressing certain portions of the early SV40 virus DNA [18]. We dissected specific portions of the nephron of a Brinster Large T transgenic mouse and cultured the segments separately on collagen-coated wells. This resulted in the production of a unique set of immortalized but non-transformed renal tubular epithelial cell culture lines: the cell lines S1 and S3, derived from early and late portions of the proximal tubule, respectively, and DCT cells, derived from the distal convoluted tubule. The cultured cells display contact inhibition as well as characteristics similar to those observed in corresponding freshly dissected nephron segments. For example, S1 and S3 cells are gluconeogenic, produce increased amounts of ammonia in response to acid challenge and appropriately respond to hormones. DCT cells display parathyroid hormone sensitivity [8, 14–16].

Cells are grown in cell culture dishes or multi-well plates in DMEM:Ham's F12 medium (Sigma) supplemented with 7% fetal bovine serum, 0.03 n*M* insulin, and 5 n*M* sodium selenite at 37 °C in 5% CO_2:35% O_2. All cell lines tested negative for mycoplasma contamination.

Cytotoxicity assay

Confluent monolayers were treated with various concentrations of DDP (Sigma) with or without 5 m*M* AA (all obtained from Sigma) for 1 h in chloride- and AA-free KRB buffer, pH 7.4 (122 m*M* Na-gluconate, 5 m*M* K-gluconate, 2 m*M* HEPES, 25 m*M* $NaHCO_3$, 1 m*M* $MgSO_4$, 1.2 m*M* NaH_2PO_4, 0.9 m*M* Ca-gluconate, 1 m*M* L-glutamine, 5 m*M* glucose; KRB buffer), which had been gassed for 5 min with 95% O_2/5% CO_2, and then washed and replenished with DMEM:Ham's F12 medium. Cytotoxicity for all studies was assayed 48 h later by incubating the cells with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) as previously described [8]. The 48-h time point was chosen to mimic the clinical situation, since the first signs of DDP nephrotoxicity become apparent after 48 h in vivo [7]. Briefly, the cell monolayers were incubated for 3 h with MTT at a final concentration of 1 mg/ml in the culture medium, and then extracted with 100% 2-propanol. MTT incorporation and metabolism to formazan dye is a measure of cell viability. The concentration of formazan dye was determined spectrophotometrically at 570 nm. The corresponding IC50 (drug concentration at 50% growth inhibition) was calculated by linear regression of the resulting logarithmic-linear dose-response curves.

Cellular accumulation of platinum

Confluent cell monolayers grown in 100-mm dishes were exposed to various concentrations of DDP for various time points up to 1 h in KRB buffer with and without 1–5 m*M* AA. Each data point was a mean of three cell monolayers. The uptake rate of DDP was linear within the 1-h time range. After incubation cell layers were rinsed once with ice cold PBS then trypsinized, detached by gentle shaking, transferred into a test tube and centrifuged for 3 min at 2000 *g* at 4 °C. The pellet was resuspended in 300 µl PBS and an aliquot of 30 µl was taken for protein determination. After recentrifugation for 3 min at 2000 *g* the pellet was digested with 270 µl 65% nitric acid overnight and assayed for elemental platinum content by atomic absorption spectroscopy [17]. The molar amount of elemental platinum is equivalent to the molar amount of incorporated DDP. The instrument settings for the Perkin Elmer 2380 Atomic Absorption Spectrophotometer with attached HGA-2100 Controller were selected as follows: drying temperature 75 °C; drying time 50 s; charring temperature 1800 °C; charring time 40 s; atomizing temperature 2700 °C; atomizing time 6 s; absorption 265.9 nm; sensitivity ± 0.3 µ*M* Pt in sample; detection minimum 0.2 µ*M* Pt in sample.

Statistics

The data were based on at least three independent experiments. The data are presented as means $\pm$ standard deviations. Data of each treatment group were subject to a one-way analysis of variance. Group comparisons were done with a Student-Newman-Keuls test with significance taken at $P < 0.05$.

