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The silkworm: A promising invertebrate diabetes model for natural drug discovery

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Abstract: As an economically important insect, the silkworm (*Bombyx mori*) occupies a central position in the silk industry. Its unique physiological characteristics make it a potential model animal for research on disease modeling and drug screening. The aim of this review was to explore the feasibility of the silkworm as a model animal for diabetes and to evaluate the potential application of this model in new drug discovery. Through high-glucose feeding and chemical induction, researchers successfully constructed silkworm models with diabetic phenotypes, which exhibited features such as hyperglycemia and insulin resistance similar to human diabetes. Subsequently, the researchers screened a variety of natural medicines and found that certain natural medicinal components were able to significantly reduce blood glucose levels and improve insulin sensitivity in the model silkworms. This review not only provides a new model animal for the pathophysiological study of diabetes, but also provides an experimental basis for the application of natural medicines in diabetes treatment, opens up a new way for the discovery of the active sub-ingredients of natural medicines, and is expected to provide a new strategy and source of medicines for the treatment of diabetes.

***Keywords*:** silkworm model, diabetes, natural medicine

Introduction

Diabetes is a global metabolic disease with a growing epidemic. According to the 10th edition of the IDF Diabetes Atlas, released by the International Diabetes Federation (IDF) on December 6, 2021, the number of adults aged 20-79 years with diabetes reached 537 million worldwide in 2021, which is equivalent to 1 in 10 people. Epidemiologic projections indicate that the number of people with the disease will climb to 643 million by 2030 and further to 783 million by 2045. Notably, between 2021 and 2045, the world population is expected to grow by 20%, while the number of people with diabetes will increase by 46% (*1*), significantly faster than the population growth rate. China, as the country with the largest number of diabetic patients in the world, has reached an epidemiologic level of prevalence in its adult population. Diabetes is characterized by persistent hyperglycemia and defective or impaired insulin secretion. Its typical clinical manifestations can be summarized as the "three more and one less" syndrome, *i.e.*, polydipsia, polyuria, polyphagia with progressive weight loss. As the disease progresses, patients may develop multi-system complications, including chronic progressive damage to target organs such as eyes, kidneys, nerves, heart and cardiovascular organs, which ultimately leads to organ failure. In addition, patients are at risk for serious complications such as acute metabolic disorders (*2*).

However, the current clinical application of antidiabetic drugs still has obvious limitations in disease management, and their adverse reactions not only affect the therapeutic effect, but also seriously threaten the health and quality of life of patients (*3*). Taking the commonly used drugs for type 2 diabetes treatment as an example: empagliflozin may trigger adverse reactions such as blood glucose fluctuation, nausea and dizziness; dagliflozin may lead to abnormal weight loss; and the use of cagliflozin has been associated with a variety of serious complications, including diabetic ketoacidosis, lower limb amputation, acute kidney injury, fungal infections, and osteomyelitis, among others. In addition, dulaglutide and somatostatin, which are glucagon-like peptide-1 receptor agonists, have good glucose-lowering effects, but their high incidence of gastrointestinal adverse reactions limits clinical application. The safety issues raised by these drugs not only aggravate the disease burden of patients, but also bring additional economic burden. Therefore, the development of new safe, economical, and efficient therapeutic drugs has become an urgent need in the current field of diabetes treatment.

In the process of searching for safe and effective therapeutic drugs, natural medicines have gradually emerged as a hotspot of research by virtue of their many advantages such as multi-target regulation and fewer side effects. Natural medicines not only have rich chemical diversity, complex structure and variety, which provide a large amount of material basis for drug screening, but also have a certain degree of biological adaptability and safety due to their natural screening. In addition, natural medicines contain the wisdom of traditional medicine as well as the treasures of ethnomedicine, which provide abundant clues and experiences for modern drug screening. These natural products originated from plants, animals and microorganisms are opening up completely new pathways for diabetes treatment with the help of modern scientific validation. For example, artemisinin and its derivatives can improve insulin resistance by inhibiting the inflammatory response. Many studies have shown that artemisinin is able to target the NF-κB signalling pathway and reduce the release of inflammatory factors, thereby alleviating insulin resistance (*4,5*): diazoxide (Catalpol) improves insulin resistance by activating the hepatic PI3K/Akt pathway (*6*); and curcumin attenuates diabetic myocardial fibrosis by regulating the MAPK/NF-κB signalling axis ($p < 0.05$) (*7*); baicalein stimulates insulin secretion in β-cell lines and human pancreatic islets (*8*); and berberine slows down glucose uptake by inhibiting α-glucosidase activity (*9*).

