Effect of Route of Administration and Dose on Diabetes-Induced Protection against Cisplatin Nephrotoxicity (44027)

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Abstract. **The kidneys of diabetic rats exhibit resistance to the actions of a variety of nephrotoxic chemicals. Using the streptozotocin (STZ) diabetic rat as a model, the experiments in this study examined the effect of the diabetic state on bioavailability, renal accumulation and renal toxicity following intraperitoneal (ip) and intravenous (iv) injection of cisplatin at various doses. Comparison of the areas under the plasma concentration versus time curves up to 220 min after cisplatin injection (5 mg/kg body wt) demonstrated that bioavailability of cisplatin was significantly impaired in STZ diabetic versus nondiabetic rats after the ip route of administration, but not after the iv route. Regardless of the route of cisplatin injection, both renal cortex platinum level and renal toxicity as quantified by blood urea nitrogen (BUN) increase, were significantly lower in STZ diabetic versus nondiabetic rats. Thus, while decreased bioavailability probably plays a role in the protection seen after ip injection, renal mechanisms must also be involved since protection was also noted after iv injection despite equal bioavailability. To determine whether the protection can be overcome by increasing the dose of cisplatin, renal platinum and BUN levels in STZ diabetics and nondiabetics were compared after ip and iv cisplatin injections at 2.5, 5, 7.5, and 10 mg/kg body wt. The results demonstrate that the diabetes-induced renal protection can be partially reversed by increased cisplatin dose, but only by the iv route. Reversal was accompanied by increased renal platinum accumulation. These results suggest that STZ diabetes alters the tubular cells in a manner that confers protection that more closely resembles tolerance than absolute inherent resistance to the nephrotoxic actions of cisplatin.**

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Current understanding of the pathogenesis of diabetic nephropathy and its progression to end-stage renal disease is far from complete. Study of the process has centered primarily on glomerular pathophysiology, with relatively less emphasis on diabetes-associated changes of tubular cell function, which include alterations in both reabsorptive and secretory transport processes (1). In addition, the kidneys of streptozotocin (STZ) diabetic rats become resistant to the actions of tubular toxins such as gentamicin (2) and cisplatin (3), a phenomenon associated with decreased accumulation of the toxin by proximal tubular cells (4, 5). The development of this protection from the nephrotoxic effects of at least one of these toxins, cisplatin, is related to the length of the diabetic state and, in fact, occurs not only in STZ diabetic rats but also in galactosemic rats which are normoinsulinemic, normoglycemic, and show a normal lipid profile (6). Taken together, the results suggest that the protection is associated with progressive biochemical changes in renal tubular cells secondary to chronic elevation of plasma hexose concentrations. While the underlying mechanisms mediating the protection phenomenon remain elusive, their ultimate definition will provide new insight into the way that experimental diabetes alters renal function. The current study was designed to extend published results by determining

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the influence of two variables, dose and route of administration, on the ability of diabetic rat kidneys to accumulate and resist the toxic action of cisplatin.

Materials and Methods

Induction of Experimental Diabetes. Male Sprague-Dawley rats with an initial body weight of 250–300 g were used. They were housed in temperature- and light-controlled quarters and given free access to food and water throughout the course of the study. Diabetes was induced by a single injection of streptozotocin (Sigma Chemical Co., St. Louis, MO) into the lateral tail vein at a dose of 65 mg/kg body wt. Each dose was prepared immediately before injection by dissolving an appropriate amount of STZ in citrate buffer (pH 4.5). Animals were considered to be diabetic if within 4 days of STZ injection, nonfasting plasma glucose levels exceeded 250 mg/100 ml (Beckman glucose analyzer) and glucosuria was present as measured semiquantitatively with Tes-Tape (Eli Lilly Co., Indianapolis, IN). Age-matched controls were injected with the citrate buffer vehicle.

