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An in vitro screening system for the nephrotoxicity of various platinum coordination complexes

A cytochemical study

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Summary. Isolated rat kidney tubules were cultured in Earle's medium with and without the platinum coordination complexes. Aliquots were taken at 0, 1, 2, 3, 4, 5, 6, and 8 h and analyzed for the amount of Na^+/K^+-ATPase, Ca^{2+}-ATPase, alkaline phosphatase, and acid phosphatase. Culture medium was also analyzed biochemically for the amounts of alkaline phosphatase present. There is a decrease in the various enzymes levels of the tubules after incubation in nephrotoxic analogues with a corresponding increase in the culture medium. These results compare favorably with in vivo studies. The alkaline phosphatase monitored in the rat kidney cross sections from both the normal and the drug-treated animals at 0, 3, 5, 10, and 20 days showed a correlation in the decrease of enzyme levels in the kidney with a corresponding increase in the urinary levels in both the Wistar and the Long Evans rats. The baseline levels were higher in the Long Evans rats than in the Wistar rats. After cisplatin (nephrotoxic) treatment the Long Evans rats had twice as much alkaline phosphatase in the urine at day 5 as the Wistar rats. Rats treated with cyclobutanedicarboxylatoplatinum (II) did have some alkaline phosphatase output in the urine in excess of the normal levels, but this increase was not so highly significant as to justify classifying the drug as nephrotoxic.

Introduction

Heavy metal platinum coordination complexes are attracting considerable attention as potential chemotherapeutic agents against a number of tumors. Of these, *cis*-dichlorodiammineplatinum II (cisplatin, CDDP) is currently being used in the treatment of ovarian and testicular cancers [23]. The drug is, however, not without certain toxic side effects in the kidney (proteinuria, morphological damage) [4, 24], intestine (diarrhea and anorexia) [24], and lymphatic system (splenic atrophy) [3, 24]. Nephrotoxicity is the most important side effect, as it is the limiting factor in the chemotherapeutic uses of the drug [13–15].

As new platinum analogues are synthesized [17] there is an increasing need for a fast, efficient system for evaluation of the chemotherapeutic potential and nephrotoxicity of these drugs [3]. Currently many laboratory animals and man-hours are required to test each drug. This process takes a long time and tends to hinder the chemotherapeutic application of these new analogues. In this study an in vitro screen system is proposed and tested. To test the nephrotoxicity, isolated kidney tubules are cultured for the duration of each experiment. Since the main functions of the kidney are transport-related [12], membrane transport enzymes (Ca^{2+}-activated ATPase, Na^+/K^+-activated ATPase, alkaline phosphatase, and 5'-nucleotidase) are demonstrated and quantitated cytochemically, then used as indicators of normal and impaired renal function [6, 29]. Similar studies were repeated in rats, and results were compared with those obtained in in vitro studies for uniformity.

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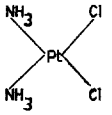
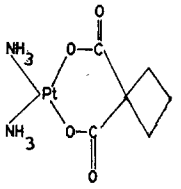
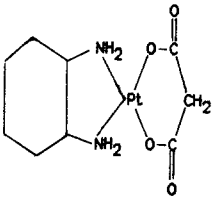
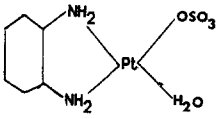
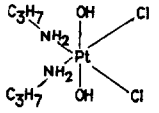
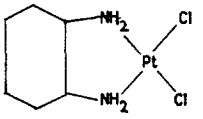
Materials and methods

In vivo studies. Outbred male Swiss Wistar (Crl:(WI)BR) and Long Evans (Crl:(LE)BR) rats (Charles River Lab.) weighing 150–300 g received IP injections of various platinum coordination complexes in the concentrations and with the injection vehicles shown in Table 1. Most of the detailed observations were based on *cis*-diammine-1,1-cyclobutanedicarboxylateplatinum (II) or *cis*-dichlorodiammine platinum (II) (Johnson Matthey Research Laboratories). The day of the injection was taken as day 0. Sampling intervals were 0, 3, 5, 10, and 20 days after injection. Ten animals were killed by cervical dislocation at each sampling interval. The kidneys from the left side were removed and mounted on cryostubs in OCT mounting medium, frozen, and kept until use. Sections 10 μm in thickness were cut using an IEC cryostat microtome for enzymatic analysis (see Table 2).

Kidneys from the right side were removed, fixed in 100% ethyl alcohol (−20 °C), and embedded in paraffin. Sections 5 μm in thickness were cut using a rotary microtome (American Optical). The sections were processed for the cytochemical localization of thiol (−SH) groups.

