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Lowering the Blood Glucose of Diabetes Mellitus Mice by Oral Administration with Transgenic Human Insulin-like Growth Factor I Silkworms

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ABSTRACT: To evaluate the biological activity of the posterior silk glands of transgenic silkworms expressing human insulin-like growth factor I (hIGF-I), we bred hIGF-I-transgenic silkworms through eight generations by continuously selecting with green fluorescence and G418. The G8 transgenic silkworms were confirmed by polymerase chain reaction and dot blotting, and their posterior silk glands were removed from the fifth instar larvae to make freeze-dried powders. Enzyme-linked immunosorbent assay results showed that the expression level of hIGF-I in the posterior silk glands of G8 transgenic silkworm is approximately 493 ng/g of freeze-dried powder. When the freeze-dried powder was administrated by gavage to diabetes mellitus (DM) mice, the blood glucose in DM mice significantly decreased ($P < 0.05$) in a time- and dose-dependent manner compared with that of DM mice orally administrated with distilled water and normal freeze-dried powders made of untreated silk glands. These results demonstrated that hIGF-I expressed in posterior silk glands of transgenic silkworms could reduce blood glucose by oral administration.

KEYWORDS: *transgenic silkworm, hIGF-I, DM mice, reducing blood glucose*

INTRODUCTION

The silk gland of the silkworm is a highly specialized organ that has the tremendous ability to synthesize and secrete silk protein. As a good bioreactor, it has extensive prospects for drug delivery applications. The technology for the production of transgenic silkworms using a *piggyBac* transposon derived vector has been developed, in which target genes are stably inherited in the descendants of engineered insects.[1]

Human insulin-like growth factor I (hIGF-I) is a 70 amino acid polypeptide hormone (7.5 kDa) similar in structure to insulin, which can be used in treating patients with type 1 and type 2 diabetes mellitus (DM) and insulin resistance syndromes,[2] and it has been successfully expressed in the silk glands of transgenic silkworms by using the sericin[3] and *Fhx/P25*[4] promoters. However, the biological activity and the medical treatment of offspring of the hIGF-I-transgenic silkworm have not been investigated. In addition, a recent study showed that the oral insulin treatment effect in individuals with confirmed for insulin autoantibody (IAA) ≥ 80 nU/mL appeared to be maintained with additional follow-up; however, once therapy stopped, the rate of developing diabetes in the oral insulin group increased to a rate similar to that in the placebo group.[5]

In this paper, we evaluated the biological activity of hIGF-I expressed in the posterior silk glands of transgenic silkworms. DM mice, induced by injection of streptozotocin (STZ), were orally administrated with the freeze-dried powder of silk glands of hIGF-I-transgenic silkworms, and the blood glucose levels were significantly decreased in a time- and dose-dependent manner ($P < 0.05$), suggesting that the expressed hIGF-I in silk glands of transgenic silkworms had biological activity and could possibly be used as a perorally administered functional food as an alternative treatment for DM patients.

MATERIALS AND METHODS

Screening of an Inbred Strain of Transgenic Silkworm. Eggs of G4 generation transgenic hIGF-I silkworms[4] were incubated and reared with mulberry leaves. On the third day of the first instar stage and the first day of the second instar stage, the larvae were fed mulberry leaves coated with an aminoglycoside antibiotic (G418) at a concentration of 10 μg/mL. The survivors developing normally were fed with untreated leaves and screened for green fluorescent protein (GFP). The fluorescent silkworms were allowed to develop into moths, which inbred and laid eggs for seed reservation. The genomic DNAs of the copulated moths were extracted and identified by polymerase chain reaction (PCR) amplification. The identification of successive passages was carried out in the same way until the G8 generation.