Bioavailability Assessment. These experiments were designed to determine the influence of the route of administration on delivery of cisplatin to the kidney. Two groups of rats were used (n = 6/group): 6-week diabetics and age-matched nondiabetics. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium at a dose of 35 mg/kg body wt. The carotid artery was cannulated with size 50 polyethylene tubing. Patency of the cannula was maintained by a slow infusion (20 μl/min) of saline. Cisplatin, dissolved in 0.9% saline, was administered at a dose of 5 mg/kg body wt by either the intraperitoneal (ip) or intravenous (iv) route. Blood samples were drawn from the carotid artery immediately before and at 2, 5, 15, 30, 60, 90, 120, and 220 min after injection of cisplatin. Plasma concentration versus time curves were constructed, with area under the curve being calculated by the trapezoidal method (7). The heparinized blood was immediately centrifuged and the plasma frozen for later platinum analysis by atomic absorption spectroscopy (AAS) as described below. The red blood cells were resuspended in saline and re-introduced into the body. At the conclusion of the experiment, the rat was sacrificed with carbon dioxide in a closed chamber and the kidneys were rapidly excised and decapsulated. A thin slice of cortex (75–100 mg wet wt, 0.3–0.5 mm in thickness) was cut from the surface of each kidney using a Stadie-Riggs microtome. The two slices were pooled and frozen for later platinum analysis by AAS.

Protein Binding. Extent of protein binding of platinum in the plasma was determined in nondiabetic and diabetic rats by centrifugation using the Centrifree micropartition system (Amicon, Danver, MA). Protein binding was expressed in terms of percentage platinum bound after correcting for platinum bound to the filter.

Effect of Dose on Toxicity. The animals were divided into two groups: 6-week diabetics and age-matched nondiabetics (n = 24 rats/group). Half of the rats in each group received an ip and the other half an iv (lateral tail vein) injection of cisplatin at a dose of either 2.5 mg/kg, 5 mg/kg, 7.5 mg/kg, or 10 mg/kg body wt (n = 3 rats/dose for each route of administration). On the first day of the experiment, the rats were weighed and an initial blood sample was taken. After cisplatin administration, the rats were returned to the animal quarters and were maintained without intervention for 4 days. Ninety-six hours after cisplatin injection, a second blood sample was obtained, after which the animals were sacrificed and the kidneys harvested and sampled as described above. Nephrotoxicity was assessed by comparing blood urea nitrogen (BUN)

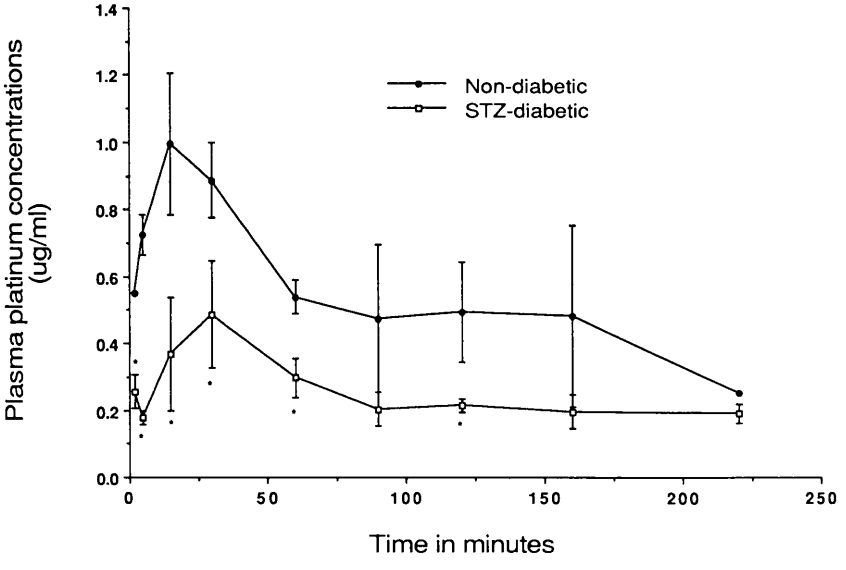

Figure 1. Plasma platinum concentrations in nondiabetic and STZ diabetic rats following intraperitoneal administration of cisplatin. Cisplatin was injected at a dose of 5 mg/kg body wt. Plasma platinum concentration is expressed as μg platinum per ml of plasma. Each point is the mean ± SD of at least three independent experiments. Statistical significance ($P < 0.05$) determined by Student's t test. *Significantly different from values observed for nondiabetic rats.

