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Comparative Nephrotoxicity of a Novel Platinum Compound,[1] Cisplatin, and Carboplatin in Male Wistar Rats

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The nephrotoxicity of three platinum-containing antitumor agents was compared at doses that approximate the LD10 (cisplatin) or the LD50 (CI-973, carboplatin) doses. Male Wistar rats were administered single iv doses of 45 mg/kg CI-973, 6.5 mg/kg cisplatin, or 65 mg/kg carboplatin and observed for 4 days. Cisplatin treatment increased blood urea nitrogen (4×), creatinine (3×), glucose, and fractional electrolyte excretions, and decreased creatinine clearance by Day 4. These parameters were not significantly altered in CI-973- and carboplatin-treated animals. Cisplatin increased urinary excretion of LDH (sixfold), GGT (twofold), and NAG (twofold); CI-973 and carboplatin increased GGT excretion (approximately twofold). Cisplatin induced the following functional changes as a consequence of direct nephrotoxicity: decreases in GFR (84%), ERPF (97%), ERBF (96%), and ERTS (95%), and increases in FF (fivefold). Functional changes, attributed to prerenal effects of CI-973, included a decrease in ERPF (35%) and an increase in FF (48%). No changes were seen following carboplatin treatment. All cisplatin-treated rats had proximal tubular necrosis in the outer stripe of the outer medulla, extending multifocally into inner cortical medullary rays. No renal lesions were detected by light or electron microscopy in the control or CI-973- or carboplatin-treated rats. Cisplatin produced marked nephrotoxicity as determined by biochemical, functional, and histopathologic endpoints. CI-973 and carboplatin were significantly less nephrotoxic than cisplatin.

Cisplatin is a potent antitumor agent with activity against a broad spectrum of human malignancies (Prestayko *et al.*, 1979). Clinical use of cisplatin is limited by nephrotoxicity and the development of drug resistance (Fillastre and Raguenez-Viotte, 1989; Daugaard and Abildgaard, 1989; Hamilton *et al.*, 1989). A multitude of platinum-containing analogues have been synthesized and evaluated in an attempt to identify new analogues which will circumvent the dose-limiting nephrotoxicity and cisplatin resistance.

Carboplatin, the only other platinum analogue currently marketed, produces myelosuppression as the dose-limiting toxicity but is relatively free of the neuro- and nephrotoxicity seen with cisplatin (Rose and Schurig, 1985; Siddik *et al.*, 1987; Canetta *et al.*, 1990). Despite the improved toxicity profile of carboplatin, cross-resistance between cisplatin and carboplatin develops *in vitro* and *in vivo* (Perez *et al.*, 1991).

CI-973 is a platinum diamine complex currently undergoing clinical evaluation. CI-973 incorporates the cyclobutanedicarboxylato ligand of carboplatin and its half-life, tissue distribution, and urinary excretion are similar to those of carboplatin (Parke–Davis, unpublished data). Preclinical data have shown that CI-973 possesses marked antitumor activity against cisplatin-resistant tumors *in vitro* and in several *in vivo* tumor models when compared to cisplatin and carboplatin (Kraker *et al.*, 1991; Kobayashi *et al.*, 1991).

The gastrointestinal tract (GI) and hematopoietic system were the major target organs identified in single- and repeated-dose preclinical studies with CI-973 in mice, rats, and dogs (Nippon Kayaku and Parke–Davis, unpublished data). GI toxicity was manifested by diarrhea, congestion and regenerative changes in large and small intestine, and necrosis of epithelial cells in the gastric glands. Hematopoietic toxicities included decreased RBC, WBC, and platelet counts and bone marrow hypoplasia.

Although mild renal lesions were found in rats and dogs in repeated dose studies, routine evaluations were inconclusive as to the nephrotoxic potential of the drug. Considering CI-973 is to be given to patients previously exposed to cisplatin who may be renal compromised, a more complete evaluation of nephrotoxic effects was deemed necessary. Therefore, the objectives of this study were to evaluate the

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renal toxicity of CI-973 administered intravenously to male rats and to compare the nephrotoxic potential relative to that of two other platinum-containing drugs, cisplatin and carboplatin.