In vitro studies. Three inbred Wistar rats weighing 150–300 g were killed, and their kidneys were removed. Kidney tubules were isolated following a modified version of the procedure developed by Nagata and Rasmussen [21]. The medulla of each kidney was excised, and the remaining cortices were then mechanically minced and placed in Hank's solution (Grand Island Biological Supply Co.). The minced cortices were then enzymatically digested in 100 ml Hank's solution which also contained

Table 1. Platinum coordination complexes tested[a]

Drug	Structure	Abbreviation	Dosage[b]	Vehicle	JM number
cis-Dichlorodiammine Pt (II)		Cisplatin, CDDP	5.0 mg/kg 9.0 mg/kg	0.15 *M* Saline	–
cis-Diammine-1,1-cyclobutane dicarboxylate Pt (II)		CBDCA	50 mg/kg	5% Glucose	8
Malonato-1,2-diammino cyclohexane Pt (II)		Malonato	80 mg/kg	DMF 1.5% $NaHCO_3$	74
Aquasulfato-1,2-diammino cyclohexane Pt (II)		Sulfato	12 mg/kg	5% Glucose	20
cis-Diisopropylammine-*trans*-dihydroxy-di-chloro Pt (IV)		CHIP	40 mg/kg	0.15 *M* Saline	9
dichloro-1,2-diammino cyclohexane Pt (II)		DACH-Cl_2	20 mg/kg	0.15 *M* Saline	–

[a] There were at least 10 animals in each sampling group
[b] These dosages were based on previous animal studies and have been proven to be effective against various tumor systems [20]

Table 2. Effects of cisplatin (CDDP) and cyclobutanedicarboxylatodiammineplatinum (II) (CBDCA) on the transport enzymes in the kidney of adult male rats[a,b]

Days after injection	Alkaline phosphatase		Ca^{2+}-ATPase		Na^+/K^+-ATPase		5'-N	
	CDDP	CBDCA	CDDP	CBDCA	CDDP	CBDCA	CDDP	CBDCA
0	++++	++++	++++	++++	++++	++++	++++	++++
1	++	++++	++	++++	++	++++	++	++++
3	±	+++	±	+++	±	+++	±	+++
5	±	+++	±	+++	±	+++	±	+++
10	+	++++	+	++++	+	++++	+	++++

[a] Dosage: CDDP 5.0 mg/kg; CBDCA 50 mg/kg
[b] ++++, very dense reaction (100%); +++, dense reaction (75%); ++, average reaction (50%); +, poor reaction (10%); ±, less than 10% reaction

40 mg collagenase (Worthington Biochemical), 100 mg hyaluronidase (Sigma T I, Sigma Chemical Co.), 25 mg streptomycin sulfate, and 180 mg glucose. The tubules were then washed and mechanically dispersed using a dispo pipet. The sample was then divided into 13 equal portions and incubated in minimal essential medium (Grand Island Biological Supply Co.). One part of the sample served as control, while platinum coordination complexes were added to the rest at the chemotherapeutic dosages shown in Table 3. On the basis of our previous experience with sarcoma-180 cells in animals, which showed that the chemotherapeutic dosages in the animals were equally ef-

Table 3. Effects of cisplatin (CDDP) and cyclobutanedicarboxylatodiammineplatinum (II) (CBDCA) on the transport enzymes in the isolated kidney tubules[a,b]

Hours after incubation	Alkaline phosphatase		Ca^{2+}-ATPase		Na^+/K^+-ATPase		5'-N	
	CDDP	CBDCA	CDDP	CBDCA	CDDP	CBDCA	CDDP	CBDCA
0	++++	++++	++++	++++	++++	++++	++++	++++
1	++	++++	++	++++	++	++++	++	++++
3	±	+++	±	+++	±	+++	±	+++
5	±	+++	±	+++	±	+++	±	+++
8	−	++	−	++	−	++	−	++

[a] Dosage: CDDP 5.0 mg/l culture medium; CBDCA 50 mg/l culture medium

[b] ++++, very dense reaction (100%); +++, dense reaction (75%); ++, average reaction (50%); +, poor reaction (10%); ±, less than 10% reaction; −, no reaction

fective in culture medium if the volume of the culture medium was taken for the total weight of the animal, in the present studies the same dosages were used in the in vitro studies as had been found successful for the treatment of cancers in animals. Samples were taken at 0, 1, 2, 3, 4, 5, 6, and 8 h, and fixed in 1% glutaraldehyde in 0.05 *M* cacodylate buffer (pH 7.4), washed in 4.5% sucrose-cacodylate buffer (pH 7.4), mixed with Tissue Tek II embedding medium (Lab Tek division of Miles Laboratories), and frozen until use. Sections 10 μm in thickness were cut using an IEC cryostat (International Equipment Company), and placed on gelatin (2.5%)-coated cover slips. Such coverslips were placed briefly in buffered glutaraldehyde (1%) to help attach the sections firmly to the cover slips, and processed for cytochemical studies described below.

Cytochemical studies. Both kidney cross sections and isolated kidney tubule sections were tested for the following phosphatases.

Ca^{2+}-activated ATPase (Ca^{2+}-ATPase) was detected by incubation in 0.1 *M* Tris-maleate buffer (pH 7.3), adenosine 5'-triphosphate (ATP) 10 m*M*, 3% lead nitrate, 1 m*M* calcium chloride, 0.2 m*M* magnesium chloride, and distilled water [8]. Incubation lasted 45 min at 37 °C. The control incubation media omitted the substrate or additionally contained 15 μg/ml quercetin (an inhibitor of Ca^{2+}-ATPase activity [11]).

Na^+-activated ATPase (Na^+-K^+-ATPase) was determined by incubation in medium composed of Tris-maleate buffer 0.1 *M* (pH 7.3), 10 m*M* ATP, 3% lead nitrate, 100 m*M* sodium chloride, 10 m*M* magnesium sulfate, 5% sucrose, and distilled water [20]. Control media used omitted ATP, sodium chloride or additionally contained 0.7 mg/ml ouabain, an inhibitor of Na^+/K^+ ATPase activity [12]. Incubations were carried out for 45 min at 37 °C.

