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ORIGINAL ARTICLE

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Inhibition of basolateral transport and cellular accumulation of cDDP and *N*-acetyl-L-cysteine-cDDP by TEA and PAH in the renal proximal tubule

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Abstract *Purpose*: The objective of this study was to determine the effect of *para*-aminohippurate (PAH) and tetraethylammonium (TEA) on basolateral cellular accumulation (C_{Pt}) and bath-to-lumen transepithelial transport rates ($J_{B\rightarrow L}$) of platinum from cisplatin (cDDP) and a conjugate of cDDP, *N*-acetyl-L-cysteine-cDDP (NAC-cDDP), in S_1, S_2, and S_3 segments of the rabbit proximal tubule. *Methods*: Cellular accumulations and transport rates were determined using the isolated perfused tubule technique and samples were analyzed by ICP-MS. *Results*: First, to establish the control data, each tubular segment was bathed in free cDDP (2 m*M*) which resulted in no observable toxicity. Next, TEA (4 m*M*) was added to the bathing solution containing cDDP. This resulted in a reduction in platinum $J_{B\rightarrow L}$ by approximately 75% in the S_1 segment and 50% in the S_2 and S_3 segments. C_{Pt} was reduced by 80–90% in relation to control values with no observable changes in toxicity. In the next experiment, exposure of the basolateral membrane to NAC-cDDP (2 m*M*) elicited pronounced toxicity after 20–30 min of perfusion. The $J_{B\rightarrow L}$ for NAC-cDDP was similar for each of the three nephron segments. There were no significant differences in the ability of these three segments to accumulate NAC-cDDP, but the conjugate increased uptake of platinum by 200–300% in the S_1 and S_2 segments, with no significant change in the S_3 segments, compared cDDP control values. The presence of PAH (4 m*M*) in the bathing solution significantly reduced $J_{B\rightarrow L}$ (by approximately 90%) for NAC-cDDP in all segments and the C_{Pt} by approximately 80%. This also abrogated the NAC-cDDP-induced toxicity. *Conclusions*: There was axial heterogeneity among the basolateral membranes of the S_1, S_2, and S_3 segments of the proximal tubule in accumulating free cDDP and transport of NAC-cDDP. Generally, the NAC-cDDP molecule was transported more avidly than free cDDP across the basolateral membrane, except in the S_3 segment, where accumulation was similar to that of free cDDP. It is concluded that a PAH-sensitive organic anion transporter is involved in the accumulation of NAC-cDDP at the basolateral membrane and a TEA-sensitive organic cation transport system is involved in the accumulation of free cDDP.

Keywords Cisplatin (cDDP) · *N*-Acetyl-L-cysteine-cDDP (NAC-cDDP) · Organic cation and anion · Basolateral transport · Proximal tubule

Abbreviations C_{Pt}: cellular accumulation of Pt · $J_{B\rightarrow L}$: bath-to-lumen · *MLC*: mean luminal concentration · *NAC*: *N*-acetyl-L-cysteine

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Introduction

Cisplatin (cDDP) is a common chemotherapeutic agent which is used successfully to treat several different cancer types, primarily solid tumors. The major side effect of succeeding treatments of cDDP is the ensuing nephrotoxicity, which has the potential to be irreversible. Impairment of renal function by cDDP has been associated with renal tubular damage of the S_3 segment of the proximal tubule in many mammalian species, including humans [10, 14, 26]. One source of cDDP toxicity are cDDP concentrations inside renal proximal tubular cells which exceed the concentration in the plasma often by a factor of 5 [15]. Therefore, efforts to minimize nephrotoxicity by preventing renal accumulation of cDDP and/

or its biotransformation products needs to be investigated fully in order to improve the efficacy of cDDP.

Renal accumulation of platinum in vivo is a rapid process that reaches a steady-state between 1 and 6 h after administration [27]. However, the form of cDDP that may be in a continuous exchange across the tubular membrane during this 1 to 6-h time period is not known [7]. Investigations have revealed that cDDP uses an organic ion transport system at the basolateral membrane to enter the cytosolic space of renal proximal tubular cells. It has been assumed, because cDDP prevents the uptake of various organic ions at the basolateral side e.g. *para*-aminohippurate (PAH) and tetraethylammonium (TEA), that cDDP utilizes an organic ion transport system [4, 5, 9, 20, 23, 29]. Furthermore, patients who are given cDDP, with a subsequent bolus injection of probenecid, have decreased secretion of cDDP. Conversely, it has been demonstrated utilizing slices of the renal cortex that the organic anions, PAH and pyrazionate, have no effect on the accumulation of cDDP, while drugs utilized by the organic cation system (OCT), mepiperphenidol, TEA, and tolazoline all significantly reduce the uptake of cDDP [27].