Confirmation of Pure Transgenic Silkworm Lines. The genomic DNAs of copulated moths of G8 generation transgenic silkworms were identified with PCR amplification using primer pairs hIGF-I (forward primer 5′-TGG ATA TCA TGG GAC CGG AGA CGC TCT GC-3′ and reverse primer 5′-ATC TCG AGA AGC TTA AGC TGA CTT GGC AGG CTT G-3′), Destabilized enhanced Green Fluorescent Protein (DEGFP) (forward primer 5′-TGG AAT TCA TGG TGA GCA AGG GCG AGG-3′ and reverse primer 5′-TTG GAT CCT TA C TTG TAC AGC TCG TCC ATG-3′), and

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Neomycin resistance gene (NEO) (forward primer 5′-AGC TCG AGA ATT CTA GCT AGA GGT CGA C-3′ and reverse primer 5′-CTG ATA TCA TGA TTG AAC AAG ATG G-3′). The genomic DNAs of the G5, G6, G7, and G8 generation moths were identified using dot blotting. DNA hybridization with a Digoxigenin (DIG)-labeled *gfp* probe, membrane washes, and signal detection were carried out according to the manufacturer's instructions (DIG DNA Labeling and Detection Kit, Roche, Mannheim, Germany). Positive (constructed transgenic vectors) and negative (genomic DNA of normal silkworm) controls were also included.

Detection of hIGF-I Expression in the Posterior Silk Glands of Transgenic Silkworms. A sample of the posterior silk glands (PSGs) of the transgenic silkworms was mixed with the proper volume of phosphate-buffered saline (PBS; 0.01 mol/L, pH 7.2), and ground, and then 2 × SDS loading buffer (0.1 mol/L TrisCl, 0.2 mol/L dithiothreitol, 4% SDS, 20% glycerol, 0.2% bromophenol blue, 4% β-mercaptoethanol) was added. After the mixture was heated in a boiling water bath for 5 min and centrifuged at 12000*g* for 3 min, the supernatant was electrophoresed on acrylamide gels; the stacking gel and the separating gel were at 5% (v/v) and 15% (v/v), respectively. The proteins in the gel for Western blotting were transferred to a poly(vinylidene fluoride) (PVDF) membrane using an electrophoretic transfer cell. Western blotting was then performed using a rabbit anti-hIGF-I primary antibody (Beijing Biosynthesis Biotechnology, Beijing) and a horseradish peroxidase (HRP)-conjugated goat antirabbit secondary antibody (Beijing Biosynthesis Biotechnology).

Also, the PSGs of transgenic larvae on the fourth day of the fifth instar stage were dissected, freeze-dried, and powdered. Then 0.1 g of powder was treated with 1000 μL of lysate solution (8 M urea, 2 M thiouracil, 4% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 20 mM Tris base, 30 mM dithiothreitol (DTT)) at 0 °C for 12 h and centrifuged at 12000*g* for 10 min. The supernatants were analyzed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&DSystems, Minneapolis, MN).

Establishing a Model of DM Mice. Male Institute of Cancer Research (ICR) mice (strain SYXK (SU) 2007-0007) were maintained in specific pathogen-free (SPF) conditions at 25 °C and 60% humidity at the Experimental Animal Resource Centre of Soochow University (Suzhou, China) on regular mouse chow and water ad libitum with 12 h day/night regimes. streptozotocin (STZ) (Cheng pulls Biological Technology Co., Shanghai) dissolved in sodium citrate buffer (0.1 mol/L, pH 4.5) was filtered with a 0.2 μm filter. Male mice aged 5−6 weeks (20 ± 2 g) received a single tail vein injection of either 0.1 mL of saline (0.9% NaCl) or STZ (100 mg/kg of body weight (bw)). To avoid a short-term drug effect, after 48 h, the blood glucose levels of the injected mice were measured to monitor the development of diabetes. Only mice showing blood glucose stably over 7.0 mmol/L in the fasting state[6] were included in the experiment.

