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Syringin attenuates insulin resistance via adiponectin-mediated suppression of low-grade chronic inflammation and ER stress in high-fat diet-fed mice

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ABSTRACT

In the treatment of type 2 diabetes, improvements in glucose control are often linked to side effects such as weight gain and altered lipid metabolism, increasing the risk of cardiovascular disease. It is therefore important to develop antidiabetic drugs that exert beneficial effects on insulin sensitivity and lipid metabolism at the same time. Here we demonstrate that syringin, a naturally occurring glucoside, improves glucose tolerance without increased weight gain in high-fat diet-induced obese mice. Syringin augmented insulin-stimulated Akt phosphorylation in skeletal muscle, epididymal adipose tissue (EAT), and the liver, showing an insulin-sensitizing activity. Syringin-treated mice also showed markedly elevated adiponectin production in EAT and suppressed expression of pro-inflammatory cytokines in peripheral tissues, indicating a significant reduction in low-grade chronic inflammation. Additionally, syringin enhanced AMP-activated protein kinase activity and decreased the expression of lipogenic genes in skeletal muscle, which was associated with reduced endoplasmic reticulum (ER) stress. Taken together, our data suggest that syringin attenuates HFD-induced insulin resistance through the suppressive effect of adiponectin on low-grade inflammation, lipotoxicity, and ER stress, and show syringin as a potential therapeutic agent for prevention and treatment of type 2 diabetes with low risk of adverse effects such as weight gain and dysregulated lipid metabolism.

1. Introduction

Obesity-induced insulin resistance is a hallmark of metabolic syndrome, predisposing to the development of type 2 diabetes and cardiovascular disease [1]. Overnutrition triggers alterations in lipid metabolism, increased low-grade inflammation and ER stress, and dysregulation of AMP-activated protein kinase (AMPK), leading to an insulin-resistant state [2]. Thus, the key strategy in the prevention of insulin resistance and metabolic syndrome is to reduce these pathogenic factors. However, one of the most common adverse effects of major anti-diabetic drugs that act as insulin sensitizer or insulin-secretion stimulator, such as thiazolidinediones (TZDs), sulfonylureas, and glinides, is body weight gain, which might increase the risk of cardiovascular diseases [3].

TZDs, which are ligands for peroxisome proliferator-activated receptor-γ, promote lipogenesis and also reduce lipid content in both liver and muscle by shifting their intracellular lipid storages to white adipose tissue (WAT), leading to amelioration of lipotoxicity and impaired insulin signaling [4]. Thus, it is paradoxical that TZD treatment improves insulin sensitivity while causing weight gain. This side effect of medication-induced weight gain can be avoided by using metformin, a biguanide suppressing hepatic glucose production through activation of AMPK, but the insulin-sensitizing effect of metformin is comparatively lower than other insulin sensitizers [5]. TZDs also have been shown to activate AMPK in the liver through up-regulation of adiponectin expression in WAT [6]. Opposite to the enhancing effect of TZDs on adiponectin production, metformin reduces adiponectin secretion in adipocytes [7], and instead, activates AMPK directly in muscle and adipose tissue. It is therefore concluded that most widely used medications for the treatment of insulin resistance exert their effect through different mechanisms, and also have different adverse effects. Thus, there is an unmet need for the search for new, safer, and more potent agents which have greater efficacy in improving insulin sensitivity and simultaneously exert beneficial effects on lipid metabolism to reduce undesirable adverse effects such as body weight gain.

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Syringin is a naturally occurring phenylpropanoid glucoside (4-[(1E)-3-hydroxyprop-1-en-1-yl]-2,6-dimethoxyphenyl β-D-glucopyranoside) that has attracted wide attention for its considerable bioactivities such as antioxidant, neuroprotective, anti-diabetic, anti-inflammatory, and anti-allergic properties [8]. It is also called eleutheroside B belonging to eleutheroside derivatives found in *Eleutherococcus senticosus* (Siberian ginseng), a woody medicinal plant native to Northeastern Asia, which has been widely used to treat a wide range of illnesses in traditional medicine [9]. A few studies have shown that syringin has anti-hyperglycemic activities in diabetic animal models. One earlier report demonstrated that eleutheroside compounds isolated from *E. senticosus* roots including syringin exerted marked hypoglycemic effects in normal and alloxan-induced hyperglycemic mice [10]. Other studies reported that both normal and streptozotocin-induced diabetic rats injected with syringin showed significantly decreased plasma glucose level, accompanied by increased level of plasma insulin [11–13]. However, these previous studies mainly focused on the stimulating effect of syringin on insulin secretion, not insulin action, and little information is available regarding its insulin-sensitizing activity. Of particular interest is thus to clarify whether syringin has the efficacy in enhancing insulin sensitivity, and if so, elucidate its underlying mechanism.

