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Uptake and metabolism of cisplatin by rat kidney

ROBERT SAFIRSTEIN, PETER MILLER, and JOSEPH B. GUTTENPLAN

Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, and Department of Biochemistry, New York University Dental Center, New York, New York

Uptake and metabolism of cisplatin by rat kidney. Cisplatin, an effective antineoplastic agent, is toxic to the kidney. Since the kidney's vulnerability to cisplatin may originate in its ability to accumulate and retain platinum to a greater degree than other organs, we studied the characteristics of the renal accumulation of platinum and investigated the nature of intracellular platinum. Cisplatin and ethylenediaminedichloroplatinum, nephrotoxic and antineoplastic liganded platinum compounds, were concentrated in rat renal cortical slices fivefold above medium concentration. Platinum uptake was energy- and temperature-dependent and could be inhibited by drugs which inhibit base transport. The organic anions para-aminohippurate and pyrazinoate did not reduce renal slice platinum uptake. Unbound platinum in the blood and urine was predominantly cisplatin but unbound platinum in kidney cytosol was not. This latter compound, in contrast to cisplatin, was not active as a mutagen. These studies suggest that the kidney accumulates platinum in part by transport or specific binding to the base transport system in the kidney and biotransforms it intracellularly. Unbound platinum in the cell is not cisplatin and may no longer be toxic.

Captation et métabolisme du cisplatine par le rein de rat. Le cisplatine, un agent anti-néoplasique efficace, est toxique pour le rein. Puisque la vulnérabilité du rein au cisplatine pourrait provenir de sa capacité d'accumuler et de conserver le platine à un degré plus élevé que d'autres organes, nous avons étudié les caractéristiques de l'accumulation rénale de platine et cherché la nature du platine intra-cellulaire. Le cisplatine et l'éthylènediamminedichloroplatine, des composés néphrotoxiques et anti-néoplasiques liés au platine, étaient concentrés dans des tranches corticales rénales de rat cinq fois plus que la concentration du milieu. La captation du platine était dépendante de l'énergie et de la température, et pouvait être inhibée par des médicaments qui inhibent le transport des bases. Les anions organiques para-aminohippurate et pyrazinoate ne diminuaient pas la captation du platine par les tranches rénales. Le platine non lié dans le sang et l'urine était de façon prédominante du cisplatine, mais le platine non lié dans le cytosol de rein n'en était pas. Ce dernier composé, contrairement au cisplatine, n'était pas actif en tant que mutagène. Ces études suggèrent que le rein accumule le platine en partie par un transport ou par une liaison spécifique au système de transport des bases dans le rein, et le biotransforme dans les cellules. Le platine non lié dans la cellule n'est pas du cisplatine et pourrait ne plus être toxique.

Cisplatin, a platinum-containing coordination complex, is an antitumor agent with proven efficacy against many solid tumors. The chief dose-limiting side effect of cisplatin is its pronounced nephrotoxicity which occurs at doses lower than those that damage other organs [1, 2]. Acute renal failure can occur with a single dose of cisplatin [3] and repeated exposure causes chronic renal failure [4, 5]. Relevant perhaps to the nephrotoxicity of cisplatin are the observations that the kidney accumulates and retains platinum to a greater extent than other organs, and it is the kidney that is the predominant excretory organ for cisplatin [6, 7]. Although the kidney's vulnerability may originate from its role in the excretion and uptake of platinum, many aspects of the renal handling of cisplatin are not understood.

In the rat, 50% of injected cisplatin is excreted in the urine 24 hr after its administration [6, 7] and most of excreted platinum appears in the urine within the first hour [8]. Although platinum is extensively bound to plasma proteins [9], free platinum in the plasma is mainly cisplatin; by virtue of its low molecular weight and uncharged character, cisplatin should be freely filtered at the glomerulus [10]. Plasma clearance of unbound platinum is at or slightly above inulin clearance [11–13]. Thus cisplatin enters the urinary space predominantly by filtration and, possibly, by secretion.

