Reduced Renal Accumulation and Toxicity of Cisplatin in Experimental Galactosemia (43610)

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Abstract. **The kidneys of streptozotocin (STZ)-diabetic rats are resistant to certain toxic effects of the antineoplastic drug cisplatin. The mechanism is unknown. This study used the galactosemic rat model to test the hypothesis that the apparent diabetes-induced protection is due to changes in the kidney secondary to chronically elevated hexose concentrations. Galactosemic rats are normoinsulinemic and are free from many of the multiple biochemical abnormalities seen in STZ diabetics. The experiments compared renal cortical platinum (Pt) and blood urea nitrogen (BUN) levels after intraperitoneal injection of 5 mg/kg of cisplatin in galactosemic, STZ-diabetic, and age-matched nondiabetic Sprague-Dawley rats. Nephrotoxicity was defined as a BUN concentration ratio (after to before cisplatin) >2.5. The results demonstrate that the kidneys of both galactosemic and STZ-diabetic rats became resistant to cisplatin-induced elevation of BUN and, further, that the development of the protection was related to the duration of the diabetic state. Although the protective effect developed more slowly in the galactosemic rats, the attenuation of the rise in BUN was ultimately comparable to that seen in STZ diabetics. Renal cortex [Pt] after cisplatin injection was significantly lower in galactosemics and STZ diabetics compared with age-matched nondiabetics, with the order nondiabetics > galactosemics > STZ diabetics. It was noted, however, that renal Pt accumulation was maximally depressed within 4 weeks of experimental diabetes, whereas the BUN ratio continued to decline with increasing duration of both galactosemia and STZ diabetes. Thus, reduced renal Pt accumulation cannot by itself explain the progressive attenuation of the toxicity. The results support the hypothesis and suggest that the galactosemic rat will be a useful model for mechanistic study of diabetes-induced protection from cisplatin nephrotoxicity.** [P.S.E.B.M. 1993, Vol 203]

Cisplatin is a widely used antineoplastic drug that produces significant toxicity to the tubular epithelial cells of the kidney cortex. Scott *et al.* (1) have recently demonstrated dramatic attenuation of cisplatin nephrotoxicity in streptozotocin (STZ)-induced diabetic rats. The mechanism of the protective effect in this model of experimental diabetes is unknown, but we have recently demonstrated that it is associated with significantly decreased platinum accumulation in diabetic kidney cortex after intraperitoneal injection of cisplatin (2). A similar correlation between renal cortical concentration and tubular toxicity has been established for the aminoglycoside antibiotics such as gentamicin. In fact, the potentiating effect of cisplatin on gentamicin's nephrotoxic action is associated with increased renal accumulation of the antibiotic (3). Because STZ specifically kills the insulin-secreting β cells in the pancreas, it produces complex and interrelated alterations in the biochemistry of carbohydrates, lipids, and proteins reflecting those observed in human Type I diabetes. Another experimental model of diabetes is the galactosemic rat in which chronic elevations in blood hexose concentration can be maintained in the presence of normal blood insulin levels, thus avoiding the multiple biochemical abnormalities associated with hypoinsulinemia seen in STZ-induced diabetes. Although galactosemia has been used to advantage for the

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study of diabetic cataract development and neuropathy (4–6), the effects of this model on kidney structure and function have been examined only recently. The results demonstrate that kidneys from galactosemic animals exhibit many but not all of the changes observed in STZ-induced diabetes. Both models of diabetes produce renal hypertrophy, enzymuria, proteinuria, and elevated creatinine clearance (6, 7). On the other hand, mesangial expansion and glomerular basement membrane thickening are seen only in the STZ-diabetic model (7, 8). The purpose of the current investigation was to compare the mitigating effects of galactosemia and STZ-induced diabetes on cisplatin nephrotoxicity under controlled experimental conditions. The experiments were designed to test the hypothesis that diabetes-induced protection from the nephrotoxicity of cisplatin is due to progressive changes in the kidney secondary to chronically elevated hexose concentrations.

Methods

Induction of Experimental Diabetes. Male Sprague-Dawley rats with initial body weights 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. After initial weighing and blood sampling from the lateral tail vein, animals were assigned to one of three experimental groups: 1, streptozotocin-induced diabetic (STZ diabetic); 2, galactosemic; or 3, age-matched nondiabetic controls.