Effect of hIGF-I Expression in PSGs on Treatment of DM Mice. The mice were randomly divided into eight groups (n = 10/group). The mice in the normal group (group N) and DM model group (group DM) were administrated intragastrically with distilled water (50 μL/g of bw). The DM mice in the insulin treatment group (group IT) were administrated intragastrically with insulin (Nanjingxinbai Pharmaceutical Co., China) at a dose of 0.0025 IU (96.15 ng of insulin)/g of bw. In the insulin glargine treatment group (group IGT) test animals were treated with insulin glargine (Sanofi-Aventis Deutschland GmbH, Germany) at 0.0025 IU (91 ng of hIGF-I)/g of bw. The DM mice in the transgenic silk gland treatment group were divided into three subgroups, namely, TSL (low dosage), TSM (medium dosage), and TSH (high dosage). The freeze-dried powder of PSGs of transgenic silkworms was orally given to the mice in the TSL group at a dose of 0.002 g (containing 1 ng of hIGF-I)/g of bw, in the TSM group at a medium dose of 0.008 g (containing 4 ng of hIGF-I)/g of bw, and in the TSH group at a high dose of 0.017 g (containing 8 ng of hIGF-I)/g of bw. In the control group (group C), the DM mice were orally administrated with the freeze-dried powder of PSGs of nontransgenic silkworms at a dose of 0.008 g (without hIGF-I)/g of bw. The treatments were carried out daily.

After fasting for 7−8 h, each mouse was weighed and its blood glucose level was measured with a glucometer (TheraSense Co. Beijing) on the second, fourth, and sixth days. Statistical analysis was performed by analysis of variance (SPSS 16.0 software) using Student's t test. Statistical significance was defined as $P < 0.05$.

Dynamic Change of the Content of hIGF-I in the Blood of DM Mice Administrated with PSGs of the Transgenic Silkworms. To verify that hIGF-I could be absorbed in the alimentary canal directly, normal mice were orally treated with 0.3 g (147.9 ng of hIGF-I) of the freeze-dried powder, and then the blood was drawn from the tail veins at 0, 1, 2, 4, 6, and 8 h postadministration and centrifuged at 8000*g* for 5 min. The supernatant was used to determine the content of hIGF-I by ELISA. Among 30 male mice only treated with distilled water, each of 3 mice was sampled at the different checkpoint times, and their mixed blood, as a control, was used to measure hIGF-I.

RESULTS

Identification of Transgenic Silkworm Lines. Screening of inbred strains of transgenic silkworms was performed. The individuals showing green fluorescence were used for seed reservation. The eggs and pupae of the G8 generation of the transgenic silkworms emitted green fluorescence under a fluorescence microscope (Figure 1A,B). To verify that the target genes are stably inherited in the descendants of engineered silkworms, the genomic DNA was extracted from G5−G8 generation moths and hybridized with a DIG-labeled *gfp* probe. Positive results showed that the *gfp* gene existed in the genomes of all the G4−G8 generation fluorescent moths (Figure 1C). Furthermore, the genomic DNA of G8 generation moths was identified with PCR amplification using primer pairs of hIGF-I, DEGFP, and NEO, and the specific bands of hIGF-I

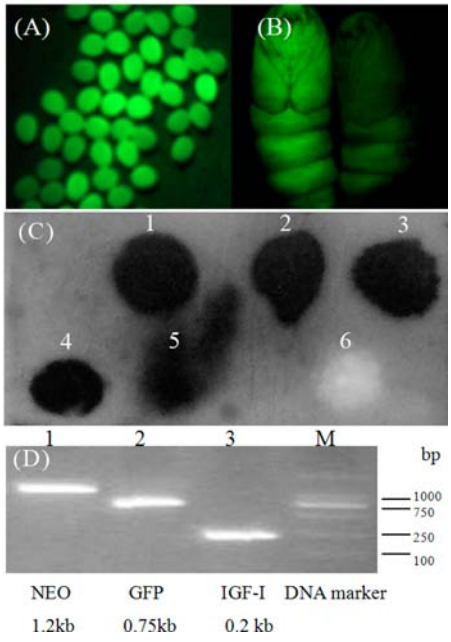

Figure 1. Observation and identification of transgenic silkworm lines: (A) eggs of the G8 generation in a fluorescent field; (B) pupae of the G8 generation in a fluorescent field; (C) dot hybridization of G5−G8 generation moths (1, vector pigA3GFP-hIGF-I-neo; 2−5, G5, G6, G7, and G8 transgenic moths; 6, normal silkworm); (D) identification of G8 generation moths with PCR amplification using primer pairs hIGF-I-1/hIGF-I-2, DEGFP-1/DEGFP-2, and NEO-1/NEO-3 (M, DNA marker; lanes 1−3, PCR amplification using primer pairs NEO-1/NEO-3, DEGFP-1/DEGFP-2, and hIGF-I-1/hIGF-I-2, respectively).