The apparent contradiction in the experimental evidence for cDDP transport described above provided the basic rationale for performing experiments to characterize transport and accumulation of free cDDP and NAC-cDDP across the basolateral membrane in the discrete tissue segments of the mammalian renal proximal tubule.

Materials and methods

Materials

cDDP was purchased from Sigma Chemicals (St. Louis, Mo.) and subsequently prepared in an artificial perfusion medium (APM, see below) and was stored in the dark at 5°C. ^{3}H-L-Glucose was purchased from NEN (Boston, Mass.) with a specific activity of 15.5 Ci mM^{-1} and was stored in ethanol-water (9:1 vol/vol) at 5°C. For these experiments, 10 μl ^{3}H-L-glucose (10 mM) was dried by nitrogen and subsequently reconstituted in 20 μl perfusion solution (perfusate) for a resulting concentration of 32.25 μM.

Solutions

The standard perfusion and bathing solutions consisted of an APM, containing (in millimoles) 145 Na^+, 140 Cl^-, 5.0 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 1.2 SO_4^{2-}, 2.0 $HPO_4^{2-}/H_2PO_4^-$, 0.5 L-glutamate, and 1.0 D-glucose The final pH was 7.4 (adjusted with 1.0 *M* NaOH or NaCl) and the osmolality was adjusted to 290 mosmol kg^{-1} of water by adding distilled water. The volume marker ^{3}H-L-glucose (32.25 μM) and the vital dye FD & C Green (250 nM) were always present in the perfusate whereas cDDP was always added only to the bathing solution.

Dissecting solution

A sucrose phosphate buffer solution (4°C) was used for tubular dissection. The buffer consisted of 125 mM sucrose, 13.3 mM NaH_2PO_4, and 56 mM Na_2HPO_4. The final pH was 7.4 (adjusted with 1.0 *M* NaOH) and the osmolality was adjusted to 290 mosmol kg^{-1} of water by adding distilled water.

Drug preparation

NAC-cDDP solutions were prepared on the day of the experiment by sequentially adding each compound until concentrations of 4 mM NAC and 2 mM cDDP were achieved. The 2:1 ratio was used to ensure that the majority of cDDP was bound to NAC, assuming that at most two molecules of NAC were bound to one molecule of cDDP. cDDP and NAC-cDDP solutions older than 1 day turned yellow and were discarded.

Animals

Female New Zealand White specific pathogen-free rabbits were purchased from Myrtles Rabbit Farm (Thompson Station, Tenn.). All rabbits were maintained on regular rabbit chow and given water ad libitum. Rabbits were anesthetized with ketamine (33 mg/kg body weight) and xylazine (33 mg/kg body weight) purchased from Butler Chemical (Bedford, Ohio). All experiments were conducted according to the NIH "Guide for the Care and Use of Laboratory Animals."

Methods

In a first group of experiments, the effects of temperature (23°C and 38°C) on the basolateral transport of free cDDP in dissected, perfused rabbit proximal tubules were determined. These experiments were control experiments for cDDP transport and accumulation. In a second group of experiments, the effects of placing the organic cation inhibitor TEA (4 mM) in the bathing solution with free cDDP (2 mM) while measuring basolateral transport and cellular accumulation of platinum at 38°C were studied. In a third group of experiments, the basolateral transport and cellular accumulation NAC-cDDP at 38°C with and without 4 mM PAH in the bathing solution were studied.

Isolated perfused-tubule technique

Methods used for the identification, dissection, and perfusion of the three segments of the rabbit proximal tubule have been described previously in detail [2, 3, 6]. To obtain tubular segments, rabbits were first anesthetized, and then the kidneys were quickly removed. The kidneys were cut into 1.0-mm thick coronal sections which were stored in a cold phosphate-sucrose buffer solution. Renal slices were always used within 8–12 h. Tubules were dissected manually from the individual slices under a stereomicroscope. Individual segments were identified as described by Barfuss et al. [2, 24].