Diabetes was induced in the first group by a single injection of STZ (Sigma Chemical Co., St. Louis, MO) into a lateral tail vein at a dose of 65 mg/kg body wt. Each dose was prepared immediately before injection by dissolving the appropriate amount of STZ in 50 m*M* citrate buffer (pH 4.5). Animals were considered to be diabetic if, within 4 days of STZ injection, nonfasting plasma glucose levels exceeded 300 mg/100 ml (Beckman glucose analyzer), osmotic diuresis was evident, and glucosuria was present as measured semiquantitatively with Tes-Tape (Eli Lilly Co., Indianapolis, IN). Galactosemia was induced and maintained by feeding the rats a diet consisting of 40% w/w D-galactose (United States Biochemical Corp., Cleveland, OH). This was provided as biscuits made with powdered standard laboratory chow, D-galactose, and water, mixed thoroughly, and oven dried at 50°C for 4 days. Presence of galactosemia was indicated by diuresis within 4 days, impaired weight gain (compared with controls), and the eventual development of cataracts as indicated by a change of eye color from red to opaque white (within 3–6 weeks). Age-matched controls were rats injected with the citrate buffer vehicle and fed a normal laboratory chow.

Cisplatin Administration. The general approach was to maintain the galactosemic, STZ-diabetic, and age-matched control rats for specified intervals of time without intervention (other than weighing and blood or urine sampling), and then inject them with the nephrotoxic dose of cisplatin. This was followed with evaluation and analysis. On the day of cisplatin injection, the rats were weighed and a tail vein blood sample was taken. Cisplatin (Alfa Products, Ward Hill, MA), dissolved immediately before injection in 0.9% saline (1.7 mg/ml), was administered intraperitoneally at a dose of 5 mg/kg body wt and the rats were returned to their cages. Unless otherwise stated, the animals were sacrificed by CO_2 administration in a closed chamber 96 hr after cisplatin administration. Blood and tissue were collected as needed.

Toxicity Assessment. Nephrotoxicity was assessed by comparing blood urea nitrogen (BUN) concentration (mg/100 ml in plasma) immediately before and 96 hr after cisplatin injection (2). BUN was quantified colorimetrically with a commercially available kit (Sigma procedure 640). The BUN level 96 hr after cisplatin injection was compared with the level in blood drawn immediately before the cisplatin injection. A BUN ratio (after to 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 (9), which is wide enough to allow a doubling before a value falls outside the normal range. No attempt was made to grade the degree of toxicity in this study.

Urine Collection. Urine volume was determined by placing the rats in individual metabolism cages arranged to allow quantitative collection of feces-free urine. Free access to food and water was allowed. The rats were typically maintained in these cages for 3 days and 24-hr urine volume recorded at the same time each morning. In some experiments, urine Pt concentrations were determined by atomic absorption spectroscopy as described below.

Platinum (Pt) Analysis. Pt levels in urine and nitric acid tissue digests were determined by flameless atomic absorption spectroscopy using a Perkin-Elmer model 380 spectrophotometer fitted with a model HGA400 programmer (2). Results are expressed as μg Pt/ml or μg Pt/g dry tissue wt. 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 following five-step program was used (temperature, ramp time in sec, hold time in sec): (i) 80°C, 15, 15; (ii) 150°C, 15, 15; (iii) 400°C, 5, 5; (iv) 1300°C, 5, 5; (v) 2650°C, 1, 5. 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 values fell within this range.

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 with Dunnet's comparison of treatments.

Results

Effect of Experimental Diabetes on Plasma Glucose, Body Weight, and Urine Output. Mean plasma glucose concentrations for the nondiabetic, age-matched controls and for the galactosemics never exceeded 165 mg/100 ml in any rat during the 16 weeks covered by this study. In contrast, all rats in the STZ group exhibited levels in excess of 400 mg/100 ml and there were no significant differences between the mean values seen 1 week after STZ injection (529 ± 62 mg/100 ml) versus 16 weeks after STZ injection (470 ± 31 mg/100 ml). Figure 1 represents mean body weights for the three groups of rats after 4, 8, and 16 weeks of treatment. Initial body weights for all rats fell within the range of 250–300 g. The expected steady weight gain was evident in the controls, but was not the case for the experimental diabetic groups. While the STZ-diabetic rats tended to lose weight, the galactosemics would be better characterized as failing to gain from their initial weight. Urine volume was significantly increased over controls in both STZ-diabetic and galactosemic rats (Fig. 2), with the effect being most pronounced in the STZ-diabetic group. This osmotic diuresis is expected in both models of experimental diabetes (1, 7, 8). All galactosemic rats, but none of the STZ-diabetic rats, had lens opacities (cataracts) within 5 weeks of initiation of the experiment, as has been reported by others (7, 10).

