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Antihyperglycemic Action of Sinapic Acid in Diabetic Rats

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ABSTRACT: Sinapic acid is a hydroxycinnamic acid contained in plants. In an attempt to know the hyperglycemic effect of sinapic acid, this study applied streptozotocin (STZ) to induce type 1-like diabetic rats and fed fructose-rich chow to induce type 2-like diabetic rats. Sinapic acid dose-dependently reduced the hyperglycemia of STZ-diabetic rats (9.8 ± 1.8%, 11.6 ± 0.7%, and 19.4 ± 3.2% at 5 mg/kg, 10 mg/kg, and 25 mg/kg, respectively). Also, sinapic acid attenuated the postprandial plasma glucose without changing plasma insulin in rats. Repeated treatment of sinapic acid increased the gene expression of GLUT4 in soleus muscle of STZ-diabetic rats. Moreover, sinapic acid enhanced glucose uptake into isolated soleus muscle and L6 cells (337.0 ± 29.6%). Inhibition of phospholipase C (PLC) using U73122 (1.00 ± 0.02 μg/mg protein) or protein kinase C (PKC) using chelerythrine (0.97 ± 0.02 μg/mg protein) attenuated the sinapic acid-stimulated glucose uptake (1.63 ± 0.02 μg/mg protein) in L6 cells. Otherwise, the reduced glucose infusion rate (GIR) in fructose-rich chow-fed rats was also raised by sinapic acid. Our results suggest that sinapic acid ameliorates hyperglycemia through PLC-PKC signals to enhance the glucose utilization in diabetic rats.

KEYWORDS: *glucose utilization, hyperglycemia, hyperinsulinemic euglycemic clamp, postprandial glucose, sinapic acid*

INTRODUCTION

Diabetes is a metabolic disease showing hyperglycemia and insulin resistance in diabetic patients.[1] Hyperglycemia is a common symptom of diabetic disorders, leading to damage to tissues and organs. Thus, control of blood sugar is critical in the handling of diabetic disorders. In clinics, metformin and others are widely used as antihyperglycemic agents.[2] Many agents, including thiazolidinedione (TZD), which activates peroxisome proliferator-activated receptors (PPARs), have been developed to treat diabetes, but applications were limited due to the side effects.[3] For better medications, alternative agents were introduced in recent years.[4] Thus, the development of new substance(s) to control hyperglycemia is helpful.

Sinapic acid, one of the phenylpropanoid family, is known to contain in many foods including rice and wheat.[5] Sinapic acid is less presented in free form, except in processed foods after freezing, sterilization, or fermentation. It has been indicated that the glycosylated derivatives or esters of quinic acid, shikimic acid, and tartaric acid are changed to bound forms.[6] The fruits, including blueberries, kiwis, plums, cherries, and apples, also contain sinapic acid of about 0.5−2 g/kg fresh weight.[7] Sinapic acid has an anti-inflammatory action through decreasing the expression of proinflammatory cytokines, including iNOS, COX-2, TNF-α, and IL-1β.[8] Inflammation is known to link with the progress of diabetic disorders.[9] The consumption of whole-grain wheat ameliorates the metabolic diseases, indicating the merit of sinapic acid in diabetic disorders.[10] However, the potential mechanism(s) regarding the antihyperglycemic action of sinapic acid remained obscure. Thus, the present study aims to investigate the action mechanism(s) of sinapic acid for lowering blood glucose in diabetic animals.

MATERIALS AND METHODS

Chemicals. Sinapic acid (purity >98%) from Sigma (St. Louis, MO, USA) was dissolved in 75% ethanol. U73122 (PLC inhibitor) and chelerythrine (PKC inhibitor) were purchased from Tocris Bioscience (Ellisville, MO, USA).

Animal Models. We purchased male Wistar rats weighing 250−300 g from the Animal Center of National Cheng Kung University Medical College to feed the standard laboratory diet. Type-1-like diabetes was induced by an intravenous injection (iv) of STZ (65 mg/kg) into rats as described previously.[11] Animals considered as diabetic using the plasma glucose concentrations reach 280 mg/dL or higher in addition with polyuria and/or diabetic features. Experiments performed at two weeks later of STZ injection. Otherwise, rats received 60% fructose chow (Teklad Laboratory Diets, Madison, WI) named as fructose-rich chow employed to induce the type-2 like diabetes showing insulin resistance as described previously.[12] Tolbutamide (10 mg/kg, ip) induced hypoglycemia was employed

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to characterize the formation of insulin resistance in rats receiving fructose-rich chow. The loss and/or marked decrease of tolbutamide-induced action occurred about eight weeks later. Then, we organized eight animals as one group in the experiments. Animal procedures performed all following the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the guidelines of the Animal Welfare Act.