METHODS

CI-973 ([SP-4-3-(*R*)]-[1,1-cyclobutanedicarboxylato(2-)](2 methyl-1,4-butanediamine-*N,N'*)platinum) was synthesized by Nippon Kayaku. Carboplatin was synthesized by a method adapted from Kraker *et al.* (1992) using cisplatin and the (di)silver salt of 1,1-cyclobutanedicarboxylic acid. Cisplatin was obtained from Sigma Chemical Co. (St. Louis, MO), and [^{3}H]inulin and [^{14}C]para-aminohippurate (PAH) were purchased from New England Nuclear (Wilmington, DE). All other chemicals, not detailed above, were obtained from commercial sources. Male Wistar rats (Charles River Laboratories, Kingston, NY), weighing approximately 165–185 g (biochemical assessment groups) or 250–300 g (renal function groups), were individually housed and maintained in an environmentally controlled room on a 12-hr light/dark cycle. Animals had *ad libitum* access to food (Purina Certified Chow, 5002) and water throughout the study. Animals were obtained from a single shipment for the definitive phase; animals in the renal function groups were 2 weeks older in order to facilitate surgical procedures.

Dose range finding. Groups of male rats ($N = 3$) were administered single intravenous doses of 35, 45, and 55 mg/kg CI-973; 6.5 mg/kg cisplatin; or 50, 65, and 80 mg/kg carboplatin. The doses of CI-973 and carboplatin represent approximate LD10, LD50, and lethal doses (Rose and Schurig, 1985; Levine *et al.*, 1981; unpublished data); the dose of cisplatin is a known nephrotoxic dose and represents the approximate LD10 (Rose and Schurig, 1985; Siddik *et al.*, 1987; Levine *et al.*, 1981). CI-973 and carboplatin were prepared in sterile D5W, and cisplatin was prepared in sterile saline; the vehicle control was sterile D5W. Blood samples were collected for analysis of hematology and biochemistry parameters on Day 4, prior to termination.

Biochemical assessment. Groups of six male rats each were administered single intravenous doses of 45 mg/kg CI-973, 6.5 mg/kg cisplatin, or 65 mg/kg carboplatin. Animals were housed in Nalgene metabolism cages throughout the study. Hematology (RBC, WBC, and platelet count) and serum biochemistry determinations (glucose, urea nitrogen, creatinine, chloride, sodium, and potassium) were measured with an automated clinical analyzer prior to dosing and on Day 4.

Water consumption and urine volume were measured daily. Samples for urinalysis were collected on ice prior to dosing and on Days 2 and Day 4. Urinary creatinine, glucose, osmolality, potassium, chloride, sodium, alkaline phosphatase (AP), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase (GGT), and *N*-acetyl-β-glucosaminidase (NAG) were measured with an automated clinical analyzer. Electrolyte/glucose excretion and enzyme excretion were calculated for Days 2 and 4. Creatinine clearance and fractional electrolyte excretion were also determined for Day 4. Urinary enzyme excretion was normalized by calculating units/day or units/mg creatinine.

Right kidneys and brains were weighed at necropsy. Left kidneys were perfusion-fixed with 2.0% glutaraldehyde in 0.1 M cacodylate buffer (Griffith *et al.*, 1967). Sections were further fixed in 10% buffered formalin and processed for light microscopy. Sections for electron microscopy were further fixed in cold 2% glutaraldehyde, washed in 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon/Araldite.