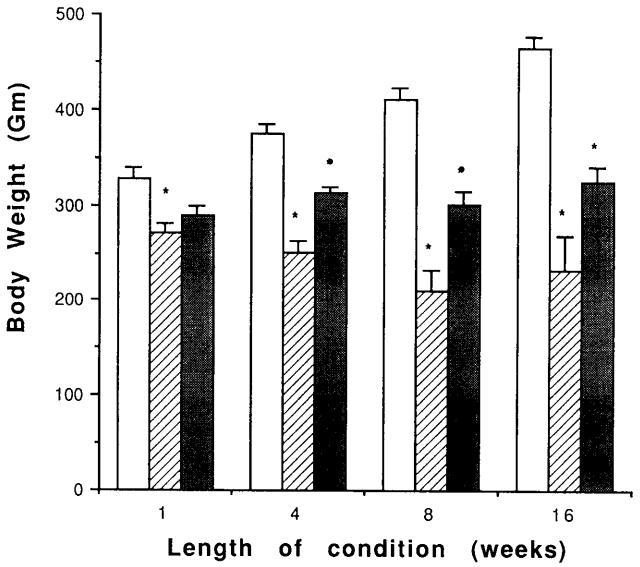

Figure 1. Comparison of body weights of nondiabetic, STZ-induced diabetic, and galactosemic rats. Values are the mean ± SD from at least four animals. An asterisk indicates significant difference from nondiabetic value. Clear bar = nondiabetic, hatched bar = STZ, and solid bar = galactosemic.

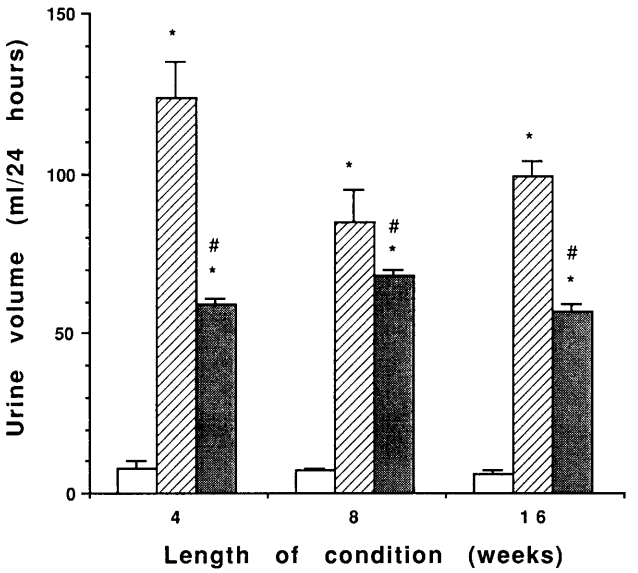

Figure 2. Comparison of daily urine volumes of nondiabetic, STZ-induced diabetic, and galactosemic rats. Animals were placed in individual metabolism cages arranged to allow quantitative collection of feces-free urine. Each value is the mean ± SD of at least four animals. Asterisk indicates significant difference from nondiabetic value. Cross-hatch indicates significant difference from STZ-diabetic value. Clear bar = nondiabetic, hatched bar = STZ, and solid bar = galactosemic.

Cisplatin Nephrotoxicity. Maximal functional disturbance in the kidneys of cisplatin-exposed rats occurs 3–5 days after intraperitoneal injection. This is commonly measured as a significant rise in the concentration of blood urea nitrogen concentration (1, 11, 12). The effect of duration of experimental diabetes on cisplatin nephrotoxicity is presented in Figure 3. In the STZ-diabetic group, the BUN ratio was not significantly different from that in the controls 1 week after induction of diabetes, but the cisplatin-induced rise in BUN level declined significantly after 4 weeks of diabetes and was virtually absent after 8 weeks. A similar pattern of progressive attenuation of toxicity was evident in the galactosemic rats, but the protective effect was slower to develop, with complete protection (no rise in BUN) evident only after 16 weeks.