Laboratory Determinations. The plasma glucose was determined following a previous report.[13] In brief, the concentration of plasma glucose was estimated through the glucose oxidase method in an analyzer (Quik-Lab, Ames; Miles Inc., Elkhart, IN, USA). The plasma insulin was measured using an insulin enzyme-linked immunosorbent assay kit (Linco, St. Charles, MO).

Assay of Postprandial Glucose. The postprandial glucose was determined mainly according to a previous report.[14] In brief, after fasting for 12 h, the basal plasma glucose was identified using samples from the tail vein of rats under temporary anesthesia with isoflurane (2%). Then, ethanol solution of sinapic acid (25 mg/kg) or the same volume of vehicle (75% ethanol) as control was administered to rats using oral intake. At 45 min later, glucose levels of blood samples (0.1 mL) from tail vein were showing 0 min. To mimic the food consumption, rats received oral intake of glucose solution (1 g glucose per mL of saline) at 5 mL/kg body weight. Blood samples (0.1 mL) were then drawn from the tail vein at 10, 60, 120, 180, and 240 min later for assay of plasma glucose. In this assay, rats were all kept under the persistent anesthesia using pentobarbital (30 mg/kg, ip).

The Hyperinsulinemic Euglycemic Clamping. We performed the hyperinsulinemic euglycemic clamping mainly according to a previous report.[15] After fasting for 12 h, rats under anesthesia with pentobarbital (30 mg/kg, ip) received the cannulation in the femoral vein for the infusion of glucose and insulin and another in the femoral artery for the sampling. Before the experiment, animals were placed in a restrainer to accustom them to the condition. In beginning, rats received the infusions of regular human insulin (Novo Industrias, Bagsvaerd, Denmark) at 40 mU/kg/min. Insulin for infusion was diluted with the saline containing 0.5% human serum albumin (Baxter, Glendale, CA, USA). The plasma glucose was measured immediately using the blood samples (10 μL) collected at 10 min intervals. Then, the plasma glucose level was maintained at 5.5 mmol/L by the infusion of 20% dextrose (Abbott, Chicago, IL). At the steady state generated within 70−90 min, the blood sample was collected to assay the glucose concentration. After the final sampling (120 min later), rats were sacrificed using a lethal dose of pentobarbital (100 mg/kg, ip). We calculated the glucose infusion rate (GIR) at the steady state using Steele's equation.[16]

Determination of Glucose Uptake into Soleus Muscle. Glucose uptake into skeletal muscle was assayed in isolated soleus muscle as described previously.[17] In brief, STZ-diabetic rats were sacrificed using a lethal dose of pentobarbital. The soleus muscle was immediately isolated for cutting into long longitudinal strips of about 35 ± 5 mg/strip. Then, strips kept in Krebs−Ringer buffer (pH 7.4) were incubated with 2-[^{14}C]-deoxy-glucose (^{14}C 2-DG) (1 μCi/mL; Perkin-Elmer Life Sciences, Inc., Boston, MA, USA) for 5 min at 37 °C. Incubation was terminated using an addition of ice-cold Krebs−Ringer buffer and samples dissolved with 1 N NaOH. After neutralization with 1 N HCl, the radioactivity in each sample was estimated using a β-counter (Beckman LS5000TA, Fullerton, CA, USA).

Cell Culture. We purchased L6 cell line from the Culture Collection and Research Center (CCRC 60083) in Food Industry Institute (Hsin-Chiu City, Taiwan). This cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM) at 37 °C under a humidified atmosphere containing 5% CO_2 and supplemented with 10% fetal bovine serum and 1% antibiotic solution containing penicillin 10000 U/mL and streptomycin 10 μg/mL. Maturation of this cell line was induced as described previously.[18] Briefly, after grown to 70% confluence, cells were exposed to DMEM supplemented with 10% horse serum. Then, cells were grown into myotubes showing multiple nuclears within 7−10 days. Before the assay of glucose uptake, cells were starved in serum-free medium for 4−6 h.