Alkaline phosphatase (AP) activity was visualized by incubation in medium containing 0.2 *M* Tris-maleate buffer (pH 8.2), sodium β-glycerophosphate (1.2%), 1% lead nitrate, 0.2 m*M* magnesium chloride and distilled water for 45 min at 37 °C according to the method of Hugon and Borgers [16]. The control incubation medium contained 50 m*M* L-phenylalanine, an inhibitor of AP activity [7].

5'-Nucleotidase (5'N). For visualization of 5'N activity, incubation in medium consisting of 0.1 *M* Tris-maleate buffer (pH 7.3), 1.4 m*M* adenosine 5'-monophosphate (AMP), 1% lead nitrate, 10 m*M* magnesium sulfate, and 5% sucrose was carried out at 37 °C for 45 min according to the procedure developed by Uusitalo and Karnovsky [27]. Control medium omitted the substrate (AMP).

Acid phosphatase (Acid P) activity was visualized by incubation in medium containing 0.2 *M* Tris-maleate buffer (pH 5.2), sodium β-glycerophosphate (1.2%), 1% lead nitrate, 0.2 m*M* magnesium chloride, and distilled water for 45 min at 37 °C [22].

Sections (10 μm) of control and drug-treated tissue after incubation in the appropriate incubation medium were treated with 1% ammonium sulfide and mounted in glycerin jelly. Slides were photographed using a Zeiss Photomicroscope II loaded with Kodak Plus X film. The reaction product was quantitated by scanning microdensitometry using a Joyce Loebl MK IIIC double-beam recording microdensitometer (JL and Company LTD, Electron House, Princesway Team Valley, Gateshead-on-Tyne II, England), and by direct microscopic visualization.

Thiol groups (SH) were localized according to the method of Engel and Zerlotti [10]. The method involves treatment of the sections with 95% ethanol, followed by staining with the azomercurial reagent 4-(*p*-dimethylaminobenzene azo) phenylmercuric acetate, and retreatment with 95% ethanol. A control was prepared with preincubation in *N*-ethyl maleimide (75 m*M*) in phosphate buffer (pH 7.0) at 37 °C for 2 h [5], blocking the thiol groups, or by controlled trypsin (protease) digestion, demonstrating that the thiol groups are protein-bound. The sections were then mounted using Permount (Fisher Chemical Company), and observed with monochromatic blue (L = 458 nm) light.

Determination of tubule viability. Tubule viability was determined by removal of control and drug-treated tubules at each sampling interval. These samples were then treated with 0.2% trypan blue solution, and counts made of the viable kidney tubules (viable tubules do not take up the stain) [9].

Biochemical assays. Spectrophotometric assays were performed on culture media from each sampling interval during the in vitro screening process, to determine the amount of alkaline phosphatase activity present in the media [19]. AP activity was detected by adding 0.1 ml culture medium to a mixture of 10 m*M* disodium-*p*-nitrophenyl phosphate

and 0.1 *M* carbonate bicarbonate buffer (pH 10.0) which had been warmed for 10 min at 30 °C. The mixture was then placed in a 5-ml cell with a 1-cm path length and checked for absorbance at 400 nm using a Beckman 25 spectrophotometer. Using the absorbance, the number of units of alkaline phosphatase/ml culture medium was determined according to Beer's law.

Rat urine was collected from five pairs each of male Wistar and Long Evans rats at 0, 1, 3, 5, 7, and 10 days after injection with CBDCA or CDDP [19]. The urine was either analyzed immediately or frozen until use without any loss of enzymatic activity. It was spun in an IEC clinical centrifuge for 8 min at high speed and dialyzed for 3 h using Spectrapore membrane tubing (VWR scientific) and distilled water at 4 °C. The urine was then assayed for the presence of both alkaline phosphatase and acid phosphatase [19]. Acid phosphatase activity was determined by incubation in medium consisting of 0.1 *M* acetate buffer (pH 4.5), 0.15 *M* substrate Na-β-glycerophosphate. Trichloroacetic acid (20%) was added after 10 min of incubation. Alkaline phosphatase activity was determined as described above for the culture medium. The amounts of acid phosphatase and alkaline phosphatase present in the urine were calculated in a similar way to that described for culture medium analysis. Correction was made for the amount of total urine excreted by each animal.

Data analysis. All data analysis was performed on a Macintosh microcomputer using a Number Cruncher Statpak. Prior to the analysis of variance data was checked for equality and normality of variance using both the F max test, and Bartlett's homogeneity test.