concentration (mg/100 ml in plasma) immediately before and 96 hr after cisplatin injection. BUN was quantified colorimetrically with a commercially available kit (Sigma procedure 640). A BUN ratio (after/before cisplatin) in excess of 2.5 was considered to be evidence of renal toxicity. This definition is based on the fact that the reported normal range of BUN in the Sprague-Dawley rat is 12–26 mg/100 ml, which is wide enough to allow a doubling before a value falls outside the normal range (8).

Platinum Analysis. Platinum (Pt) levels in plasma and nitric acid tissue digests were determined by electrothermal atomic absorption spectroscopy using a Perkin-Elmer model 380 spectrophotometer fitted with a Model HGA400 programmer (6). A standard curve was constructed for each analytical run using appropriate nitric acid dilutions of a commercially available Pt atomic absorption spectroscopy standard solution (1000 μg/ml; Sigma). The curve was consistently linear in the range of 100–500 μg/ml and all samples were diluted as needed with 0.2% nitric acid so as to fall within this range. Results are expressed as microgram per milligram or per gram wet tissue weight.

Statistical Analysis. All data are expressed as mean ± SD unless otherwise stated. Significance ($P < 0.05$) was assessed using either Student's t test or one-way analysis of variance (ANOVA) with Scheffe's test using StatQuik for Macintosh (Lunden Software, Chagrin Falls, OH).

Results

Effect of Route of Administration on Plasma Platinum Concentration Over Time. Figure 1 shows plasma platinum concentrations versus time profiles in nondiabetic and STZ diabetic rats injected with cisplatin by the ip route. Peak platinum concentrations were observed approximately 20 min after injection in both nondiabetic and STZ diabetic animals. However, the maximum plasma platinum concentration for the STZ diabetic rats was 0.5 μg/ml versus 1 μg/ml in the age-matched nondiabetics. In fact, plasma platinum levels were at least 50% lower in the STZ diabetic rats at all time points up to 180 min compared with levels observed in the nondiabetic rats. A comparison of the concentration versus time curves for the two groups shows that for STZ diabetics, the area under the curve was only 47% of the nondiabetic value. In contrast to the ip injection pattern, the plasma platinum versus time curves for the STZ diabetics and the nondiabetics after iv injection were virtually superimposable (Fig. 2). A comparison of Figure 1 and 2 makes it evident that injection of cisplatin by the iv route at 5 mg/kg resulted in higher plasma platinum concentrations at all time points. Percentage of total platinum bound to proteins in plasma drawn 220 min was not significantly different between nondiabetic and STZ diabetic rats for a given route of administration (Table I). Although there was no statistically significant effect of route of administration on protein binding, a trend toward a slightly lower degree of binding in the iv injected animals was evident. It would follow that, as a result of decreased delivery of cisplatin to blood from the site of injection, one can expect that kidney platinum concentration will be lower in the STZ diabetics than in the nondiabetics after ip cisplatin injection. That this was indeed the case is shown in Table II. Renal cortex platinum concentration at 220 min was 71% less in the STZ diabetic rats compared with nondiabetic rats following ip cisplatin injection. On the other hand, if reduced toxin bioavailability was the only factor in diabetes-associated protection from cisplatin, one would expect to observe the same degree of renal accumula-