The process by which cisplatin enters the renal cell is unknown. The renal accumulation of platinum in vivo is a rapid process which is completed 1 to 6 hr after it is given [6, 14]. Twenty-four hours after a 5 mg/kg of body wt dose of cisplatin to the rat, its kidney contains nearly 1% of the administered dose [15] and concentrates platinum eightfold above the plasma concentration [6]. Platinum can be found in many subcellular sites with most of it present in the cytosolic compartment [14]. A surprisingly large amount of intracellular platinum is not protein bound (30 to 50%) and neither the chemical form nor biologic activity of this portion of cell platinum are known at the present time [14].

The present studies were performed to characterize further the process of renal accumulation of platinum and also to investigate the chemical form and biologic activity of unbound platinum in the renal cell.

Methods

Isotopes. Two radiolabeled platinum compounds were used. (^{195m}Pt) Cisplatin in isotonic saline (sp act 145 mCi/mmole) was made available through J. D. Hoeschele, Ph.D., and F. F. Knapp, Jr., Ph.D. at Oak Ridge National Laboratory, Oak Ridge, Tennessee. Nonradioactive cisplatin was obtained through the drug synthesis and chemistry branch, National Cancer Institute, Bethesda, Maryland. Since ^{195m}Pt has a short half-life and radiolabeled cisplatin was synthesized at irregular intervals, ^{14}C-labeled ethylenediamminedichloroplatinum, Pt(^{14}C-en)Cl_2, was used to facilitate the in vitro studies. This

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platinum-containing compound is both nephrotoxic and antineoplastic [16], undergoes similar nucleotide reactions [17], and is retained by the kidney to a similar extent as cisplatin [15]. It differs from cisplatin in that it contains an ethylene bridge between the primary amines of cisplatin. Pt(^{14}C-en)Cl_2 was prepared from ^{14}C-labeled ethylenediamine, sp act 20 to 40 mCi/mmole (Amersham, Arlington Heights, Illinois) and PtK_2Cl_4 by the route suggested by Dhara [18]. Pt(^{14}C-en)Cl_2 was prepared with a specific activity of 300 to 350 μCi/mM; its purity was confirmed by ascending paper chromatography or HPLC (see below). [^{3}H-methoxy]-inulin was obtained with a specific activity of 100 to 500 mCi/g (New England Nuclear Corp., Boston, Massachusetts).

Kidney slice studies. Rat cortical tissue slices (15 to 30 mg) were prepared using a hand-held microtome, weighed and incubated in a Cross and Taggart buffer system consisting of sodium chloride (77 mM), potassium chloride (36 mM), sodium acetate (10 mM), sodium phosphate (7 mM, pH 7.4), and calcium chloride (1 mM) [19]. In most experiments PAH (6.7×10^{-5} M) was included in the incubation medium.

The slices were incubated in a water-bath shaker (New Brunswick, Scientific, New Brunswick, New Jersey) set at 90 to 100 cycles/min under 100% oxygen atmosphere at 23°C. Each incubation vial contained at least 1 μCi of ^{14}C-labeled Pt(en)Cl_2 or (^{195m}Pt)-cisplatin. The uptake of the platinum analogues was studied over a concentration range of 10^{-6} to 10^{-3} M. At 30, 60, 120, and 240 min the vials were removed and placed on ice. The slices were reweighed and homogenized in distilled water. This protocol was used to observe the effects of reduced temperature (4°C), anoxia (N_2 atmosphere), and pH (6–8) on uptake. In experiments where uptake was measured in the presence of inhibitors, the slices were incubated for 2 hr in media modified by adding inhibitors in a phosphate buffer (67 mM) and adjusted to pH 7.4 after the addition of the inhibitor. Control slices were incubated in media containing equal volumes of this buffer without an added drug. Each of the platinum analogues was stable in the incubation medium with or without the added inhibitors as determined by HPLC (see below). ^{3}H-inulin was added to the incubation medium in one study to correct for slice extracellular fluid platinum content. ^{195m}Pt or ^{14}C activity in the tissue and medium was determined in a spectrophotometer (Packard TRI-CARB, Downers Grove, Illinois) using a commercial scintillant (Budget Solve, Research Products International, Elks Grove, Illinois) and the ^{14}C window of the spectrophotometer. The counting efficiency for [^{195m}Pt] under these conditions exceeds 90% [10]. Platinum bound to tissue proteins was determined in decolorized Protosol (New England Nuclear Corp., Boston, Massachusetts) digests of 7% trichloroacetic acid precipitates of the slice homogenates. PAH in homogenate and medium was determined by a modification of the method of Bratton and Marshall [20]. Tissue uptake was calculated as the ratio of tissue (CPM/g tissue weight) to medium (CPM/ml) platinum concentration.

