NEURAL REGENERATION RESEARCH
Volume 8, Issue 19, July 2013

www.nrronline.org

doi:10.3969/j.issn.1673-5374.2013.19.003 [http://www.nrronline.org; http://www.sjzsyj.org]
Chen ZH, Yang SH, He YQ, Song CJ, Liu YP. Effect of sericin on diabetic hippocampal growth hormone/insulin-like growth factor 1 axis. Neural Regen Res. 2013;8(19):1756-1764.

Effect of sericin on diabetic hippocampal growth hormone/insulin-like growth factor 1 axis

Zhihong Chen[1], Songhe Yang[1], Yaqiang He[1], Chengjun Song[1], Yongping Liu[2]

1 *Department of Human Anatomy, Chengde Medical College, Chengde 067000, Hebei Province, China*
2 *Department of Biochemistry, Chengde Medical College, Chengde 067000, Hebei Province, China*

Abstract

Previous studies have shown that sericin extracted from silk cocoon significantly reduces blood glucose levels and protects the nervous system against diabetes mellitus. In this study, a rat type 2 diabetes mellitus model was established by intraperitoneal injection of 25 mg/kg streptozotocin for 3 successive days, following which the rats were treated with sericin for 35 days. After treatment, the blood glucose levels of the diabetic rats decreased significantly, the growth hormone level in serum and its expression in the hippocampus decreased significantly, while the insulin-like growth factor-1 level in serum and insulin-like growth factor-1 and growth hormone receptor expression in the hippocampus increased significantly. The experimental findings indicate that sericin improves disorders of the growth hormone/insulin-like growth factor 1 axis to alleviate hippocampal damage in diabetic rats.

Key Words

neural regeneration; traditional Chinese medicine; sericin; type 2 diabetes mellitus; hippocampus; growth hormone; insulin-like growth factor 1; growth hormone receptor; growth hormone/insulin-like growth factor 1 axis; streptozotocin; blood glucose; western blot assay; reverse transcription-PCR; grants-supported paper; neuroregeneration

Research Highlights

(1) The growth hormone/insulin-like growth factor 1 axis is an important anabolic-conditioning system that plays a critical role in the growth and development of the central nervous system.
(2) Enzyme-linked immunosorbent assay, western blot and reverse transcription-PCR were used to demonstrate the role of sericin in regulating the expression of hippocampal growth hormone/insulin-like growth factor 1 in diabetic rats.
(3) Sericin can alleviate diabetic hippocampal damage through improving disorders in the growth hormone/insulin-like growth factor 1.

Zhihong Chen, Ph.D., Professor.

Corresponding author: Yongping Liu, Master, Professor, Department of Biochemistry, Chengde Medical College, Chengde 067000, Hebei Province, China

Received: 2013-01-11
Accepted: 2013-04-07
(N20111004001)

Acknowledgments: We thank the Institute of Basic Medical Science, Chengde Medical College, for providing laboratory space and equipment.
Funding: This study was financially sponsored by the Natural Science Foundation of Hebei Province, H2012406018, H2013406096 and a grant from Hebei Province Department of Education, No. 2006301.

Author contributions: Chen ZH was in charge of the funding, developed the experimental concept and design, performed some of the experiments, analyzed the data and wrote the paper. Yang SH was responsible for animal feeding and processing, and provided information support. He YQ performed some of the experiments. Song CJ performed the statistical analysis. Liu YP supervised the study. All authors approved the final version of the manuscript.

Conflicts of interest: None declared.

INTRODUCTION

Diabetes mellitus is an endocrine and metabolic disease caused by absolute or relative insulin deficiency. Diabetes mellitus is characterized by hyperglycemia and highly prevalent complications caused by the low function of insulin secreting cells and inefficient insulin work capacity[1]. With the progress of society and the improvement of living standards, the incidence of diabetes mellitus is increasing yearly. Diabetes mellitus is regarded as equal to cardiovascular disease and malignant tumors as the most serious health risk, showing a high morbidity and mortality[2-3]. Type 2 diabetes mellitus is dominant and is characterized by elevated blood glucose, insulin resistance and impaired islet β-cell function[4-5]. In the

Ethical approval: This study was approved by Animal Ethics Committee of Hebei Province, China.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application/funding source disputations.

treatment of type 2 diabetes mellitus, it is crucial to reduce blood glucose levels and promote insulin secretion from β-cells.