Assay of 2-NBDG Uptake into L6 Cells. We used 2-NBDG as a fluorescence indicator to assay the glucose uptake mainly as described previously.[19] After cultured for 48 h, we prepared 1 × 10^6 cells/mL of L6 cells for each assay. The cells were washed gently with phosphate-buffered saline (PBS) instead of medium. Cells were detached with trypsin to suspend in PBS containing 0.2 mM 2-NBDG and the test compound at the indicated concentration and then incubated in a water bath with 37 °C for 60 min under dark conditions. After centrifugation (4 °C, 5000*g*, 10 min), the obtained pellet was washed three times with cold PBS and maintained under ice cooling. The pellet was then suspended in 1 mL of PBS for determination of the fluorescence intensity in a fluorescence spectrofluorometer (Hitachi F-2000, Tokyo, Japan), using excitation and emission wavelengths at 488 and 520 nm, respectively. The intensity of fluorescence was used to calculate the uptake of 2-NBDG into cells.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). We used Trizol reagent (Invitrogen) to extract total RNA from soleus muscle. In the reverse transcription reaction, 2 μg of the obtained total RNA were applied in addition with Superscriptase II (Invitrogen), oligo-dT, and random primers as described previously.[20] The GLUT4 primer sequences were 5′-tttctgttggtatgcataatttgtaat-3′, and 5′-ccagtagaggaggtcaacaacc-3′, which were selected using the web-based assay design software from Universal Probe Library Assay Design Center. Reactions were carried out in a mixture (20 μL) containing PCR buffer (13.4 μL), each probe (0.2 μL at 20 μmol/L), LightCycler TaqMan (4 μL), and template cDNA (2 μL). Under the LightCycler detection system (Roche Applied Science), PCR reaction was processed using one cycle of 95 °C for 10 min, 45 cycles of 94 °C for 10 s, 60 °C for 20 s, and 72 °C for 1 s. The crossing point for each amplification curve was obtained from the second derivative maximum method. Concentration of each gene was then obtained from LightCycler software after calculation with the appropriate standard curve. The relative mRNA expression was identified using the ratio of concentrations from target gene and housekeeping gene 36B4.

Western Blot Analysis. Western blot analysis was carried out following a previous report.[21] At first, the homogenates (50 μg) of isolated soleus muscle were separated using sodium dodecyl sulfate−polyacrylamide gel electrophoresis. Western blotting analysis was then carried out using an antirat GLUT4 antibody (Abcam, Cambridge, U.K). The goat polyclonal actin antibody (Millipore, Billerica, MA, USA) was also probed with samples as internal control. After incubation with the peroxidase-conjugated secondary antibodies, the blots were developed in ECL-Western blotting system. The densities of the blots at 45 KDa for GLUT4 and 43 KDa for actin were then quantified using a laser densitometer, and the quantification was performed in three experiments.

Statistical Analysis. Data showing as the mean ± SEM were obtained from the number (n) of samples in each group. Difference was compared using the repeated measures analysis of variance (ANOVA) and the Newman−Keuls posthoc analysis. A p-value of 0.05 or less was considered significant.

RESULTS

Sinapic Acid Attenuated Hyperglycemia in STZ-diabetic Rats. Oral administration of sinapic acid produced plasma glucose-lowering activities at 5, 10, and 25 mg/kg about 9.8 ± 1.8%, 11.6 ± 0.7%, and 19.4 ± 3.2% in STZ-diabetic rats, respectively, 90 min later (n = 8). As shown in Figure 1, sinapic acid dose-dependently attenuated hyperglycemia while the plasma glucose was markedly reduced to 337.4 ± 15.4 mg/dL ($p < 0.001$; n = 8) by sinapic acid at 25 mg/kg.