Cisplatin metabolism. A total of eight male Sprague-Dawley rats were given 5 mg/kg cisplatin (1 mg/ml) 0.9% NaCl, 10 to 50 μCi [^{195m}Pt]-cisplatin/ml) i.p. The animals were placed in metal metabolic cages and urine samples were collected for up to 24 hr after injection. In preliminary experiments performed in five rats given 5 mg/kg cisplatin, as above, platinum in the cortical homogenates represented 97.1 ± 0.4% of the total platinum in the kidney 24 hr after the cisplatin injection. We, therefore, turned our attention to the cortical form of the nonprotein bound platinum. In these preliminary studies no difference in the chromatographic appearance and biologic activity of excreted platinum was observed when collected under oil or kept on ice during this 24-hr period. The rats were anesthetized and the kidneys were removed after intra-arterial perfusion of 10 to 20 ml of cold 0.25 M sucrose in 0.1 M phosphate buffer (pH 7.4). Homogenates of rat kidney cortex were prepared in the same buffer (3:1 W/V) in the cold using teflon pestles and glass tubes (TRI-R Instruments, Inc., Rockville Center, New York). The homogenate was centrifuged (Sorvall RC2B, Newton, Connecticut) at 4°C at ×800*g* × 2, and ×12,000*g* × 2 for 10 min each. The supernatant was then spun at ×105,000*g* (Beckman L5-75, Beckman Instruments, Palo Alto, California) for 60 min. An ultrafiltrate of the postmicrosomal supernatant fraction (the fraction containing the most platinum) was prepared in a cold room using a micropartition system and YMP filters, molecular weight cutoff 30,000 daltons (Amicon MPS-1, Amicon Corp, Danvers, Massachusetts). These membranes retain 93% of the proteins present in the cytosolic fractions. In some experiments these ultrafiltrates were filtered again through 500-dalton cutoff filters (Amicon UM05) using a stirred ultrafiltration cell (Amicon Model 3). Greater than 95% of cisplatin added to ultrafiltered cytosols of untreated animals is passed chromatographically unchanged through these filters. When cisplatin was added to kidney cytosol of control rats and an ultrafiltrate was prepared immediately after its addition, 87% of the platinum added was recovered chromatographically unchanged in the ultrafiltrate.