The existing treatments for diabetes mellitus include drugs, which constitute the main treatment, insulin injection, cell transplantation and gene therapy. Several drugs are available to regulate blood glucose, delay disease progression and reduce complications, including sulfonylureas, biguanides, α-glucosidase inhibitor, and insulin sensitizers. However, these drugs still produce side effects to varying degrees and fail to improve insulin resistance and protect islet cells, while long-term application leads to adverse reactions and drug resistance[6-8]. Although injection of exogenous insulin is a commonly used method to regulate blood glucose in the later stages of diabetes mellitus, the insulin injection is slowly absorbed and requires daily administration, leading to insufficient feedback regulation of blood glucose. Furthermore, the treatment effect is not satisfactory and is inconvenient to the patients[9-10]. Pancreas transplantation and islet transplantation have been applied for the treatment of diabetes mellitus (mainly type 1), but did not achieve satisfactory effects because of the shortage of transplant donors and transplant rejection[11]. The rapid development of recombinant DNA and transgenic technologies, particularly diabetes mellitus-related gene cloning and viral vector-based gene transfer technology, has enabled great progress to be made in gene therapy for diabetes mellitus, mainly focusing on type 1 diabetes mellitus[12]. Gene therapy is a commonly used treatment targeting type 1 diabetes mellitus; however, it is problematic because of the lack of an effective insulin gene transfer system, auto-immune destruction of the target cells, persistent gene expression, and the long-term impact on the body[13-14]. Therefore, increasing attention is being paid to the screening of Chinese herbal medicines for hypoglycemic natural substances with good absorption efficacy and an absence of toxic side effects.

Diabetes mellitus is regarded as wasting-thirst disease, and the treatment principles are based on the production of sperm, clearing heat, nourishing *yin* and moistening dryness. Chinese ancient medical masters have accumulated rich experience in the prevention and treatment of diabetes mellitus, which has been frequently documented in classic medical books including *Synopsis of Golden Chamber*, *Medical Secrets of An Official*, *Thousand Golden Prescriptions* and *Internal Doctrine of the Yellow Emperor*. Currently, treatment of diabetes mellitus with Chinese herbal medicine is based on the differentiation of symptoms and signs, and can be regulated according to the *yin* and *yang* in blood, cold-heat and deficiency-excess, and internal and external toxins. However, these herbal therapies do not meet clinical needs.

The cocoon shell of the silkworm, Bombyx mori, is sweet, warm in nature and non- toxic, and it can nourish *yin*, moisturize dryness, enhance sperm production, quench thirst and promote granulation. It can also be used to treat polydipsia, kidney consumption and cloudy urine, as well as polyphagia and emaciation[15-16]. A piece of floss is bonded by two monofilament fibers comprising central silk fibroin and peripheral sericin. The silk industry originated in China and has developed for thousands of years. The majority of silk applications involve silk fibroin, which is used for clothing, while sericin, which accounts for 30% floss, is discarded. Increasing attention has been paid to the effective use of a large number of high-quality proteins, and sericin possesses many advantages for beauty, skin care, nutrition, anti-oxidation, and anti- cancer treatment[17-21]. The silk cocoon soaked in water is a prescription for regulating blood glucose levels, and sericin can effectively protect islet cells, gonads and kidney[22-27]. Preliminary studies by our research group have shown that sericin might improve aberrant Akt signaling, decrease heme oxygenase-1 expression in the hippocampus

and cerebral cortex, and reduce the apoptosis of hippocampal neurons in diabetic rats, thus protecting the nervous system[28-29].

The growth hormone/insulin-like growth factor 1 axis is an important anabolic-conditioning system that plays a critical role in the growth and development of the central nervous system. The hippocampal expression of insulin-like growth factor 1 can promote synaptic regeneration, neuroprotection, myelin formation, neuroregeneration and dendritic branch formation after hippocampal injury[30-31]. The growth hormone/insulin-like growth factor 1 axis undergoes abnormal changes in diabetes mellitus, which aggravate the disease progression and trigger complications[32].

To improve diabetes mellitus and its chronic complications, improvement of growth hormone/insulin-like growth factor 1 axis anomalies is one of the treatment strategies. The present study aimed to explore the protective effect of the hippocampal growth hormone/insulin-like growth factor 1 axis in type 2 diabetic rats.

RESULTS

Quantitative analysis of experimental animals

Thirty Sprague-Dawley rats were used, 10 of which were randomly assigned to a control group that received no treatment. The remaining 20 rats were used to establish a model of type 2 diabetes mellitus, and then randomly divided into a model group and a sericin group. The model group received no treatment and the sericin group was given sericin *via* intragastric administration. All rats entered the final analysis without any losses.

Sericin significantly reduced blood glucose levels in diabetic rats

A glucose oxidase test showed that blood glucose levels in the diabetic model rats were significantly increased compared with the control group ($P < 0.01$). After sericin administration for 35 days, the blood glucose levels in the diabetic rats decreased significantly ($P < 0.01$; Table 1).

Effect of sericin on serum growth hormone and insulin-like growth factor 1 levels in diabetic rats

Enzyme-linked immunosorbent assay showed that the levels of serum growth hormone were significantly increased in diabetic rats, while the levels of insulin-like growth factor 1 were significantly decreased compared with the control group ($P < 0.01$). After sericin administration for 35 days, the serum growth hormone levels were significantly lower, and the insulin-like growth factor-1 levels were significantly higher in the diabetic rats ($P < 0.01$; Table 1).