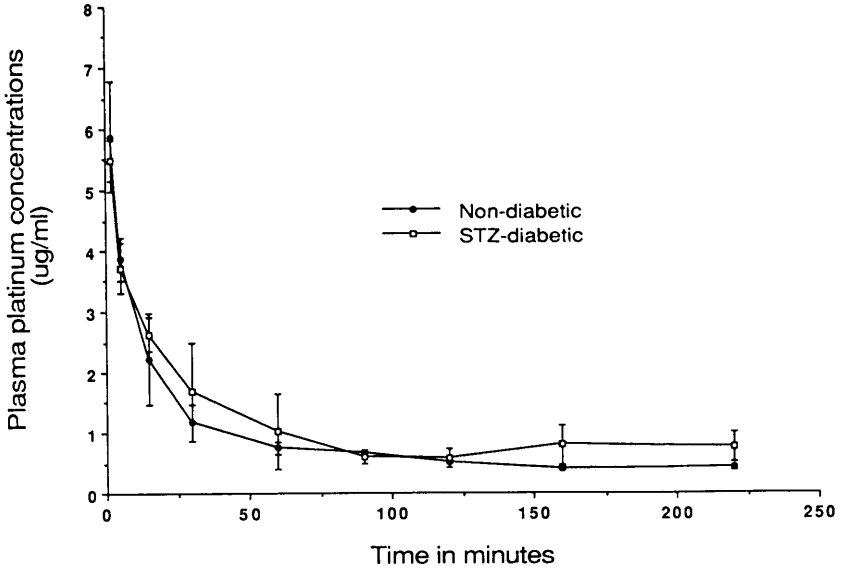

Figure 2. Plasma platinum concentrations in nondiabetic and STZ diabetic rats following intravenous administration of cisplatin. Cisplatin was injected at a dose of 5 mg/kg body wt. Plasma platinum concentration is expressed as μg platinum per ml of plasma. Each point is the mean ± SD of at least three independent experiments. Statistical significance ($P < 0.05$) determined by Student's t test. There was no significant difference between the values obtained from nondiabetic and diabetic rats at any time point.

Table I. Extent of Platinum Binding to Plasma Proteins in Nondiabetic and STZ Diabetic Rats 220 min after Cisplatin Administration

Group	Intraperitoneal route (%)	Intravenous route (±)
Nondiabetic	95.67 ± 1.15	93.33 ± 1.15
STZ diabetic	95.00 ± 1.73	92.00 ± 1.73
P	>0.05	>0.05

Note. Cisplatin was administered either intraperitoneally or intravenously at a dose of 5 mg/kg body wt. Binding was assessed by centrifugation using the Centrifree micropartition systems. Protein binding is expressed in terms of percentage platinum bound to plasma proteins. Each value represents the mean ± SD of at least three independent experiments. Statistical significance ($P < 0.05$) was assessed by Student's *t* test. No significant differences were noted.

tion of platinum in both groups of rats after iv injection. However, Table II also shows that the STZ diabetics accumulated 53% less platinum than did the nondiabetics despite comparable bioavailability. Given the higher plasma levels of platinum after iv injection of cisplatin, it is not surprising that platinum levels were also higher in the kidneys of the animals injected by the iv route compared with the ip route.