Animal model construction has become an irreplaceable experimental paradigm in the study of pathological mechanisms of diabetes and its complications. According to the evolutionary status of species and research needs, three main types of experimental systems are currently used:

Classical mammalian models: rodents (*e.g.*, rats and mice) (*10*) are widely used by virtue of their clear genetic backgrounds and short reproductive cycles; rabbits (*11*) occupy an important position in diabetes research by virtue of their unique metabolic characteristics; and pigs (*12*) have become an ideal large-animal model of diabetes because of the high degree of similarity between their physiological structures and those of humans.

Novel model organism system: With the development of histological technology, lower biological models show unique value: drosophila (*13*) provides a new perspective for diabetes research with its simple genome and short life cycle; nematode (*14*) becomes an ideal model for studying metabolic regulation mechanism with its complete insulin signalling pathway; zebrafish (*15*) plays an important role in diabetes research with its advantages of embryonic transparency and easy genetic manipulation for diabetes research.

Breakthroughs in characteristic resource organisms: as a model organism originated in China, the silkworm (*Bombyx mori*) (*16*) has attracted attention from academics for its unique regulatory mechanism of glucose metabolism and its advantages in large-scale rearing. Studies have shown that the hemolymph glucose homeostatic regulatory system of the silkworm is highly homologous to that of humans, which, coupled with its short life cycle (about 50 days) and well-defined developmental stages, provides a novel platform for the study of diabetes dynamics. Systematic comparisons show that the silkworm exhibits significant advantages in terms of the cost of glucose tolerance experiments (90% lower than that of mammals) and the convenience of phenotypic observation.

Advantages of the silkworm as a model animal

The silkworm, also known as the silkworm moth, is an economically important insect in the Lepidoptera family of silkworm moths. By virtue of its unique biological characteristics, the silkworm has developed into a valuable model organism in the field of life science research, showing broad application prospects in several research directions. Currently, researchers have successfully constructed a variety of disease models (*e.g.*, gout model (*17*), microbial infection model (*18,19*), Parkinson's mode (*16*), diabetes model, virus infection model (*20*), *etc.*). In addition, the silkworm also plays an important role in toxicology studies (*21,22*), environmental monitoring (*23,24*), and drug screening (*19,25*), providing an ideal experimental platform for related research (Table 1).

Physiological characteristics suitable for research

As a model organism, the silkworm exhibits unique advantages in experimental manipulation. Compared with mammals (*e.g.*, mice, rabbits), the silkworm does not require complex fixation devices, which significantly improves the efficiency of experiments. Compared with other insect models (*e.g.*, drosophila), the silkworm is moderately active and not easy to escape, which facilitates experimental manipulation and continuous observation, and this characteristic greatly improves the controllability and reproducibility of the experiments. Compared with invertebrates (*e.g.*, nematodes), the domestic silkworm has a moderate body size, and its organs and tissues are clearly recognizable, which facilitates precise anatomical isolation and sample collection, providing ideal conditions for in-depth mechanistic studies. These unique physiological characteristics make the silkworm a valuable model organism for metabolic disease research.

Low feeding cost and short cycle time

The silkworm has a unique developmental biology, its life cycle goes through four developmental stages: egg, larva, pupa and adult, and it only takes 23-29 days from hatching to 5th instar larvae (Figure 1). This short life