Table 1 Effect of sericin on blood glucose (mmol/L), serum growth hormone (ng/mL) and insulin-like growth factor 1 (ng/mL) levels in diabetic rats

Group	Blood glucose	Growth hormone	Insulin-like growth factor 1
Control	11.12±2.22	1.36±0.53	1 124.75±186.37
Model	29.00±5.39[a] (Q = 13.875)	2.56±1.13[a] (Q = 4.790)	520.20±121.81[a] (Q = 11.543)
Sericin	14.03±3.98[b] (Q = 11.616)	1.36±0.57[b] (Q = 4.790)	981.10 ± 180.88[b] (Q = 8.800)
F	55.42	7.65	36.37
P	< 0.01	< 0.01	< 0.01

Data are expressed as mean ± SD for 10 rats in each group. Comparisons between groups were tested by one-way analysis of variance and pairwise comparison was performed using the *q* test. [a]$P < 0.01$, *vs*. control group; [b]$P < 0.01$, *vs*. model group.

Effect of sericin on growth hormone and growth hormone receptor protein expression in the hippocampus of diabetic rats

Western blot analysis demonstrated that growth hormone protein expression levels in the hippocampus of diabetic rats were significantly increased, while the growth hormone receptor protein expression levels were significantly decreased compared with the control group ($P < 0.01$). After sericin administration for 35 days, the hippocampal growth hormone expression levels in diabetic rats were significantly decreased, and the growth hormone receptor protein expression levels were significantly increased ($P < 0.01$; Figure 1, Table 2).

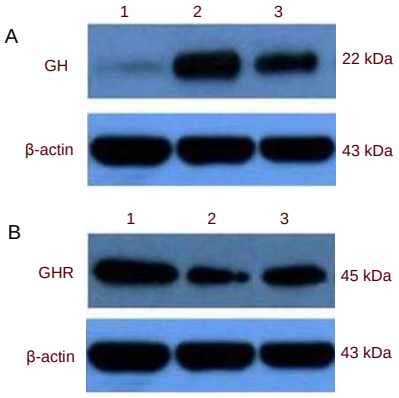

Figure 1 Growth hormone (GH; A) and growth hormone receptor (GHR; B) expression in rat hippocampus (western blot assay).

The growth hormone expression levels were higher and the growth hormone receptor expression levels were lower in the model group than in the control group. In the sericin group, the growth hormone expression levels decreased, while the growth hormone receptor expression levels increased.

1: Control group; 2: model group; 3: sericin group.

Table 2 Effect of sericin on growth hormone and growth hormone receptor protein expression in the hippocampus of diabetic rats (western blot assay)

Group	Growth hormone	Growth hormone receptor
Control	0.157±0.016	0.942±0.013
Model	0.925±0.029[a]	0.355±0.017[a]
	(Q = 101.620)	(Q = 123.438)
Sericin	0.565±0.025[b]	0.539±0.015[b]
	(Q = 47.686)	(Q = 38.779)
F	2 584.90	3 984.64
P	< 0.01	< 0.01

Expression of the target proteins is represented as the ratio of target band absorbance to β-actin absorbance. Data are expressed as mean ± SD for 10 rats in each group. Comparisons between groups were tested by one-way analysis of variance and pairwise comparison was performed using the *q* test. [a]*P* < 0.01, *vs*. control group; [b]*P* < 0.01, *vs*. model group.

Effect of sericin on growth hormone, growth hormone receptor and insulin-like growth factor 1 mRNA expression in the hippocampus of diabetic rats

Reverse transcription-PCR analysis showed that, compared with the control group, hippocampal growth hormone mRNA expression levels in the model group were significantly increased, while the growth hormone receptor and insulin-like growth factor 1 mRNA expression levels were significantly decreased (*P* < 0.01). After sericin administration for 35 days, the growth hormone mRNA expression levels in the rat hippocampus were significantly reduced, while the growth hormone receptor and insulin-like growth factor 1 mRNA expression levels were significantly increased (*P* < 0.01; Figure 2, Table 3).

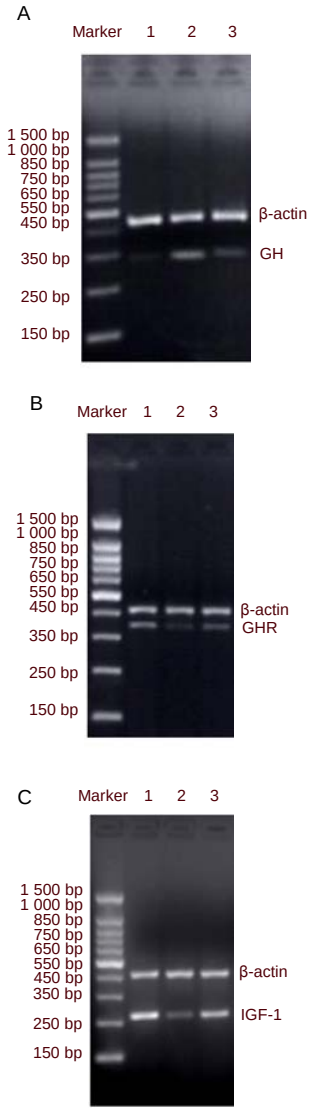

Figure 2 Effect of sericin on the mRNA expression of growth hormone (GH; A), growth hormone receptor (GHR; B) and insulin-like growth factor 1 (IGF-1; C) in the hippocampus of diabetic rats.