Dissected tubules were transferred to a Lucite chamber mounted on the stage of an inverted microscope and perfused in vitro by techniques modified in this laboratory and others [2, 3, 24]. Briefly stated, tubules were suspended between two sets of pipettes, one set to perfuse and the other to collect the perfused fluid. The perfusion rate was maintained at about 7 nl min^{-1} by hydrostatic pressure and perfused solutions were collected in a constant-volume pipette (40–80 nl). Collections were timed to determine a collection rate for each sample. During all experiments, the bathing fluid was pumped into the bathing chamber (0.3 ml) at 0.26 ml min^{-1} and was continuously aspirated and collected directly into a scintillation vial at 5-min intervals.

Toxicity criteria

Each tubule was assessed for toxicity by the ^{3}H-L-glucose lumen-to-bath flux rate (leak, femtomoles per minute per millimeter tubule length) and changes in visual appearance of the tubular epithelium.

The greater the leak rate of ^{3}H-L-glucose, compared to the 3 fmol min^{-1} mm^{-1} leak rate of a normal tubule, the greater the magnitude of cellular toxicity [3]. Toxicity in tubular segments was apparent as cellular dye uptake (cells appeared green), cellular swelling and blebbing of the luminal membrane.

Tubular platinum content

At the end of each bath-to-lumen experiment, the tubular segment was removed from the bathing solution by grabbing it near the perfusion pipette with a pair of fine forceps and rapidly pulling it free from the pipettes. Next, the tubule was placed in 10 µl APM solution for 1–2 min to rinse excess bathing solution from the tubule, which may have contained residual cDDP or NAC-cDDP. Tubules were then transferred to an extraction chamber containing 10 µl trichloroacetic acid (3% TCA) for 2–3 min. The 3% TCA made the tubule rigid and opaque in appearance and released the cellular contents. The perceptibly opaque tubular fraction was then removed with a fine glass needle and subsequently placed in 2 ml 2% 1 *N* nitric acid for digestion and platinum analysis. Lastly, the 10 µl 3% TCA solution containing the non-perceptibly opaque tubular fraction was removed and rinsed in the 10 µl extraction chamber three times with subsequent placement of the eluent in 2 ml 2% 1 *N* nitric acid, also for platinum analysis.

Platinum analysis

Three of the six collectate, perfusate, and bathing solution samples, along with the perceptible and non-perceptible tubular extract samples, from each perfused tubule were measured for platinum content using an inductively coupled plasma mass spectrometer. All samples were spiked with indium (25 ng/ml) to serve as an internal standard. Platinum analyses for this study were calibrated against a set of working platinum standard solutions.

Results

cDDP accumulation, transport, and toxicity

Cellular accumulation of platinum (C_{Pt}) and the bath-to-lumen transepithelial transport rate ($J_{B\rightarrow L}$) of platinum were measured in perfused S_1, S_2, and S_3 segments at 23°C. The values for these two parameters, in all segments, were not significantly different from zero transport and accumulation at 23°C (data not shown). To determine how free cDDP was transported at the basolateral membrane, all the three nephron segments were perfused with APM and bathed in cDDP (2 m*M*) at 38°C. Each segment avidly accumulated free cDDP (S_1 800, S_2 750, S_3 1900 µ*M*) across their basolateral cell membranes. The S_3 segment accumulated the greatest amount of platinum (approximately 400% more than S_1 and S_2, Fig. 1).

All nephron segments secreted platinum into the luminal fluid (S_1 335 ± 39, S_2 84 ± 32, S_3 81 ± 18 fmol min^{-1} mm^{-1}) with the greatest luminal appearance rate and mean luminal concentration (MLC) confined to the S_1 segment (Fig. 2, Table 1).

The contribution of the organic cation transport system to cDDP transport was analyzed by adding the organic cation TEA (4 m*M*) to the bathing solution with cDDP (2 m*M*). The following results indicate that TEA