In this study, we evaluated the anti-hyperglycemic activity of syringin using high-fat diet (HFD)-induced obese mice. Interestingly, glucose tolerance and insulin sensitivity were significantly improved in syringin-treated mice without increased weight gain. Syringin administration promoted adiponectin production, accompanied by reduced low-grade chronic inflammation in the liver, adipose tissue and skeletal muscle. In addition, syringin-treated mice showed an increased AMPK activation and a commensurate decrease of lipogenic gene expressions in skeletal muscle, leading to alleviation of ER stress. These findings show syringin as an alternative insulin-sensitizing agent with positive influence on lipid metabolism that has potential to prevent and treat diabetes while reducing the risk of side effect of increased adiposity.

2. Materials and methods

2.1. Animals

Four-week-old male mice were purchased from Hyochang Bioscience (Daegu, Korea) and maintained in humidity and temperature-controlled environment (22 ± 1 °C and 45 ± 10%) on a 12 h light/dark cycle. Mice were assigned to two groups fed with normal chow diet (2018S, Harlan Laboratories, Indianapolis, IN) or high-fat (HF) diet (60%kcal from fat, D12492, Research Diets, Inc., New Brunswick, NJ) for 4 weeks. HF diet-fed mice were then randomly split into 2 groups again, syringin-treated and untreated control group, and given an every-other-day intraperitoneal injection of syringin (5 mg/kg) or PBS, respectively, for additional 4 weeks. All mice were fasted for 4 h prior to sacrifice. Tissues, including subcutaneous adipose tissue, epididymal adipose tissue, liver, and quadriceps skeletal muscle were rapidly excised, snap-frozen in liquid nitrogen, and stored at −75 °C until processed for experiments.

2.2. Glucose tolerance test & insulin tolerance test

After 3 weeks of syringin treatment, mice were fasted for 16 h and followed by intraperitoneal injection of glucose (2 g/kg). For insulin tolerance test, mice were fasted for 4 h and injected intraperitoneally with insulin (0.75 U/kg). Blood samples were obtained by tail-bleeding, and blood glucose levels were checked at 0, 15, 30, 60, 90, and 120 min after glucose injection by Accu-Check Go (Roche Diagnostics GmbH, Basel, Switzerland).

2.3. Western blot analysis

Tissues were homogenized in ice-cold PRO-PREP protein extraction solution (iNtRON Biotechnology, Seongnam, Korea), and centrifuged at 16,600*g* for 10 min at 4 °C. Supernatants were collected, boiled, separated by 10% SDS-PAGE, and transferred to PVDF membranes. Membranes were blocked, and incubated with primary antibodies against total Akt, phospho-Akt (Ser473), total AMPK, phosphor-AMPK (Thr172), adiponectin and BiP (Cell signaling technology, Beverly, MA). Membranes were washed, and incubated with anti-rabbit IgG conjugated with horseradish peroxidase (Cell signaling). Signal were amplified by enhanced chemiluminescence, and analyzed by AlphaImager 2200 (ProteinSimple, Santa Clara, CA).

2.4. ELISA

Measurement of serum insulin was performed with UltraSensitive Mouse Insulin ELISA kit (Morinaga Institute of biological Science Inc., Yokohama, Japan) according to the manufacturer's instructions.

2.5. Real-time RT PCR

Total RNA was extracted using TRI reagent® (Molecular Research Center, Cincinnati, OH) and reverse transcribed with oligo (dT) primer and GoScript™ reverse transcriptase (Promega, Madison, WI). Gene expression was analyzed using SYBR Premix Ex Taq™ (Tli RNaseH Plus) kit (Takara Bio Inc., Shiga, Japan) on ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA). Quantification of gene transcripts for acetyl-CoA carboxylase (ACC), binding immunoglobulin protein (BiP), CD36, CCAAT-enhancer-binding protein homologous protein (CHOP), fatty acid synthase (FAS), interferon γ (IFNγ), interleukin-1β (IL-1β), IL-6, IL-10, IL-12, IL-13, low-density lipoprotein receptor (LDLR), monocyte chemoattractant protein1 (MCP1), protein disulfide-isomerase (PDI), peroxisome proliferator-activated receptor γ (PPARγ), stearoyl-CoA desaturase-1 (SCD1), sterol regulatory element-binding protein1c (SREBP1c), tumor necrosis factorα (TNFα) and tribbles homolog 3 (TRB3) was performed by using gene-specific primers. Primer sequences are available upon request. Results were presented as mean ± S.D. normalized to expression of β-actin using the ΔΔCt method.

2.6. Statistical analysis

All experimental data are represented mean ± S.D. The significance of differences between groups was evaluated using ANOVA or two-tailed Student's *t*-test. Statistical significance was set at p values < 0.05.

3. Results

3.1. Syringin improves glucose intolerance in HFD-induced obese mice

To initially examine whether syringin reverses HFD-induced insulin resistance, mice on a HFD for 8 weeks was administered with syringin for the latter 4 weeks. HFD-induced glucose intolerance was significantly improved in syringin-treated mice compared to untreated controls, whereas importantly, body weight gain was not significantly different between the two groups (Fig. 1A and B).

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