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Effects of Silkworm Extract on Streptozotocin-induced Diabetic Rats

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This study was undertaken to find effects of ethanol extracts from *Bombyx mori* on the hyperglycemia induced by streptozotocin in rats. Blood glucose level of rats increased depending on experimental days after streptozotocin treatment. But there was no difference in blood glucose level between the rats injected with ethanol extracts of *B. mori* and normal rats. There was no significant difference in the kidney fibrosis between the rats injected with ethanol extrscts of *B. mori* and normal rats at all ages examined. The transforming growth factor-β1 (TGF-β1) protein was detected in the glomerular endothelial cells and tubular epithelial cells of streptozotocin rats at 14 days of age. In the rats injected with ethanol extracts of *B. mori* to the kidneys, the TGF-β1 protein was detected very faintly. The TGF-β1 mRNA of streptozotocin rats increased at 14 days of age, and this was higher than the rats injected with ethanol extrscts of *B. mori*. Taken these together, the results suggest that the ethanol extracts of *B. mori* ameliorate hyperglycemia of the rats induced by streptozotocin.

Key words: *Bombyx mori*, Streptozotocin, Diabetic nephropathy

Introduction

Diabetic nephropathy is one of the most common causes of end-stage renal failure in the world. Diabetic nephropathy is characterized by a rapid phase of renal growth following the onset of hyperglycemia so that both whole kidney weight and glomerular volume are increased (Mackay *et al.*, 1990). Later, diabetic nephropathy is characterized by progressive mesangial expansion and a decrease in glomerular filtration rate (Steffes *et al.*, 1989; Kreisberg and Ayo, 1993; Ziyadeh, 1993). The mechanisms responsible for diabetic glomerular hypertrophy and injury have not been completely elucidated, but a number of studies suggest that transforming growth factor-β1 (TGF-β1) plays a central role in these processes (Roberts *et al.*, 1992; Border and Ruoslahti, 1992). TGF-β1, a multifunctional cytokine, plays an important role in regulating tissue repair and remodeling following injury. One of the most important biological actions of TGF-β1 is the regulation of extracellular matrix deposition (Klahr, 1998; Schreiner *et al.*, 1988).

By inhibiting TGF-β1 using a neutralizing antiserum (Terrell *et al.*, 1993) or neutralizing proteoglycan decorin (Isaka *et al.*, 1993), Border *et al.* (1992) have estabiliched a role for TGF-β1 in the anti-Thy-1 model of glomerulonephritis. In streptozotocin (STZ)-induced diabetic rats, TGF-β1 mRNA levels detected by polymerase chain reaction (PCR) are also increased in the renal cortex and isolated glomeruli as early as 24 hrs after STZ administration (Shankland and Scholey, 1994).

In traditional oriental medical application in Korea, Chung *et al.* (1995) reported that the extracts of *B. mori* have ablity to decrease the blood glucose level and it can control the post prandial blood glucose by inhibition of α-glucosidase, the catalase of carbohydrates.

We have been investigating the potential of ethanol extracts from silkworm larva to prevent progression of diabetic nephropathy in the streptozotocin induced rats.

Materials and methods

Extract preparation from *Bombyx mori*

The 3 rd day of 5 th instar silkworm larvae (*Bombyx mori*) reared by natural mulberry leaves were subjected

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to extraction by boiling in water for 3 hrs. Then, the extract was evaporated under reduced pressure by 99% ethanol solution. The silkworm extracts were diluted with 0.9% NaCl and filtered. The silkworm extract was stored at 4°C.

Animal model

A chronic hyperglycemic state was induced in Sprague-Dawley male rats by a single intraperitoneal injection of STZ (Sigma-Aldrich Canada, Oakvile, Ontario, Canada). The STZ was prepared in the concentration of 70 mg/kg of body weight dissolved in citrate buffer (0.1 mol/l, pH 4.5). Blood samples of tail vein were obtained at 12 hrs after administration of STZ for determination of blood glucose concentration with reagent strips (Haemoglukotest, Boehringer Mannheim, Germany). After the onset of hyperglycemia, defined as blood glucose levels greater than 15 mmol/liter, the blood glucose level was measured daily. Rats were excluded if their blood glucose levels were below 15 mmol/liter. STZ induced diabetic rats were divided into two groups. One group was injected with silkworm extracts and the other group (control) was not. To evaluate the effect of silkworm extracts on the disease development in rats, silkworm extracts were administrated i. m. at a dose of 1 g/kg/day. Age-matched STZ control rats received an injection of the citrate buffer. The glycaemic state of the rats was assessed during 21 days the experiment using reagent strips. Control and silkworm extracts injected rats were killed at the days of 1, 3, 7, 14 and 21 kidney at both sides were harvested.

