Effect of Antihypertensive Therapy on Renal Injury in Type 2 Diabetic Rats With Hypertension

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Abstract—In a previous study, we demonstrated that doxazosin (DZN), an α1-adrenergic blocker, prevented proteinuria in streptozotocin diabetic rats. In this study, we investigated whether DZN would lower established proteinuria by improving glomerular sclerosis in spontaneously hypertensive corpulent rats with type 2 diabetes mellitus. DZN treatment was compared with treatment with angiotensin-converting enzyme inhibitor, lisinopril (LIS) alone, and DZN in combination with LIS. Combination therapy was used to examine any additive effect of either drug alone in the reduction of proteinuria and glomerular sclerosis. Both male and female rats age 6 months with established proteinuria were used. The rats were allocated randomly to 1 of 4 groups: untreated, DZN treated, LIS treated, or a combination of DZN and LIS treatment. Drug treatment was continued for 16 weeks. The results show that (1) either drug alone or in combination significantly lowered systolic blood pressure; (2) DZN, LIS, or combination therapy reduced albuminuria at 16 weeks of treatment from baseline by 38.61±5.77%, 30.70±4.21%, and 42.17±4.77% (mean±SE), respectively. No difference in albuminuria was observed among the 3 groups of rats; (3) the fractional mesangial area, which was 20.55±3.77% in untreated rats, was significantly reduced to 11.18±1.32% in DZN-treated rats, with a further reduction to 8.72±0.64% in LIS-treated rats and to 3.48±0.35% in rats treated with DZN+LIS; and (4) DZN but not LIS significantly improved plasma glucose levels in spontaneously hypertensive corpulent rats (untreated 21.06±0.97 mmol/L versus DZN treated 15.81±0.93 mmol/L or DZN+LIS treated 17.38±1.10 mmol/L; P<0.025 to 0.005). Thus, the data suggest that 16-week treatment with either DZN or LIS improves established proteinuria and glomerular sclerosis, but combination therapy is superior to either DZN or LIS alone in preventing glomerular sclerosis in type 2 diabetic rats with hypertension. (Hypertension. 2000;36:233-238.)

Key Words: diabetic nephropathy □ rats, spontaneously hypertensive □ adrenergic receptor blocker □ antihypertensive agents □ angiotensin-converting enzyme inhibitors □ albuminuria □ glomerulosclerosis

Diabetes mellitus is the leading cause of end-stage renal disease in the United States.1 Both type 1 and type 2 diabetic patients are at risk for the development of nephropathy because of hyperglycemia and its consequences. Morphologically, either diffuse or selective expansion of the mesangial matrix, which causes obliteration of the capillary lumen and loss of filtration surface area, is characteristic of diabetic nephropathy. Clinically, microalbuminuria, which is defined as urinary albumin excretion of 30 to 300 mg/d, is the hallmark of diabetic nephropathy. Several studies have shown that microalbuminuria can predict the later development of diabetic nephropathy in both types of diabetic subjects.2,3 Prevention of microalbuminuria by good glycemic control was found to preserve renal function and to delay the progression of diabetic nephropathy in animals and human subjects.4–6 In addition to hyperglycemia, systemic hypertension can aggravate diabetic nephropathy. Several studies have shown that control of hypertension not only improves proteinuria but also delays the progression of diabetic nephropathy.7–9 Among antihypertensive drugs, the angiotensin-converting enzyme (ACE) inhibitors were found to be therapeutically more efficacious in protecting the kidney than other groups of antihypertensive drugs.2,10–13 However, some calcium blockers,14–16 α1-receptor blockers,17,18 and β-blockers12,19 were also found to prevent proteinuria in diabetic patients and animals.

We previously reported that doxazosin (DZN), an α1-adrenergic blocker, improves albuminuria by preventing glomerular loss of heparan sulfate proteoglycan in streptozotocin diabetic rats.17 In addition, DZN was found to lower plasma glucose in these diabetic rats. Drug treatment began 1 week after induction of diabetes; therefore, DZN improved albuminuria and plasma glucose by attenuating the rise in blood pressure. It is, however, not known whether DZN improves proteinuria and plasma glucose in type 2 diabetic rats with established proteinuria and hypertension.

