Effect of Hyperlipidemia on Nephropathy in Streptozotocin-Induced Diabetic Rats — Electron Microscopy —

Hisako Murakami¹², Akiko Murata¹, and Kosaku Fujiwara²

¹Biological Research Laboratories, Lederle (Japan), Ltd., ²Department of Pathobiology, Nihon University School of Veterinary Medicine

Abstract: The effect of hyperlipidemia induced by cholesterol (CHS)-feeding or Triton WR-1339 (Triton) injection on renal glomerular lesions in the streptozotocin (STZ)-induced diabetic rats was ultrastructurally studied. In non-diabetic and hyperlipidemic rats, mesangial cells contained electron-lucid vacuoles or droplets but showed no cellular proliferation or increase in type IV collagen and fibronectin positive areas. In STZ-diabetic rats, however, either with or without lipidemia mesangial cells were significantly increased in number, showing "mesangial interposition" between the capillary lamina densa and endothelial cells. The mesangial cells and the capillary basement membrane were positive for type IV collagen, and the former was also positive for fibronectin. Some mesangial cells were packed with a number of electron dense droplets or vacuolation, narrowing the capillary lumen. The basement membrane was thicker and type IV collagen– as well as fibronectin-positive areas were expanded in STZ-diabetic and hyperlipidemic rats. (J Toxicol Pathol 1997; 10: 219–224)

Key words: Diabetic nephropathy, Electron microscopy, Hyperlipidemia, Rat, Streptozotocin

Introduction

Glomerular sclerosis and hyalinization finally caused severe renal failure in diabetic nephropathy, possibly because of synthetic disorders in the glomerular basement membrane (GBM)³–⁴. In the pathogenesis of progressive glomerulosclerosis the potential role of lipids has been noticed⁵–¹⁰, and endogenous or exogenous hyperlipidemia was shown to enhance progressive renal injury in annual models¹¹. In addition, the renal lesions were reported to be aggravated after cholesterol feeding in cases of hypertension¹², puromycin nephrosis¹³, or nephrectomy¹⁴,¹⁵.

The mesangial matrix contains glycoproteins, fibronectin, and laminin, as well as proteoglycans, decorin, and collagen. In the GBM¹⁶ and mesangial matrix¹⁷ of streptozotocin (STZ)-diabetic rats with cholesterolemia¹⁸, type IV collagen and fibronectin were shown to be increased.

This study is to see effects of either treating with hyperlipidemia-inducing by Triton WR-1339 (Triton) or cholesterol (CHS) feeding on ultrastructural changes of renal glomeruli in the STZ-treated rats.

Materials and Methods

Animals and feeding or treating with chemicals

Sprague-Dawley (SD) rats were bred at this laboratory. Seven-week-old male rats weighing 221.0 to 281.8 g were housed individually in a stainless steel cage kept in a facility controlled at 23±2°C, 50±10% relative humidity and daily lighting for 12 hr. They were freely given CE-2 (CLEA, Tokyo) pellets with or without 1% CHS and tap water filtered through a Millex-HA® (pore size 0.22 µm; Millipore Corp., Tokyo).

STZ and Triton were from Wako Pure Chemical Industries, Osaka and Ruger Chemical Inc., Tokyo, respectively. STZ (50 mg/kg/day) was injected intraperitoneally for two consecutive days, and 24 hr after the second injection when 10 of 13 animals showed glucosuria, daily feeding of 1% CHS diet was started in 3 of 10 diabetic animals, while the other group of 4 diabetic animals was injected intraperitoneally with Triton (400 mg/kg) twice a week for 16 weeks. The remaining 3 diabetic animals were fed CE-2 diet throughout the experimental period without CHS feeding nor Triton injection. The other two groups of 2 intact animals each were treated with either CHS or Triton as in STZ-induced diabetic rats, and 2 animals remained without any treating.

Electron microscopy

Under ether anaesthesia the kidneys of animals were irrigated at 4 ml/min with physiological saline and then with periodate-lysine–paraform–aldehyde (PLP) solution¹⁹ through the abdominal aorta. Tissue samples were fixed with 1.25% glutaraldehyde for 2 hr and 1% osmium tetroxide for 2 hr and embedded in epoxy resin. Ultrathin sections were made and stained with uranyl acetate and lead nitrate. For immuno-electron microscopy, tissues were fixed with PLP solution for 4 hr and washed with sucrose (Wako Pure Chemicals, Tokyo) and frozen. Six µm frozen sections were treated with 10% fetal bovine sera (Sigma Chemical, St. Louis) in phosphate buffered saline solution (PBS) and then overnight at 4°C with antibodies to fibronectin (1:200; Dako, Kyoto) or type IV collagen (1:200; Cosmo Bio., Tokyo). After rinsing in PBS, the sections were incubated with peroxidase-conjugated Fab fragment of anti–rabbit goat IgG (1:75; MBL, Tokyo) at 4°C overnight and then fixed with 1% glutaraldehyde, rinsed in PBS, and treated with 3-3′-diaminobenzidine (DAB) solution for 30 min. Then they were treated with DAB-H2O2 solution, rinsed with PBS and fixed with 1% osmium tetroxide for 2 hr and embedded in
Fig. 1. Glomerular mesangial cell of a non-diabetic and CHS-fed rat on Day 112, containing a small amount of lipid deposition (arrows). Bar = 2 μm.

Fig. 2. Vacuoles (arrows) in a renal glomerular mesangial cell in a non-diabetic Triton-injected rat on Day 112. Bar = 2 μm.
Fig. 3. Proliferated glomerular mesangial cells of an STZ-diabetic rat on Day 112, showing "mesangial interposition" ingrowing the capillary lamina densa and an endothelial cell (a), the thickened basement membrane (b), fibronectin positive area within a mesangial cells (c), and type IV collagen positive basement membrane (d). Bar=2 μm.