1: Control group, 2: model group, 3: sericin group. The bands for growth hormone, growth hormone receptor, insulin-like growth factor 1 and β-actin were 368, 394, 280 and 445 bp, respectively.

DISCUSSION

The growth hormone/insulin-like growth factor 1 axis is a critical anabolic-conditioning system that plays an important role in tissue differentiation, proliferation and metabolism[33-34]. Growth hormone, insulin-like growth factor 1 and its receptor are present in brain tissue, and insulin-like growth factor 1 in the blood may bind to insulin-like growth factor 1 receptor in brain tissue after passing through the blood-brain barrier[35]. Brain tissue also secretes a small amount of growth hormone and insulin-like growth factor 1[36-37]. In addition to the effect of growth hormone/insulin-like growth factor 1 axis on growth and development, increasing attention has been paid to the regulatory effect of the growth hormone/ insulin-like growth factor 1 axis on the central nervous system.

Both type 1 and type 2 diabetes mellitus are characterized by growth hormone/insulin-like growth factor 1 axis abnormalities, which are closely associated with hippocampal damage[35, 38]. Sustained hyperglycemia may decrease the hypothalamic regulation of glucose and weaken the hypothalamic inhibition of growth hormone, thus leading to an increase in growth hormone levels[39]. The elevated growth hormone levels are also the result of growth hormone resistance, reduced insulin-like growth factor 1 levels and suppressed negative feedback in the pituitary gland[40].

Table 3 mRNA expression of rat hippocampal growth hormone, growth hormone receptor and insulin-like growth factor-1 (reverse transcription-PCR)

Group	Growth hormone	Growth hormone receptor	Insulin-like growth factor 1
Control	0.179±0.016	0.618±0.020	1.502±0.028
Model	0.505±0.014[a] (Q = 53.876)	0.322±0.012[a] (Q = 60.721)	0.544±0.027[a] (Q = 118.066)
Sericin	0.314±0.026[b] (Q = 31.556)	0.498±0.013[b] (Q = 36.150)	1.032±0.022[b] (Q = 60.197)
F	732.76	932.94	3 485.33
P	< 0.01	< 0.01	< 0.01

The mRNA expression levels are represented as the ratio of the target band absorbance to β-actin absorbance. Data are expressed as mean ± SD for 10 rats in each group. Comparisons between groups were tested by one-way analysis of variance and pairwise comparison was performed using the *q* test. [a]*P* < 0.01, *vs.* control group; [b]*P* < 0.01, *vs.* model group.

The elevated growth hormone levels make blood glucose levels difficult to control and influence the occurrence and development of chronic diabetic complications. Tissue sensitivity to growth hormone is associated with growth hormone receptor and growth hormone binding protein; growth hormone receptor is the key functional factor in the growth hormone/insulin-like growth factor 1 axis, and growth hormone receptor gene expression may be influenced by diabetes mellitus[41-42]. Recombinant human growth hormone can upregulate the growth hormone binding capacity and growth hormone receptor mRNA expression[43]. Excessive growth hormone inhibits the expression of growth hormone receptor, which suggests a regulatory feedback mechanism between growth hormone and growth hormone receptor[44]. Therefore, diabetes mellitus is accompanied by elevated growth hormone levels, decreased growth hormone receptor expression, reduced tissue sensitivity to growth hormone, and reduced growth hormone function. Decreased growth hormone receptor levels lead to growth hormone resistance and reduce growth hormone-mediated insulin-like growth factor 1 levels[45-46]. Insulin-like growth factor 1 levels are also decreased in diabetes mellitus, and accordingly growth hormone function is attenuated[47] and the anti-apoptotic effects of insulin-like growth factor 1 are reduced in the central nervous system, leading to hippocampal neuronal apoptosis and cognitive function deficits[48-51]. Insulin-like growth factor system abnormalities are the key factors contributing to hippocampal neuronal apoptosis, and may be involved in the development of cognitive dysfunction[52]. Li *et al*[53] found that insulin-like growth factor 1 expression was significantly decreased in the brains of diabetic mice and was accompanied by hippocampal neuronal apoptosis, regardless of whether the disease duration was 2 or 8 months.

Sericin was shown to significantly reduce serum growth hormone levels, downregulate hippocampal growth hormone expression, increase serum insulin-like growth factor 1 levels, and upregulate expression of insulin-like growth factor 1 and growth hormone receptor in the hippocampus of diabetic rats. These results indicate that sericin can improve disorders of the hippocampal growth hormone/insulin-like growth factor 1 axis and may alleviate hippocampal damage.

MATERIALS AND METHODS

Design

A randomized, controlled animal experiment.

Time and setting

The experiments were performed from October 2009 to December 2010 at the Institute of Basic Medical Sciences, Chengde Medical College, China.