Results

In the present studies, although we have tested a number of platinum coordination complexes details will be restricted to cisplatin and CBDCA administered to two dif-

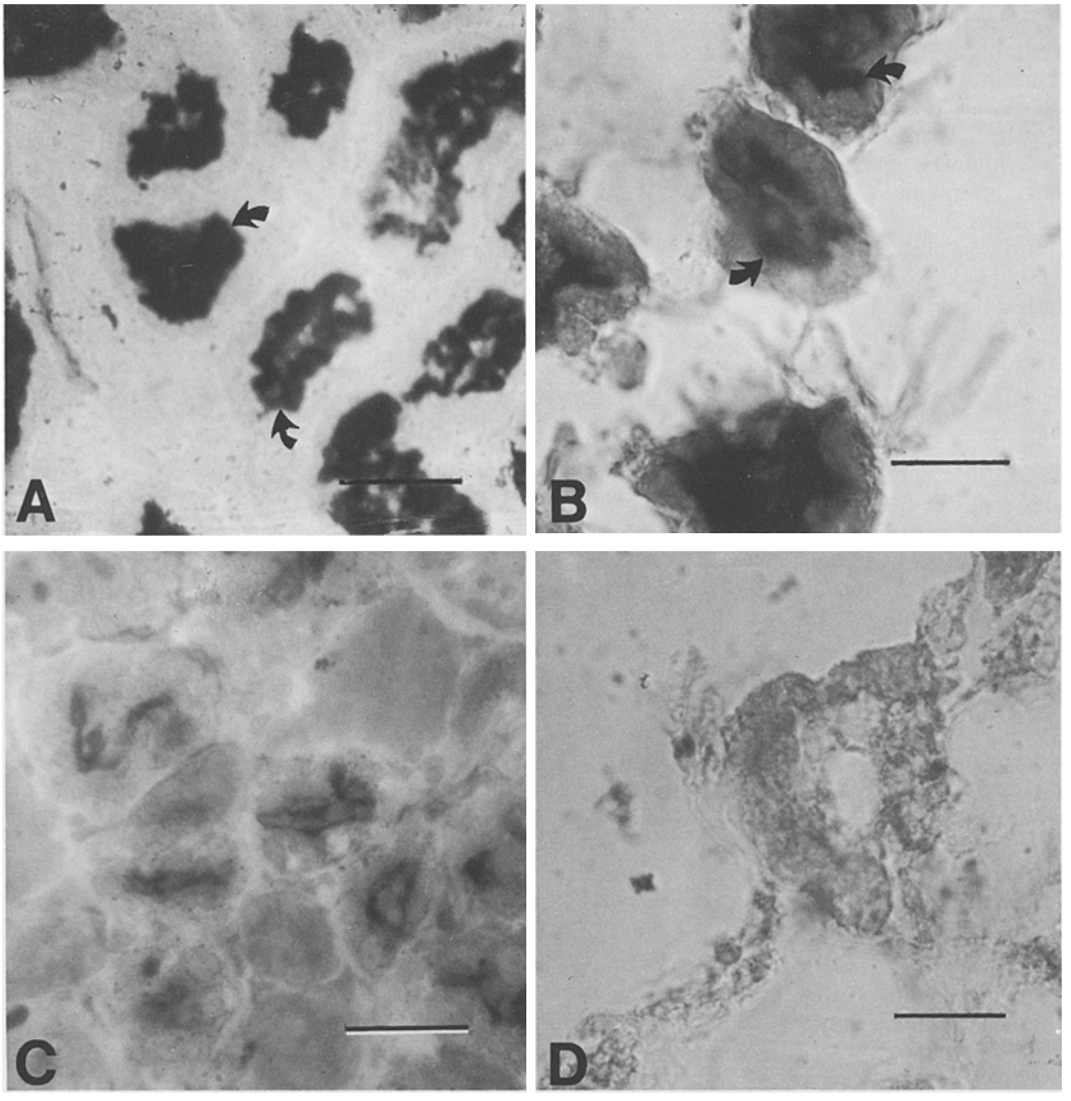


Fig. 1. **A** Cross section of a male Wistar rat kidney showing alkaline phosphatase reaction product (r. p.) *(arrows)* in a normal animal (Original magnification X250; *bar*= 100 μm) **B** Cross section of isolated rat kidney tubules showing alkaline phosphatase activity *(arrows)* (Original magnification X950; *bar*= 25 μm) **C** Cross section of male Wistar rat kidney 5 days after cisplatin (5 mg/kg) treatment, showing only part of the kidney tubules positive for alkaline phosphatase activity (Original magnification X250; *bar*= 100 μm) **D** Cross section of isolated rat kidney tubules 5 h after CDDP (5 mg/kg) treatment, showing no alkaline phosphatase reaction product (Original magnification X950; **bar** = 25 μm)

ferent strains of rats. Any significant differences in the enzyme distribution in the kidney tubules using other compounds will be pointed out.

Cytochemical localization of alkaline phosphatase in a cross section of a normal rat kidney (Fig. 1 A) or of the isolated kidney tubules (Fig. 1 B) shows a normal distribution of the enzyme, mostly on the cell border lining the lumen of the tubules. However, cross sections of the kidneys taken from rats after 5 days of cisplatin treatment (Fig. 1 C) and cross sections of kidney tubules in in vitro experiments after 5 h of cisplatin treatment showed little or no reaction product on the brush borders (Fig. 1 D). A similar lack of reaction product was seen in negative control sections that were incubated with either phenylalanine or levamisole. Sodium ATPase and calcium ATPase had a distribution similar to that of alkaline phosphatase, with the addition of strong intensity on the basolateral cell borders of the kidney tubules. Following CDDP treatment both calcium and sodium ATPase were affected in the same way as alkaline phosphatase.

