Effects of Hypertriglyceridemia on Streptozotocin-Induced Diabetic Nephropathy in Rats

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Abstract: The effect of hypertriglyceridemia was examined on streptozotocin (STZ)-induced diabetic nephropathy in rats. After repeated intraperitoneal injection with Triton WR-1339 (Triton) in STZ-diabetic rats, food and water consumption and urine volume were significantly decreased and albumin/globulin ratio lowered compared with non-injected Triton and STZ-diabetic rats. Between STZ-diabetic rats with Triton injection and those without, no significant difference was observed in the extent of mesangial matrix and PAS, type IV collagen, fibronectin, and laminin positive areas. The results suggested that the proliferation of mesangial cells might be inhibited by the high levels of low and very low density lipoproteins, and diabetic nephropathy was not affected by the inducing of hypertriglyceridemia. (J Toxicol Pathol 1997; 10: 157-164)

Key words: Diabetes, Hypertriglyceridemia, Nephropathy, Rat, Streptozotocin

Introduction

The coronary arterial disorders, arteriosclerosis, nephropathy, neuropathy, and retinopathy are known to be diabetic complications in man¹⁻⁵. The diabetic nephropathy can be aggravated with hyper-glycemia, hypertension, and excessive protein intake. On the other hand, hyperlipidemia constitutes a major risk for atherosclerotic lesions and recently, it was reported to injure the renal function using animal models⁶⁻⁹. However, there has been no evidence of causal relation of hyperlipidemia to diabetes.

A compound [2-deoxy-2-(3-methyl-3-nitrosourea) D-glucopyranose, C₈H₁₅N₃O₇], streptozotocin (STZ) is a culture extract of *Streptomyces acromogenes* injuring pancreatic beta cells and inducing diabetes in many species of animals^{10,11}. In our previous report¹², hypercholesterolemia was shown to promote the progression of STZ-induced diabetic nephropathy in rats, but the renal injury caused by hypertriglyceridemia remains unstudied. A lipoprotein lipase (LPL) receptor blocker, Triton WR-1339 (Triton), was described to elicit hypertriglyceridemia rapidly and dose-dependently after intravenous or intraperitoneal administration¹³. The present study is to see the effect of Triton-induced hypertriglyceridemia on the STZ-induced diabetic nephropathy in rats.

Materials and Methods

Animals

Sprague-Dawley (SD) rats were bred at this laboratory, and 7-week-old male rats weighing 187.8 to 298.8 g, were

individually housed in a stainless steel cage. They were freely given CE-2 pellets (CLEA, Tokyo) and tap water filtered through a Millex-HA® (pore size 0.22 µm; Millipore Corp., Tokyo). The environment was controlled at 13±2°C, 50±10% relative humidity, and daily lighting for 12 hr.

Chemicals

STZ and Triton were from Wako Pure Chemical Industries, Osaka and Ruger Chemical Inc., respectively. Rats were injected intraperitoneally (i.p.) with 50 mg/kg/day STZ for two consecutive days and 24 hr after the second STZ injection when animals showed glucosuria, a half of STZ-treated diabetic rats as well as non-treated controls received i.p. injection of 400 mg/kg Triton twice a week for 16 weeks.

Fig. 1. Body weight. STZ: Streptozotocin 50 mg/kg i.p. on Days -2 and -1. Triton: Triton WR1339 400 mg/kg i.p. twice a week throughout the experimental period. Significantly different from the non-treated control group at $p < 0.01$ (*); from the STZ group at $p < 0.05$ (#).

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Observation

Animals were weekly examined for body weight and food consumption, and water consumption and urine volume for a 16-hr-period were measured monthly. The blood and urine were chemically analyzed monthly. Serum samples were kept at -40°C and examined for glucose, total-cholesterol, triglycerides, total protein, albumin, and albumin/globulin (A/G) ratio using Clinitek® 200 (Miles-Sankyo, Tokyo). Urine samples were examined for glucose, creatinine, total protein, and N-acetyl- β -D-glucosaminidase (NAG) using N-Multistix® SG and Clinitek® 200 (Miles-Sankyo, Tokyo). Urine sediments were subjected to microscopy after staining of Sternheimer. The data were statistically analyzed for using Dunnett's multiple comparison test and *t*-test. Statistical significance was set at $p < 0.05$.