Histological analysis

Kidney was fixed in 10% paraformaldehyde (PFA) overnight. The tissues were dehydrated through a graded ethanol series and then embedded in paraffin. Kidney tissues were dissected into 2 μm sections for hematoxylin and eosin (HE) staining.

Immunohistochemical staining

To examine the level of TGF-β1 protein, immunohistochemical analysis was performed with rabbit anti-pocrine TGF-β1 polyclonal antibody (Cellscience, USA). Two μm thick sections were deparaffinized in xylene and hydrated to ethanol. Hydrated sections were treated with 3% H_2O_2 in methanol for 30 min to block endogenous peroxidase and washed with 0.01 M phosphate buffer for 10 min. After through washing, sections were processed by an indirect immunoperoxidase technique using a commercial kit (ABC kit, DAKO, USA) with secondary antibodies. The section was counterstained with Mayers hematoxylin.

Reverse transcription polymerase chain reaction (RT-PCR)

Isolated kidney was homogenized with a Polytron homogenizer (Kinematica, Switzerland) in RNAzol B (TEL-TEST, USA). Complementary DNA (cDNA) was synthesized using a commercial kit (Promega, USA) following the standard protocol. PCR was performed with the following primers: (i) 5' primer, GTG GAC ATT GTT GCC ATC AAC G, and 3' primer, AGG GAG TTG TCA TAT TTC TCG for GAPDH, (ii) 5' primer, CCT GCT GCT TTC TCC CTC AAC C, and 3' primer, CTG GCA CTG CTT CCC GAA TGT C for TGF-β1. Amplification was carried out at programmable temperature control system through 20 to 40 cycles of denaturation at 95°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for one min. Amplication of the housekeeping gene glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as a positive control for intact RNA and for measuring the efficiency of RT-PCR. The PCR products were subjected to electrophoresis through 1.5% agarose gels, and stained with ethidium bromide. The band intensities were determined by an image analysis using a Image Pro (Image Plus, USA).

Results

As shown in Fig. 1, there was difference in blood glucose level between silkworm extracts injected and control rats. Blood glucose levels of only STZ-control rats increased significantly with aging and were significantly higher than that of silkworm extracts injected rats.

Histologically, the silkworm extracts injected kidney

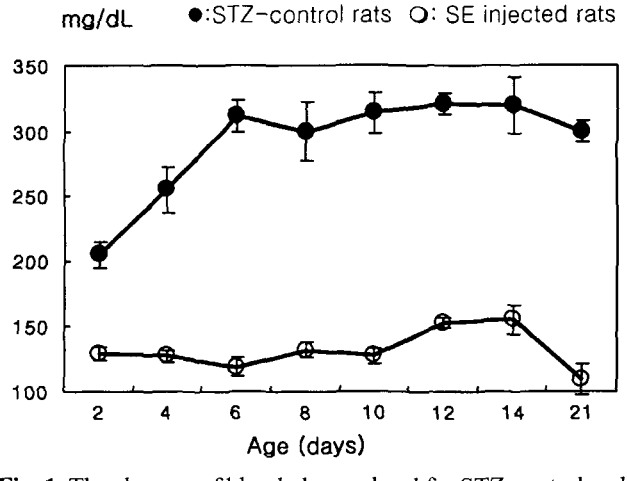

Fig. 1. The changes of blood glucose level for STZ-control and silkworm ethanol extracts injected rats. Values are means ± SD.

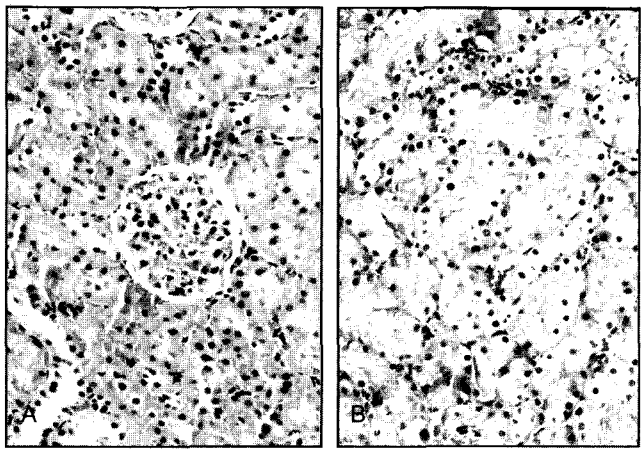

Fig. 2. The light micrograph of kidney from the rats injected with silkworm ethanol extracts (A) shows unremarkable changes, but that of STZ-control rats (B) at 21 days of age shows evidence of tubular necrosis (HE stain, X 200) (arrow is marked tubular atrophy).