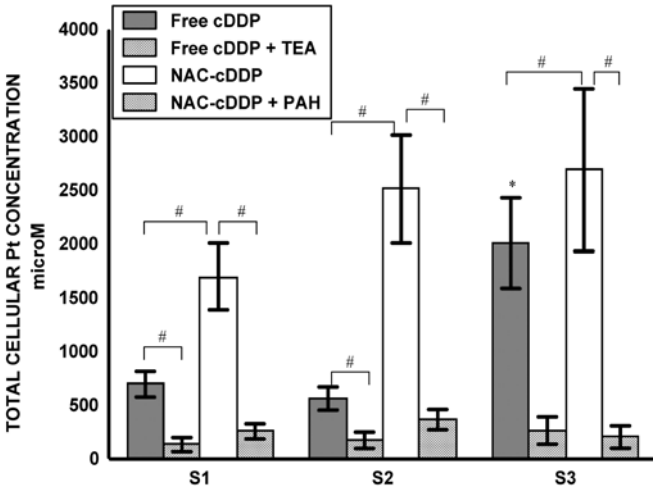

Fig. 1 Cellular concentration of platinum in S_1, S_2, and S_3 segments of rabbit proximal tubules bathed in APM containing cDDP (2 m*M*), cDDP (2 m*M*) plus TEA (6 m*M*), cDDP (2 m*M*) plus NAC (4 m*M*) and those bathed in APM containing cDDP (2 m*M*), NAC (4 m*M*) and PAH (4 m*M*). $^*P < 0.005$ between segments, $^{\#}P < 0.005$ within segments

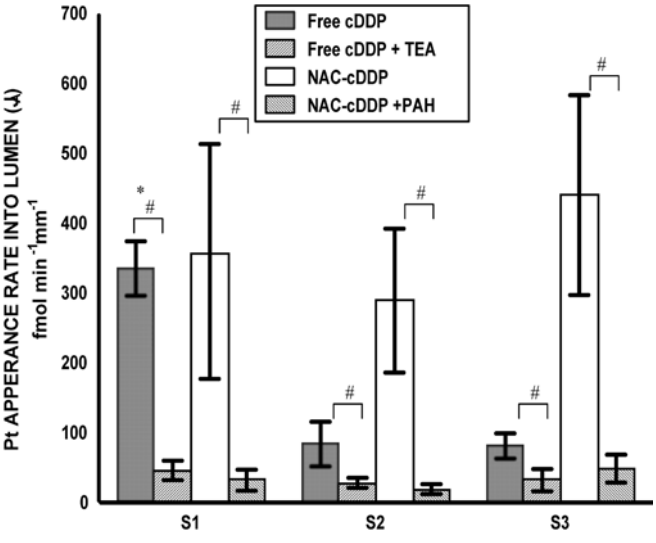

Fig. 2 Bath-to-lumen secretion rates of platinum in S_1, S_2, and S_3 segments of rabbit proximal tubules bathed in APM containing cDDP (2 m*M*), cDDP (2 m*M*) plus TEA (6 m*M*), cDDP (2 m*M*) plus NAC (4 m*M*) and those bathed in APM containing cDDP (2 m*M*), NAC (4 m*M*) and PAH (4 m*M*). $^*P < 0.005$ between segments, $^{\#}P < 0.005$ within segments

Table 1 Leak rates, MLC, and perfusion rates for cDDP flux in S_1, S_2 and S_3 segments of rabbit proximal tubule with cDDP in the bathing solution. Values are means ± SE for each group of four or more tubules

Tissue segment	Leak rate (fmol min^{-1} mm^{-1} ^{3}H-L-glucose)	MLC (µ*M* Pt)	Perfusion rate (nl/min)	*N*
S1	4.81 ± 0.9	11 ± 4.0	7.72 ± 1.56	4
S2	7.85 ± 5.5	4 ± 1.0	8.86 ± 1.10	5
S3	12.1 ± 2.9	4 ± 1.3	8.94 ± 0.38	5

inhibited free cDDP accumulation: (1) C_{Pt} significantly decreased to values less than controls in all three tissue segments (S_1 100, S_2 120, S_3 250 fmol min^{-1} mm^{-1}; Fig. 1); (2) the effects of TEA on the transepithelial secretion of platinum was lowered substantially in the S_1 segment (50 fmol min^{-1} mm^1) compared to the control $J_{B\rightarrow L}$ in the S1 segment (325 fmol min^{-1} mm^1); and (3) the S_2 and S_3 segments each showed a significant reduction in the secretion rate of platinum, but not to the extent of the S_1 segment (Fig. 2).

Photomicrographs indicated no noteworthy levels of toxicity when free cDDP was placed at the basolateral membrane of isolated perfused tubular segments (Fig. 3). The leak rate of the volume marker, ^{3}H-L-glucose, for all three segments was significantly greater than 3 fmol min^{-1} mm^{-1} for a normal tubule [3]. However, the leak rate was not significantly different from the leak rate for a control tubule when TEA and free cDDP where applied to the basolateral membrane (Table 2). Tubules bathed with free cDDP and TEA showed no visual toxicity (data not shown).