Results

Reduction of cisplatin toxicity by amino acids

Certain AAs have been shown to reduce DDP nephrotoxicity in vivo. The mechanism of protection, however, is not well understood. Here we screened the following AAs for their effects on DDP cytotoxicity on RTE cells: AAs with non-charged hydrophobic side chains (Ala, Val, Leu, Ile, Pro, Phe, Trp, Met); AAs with non-charged hydrophilic side chains (Gly, Ser, Thr, Cys, Tyr, Asn, Gln); acidic AAs (Asp, Glu); and basic AAs (Lys, Arg, His). In addition, we tested the Cys analogs DL-homocysteine and *N*-acetylcysteine. In an initial screening, cells were exposed concurrently to various DDP concentrations and 1 m*M* AA in KRB buffer for 1 h. Cytotoxicity was assayed 48 h later by the ability of the cells to convert MTT into formazan (MTT assay). None of the AAs was toxic by itself. No cytoprotection was observed for any of the AAs except for the sulfur-containing AAs Cys, Met, *N*-acetylcysteine and DL-homocysteine at 1 m*M* (data not shown). This effect was enhanced at an AA concentration of 5 m*M*, and at the higher concentration the differences in their potencies became clearer. Physiological concentrations of AAs in the luminal fluid exceed 5 m*M*. Table 1 summarizes the effects of 5 m*M* Cys, Met, *N*-acetylcysteine, and DL-homocysteine on the cytotoxicity of 0.25 m*M* DDP. The single measure of cell viability was chosen to demonstrate the relative differences between the cell lines and the molecules tested under comparable conditions in the same experiment. Since mitochondrial activity is compromised by DDP, we believe the MTT assay to be a particularly sensitive method for measuring the differential effects of DDP in mitochondria-rich tissues, such as renal cells. The average cytotoxicity of 0.25 m*M* DDP exposure for 1 h, measured after 48 h was: 80 ± 9.6% for S_1 cells, 85.5 ± 2% for S_3 cells, and 51.3 ± 6.3% for DCT cells ($P < 0.05$). The decrease in cytotoxicity was strongest for Cys and was followed in decreasing order by Met, DL-homocysteine and *N*-acetylcysteine.

Cisplatin uptake is inhibited by sulfur-containing amino acids

The effects on DDP uptake were studied for all four sulfur-containing AAs. Cells were exposed concurrently to 0.4 m*M* DDP and 1 m*M* AA in KRB buffer for 1 h, which proved to be a suitable combination for evaluating the differential effects of AAs in uptake experiments. The platinum content of the cells was then determined by atomic absorption spectroscopy. The results are shown in Table 2. All four AAs inhibited DDP uptake, but differed in their ability to do so. The percent inhibition was also cell line dependent: in general S3 cells seemed to be most protected against cytotoxicity as well as uptake of DDP. Again, Cys proved to be the most potent inhibitor of uptake in all three cell lines. Because Cys seemed to have the greatest potency in terms of inhibiting cytotoxicity as well as uptake of DDP, the following experiments were done with Cys alone.

Cisplatin-cysteinyl conjugates are taken up at a greater rate

To investigate the effects of possible complexation of Cys and DDP during the uptake experiments, we pre-

Table 1 Inhibition of cytotoxicity in S_1, S_3, and distal convoluted tubule (*DCT*) cells by cysteine, methionine, *N*-acetylcysteine, and DL-homocysteine. Confluent cell layers were incubated with 0.25 m*M* cisplatin (*DDP*) alone or concurrent with 5 m*M* amino acid for 1 h. Percentage represents the difference in cytotoxicity compared with DDP toxicity alone ($P < 0.05$)

Amino acid (5 m*M*)	Δ% DDP cytotoxicity compared with absolute toxicity of 250 μ*M* DDP			
	S_1 cells	S_3 cells	DCT cells	*P* value
L-Cysteine	−19 ± 3.6	−79 ± 3.0	−78 ± 12.2	<0.05
N-Acetylcysteine	−6 ± 3.8	−11 ± 3.6	0 ± 7.1	<0.05
DL-Homocysteine	−11 ± 2.0	−44 ± 5.7	−56 ± 5.1	<0.05
L-Methionine	−17 ± 1.8	−74 ± 3.0	−50 ± 12.0	<0.05