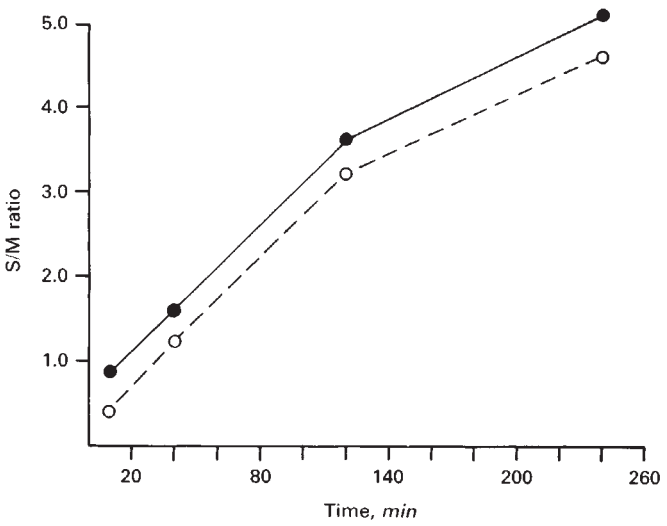

Fig. 1. *Pt(^{14}C-en)Cl_2 uptake by kidney slices incubated for up to 240 min in the presence of 60 μM Pt(^{14}C-en)Cl_2.* Each *point* is the mean of four determinations. The *lower curve* is the Pt(^{14}C-en)Cl_2 slice with uptake corrected (○---○) for extracellular Pt(^{14}C-en)Cl_2 determined using ^{3}H-inulin as an extracellular marker; ●—● refers to uncorrected uptake.

High performance liquid chromatography (HPLC). HPLC was performed on a system consisting of a solvent delivery system (Waters, M45, Milford, Massachusetts) using a partisil-10 SCX strong cation exchange column (Whatman, Clifton, New Jersey). The details of this system have been published

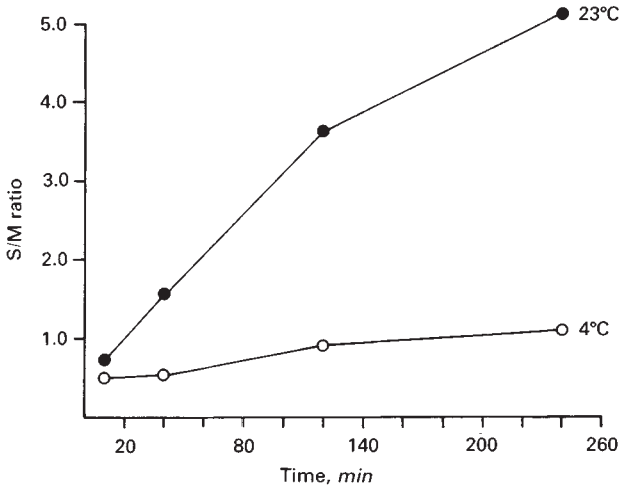

Fig. 2. *Effect of temperature on Pt(^{14}C-en)Cl$_2$ uptake by renal tissue slices.*

previously [10]. Samples were injected. The column was eluted with 0.012 M sodium acetate, pH 3.5 at a flow rate of 0.5 ml/min and fractions were collected every 0.4 min using a fraction collector (Model 2112, LKB, Rockville, Maryland). ^{195m}Pt activity was determined in the fractions using a scintillation counter.

Mutagenesis. Bacterial mutagenesis was assayed by the standard Ames test without any metabolic activation system, using *Salmonella typhimurium* TA 100 (kindly supplied by Dr. B. Ames, Berkeley, California) [21]. This mutant strain has an absolute requirement of histidine for growth. The test measures reversion to histidine independence. Bacteria (0.1 ml of an overnight culture) are mixed with a test substance and overlaid onto histidine-poor agar. The plates are incubated at 37°C for 2 days and each revertant gives rise to a visible colony. Colonies are counted using a colony counter (Artek Systems Corporation, East Farmingdale, New York). Spontaneous revertants, which arise on control plates without added mutagen, are subtracted from all revertant numbers.

Ultrafiltrates of cell cytosol were prepared as above using (Amicon YMB) filters which were washed beforehand with the homogenate buffer. This washing step was necessary to remove the sodium azide, a mutagen, used as a preservative in these filters. In addition to the mutagenesis assay, the ultrafiltrates were combined with diluted bacteria (5×10^6 dilution) and placed onto histidine-containing minimal agar to determine toxicity. Toxic fractions were diluted and mutagenesis reassayed under conditions of minimal (10%) toxicity. This procedure was necessary since a reduction in the number of viable colonies may result in a reduction in the numbers of revertant colonies observed.