Effects of Sinapic Acid on Postprandial Plasma Glucose in Wistar Rats. From the data in Figure 1, sinapic acid at 25 mg/kg showed the maximum action to lower plasma glucose. Thus, 25 mg/kg of sinapic acid or the same volume of vehicle was applied to oral intake into normal rats. After 45 min, the plasma glucose values were indicated as 0 min as

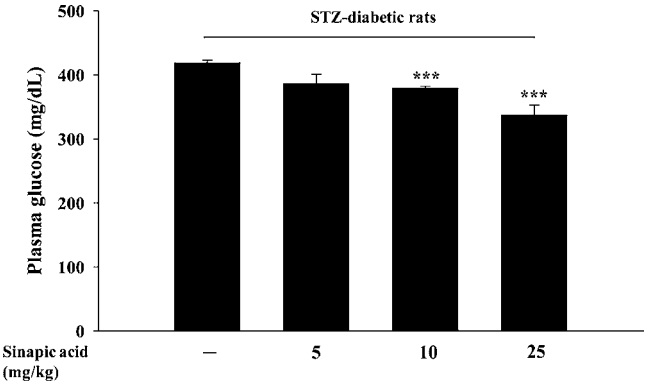

Figure 1. Attenuation of hyperglycemia by sinapic acid through oral administration into STZ-diabetic rats. Data showing mean and SEM (bar) were obtained from eight rats in each group. The same volume of vehicle (75% ethanol) was treated as the control. ***, $p < 0.001$ vs control.

described previously.[22] Then oral intake of glucose solution (1 g glucose per mL saline) at 5 mL/kg of body weight was used to mimic the food consumption. Different from the vehicle, as shown in Figure 2A, sinapic acid at 25 mg/kg markedly lowered the plasma glucose at 10, 60, 120, and 180 min. Otherwise, hyperglycemia is known to induce the insulin secretion. Thus, plasma insulin was raised in parallel with the higher of postprandial glucose. In the present study, the postprandial glucose peaked at 10 min but the plasma insulin at same time point was not different between the sinapic acid-treated group and the vehicle-treated group (Figure 2B). Thus, the lowering of postprandial glucose by sinapic acid seems not to be associated with the insulin secretion.

Effect of Sinapic Acid on Expressions of GLUT4 in Soleus Muscle of STZ-diabetic Rats. Sinapic acid treated STZ-diabetic rats at the effective dose (25 mg/kg) through oral intake three times daily for 3 days, as described previously,[22] may result in a reduction of plasma glucose from 411.6 ± 5.5 to 325.8 ± 5.2 mg/dL. Then, the soleus muscle was isolated from these STZ-diabetic rats. A marked higher GLUT4 mRNA expression in soleus muscle was observed in sinapic acid treated STZ-diabetic rats (Figure 3A). Also, increase in protein level of GLUT4 in soleus muscle by sinapic acid was identified using Western blotting analysis (Figure 3B). Thus, increase of GLUT4 gene expression by sinapic acid in soleus muscle has been characterized.

Increase of Glucose Uptake by Sinapic Acid in Soleus Muscle. Using [^{14}C]2-DG uptake as the tracer, glucose uptake into soleus muscle isolated from STZ-diabetic rats was concentration-dependently raised by sinapic acid that reached the plateau between 1 and 10 μM (Figure 4). Otherwise, as a positive control, insulin (1 nM) stimulated [^{14}C]2-DG uptake (337.0 ± 29.6%, $n = 8$) markedly.

Increase of Nonradioactive Glucose Uptake by Sinapic Acid in L6 Cells. In L6 cells incubated with high glucose (30 mM), using 2-NBDG as a tracer, sinapic acid (0.1 μM-10 μM) concentration-dependently enhanced glucose uptake that reached the plateau between 1 and 10 μM as shown in Figure 5.

Mediation of the PLC-PKC Signals in Sinapic Acid-Induced Glucose Uptake. Role of the PLC-PKC pathway was investigated in the enhancement of glucose uptake by sinapic acid using 2-NBDG as a tracer. The phospholipase C (PLC) inhibitor U73122 or the PKC inhibitor chelerythrine