Histopathology

At the end of the experiments, all rats were exsanguinated from the carotid vein under ether anesthesia, and the kidneys were sampled and fixed in 10% phosphate buffered formalin. Paraffin sections $3\text{ }\mu\text{m}$ thick were stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS). A total of 40 glomeruli from each animal were measured for the mesangial PAS positive matrix areas using MacScope® (Mitani, Tokyo) and Desktop Computer Macintosh Quadra 840AV (Apple Computer, USA)¹⁴.

Fig. 2. Food consumption. STZ, Triton: See Fig. 1. Significantly different from the non-treated control group at $p < 0.01$ (*); from the STZ group at $p < 0.05$ and $p < 0.01$ (#, ##), respectively.

For immunostain paraffin sections were treated with 0.05% protease type XXVII (Sigma, Tokyo) in Tris-HCl buffer (pH 7.6) at 37°C and then with 0.3% H_2O_2 in methanol and rinsed in PBS. After blocking with 10% fetal bovine sera (Sigma, Tokyo) in PBS, the sections were incubated at 4°C overnight with antibody to fibronectin (1:2,000; Dako, Kyoto), laminin (1:400; Dako, Kyoto) or type IV collagen (1:2,000; Cosmo Bio., Tokyo). Thereafter sections were incubated with biotinylated rabbit immunoglobulin (Dako, Kyoto, 1:600) at room temperature for 30 min and then

Fig. 3. Water intake. STZ, Triton: See Fig. 1. Significantly different from the non-treated control group at $p < 0.01$ (*); from the STZ group at $p < 0.05$ (#).

Fig. 4. Urine volume. STZ, Triton: See Fig. 1. Significantly different from the non-treated control group at $p < 0.01$ (*) and from the STZ group at $p < 0.01$ (#).

Table 1. Blood Chemistry on Day 112

Group	Treating	Number of animals	Glc (mg/dl)	Chol (mg/dl)	TG (mg/dl)	TP (g/dl)	Alb (g/dl)	A/G
A	—	5	122 ± 19	99 ± 23	176 ± 66	7.87 ± 0.69	2.91 ± 0.16	0.61 ± 0.07
B	STZ [‡]	4	556 ± 110**	163 ± 28	303 ± 238	5.32 ± 1.24	2.19 ± 0.19	0.86 ± 0.55
C	Triton ^{§§}	7	131 ± 37	878 ± 479**	2,540 ± 896**	16.80 ± 5.68**	3.82 ± 1.19	0.31 ± 0.06**
D	STZ + Triton	5	553 ± 40**	264 ± 166	537 ± 326	6.13 ± 0.96	1.82 ± 0.36*	0.44 ± 0.15 [‡]

[‡]: Streptozotocin (50 mg/kg i.p.) on Days -2 and -1.

^{§§}: Triton WR1339 (400 mg/kg i.p.) twice a week throughout the experimental period.

*, **: Significantly different from A at $p < 0.05$ and $p < 0.01$, respectively.

[‡]: Significantly different from B at $p < 0.05$.

treated with avidin-biotin complex (ABCComplex, Dako, Kyoto) and diaminobentidin (DAB, Wako Pure Chemicals, Tokyo)-H₂O₂. They were then counterstained with hematoxylin.

Results

As shown in Figs. 1 to 4, significant difference was seen throughout the study in body weight, food, and water consumption, and urine volume throughout the study between STZ-diabetic rats and non-diabetic ones. In STZ-diabetic and Triton-treated group, food and water consumption and urine volume were significantly decreased as compared with those without Triton, while Triton had no significant effects on non-diabetic rats.

As shown in Table 1, the albumin level and A/G ratio in STZ-diabetic and Triton-injected rats were significantly lower as compared with those without Triton. Serum cholesterol, triglyceride, and total protein levels on Day 112 were remarkably higher and the A/G ratio was lower in non-diabetic and Triton injected rats as compared with those without Triton. As shown in Table 2, urine glucose values were much greater in STZ-diabetic and Triton-injected rats than those without Triton. In STZ-diabetic rats either with or without Triton injection, urine total protein and creatinine were lower than in non-diabetic ones.

No significant difference was observed between those with and without Triton injection in the extent of mesangial matrix, PAS positive areas, and type IV collagen, fibronectin, and laminin positive areas. In STZ-diabetic rats the areas were greater in extent than in non-diabetic ones (Table 3).

In Triton injected either diabetic or non-diabetic animals, foamy cells appeared in the mesangium, and clear cells were seen in the proximal and distal convoluted tubules of diabetic animals (Figs. 5 to 8). No significant effects of

Triton injection were recognized on staining for collagen IV, fibronectin, and laminin in the glomeruli of STZ-diabetic rats.