Renal function. Groups of four male rats each were administered single intravenous doses of D5W, CI-973, cisplatin, and carboplatin as described above. Renal clearance of inulin and PAH were determined 4 days following dosing. Each rat was anesthetized with sodium pentobarbital and placed on a heated pad to maintain body temperature at 37°C. A tracheostomy was performed, and then the carotid artery, jugular vein, and urinary bladder were each catheterized. Animals were infused with 0.9% saline containing 12 mg/ml [^{3}H]inulin (0.5 μCi/mL) and 10 mg/mL [^{14}C]PAH (0.5 μCi/mL) at a rate of approximately 0.08 ml/min/kg (Welch *et al.*, 1987; Pegg *et al.*, 1976). After a 90-min equilibration period, three consecutive 30-min urine samples were collected. Arterial blood samples were obtained at the midpoint of each urine collection. [^{3}H]Inulin and [^{14}C]-PAH in plasma and urine were simultaneously determined using a dual-label program on a Beckman (LS 9800) liquid scintillation spectrometer. Plasma and urine sodium and chloride were determined. The following renal function parameters were calculated: glomerular filtration rate (GFR), estimated renal plasma/blood flow (ERPF/ERBF), filtration fraction (FF), estimated renal tubular secretion (ERTS), and fractional excretion (FE) of sodium and chloride.

Statistical analyses. All data were expressed as means ± standard errors. Each measurement after drug administration was adjusted for baseline by subtracting the pretest measurement (where applicable) from the postdose measurement. Data collected only on Day 4 were analyzed in a pairwise manner within a one-factor analysis of variance. Data collected at more frequent intervals were compared between groups over time using standard repeated measures analysis of variance. Differences were considered statistically significant at the two-tailed $p < 0.05$ level.

TABLE 1
Range-Finding Study: Mean Body Weight, Hematology, and Biochemistry Values

Drug	Dose (mg/kg)	Body weight (% change)	WBC (thousand/mm^3)	Platelet (thousand/mm^3)	BUN (mg/dl)	Creatinine (mg/dl)
Control	0	+12	6.2 ± 0.3	938 ± 150	17 ± 0	0.5 ± 0.0
CI-973	35	+8	1.6 ± 0.3	742 ± 87	16 ± 1	0.5 ± 0.1
CI-973	45	−4	1.9 ± 0.5	825 ± 70	13 ± 3	0.5 ± 0.0
CI-973	55	−12	1.3 ± 0.1	854 ± 40	25 ± 13	0.5 ± 0.1
Cisplatin	6.5	−2	3.5 ± 0.4	860 ± 38	73 ± 15	1.5 ± 0.3
Carboplatin	50	+4	3.6 ± 0.9	1177 ± 85	18 ± 1	0.6 ± 0.0
Carboplatin	65	+4	2.7 ± 0.8	900 ± 33	14 ± 1	0.5 ± 0.0
Carboplatin	80	−3	2.8 ± 1.3	508 ± 27	14 ± 2	0.5 ± 0.0

Note. Animals received a single dose of drug iv. Parameters measured on Day 4. Values represent mean ± SE from three animals.

RESULTS

Dose Range-Finding Study

No clinical signs were observed in animals receiving CI-973 at doses of 35 or 45 mg/kg. At 55 mg/kg, diarrhea occurred in all animals on Day 4 and one animal died on Day 4. Cisplatin-treated animals were asymptomatic and only one 80 mg/kg carboplatin animal had soft feces. Body weight loss was seen at 45 and 55 mg/kg CI-973, with cisplatin, and at 80 mg/kg carboplatin. All other treated groups exhibited body weight gain suppression (Table 1). All three drugs caused decreases in WBC at the doses administered but there were no changes in RBC (data not shown). An 80 mg/kg dose of carboplatin also decreased platelet counts relative to controls (Table 1). No changes in serum urea nitrogen (BUN) or creatinine were found at any dose of CI-973 with the exception of an elevated BUN value in a moribund animal at 55 mg/kg. Cisplatin-treated animals exhibited a 4.3-fold increase in BUN and a 3-fold increase in serum creatinine. Carboplatin did not alter these parameters (Table 1).

Cisplatin administration induced marked diffuse proximal tubular necrosis in the outer stripe of the outer medulla. In several foci, proximal tubular necrosis extended from the outer stripe into medullary rays. No renal lesions were detected at any dose in CI-973- or carboplatin-treated animals.