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(0.2 kb), GFP (0.75 kb), and NEO (1.2 kb) were successfully amplified (Figure 1D).

Expression Levels of hIGF-I in PSGs. A sample of PSGs was subjected to sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and Western blotting to verify the expression of hIGF-I in the PSG of a G8 transgenic silkworm, and a specific band was detected, suggesting hIGF-I was successfully expressed (Figure 2).

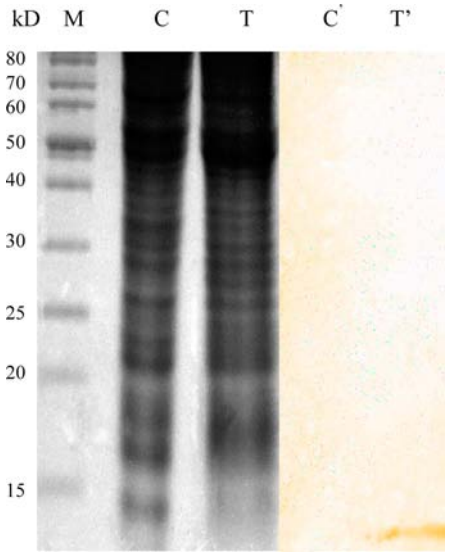

Figure 2. SDS−PAGE (left) and Western blotting (right) of the PSG of a transgenic silkworm. Lane M shows the protein marker. Lane C shows the protein analysis of the PSG of a normal silkworm. Lane T shows the protein analysis of the PSG of a transgenic silkworm. Lane C′ shows the Western blotting corresponding to lane C. Lane T′ shows the Western blotting corresponding to lane T. In the Western blotting, the primary antibody was rabbit anti-hIGF-I and the secondary antibody was HRP-conjugated goat antirabbit immunoglobulin G.

The expression level of hIGF-I in the PSGs of G8 fifth instar transgenic silkworms was approximately 493 ng/g of freeze-dried powder as measured by ELISA. This result revealed that exogenous proteins could be stably produced in the PSGs of the transgenic silkworms.

PSGs of the hIGF-I-Transgenic Silkworms Improve the Blood Glucose and Body Weight of DM Mice. Blood glucose levels of STZ-induced diabetic male mice were measured on the second, fourth, and sixth days. The blood glucose levels of normal mice (group N) were maintained within normal ranges. The blood glucose levels of mice in group DM were elevated and were higher than those of group N, indicating that the DM mice were established successfully. There was no obvious difference in the blood glucose levels of mice between group C and group DM. Compared with group DM, the blood glucose levels of mice in groups IGT, IT, TSH, and TSM were significantly decreased (Table 1). The lower ranges of blood glucose levels in groups TSH and TSM were lower than those in groups IGT and IT. The blood glucose levels of mice in groups DM and TSL were elevated by 27.59% and 12.43% on the sixth day, respectively. Blood glucose levels in groups TSH and TSM declined by 12.85% and 6.51%, respectively, compared with pretreatment data. These results showed that intragastric administration with the silk glands containing expressed hIGF-I could decrease the blood glucose levels in a dose-dependent manner. Furthermore, the blood glucose levels of mice in group TSH on the second, fourth, and sixth days were decreased by 9.38%, 11.12%, and 12.85%, respectively, and those in group TSM were decreased by 3.45%, 5.40%, and 6.51% compared with pretreatment data. These results also suggest that intragastric administration of the silk glands containing expressed hIGF-I could decrease the blood glucose levels in a time-dependent manner (Figure 3, data source from the right part of Table 1).

STZ-induced diabetic male mice, orally administered with the freeze-dried PSG powder of G8 generation transgenic silkworms, were weighed on the second, fourth, and sixth days (Table 2). There were obvious differences among the six treatment groups in body weight. Up to the sixth day, the body weights of mice in group N had increased by 20.35%, those of mice in group DM had declined by 12.11%, and the body weights of mice in groups IT and IGT had increased by 3.87% and 3.08%, respectively. The body weights of mice in group C had declined by 6.82%, and those in group TS declined by only 1.07%. These results showed that the body weights of DM mice were improved by orally administrating PSG powder of transgenic hIGF-I silkworms.