Table 1. Application of silkworm disease model in newdrug discovery

Silkworm model types	Characterization	Mechanism exploration	Success case
Diabetes (*26*)	Blood sugar levels rise markedly, resulting in the emergence of impaired glucose tolerance, which can be mitigated by hypoglycemic medications.	Increased JNK phosphorylation levels lead to insulin resistance.	1. YM0831 (*25*) 2. Extract of *Salacia reticulata* (*49*) 3. Jasmine crude polysaccharide (*27*) 4.Total flavonoids of *Paederia scandend* (*27*) 5.Total flavonoids of hibiscus flowers (*27*) 6.Total flavonoids of chamomile (*27*)
Bacteria			
Staphylococcus aureus (*28*)	After injecting bacteria, the hemolymph of silkworm resulted in a significant decrease in their survival within 24-48 h. Glycopeptide antibiotics were non-toxic to larvae and cured *S. aureus* infection.	The silkworm is infected by a variety of pathogenic bacteria, but can be effectively treated with clinical antibiotics (which have similar ED_{50} values). In addition, parameters such as drug half-life and volume of distribution in the silkworm are consistent with mammalian models (*29*).	Lysocin E (*19*)
Pseudomonas aeruginosa (*18*) *Vibrio cholerae* (*18*)	When injected into the bloodstream of 5th instar silkworm larvae, more than 90% of the larvae died within 2 days.		Silkworms could be used in anti-bacterial drug development.
Fungus			
Candida albicans (*31*) *Candida tropicalis* (*31*) *Candida glabrata* (*31*) *Cryptococcus neoformans* (*31*) *Aspergillus fu*migatus (*31*)	After injecting fungi, silkworms died.	Antifungal drugs exhibit a high protein binding capacity in the hemolymph of the silkworm, similar to that of mammalian serum, which can lead to reduced therapeutic efficacy. The silkworm recognizes not only bacterial peptidoglycans and lipopolysaccharides, but also the fungal cell wall component β-glucan, which activates the innate immune system (30, 31).	Silkworm have potential for antifungal drug discovery. ASP2397 (*32*)
Virus			
Bombyx mori nucleopolyhedrovirus	Antiviral drugs inhibit the proliferation of baculoviruses in the body fluids of the silkworm, and have a therapeutic effect.	Cinnzeylanine inhibits the proliferation of herpes simplex virus in Vero cells.	Cinnzeylanine (*20*)
Innate immunity	Upon injecting peanut oil into the body of a silkworm, melanin is generated within its system, causing the blood to turn black and the muscles to contract.	After chemical treatment (saponification reaction) to destroy the structure of TAGs, their immune activation effect is significantly weakened.	Triacylglycerol (*33*)
Toxicology (*34*)	The half-life of the drug is similar in both silkworms and mice.	The lethal dose of cytotoxic chemicals for silkworms is consistent with that for mammals, and the metabolic pathways of drugs in silkworms and mice are consistent.	Silkworms have potential for assessing drug toxicity and studying metabolic mechanisms.
Parkinson's disease (*16*)	Motor dysfunction, dopaminergic neuron degeneration, and decreased dopamine levels.	50 proteins were severely downregulated, and mechanical damage was observed in silkworm tissues	Silkworms have potential for Parkinson's disease mechanism research and drug discovery.
Gout (*17*)	Elevated uric acid Levels.	When *Bm5'N* mutation occurs in silkworms, the level of uric acid in the epidermis significantly increases.	Silkworms may be a suitable model for studying uric acid metabolism pathways associated with human diseases.

cycle combined with its strong reproductive ability can rapidly obtain a large number of offspring, significantly shortening the experimental cycle and improving research efficiency. In terms of feeding conditions, the silkworm only needs mulberry leaves or mulberry leaf-based artificial diets to meet its nutritional needs, and feeding management is simple. Compared with mammalian models, the requirements of laboratory rearing facilities for silkworms are simple, without need for complex incubators and specialized animal rooms, and a single incubator for silkworms can accommodate hundreds to thousands of individuals at the same time, which greatly saves experimental space. These advantages make establishment and maintenance costs of silkworm models significantly lower than those of traditional mammalian models, providing an economically feasible option for large-scale experimental research.

The main stages of laboratory silkworm rearing are shown in Figure 1 above. The number of days required for each stage: eggs 7–10 days, 1st instar larvae 3–4 days, 2nd instar larvae 3–4 days, 3rd instar larvae 4–5 days, 4th instar larvae 6–7 days, 5th instar larvae 7–9 days.

Pathophysiological similarities with human diabetic disease

The silkworm, has unique molecular biological advantages as a model for diabetes research. Studies have shown that the primary structure of the silkworm is significantly homologous to human insulin, and its A and B chains are highly conserved with human insulin (*35*), which makes it an ideal model for studying the pathogenesis of diabetes mellitus, its complications, and the screening of hypoglycemic drugs. In addition, the silkworm can exhibit typical diabetes-like symptoms such as metabolic disorders due to hyperglycemia under pathological conditions. It is noteworthy that a special peptide hormone, bombyxin, exists in the silkworm, and its three-dimensional structure is highly similar to that of human insulin (*36*), which provides an important molecular basis for study of the insulin signaling pathway and related metabolic regulation mechanisms.

Genetic similarity

The silkworm exhibits significant evolutionary conservation of genetic mechanisms with humans, and its genome structure is highly homologous to the human genome in several functional regions. This conservatism enables certain mutants of the silkworm to mimic the phenotypic characteristics of human genetic diseases (*37*). It provides a unique model organism platform for human research on genetic diseases. Through the silkworm model, researchers can systematically analyze the functional network of related genes, mutation mechanisms and their pathogenesis, which not only deepens our understanding of the molecular basis of genetic diseases, but also provides important theoretical support for early diagnosis of diseases and development of targeted therapies and preventive strategies, as well as potential intervention targets. Therefore, as a model organism for study of human genetic diseases, the silkworm has an irreplaceable value in both basic research and translational medicine (*38*).