Table 2 Inhibition of cisplatin (*DDP*) uptake by cysteine, methionine, *N*-acetylcysteine, and DL-homocysteine in S_1, S_3, and distal convoluted tubule (*DCT*) cells. Confluent cell layers were incubated for 1 h with 0.4 m*M* DDP alone or concurrent with 1 m*M* amino acid in Krebs-Ringer bicarbonate buffer. Platinum content of the cells was determined with atomic absorption spectroscopy. Average uptake was 5.28 ± 0.9 pmol/h per microgram protein for S_1 cells, 7.33 ± 0.4 for S_3 cells, and 3.56 ± 0.8 for DCT cells ($P < 0.05$)

Amino acid (1 m*M*)	Δ% DDP uptake compared with absolute uptake of 400 μ*M* DDP			
	S_1 cells	S_3 cells	DCT cells	*P* value
L-Cysteine	−23.5 ± 4.5	−39.0 ± 3.9	−32.0 ± 6.0	<0.05
N-Acetylcysteine	−19 ± 1.6	−17 ± 3.0	−14 ± 0	<0.05
DL-Homocysteine	−6.0 ± 2.4	−20.0 ± 3.3	−12.0 ± 3.5	<0.05
L-Methionine	−3.0 ± 6.0	−14.0 ± 4.0	−12.0 ± 4.5	<0.05

incubated DDP with Cys overnight at molar ratios of 1:1 and 1:2 (DDP/Cys) and measured the uptake of cisplatin-cysteinyl complexes. Interestingly, as shown in Fig. 1, the uptake of cisplatin-cysteinyl complexes was greatly enhanced compared with native DDP in all three cell lines. The uptake rates of complexes that were formed at a 1:1 ratio were greater than at the 1:2 ratio, indicating that different complexes may have formed depending on the ratio. At the 1:2 ratio, DDP uptake was enhanced by 4.4-fold in S_1 cells, by 1.6-fold in S_3 cells, and by 2.6-fold in DCT cells. The 1:1 ratio produced an increase in uptake of 29-fold in S_1, 15-fold in S_3 cells, and 8-fold in DCT cells. In addition we were able to show that the uptake of cisplatin-cysteinyl conjugates (1:2) could be inhibited greatly by the concurrent addition of 0.5 m*M* free Cys. The percent inhibition of the uptake of 0.1 m*M* cisplatin-cysteinyl complex, formed at a 1:2 ratio, was 96 ± 5% for S_1, 99 ± 2% for S_3, and 90 ± 6% for DCT cells ($P < 0.05$). These findings suggest that complexation of DDP with Cys may not be the mechanism of uptake inhibition by Cys. Despite their greatly enhanced uptake, cisplatin-cysteinyl complexes proved to be virtually non-toxic to all three cell lines (Table 3**).** Survival of cells incubated for 1 h with 0.3 m*M* DDP pre-complexed overnight with Cys at a ratio of 1:2, was 100%, while significant toxicity was observed with 0.3 m*M* native DDP.

Cystein may competitively inhibit cisplatin uptake

To further investigate the nature of uptake inhibition by Cys, the uptake rate of DDP was measured as a function of three different DDP concentrations with and without two different Cys concentrations. Cys concentration was kept constant for various DDP concentrations. A double-reciprocal Lineweaver-Burk plot of these variables was used to determine the nature of inhibition. Figure 2 shows Lineweaver-Burk plots for all three cell lines. After applying linear regression to the data points, it became apparent that the lines generated for S_1 and DCT cells intersected close to the y-axis. For S_3 cells the lines intersected in the −x/−y quadrant. The plots suggest that DDP uptake may be competitively inhibited by Cys in S_1 and DCT cells. In S_3 cells the inhibition may occur through a more complex mechanism.

