ORIGINAL ARTICLES

Alterations of glomerular and extracellular levels of glutathione peroxidase in patients and experimental rats with diabetic nephropathy

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To investigate the status and role of glutathione peroxidase (GPX) in diabetic nephropathy, we measured GPX in the plasma and urine of 14 patients with diabetic glomerulosclerosis (DGS) and measured glomerular GPX immunostaining in these patients and in rats with streptozotocin-induced diabetes of varying duration. Plasma GPX levels were significantly lower in DGS patients than in diabetic patients without nephropathy ($P < .05$) or normal controls ($P < .01$). Urinary GPX concentrations were also significantly lower in DGS patients than in diabetic patients without nephropathy or normal controls (both $P < .05$). Immunostaining of glomerular GPX was significantly less in DGS patients than in normal controls ($P < .05$) and was negatively correlated with the glomerular sclerosis score and the index of mesangial expansion. Serial examination of glomerular GPX in diabetic rats showed that immunostaining scores for glomerular GPX in rats were significantly lower than those in normal control rats after 1 and 3 months' duration of diabetes, and staining scores were also significantly lower in rats killed after 3 months of diabetes than in those killed after 1 week. In conclusion, our study demonstrates that GPX concentrations in plasma, urine, and glomeruli are decreased in individuals with DGS and that the immunostaining of glomerular GPX decreases progressively. (J Lab Clin Med 2005;145:181–6)

Abbreviations: CCR = creatinine-clearance rate; DGS = diabetic glomerulosclerosis; EDTA = ethylenediaminetetraacetate; GPX = glutathione peroxidase; HbA_{1c} = hemoglobin A_{1c}; ROS = reactive oxygen species; SOD = superoxide dismutase

The number of individuals with diabetes and end-stage renal disease is increasing dramatically worldwide, and in many countries diabetes has become the single most frequent cause of end-stage renal disease.[1–3] Its progressive nature and poor response to renal replacement therapy has led nephrologists to seek a better understanding of its pathogenesis. We recently found that ROS are involved in the pathogenesis of diabetic nephropathy.[4] ROS induce lipid peroxidation,[5] mediate tissue injuries induced by cytokines and growth factors,[6] induce oxidation of low-density lipoprotein,[7] and inactivate vasodilating nitric oxide.[8] Depletion of antioxidants and the resulting ROS imbalance may be as important as increased ROS activity in ROS-related injury.[9] Reduced antioxidant activity and increased ROS production result in increased oxidative stress and may contribute to the development of cardiovascular complications in individuals with diabetes.[10]

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A recent study by Hartnett et al has demonstrated decreased serum GPX levels in patients with diabetic retinopathy.[11] However, no reports on the status of GPX in the kidneys of individuals with diabetes have been published. GPX is an important antioxidant enzyme that catalyzes the reduction of organic hydroperoxides and hydrogen peroxide, using glutathione as the reducing agent. The findings of 1 recent study involving rats indicate that plasma DPX is synthesized mainly in the kidney.[12] Because GPX plays an important role in the protection of extracellular fluid components and cell surfaces against peroxide-mediated damage,[13] any reduction in its activity may therefore result in increased lipid peroxidation and potentially in an increased risk of atherosclerosis. In this study we therefore assessed glomerular immunostaining of GPX and measured the GPX concentrations in the plasma and urine of patients with DGS, then compared our findings with those from diabetic patients without proteinuria and normal controls. We also studied rats with streptozotocin-induced diabetes.

METHODS

Study population. We studied plasma and urine specimens from 14 patients with type 2 diabetes and biopsy-proved DGS, 16 patients with type 2 diabetes but no proteinuria, and 16 normal controls.

The 14 patients with DGS comprised 8 men and 6 women. Their mean ± SD serum creatinine concentration was 94 ± 9.6 μmol/L, and the CCR was 98 ± 9 mL/min. Their mean age was 45 ± 8 years old, the known duration of diabetes was 12 ± 5 years, and daily protein loss was 1.9 ± 1.0 g/d. All patients had retinopathy at the time of diagnosis.