Figure 1. Standardized laboratory feed system for silkworms.

Metabolic relevance

The silkworm shows remarkable similarity with mammals in terms of toxicity response and drug metabolism. On the one hand, the lethal dose levels of cytotoxic chemicals in silkworms are basically the same as those in mammals; on the other hand, the half-life of 4-methylumbelliferone in the hemolymph of silkworm larvae is 7.0 ± 0.1 min, which is similar to that in the blood of mice, according to a study using 4-methylumbelliferone as a model drug. More interestingly, at the level of metabolic mechanisms, silkworms and mammals share commonalities: both can metabolise chemicals by reacting with cytochrome P450 enzymes, binding to hydroxylated compounds, and ultimately excreting them (*34*).

Fewer ethical issues, in line with the 3R principle of animal experimentation

The ethical review of experimental animals is an important mechanism to ensure the scientific and ethical nature of animal experiments, the core of which lies in the strict adherence to the "3R" principle (Replacement, Reduction, Refinement) (*39*). According to internationally accepted ethical guidelines for animal experiments, researchers should preferentially select lower animals as experimental subjects, minimize the number of animals used by optimizing the experimental design, and improve the experimental methods to minimize animal suffering (*40*). In this context, the silkworm, as an invertebrate model organism, has obvious ethical advantages over higher mammals: its nervous system is relatively simple and its perceptual ability is limited, so there are fewer ethical controversies involved in the experimental process, and the restrictions on ethical review are relatively loose, which provides convenient conditions for carrying out large-scale experimental research.

Methods for modeling diabetes in the silkworm

The construction of the silkworm diabetes model is an important experimental platform to study the pathogenesis of diabetes and drug screening. At present, commonly used model construction methods mainly include the high sugar feed induction method and chemical drug induction method. These two methods, their principles of operation, and their advantages and disadvantages will be introduced separately in the following.

High-sugar diets induction

High sugar feed induction refers to the induction of a pathological state similar to diabetes or other metabolic disorders in experimental animals or biological models by feeding high-sugar diets for a long or short period of time, which causes a sustained increase in blood glucose levels. The human diabetic state is mimicked by feeding silkworms a mulberry leaf or artificial diets containing high concentrations of glucose, sucrose, fructose, or other sugars to elevate their blood glucose levels (*26,41-42*). A model of type 2 diabetes was successfully established after rearing 5th instar larvae for 18 h on a diet containing 10% glucose (high-glucose diet), and the silkworms showed typical diabetic characteristics: the total sugar level in the hemolymph of silkworms on the high-glucose diets was 2.4-fold higher than that of normal silkworms, their glycaemic content was significantly elevated ($p < 0.01$) and a glucose tolerance test showed impaired glucose clearance capacity. Phosphorylated JNK levels were found to be significantly increased in the fat bodies of hyperlipidemic silkworms fed a high-sugar diet for 18 h as compared to a normal-diet silkworms by Western blot analysis (*26*).

In terms of sugar selection, there were significant differences in the regulation of blood glucose in the model animals using different sugars. Glucose, as a monosaccharide, can be directly absorbed into the blood circulation, leading to a rapid increase in blood glucose; sucrose, as a disaccharide, needs to be decomposed into glucose and fructose in the body before being absorbed and utilized (*43*), so its rate of glucose increase is relatively slow; and fructose, although the same as other monosaccharides, is converted into glucose mainly through hepatic metabolism, so its glucose-raising effect is not as significant as that of glucose.

However, the method faces challenges in its implementation. There is an obvious seasonal limitation of mulberry leaves, the traditional food of silkworms, especially in the winter when the supply is insufficient, which may affect the continuity and reproducibility of the experiments. To overcome this limitation, researchers have developed artificial diets for the silkworm, among which Japanese scholars were the first to realize the breakthrough of an artificial diet feeding the 3rd instar (*44*), and the technology of feeding silkworms at the 5th instar on artificial diets is now very mature.