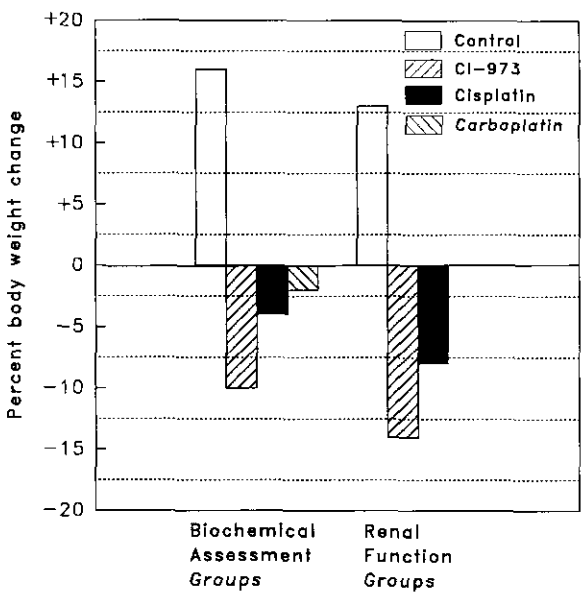

FIG. 1. Effects of CI-973, cisplatin, and carboplatin treatment on body weight. Rats in the biochemical assessment groups averaged between 168 and 184 g at the time of treatment, and rats in the renal function groups averaged between 245 and 298 g. Data are expressed as the percent body weight change between predose and termination weights, $n = 6$. All treated groups are statistically different from respective controls ($p < 0.05$).

Definitive Studies

Doses for the definitive studies were selected to compare CI-973 and carboplatin at equitoxic doses to a known nephrotoxic dose of cisplatin. Doses of CI-973 and carboplatin were considered maximally tolerated doses over the time frame used in this study, thus maximizing the possibility of nephrotoxic injury.

Clinical signs. Four of six animals receiving 45 mg/kg CI-973 in the biochemical assessment phase exhibited soft feces at termination. Animals given the same dose of CI-973 in the functional assessment phase appeared more severely affected with diarrhea in five of six animals on Day 4 and/or termination. One animal receiving 45 mg/kg CI-973 died on Day 4. Animals receiving cisplatin or carboplatin exhibited no adverse clinical signs except soft feces in a single animal treated with 65 mg/kg carboplatin.

Mean body weight gains of 16 and 13% occurred in the control groups from the biochemical and functional phases, respectively (Fig. 1). In contrast, animals given CI-973 lost an average of 10 or 14% of body weight over 4 days in the respective phases. Animals receiving cisplatin also lost weight in these studies, averaging 4 and 8% body weight loss. Minimal weight loss of 2% or less was observed in carboplatin-treated animals.

Biochemical Assessment Study

Hematology. WBC counts were decreased 63 and 44%, respectively, in animals receiving CI-973 and carboplatin (Table 2). Platelet counts decreased approximately 25% compared to control in CI-973- and carboplatin-treated groups. No significant changes in RBC counts were seen in any group.

Biochemistry. Serum urea nitrogen and creatinine were elevated in all animals receiving cisplatin (Fig. 2). Mean values for BUN and creatinine were not affected by CI-973 or carboplatin treatment. Two animals receiving CI-973 had BUN values of 30 and 44 mg/dl without changes in creatinine. Due to the poor clinical condition of these ani-

TABLE 2
Definitive Study: Mean Hematology Values

Drug	Dose (mg/kg)	WBC (thousand/mm^3)	RBC (million/mm^3)	Platelet (thousand/mm^3)
Control	0	5.2 ± 0.8	5.6 ± 0.5	936 ± 94
CI-973	45	1.9 ± 0.2*	6.1 ± 0.7	710 ± 54*
Cisplatin	6.5	5.0 ± 0.8	5.3 ± 0.4	896 ± 87
Carboplatin	65	2.9 ± 0.5*	5.4 ± 0.1	706 ± 40*

Note. Values represent the mean ± SE from four to six animals.
* Significantly different from control, $p \leq 0.05$.