Effect of Increased Dose of Cisplatin on Nephrotoxicity. Figure 3 presents the results of experiments designed to quantify the dose dependence of diabetes-induced protection from the nephrotoxic effect of cisplatin. Nondiabetic rats injected with cisplatin by the ip route demonstrated a dose-dependent increase in renal toxicity as indicated by mean BUN ratios which ranged from 0.94 for the 2.5 mg/kg dose to 14.4 for the 10 mg/kg dose. In contrast, the BUN ratios in STZ diabetic rats did not exceed 1.6 at any ip dose of cisplatin. Thus, ip injection of increasing doses of cisplatin to a maximum of 10 mg/kg body wt could not overcome the diabetes-induced protection against nephrotoxicity. Administration of cisplatin to nondiabetic rats by the iv route produced a pattern of dose-dependent renal toxicity that was qualitatively similar to that seen after ip injection in this group. As was the case with the ip route, there was no significant difference in the BUN ratios of nondiabetic and STZ-diabetic rats given 2.5 mg/kg body wt cisplatin. For the 5 and 7.5 mg/kg doses, the degree of toxicity as measured by BUN ratio was greater than that seen in the corresponding ip injected animals (Fig. 4). After the 10 mg/kg dose, all rats died within 24 hr of iv injection of cisplatin precluding BUN determination. A comparison of the effect of increasing doses of cisplatin by the iv route in the STZ diabetic rats shows a toxicity pattern that differs qualitatively as well as quantitatively from that seen after ip injection. Unlike the observation for the ip route, iv injection of 5 and 7.5 mg/kg cisplatin to STZ diabetic rats produced corresponding increases in renal toxicity. This indicates that protection can be partly overcome by increased dosing by the iv route. After the 10 mg/kg dose, all rats died within 24 hr of iv injection of cisplatin so that no renal toxicity assessment was available. The actual cause of death was not investigated in this study. A notable observation is that at a given dose of cisplatin by either route, the BUN ratio was always significantly lower in the STZ diabetic rats.

Effect of Increased Dose of Cisplatin on Renal Platinum Concentration. Table III presents the mean renal cortex platinum concentrations from STZ diabetic and nondiabetic rat kidneys sampled 4 days after cisplatin injection by the ip and iv routes. These data were obtained fro the same rats used in the toxicity experiments discussed above. It is evident that both the route of administration and the diabetic state had a major influence on cisplatin distribution to the kidneys. All of the rats injected with the 10 mg/kg dose of cisplatin by the iv route died within 24 hr. While iv injection resulted in an expected dose-dependent increase in renal cortex platinum level, ip administration did not. Regardless of the route of administration, platinum accumulation was significantly lower in the STZ diabetic kidney cortex. This was most notably evident in the kidney cortex from the ip injected STZ diabetics which accumulated at all three dose levels, less than 8% of the platinum accumulated by the corresponding nondiabetics. For the iv injected animals, the value was 45% lower in the STZ diabetics versus the nondiabetics. Comparison of renal platinum levels four days after administration of 5 mg/kg body wt cisplatin (Table III) to levels measured 220 min following cisplatin injection at the same dose (Table II) shows that renal cortex platinum tended to decrease over the 4-day time frame. The exception was an increase in the value for the nondiabetic rats injected with cisplatin by the ip route.

Discussion

Cisplatin's effects on renal morphology and function in the normal rat have been well-described previously (9–11). A commonly used and convenient indicator for cisplatin-induced nephrotoxicity is a dose-dependent increase in BUN concentration that peaks 3–5 days after injection of a nonlethal, nephrotoxic dose (e.g., 5 mg/kg) and thereafter slowly declines. Scott *et al.* (3) were the first to note that in STZ diabetic rats, the characteristic increase in BUN concentration is not evident after ip injection of 5 mg/kg cisplatin. We subsequently showed, using the same dose and route of administration of cisplatin in the STZ diabetic rat, that the protection from renal toxicity, as indicated by an absence of increase in BUN concentration, is associated with decreased accumulation of platinum in the renal cortex (5). To date the mechanism of the decreased accumulation is undefined.

Table II. Platinum Accumulation in Renal Cortex of Nondiabetic and STZ Diabetic Rats 220 min after Cisplatin Administration

Group	Intraperitoneal route (μg/g wet wt)	Intravenous route (μg/g wet wt)	P^a
Nondiabetic	6.60 ± 1.0	12.30 ± 2.7	<0.05
STZ diabetic	1.90 ± 0.7	6.10 ± 1.7	<0.05
Ratio (diabetic/control)	0.29	0.47	<0.05
P^b	<0.05	<0.05	

Note. Cortical slices from kidneys harvested 220 min after intraperitoneal or intravenous cisplatin administration (5 mg/kg body wt) were dried, dissolved in concentrated nitric acid, and analyzed for platinum content by AAS. Renal platinum concentration is expressed as μg platinum/g wet wt of tissue. Each value represents the mean ± SD of at least three independent experiments. Statistical significance ($P < 0.05$) was assessed by paired *t* test.