Materials

Animals

Thirty healthy, clean, male Sprague-Dawley rats, aged 3 months and weighing 200–250 g, were provided by the Experimental Animal Center of Hebei Medical University (license No. 712024). All animals were reared in a clean animal laboratory at constant temperature (20 ± 2°C) and constant humidity (40–70%). Experimental disposals were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals* formulated by the Ministry of Science and Technology of China[54].

Drugs

Silk cocoon was soaked in water, decocted, filtered and concentrated to prepare sericin[26]. Sericin was provided by the Sericultural Institute of Chengde Medical College, and extracted at the Institute of Basic Medical Sciences, Chengde Medical College, China.

Methods

Establishment of type 2 diabetes mellitus models

Rats in the model group and the sericin group were injected with 2% streptozotocin (Sigma, St. Louis, MO, USA) *via* intraperitoneal injection (25 mg/kg) for 3 successive days to induce type 2 diabetes mellitus. The success of model induction was defined by fasting plasma glucose ≥ 16.7 mmol/L[55-56]. Rats in the control group were regularly fed with no treatment.

Sericin intervention

Immediately after model establishment, rats in the sericin group received intragastric administration of sericin (2.4 g/kg) once a day for 35 days[57].

Blood samples

Rats were fasted for 12 hours after drug administration and then anesthetized by intraperitoneal injection of 4% chloral hydrate. A 3 mL volume of inner canthus blood was collected and centrifuged for 20 minutes at 3 000 r/min, and the serum was collected and stored at –20°C.

Determination of blood glucose levels using the glucose oxidase test

Blood glucose levels were determined using a Boehringer Mannheim in/Hitachi 717 automatic clinical biochemistry analyzer (Hitachi, Tokyo, Japan) and a glucose detection kit (batch No. 20071030, Baoding Great Wall Clinical Reagent Co., Ltd., Baoding, Hebei Province, China)[58-59].

Analysis of serum growth hormone and insulin-like growth factor 1 levels by enzyme-linked immunosorbent assay

The serum growth hormone and insulin-like growth factor 1 levels were determined, according to the instructions of assay kits for growth hormone (Rb, West Hills, CA, USA) and insulin-like growth factor 1 (Wuhan Boster Biotechnology Co., Ltd., Wuhan, Hubei Province, China), and the plates were read on a Multiskan MK3 Micro-plate reader (Thermo, Waltham, MA, USA)[60-61]. Samples (10 μL) were diluted with sample diluent (40 μL), and blank control wells without reagent or samples were also included. The plates were incubated at 37°C for 30 minutes, rinsed five times for 30 seconds each, and further incubated with 50 μL of reagent (except for blank control wells) and washed. The samples were developed with 50 μL of chromogenic agents A and B at 37°C in the dark for 15 minutes, and the reactions were terminated with termination solution. The absorbance value was read at a wavelength of 450 nm.

Hippocampal tissue

Rats were decapitated and the brains were rapidly harvested, from which the hippocampi were isolated and stored in liquid nitrogen[27, 62].

Analysis of hippocampal growth hormone and growth hormone receptor protein expression by western blot assay

Total hippocampal protein was extracted and the protein concentration was determined using the BCA protein assay kit (Beijing Taigemei Science and Technology Co., Ltd., Beijing, China). The proteins were separated by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were incubated with mouse anti-rat growth hormone monoclonal antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-rat growth hormone receptor polyclonal antibody (1:200; Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China) and mouse anti-rat β-actin monoclonal antibody (1:1 000; Santa Cruz Biotechnology) at 4°C overnight. Subsequently, the membranes were incubated with horseradish peroxidase conjugated goat anti-rabbit IgG (1:5 000; KPL Corp., Gaithersburg, MD, USA) at room temperature for 1 hour, and bound antibodies were detected with Super ECL Plus ultra-sensitive luminescent solution (Beijing Taigemei). The films were scanned with an EPSON scanner (Epson (China) Co., Ltd., Beijing, China) and the developing strip was analyzed using Quantity One-4.6.2 software (BIO-RAD, Hercules, CA, USA). The relative expression levels of target protein were represented as the ratio of target band absorbance to β-actin absorbance[63-64].

Analysis of hippocampal growth hormone, growth hormone receptor and insulin-like growth factor-1 mRNA expression by reverse transcription-PCR

Total hippocampal RNA was extracted with Trizol according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into cDNA. PCR primers were synthesized by Sangoniotech (Shanghai) Co., Ltd., Shanghai, China.

The PCR primer sequences are as follows:

Gene	Primer sequence	Tm (°C)	Cycle	Product length (bp)
Growth hormone	F: 5′-TGA CAC CTA CAA AGA GTT CGA GCG-3′ R: 5′-TGT TGG CGT CAA ACT TGT CAT AGG-3′	65	33	368
Growth hormone receptor	F: 5′-CTG GGT TGA GTT CAT TGA GCT GGA T-3′ R: 5′-TGT AGA GGG GAG TTG GTG GGT TGA C-3′	62	31	394
Insulin-like growth factor 1	F: 5′-CTG GTG GAC GCT CTT CAG TTC G-3′ R: 5′-TCC TTC TCC TTT GCA GCT TCC-3′	59	31	280
β-actin	F: 5′-GAG GGA AAT CGT GCG TGA C-3′ R: 5′-CTG GAA GGT GGA CAG TGA G-3′	55	29	445

Tm: Temperature; F: forward primer; R: reverse primer.