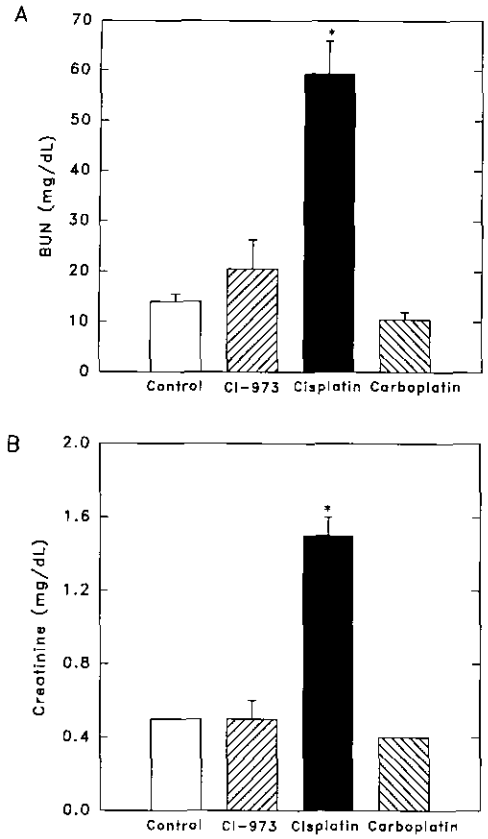

FIG. 2. Effect of drug treatment on (A) BUN and (B) serum creatinine. Parameters were determined on Day 4 and are expressed as means ± SE, $n = 6$. *Significantly different from control ($p < 0.05$).

mals, these BUN elevations were considered prerenal. No significant changes in serum electrolytes, glucose, or protein were noted (data not shown).

Urine volume (14 ± 1 ml/day) and water consumption (32 ± 1 ml/day) were consistent throughout the study in control animals. On Day 4 urine volume (9 ± 1 ml) and water consumption (15 ± 3 ml) were decreased in CI-973-treated animals. Urine volume and water consumption were highly variable in cisplatin-treated animals on Days 1–3 but were significantly increased on Day 4 (23 ± 5 and 41 ± 5 ml/day, respectively). Urine volume and water consumption in carboplatin-treated animals were similar to the control group. No significant changes in urinary pH were seen. Occult blood, glucose, protein, WBCs, and/or RBCs were consistently observed in the urine of cisplatin-treated rats (data not shown).

The renal excretion of electrolytes (mEq/day of Na^+, K^+, Cl^-) was significantly reduced in all treated groups on Days 2 and 4, except for the Cl^- excretion in the carboplatin group on Day 2 (Table 3). Urinary osmolar excretion (mosmol/day) was decreased following cisplatin treatment on Days 2 and 4, whereas CI-973 and carboplatin treatment decreased osmolar excretion only on Day 4. Fractional excretion of electrolytes was decreased in CI-973 and carboplatin-treated animals; in contrast, cisplatin induced significant increases in fractional excretion of electrolytes (Table 4). Renal excretion and fractional excretion of glucose were increased on Days 2 and 4 and clinically estimated GFR (creatinine clearance) was significantly decreased on Day 4 in animals given cisplatin, but unchanged in other groups.

Compared to controls, cisplatin treatment caused increased LDH excretion on Days 2 and 4 (2.5- and 5.7-fold, respectively; Fig. 3), increased GGT excretion on Days 2 and 4 (1.9- and 2.0-fold), and increased NAG excretion 1.6-fold on Day 4; urinary AP was not significantly increased. CI-973 and carboplatin urinary enzyme excretion values were unchanged from controls on Day 2. On Day 4, urinary GGT was elevated in the CI-973 (2.0-fold) and carboplatin (1.7-fold) groups, although the increase was not statistically significant in the carboplatin-treated animals. A minimal, although statistically significant, increase in NAG was observed on Day 4 in the carboplatin group (1.2-fold). Similar patterns, but of greater magnitude, were observed when urinary enzyme excretion was expressed as units/mg creatinine. On Day 4, cisplatin increased urinary LDH 11.2-fold, GGT 4.0-fold, and NAG 3.0-fold; CI-973 increased GGT 4.0-fold, and carboplatin increased GGT 4.0-fold and NAG 1.6-fold.