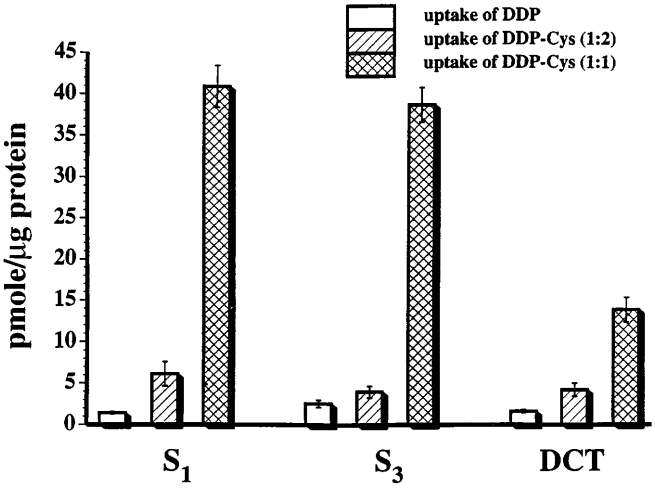

Fig. 1 Uptake of native cisplatin (*DDP*) compared with DDP-Cys formed at a 1:1 and 1:2 ratio. Confluent cell layers of S_1, S_3, and distal convoluted tubule cells were incubated for 1 h with 0.1 m*M* DDP or DDP complexes in Krebs-Ringer bicarbonate buffer. Platinum content of the cells was determined with atomic absorption spectroscopy. *Cys* cysteine

Table 3 Cytotoxicity of native cisplatin (*DDP*) compared with DDP-Cys formed at a 1:2 ratio. Confluent cell layers were incubated with 0.3 m*M* of DDP or DDP-Cys for 1 h in Krebs-Ringer bicarbonate buffer. Cytotoxicity was assayed 48 h later with an MTT assay. *Cys* cysteine, *DCT* distal convoluted tubule, *MTT* 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

Drug	% Survival 48 h after cisplatin treatment			
	S_1 cells	S_3 cells	DCT cells	*P* value
Cisplatin	31 ± 4.1	38 ± 5.4	55 ± 4.1	< 0.05
Cisplatin-cysteinyl complex	107 ± 13.3	106 ± 4.5	111 ± 30.5	< 0.05

Discussion

We investigated the modulation of DDP cytotoxicity by AAs in renal tubule epithelial cells. Although recently glycine and arginine have been shown to prevent nephrotoxicity in vivo, we found that DDP cytotoxicity was only reduced by sulfur-containing AAs in vitro at a concentration of 1 m*M*. Since we focused on strong inhibitors of DDP accumulation, non-sulfur-containing AAs were not further tested at higher concentrations. The molecules of Cys, Met, DL-homocysteine, and *N*-acetylcysteine displayed different potencies for inhibiting cytotoxicity, with Cys being the most potent inhibitor followed by Met. The ability of sulfur-containing AAs to inhibit DDP cytotoxicity in RTE cells roughly correlated with their potencies in inhibiting uptake. The inhibition of cytotoxicity as well as DDP uptake by sulfur-containing AAs occurred in a differential fashion, depending on the cell type. S3 cells were most protected by all four AAs against DDP toxicity and uptake. This finding is important, since the S3 segment of the nephron is a major site of DDP nephrotoxicity. In terms of reduction of DDP cytotoxicity and uptake Cys was the most potent molecule, and exerted its greatest effects in S_3 cell, followed by DCT cells and then S_1 cells. The differential reduction of DDP cytotoxicity seemed to be directly related to the differential DDP uptake rate: S_3 cells accumulated the greatest amount of DDP, followed again by the other cell lines in the same order. We conclude that the inhibition of DDP uptake plays an important role in the