The PCR conditions were: 94°C for 2 minutes, 94°C for 30 seconds, 50–65°C for 30 seconds, and 72°C for 1 minute. Amplification products (5 μL) and DNA Ladder (5 μL; Beijing Taigemei) were subjected to 2% agarose gel electrophoresis (90 V, 40 minutes) and visualized using a ZF UV transmittance and reflection analyzer (Jiapeng Technology Co., Ltd., Shanghai, China). Quantitative analysis was performed using Quantity One-4.6.2 software, and the relative mRNA expression levels were represented as the ratio of target band absorbance to β-actin absorbance[24, 65-66].

Statistical analysis

Data were analyzed using SPSS 15.0 software (SPSS, Chicago, IL, USA) and are expressed as mean ± SD. Differences between groups were compared using one-way analysis of variance, and pairwise comparisons were performed using the *q* test. A *P* value < 0.05 was regarded as statistically significant.

REFERENCES

[1] Lu ZF, Zhong NS. Internal Medicine. 7th ed. Beijing: People's Medical Publishing House. 2008.

[2] Wilke T, Ahrendt P, Schwartz D, et al. Incidence and prevalence of type 2 diabetes mellitus in Germany: an analysis based on 5.43 million patients. Dtsch Med Wochenschr. 2013;138(3):69-75.

[3] Kota SK, Meher LK, Jammula S, et al. Genetics of type 2 diabetes mellitus and other specific types of diabetes; its role in treatment modalities. Diabetes Metab Syndr. 2012; 6(1):54-58.

[4] Jelenik T, Roden M. Mitochondrial plasticity in obesity and diabetes mellitus. Antioxid Redox Signal. in press.

[5] Consoli A, Di Biagio R. Protective effects of glucagon-like peptide-1 on beta-cells: preclinical and clinical data. G Ital Cardiol (Rome). 2011;12(12 Suppl 2):5-9.

[6] Bodmer M, Meier C, Krähenbühl S, et al. Metformin, sulfonylureas, or other antidiabetes drugs and the risk of lactic acidosis or hypoglycemia: a nested case-control analysis. Diabetes Care. 2008;31(11):2086-2091.

[7] Correia S, Carvalho C, Santos MS, et al. Mechanisms of action of metformin in type 2 diabetes and associated complications: an overview. Mini Rev Med Chem. 2008; 8(13):1343-1354.

[8] McAlister FA, Eurich DT, Majumdar SR, et al. The risk of heart failure in patients with type 2 diabetes treated with oral agent monotherapy. Eur J Heart Fail. 2008;10(7): 703-708.

[9] Pozzilli P, Raskin P, Parkin CG. Review of clinical trials: update on oral insulin spray formulation. Diabetes Obes Metab. 2010;12(2):91-96.

[10] DeWitt DE, Hirsch IB. Outpatient insulin therapy in type 1 and type 2 diabetes mellitus: scientific review. JAMA. 2003;289(17):2254-2264.

[11] Valdés-González RA, White DJ, Dorantes LM, et al. Three-yr follow-up of a type 1 diabetes mellitus patient with an islet xenotransplant. Clin Transplant. 2007;21(3): 352-357.

[12] Yoon JW, Jun HS. Recent advances in insulin gene therapy for type 1 diabetes. Trends Mol Med. 2002;8(2): 62-68.

[13] Calne RY, Gan SU, Lee KO. Stem cell and gene therapies for diabetes mellitus. Nat Rev Endocrinol. 2010;6(3): 173-177.

[14] Wu WJ, Zou DJ. Research advances of gene therapy for diabetes mellitus. Zhongguo Linchuang Kangfu. 2003; 24(7):3356-3357.

[15] Li SZ. Bencai Gangmu. Beijing: People's Medical Pubishing House. 1985:1045-1052.

[16] Bao XA. Yanfang Xinbian. Tianjin: Tianjin Science and Technology Publishing House. 1993.

[17] Manosroi A, Boonpisuttinant K, Winitchai S, et al. Free radical scavenging and tyrosinase inhibition activity of oils and sericin extracted from Thai native silkworms (Bombyx mori). Pharm Biol. 2010;48(8):855-860.

[18] Kim H, Lim YJ, Park JH, et al. Dietary silk protein, sericin, improves epidermal hydration with increased levels of filaggrins and free amino acids in NC/Nga mice. Br J Nutr. 2012;108(10):1726-1735.

[19] Seo CW, Um IC, Rico CW, et al. Antihyperlipidemic and body fat-lowering effects of silk proteins with different fibroin/sericin compositions in mice fed with high fat diet. J Agric Food Chem. 2011;59(8):4192-4197.