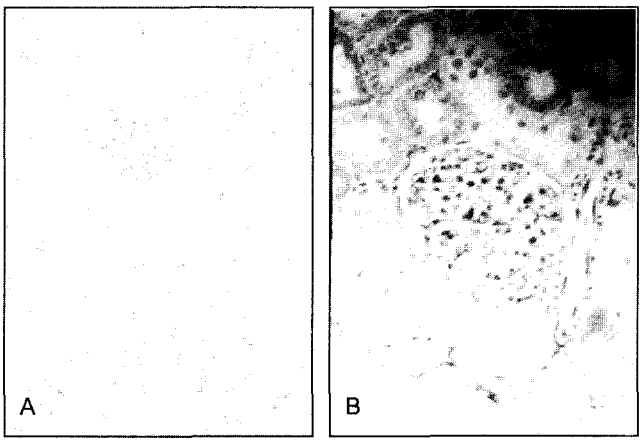

Fig. 3. The immunohistochemical stains in kidney of 14-day-old silkworm ethanol extract injected rats. (A) show faintly expression of TGF-β1 mostly and that of STZ-control rats (B) show increased strongly expression of TGF- β1 at the tubular epithelium and the glomerulus (X 200).

had a normal appearance (Fig. 2A). In the only kidney of STZ-control rat at day 21, collecting ducts and distal tubules displayed tubular atrophy and epithelial flattening with marked tubulointerstitial fibrosis (Fig. 2B).

Immunohistochemical results in Fig. 3 show that immunoreactive TGF-β1 protein was only faintly detected in glomeruli of silkworm extracts injected kidney at all ages examined. Conversely, marked staining for TGF-β1 was seen in glomerular and tubular cells of only 14-day-old STZ-control rats.

TGF-β1 mRNA levels in the rat kidney injected silkworm extracts rats were 2.0-fold lower than those of STZ-

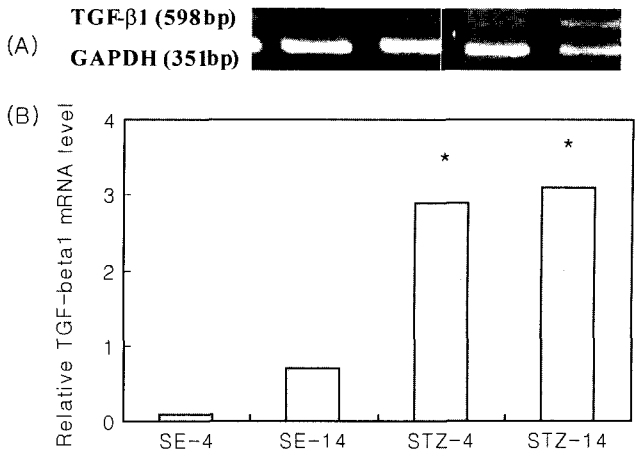

Fig. 4. The expression level of mRNA for the TGF- β1 in STZ-control (STZ) and silkworm extract injected rats (SE) at different ages of 4 and 14-day-old. (A) Representative RT-PCR showing mRNA for the TGF-β1. (B) RT-PCR results are quantified by scanning densitometry. Data are normalized with GAPDH mRNA level and are presented as relative values. Significant differences: *$p < 0.05$.

control rats at the same age (Fig. 4). There was no significant difference in TGF-β1 mRNA levels between normal rats and silkworm extracts injected rats.