NAC-cDDP accumulation, transport, and toxicity

In the next group of experiments, the transport of NAC-cDDP at the basolateral membrane in the three nephron segments of the proximal tubule was investigated. All segments showed substantial cellular accumulation of platinum (S_1 1700 ± 309, S_2 2512 ± 503, S_3 2690 ± 756 μM; Fig. 1). The luminal appearance rate of platinum indicated moderate transepithelial transport (S_1 355 ± 158, S_2 289 ± 103, S_3 440 ± 143 fmol min^{-1} mm-^{1}) when NAC-cDDP was present in the bathing solution (Fig. 2). In addition, the MLC of platinum rose concomitantly with the greater secretion rates, as would be expected (Table 3). The cellular accumulation, secretion, and MLC of platinum were all significantly greater than control values for free cDDP at 38°C. Photomicrographs of all three segments (representatives of which are shown in Fig. 3B) showed evidence of marked acute toxicity that developed over the 30 to 45-min perfusion period.

In the final group of experiments, the effects of PAH present in the bathing solution on the basolateral accumulation and secretion of NAC-cDDP were investigated. As shown in Figs. 1 and 2, cellular accumulation of platinum was substantially lowered (S_1 258 ± 70, S_2 368 ± 94, S_3 205 ± 105 μM) as was $J_{B\rightarrow L}$ (S_1 32 ± 15, S_2 19 ± 7, S_3 49 ± 20 fmol min^{-1} mm^{-1}), with subsequent reductions in MLC (Table 4). The greater leak of ^{3}H-L-glucose for tubules bathed in NAC-cDDP was abrogated in those tubules that were concurrently bathed in PAH (Table 4). In addition, there was no visual evidence of any toxicity developing during the period of perfusion when NAC-cDDP and PAH were present in the bathing solution together (Fig. 3C).

Discussion

Basolateral cisplatin uptake was sensitive to temperature in each of the three nephron segments of the rabbit renal proximal tubule since the accumulation of platinum at 23°C was not significantly different from a transport value of zero. Therefore, tubules perfused at a temperature of 38°C accumulated greater amounts of platinum than those perfused at 23°C (Fig. 1). These data indicate that cDDP accumulation occurs primarily by a carrier-mediated process at the basolateral membrane and not by binding to the plasma membrane, which is in agreement with the findings of other studies [19, 27].

The transepithelial bath-to-lumen transport rate of free cDDP occurred also as a temperature-dependent process, since its flux rate had essentially a value of zero when tubules were perfused at 23°C, but rose to levels significantly greater than zero for tubules perfused at 38°C. Therefore, bath-to-lumen transport at 38°C of cDDP occurs across the basolateral membrane, with subsequent secretion at the apical membrane, by cellular transport mechanisms and not to any notable levels by paracellular routes.