[20] Isobe T, Ikebata Y, Onitsuka T, et al. Effect of sericin on preimplantation development of bovine embryos cultured individually. Theriogenology. 2012;78(4):747-752.

[21] Manosroi A, Boonpisuttinant K, Winitchai S, et al. Free radical scavenging and tyrosinase inhibition activity of oils and sericin extracted from Thai native silkworms (Bombyx mori). Pharm Biol. 2010;48(8):855-860.

[22] Chen ZH, Song CJ, Fu XM, et al. Effects of sericin pretreatment on the expression of ECM associated protein in the kidney of diabetic nephropathy rats. Zhongguo Yike Daxue Xuebao. 2010;39(2):112-115.

[23] Fu XM, Ma HW, Fu WL, et al. Protective effects of sericin on pancreatic islet cells of type II diabetic rat. Jiepou Xue Zazhi. 2010;33(2):161-164.

[24] Fu WL, Fu Xm, Zhong MR, et al. Effects of sericine on growth hormone/insulin-like growth factor-1 axis of testis in type 2 diabetes mellitus rats. Jiepou Xuebao. 2011; 42(1):104-109.

[25] Fu WY, Zhong MR, He YQ, et al. Effects of sericine pretreatment on IGF-1 expression in testes of diabetes mellitus rat model. Zhongguo Zuzhi Huaxue yu Xibao Huaxue Zazhi. 2010;19(4):361-364.

[26] Hao XJ, Yu XM, Song CJ, et al. Effects of sericin on ERK signaling pathway of diabetic rats' kidney. Zhongguo Yike Daxue. 2013;42(1):60-64.
[27] Liu XY, Fu XM, Gao Y, et al. Protective effects of sericin on islet cells apoptosis of type 2 diabetes rats. Zhongguo Laonian Xue Zazhi. 2013;32(2):2525-2527.
[28] Chen ZH, He YQ, Fu WL, et al. Effects of sericin on heme oxygenase-1 expression in the hippocampus and cerebral cortex of type 2 diabetes mellitus rats. Neural Regen Res. 2011;6(6):423-427.
[29] Chen ZH, He YQ, Song CJ, et al. Sericin can reduce hippocampal neuronal apoptosis by activating the Akt signal transduction pathway in a rat model of diabetes mellitus. Neural Regen Res. 2012;7(3):197-201.
[30] Shangguan FF, Shi JN. Effect of growth hormone/IGF-1 on cognitive function. Zhongguo Xinli Weisheng Zazhi. 2007;21(8):568-570.
[31] Hua K, Forbes ME, Lichtenwalner RJ, et al. Adult-onset deficiency in growth hormone and insulin-like growth factor-I alters oligodendrocyte turnover in the corpus callosum. Glia. 2009;57(10):1062-1071.
[32] Holt RI, Simpson HL, Sönksen PH. The role of the growth hormone-insulin-like growth factor axis in glucose homeostasis. Diabet Med. 2003;20(1):3-15.
[33] Gibney J, Healy ML, Sönksen PH. The growth hormone/insulin-like growth factor-I axis in exercise and sport. Endocr Rev. 2007;28(6):603-624.
[34] Reinecke M. Influences of the environment on the endocrine and paracrine fish growth hormone-insulin-like growth factor-I system. J Fish Biol. 2010;76(6):1233-1254.
[35] Kim E, Sohn S, Lee M, et al. Differential responses of the growth hormone axis in two rat models of streptozotocin-induced insulinopenic diabetes. J Endocrinol. 2006;188(2):263-270.
[36] Ghigo E, Arvat E, Gianotti L, et al. Hypothalamic growth hormone-insulin-like growth factor-I axis across the human life span. J Pediatr Endocrinol Metab. 2000;13 Suppl 6:1493-1502.
[37] Cao P, Maximov A, Südhof TC. Activity-dependent IGF-1 exocytosis is controlled by the Ca(2+)-sensor synaptotagmin-10. Cell. 2011;145(2):300-311.
[38] Chiarelli F, Giannini C, Mohn A. Growth, growth factors and diabetes. Eur J Endocrinol. 2004;151 Suppl 3:U109-117.
[39] Zai GT. The relationship between growth hormone and chronic complications of type 2 diabetic patients. Xiandai Yiyao. 2004;20(14):1325-1326.
[40] Tsugane S, Inoue M. Insulin resistance and cancer: epidemiological evidence. Cancer Sci. 2010;101(5):1073-1079.
[41] Schwartzbauer G, Menon RK. Regulation of growth hormone receptor gene expression. Mol Genet Metab. 1998;63(4):243-253.
[42] Filopanti M, Giavoli C, Grottoli S, et al. The exon 3-deleted growth hormone receptor: molecular and functional characterization and impact on GH/IGF-I axis in physiological and pathological conditions. J Endocrinol Invest. 2011;34(11):861-868.
[43] Wang HT, Deng MH, Ou QJ, et al. Effects of rhGH on the expression of growth hormone receptor of liver cells in a murine experimental cirrhosis model. Zhonghua Putong Waike Zazhi. 2002;17(2):93-95.
[44] Meinhardt U, Eblé A, Besson A, et al. Regulation of growth-hormone-receptor gene expression by growth hormone and pegvisomant in human mesangial cells. Kidney Int. 2003;64(2):421-430.
[45] Chandrashekar V, Dawson CR, Martin ER, et al. Age-related alterations in pituitary and testicular functions in long-lived growth hormone receptor gene-disrupted mice. Endocrinology. 2007;148(12):6019-6025.
[46] Le Bouc Y, Brioude F. Is there a relationship between the growth hormone dose and tumoral or cardiovascular complications? Bull Acad Natl Med. 2012;196(1):127-137.
[47] Garrido MJ, Cendrós JM, Ramis J, et al. Pharmacodynamic modeling of the effects of lanreotide Autogel on growth hormone and insulin-like growth factor 1. J Clin Pharmacol. 2012;52(4):487-498.
[48] Arroba AI, Alvarez-Lindo N, van Rooijen N, et al. Microglia-mediated IGF-I neuroprotection in the rd10 mouse model of retinitis pigmentosa. Invest Ophthalmol Vis Sci. 2011;52(12):9124-9130.
[49] Wine RN, McPherson CA, Harry GJ. IGF-1 and pAKT signaling promote hippocampal CA1 neuronal survival following injury to dentate granule cells. Neurotox Res. 2009;16(3):280-292.
[50] Trejo JL, Carro E, Garcia-Galloway E, et al. Role of insu-lin-like growth factor I signaling in neurodegenerative diseases. J Mol Med (Berl). 2004;82(3):156-162.
[51] Cardona-Gómez GP, Mendez P, DonCarlos LL, et al. Interactions of estrogens and insulin-like growth factor-I in the brain: implications for neuroprotection. Brain Res Brain Res Rev. 2001;37(1-3):320-334.
[52] Zhang B, Li JJ. Insulin-like growth factor and diabetic encephalopathy. Guowai Yixue: Laonian Yixue Fence. 2006;27(4):181-185.
[53] Li ZG, Zhang W, Grunberger G, et al. Hippocampal neuronal apoptosis in type 1 diabetes. Brain Res. 2002;946(2):221-231.
[54] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.
[55] Chen P, Chen JB, Chen WY, et al. Effects of quercetin on nuclear factor-κB p65 expression in renal ubiquitin-proteasome system of diabetic rats. Zhonghua Nei Ke Za Zhi. 2012;51(6):460-465.
[56] Liu Y, Qi H, Wang Y, et al. Allicin protects against myocardial apoptosis and fibrosis in streptozotocin- induced diabetic rats. Phytomedicine. 2012;19(8-9):693-698.
[57] Zhan YL, Huang CG, Chen GL. Hypoglycemic activity of the decoction of cocoon shell on alloxan-diabetic model mice. Canye Kexue. 2003;29(4):446-448.