Table 1. Changes of Blood Glucose Values of the Experimental Mice[a]

group	initial mean blood glucose (mmol/L)	mean measured value of blood glucose (mmol/L)				change of blood glucose[b]			
		0 d	2 d	4 d	6 d	0 d	2 d	4 d	6 d
N	5.6 ± 0.5	5.2 ± 1.3	5.3 ± 1.7	5.7 ± 2.2	5.5 ± 2.4	0	0.1	0.5	0.3
DM	5.6 ± 0.6	16.7 ± 5.5	18.1 ± 5.0	19.5 ± 4.5	21.3 ± 3.5	0	1.41	2.85	4.6
C	5.9 ± 0.6	18.5 ± 2.4	19.6 ± 2.8	19.9 ± 3.0	20.8 ± 3.3	0	1.12	1.4	2.3
IGT	5.8 ± 0.5	19.2 ± 2.0	17.9 ± 1.8 ab	16.4 ± 1.7 ab	14.4 ± 1.5 ab	0	−1.3	−2.8	−4.8
IT	5.3 ± 0.7	21.7 ± 3.3	18.2 ± 3.1 ab	16.2 ± 2.8 ab	15.2 ± 2.4 ab	0	−3.5	−5.5	−6.5
TSH	5.9 ± 0.6	17.3 ± 3.3	15.7 ± 2.8 abc	15.4 ± 2.9 abc	15.1 ± 3.0 abc	0	−1.62	−1.92	−2.22
TSM	5.6 ± 0.7	18.0 ± 4.1	17.4 ± 3.7 abcd	17.0 ± 3.7 abcd	16.8 ± 3.7 abcd	0	−0.62	−0.97	−1.17
TSL	5.4 ± 0.7	18.9 ± 5.0	18.9 ± 4.7 bcd	19.1 ± 3.9 cd	19.8 ± 4.3 cd	0	0.06	0.26	0.93

[a]Key: a, compared with group DM ($P < 0.001$); b, compared with group C ($P < 0.001$); c, compared with group IT ($P < 0.05$); d, compared with group IGT ($P < 0.05$). All comparisons were calculated by Dunnett. Means ± SEM are shown ($n = 10$ in each group). Groups IGT, IT, and C were orally administrated with insulin glargine (91 ng of hIGF-I/g of bw), insulin (96.15 ng of insulin/g of bw), and PSG powders of nontransgenic silkworms (0.008 g/g of bw), respectively. Groups TSL, TSM, and TSH were orally administered with PSG powders of transgenic silkworms, 0.002 g (containing 1 ng of IGF-I)/g of bw, 0.008 g (containing 4 ng of hIGF-I)/g of bw, and 0.017 g (containing 8 ng of hIGF-I)/g of bw, respectively. Groups N and DM were orally given distilled water. The values at 0 d were detected before oral administration. [b]Change of blood glucose = measured value on the day − measured value on day 0.

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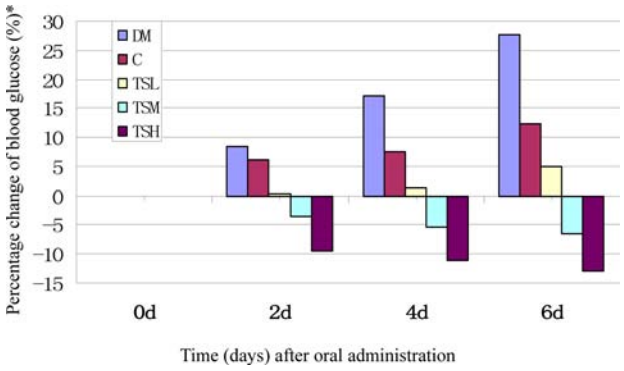

Figure 3. Percentage changes of blood glucose levels in groups DM, C, TSL, TSM, and TSH. Percentage change of blood glucose = [(measured value on the day − measured value on day 0)/measured value on day 0] × 100. DM = DM mice administrated intragastrically with distilled water. C = DM mice orally administrated with the freeze-dried powder of PSGs of nontransgenic silkworms (without hIGF-I). TSL = DM mice orally administrated with the freeze-dried powder of PSGs of transgenic silkworms containing a low dosage of hIGF-I. TSM = DM mice orally administrated with the freeze-dried powder of PSGs of transgenic silkworms containing a medium dosage of hIGF-I. TSH = DM mice orally administrated with the freeze-dried powder of PSGs of transgenic silkworms containing a high dosage of hIGF-I.