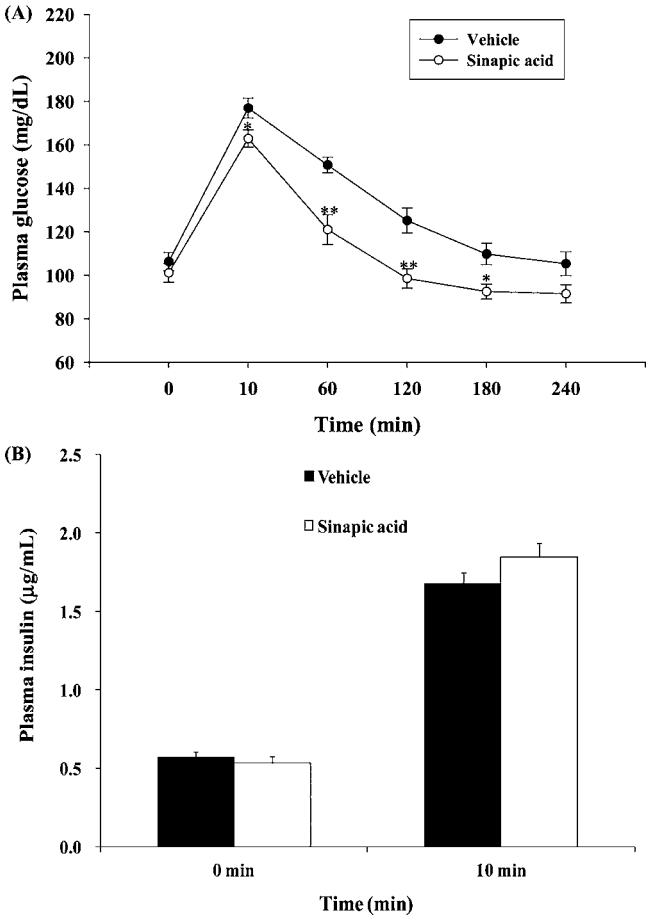

Figure 2. (A) Changes of postprandial glucose by sinapic acid in normal rats. Rats received oral intake of sinapic acid at 25 mg/kg or the same volume of vehicle for 45 min, and the obtained plasma glucose was indicated as 0 min. The saline solution containing glucose in 1 g/mL was then oral intake at 5 mL/kg body weight to each rat. Plasma glucose at the indicated times were used to compare the difference of sinapic acid-treated group (open circles) with the vehicle-treated control (closed circles). (B) Changes of plasma insulin in normal rats after oral intake of sinapic acid. Data showing mean and SEM (bar) were grouped from eight rats. The same volume of vehicle (75% ethanol) was treated as the control. *, $p < 0.05$ and **, $p < 0.01$ vs the control at same time point.

was pretreated with L6 cells at the effective concentration, as described previously,[23] to determine the influence on glucose uptake induced by sinapic acid at 10 μM that was effective from Figure 5. The increased glucose uptake by sinapic acid was markedly reduced by U73122 in a concentration-related manner (Table 1). Chelerythrine also inhibited the increase of glucose uptake by sinapic acid in same fashion (Table 1).

Sinapic Acid Improved Insulin Resistance in Fructose-Rich Chow-Fed Rats Identified by Hyperinsulinemic Euglycemic Clamping. We performed hyperinsulinemic euglycemic clamping to investigate whether the improvement of insulin resistance is involved in action of sinapic acid. Using the hyperinsulinemic euglycemic clamp, the obtained glucose infusion rate (GIR) was widely applied to identify the insulin resistance. Actually, fructose chow-fed rats showed a lower GIR than normal rats, indicating insulin resistance (Figure 6). Also, after the oral intake of 25 mg/kg in fructose chow-fed rats, sinapic acid markedly reversed GIR value to a higher level, indicating an amelioration of insulin resistance.