Chemical induction

Chemically induced models are modelling approaches in which specific chemicals are given to experimental animals to induce changes in the target disease or pathophysiology. In the field of diabetes modelling in mammals (*e.g.*, rats, mice), tetroxine (Alloxan) and streptozotocin (STZ) are two classical chemoinducers (*45*). Similarly, some researchers have used a co-injection technique in silkworm experiments, where silkworms were synchronously injected with a 40% glucose-containing solution (0.5 mL) and an 80 mg/kg solution of streptozotocin (0.5 mL), and it was found that

the glucose content of the experimental subjects showed a significant trend of glucose elevation. Streptozotocin, as an aminoglucose-nitrosourea derivative, is able to enter cells through glucose transporter protein 2 (Glut2), leading to the necrosis of insulin-producing cells (pancreatic islet β-cells). In the silkworm, glucose levels in the hemolymph are regulated mainly by a neurosecretory hormone, bombyxin, which is associated with the insulin signalling pathway. All streptozotocin may affect glucose regulation mechanisms in the silkworm by interfering with the secretion of homeoboxin or its signalling pathway, which in turn affects glucose regulation mechanisms in the silkworm (*46*).

Application of the silkworm in diabetes research

Drug screening and efficacy evaluation

Japanese researchers have found that silkworms share a conserved blood glucose regulation mechanism with mammals, and that the AMP-activated protein kinase (AMPK) and insulin signalling pathways in their hemolymph are involved in the regulation of glucose metabolism. The study demonstrated that 5-aminoimidazole-4-carboxamide-1-b-D-ribofuranoside (AICAR), an AMPK activator, and human insulin could effectively correct the growth phenotype of hyperglycemic silkworms by down-regulating the total glucose level in the hemolymph (*47,48*). This finding provides a theoretical basis for the use of silkworm models to screen for antidiabetic drugs targeting the AMPK/insulin signalling pathway. In addition, the Japanese team found that *Enterococcus faecalis* YM0831 significantly inhibited the sucrose-induced hyperglycaemic effect. When it was combined with the extract of the Traditional Chinese medicine *Kothala himbutu* (*Salacia reticulata*), it exhibited synergistic hypoglycaemic effects in the system of the silkworm (*25,49*), which provides a new idea for the development of novel complex hypoglycaemic agents. In addition, in studies using the silkworm as a model animal, a variety of herbs have been found to have hypoglycemic effects, such as jasmine crude polysaccharides, total flavonoids of *Paederia scandend*, total flavonoids of hibiscus flowers and total flavonoids of chamomile, all of which have been demonstrated to exert significant hypoglycemic efficacy by inhibiting the activity of α-glucosidase (*27*).

In terms of pharmacodynamic evaluation, the researchers successfully induced a type 2 diabetes model in silkworms by feeding them high glucose diets for 18 hours. The clinically used hypoglycaemic drugs pioglitazone and metformin both showed significant improvement in glucose tolerance in this model, which verified the reliability of this model for the evaluation of drugs used in the treatment of type 2 diabetes (*26*).

Study on the diabetes model of the silkworm, and the pathogenesis of its complications

The high glucose diet induced silkworm model (20 g of artificial diets with 10% glucose, fed continuously for 72 hours) mimics the following pathological features of diabetic reproductive system complications (*50*):

Oxidative stress markers: hemolymph malondialdehyde (MDA) levels were significantly elevated ($p < 0.01$) and superoxide dismutase (SOD) activity was significantly reduced ($p < 0.05$), suggesting systemic oxidative damage.

Reproductive system lesions: Testicular histopathology showed retarded testicular development, necrosis of spermatogonia and seminal vesicles, and obvious pathological changes, with pathological similarities to human diabetic hypogonadism.

Functional analysis of glucose metabolism-related genes in the silkworm

As an invertebrate model organism, the unique glucose metabolism regulation genes of the silkworm provide a new perspective for diabetes research:

BBX-B8 gene: a member of clade B in the gene family of insulin-like peptides in the silkworm, which affects organ development, alginate metabolism, reproduction, stress and antioxidant enzyme activities in the silkworm, whereas overexpression of the gene leads to alginate accumulation (*51*).

BmSuc1 gene: the first β-fructofuranase identified in the silkworm, and knockout of this gene decreased midgut glucose content accompanied by abnormally elevated maltase and alginase activities and impaired glycogen synthesis (*52*).

BmTre gene: *BmERR* (estrogen receptor) overexpression promotes expression of *BmTreh* in the midgut, which then accelerates conversion of alginate to glucose and increases the glucose content of hemolymph in the silkworm, suggesting that it affects energy metabolism through regulation of glycolytic genes (*53*).