Discussion

Although the pathogenesis of diabetic nephropathy has not been fully elucidated, a number of studies suggest that the growth factor/cytokine TGF-β1 plays an important role (Yamanoto *et al.*, 1993; Shankland and Scholey, 1994; Shrma and Ziyadeh, 1994; Sharma *et al.*, 1996). In vitro, TGF-β1 stimulates cell hypertrophy and extracellular matrix protein synthesis by mesangial cells and proximal tubule epithelial cells, and high glucose concentrations increase TGF-β1 mRNA levels in mesangial cells and proximal tubule cells (Rocco *et al.*, 1992). In accord with these observations, Border and colleagues have shown that there are sustained increases in TGF-β1 mRNA levels and TGF-β1 immunostaining in diabetic glomeruli, and enhanced TGF-β1 expression correlates with increased extacellular matrix protein accumulation in the mesangium (Yamanoto *et al.*, 1993; Shankland and Scholey, 1994; Shrma and Ziyadeh, 1994). Increased TGF-β1 expression is associated with increased TGF-β1 bioactivity in experimental diabetes mellitus, treatment with TGF-β1 neutralizing antibodies lowers extracellular matrix protein mRNA levels in the kidneys of streptozotocin-induced diabetic mice and attenuates hypertrophy (Sharma *et al.*, 1996).

This study was investigated to determine the effects of the silkworm ethanol extract in lowering blood glucose level and TGF-β1 mRNA expression in treating diabetes. The development of diabetic nephropathy in silkworm ethanol extract rats was prevented by STZ treatment in our experiments

References

Border, W. A. and E. Ruoslahti (1992) Transforming growth factor-β1 in disease: The dark side of tissue repair. *J. Clin. Invest.* **90**, 1-7.

Isaka, Y., Y. Fujiwara, N. Ueda, Y. Kaneda, T. Kamada and E. Imai (1993) Glomerulosclerosis induced by *in vivo* transforming growth factor β1 or platelet-derived growth factor gene into the rat kidney. *J. Clin. Invest.* **92**, 2597-2601.

Chung, S. H., J. H. Yu, E. J. Kim and K. S. Ryu (1996) Blood glucose lowering effect of silkworm. *Bull. K. H. Pharma. Sci.* **24**, 95-100.

Klahr, S. (1998) Obstructive nephropathy. *Kidney Int.* **54**, 286-300.

Kreisberg, J. I. and S. H. Ayo (1993) The glomerular mesangium in diabetes mellitus. *Kidney Int.* **43**, 109-113.

Mackay, K., A. R. Robbins, M. D. Bruce and D. Danielpour (1990) Identification of disulphide-linked transforming growth factor-β1 specific biding proteins in rat glomeruli. *J. Biol. Chem.* **265**, 9351-9356.

Roberts, A. B., B. K. Mccune and M. B. Sporn (1992) TGF-β1: Regulation of extracellar matrix. *Kidney Int.* **43**, 109-113.

Rocco, M. V., Y. Chen, S. Goldfarb and F. N. Zlyadeh (1992) Elevated glucose stimulates TGF-β gene expression and bioactivity in proximal tubule. *Kidney Int.* **41**, 107-114.

Schreiner, G. F., H. P. G. Hrris, M. L. Purkerson and R. Klahr (1988) Immunological aspects of acute ureteral obstruction: Immune cell infiltrate the kidney. *Kidney Int.* **34**, 487-493.

Shankland, S. J. and J. W. Scholey (1994) Expression of transforming growth factor β1 during diabetic renal hypertrophy. *Kidney Int.* **43**, 109-113.

Sharma, K. and F. N. Ziyadeh (1994) Renal hypertrophy is associated with upregulation of TGF-β1 gene ecpression in diabetic BB rats and the NOD mouse. *AM. J. Physiol.* **267**, F1094-F1101.

Sharma, K., Y. Jin, J. Guo and F. N. Ziyadeh (1996) Neutralization of TGF-β by anti-TGF-β antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes* **45**, 522-530.

Steffes, M. W., R. Osterby, B. Chavers and S. Mauer (1989) Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. *Diabetes* **38**, 1077-1081.

Terrell, T. G., P. K. Working, C. P. Chow and J. D. Green (1993) Pathology of recombinant human transforming growth factor β1 in rats and rabbits. *Int. Rev. Exp. Pathol.* **34**, 43-67.

Yamamoto, T., T. Nakamura, N. A. Noble, E. Ruoslahti and W. A. Border (1993) Expression of transformin growth factor β is elevated in human and experimental diabetic nephropathy. *Proc. Natl. Acad. Sci. USA* **90**, 1814-1818.

Ziyadeh, F. N. (1993) The extracellular matrix in diabetic glomerulopathy. *Am. J. Kidney Dis.* **22**, 736-744.