Renal Cortex Platinum Level. The influence of duration of experimental diabetes on the uptake/retention of platinum by the renal cortex is shown in Figure 4. Levels measured 96 hr after injection are presented. The mean renal cortex platinum concentration in the 1-week STZ-diabetic rats was not significantly different from that measured in the controls. After 4 weeks, however, the concentrations in the STZ-diabetic rat kidneys were only 34.1% of that of the age-matched controls. In contrast, galactosemic rat kidneys contained a higher platinum concentration compared with the STZ diabetics, but this still represented a significantly lower percentage (78.5%) of the amount seen in the controls. The data show also that the significantly

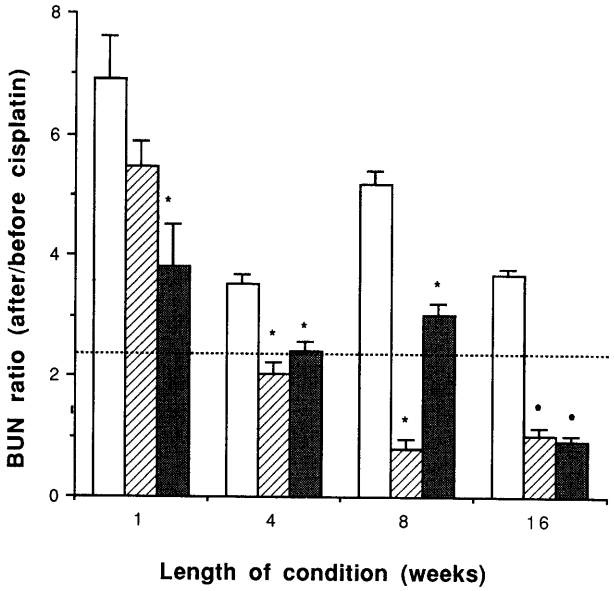

Figure 3. Protective effect of experimental diabetes from cisplatin-induced nephrotoxicity. Each value is the mean ± SD of four to six animals. BUN ratio is a comparison of the BUN concentration 96 hr after cisplatin injection (5 mg/kg body wt) to that obtained immediately before injection. A ratio value less than 2.5 (dotted line) was considered to be evidence of protection (see Methods). An asterisk indicates significant difference from nondiabetic value. Clear bar = nondiabetic, hatched bar = STZ, and solid bar = galactosemic.

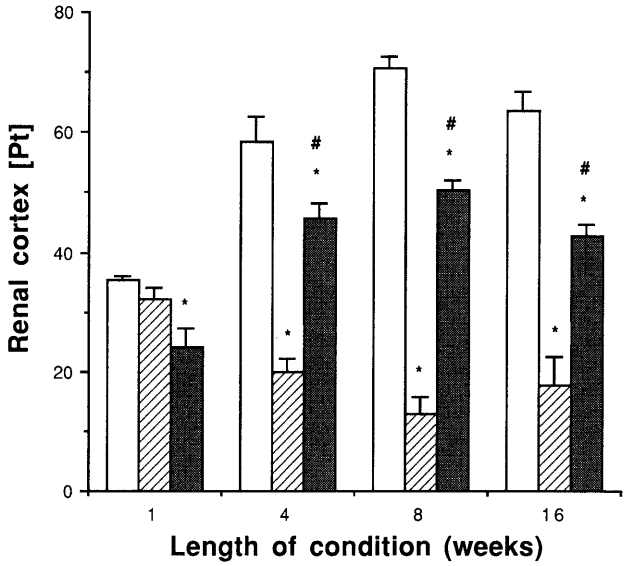

Figure 4. Effect of duration of experimental diabetes on rat renal cortex Pt accumulation after cisplatin administration. Rats were sacrificed 96 hr after intraperitoneal injection of cisplatin (5 mg/kg body wt) and renal cortex was sampled. Cortical Pt (μg/g dry wt) was quantified using flameless atomic absorption spectroscopy. Each value is the mean ± SD of at least four animals. Asterisk indicates significant difference from nondiabetic value and cross-hatch indicates significant difference from STZ-diabetic value. Clear bar = nondiabetic, hatched bar = STZ, and solid bar = galactosemic.

decreased renal cortical uptake and/or retention of platinum after cisplatin injection was virtually maximal within 4 weeks of induction of either STZ diabetes or galactosemia. In light of these results, the question arises as to whether the reduced levels of platinum 4 days after cisplatin injection reflect decreased initial accumulation or subsequent redistribution. Accordingly, we compared renal cortex platinum levels in 16-week STZ diabetics, 16-week galactosemics, and age-matched controls 30 min after intraperitoneal injection of 5 mg/kg of cisplatin (n = 3 rats/group). Renal cortex platinum concentration in the STZ diabetics was less than half that of the controls (27.1 μg/g dry tissue wt vs 62.7 μg/g dry tissue wt), with the level in the galactosemics intermediate (51.5 μg/g dry tissue wt). This reflects the hierarchy seen 4 days after cisplatin injection.