Study of antidiabetic active ingredients of silkworm origin

Deoxynojirimycin (1-Deoxynojirimycin, DNJ)

DNJ is a natural polyhydroxypiperidine alkaloid, mainly enriched in the branches, leaves, roots of Moraceae (*Morus*) plants and the hemolymph of silkworms. Its hypoglycemic mechanism is mainly through competitive inhibition of α-glucosidase, which delays hydrolysis and absorption of intestinal carbohydrates (*54*). Studies have shown that male larvae of the 5th instar day 3 of the silkworm, fed with high DNJ mulberry leaves (DNJ content ≥ 0.125%), have a hemolymph DNJ concentration of up to 0.465%, which is significantly

higher than that of other developmental stages, suggesting that this period is the optimal window for obtaining DNJ on a large scale (*55,56*). Miglitol, which was developed based on the structural modification of DNJ, has been successfully used in clinical applications, and its blood glucose lowering effect was stronger than that of DNJ ($p < 0.01$) (*57*).

In addition, DNJ has multi-target therapeutic potential:

i) Improvement of metabolic disorders: it enhances insulin sensitivity by activating the PI3K/AKT pathway with elevated phosphorylation levels ($p < 0.01$) (*58,59*), and also has an effect on adipocyte metabolism *via* intestinal flora, which improves adipose metabolism-associated disease states (*60*);

ii) Nephroprotective effects: in a diabetic nephropathy model, DNJ intervention reduced the urinary protein excretion rate and inhibited glomerular thylakoid matrix expansion (*61,62*);

iii) Cardioprotective potential: *in vitro* experiments confirmed that DNJ concentration up to 30 μM could reverse mitochondrial membrane potential attenuation ($p < 0.001$), providing new ideas for the treatment of hypertrophic cardiomyopathy (*63*).

Bombyxin

Bombyxin is the first insulin-like heterodimeric peptide (molecular weight ~5 kDa) found in insects, and its A and B chains share 50% and 30% sequence homology with human insulin, respectively (*35,64*). Functional studies have shown that neck ligation of homeopathic silkworm larvae injected with homeopathin reduced hemolymph alginate concentration and elevated alginase activity (*65,66*); and *in vitro* cellular experiments confirmed that Homeopathy regulates glucose uptake and glycogen synthesis in HepG2 cells *via* the PI3K/Akt pathway, which in turn affects energy storage and utilization homeostasis (*67*).

Core regulatory pathway of silkworm diabetes model

Diabetes is a chronic metabolic disease with multifactorial interactions, and its pathogenesis involves complex factors such as genetic susceptibility (*68*), obesity, dietary imbalance (*69*), circadian rhythm disruption, viral infections (*70*), and drug side effects (*71*). In recent years, through the analysis of key signaling pathways and their application in silkworm models, researchers have further revealed the molecular mechanism of diabetes and accelerated development of new hypoglycemic drugs (Figure 2).

AMPK signaling pathway

AMPK is a central regulator of energy metabolism that is activated by sensing changes in the intracellular AMP/ATP ratio. It regulates metabolic homeostasis