To accomplish this objective, we used spontaneously hypertensive/NIH-corpulent rats (SHR/N-cp) with type 2 diabetes. This rodent model was developed by Dr Carl T. Hansen at the National Institutes of Health (NIH); it was...
Initially bred by mating an obese Kolletsky rat to a female SHR of the Okamoto strain. After several backcrosses, the resultant SHR/N-cp rats were hypertensive with hyperglycemia, hyperinsulinemia, proteinuria, and glomerular lesions resembling diabetic glomerular sclerosis in humans. Thus, this rat represents a suitable animal model for the study of proteinuria and associated glomerular sclerosis observed in humans with type 2 diabetes and hypertension. An ACE inhibitor, perindopril, was found to ameliorate glomerular and tubulointerstitial injury in this rat model. However, the effect of combination therapy with DZN and an ACE inhibitor on established proteinuria and glomerular sclerosis has not been studied. The purpose of this study was, therefore, to: (1) examine whether DZN and an ACE inhibitor (lisinopril, LIS) lower blood pressure in SHR/N-cp rats; (2) investigate whether these antihypertensive drugs improve proteinuria, glomerular sclerosis, and plasma glucose; and (3) study whether a combination of DZN and LIS is superior to either drug alone in improving proteinuria and glomerular sclerosis.

Methods

Animals

A total of 33 male and female SHR/N-cp rats were used in the study. These rats were initially obtained from Dr Carl T. Hansen. In our laboratory, we selectively inbred rats with plasma glucose levels >12 mmol/L and urine albumin >10 mg/dL. After 4 generations of inbreeding, both male and female rats age 24 weeks were randomly divided into 4 groups and allocated to various regimens as follows: 9 rats on DZN (32 mg/100 mL) in tap water, 7 rats on LIS (7 mg/100 mL) in tap water, 8 rats on both DZN and LIS in the above dosages, and 9 rats on tap water. DZN and LIS were first dissolved in distilled water and were made to the required volume with tap water. Water was changed daily for all groups of rats. The daily consumption of DZN and LIS sufficient to lower blood pressure was 12.30±0.87 and 4.37±0.26 mg (mean±SE), respectively. Drug treatment was continued for 16 weeks. All groups of rats were fed Purina rodent chow 5001 ad libitum with the following composition by weight: protein 23.4%, total fat 10%, carbohydrate 49%, and minerals (ash and vitamins) 17.6%. The energy provided was 4.00 kcal/g. The protocol was approved by the Institutional Animal Care and Use Committee (University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark).

Blood Pressure and Urine Collection

Systolic blood pressure was determined in conscious rats by the tail-cuff method at the beginning of the study and every 4 weeks until euthanization. At the start and end of the study, each rat was placed in a metabolic cage for 24-hour urine collection, at which time body weight and food and water intakes were recorded. After total volume was measured, the urine was centrifuged and used for the determination of albumin.

Plasma Glucose and Urinary Albumin

Blood glucose was determined from tail bleeding at the beginning of the study and every 4 weeks thereafter by the glucose oxidase method with reagents supplied by Sigma Chemical Co. Urinary albumin was determined by the radioimmunoassay method of Brodows et al.

Glomerular Morphometry

Coronal sections of renal tissue (2 μm thick) were stained with periodic acid–Schiff stain and were examined by light microscopy in a blinded fashion. Glomerular volume and fractional mesangial area measurements were performed by digital image analysis with the Image-ProPlus (version 3.0) software system developed by Media Cybernetics. Instrumentation consisted of a microscope, a microscope-mounted CCD camera (768×493 resolution), and an IBM computer with a color video screen for projecting and manipulating images. A minimum of 10 glomeruli were selected randomly for morphometric analysis. Images of glomeruli at ×400 magnification were digitized, presented in pseudocolor, and saved. The digitized images were then projected on the computer screen and analyzed at a resolution of 768×493 pixels. The glomerular area or volume, defined as the cross-sectional area of the renal corpuscle bounded by the Bowman’s capsule, was determined by manually outlining the Bowman’s capsule on the image screen with the cursor, and the area was automatically calculated by the computer. The area of the mesangial matrix was measured by a pseudocolor image aid visualization of the mesangium. The fractional mesangial area was expressed as the percentage of the glomerular area.

Statistical Analysis

Data were analyzed by 1-way ANOVA for comparisons between groups and expressed as mean±SE. Significances among groups were further verified by the Tukey test. Differences within a group before and after treatment were assessed by Student’s paired t test. Values were considered significant at P<0.05.

Results

Table 1 shows pertinent information in various groups of rats at the time of euthanization. No significant differences were found in body weight, kidney weight, daily water intake, food consumption, and urine output between untreated and treated diabetic rats. However, plasma glucose was significantly lower in DZN-treated (P<0.005) and DZN+LIS-treated (P<0.025) groups than in untreated diabetic rats.