The 16 patients with diabetes but no proteinuria comprised 7 men and 9 women. Their serum creatinine concentration was 81 ± 17 μmol/L, and the CCR was 104 ± 12 mL/min. Their mean age was 52 ± 9 years old, the known duration of diabetes was 11 ± 6 years, and daily protein loss was less than 150 mg/d.

All diabetic patients were following a prescribed diet and therapy with hypoglycemic agents; none of the study subjects was taking antioxidant supplementation or had clinically symptomatic macroangiopathy.

The normal controls comprised 7 men and 9 women with a mean age of 42 ± 9 years. Five kidneys obtained from patients who had undergone nephrectomy as part of the treatment of renal cancer served as normal controls for renal changes. All subjects were nonsmokers, and patients with chronic hepatitis, hematologic or inflammatory disorders, or cancer; alcohol abusers; and individuals undergoing immunosuppressive therapy were excluded. Informed consent was obtained from all subjects, and the study was approved by the Human Ethics Committee of Kaohsiung Medical University.

Venous blood (10 mL) and urine from patients and controls was collected in standard tubes containing 5 mmol/L EDTA. After centrifugation at 600*g* for 10 minutes at 4°C, plasma was stored at −80°C until it could be assayed. All biochemical analysis was performed immediately after sample collection. We assayed plasma glucose using the glucose-oxidase method, HbA_{1C} was measured with the use of affinity chromatography, and cholesterol and triglyceride concentrations were measured enzymatically with the use of a clinical chemistry analyzer. All chemicals were purchased from Sigma-Aldrich (St Louis, Mo) unless otherwise indicated.

Glomerular immunoreactivity of GPX was also measured in diabetic rats. Male Wistar rats weighing 200 to 250 g were injected intraperitoneally with 55 mg/kg streptozotocin and used for experiments after diabetes mellitus was diagnosed. Rats were classified into 3 groups, each containing 9 rats: Group 1 rats were killed 1 week after the induction of diabetes, group 2 rats were killed 1 month after the induction of diabetes, and group 3 rats were killed 3 months after the induction of diabetes. In the cases of all 3 groups, the same number of normal control rats was killed at the same time as the experimental animals. We injected rats in groups 2 and 3 with insulin (heat-treated bovine ultralente insulin; Novo-Nordisk, Copenhagen, Denmark) every day to maintain a poorly controlled diabetic state. Plasma glucose was regularly checked and maintained at a level greater than 450 mg/dL. All rats were allowed food and water ad libitum. The study was approved by the Animal Care and Treatment Committee of our institution.

Tissue processing, scoring of glomerulosclerosis, and index of mesangial expansion. Kidney tissues from both human subjects and rats were immediately fixed in 10% neutral buffered formaldehyde overnight, then dehydrated with alcohol and embedded in paraffin for light microscopy. The tissues were also immediately fixed in 2.5% glutaldehyde and 1% OsO_4, dehydrated in graded alcohols, and embedded in spur resin for electron microscopy. The glomeruli demonstrating sclerosis were counted and scored on a scale of 0 to 4, according to the percentage of glomeruli involved (0% = 0, 1%–25% = 1, 25%–50% = 2, >50% = 3, global sclerosis = 4). We counted 25 glomeruli to get the final score, which ranged from 1 to 100 for each patient. The index of mesangial expansion was determined with the use of a semiquantitative estimate of the width of mesangial zones in each glomerulus,[14] with some modification. In brief, 0 was used to designate a normal area, 1.0 was twice the normal area, and 2.0 was 3 times the normal area, and so on. Half grades were assigned where appropriate. The total grade for 25 glomeruli in each patient represents the score of mesangial expansion in each patient. This light-microscopic parameter of the index of mesangial expansion has been reported to correlate highly with the percentage of total mesangium, the percentage of mesangial matrix, and the percentage of cellular mesangium.[14]

Measurement of plasma and urinary GPX. We measured GPX in both plasma and urine using a commercial kit (Calbiochem, Darmstadt, Germany). In brief, 100 μL of sample was added to microplates coated with polyclonal antibody specific for human plasma–specific GPX, incubated for 2 hours, and washed with buffer 5 times. After washing, 100 μL of anti–plasma-specific GPX was added, after which the preparation was incubated for 1 hour and washed 5 more