Results

No death occurred within 4 months after starting STZ injection, when all test animals were sacrificed.

In non-diabetic and CHS-fed rats, there was neither "mesangial interposition" nor mesangial proliferation, and a small number of lipid granules 0.1~0.8 μm in size were present within mesangial cells (Fig. 1). Type IV collagen and fibronectin positive areas remained unchanged.

In non-diabetic and Triton-injected rats, electron-lucid vacuoles 0.5~1 μm in diameter (Fig. 2) were seen in mesangial cells showing neither proliferation nor increase in type IV collagen and fibronectin positive areas.

In STZ-diabetic rats without CHS feeding nor Triton injection, mesangial cells were significantly increased showing "mesangial interposition" between the capillary lamina densa and endothelial cells and producing a dual structure of the partially thickened basement membrane, as shown in Fig. 3. Some mesangial cells were slightly enlarged containing cytoplasmic electron-dense droplets 0.3~0.6 μm in diameter. The capillary endothelial cells lost pores into which "mesangial interposition" occurred. The mesangial cells and capillary basement membrane were positive for type IV collagen, and the former was also positive for fibronectin.

In STZ-diabetic and CHS-fed rats, mesangial cells were increased in number, showing "mesangial interposition" as seen in STZ-diabetic rats without CHS feeding. Some mesangial cells had a number of electron-dense droplets 0.3~1.3 μm in size (Fig. 4a). The capillary lumen was narrowed by enlarged mesangial cells packed with electron dense droplets. The endothelial cells were pore-less with the thick basement membrane. The type IV collagen- and fibronectin-positive areas of mesangial cells were larger than those without CHS feeding (Fig. 4b, c).

In STZ-diabetic and Triton-injected rats, "mesangial interposition" occurred between the capillary lamina densa and endothelial cells. Some mesangial cells contained electron-lucid vacuoles 0.6~8 μm and electron-droplets 0.2~1.5 μm in diameter. The accumulation of these vacuoles and droplets in mesangial cells of diabetic and lipidemic rats, was more severe as compared with non-diabetic hypertriglyceridemic rats, and enlarged mesangial cells with vacuoles and droplets narrowed the capillary lumen (Fig. 5a). The GBM was thicker in STZ-diabetic and hypertriglyceridemic rats, and it was positive for type IV collagen and fibronectin (Fig. 5b, c). The basement membrane of capillary vessels was positive for type IV collagen.

In all STZ-diabetic rats with or without Triton-injection or CHS-feeding, the mesangial matrix was not significantly expanded.
Discussion

Mauer et al. described that diabetic nephropathy might result in glomerular sclerosis with GBM thickening and increase in mesangial cells and matrix. In diabetic nephropathy fibronectin is produced by mesangial and endothelial cells, type IV collagen is produced by mesangial and epithelial cells increased in the mesangial matrix and GBM. Also in this study, the mesangial matrix and GBM were strongly positive for type IV collagen and fibronectin.

Mesangial cells incubated with glucose rich medium is known to actively secrete fibronectin, laminin, and type IV collagen, as seen in vivo. Diabetic human patients as well as STZ-diabetic rats were shown to have increased level of serum 7s collagen, which might respond to insulin injection, and the serum collagen level was reported to be in parallel with the production of collagen in GBM. No difference was observed in 7s collagen clearance between STZ-diabetic and non-diabetic rats. In STZ-diabetic rats, the increased production of type IV collagen mediated by a chain mRNA was suggested to induce GBM thickening. In this study, type IV collagen and fibronectin-positive areas were shown to be enlarged with the increasing number of glomerular mesangial cells as well as with GBM thickening in STZ-diabetic rats.
Either glycoproteins such as fibronectin and laminin or proteoglycans such as decorin and collagen in GBM or mesangial matrix, can be glycosylated in the hyperglycemic condition\textsuperscript{39}, and glycosylated collagen might be readily combined with albumin, IgG, VLDL, or LDL, inducing the expanded mesangial matrix and thickened GBM\textsuperscript{31}. Brownlee et al.\textsuperscript{32} reported that collagen showed covalent linkage with albumin, IgG, and LDL after prolonged incubation with glucose, resulting in tissue damage by complement activation. Glycosylated fibronectin might induce GBM thickening\textsuperscript{31,33}, showing modified affinity with collagen or heparin.

In diabetic conditions with decreased lipoprotein lipase activity, increased lypolysis might induce the elevated level of serum free fatty acids in the liver accelerating VLDL production, and the elimination of VLDL-triglyceride from circulating blood might be seriously affected. The hypertglycemia is induced by dissimilation of chylomicrons and VLDL in cases of decreased level of LPL. The STZ-diabetic rats did not show a high level of triglyceride probably because of an insulin reserve suppressing LDL and VLDL\textsuperscript{30} synthesis and secretion, but the LDL receptor might be less active lacking insulin resulting in decreased rate of dissimilation of glycosilated LDL and causing hypercholesterolemia in diabetic patients\textsuperscript{44,39}. In such hyperlipidemic conditions induced in diabetic patients or animals, lipid can be accumulated within glomerular mesangial cells\textsuperscript{43-44}.

The accumulation of advanced glycation end products (AGE) in the basement membrane of blood vessels, induces activation of macrophages and monocain secretion in diabetic patients\textsuperscript{42}. Thus the syntheses of fibronectin and laminin were increased in glomerular mesangial cells with enhanced secretion of type IV collagen\textsuperscript{43}. In this study, the accelerated production of type IV collagen and fibronectin in the thickened GBM was evidenced in STZ-diabetic and CHS- or Triton-treated rats with hyperglycosemia and hyperlipidemia.

References