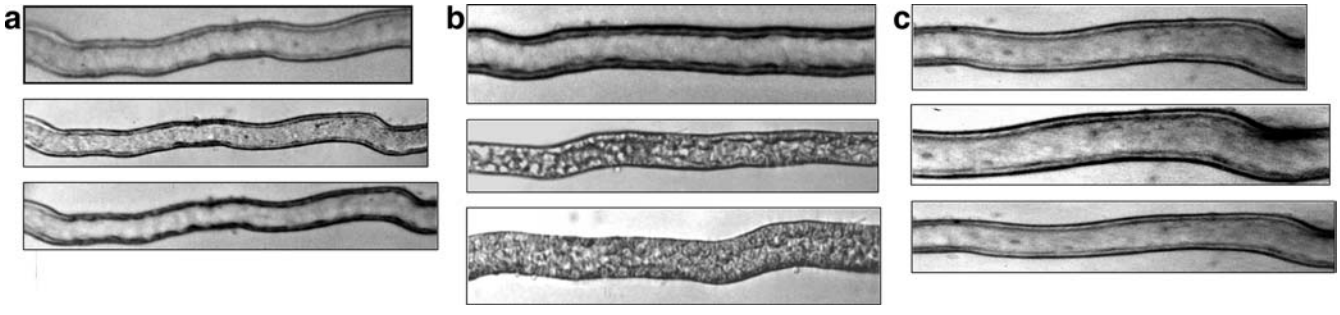

Fig. 3 **A** Sequential photomicrographs of an S_2 segment bathed in APM containing 2 m*M* free cDDP. There were marginal or no deleterious effects of free cDDP on the basolateral membranes during the approximate 45-min perfusion, as seen at 0, 15 and 30 min. **B** Sequential photomicrographs of an S_2 segment bathed in APM containing 2 m*M* free cDDP and 4 m*M* NAC. Toxicity occurred in a progressive manner, which had the visual characteristics of cellular dye uptake, cellular swelling, and blebbing of the cellular membrane as seen at 0, 15 and 30 min. **C** Sequential photomicrographs of an S_2 segment bathed in APM containing 2 m*M* free cDDP, 4 m*M* NAC, and 4 m*M* PAH. Toxicity was eliminated in these experiments, in which the tubular epithelium had the visual characteristics of decreased cellular dye uptake, cellular swelling, and blebbing of the cellular membrane, as seen at 0, 15 and 30 min

Table 2 Leak rates, MLC, and perfusion rates for cDDP with TEA in the bathing solution in S_1, S_2, and S_3 segments of rabbit proximal tubule. Values are means ± SE for each group of four or more tubules

Tissue segment	Leak rate (fmol min^{-1} mm^{-1} ^{3}H-L-glucose)	MLC (μM Pt)	Perfusion rate (nl/min)	*N*
S1	10.12 ± 2.44	2 ± 0.3	10.38 ± 1.48	5
S2	5.44 ± 1.67	1 ± 0.3	7.87 ± 0.83	5
S3	9.74 ± 2.7	1 ± 0.6	8.64 ± 0.37	5

Table 3 Leak rates, MLC, and perfusion rates for cDDP-NAC in S_1, S_2, and S_3 segments of rabbit proximal tubule. Values are means ± SE for each group of four or more tubules

Tissue segment	Leak rate (fmol min^{-1} mm^{-1} ^{3}H-L-glucose)	MLC (μM Pt)	Perfusion rate (nl/min)	*N*
S1	22.90 ± 4.47	42 ± 30	7.23 ± 1.45	4
S2	39.36 ± 6.85	61 ± 37	7.80 ± 1.03	5
S3	48.28 ± 9.82	28 ± 8	7.09 ± 1.18	5

Table 4 Leak rates, MLC, and perfusion rates for cDDP-NAC with PAH in the bathing solution in S_1, S_2, and S_3 segments of the rabbit proximal tubule. Values are means ± SE for each group of four or more tubules

Tissue segment	Leak rate (fmol min^{-1} mm^{-1} ^{3}H-L-glucose)	MLC (μM Pt)	Perfusion rate (nl/min)	*N*
S1	13.20 ± 0.9	0.05 ± 0.02	7.37 ± 1.02	5
S2	2.09 ± 1.03	0.0	9.35 ± 0.07	5
S3	5.49 ± 2.19	0.0	7.23 ± 0.04	5

The data for the basolateral temperature-dependent transport of free cDDP established control data for our studies and confirmed this particular technique to be adequate for investigating how the renal proximal tubule handles cDDP. Data from our temperature-dependent studies provide novel evidence that cDDP transport is specifically carrier-mediated in each of the three tissue segments of the mammalian proximal tubule, evidence previously unrecorded in the literature, but important in providing a detailed description of cDDP transport along the mammalian proximal tubule.

The basolateral transport of free cDDP, since it occurs in a temperature-dependent process, most likely occurs by transport proteins. A logical supposition is that free cDDP is utilizing an organic cation transporter, possibly OCT1 or OCT2, to gain access to the cytosol. The sensitivity of these transport systems to TEA made these experiments a likely starting point for investigating the mechanisms for the basolateral transport of free cDDP. The specific inhibitor of OCT2, disprocynium24 [11], may be able to more clearly define the role of OCT1 and OCT2 in transporting free cDDP at the basolateral membrane.

Each of the three nephron segments, S_1, S_2, and S_3, accumulated free cDDP, with the S_3 segment amassing significantly greater amounts of platinum across its basolateral membrane (Fig. 1). Therefore, cellular accumulation of free cDDP into the epithelium by the proximal tubule's basolateral membrane occurs as a heterogeneous process along the proximal tubule. Exposure of the basolateral membrane to TEA significantly decreased both the cellular accumulation and basolateral-to-apical transepithelial transport of free cDDP (Figs. 1 and 2). Our results suggest that free cDDP transport occurs via the organic cation transport system(s) across the basolateral membrane or some other transport system sensitive to TEA.