Results

The results of the slice uptake of Pt[^{14}C-en]Cl$_2$ are shown in Figure 1. Platinum content of the slice was fivefold above medium content at 4 hr. Nearly half (48.8 ± 1.5%) of the total Pt[^{14}C-en]Cl$_2$ content was protein-bound. When uptake was corrected for slice extracellular fluid and medium trapping little change in the slice to medium ratios was found. In experiments using [^{195m}Pt]-cisplatin, 2-hr uptakes did not differ significantly

Table 1. Effect of anoxia and various inhibitors on kidney uptake of Pt(^{14}C-en)Cl$_2$ (60 μm)

Incubation conditions M	S/M ratio[a] Control	Inhibitor	*P*
N_2	2.61 ± 0.13 (8)	1.69 ± 0.4 (8)	< 0.001
2,4 Dinitrophenol (10^{-3})	2.50 ± 0.26 (4)	1.62 ± 0.09 (6)	< 0.001
Carbonylcyanide m-chlorophenyl-hydrazone (10^{-4})	2.73 ± 0.06 (4)	1.60 ± 0.06 (4)	< 0.001
Pyrazinoate (10^{-2})	3.33 ± 0.31 (4)	3.24 ± 0.25 (4)	NS
p-Aminohippurate (10^{-2})	3.34 ± 0.39 (4)	3.31 ± 0.05 (4)	NS
Probenecid (10^{-2})	2.99 ± 0.12 (7)	1.63 ± 0.08 (7)	< 0.001
Mepiperphenidol (10^{-2})	3.33 ± 0.15 (4)	1.58 ± 0.02 (4)	< 0.001
Tolazoline (10^{-2})	3.33 ± 0.15 (4)	1.73 ± 0.04 (4)	< 0.001
Thiamine (10^{-2})	3.32 ± 0.27 (4)	2.38 ± 0.08 (4)	< 0.025
Tetraethylammonium (10^{-2})	3.33 ± 0.15 (4)	2.72 ± 0.17 (4)	< 0.05

[a] S/M is slice-to-medium ratios (mean ± SEM) after 2 hr of incubation; the number in parenthesis refers to the number of slices.

from those observed with Pt(en)Cl$_2$ (results not shown). Tissue uptake of Pt(en)Cl$_2$ or cisplatin showed no saturation at drug concentrations up to 3 mM. It was difficult to study higher concentrations of the liganded platinum compounds because of their relative insolubility at higher concentrations. Slice:medium ratios of cisplatin at pH 7 (2.70 ± 0.12) were not different from values at pH 6 (2.60 ± 0.02) or 8 (2.74 ± 0.12). Platinum uptake was markedly reduced when slices were incubated at 4°C (Fig. 2).

Table 1 summarizes the results of the inhibition studies. Dinitrophenol (DNP) 10^{-3} M and carbonylcyanide m-chlorophenylhydrazone (CCCP) 10^{-4} M, uncouplers of oxidative phospharylation, reduced Pt(en)Cl$_2$ uptake by 30 to 40%, as did incubating tissue slices under nitrogen. *p*-Aminohippurate (PAH) and pyrazinoate, organic anions, failed to affect Pt(en)Cl$_2$ uptake at concentrations up to 10^{-2} M. A series of organic bases, tolazoline, TEA, mepiperphenidol and thiamine, inhibited Pt(en)Cl$_2$ uptake to varying degrees. Probenecid inhibited Pt(en)Cl$_2$ uptake. At doses of 10^{-2} M, while probenecid and mepiperphenidol virtually eliminated PAH uptake, tolazoline inhibited Pt(en)Cl$_2$ and cisplatin uptake without affecting PAH uptake (Table 2).

Table 3 summarizes the results of experiments designed to detect inhibition of Pt(^{14}C-en)Cl$_2$ uptake into kidney slices using lower doses of the inhibitors identified in Table 1. It can be seen that all inhibitors reduce uptake at 10^{-3} M. Of particular interest is the effect of mepiperphenidol which inhibits uptake at 10^{-3} and 10^{-4} without reducing PAH uptake. Thus, both tolazoline and mepipheridol reduce platinum uptake selectively without reducing PAH uptake.