A decrease in absolute kidney weights in CI-973-treated animals correlated with lower body weights in these animals. A 19% increase in the kidney/body weight ratio was observed in animals given cisplatin. No changes in organ weights were seen in animals receiving carboplatin (data not shown).

Renal lesions were seen only in rats given cisplatin. All six cisplatin-treated rats had moderate to marked proximal tubular necrosis in the outer stripe of the outer medulla. In five of these rats the necrosis extended multifocally into inner cortical medullary rays. Some rats also had tubular hyaline casts, margination of leukocytes in small vessels, and mixed cell infiltrates in tubules. There were no renal lesions in controls or in CI-973- or carboplatin-treated rats. Electron microscopy revealed that kidneys from cisplatin animals had multifocal necrosis of proximal tubular epithelial cells with sloughing into the lumina and thinning and rarefaction of remaining cells. Kidneys from rats given CI-973 or carboplatin were again similar in appearance to controls.

Renal Function Study

Cisplatin significantly altered all renal functional parameters, causing decreases in GFR, ERPF, ERBF, and ERTS, and increases in FF, FE-Na^+, and FE-Cl^- (Table 5). Cisplatin-induced changes were significantly different from all other treated groups as well as compared to controls. Functional changes following CI-973 treatment included a lesser

TABLE 3
Renal Excretion

	Day	Control	CI-973 (45 mg/kg)	Cisplatin (6.5 mg/kg)	Carboplatin (65 mg/kg)
Glucose (mg/day)	2	2.63 ± 0.10	2.51 ± 0.25	5.54 ± 1.39*	2.78 ± 0.34
	4	2.12 ± 0.12	1.03 ± 0.16	134.6 ± 15.3*	1.73 ± 0.32
Cl^- (mEq/day)	2	3.37 ± 0.21	2.32 ± 0.11*	1.72 ± 0.12*	2.92 ± 0.14
	4	3.39 ± 0.16	0.56 ± 0.37*	1.70 ± 0.31*	1.53 ± 0.23*
K^+ (mEq/day)	2	4.15 ± 0.16	3.17 ± 0.10*	2.13 ± 0.13*	3.54 ± 0.19*
	4	3.89 ± 0.22	0.80 ± 0.30*	1.74 ± 0.16*	2.14 ± 0.32*
Na^+ (mEq/day)	2	2.44 ± 0.16	1.42 ± 0.10*	0.92 ± 0.05*	1.81 ± 0.12*
	4	2.47 ± 0.11	0.48 ± 0.26*	1.23 ± 0.18*	1.25 ± 0.20*
Osmoles (mosmol/day)	2	14.59 ± 0.93	13.06 ± 1.06	9.72 ± 1.52*	15.67 ± 0.89
	4	16.53 ± 0.95	7.97 ± 0.90*	9.45 ± 0.96*	10.48 ± 1.28*

Note. Animals received a single dose of drug iv. Parameters measured on Days 2 and 4. Values represent mean ± SE from four to six animals per group.
* Significantly different from control, $p < 0.05$.

decrease in ERPF and increase in FF when compared to control values and these effects were caused primarily by two of four animals in poor clinical condition at the time of measurement; other functional parameters were similar to controls. No functional changes were observed following 65 mg/kg carboplatin.

DISCUSSION

Recommended human doses of CI-973, cisplatin, and carboplatin are 150–300, 50–100, and 275–425 mg/m^2, respectively (unpublished data; Daugaard and Abildgaard, 1989; Canetta *et al.*, 1990). Doses administered to rats in this study correspond to 270, 39, and 390 mg/m^2 of CI-973, cisplatin, and carboplatin. From the results of this study it is readily apparent that cisplatin was nephrotoxic at a dose below that recommended for human use, whereas CI-973 and carboplatin were relatively free of renal effects at doses approaching the upper end of the therapeutic range.