CBDCA treatment has a very insignificant effect on the distribution of the various enzymes in question (Tables 2, 3). Acid phosphatase is present in lysosomes throughout the kidney and increases substantially in quantity after CDDP treatment, but only a slight increase was observed after CBDCA treatment. The activity of 5'-nucleotidase (5'N) shows a distribution similar to that of alkaline phosphatase and is affected in a similar manner after drug treatment. The decreases correlate for both in vivo and in vitro studies.

Reaction densities as viewed through the light microscope are shown for in vivo studies in Table 2, and those for in vitro studies with CDDP and CBDCA in Table 3. These results were further analyzed and quantified using a Joyce Loebl scanning microdensitometer.

Further conformation of the in vivo studies was provided by measuring alkaline phosphatase (AP) and acid phosphatase (Acid P) in the urine of both Wistar (Figs. 2 and 3) and Long Evans (Figs. 4 and 5) rats. Urine analysis for AP activity revealed significantly ($P<0.01$) higher levels, than normal at 3 and 5 days after 5 mg/kg CDDP and at 3, 5, and 7 days after 9 mg/kg CDDP, the maximal increase occurring at 3–5 days. AP levels increased 3 days after CBDCA treatment ($P<0.01$), but this increase was considerably less than that caused by either (5 mg or 9 mg/kg) CDDP injection. Urine Acid P activity was significantly higher at 3 and 5 days ($P<0.01$) and at 7 days ($P<0.05$) after 5 mg/kg CDDP, while 9 mg/kg yielded a larger increase ($P<0.01$) at 3, 5, 7, and 10 days, with a maximal increase at 5 days. Acid P activity did not significantly increase after CBDCA treatment.

Urine volumes were also monitored after injection (Figs. 6 and 7). A significant increase in urine volumes occurred at days 3 and 5 ($P<0.01$) and at 7 days ($P<0.05$) after 5 mg/kg CDDP, with a larger increase ($P<0.01$) at 3, 5, 7, and 10 days after 9 mg/kg CDDP, the maximal increase being at 5 days. A significant decrease ($P<0.05$) in urine volumes was shown at 5, 7, and 10 days after CBDCA treatment.

Further examination of the data (Figs. 2–7) indicates

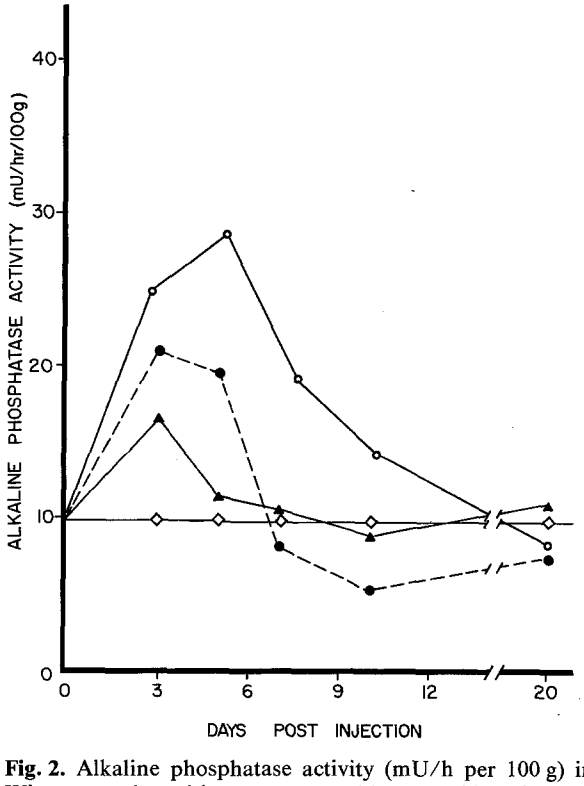


Fig. 2. Alkaline phosphatase activity (mU/h per 100 g) in male Wistar rat urine with no treatment (◇———◇) and with CDDP 5 mg/kg (●———●), CDDP 9 mg/kg (○———○), and CBDCA 50 mg/kg (—▲—)

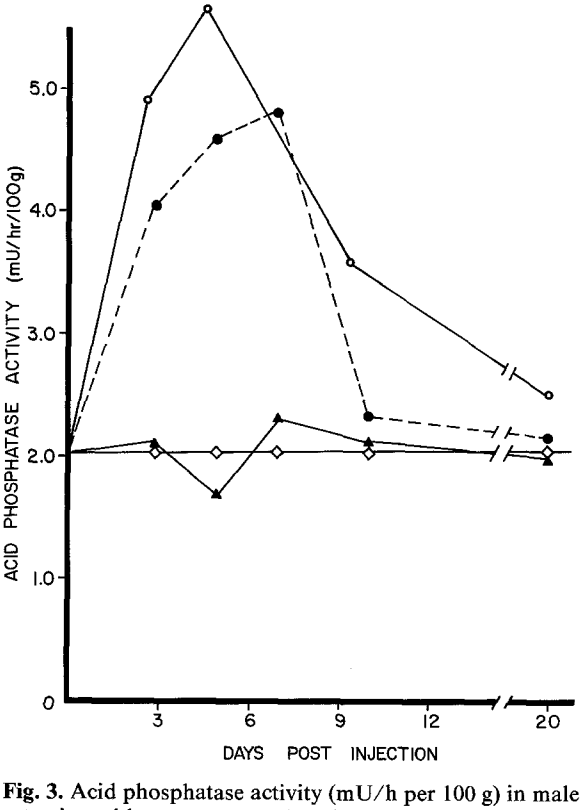


Fig. 3. Acid phosphatase activity (mU/h per 100 g) in male Wistar rat urine with no treatment (—◇—) and with CDDP 5 mg/kg (—●—), CDDP 9 mg/kg (—○—), and CBDCA 50 mg/kg (—▲—)