Figure 3 depicts the chromatographic pattern of administered ^{195m}Pt in kidney tissue and urine. The chromatographic behavior of platinum in the urine is the same as that of cisplatin. ^{195m}Pt in the cytosolic ultrafiltrate on the other hand eluted earlier than cisplatin. Another platinum containing peak

Table 2. Effect of mepiperphenidol and tolazoline on cisplatin and PAH uptake in kidney slices

	S/M ratio	
	(^{195m})Pt cisplatin	PAH
Control	2.77 ± 0.11 (8)	12.3 ± 0.6 (4)
Mepiperphenidol (10^{-2} M)	2.08 ± 0.05[a] (8)	2.54 ± 0.03[a] (4)
Tolazoline (10^{-2} M)	2.17 ± 0.08[a] (8)	13.0 ± 1.2 (4)

[a] $P < 0.001$ compared to control.

amounting to less than 15% of the recovered platinum eluted later than the principal platinum peak. This later peak was not the monoaquo or diaquo species of platinum which elutes at a later time [10]. More than 95% of the platinum present in the original ultrafiltrate passed through a 500-dalton cutoff filter.

The biologic behavior of platinum in kidney cytosolic ultrafiltrates was different from excreted platinum or cisplatin. In contrast to cisplatin [22] and excreted platinum [10], unbound intracellular platinum was no longer mutagenic (line 2, Table 4). Ultrafiltrates of kidney cytosols from untreated rats augmented cisplatin mutagenicity (compare lines 3 and 4, Table 4). To determine if the mutagenic activity of cisplatin was lost during the preparation of the cytosolic ultrafiltrates, cisplatin was added to kidney homogenates of untreated rats, and cytosolic ultrafiltrates were prepared in the same manner as before. The ultrafiltrates were then plated and mutagenicity was determined. The mutagenic activity was the same as that expected for cisplatin at a concentration equivalent to the platinum concentration in the sample. Therefore, the workup procedures for the kidney cytosolic ultrafiltrate did not lead to the deactivation of cisplatin.

Discussion

These studies demonstrate that renal slices accumulate cisplatin and a related platinum containing analogue, $Pt(en)Cl_2$, up to five times above their extracellular concentrations. Four characteristics of this renal accumulation were observed: *(1)* maximum platinum uptake depends on the presence of normal oxygen utilization; *(2)* a significant portion of the tissue platinum is protein-bound; *(3)* platinum uptake was not saturable, at least up to 3 mM concentrations; and *(4)* platinum uptake could be inhibited partially and specifically by drugs. Drugs which compete in the kidney for the transport of organic acids, PAH and pyrazinoate, had no effect on the transport of cisplatin or $Pt(en)Cl_2$. On the other hand, drugs known to be transported by the organic base transport system in the kidney such as mepiperphenidol, tetraethylammonium, tolazoline, and thiamine all reduced cisplatin uptake significantly. Of interest is the rather specific inhibitory effect observed with tolazoline and mepiperphenidol on platinum uptake since they alone inhibited cisplatin uptake without reducing PAH uptake.

Probenecid, like many of the drugs tested, inhibited both PAH and cisplatin uptake. While probenecid reduces the uptake of the bases 1-methorphan [23] and tetraethylammonium [24], and thus might be expected to reduce cisplatin uptake on that basis, other effects of probenecid should be considered. Probenecid (1 mM) can inhibit glycine conjugative reactions [25], inhibit acetylation of choline and sulfanilamide and cholinesterase, a serine protease [26] and inhibit 15-hydroxyprostaglandin dehydrogenase [27]. Of interest are experiments which demonstrate that the reduction by probenecid of amino acid uptake by kidney slices [24] and digitoxin uptake by liver slices [28] is due to an inhibitory effect on their metabolism rather than inhibition of their transport. Thus, probenecid may reduce platinum uptake by interfering with its biotransformation by the cell (see below). Taken together, the results of the uptake studies and the effect of the various inhibitors suggest that cisplatin accumulation by the kidney may involve a specific interaction with the base carrier system as well as intracellular biotransformation of cisplatin.