Dynamic Change of the hIGF-I Content in the Blood Serum of DM Mice Administered with PSGs of Transgenic Silkworms. One of the normal SPF mice was orally treated with 0.3 g (147.9 ng of hIGF-I) of the freeze-dried powder of PSG transgenic silkworms. The concentration of hIGF-I in the blood was determined at 0, 1, 2, 4, 6, and 8 h postadministration. The results showed that the expressed hIGF-I in silk glands could be absorbed into the blood of the mouse and the maximum concentration of hIGF-I presented at 2 h post-treatment declined slowly (Figure 4).

DISCUSSION

Lepidopterous *piggyBac* transposon established transgenic silkworm lines screened by EGFP could inherit the phenotype stably for more than six generations.[7] In 2011, Liang et al. using a secretory expression approach expressed hIGF-I under the control of the fibroin heavy chain promoter of the silkworm; the expressed hIGF-I was then secreted into the silk gland lumen. The transgenic silkworm inherited for seven generations, from which the PSG powder was found to reduce the blood glucose of DM mice in a dose-dependent manner.[8] In this study, after a series of screening procedures, an inbred

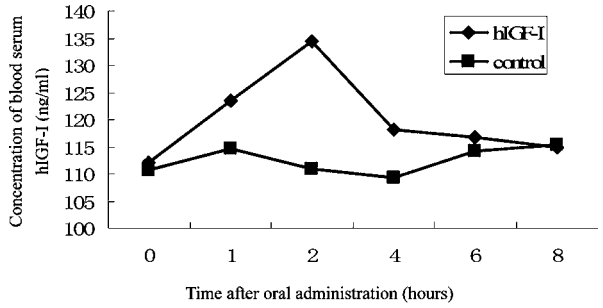

Figure 4. Change of hIGF-I concentrations in blood serum with time. Blood from one of the DM mice orally administrated once with 0.3 g of freeze-dried powder (containing 147.6 ng of hIGF-I) was collected at 0, 1, 2, 4, 6, and 8 h postadministration. The value at 0 h was detected before oral administration. A mixture of blood from three male mice treated with distilled water was used as the control.

strain of transgenic silkworms, in which hIGF-I was driven by the *Fhx/P25* promoter, was obtained. The expressed hIGF-I is in the cytoplasm, and the expression level of hIGF-I in PSGs of G8 transgenic silkworms is 493 ng/g of freeze-dried powder, which is equivalent to 148 ng/g of fresh PSGs of G4 generation fifth instar transgenic silkworms.[4] These results indicate that exogenous genes could be inherited and expressed stably through several passages in transgenic silkworms. Moreover, as the functional food, the freeze-dried powder from the transgenic silkworm could reduce blood glucose in a time- and dose-dependent manner. Furthermore, in the experiment, the freeze-dried powder was made from the whole PSGs of the silkworms, and had the contents in the PSG lumen been eliminated, the content of hIGF-I would be appropriately elevated.

Oral delivery of peptides or proteins is an attractive alternative to subcutaneous injection, as it is the easiest and most widely used route of drug delivery, especially when repeated or routine dosing is necessary, and it is less invasive and cheaper.[9,10] Oral delivery of small peptides or proteins is generally not feasible because of presystemic enzymatic degradation and poor penetration of the intestinal membrane.[11,12] Previous reports have shown that blood glucose levels were reduced in mice given oral doses of hIGF-I, expressed using recombinant baculovirus in silkworms.[13]

The oral meal test with the transgenic mice expressing hIGF-I acutely reduced blood glucose levels in streptozotocin-induced and Zucker diabetic rats.[14] It has be documented that IGF-I could be absorbed intact in receptor-active form into the portal circulation and might exert effects on the liver and other