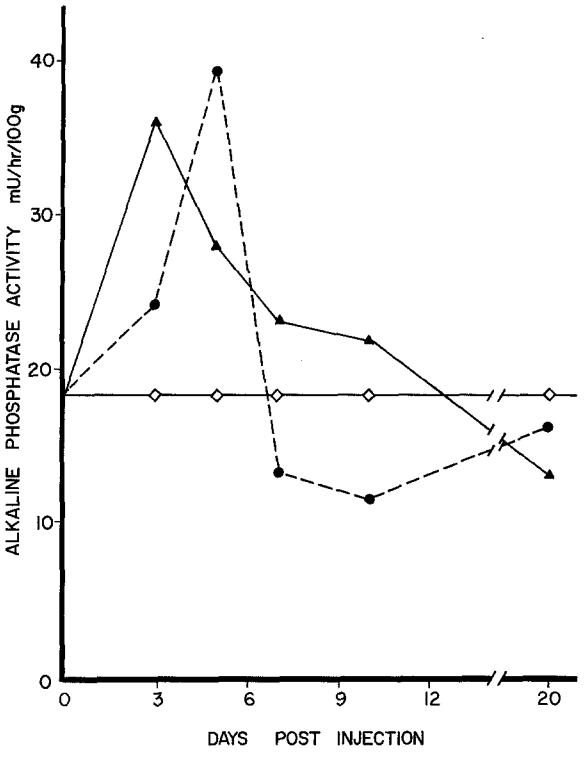


Fig. 4. Alkaline phosphatase activity (mU/h per 100 g) in male Long Evans rat urine with no treatment (—◇—) and with CDDP 5 mg/kg (—●—) and CBDCA 50 mg/kg (— ▲—)

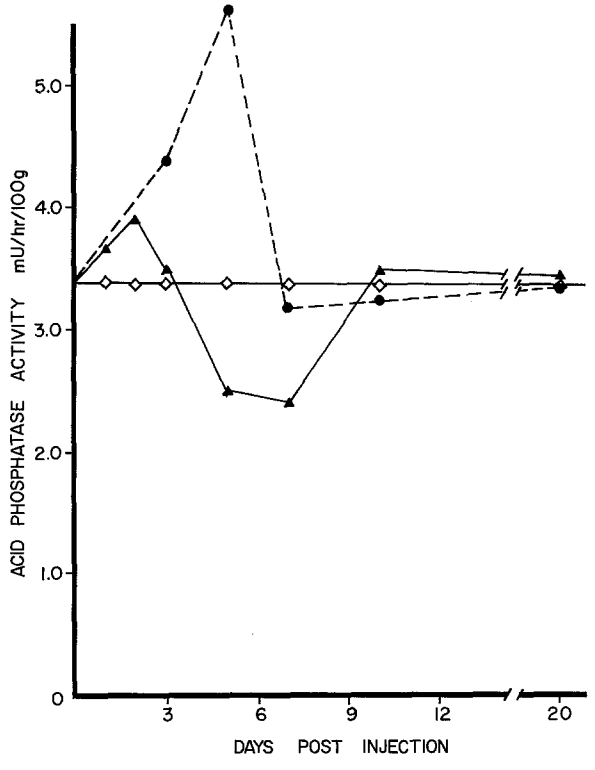


Fig. 5. Acid phosphatase activity (mU/h per 100 g) in male Long Evans rat urine with no treatment (—◇—) and after CDDP 5 mg/kg (—●—) and CBDCA 50 mg/kg (———▲———)

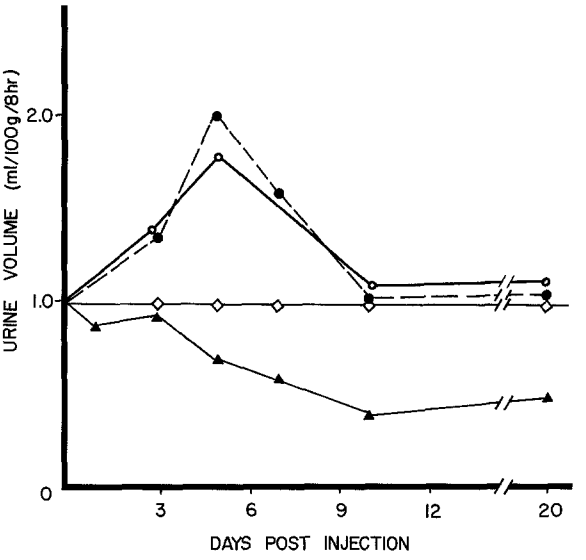


Fig. 6. Urine volume (ml/100 g) per 8-h sample for male Wistar rats without treatment (—◇—) and after CDDP 5 mg/kg (—●—) CDDP 9 mg/kg (—○—), and CBDCA 50 mg/kg (—▲—)