The transepithelial bath-to-lumen transport rates at 38°C for free cDDP were marginal in the S_2 and S_3 segments, but substantially greater, about 300%, in the S_1 segment (Fig. 2). Therefore, the bath-to-lumen transport of free cDDP across the epithelium occurs heterogeneously along the rabbit proximal tubule. Addition of TEA to the bathing solution reduced secretion of platinum by 80–85% in each of the three nephron segments of the renal proximal tubule (Fig. 2). Thus, the transepithelial transport of free cDDP likely involves the classical organic cation transport system in isolated perfused rabbit proximal tubules. The exit step for free cDDP across the apical membrane is not clearly defined, but could possibly use a proton/organic cation exchanger, OCTN or the multidrug resistance ATPase. A more definitive description of the cytosol-to-luminal transport of free cDDP is necessary to confirm the exit step for free cDDP, but was not possible using this experimental approach.

No observable and little measurable toxicity occurred when free cDDP was applied to the basolateral membrane, as indicated by our toxicity criteria (Fig. 3A and Table 1). This is strong evidence for free cDDP not being acutely toxic. cDDP concentrations used in these basolateral experiments were relatively high compared to those estimated in a clinical situation. Therefore, even at this high concentration of 2 mM, there was no acute toxicity. Here, the only evidence for toxicity was a minimal increase in leak rates that were significantly greater than the 3 fmol min^{-1} mm^{-1}, indicating alterations in the tight junction. The lack of any substantial toxicity was expected since it is well documented that cDDP nephrotoxicity does not occur in the whole animal until 2–3 days after the initial administration.

The major requirements for a molecule to be transformed by the mercapturic acid pathway are to be an organic cation, electrophilic, and moderately hydrophobic, all of which are properties of free cDDP. Since there are no obvious reasons why free cDDP is not modified by this pathway, the mercaptide of cDDP should exist. Therefore, NAC-cDDP transport and accumulation and toxicity was investigated in this study.

An inference can be made that it is not the free form of cDDP that elicits the toxicity, but a biotransformation product of cDDP, a product that may take several hours to many days to be formed. It is this observation that cDDP is not acutely toxic that precipitated further assumptions that a conjugate of cDDP is the insulting compound in cDDP treatment. Recently reported

evidence concerning cDDP nephrotoxicity led to the conjecture about the possible involvement of the mercapturic acid pathway, because of previous inferences made about the role of glutathione and gamma-glutamyl transpeptidase (GGT) inhibitors [1, 12, 25] in the renal metabolism of cDDP. In one experiment it was found that the incidence of cDDP-induced nephrotoxicity was lower in GGT-deficient mice than in wild-type mice [30]. In fact, recent evidence has clearly shown that inhibitors of GGT and cysteine S-conjugate β-lyase decrease the activation of cDDP to a reactive compound [13].

There are some problems hindering a complete development of this hypothesis that a transformation product is the insulting agent. Mainly, the hypothesis assumes that it is a slow transformation and accumulation process of a conjugate of cDDP, such as NAC-cDDP, that triggers the toxicity found in the proximal tubule after cDDP administration. The idea that cDDP transformation is a slow process practically eliminates the ability to find NAC-cDDP in the blood, due to the very small concentrations of cDDP used in a clinical situation. Tissue NAC-cDDP may be difficult to measure as well when considering the high metabolic activity of the proximal tubule. The end products of cDDP biotransformation, e.g. cDDP mercaptides, are likely candidates for measurement within the cytosol of proximal tubular cells to demonstrate more clearly the role of the mercapturic acid pathway in cDDP-induced nephrotoxicity.

The NAC-cDDP conjugate, an organic anion, accumulated avidly across the basolateral membrane of all three nephron segments (Fig. 1). In addition, the S_1 and S_2 segments accumulated less cDDP than NAC-cDDP, and there was less difference between the S_3 segment's ability to accumulate free cDDP and NAC-cDDP. The marked ability for the S_3 segment to accumulate platinum in the form of free cDDP and the organic conjugate, NAC-cDDP, may explain why this particular segment is most vulnerable to cDDP toxicity. The cellular accumulation of NAC-cDDP decreased to minimal levels in all three nephron segments when PAH was added to the bathing solution (Fig. 1). This is strong evidence for NAC-cDDP using an organic anion transporter at the basolateral membrane to gain entry into the cell. The transepithelial bath-to-lumen transport rates for NAC-cDDP were approximately the same in the S_1 and S_2 segments, but were highest for the S_3 segment (Fig. 2). Thus, secretion of NAC-cDDP into the luminal fluid occurs heterogeneously along the nephron, with the S_3 segment having the more prominent $J_{B\rightarrow L}$ value (Fig. 2). The higher $J_{B\rightarrow L}$ value recorded for the S_3 segments may be due to the number of organic anion transporters and/or their specificity for NAC-cDDP secretion at the apical membrane.