[a] Significantly different from the values for different route of administration within the same group.

[b] Significantly different from respective nondiabetic value.

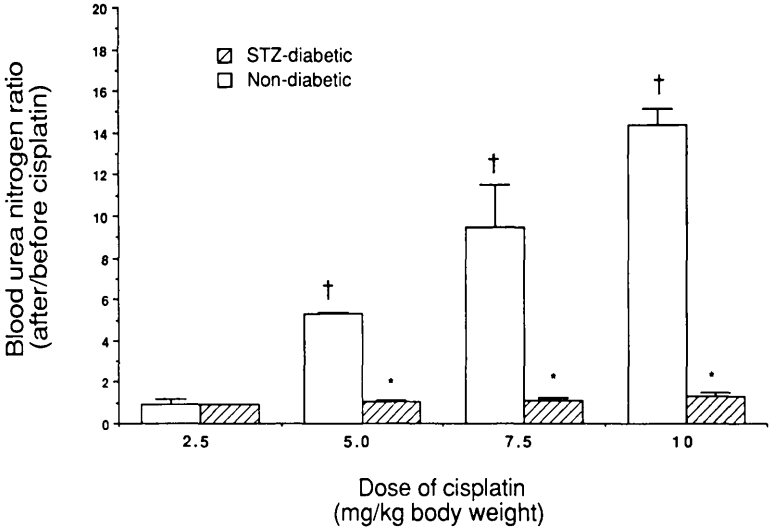

Figure 3. Effect of intraperitoneal doses of cisplatin on blood urea nitrogen (BUN) ratios in nondiabetic and STZ diabetic rats. Cisplatin was administered intraperitoneally at doses of 2.5, 5, 7.5, and 10 mg/kg body wt. BUN levels were determined in plasma samples obtained before and 96 hr after cisplatin administration. A BUN ratio (after/before cisplatin) >2.5 was considered to be evidence of renal toxicity. Each value represents the mean ± SD of at least three independent experiments. Values were statistically assessed by one-way ANOVA and tested for significance ($P < 0.05$) using Scheffe's test. *Significant difference from nondiabetic value at same dose; †significant difference in value from next lowest dose within same group.

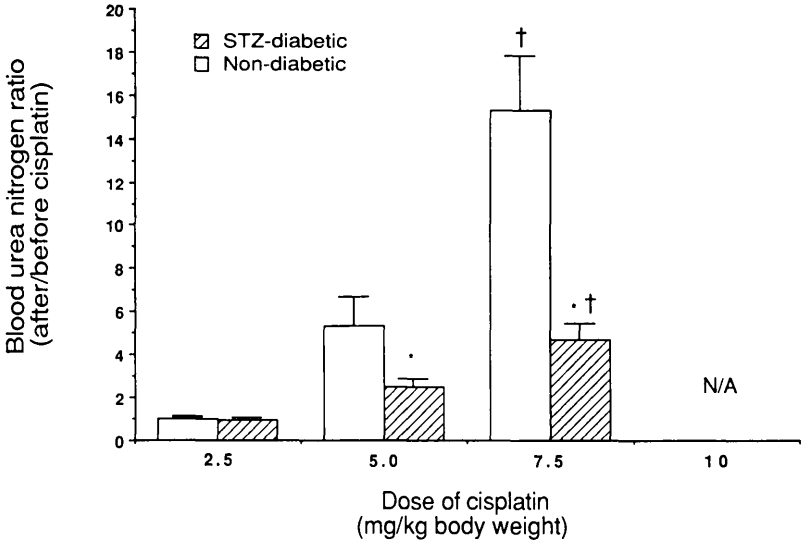

Figure 4. Effect of intravenous doses of cisplatin on blood urea nitrogen (BUN) ratios in nondiabetic and STZ diabetic rats. Cisplatin was administrated intravenously at doses of 2.5, 5, 7.5, and 10 mg/kg body wt. BUN levels were determined in plasma samples obtained before and 96 hr after cisplatin administration and expressed in terms of ratio of after/before cisplatin. A BUN ratio (after/before cisplatin) >2.5 was considered to be evidence of renal toxicity. Each value represents the mean ± SD of at least three independent experiments. Statistical significance ($P < 0.05$ assessed by a paired *t* test). *Significant difference from nondiabetic value at same dose; †significant difference in value from next lowest dose within same group.