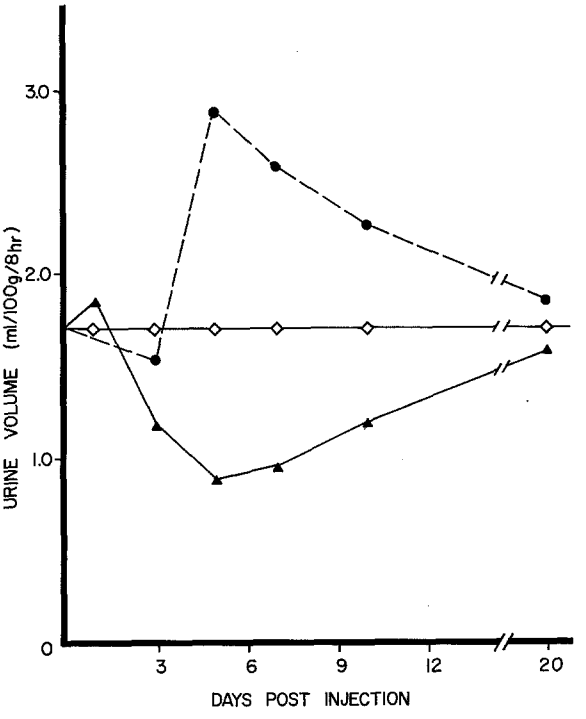


Fig. 7. Urine volume (ml/100 g) per 8-h sample for male Long Evans rats without treatment (—◇—) and after CDDP 5 mg/kg (—●—) and CBDCA 50 mg/kg (—▲—)

strain-specific differences in baseline enzyme and urine levels. Control AP and Acid P levels were 19 mU/h per 100 g and 3.5 mU/h per 100 g for Long Evans rats, as opposed to 10 mU/h per 100 g and 2.0 mU/h per 100 g for Wistar rats. Baseline urine volumes also differed, with 1.8 ml/100 g per 8 h for Long Evans rats and 1.0 ml/100 g per 8 h for Wistar rats.

Table 4. Relative nephrotoxicity of various platinum coordination complexes as measured by their inactivation of various membrane associated transport enzymes

Rank order	Complex and dosage	Toxicity grading[a]
1	CDDP 50 mg/kg	+ + + + +
2	DACH-Cl_2 20 mg/kg	+ + + +
3	SULFATO 12 mg/kg	+ + + +
4	CBDCA 500 mg/kg	+ + + +
5	CDDP 5.0 mg/kg	+ + +
6	Malanato 80 mg/kg	+ +
7	CHIP 40 mg/kg	+ +
8	CBDCA 50 mg/kg	+

[a] Ranking from 1 (+ + + + +) most to 8 (+) least toxic

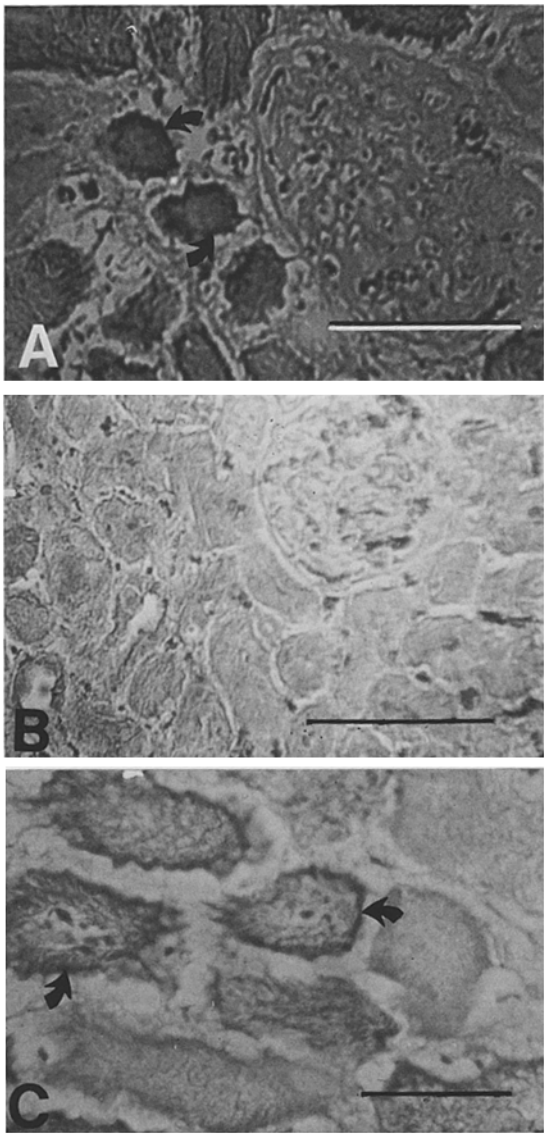


Fig. 8. A Cross section of a male Wistar rat kidney, showing the distribution of thiols *(arrows)* in a normal section. (Original magnification X250; *bar*= 100 μm) **B** Cross section of a male Wistar rat kidney, showing the lack of thiols 5 days after CDDP (5 mg/kg) treatment. (Original magnification X250; *bar*= 100 μm) **C** Cross section of a male Wistar rat kidney, showing the distribution of thiols 5 days after CBDCA (50 mg/kg) treatment. (Original magnification X400; *bar*= 50 μm)

In vitro studies were similarly confirmed using both culture media assayed for AP activity and tubules assayed for viability. There was an increase in the AP activity in the culture medium, which corresponded to a similar decrease in the enzyme levels in the tubule cells. The dead tubules did not show any changes in the enzyme levels. Based on urine analysis, culture medium analysis for AP and the transport enzyme analysis of the kidney cross sections or the isolated tubule cross sections, the various analogues tested can be ranked from the most, to least toxic relative to damage to the membrane enzymes (Table 4).