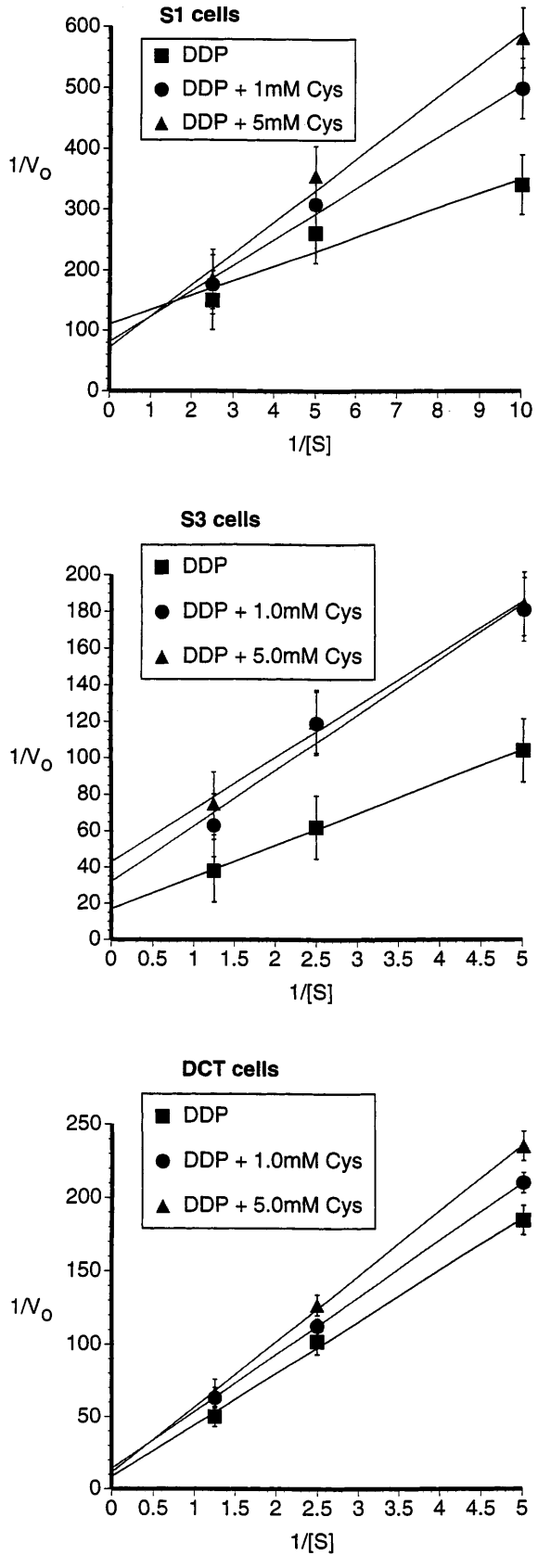

Fig. 2 The uptake rate of cisplatin (*DDP*) was measured as a function of three different DDP concentrations with and without two different cysteine (*Cys*) concentrations. Cys concentration was kept constant for various DDP concentrations. Double-reciprocal Lineweaver-Burk plots of these variables were used to determine the nature of inhibition of DDP uptake in S_1, S_3 and distal convoluted tubule (*DCT*) cells

cytoprotective action of sulfur-containing AAs. Also, our data suggest that the potencies of the different molecules in preventing cytotoxicity correlate with their ability to inhibit DDP uptake. We believe that the targeted inhibition of DDP accumulation in renal cells could lead to a new therapeutic approach to preventing nephrotoxicity.

Another potential mechanism for the inhibition of DDP uptake could be the complexation of DDP outside of the cell which could render it unsuitable to pass the cell membrane. However, our studies with pre-complexed DDP showed that the uptake of cisplatincysteinyl conjugates was greatly enhanced in all three cell lines. These data suggest that pre-complexation of DDP with sulfur-containing AAs may not be responsible for the inhibition of its uptake. Since our cisplatin-cysteinyl complexes were formed over a period of 16 h, we believe that the formation of complexes during a 1-h incubation period is negligible.

The uptake of cisplatin-cysteinyl conjugates was almost completely preventable by the addition of free cysteine to the incubation buffer. This data could suggest that cisplatin-cysteinyl conjugates may be taken up by a very specific uptake mechanism, such as a transporter-mediated process, and that passive diffusion through the cell membrane may play a negligible role. Despite their greatly enhanced uptake rate, cisplatin-cysteinyl conjugates proved to be virtually non-toxic. Thus, complexation outside of the cell cannot explain the correlation between DDP uptake and cytotoxicity. Our results, however, do not exclude the possibility of the formation of complexes inside the cell, which may, in part, account for the reduction in cytotoxicity.

In order to further characterize the type of DDP uptake inhibition through Cys, experiments were performed to yield data for double-reciprocal Lineweaver-Burk plots. Within the limits of experimental data, these plots suggest that Cys may competitively inhibit DDP uptake in S_1 and DCT cells. In S_3 cells the inhibition of uptake may occur through a more complex mechanism of a 'non-competitive' type.