Discussion

Hyperlipidemia is known to cause lipid deposition and production of foamy cells in the renal glomeruli in human cases of nephrotic syndrome and diabetic nephropathy¹⁵ as well as in abnormal lipid metabolism¹⁶. Also in animal models for dietary and endogenous hyperlipidemia, hypercholesterolemia has been reported to induce renal failure^{6,8,9,17}. Blood coagulation factors X and VII have been described to correlate with serum triglyceride value, suggesting the involvement of hypertriglyceridemia in thrombogenesis¹⁸. In our previous study¹², hypercholesterolemia was shown to promote the progression of STZ-induced diabetic nephropathy in rats, and this study is to see the effect of hypertriglyceridemia on nephropathy in STZ-induced diabetes rats.

Triton is known to induce elevation of serum triglyceride and cholesterol levels, but in STZ-diabetic and Triton injected rats the levels were not so high probably because of the insulin shortage resulting in decreased synthesis and secretion of low density lipoprotein (LDL) as well as very low density lipoprotein (VLDL), while the elimination of VLDL and LDL might be inhibited in diabetic rats¹⁹. The total protein and albumin levels and A/G ratio remained unchanged after Triton treating in STZ-diabetic animals as in non-diabetes ones. The lower serum levels of LDL and VLDL have been reported to induce increased number of mesangial cells *in vitro*²⁰, whereas the higher levels inhibit their proliferation. In this study, Triton-induced hypertriglyceridemia might have resulted in a high level of VLDL, inhibiting not only the proliferation of mesangial cells but

Table 2. Urine Chemistry on Day 112

Group	Treating	Number of animals	Glc (mg/dl)	TP (mg/dl)	Cr (mg/dl)	NAG (U/l)
A	—	5	23±9	1,080±400	109±51	25±15
B	STZ	4	2,321±1,703**	376±253*	18±5**	10±6*
C	Triton	7	18±4	891±885	89±21	29±11
D	STZ+Triton	5	6,099±4,307***	296±409***	27±28**	13±9

See the foot-notes on Table 1.

*,**: Significantly different from A at p<0.05 and p<0.01, respectively.

*,#: Significantly different from B at p<0.05 and p<0.01, respectively.

Table 3. Extent of Glomerular PAS Positive Areas

Group	Treating	Number of animals	Glomerulus (μm ²)	PAS positive area (μm ²)	PAS positive area/Glomerulus (%)
A	—	5	20,157±1,131**	3,541±126	17.7±0.9
B	STZ [†]	4	21,895±2,096	5,149±753**	24.7±1.7**
C	Triton [‡]	7	20,338±2,451	3,324±716*	16.3±2.8
D	STZ+Triton	5	20,620±2,143**	4,914±341**	24.1±2.3**

See the foot-notes on Table 1.

*,**: Significantly different from A at p<0.05 and p<0.01, respectively.

Fig. 5. Renal glomerular structures of a non-treated control rat on Day 112. a) HE, b) PAS, c) collagen IV, d) laminin, and e) fibronectin. Bar=100 μm .

Fig. 6. Renal glomerular lesions of an STZ-diabetic rat showing increased mesangial matrix. Day 112. a) HE, b) PAS, c) collagen IV, d) laminin, and e) fibronectin. Bar = 100 μ m.

Fig. 7. Renal glomerular lesions of a rat injected with Triton (400 mg/kg, i.p.) twice a week throughout the experimental period showing appearance of foamy cells in the mesangium. Day 112. a) HE, b) PAS, c) collagen IV, d) laminin, and e) fibronectin. Bar = 100 μ m.

Fig. 8. Renal glomerular lesions of STZ-diabetic and Triton injected rat showing increased mesangial matrix and appearance of foamy cells in the mesangium. Day 112. a) HE, b) PAS, c) collagen IV, d) laminin, and e) fibronectin. Bar=100 μ m.

also the production of type IV collagen, laminin, and fibronectin without causing any renal injuries. On the other hand, the higher concentration of VLDL has been described to enhance the incorporation of apolipoproteins-B and -E into the glomerular epithelial cells, and the overproduction of VLDL-apolipoprotein-B complex might cause atherosclerosis in diabetic patients with lipidemia²¹⁻²³. Thus, the control of blood lipid levels might be important, although diabetic nephropathy did not take turn for the worse in hypertriglyceridemia in this study.

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