After cisplatin entered the renal cell its form was altered. Unbound cell platinum behaved both chromatographically and biologically different from administered or excreted cisplatin [10]. The stability of cisplatin in the extracellular fluid as opposed to its instability in the intracellular fluid seems to fit well with the known chemistry of cisplatin. Cisplatin is a planar coordination complex of platinum with platinum in the +2 oxidation state. The chloride ligands are labile and it is the lability of these chloride ligands which is important to the reactivity, and toxicity, of the cisplatin molecule [16]. At the relatively low cell concentrations of chloride, about 20 mM [29], substitution of the chloride ligands of the cisplatin molecule with water is facilitated converting cisplatin to positively charged reactive and toxic aquated derivatives [30–32]. The chromatographic behavior of unbound cell platinum was indeed different from the parent compound. The early elution of this compound from the cationic exchange column at the pH of the elution buffer suggests that the compound in the cell is neutral and formed by nucleophilic substitution of the chloride without change in charge. Although glutathione would seem to be a likely candidate for such interaction, a recent publication reports that only a small fraction (30%) of cellular platinum was associated with glutathione [33].

Another characteristic of this unbound platinum metabolite is its lack of mutagenicity (Table 4). Mutagenic compounds usually react with or can be metabolically converted to compounds which react with electron-rich centers, such as neutral nitrogens or sulfhydryl groups. Several of these electron-rich centers exist in DNA and can react with mutagenic compounds to form DNA adducts. These adducts by a variety of mechanisms cause errors during DNA replication that lead to mutations [21]. Since the electrophilic reactivity of cisplatin is believed to be responsible for its mutagenic activity and since the toxicity of platinum coordination complexes is correlated with their mutagenicity [34], the loss of such activity may indicate that the intracellular platinum compound is no longer toxic. Confirmation of this will require isolation and identification of this compound found in the cell.

The results of these experiments suggest two possible approaches to reducing cisplatin nephrotoxicity: First, it seems possible to reduce platinum uptake in vivo and thereby decrease toxicity. Second, and more speculative, if the inactive platinum compound found in renal cells is formed by an enzyme-mediated process, the levels of this enzyme might be enhanced by pretreatment with appropriate agents. This approach has been reported to enhance the levels of renal cell ligandin and ameliorate the nephrotoxicity of $HgCl_2$ [35].

Table 3. Dose-dependent inhibition of kidney slice Pt(^{14}C-en)Cl_2 and PAH uptake by tolazoline mepiperphenidol, thiamine, and probenecid

Inhibitor	Conc. M	No. of slices	Pt(^{14}C-en)Cl_2 S/M ratio	*P*	No. of slices	PAH S/M ratio	*P*
Control		20	3.12 ± 0.10		14	12.9 ± 1.01	
Tolazoline	10^{-2}	7	1.60 ± 0.08	< 0.001	7	13.2 ± 1.9	NS
	10^{-3}	4	2.62 ± 0.06	< 0.05	3	15.3 ± 3.4	NS
	10^{-4}	4	3.14 ± 0.19	NS	3	22.2 ± 4.5	< 0.01
Mepiperphenidol	10^{-2}	8	1.76 ± 0.05	< 0.001	11	0.49 ± 0.13	< 0.001
	10^{-3}	4	2.49 ± 0.11	< 0.025	3	13.1 ± 0.70	NS
	10^{-4}	4	2.64 ± 0.12	< 0.05	3	13.2 ± 1.40	NS
Probenecid	10^{-2}	8	1.63 ± 0.07	< 0.001	8	Not detected[a]	
	10^{-3}	4	2.57 ± 0.17	< 0.05	4	Not detected	
	10^{-4}	4	3.14 ± 0.19	NS	4	Not detected	
Thiamine	10^{-2}	4	2.38 ± 0.08	< 0.005		Not assessed[b]	
	10^{-3}	4	2.51 ± 0.10	< 0.025		Not assessed	
	10^{-4}	4	2.71 ± 0.05	NS		Not assessed	

Abbreviations: S/M ratio, slice-to-medium ratios (mean ± SEM) after 2 hr of incubation; NS, not significant.
[a] PAH concentration in homogenates fell below the level of detection.
[b] PAH was not added to the incubation medium.