Discussion

The present results demonstrate that both models of experimental diabetes attenuate the cisplatin-induced rise in BUN and that the apparent protection is related to the duration of the treatment. Although the effect is slower to develop in galactosemia than in STZ diabetes, the attenuation of the toxicity is ultimately comparable in the two conditions. Cogent interpretation of these results requires consideration of the differing metabolic effects of the two models. STZ injection destroys most of the insulin-secreting β cells of the pancreas, causing major alterations in carbohydrate, lipid, and protein metabolism closely resembling those typical of human Type I diabetes mellitus. By contrast, galactosemia is a simplified model of diabetes in which a high blood sugar concentration is maintained in the presence of normal insulin levels and without the supranormal blood levels of glucose, cholesterol, nonesterified fatty acids, branched chain amino acids, and fibrinogen typical of STZ diabetes (8, 13). The galactosemia-induced protection eliminates STZ as the causative agent. Thus, our data support the hypothesis that attenuation of cisplatin-induced nephrotoxicity in experimental diabetes develops primarily as a result of renal changes secondary to chronically elevated plasma hexose levels and also show that the hexose does not have to be glucose.

The observable functional correlate seen in both models was a significant reduction in renal cortical platinum content after cisplatin injection compared with that seen in the kidneys of the age-matched control rats. It must be noted, however, that comparison of the influence of duration of diabetes on BUN ratio (Fig. 3) with the time-dependent change in renal platinum uptake/retention (Fig. 4) shows that while the latter was virtually maximally depressed when compared with controls within 4 weeks of treatment in both STZ-diabetic and galactosemic rats, the BUN ratio continued to decline with increasing duration of experimental diabetes. This was most striking after 16 weeks of treatment when cisplatin injection produced no significant rise in BUN levels in either STZ diabetics or

galactosemics, compared with a near quadrupling in the age-matched control rats despite widely differing renal platinum concentrations. This may mean that a toxicity threshold lies between the platinum levels seen in the protected 16-week galactosemics (43.1 μg/g dry tissue wt) and the unprotected controls (63.5 μg/g dry tissue wt). However, recent studies by Blisard *et al.* (14) suggest that renal platinum level does not correlate directly with cisplatin toxicity. These researchers compared the effects of cisplatin and its nontoxic isomer transplatin on the rat kidney. It was found that kidney platinum concentrations were significantly higher after transplatin injection. This suggests that the protection observed in the current study was more likely a consequence of diabetes-related alteration of intracellular platinum speciation and/or disposition than of reduced total platinum concentration *per se.* This deserves further investigation.

Although the current experiments were not designed to identify the link between chronically elevated blood hexose level and reduced renal toxicity of cisplatin, the results do indicate that the effect is not dependent upon glucose-specific biochemical changes in diabetic kidney. Valentovic *et al.* (15) have documented a significant increase in the rate and extent of Pt excretion after cisplatin administration to STZ-diabetic versus control Fischer 344 rats. While the relationship of this to protection from the nephrotoxic effect of cisplatin is undefined, it is possible that this was a factor in protection induced by galactosemia. This needs to be further investigated.