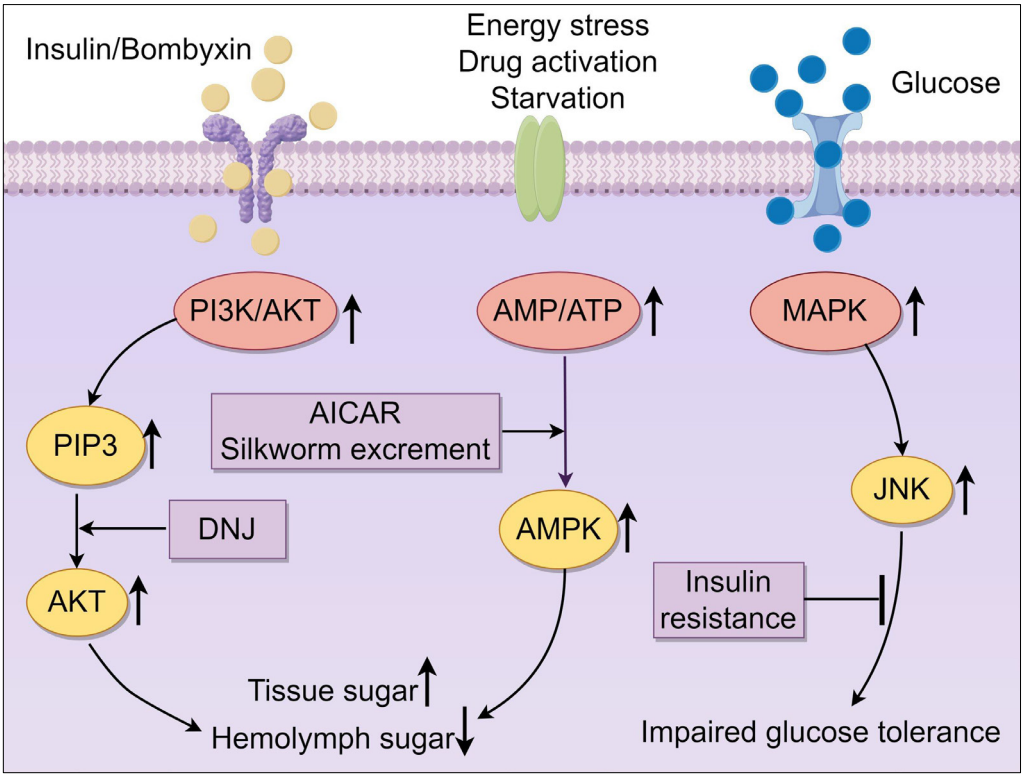

Figure 2. Key signaling pathways in the silkworm diabetes model. The figure shows the interactions between the insulin signaling pathway, PI3K/Akt pathway, AMPK pathway, and MAPK pathway in the silkworm diabetes model. These pathways are activated through molecular cascades and jointly regulate sugar levels in tissues and hemolymph, revealing the mechanism of insulin resistance.

through a dual action: inhibiting glycolipid synthesis (*e.g.*, inhibition of acetyl coenzyme A carboxylase) and promoting catabolism (*e.g.*, enhancing fatty acid oxidation) (*72-74*). In diabetic silkworms, activation of AMPK in response to energetic stress, drug (*e.g.*, AICAR) stimulation results in an increase in tissue glucose levels and a decrease in hemolymph glucose levels (*47*). Notably, extracts of silkworm excrement (*i.e.*, a kind of Tradition Chinese Medicine) have also been shown to exert hypoglycemic effects by upregulating AMPK phosphorylation levels (*75*).

PI3K/Akt signaling pathway

As the core pathway of insulin signaling, homeopathins and insulin activate phosphatidylinositol 3-kinase (PI3K), which catalyzes the generation of phosphatidylinositol 3-phosphate (PIP3), which then phosphorylates and activates Akt (protein kinase B). Activated Akt enhances glucose uptake and hepatic glycogen synthesis in peripheral tissues (*48,76*).

MAPK signaling pathway

The MAPK (mitogen-activated protein kinase) pathway is generally involved in insulin resistance by regulating chronic inflammatory responses. Activation of the MAPK pathway, followed by enhanced phosphorylation of JNK in the silkworm, usually in a high glucose environment, leads to impaired glucose tolerance and insulin resistance in the silkworm (*26,48*).

Natural drug development paradigm based on silkworm modeling

Our research team proposes a stepwise development strategy that integrates the silkworm model with histological techniques:

Initial screening phase (*i.e.*, screening *in vivo*): Referring to the data obtained by previous researchers (*42,50*), we used 10% high-glucose diets to induce the modeling for 18 h. Modeling was initially considered successful with a blood glucose value > 11.1 mmol/L, and then the candidate compounds were screened by *in vivo* efficacy evaluations (*e.g.*, blood glucose value determination, glucose tolerance test, glucose-lowering rate, *etc.*).

Validation phase (*i.e.*, validation *in vitro*): In order to deeply investigate the glucose-lowering potential of the candidate compounds in the *in vitro* environment, a model of insulin resistance can be constructed by using human hepatocellular carcinoma cells (HepG2 cells). Co-cultivating the HepG2 cells with insulin and high glucose concentration to establish an *in vitro* insulin-resistant cell model, and then performing glucose consumption and glycogen synthesis assays (*77,78*) and then assessing whether candidate compounds are able to effectively improve insulin resistance or not, and whether the candidate compounds could effectively improve glucose uptake and metabolism in insulin-resistant cells.

Separation and purification of target chemical monomers and spectral analysis: separation and purification of target chemical monomers is to precisely separate and purify the target monomers based on the difference in physicochemical properties between monomers and impurities by using chromatography, extraction and other techniques. Afterwards, the structural information of the target monomer, such as functional groups, chemical bonding, molecular weight and spatial configuration, can be analyzed by ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS), high-speed countercurrent chromatography (HSCCC) and other spectroscopic means (*79*). Structural identification of the active ingredients can also be accomplished by UPLC/Q-TOF-MS/conjugated NMR (*80*). Secondary validation of hypoglycemic activity: after obtaining the monomer compounds, confirmatory experiments for hypoglycemic activity were again performed *in vivo* (silkworms) and *in vitro* (HepG2 cells).