Based upon experimental data over the past 20 years, it has been proposed that DDP enters the cell through both passive diffusion and a transporter-mediated process [6]. Here we present data that suggest for the first time that DDP may share a renal cell transporter that is specific for the transport of Cys and related sulfur-containing AAs. In direct comparisons, Cys and Met were significantly more effective in the inhibition of DDP uptake than DL-homocysteine and *N*-acetylcysteine. This suggests that there could be a structure-function

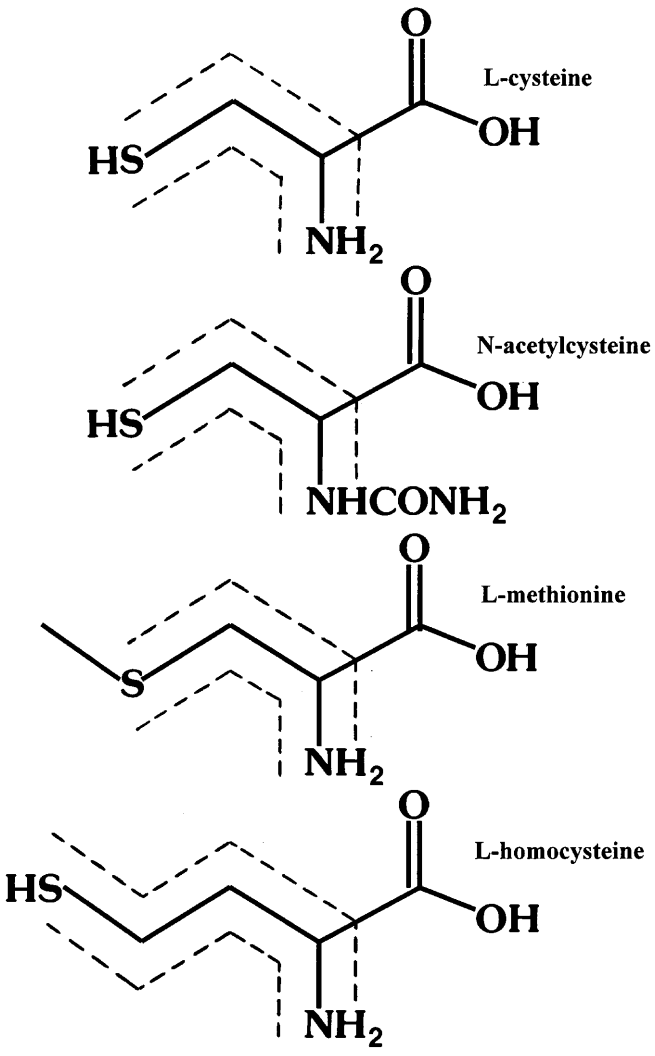

Fig. 3 Structure of L-cysteine, *N*-acetylcysteine, L-methionine, and L-homocysteine. *Broken line* indicates similar structural key elements that seem to be of importance for the inhibition of cisplatin uptake in renal cells

relationship for the inhibition of DDP and not merely a generic effect of the sulfur group. From our experiments the following observations can be made. Firstly, at a concentration of 0.5 m*M*, only sulfur-containing AAs can prevent DDP toxicity and uptake, indicating that the sulfur atom plays an important role. This effect could not be linked to a pre-complexation of DDP, but rather to an inhibition of DDP uptake. Secondly, both Cys and Met are strong inhibitors of DDP uptake. As shown in Fig. 3, Cys has a sulfhydryl group, whereas Met possesses a methylated sulfur atom. This indicates that a sulf*hydryl* group is not crucial to the inhibition of uptake, but that the properties of the sulfur atom itself are of importance. Thirdly, in comparison with Cys, N′-acetylcysteine is a much weaker inhibitor of DDP uptake. The only difference between the two molecules is an acetyl group ($-COCH_3$) bound to the amino group of Cys. Since the acetyl group alters the molecule on the amino group, we conclude that the amino group in proximity to the sulfur atom plays an important role. Since non-sulfur-containing AAs did not produce any measurable effect on cytotoxicity at 0.5 m*M*, we suggest that the combination of a sulfur atom in close proximity to an amino group, either two or three carbon atoms in between, is a key feature for inhibitors of DDP uptake. For the same reason the carboxyl group −COOH may not be important for the inhibition of DDP uptake. The resulting structural key feature R-CH(NH_2)-[CH_2]$_{1-2}$-S-R, as indicated in Fig. 3, is shared by the four sulfur-containing AAs that were investigated, and also by 'amifostine' (ethanethiol-2-[(3-aminopropyl)amino]-dihydrogen phosphate), an FDA-approved drug for the inhibition of cumulative nephrotoxicity after repeated rounds of high-dose DDP in patients with advanced ovarian carcinoma and non-small-cell lung cancer [9]. Since DDP may enter renal cells through a transporter-mediated process, the transporters involved may have a receptor site that recognizes a structural key feature, as identified in our experiments. It may be possible to identify an even more potent inhibitor of DDP uptake by altering the structures of the side groups -R.

