Potential Role of Angiotensin-Converting Enzyme Inhibitors and Statins on Early Podocyte Damage in a Model of Type 2 Diabetes Mellitus, Obesity, and Mild Hypertension

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Background: Experimental findings suggest that the obese Zucker rat (OZR) is a model of type 2 diabetes–related nephropathy with several metabolic abnormalities. However, the exact mechanisms by which these factors cause early glomerulosclerosis and proteinuria remain unclear. Furthermore, structural abnormalities and regulation of podocytes have recently emerged as prominent underlying factors in proteinuria. The aim of this study was to evaluate the potential role of angiotensin-converting enzyme inhibitors and statins on early podocyte damage in an experimental model of type 2 diabetes mellitus.

Methods: We used OZR to evaluate some of the pathogenic mechanisms and the effects of two drugs, an angiotensin-converting enzyme (ACE) inhibitor (quinapril) and a statin (atorvastatin), involved in the development of proteinuria and especially podocyte damage. We studied glomerular and tubulointerstitial injury by assessing inflammation mediators (murine monoclonal antibody against CD68 [ED1$^+$], interleukin-8 [IL-8], interferon-γ–inducible protein 10 [IP-10]) and podocyte damage markers using desmin staining and electron microscopy.

Results: Glomerular lesions were correlated with cholesterol ($r = 0.676$), proteinuria ($r = 0.804$), triglycerides ($r = 0.593$), insulin ($r = 0.345$), creatinine ($r = 0.266$), and glucose ($r = 0.245$). In addition, podocytes from OZR showed positive staining for desmin. Use of the ACE inhibitor quinapril normalized proteinuria, cholesterol levels, glomerular lesions, and podocyte morphology. In contrast, atorvastatin ameliorated but did not normalize renal damage, with a partial reduction in desmin staining and podocyte morphology. Treatment with both drugs resulted in only a slight reduction in IL-8 and IP-10 in the tubulointerstitium.

Conclusions: In the OZR, cholesterol was an important determinant of renal injury. Most notably, glomerulosclerosis in the OZR Is characterized by early podocyte damage and tubulointerstitial injury. In addition, our findings showed that quinapril primarily normalized podocyte morphology, whereas atorvastatin ameliorated renal lesions through the diminution of lipids and by its lipid-independent pleiotropic effect. Am J Hypertens 2005;18:557–565 © 2005 American Journal of Hypertension, Ltd.

Key Words: Zucker rats, diabetic nephropathy, podocytes, cholesterol, angiotensin-converting enzyme inhibitors, statins.

Experimental findings suggest that the obese Zucker rat (OZR) is a model of type 2 diabetes and glomerulosclerosis in which lipid abnormalities and insulin levels could have an important role.[1] Dyslipidemia is one of the non-immunologic factors that contributes to the progression of glomerulosclerosis as well as to kidney disease associated with diabetes mellitus and the nephrotic syndrome.[2] However, the exact mechanisms by which these factors cause glomerulosclerosis or contribute to diabetic nephropathy remain unclear. In particular, it is unclear whether renal injury in patients with proteinuric diseases is associated with damaged podocytes. Furthermore, structural abnormalities and regulation of podocytes have recently emerged as prominent underlying factors in proteinuria and glomerulosclerosis.[3,4]

We previously reported the protective effect of angiotensin-converting enzyme (ACE) inhibition, which diminishes proteinuria and cholesterol and also delays the

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Table 1. Experimental design

Group	Dose	Treatment	16 Weeks of age	24 Weeks of age	32 Weeks of age
LZR untreated	—	—	8	8	8
OZR untreated	—	—	8	8	8
OZR quinapril	10 mg/kg/day	Quinapril	—	8	8
OZR atorvastatin	10 mg/kg/day	Atorvastatin	—	8	8

LZR = lean zucker rats; OZR = obese zucker rats.
Numbers are numbers of animals in each group.

evolution of nephropathy, probably through its effects on angiotensin II.[5] In addition, the administration of lipid-lowering agents such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors decreases serum cholesterol, proteinuria and focal glomerulosclerosis in experimental animals and in patients with the nephrotic syndrome. O'Donnell et al[1] reported that lovastatin slowed the progression of established glomerular disease in OZR. Moreover, it has recently been demonstrated that statins may reduce proteinuria in the diabetic population.[6] However, there are no studies in which podocyte damage have been correlated with statins.