All three platinum analogues caused a decrease in body weight or body weight gain suppression (CI-973 > cisplatin > carboplatin). Generalized toxicity was more evident in CI-973 animals than in carboplatin animals, and cisplatin had few nonrenal effects. Although these drugs were tested at equitoxic doses, deaths from CI-973 occur earlier, between 5 and 11 days (unpublished data), compared to those from carboplatin between 8 and 12 days (Levine *et al.*, 1981; Siddik *et al.*, 1987). The greater weight loss and possible electrolyte loss resulting from diarrhea in CI-973-treated animals had effects, not reflective of nephrotoxicity, on several measured renal parameters.

BUN and serum creatinine are frequently monitored, but these parameters are not early indicators of nephrotoxicity as extensive renal damage is required to cause measurable increases (Sharratt and Frazer, 1963). CI-973- and carboplatin-treated animals had no elevations in BUN or creatinine which correlated with the absence of renal lesions in these animals. Cisplatin-induced elevations of BUN and creatinine as seen in this study have been previously reported in other strains of rats (M. A. Smith *et al.*, 1988; Lelieveld *et al.*, 1984; Goldstein *et al.*, 1981). The cisplatin-induced renal lesions were similar in nature to those previ-

TABLE 4
Calculated Urinary Excretion Parameters: Fractional Excretion and GFR

Parameter	Vehicle	*CI-973* (45 mg/kg)	*Cisplatin* (6.5 mg/kg)	*Carboplatin* (65 mg/kg)
GFR (ml/min)	1.04 ± 0.13	0.73 ± 0.15	0.18 ± 0.03*	1.24 ± 0.23
Glucose (%)	0.11 ± 0.01	0.09 ± 0.01	43.6 ± 3.87*	0.09 ± 0.02
Cl^- (%)	2.15 ± 0.14	0.33 ± 0.21*	6.16 ± 1.45	0.81 ± 0.12*
K^+ (%)	56.7 ± 5.19	18.8 ± 5.15*	153.3 ± 19.8*	27.0 ± 2.70*
Na^+ (%)	1.17 ± 0.09	0.21 ± 0.12*	3.32 ± 0.62*	0.48 ± 0.05*

Note. Animals received a single dose of drug iv. Parameters measured on Day 4. GFR estimated by creatinine clearance. Values represent mean ± SE from four to six animals per group.
* Significantly different from control, $p < 0.05$.

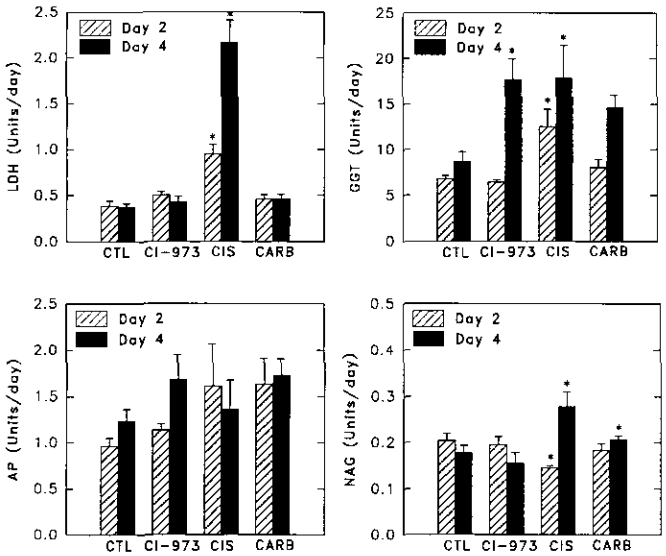

FIG. 3. Effect of drug treatment on urinary enzyme excretion. Twenty-four-hour urine collections on ice were made on Days 2 and 4 from rats housed in metabolism cages. Abbreviations used: CTL, control; CIS, cisplatin, and CARB, carboplatin. Data are expressed as means ± SE, $n = 5$–6. *Significantly different from control ($p < 0.05$).

ously described in the literature (Ward and Fauvie, 1976; Dobyan *et al.*, 1980; J. H. Smith *et al.*, 1988) and correlated with increases in BUN and creatinine.