The differential effect of Cys on DDP cytotoxicity and uptake may suggest that the ratio between passive diffusion versus active transport is different in different cell lines. Whereas S_3 cells may display a higher expression of transporters, it may be decreased in DCT and S_1 cells. An improved understanding of the DDP uptake mechanism may prove to be essential for creating better methods to prevent nephrotoxicity.

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References

1. Appenroth D, Winnefeld K, Schroter H, Rost M (1993) Beneficial effect of acetylcysteine on cisplatin nephrotoxicity in rats. J Appl Toxicol 13: 189
2. Blachley JD, Hill JB (1981) Renal and electrolyte disturbances associated with cisplatin. Ann Int Med 95: 628
3. Bogin E, Marom M, Levi Y (1994) Changes in serum, liver and kidneys of cisplatin-treated rats; effects of antioxidants. Eur J Clin Chem Clin Biochem 32: 843
4. Colvin M (1993) Alkylating agents and platinum antitumor compounds. In: Holland JF, Frei E, III, Bast RC, Jr, Kufe DW, Morton DL, Weichselbaum RR (eds) Cancer medicine. Lea and Febiger, Philadelphia, p 733
5. Deegan PM, Pratt IS, Ryan MP (1994) The nephrotoxicity, cytotoxicity and renal handling of a cisplatin-methionine complex in male Wistar rats. Toxicology 89: 1
6. Gately DP, Howell SB (1993) Cellular accumulation of the anticancer agent cisplatin: A review. Br J Cancer 67: 1171
7. Gonzales-Vitale JC, Hayes DM, Cvitkovic E, Sternberg SS (1977) The renal pathology in clinical trials of cis-platin (II) diamminedichloride. Cancer 39: 1362
8. Kaunitz JD, Cummins VP, Mishler D, Nagami GT (1993) Inhibition of gentamicin uptake into cultured mouse proximal tubule epithelial cells by L-lysine. J Clin Pharmacol 33: 63

9. Kemp G, Rose P, Lurain J, Berman M, Manetta A, Roullet B, Homesley H, Belpomme D, Glick J (1996) Amifostine pretreatment for protection against cyclophosphamide-induced and cisplatin-induced toxicities: results of a randomized control trial in patients with advanced ovarian cancer. J Clin Oncol 14: 2101
10. Kröning R, Katz D, Lichtenstein AK, Nagami GT (1999) Differential effects of cisplatin in proximal and distal renal tubule epithelial cell lines. Br J Cancer 79: 293
11. Li Q, Bowmer CJ, Yates MS (1994) Effect of arginine on cisplatin-induced acute renal failure in the rat. Biochem Pharmacol 47: 2298
12. Li Q, Bowmer CJ, Yates MS (1995) Amelioration of cisplatin nephrotoxicity with glycine: dose dependency in rats. J Pharm Pharmacol 47: 223
13. Loehrer PJ, Einhorn LH (1984) Cisplatin. Ann Int Med 100: 704
14. Nagami GT, Warech EM, Mischler DR (1990) Cells derived from S1, S2 and S3 proximal tubule segments of a transgenic mouse. J Am Soc Nephrol 1: 656
15. Nagami GT, Warech EM, Mischler DR (1991) Effect of angiotensin II on ammonia production by mouse S1 and S3 proximal tubule cell lines. J Am Soc Nephrol 2: 707
16. Nagami GT, Warech EM, Mischler DR (1992) Ammonia production and transport by cultured proximal tubule cells grown on permeable supports. J Am Soc Nephrol 3: 783
17. Parti R, Wolf W (1990) Quantitative subcellular distribution of platinum in rat tissues following i.v. bolus and i.v. infusion of cisplatin. Cancer Chemother Pharmacol 26: 188
18. Palmiter RD, Chen HY, Messing A, Brinster RL (1985) SV 40 enhancer and large-T antigen are instrumental in development of choroids plexus tumors in transgenic mice. Nature 316: 457
19. Tokunaga J, Kobayashi M, Kitagawa A, Nakamura C, Arimori K, Nakano M (1996) Protective effects of N-benzoyl amino acids on cisplatin nephrotoxicity in rats. Biol Pharm Bull 19: 1451