Glucose-lowering mechanism studies: glucose uptake assay, glycogen synthesis assay, protein expression analysis related to insulin signaling pathway (*e.g.*, protein expression and phosphorylation levels can be detected by protein immunoblotting (Western blot) (*81*) and also by detecting the expression level of insulin genes by using real-time quantitative polymerase chain reaction (qPCR), *etc.* (*82*).

Cross-species validation: efficacy confirmation in mammals, including glucose tolerance test, pharmacokinetic parameters ($t_{1/2}$, CL, V_{ss}) (*83*), dose-response curves, protein expression analyses, toxicity assessment (LD_{50}), AUC analyses, and correlation analyses comparing sensitivities and differences between silkworms and mammals to the drug.

Conclusion

Despite the unique advantages of the silkworm model in diabetes research, its biological limitations need to be recognized objectively: the silkworm lacks the adaptive immune system (*e.g.*, T/B lymphocyte network) and the complex endocrine regulatory axes (*e.g.*, hypothalamus-pituitary-adrenal axis) that are unique to mammals, and its diabetes pathology is difficult to completely mimic the chronic complications in humans (*e.g.*, diabetic nephropathy with microvascular pathology). In addition, issues such as stability of model building and the damaging nature of the model animals caused by the chemicals when induced need to be further improved and adapted.

In the future, we can strive to further optimize the diabetes model of the silkworm, so as to enhance reliability and applicability of the model. For example,

gene editing technology can be used in 2004, a genome-wide test led by Southwest University of China revealed the functional annotations of about 18,510 genes in the silkworm (*84*), a landmark study that not only provided key data for invertebrate developmental biology, but also made the silkworm an ideal model organism for gene editing. Based on this technology platform, researchers have been able to accurately mimic the pathological process of diabetes by targeting the editing of genes related to pancreatic β-cell function (*e.g.*, *HNF4A, HNF1A, PTPN22R620W, INS*, *etc.*) or key nodes of the insulin signalling pathway (*e.g.*, PI3K-Akt, cAMP).

It is worth noting that the insulin signaling pathway is highly conserved between silkworms and humans, which makes the results of related studies in model organisms widely applicable and of important reference value. In terms of experimental strategy, the researchers constructed a dual-track technology system. On the one hand, CRISPR-Cas9-mediated gene editing technology was used to precisely target the key nodes in the insulin signaling pathway and edit the related genes; and on the other hand, the key links in the insulin signaling pathway were focused on, and the regulatory mechanism was explored in depth. This combination of technologies can flexibly realize the precise regulation and in-depth analysis of the insulin signaling pathway (*85,86*).

Although gene editing technology can accurately reproduce single-gene diabetes subtypes such as neonatal diabetes (*87*), its application still faces multiple challenges: firstly, gene editing technology is difficult to operate, requiring a professional technical team and sophisticated experimental equipment to ensure the accuracy of the operation, secondly, gene editing experiments are extremely demanding on the environment and conditions, which need to be carried out in highly sterile and stable environments, and furthermore, the cost of gene editing is high, including the acquisition of equipment, reagents and consumables, as well as the cost of labor, and finally, if editing errors occur during the gene editing process, it may lead to unpredictable consequences and even irreversible damage to the genome of the experimental organisms (*88*).

However, gene editing technology still has a broad application prospect in the study of diabetes modeling in the silkworm. Through gene editing, specific genes can be precisely modified to accurately model the pathogenesis of human monogenic diabetes. This not only contributes to an in-depth understanding of the pathophysiological process of diabetes, but also provides strong support for the development of new therapeutic approaches.

At the same time, the similarities and differences between the silkworm and human diabetes should be explored in depth, so as to explore new ideas and methods for the treatment of diabetes. In addition, the application of the silkworm to the field of diabetes personalized medicine should be strengthened to provide a strong basis for the realization of precise treatment.

In conclusion, the use of the silkworm as a model animal for diabetes research has great potential in the study of diabetes disease pathogenesis, screening of diabetes drugs, evaluation of pharmacological efficacy, and toxicological studies. And as a valuable research resource, natural medicines are rich in medicinal potential and provide a source of innovation for modern medicine. The silkworm may be expected to become a key model animal for the development of new natural drugs for the treatment of diabetes, bringing new breakthroughs in the treatment of diabetes.

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