At start, systolic blood pressure was significantly higher in rats allocated to LIS or combination therapy compared with those rats allocated for DZN treatment or for those untreated. In the untreated group, there was a gradual increase in systolic blood pressure (Figure 1). This increase was significantly blunted in rats treated with antihypertensive drugs. A significantly lower systolic blood pressure was observed in LIS-treated rats and in rats that received combination therapy compared with DZN-treated rats; however, no difference was noted between LIS-treated rats and those that received com-
combination therapy. The percent change in albuminuria from baseline at 16 weeks of treatment in various groups is shown in Figure 2. There was an increase of 44.25 ± 21.06% in albuminuria in untreated rats. In contrast, antihypertensive drugs lowered albuminuria by 38.61 ± 5.77%, 30.70 ± 4.21%, and 42.17 ± 4.77%, respectively, in DZN-, LIS-, and DZN+LIS-treated rats. No difference in albuminuria was noted among the 3 groups of diabetic rats.

Figure 3 shows the fractional mesangial area and glomerular volume in various groups of animals. In untreated rats, this fractional mesangial area composed 20.55 ± 3.77% of the glomerulus, which was significantly reduced to 11.18 ± 1.32% in DZN-treated rats (P < 0.05) with further reduction to 8.72 ± 0.64% in LIS-treated rats (P < 0.005). The improvement in fractional mesangial area in LIS-treated rats was significant (P < 0.001) compared with DZN-treated rats. Combination therapy significantly (P < 0.001) reduced the fractional mesangial area to 3.48 ± 0.35% compared with either untreated or treated rats with DZN or LIS. No difference in glomerular volume was observed among the various groups of rats.

Representative glomeruli from various groups of rats are shown in Figure 4. Untreated diabetic rats demonstrated more deposition of periodic acid–Schiff–positive material than other groups of treated rats. Also, glomerular capillaries were better preserved in treated rats than in untreated rats.

Discussion

In this study, we demonstrated that (1) both DZN and LIS lower blood pressure in SHR/N-cp rats with type 2 diabetes at a mean concentration of 12.30 ± 0.87 and 4.57 ± 0.26 mg/d, respectively; (2) treatment for 16 weeks with DZN, LIS, or a combination of these agents improves established albuminuria; (3) both DZN and LIS decrease glomerular sclerosis significantly, but this decrease is more prominent in rats treated with combined therapy; and (4) only DZN but not LIS lowers plasma glucose levels in these diabetic rats.

This is the first study that showed an improvement in glomerular sclerosis in diabetic rats by α₁-blockade. Although the mechanism of this improvement is not understood, a recent study demonstrated focal tubulointerstitial fibrosis and upregulation of transforming growth factor-β1 (TGF-β1) in rats with chronic infusion of phenylephrine. It is possible that α₁-blockade decreased the expression of TGF-β1 in glomeruli of diabetic rats and thus improved glomerular sclerosis. This hypothesis is consistent with the observation of Takahashi et al., who showed the suppression of TGF-β1 mRNA expression by prazosin (another α₁-blocker) in ventricular myocytes from hypertrophied hearts. The finding that LIS also improved glomerular sclerosis is not unexpected, because ACE inhibitors were found to ameliorate renal injury in both diabetic and nondiabetic renal diseases with downregulation of TGF-β1 mRNA levels. Our data support the observations of Velasquez et al., who demonstrated the amelioration of both glomerular and tubulointerstitial fibrosis by another ACE inhibitor (perindopril) in these hypertensive diabetic rats, and the observations of Verseput et al. in fawn-hooded rats treated with LIS.

Of interest is the demonstration of minimal glomerular sclerosis with combination therapy. How this combination therapy with similar blood pressure control is superior to either DZN or LIS treatment alone is unclear. However, it is possible that combination therapy may have resulted in normalization of TGF-β1 and extracellular matrix production. Recent studies have shown that combination therapy (an ACE

Figure 1. Systolic blood pressure (mean ± SE) measured at 4-week intervals in untreated (n=9), DZN-treated (n=9), LIS-treated (n=7), and DZN+LIS-treated (n=8) diabetic rats. The SEs of the mean are not shown because they are on the order of the dimensions of the symbols. Asterisks denote statistical significance (P < 0.001) between untreated and DZN-treated rats or between DZN-treated and LIS-treated rats or combination therapy.

Figure 2. The percent change (mean ± SE) from baseline in albuminuria at 16 weeks in untreated (n=9), DZN-treated (n=9), LIS-treated (n=7), and DZN+LIS-treated (n=8) diabetic rats.