The results from the current study demonstrate that both route of administration and dose of cisplatin influence diabetes-induced protection from nephrotoxicity. It is probable that initiation of nephrotoxicity occurs within the first 4 hr after cisplatin administration (12, 13). Thus, plasma platinum levels observed during the first few hours after injection correspond to the phase wherein cisplatin initiates a cascade of events in the kidneys that lead to the characteristic manifestations of cisplatin-induced renal toxicity that

Table III. Effect of Cisplatin Dose on Platinum Accumulation in Renal Cortex of Nondiabetic and STZ Diabetic Rats

Group	Intraperitoneal injection of cisplatin (mg/g body wt)				Intravenous injection of cisplatin (mg/kg body wt)			
	2.5	5	7.5	10	2.5	5	7.5	10
Nondiabetic	2.45 ± 0.37	10.09 ± 0.58	8.58 ± 2.12	12.37 ± 1.53	2.25 ± 0.51	5.93 ± 0.39	9.53 ± 0.69	N/A[a]
STZ-Diabetic	0.77 ± 0.25	0.76 ± 0.06	0.42 ± 0.12	0.38 ± 0.06	1.09 ± 0.11	3.27 ± 0.55	5.29 ± 0.85	N/A[a]
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	

Note. Kidneys were harvested 96 hr after intraperitoneal or intravenous cisplatin administration at doses of 2.5, 5, 7.5, and 10 mg/kg body wt. Cortical slices were dried, dissolved in concentrated nitric acid, and analyzed for platinum content by AAS. Renal platinum concentration is expressed as μg platinum/g wet weight of renal tissue. Each value represents the mean ± SD of at least three independent experiments. Statistical significance ($P < 0.01$) was assessed by two-tailed *t* test.

become progressively evident over the next several days. A key observation in the current study was significantly reduced bioavailability of cisplatin administered by the ip route in the STZ diabetic rat. It is not clear whether this is unique to this particular drug or whether it is more generally applicable to other agents. Should the latter be true, it suggests diabetes-related changes in the peritoneal membrane or it's blood supply. An extensive literature search yielded no information on this. This needs to be further investigated. The area under the concentration versus time curve is a commonly used index of drug bioavailability. The fact that the area under the curve after ip cisplatin administration in the STZ-diabetics was less than half that seen in nondiabetics indicates that, at least for cisplatin, some of the protection can be attributed to reduced bioavailability during this critical early period after cisplatin injection. Most reports on the protection phenomenon for cisplatin in STZ diabetic rats describe experiments carried out using the ip route of administration. The current results suggest that conclusions drawn from such experiments need to be reexamined.

In contrast, following iv injection of cisplatin in STZ-diabetic rats, the plasma platinum levels were not significantly different from those observed in nondiabetic rats, indicating that there was equal bioavailability (Fig. 2). This conclusion is supported not only by the virtually equivalent arterial blood levels in the nondiabetic and STZ diabetic rats at all time points, but also by the lack of effect of diabetes on cisplatin protein binding (Table I). The fact that, despite equal bioavailability following iv administration of cisplatin, the STZ rat kidney cortex platinum levels were significantly lower than those seen in the nondiabetics (Table II), suggests that kidney-based mechanisms also have an important role in the development of protection in the diabetic kidney.