As seen in Table 3 and Fig. 3B, NAC-cDDP was acutely toxic. NAC-cDDP was nephrotoxic in each of the three tissue segments with toxicity being most severe in the S_3 segment. It should be noted that the photomicrographs are representative of nephrotoxicity that occurred in all three nephron segments. However, there was substantial protection against NAC-cDDP-induced nephrotoxicity when PAH was added to the bathing solution, as shown by a significant decrease in leak rate of ^{3}H-L-glucose (Table 4) and elimination of toxicity seen in the photomicrographs (Fig. 3C). The protective effects of PAH support the hypothesis that the NAC-cDDP conjugate is transported by the organic anion transport system, since it is well documented that PAH utilizes an organic anion transporter [8, 16, 18, 22, 23].

The enhanced transport and accumulation of cDDP that occurred when both cDDP and NAC were present in the bathing solution, suggests that the two molecules are binding in the bathing solution. It is possible that NAC enhances the transport of cDDP by altering the cation transport system utilized by cDDP, but as demonstrated in control experiments, cDDP alone was not acutely toxic in our system. The ability of cDDP to bind to free sulfhydryl groups in the plasma and within the cell is well documented [17, 21, 28, 31, 32]. Since NAC has a free sulfhydryl group, it is very probable that the two molecules form a conjugate rapidly in solution, especially considering that there are no other free sulfhydryl groups in the APM solution. In our experiments we did not verify the actual presence of NAC-cDDP but did verify using HPLC that cDDP could bind to cysteine in our APM. However, in our current study, experiments were performed with only NAC (4 m*M*) in the APM bathing solution and no toxicity was observed. This ruled out the possibility that the toxic effect of NAC-cDDP is due to the high NAC concentrations. This does not rule out the possibility that NAC is transported independently of cDDP and a biotransformed NAC molecule binds to intracellular cDDP forming a toxic compound in the cytosolic compartment.

The rapidity with which NAC-cDDP triggers a toxic response was probably exacerbated in our system, but the fact that NAC-cDDP is toxic, even at the concentrations used in our experiments, demonstrates the potential for NAC-cDDP to be a toxic compound. In all likelihood, the in vivo formation of NAC-cDDP would occur much more slowly and would not have the intense and acute toxic effects demonstrated here.

Another feature of this work worth highlighting is the surprising lack of acute toxicity with high concentrations of cDDP (2 m*M*). This concentration in all likelihood greatly exceeds the expected clinical dose, further suggesting that a biotransformation product of cDDP and not free cDDP most likely mediates the toxic effect associated with this drug. Furthermore, this is the first experiment in which a functioning piece of intact proximal tubule has been used to characterize cDDP transport, differing substantially from the majority of experiments reported in the literature, in which poorly characterized and dedifferentiated cell lines were utilized in describing cDDP transport.

In conclusion, free cDDP was transported at the basolateral membrane of isolated perfused rabbit prox-

imal tubule in a temperature-dependent process and in a heterogeneous manner. Free cDDP is likely transported at the basolateral membrane by the organic cation transport system, possibly OCT1 and OCT2. A more detailed delineation of this transport system is imperative for a proper description of free cDDP transport into renal tissue. The other major finding is that the mercaptide of cDDP, NAC-cDDP, was avidly transported and accumulated in rabbit renal proximal tubules, also in a heterogeneous process. NAC-cDDP transport in our system occurs by a PAH-sensitive organic anion transport system. Because NAC-cDDP is extremely toxic, as outlined above, targeting the OCT family of proteins, OCT1/OCT2 and OAT1/OAT3, with more specific inhibitors at the basolateral membrane would be a reasonable approach to preventing cDDP nephrotoxicity. Further experiments need to be done to clearly elucidate the insulting agent in cDDP therapy, but it would appear from our experiments that the mercaptide of cDDP, NAC-cDDP, is a potential candidate.

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