For the above-mentioned reasons, the present article focuses on some of the molecular and morphologic mechanisms potentially underlying the development of proteinuria and glomerulosclerosis, using OZR with genotype-related diabetic nephropathy similar to type II diabetes mellitus. We examined podocyte damage, glomerular cytokine expression, and tubulointerstitium injury during this early nephropathic state. In contrast to most studies that have focused on the role of the mesangium, we also examined the contribution of podocyte cells to the development of glomerulosclerosis in an experimental plurimetabolic model showing significant proteinuria.

We also evaluated the effects of two drugs, an ACE inhibitor (quinapril) and an inhibitor of HMG-CoA reductase (atorvastatin) widely used in diabetic nephropathy, along with the evolution of the glomerulosclerosis and role that these agents play in reducing serum lipids. Biochemical and histologic studies were performed to determine the stages of nephropathy. In addition, we also studied glomerular and tubulointerstitial injury by assessing inflammation mediators (murine monoclonal antibody against CD68 [ED1$^+$], interleukin-8 (IL-8), interferon-γ–inducible protein–10 [IP-10]) and podocyte damage markers using desmin staining [8] and electron microscopy[7].

Methods

Experiments were performed in Zucker rats that were obtained from an established colony at the animal breeding center of IFFA CREDO (Lyon, France). They were kept in accordance with the Catalan Royal Decree 214/1997 (DOGC 30/7/1997) concerning the protection of experimental animals. The animal center maintained constant humidity, temperature, and air ventilation with a light–dark cycle of 12 h.

The animals were randomly assigned into four groups (Table 1). Two control groups comprised lean Zucker rats (LZR) (n = 24) and obese Zucker rats (OZR) (n = 24) that received tap water ad libitum. The third group consisted of OZR (n = 16) that received quinapril (Pfizer, Madrid, Spain) at 10 mg/kg/day from 8 weeks of age. The drug was dissolved in drinking water and the concentration was adjusted for daily water intake. The fourth group consisted of OZR (n = 16) that received atorvastatin in the pellet chow (Pfizer, Madrid, Spain) at 10 mg/kg/day from 8 weeks of age. The rats ingested a standard chow diet (A04, Panlab, Barcelona, Spain) throughout the study period.

Eight animals from each of the two untreated groups were killed at 16 weeks of age. In addition, eight animals from each of the four groups were killed at 24 and 32 weeks of age. Body weight was recorded once per week. Diuresis with 24-h urine collection and blood pressure (BP) measurement by the tail-cuff method were performed once per month.

Measurements of metabolic and renal parameters were performed at three time points as follows. Systolic BP (n = 136 measurements) was measured by the tail-cuff technique under nonstressed conditions with a validated pressure monitor (LE-5001, Letica Scientific Instruments, Barcelona, Spain). Biochemical studies (glucose, cholesterol, triglycerides, creatinine, and total proteins) (n = 80 measurements) were measured in fasting serum samples obtained from a central arterial with a clinical analyzer (Technicon Instruments, Tarrytown, NY). In the 24-h urine samples (n = 136 measurements), urine protein concentration (mg/day) was assessed by precipitation with sulfosalicylic acid. Insulin plasma concentrations were determined using a rat insulin radioimmunoassay kit (Linco, St. Charles, MO).

Morphologic Studies

On the day that they were killed, animals were anesthetized with an intraperitoneal injection of a mixture of atropine (1 mg), diazepam (5 mg), and Ketolar (Pfizer, Madrid, Spain) (20 mg), one dose per 400 g of body weight. The abdominal cavity was opened and the aorta was cannulated just above the bifurcation. Kidneys were perfused with 20 to 30 mL of a cold saline solution, removed, and stored in cold phosphate-buffered