Ramsammy *et al.* (4) have demonstrated that STZ diabetic rats are not absolutely resistant to gentamicin-induced nephrotoxicity by showing that increased doses resulted in restoration of toxicity (i.e., loss of protection). Thus, the kidneys from the diabetic animals seemed to have developed something more akin to tolerance rather than absolute inherent resistance to the toxicant. The results of the current study indicate that not only is the same true of the protection from cisplatin-induced nephrotoxicity, but also that the reversal of protection depends upon the route of administration. Increasing doses of cisplatin by both the ip and iv routes caused dose-dependent increases in BUN ratios in nondiabetic rats, but this was not so for the STZ-diabetics. While iv cisplatin administration also produced a dose-dependent increase in BUN ratios in STZ-diabetic rats (albeit to a significantly lower degree than in the nondiabetics), this was not true after ip injection of cisplatin even after the 10 mg/kg dose which killed all iv injected rats. Comparison of renal platinum levels at the time of sacrifice of these rats (i.e., 4 days after cisplatin injection, Table III) suggests that, even at the highest given dose, the kidneys of the ip injected STZ diabetic rats failed to accumulate sufficient cisplatin to cause measurable damage. Although this study was not designed to define the renal toxicity threshold for cisplatin in the rat, it would appear to be above the approximately 2 μg/g of tissue accumulated by diabetic kidney during the 220 min post injection of the 5 mg/kg dose of cisplatin (Table II).

While reduced accumulation of cisplatin seems to be a key factor in the development of protection in experimental diabetes, data from the current study suggest that it may not be the sole factor. If reduced accumulation is the only factor mediating protection, it is reasonable to expect that similar renal cortex platinum concentrations in nondiabetic and STZ diabetic rats should result in the animals having similar toxicity. Furthermore, if the evidence (10) supporting the conclusion that the initiating toxic event occurs with 4 hr of cisplatin administration is accepted, it follows that the relevant tissue concentrations for comparison in the current study are those presented in Table II, which were measured 220 min after cisplatin injection. It can be seen that the kidneys harvested from nondiabetic rats injected with 5 mg/kg cisplatin by the ip route and those harvested from STZ diabetic rats injected by the iv route with this dose accumulated vir-

tually the same concentration of platinum. Because there is no consistently quantifiable marker of nephrotoxicity so soon after cisplatin injection, a direct comparison of toxicity cannot be made with these rats However, the BUN data of Figure 3 and 4 obtained from identical rats sacrificed 4 days after injection of 5 mg/kg cisplatin demonstrate that, while there was an increased BUN concentration exceeding 5-fold over baseline in the ip injected nondiabetics (Fig. 3), no significant increase over baseline BUN level was observed in STZ diabetics injected with cisplatin by the iv route. While clearly not direct evidence, these observations are provocative in that they suggest that 6 weeks of uncontrolled STZ diabetes in rats may increase the renal toxicity threshold for cisplatin in addition to causing decreased accumulation.

The results suggest that the protection involves decreased uptake and/or enhanced efflux processes in proximal tubular cells. We have recently presented results from incubated renal cortex slice experiments that lend further support to this suggestion (14). It is also conceivable that STZ diabetes alters the intracellular disposition of cisplatin or the mix of its biotransformation products which were not measured in this study. Definition of differences in the species of platinum in diabetic versus nondiabetic kidney after cisplatin injection may provide insight into diabetes-related alterations mediating the protection phenomenon.

The precise mechanism for the decreased vulnerability of the kidneys to nephrotoxins in experimental diabetes remains to be resolved. Our previous demonstration that galactosemic rat kidneys also develop protection from cisplatin toxicity suggests that the phenomenon is related to hyperglycemia (6). Abnormal glycosylation of the cellular proteins is hypothesized to be an important component of end organ damage in diabetes. Indeed, quantification of glycosylated hemoglobin (a model intracellular protein) is well accepted as a valid indicator of the progression of the disease. To date, the role of protein glycosylation in diabetic nephropathy in general and tubular cell changes in particular remains undefined.

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