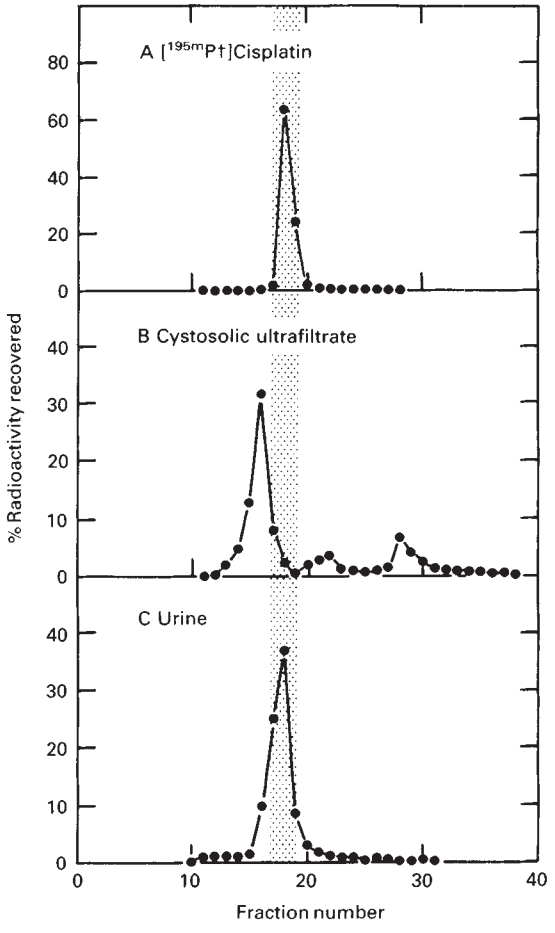

Fig. 3. *Chromatogram of ^{195m}Pt in injectate* (panel **A**), *tissue* (panel **B**), *and urine* (panel **C**). *Panel* **A**—*150 μl injection of 1.8 μg/ml solution of [^{195m}Pt]-cisplatin in 0.9% NaCl eluted with 0.012* M *Na acetate, pH 3.5, at 0.5 ml/min.* Fractions were collected at 0.4-min intervals. *Panel* **B**—*100 μl injection of ultrafiltrate (1.81 μg cisplatin equivalent). Panel* **C**—*50 μl injection of urine containing platinum (142.4 μg/ml cisplatin) collected 24 hr after the administration of cisplatin.*

Table 4. Effects of kidney cytosolic ultrafiltrates from untreated and cisplatin-treated rats on revertant numbers in *Salmonella typhimurium* TA100

Kidney fraction	Treatment	Addition	Revertants
UF	None	None	262
UF	Cisplatin	None	307
UF	None	Cisplatin	1034
None	None	Cisplatin	643

Abbreviations: UF, ultrafiltrate of kidney cytosol; treatment, treatment of the animals with cisplatin 5 mg/kg body wt 24 hr prior to isolation of kidneys [the platinum content of each plate was 3.4×10^{-9} moles of platinum (1.02 μg cisplatin equivalent in 0.6 ml of ultrafiltrate)]; addition, the addition of 3.3×10^{-9} moles of platinum (1.00 μg cisplatin equivalent) to ultrafiltrate aliquot (0.6 ml) or directly to incubate prior to plating; revertants, spontaneous revertants already subtracted (precision between duplicate plates was within 25%).

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Reprint requests to Dr. R. Safirstein, Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, New York 10029, USA

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