Urinary osmolar, electrolyte, and glucose excretion were useful in evaluating the concentrating ability of the kidney following drug treatment. Decreased osmolar and electrolyte excretion following CI-973 administration may have resulted from conservation of electrolytes, compensating for losses through the GI tract. Since glucose excretion was unchanged and fractional excretion of electrolytes were decreased or unchanged, renal tubules appeared to maintain normal resorptive capacity. Carboplatin had very similar effects to CI-973, with decreased or unchanged excretion of electrolytes and no change in glucose excretion. As anticipated from previous studies (Goldstein *et al.*, 1981; J. H. Smith *et al.*, 1988), animals receiving cisplatin had increased output of dilute urine with decreased osmolality and electrolyte concentration. The marked increase in glucose excretion indicated that proximal tubular resorption of glucose was severely impaired. Increased fractional excretion of electrolytes following cisplatin treatment was also indicative of impaired proximal tubular function.

Enzymuria has been frequently utilized to assess nephrotoxicity (M. A. Smith *et al.*, 1988; Price, 1982). Increases in urinary LDH and GGT activities have been found to be the most sensitive predictors of proximal tubular toxicity (Hofmeister *et al.*, 1986). Litterst *et al.* (1985) reported AP, GGT, and NAG elevations following doses of cisplatin as low as 2 mg/kg. The cytosolic enzyme, LDH, was significantly increased by cisplatin treatment but was not significantly altered by the other platinum agents. LDH appeared to be the most sensitive and clear indicator of cytotoxicity. Excretion of the brush border enzyme GGT was moderately elevated by all three agents; however, AP was not substantially elevated by any agent. The presence of GGT in the urine of CI-973 and carboplatin animals was likely of renal origin, but there were no correlative histopathologic or ultrastructural changes to this enzyme elevation. NAG, a lysosomal enzyme, was significantly elevated by cisplatin and carboplatin, but the increase was less than twofold greater than the control values.

Nonrenal effects may account for the decrease in renal plasma flow and the increase in filtration fraction observed following CI-973 treatment. Probable water loss through the GI tract (diarrhea) without a compensatory increase in water consumption is likely responsible for the decreased renal plasma flow. Although changes in these parameters were attributed to drug treatment, the changes were likely due to prerenal effects and were minor in comparison with cisplatin-induced functional changes.

In conclusion, cisplatin produced marked nephrotoxicity as determined by biochemical, functional, and histopatho-

TABLE 5
Renal Function Parameters

Parameter	Vehicle	CI-973 (45 mg/kg)	Cisplatin (6.5 mg/kg)	Carboplatin (65 mg/kg)
GFR (ml/min)	1.66 ± 0.29	1.58 ± 0.28	0.27 ± 0.15*	1.79 ± 0.21
ERPF (ml/min)	9.29 ± 1.50	6.02 ± 1.13*	0.32 ± 0.18*	9.56 ± 1.49
FF (%)	17.9 ± 0.3	26.5 ± 0.9*	89.6 ± 3.7*	18.9 ± 0.63
ERBF (ml/min)	16.26 ± 2.62	11.11 ± 1.68	0.60 ± 0.35*	15.95 ± 2.57
ERTS (μg/min)	171 ± 21	146 ± 16	9 ± 6*	204 ± 14
FE-Na (%)	0.49 ± 17	0.62 ± 0.28	11.37 ± 1.14*	0.40 ± 0.13
FE-Cl (%)	0.86 ± 0.25	0.84 ± 0.40	17.41 ± 3.63*	0.54 ± 0.14

Note. Animals received a single dose of drug iv. Parameters measured on Day 4. Data represent the mean ± SE of four animals.
* Significantly different from control ($p < 0.05$).

logic assessments in rats. CI-973 and carboplatin were less nephrotoxic relative to cisplatin following administration of maximally tolerated single intravenous doses.

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