[58] Fu XM, Xue JF, Fu WL, et al. Effects of sericin pretreatment on the expression of pancreatic islet cell proteins in diabetes rats. Zhongguo Yike Daxue Xuebao. 2012;41(11): 1007-1010.

[59] Song CJ, Fu XM, Li J, et al. Effects of sericine on TGF-beta1/Smad3 signal pathway of diabetic mephropathy rats kidney. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2011; 27(1):102-105.

[60] Ma Q, Liu SF, Zhuang ZM, et al. Molecular cloning, expression analysis of insulin-like growth factor I (IGF-I) gene and IGF-I serum concentration in female and male Tongue sole (Cynoglossus semilaevis). Comp Biochem Physiol B Biochem Mol Biol. 2011;160(4):208-214.

[61] Tsai CY, Lai CH, Chang MH, et al. IGF-II and MMP9 as surgical repair indicators of ventricular septal defects. Clin Chim Acta. 2011;412(9-10):761-765.

[62] Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 5th ed. London: Academic Press. 2005.

[63] Lu M, Xu L, Li B, et al. Protective effects of grape seed proanthocyanidin extracts on cerebral cortex of streptozotocin-induced diabetic rats through modulating AGEs/RAGE/NF-kappaB pathway. J Nutr Sci Vitaminol (Tokyo). 2010;56(2):87-97.

[64] Li Q, You C, Liu L, et al. Craniopharyngioma cell growth is promoted by growth hormone (GH) and is inhibited by tamoxifen: involvement of growth hormone receptor (GHR) and IGF-1 receptor (IGF-1R). J Clin Neurosci. 2013;20(1): 153-157.

[65] Zhan LB, Sui H, Lu XG, et al. Effects of Zibu Piyin recipe on SNK-SPAR pathway in neuron injury induced by glutamate. Chin J Integr Med. 2008;14(2):117-122.

[66] Kelly BN, Haverstick DM, Vance ML, et al. Quantification of growth hormone mRNA in blood. Clin Chim Acta. 2012;414:206-210.

(Reviewed by Lodge A, Norman C, Shen LX, Zeng XN)
(Edited by Yu J, Yang Y, Li CH, Song LP, Liu WJ, Zhao M)