Figure 3. Glomerular morphometry (mean ± SE) in various groups of rats. Numbers in parentheses represent the number of animals in each group.
inhibitor plus a calcium antagonist) is more effective than either drug alone in ameliorating glomerular sclerosis in animals or albuminuria in diabetic patients.

Albuminuria, the clinical hallmark of diabetic nephropathy, was equally reduced by DZN, LIS, and combination therapy. This suggests that not only ACE inhibitors but also \( \alpha_1 \)-blockers are effective in reducing established proteinuria in hypertensive type 2 diabetic rats. The mechanisms for the improvement in proteinuria may relate to the preservation of glomerular heparan sulfate, as shown with DZN and ACE inhibitors. Mechanisms such as control of glomerular hypertension and maintenance of glomerular capillary permeability and renal autoregulation may have played a role in reducing albuminuria. Also, amelioration of glomerular sclerosis may in part be responsible for the improvement in proteinuria. DZN and prazosin have been shown to improve albuminuria in hypertensive patients and in patients with kidney disease, although a recent short-term (4 months of treatment) study failed to demonstrate a reduction in albuminuria in diabetic patients treated with DZN. However, in this study, when DZN was combined with an ACE inhibitor (cilazapril), the reduction in albuminuria was much greater than with either drug alone. The discrepancy between our study and that of Rachmani et al. appeared to be related to the duration of treatment, because >4 months of treatment in type 2 diabetics causes a significant reduction in proteinuria by DZN.

Blood pressure reduction seems to be the major intervention in ameliorating both albuminuria and glomerular sclerosis in DZN- and LIS-treated rats. However, the improvement in glomerular sclerosis with combination therapy compared with LIS therapy with similar blood pressure reduction (Figure 1) implies mechanisms other than blood pressure reduction in preserving renal function. In our study, we measured only systolic blood pressure at 4-week intervals. The use of 24-hour blood pressure monitoring, with telemetry similar to that of Bakris et al., may have yielded better blood pressure values in various groups of rats.

A significant decrease in plasma glucose concentration by DZN and not by LIS confirms our similar observation in streptozotocin diabetic rats. The mechanism by which DZN improves plasma glucose is unclear. However, DZN has been shown to lower plasma glucose and insulin levels without altering glycosylated HbA\(_1\) in type 2 diabetics with hypertension after 6 weeks of treatment. Similar results were observed in essential hypertensive subjects treated with DZN.

Figure 4. Representative glomeruli from untreated (A), DZN-treated (B), LIS-treated (C), and DZN+LIS-treated (D) rats. Periodic acid–Schiff stain; magnification \( \times 400 \).
for 26 weeks. Lithell and Kageyama et al reported improved insulin sensitivity with euglycemic insulin clamp studies in essential hypertensive subjects after DZN therapy. Giordano et al reported increased total body glucose uptake in type 2 diabetic patients with hypertension who were treated with DZN for 12 weeks. These authors reported no effect on fasting plasma glucose of HbA1c concentrations, but the response of glucose to an oral glucose load was significantly improved. This is consistent with studies that showed improved insulin action in type 2 diabetic patients with hypertension. In contrast, Maheux et al demonstrated no effect of DZN on either insulin-mediated glucose disposal or plasma insulin levels in type 2 diabetics with hypertension. However, in nondiabetic hypertensive patients, DZN treatment was associated with a significant improvement in insulin sensitivity with euglycemic insulin clamp studies. Although other studies indicate that DZN improves glucose metabolism was associated with a significant improvement in fasting plasma glucose of HbA1c concentrations, but the effect of DZN on either insulin-mediated glucose disposal or plasma insulin levels in type 2 diabetics with hypertension. However, in nondiabetic hypertensive patients, DZN treatment was associated with a significant improvement in insulin-mediated glucose disposal. Except for this report, the other studies indicate that DZN improves glucose metabolism in patients with type 2 diabetes. Our results of improved circulating glucose levels are consistent with these studies.

In conclusion, the data suggest that DZN and LIS treatments are effective in reducing even established proteinuria, possibly by preserving the diabetes-induced decrease in glomerular heparan sulfate and by ameliorating glomerular sclerosis in hypertensive rats with type 2 diabetes. However, combination therapy was superior to either DZN or LIS treatment in preventing glomerular sclerosis in these rats. The observed effect of DZN in lowering blood glucose levels in the present as well as a previous study is an important finding that warrants extensive investigation in diabetic subjects.

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References


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