Photomicrographs depicting in vivo thiol group localization are shown for normal animals (Fig. 8A) and for others after 5 days of CDDP treatment (Fig. 8B) and 5 days of CBDCA treatment (Fig. 8C). The thiol groups appear to be localized on the brush and basolateral borders of kidney tubule cells. The photomicrographs indicate a decrease in thiol groups following CDDP treatment, but like AP, these groups showed little decrease after CBDCA treatment.

Discussion

This study addresses itself to two main issues. First and foremost is the proposal and testing of an in vitro nephrotoxicity assay system. This is particularly appropriate, since it has been established that nephrotoxicity is one of the most severe side effects of CDDP chemotherapy [13]. Second, through the in vivo screening procedures an attempt is made to determine whether there are strain-specific differences in both the normal quantity of rat urine enzymes and the physiologic response to drug compromise.

Transport enzymes are of major biological importance within the kidney [12] and elsewhere throughout the organism. They are responsible for metabolite exchange and have been implicated in the determination of normal and metastatic phenotypic states [18]. The transport enzymes have also been thought to interfere in mitotic apparatus assembly and disassembly, thus interfering with cytokinesis [1, 2]. Inactivation of these enzymes would tend to alter both the function and the viability of kidney tubules.

The mechanism of interaction/inactivation of membrane enzymes may be manyfold. Depending on the strength of membrane attachment the enzymes can be removed yet remain functional; inactivated, but not removed (ATPases); or be removed and inactivated; or stimulated to increase in quantity (acid phosphatase). Inactivation of membrane ATPase would tend to lead to an ionic imbalance, which is probably responsible for cellular mortality [26].

In the case of acid phosphatase, lysosomal buildup occurs until the cells lyse, releasing their enzymes into the urine in a functional form. This is indicated by the delayed peaks at 6–7 days as compared to the release of alkaline phosphatase, which shows a peak at 3–5 days. This increase in urinary alkaline phosphatase corresponds to a decrease in the membrane enzyme levels in the kidney, suggesting a possible discharge into the urine. This increase in the discharge of enzyme was also found in the cultured kidney tubules, showing that a similar situation occurs in the in vitro model.

A naturally occurring difference in the quantity of various rat urinary enzymes is seen in Long Evans and Wistar

rats. This is not suprising, in that currently differences in gene family functional products (isozymes) between species are being used to construct genetically determined phylogenies for various organisms and even to show genetic variation within a species. The discovery of strain-specific baselines for alkaline phosphatase activity, acid phosphatase activity, urine volume, and daily weight gains are clearly shown. These findings indicate that such differences must be taken into account when choosing animals for toxicological study: a specific animal's sensitivity should be considered in experimental design and should also be accounted for in the presentation of data.

The mechanism of action of CDDP has puzzled the scientific community since the discovery of its antimetastatic and nephrotoxic properties. CDDP tends to show significant decreases of transport enzyme activity within the cell membranes, as well as corresponding increases in similar urinary enzyme activity. Treatment with a second-generation analogue, CBDCA, does not show nearly the same effect on urine alkaline phosphatase activity, although there is a slight, statistically insignificant increase in acid phosphatase activity after CBDCA injection.

The increase in acid phosphatase activity following CBDCA injection is not surprising, since it is a lysosomal enzyme and lysosomes respond to a foreign substance in the body. The increase in alkaline phosphatase activity in the urine, however, does indicate that while both drugs have a nephrotoxic effect, CBDCA has much less of an effect. This is documented by a comparison with both in vitro and in vivo enzyme reaction product densities.

CBDCA has been shown to have antimetastatic activity similar to that of CDDP, although the chemotherapeutic dosage is ten times that of CDDP. It has been demonstrated that CBDCA does not dissociate as rapidly as CDDP and is largely excreted unchanged [25]. CBDCA has additionally been shown to cause a significant increase in red blood cell hemolysis, probably through the creation of an artificial osmotic gradient, while CDDP does not have as great an effect on red blood cell hemolysis [25].

Results of thiol cytochemistry indicate a decrease in membrane-bound thiols after CDDP treatment, although after CBDCA treatment there is not as drastic a reduction in thiol levels. This is important in that thiols located within proteins have been shown to be responsible for secondary structure and interchain attachments [25] (insulin and immunoglobulin G, both similar in size to alkaline phosphatase) and may be implicated in the linkage of enzymes such as alkaline phosphatase to the membrane. Thiols have also been implicated as key structural units in the binding sites of some enzymes.

The results in this study indicate that CBDCA is a promising second-generation platinum analogue with respect to decreased toxicity without substantial loss of antimetastatic potential, although not all of the second-generation analogue are as promising [28].

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