Excessive activity of the aldose reductase-dependent polyol pathway which converts aldoses to corresponding alcohols (i.e., glucose to sorbitol and galactose to galactitol) is a clearly identified consequence of both STZ diabetes and galactosemia (5, 8, 13). The exaggeration of the reactions of the pathway cause intracellular buildup of largely inert but osmotically active sugar alcohols and, consequently, disruptive cellular swelling. The reductase enzyme involved in the polyol pathway is localized in cells of the proximal tubules (i.e., the site of cisplatin-induced damage), but the relationship of polyol accumulation in the cells of diabetic rats to tubular cell changes is not clear. Although we did not quantify renal sorbitol and galactitol levels in these experiments, Lorentz *et al.* (8) compared renal cortical polyol concentrations in 7-week duration STZ-diabetic and galactosemic rats. They found that polyol levels were significantly increased to about the same extent over those of control rats in both diabetic models. The site of the polyol accumulation (i.e., glomerular versus tubular) was not reported. Bank *et al.* (13) subsequently reported that the glomerular hyperperfusion associated with galactosemia was prevented by the aldose reductase inhibitor sorbinil. Influence of this drug on other aspects of renal function in this model was not reported. It would be interesting to determine the effect of sorbinil treatment on development of protection from cisplatin-induced renal tubular toxicity. An alternative explanation is that attenuation of cisplatin-induced kidney damage as measured by a rise in BUN developed as a result of nonenzymatic glycosylation of renal proteins in the presence of chronically elevated hexose concentrations. A growing body of evidence suggests that end organ dysfunction in diabetes is associated with attachment of glucose to free amino groups in proteins. Initially reversible, the glycosylation process progresses to form advanced glycosylation end products that can irreversibly cross-link protein molecules, presumably leading to functional alterations (16). Glycosylation of hemoglobin is well known in human diabetes and has been shown to occur also in both galactosemic (8) and STZ-diabetic rats (7). Although glycosylation of renal proteins in STZ-diabetic rats has been reported (17), the identity of the proteins involved and a relationship to functional alterations in the kidney are unresolved. Glycosylation of renal proteins in galactosemic rats has not been reported to our knowledge. It is conceivable that glycosylation of tubular cell proteins could alter uptake and/or cellular distribution of cisplatin in experimental diabetes, thus altering response to the toxin.

1. Scott LA, Madan E, Valentovic MA. Attenuation of cisplatin nephrotoxicity by streptozotocin-induced diabetes. Fund Appl Toxicol **12**:530–539, 1989.
2. Cacini W, Singh Y. Renal metallothionein and platinum levels in diabetic and nondiabetic rats injected with cisplatin. Proc Soc Exp Biol Med **197**:285–289, 1991.
3. Kacew S, Bergeron MG. Pathogenic factors in aminoglycoside-induced nephrotoxicity. Toxicol Lett **51**:241–259, 1990.
4. Engerman RL, Kern TS. Experimental galactosemia produces diabetic-like retinopathy. Diabetes **33**:97–100, 1984.
5. Cheng H-M, Xiong H, Xiong J, Tsubota K. Metabolic studies of galactosemic cataract. Exp Eye Res **51**:345–349, 1990.
6. Sharma AK, Thomas PK, Baker RW. Peripheral nerve abnormalities related to galactose administration in rats. J Neurol Neurosurg **39**:794–802, 1976.
7. Kern TX, Engerman RL. Kidney morphology in experimental hyperglycemia. Diabetes **36**:244–249, 1987.
8. Lorentz WB, Shihabi ZK, Weidner N. Galactosemic nephropathy in the rat. Clin Physiol Biochem **5**:261–267, 1987.
9. Ringler DH, Dabich L. Hematology and clinical biochemistry. In: Baker HJ, Lindsey JR, Weisbroth SH, Eds. The Laboratory Rat: Biology and Diseases. New York: Academic Press, p112, 1979.
10. Daniels BS, Hostetter TH. Functional and structural alterations of the glomerular permeability barrier in experimental galactosemia. Kidney Int **39**:1104–1111, 1991.
11. Boogaard PJ, Lempers ELM, Mulder GH, Meerman JHN. 4-Methylthiobenzoic acid reduces cisplatin nephrotoxicity in rats without compromising antitumour activity. Biochem Pharmacol **41**:1997–2003, 1991.
12. Jones MM, Basinger MA, Holscher MA. Relative effectiveness of some compounds for the control of cisplatin-induced nephrotoxicity. Toxicology **68**:227–247, 1991.

13. Bank N, Coco M, Aynedjian HS. Galactose feeding causes glomerular hyperperfusion: Prevention by aldose reductase inhibition. Am J Physiol **256**:F994–F999, 1989.
14. Blisard KS, Harrington DA, Long DA, Jackson JE. Relative lack of toxicity of transplatin compared to cisplatin in rodents. J Comp Pathol **105**:367–376, 1991.
15. Valentovic MA, Scott LA, Madan E, Yokel RA. Renal accumulation and urinary excretion of cisplatin in diabetic rats. Toxicology **70**:151–162, 1991.
16. Brownlee M. Glycosylation products as toxic mediators of diabetic complications. Annu Rev Med **42**:159–166, 1991.
17. Yu DK, McLennan S, Turtle JR. Non-enzymatic glycosylation of tissue protein in diabetes in the rat. Diabetologia **24**:377–381, 1983.