Table 2. Changes in Body Weights of Mice during the Experimental Period[a]

		body weight (g)			
group	initial body weight (g)	0 d	2 d	4 d	6 d
N	18.5 ± 1.0	20.3 ± 1.2	21.6 ± 1.8	22.7 ± 1.8	24.4 ± 1.4
DM	19.2 ± 1.4	18.4 ± 3.1	18.0 ± 2.9	17.5 ± 2.8	16.9 ± 2.7
C	19.8 ± 1.5	17.0 ± 2.5	16.7 ± 2.4	16.5 ± 2.1 a	15.9 ± 2.4
TSM	19.8 ± 0.9	17.8 ± 2.2	17.7 ± 2.5 a	17.8 ± 3.0 a	17.7 ± 2.9 a
IT	20.3 ± 1.3	17.6 ± 2.6	17.7 ± 2.2 a,b	18.0 ± 2.5 a.b	18.3 ± 2.1 a.b
IGT	19.8 ± 1.1	17.9 ± 1.9	17.9 ± 2.1 a,b	18.3 ± 1.7 a.b	18.4 ± 2.0 a.b

[a]Key: a, compared with group DM ($P < 0.01$); b, compared with group TSM ($P < 0.05$). All comparisons were calculated by Dunnett. Means ± SEM are shown ($n = 10$ in each group). Groups IGT, IT, TSM, and C were orally administered with insulin glargine (91 ng of hIGF-I/g of bw), insulin (96.15 ng of insulin/g of bw), PSG powders of transgenic silkworms (4 ng of hIGF-I/g of bw), and PSG powders of nontransgenic silkworms (0.008 g/g of bw), respectively. The values at 0 d were detected before oral administration.

peripheral tissues.[15] This result was also confirmed in neonatal piglets.[16]

In our study, the results showed that when the mouse was orally administrated with PSGs of the transgenic silkworms expressing hIGF-I, the concentration of hIGF-I in the blood increased within the first 2 h, suggesting that hIGF-I could be absorbed by oral administration. Furthermore, transgenic silk glands containing hIGF-I decreased blood glucose levels in DM mice in a dose-dependent manner, even when the amount of hIGF-I in transgenic silk glands was less than the amount of insulin or insulin glargine. This indicated that, using the same amount of hIGF-I in transgenic silk glands, insulin, and insulin glargine, the level of blood glucose of DM mice could be reduced effectively by orally administrating transgenic silk glands containing expressed hIGF-I. Moreover, the dose of hIGF-I is lower than those of previous reports.[17,18] The increased rate of blood glucose levels in group TSL is lower than that in group DM, which may be the result of fibroin hydrolysate lowering blood glucose in ob/ob mice.[19] Another possible explanation was that fibroin could act as an oral adjuvant to reduce blood cholesterol levels in fibroin-fed rats.[20]

Pardina et al.[21]found that hIGF-I could induce body mass loss (P = 0.0025) in morbidly obese patients. Similarly, the weight of mice in group TSM decreased by 1.07%, which was less than that of groups NS (down 6.82%) and DM (down 12.11%). These results implied that low doses of transgenic silk glands containing hIGF-I could retard the decline of the weight of DM mice.

Oral administration of insulin can delay the onset and reduce the incidence of diabetes in traditional inbred mice (NOD) mice over a 1 year period in animals administered 1 mg of porcine insulin orally twice a week for 5 weeks and then weekly until 1 year of age.[22] In addition, in this study, the blood glucose levels of DM mice in group IT were reduced by oral administration when treated once for 1 day.

In our study, dynamic changes of hIGF-I concentrations in the blood serum of DM mice were observed, which showed that the levels in blood serum at 8 h post-treatment were similar to the pretreatment levels. The metabolic half-life of hIGF-I by oral administration is 3.7 h, which is longer than that by injection.

In summary, we used transgenic silk glands containing hIGF-I to reduce the blood glucose of DM mice successfully. In the future, it may be used as an oral glucose-lowering food in clinical trials.

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Notes

The authors declare no competing financial interest.

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