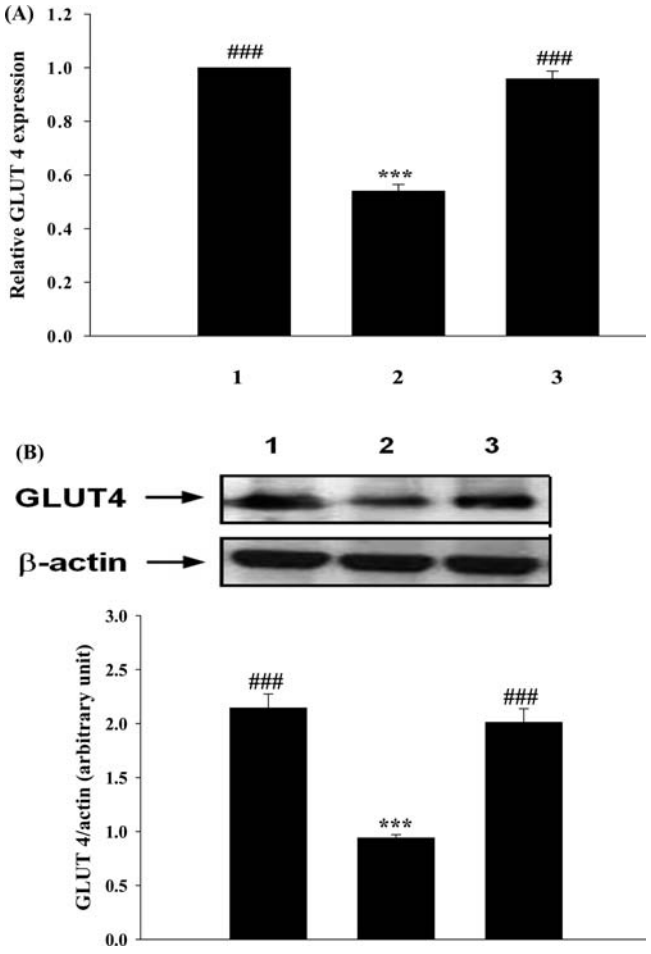

Figure 3. (A) GLUT4 expressions in soleus muscle from STZ-diabetic rats received sinapic acid as described in Materials and Methods. Lane 1, vehicle-treated Wistar rats; lane 2, vehicle-treated STZ-diabetic rats; lane 3, sinapic acid (25 mg/kg)-treated STZ-diabetic rats. The mRNA levels were assayed using qRT-PCR. Data showing the mean with standard error (SE) (n = 8) are presented in each column. ***, p < 0.001 vs lane 1. ###, p < 0.001 vs lane 2. (B) Upper panel shows protein level of the GLUT4 or actin in soleus muscle from STZ-diabetic rats received sinapic acid as described in Materials and Methods. Lane 1, vehicle-treated Wistar rats; lane 2, vehicle-treated STZ-diabetic rats; lane 3, sinapic acid (25 mg/kg)-treated STZ-diabetic rats. Each column showing the ratio of GLUT4/actin as the mean with standard error (SE) (n = 8) is indicated in the lower panel. ***, p < 0.001 vs lane 1. ###, p < 0.001 vs lane 2.

DISCUSSION

In the present study, we found that sinapic acid dose-dependently attenuates hyperglycemia in STZ-diabetic rats (Figure 1). Sinapic acid also lowered postprandial plasma glucose in rats without altering plasma insulin (Figure 2). Sinapic acid markedly enhanced glucose uptake in isolated soleus muscle and cultured L6 cells (Figures 4 and 5). Moreover, sinapic acid improved the insulin resistance in fructose chow-fed rats (Figure 6). Thus, sinapic acid is identified as an antihyperglycemic agent in diabetic animals.

Sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) is known to be contained in the edible plants including cereals, nuts, oil seeds, rapeseed, and berries.[24] Sinapic acid is introduced as antioxidant, with an efficacy higher than ferulic acid and comparable to caffeic acid.[25] Sinapic acid strongly inhibited tyrosine nitration induced by pernitrate.[26] Addition-

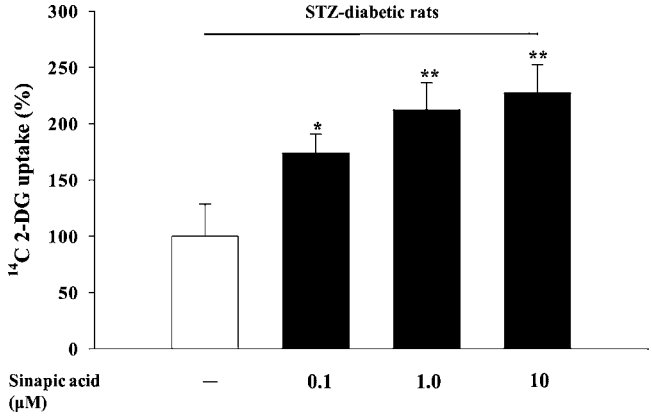

Figure 4. Effect of sinapic acid on glucose uptake using the isolated soleus muscle. After treatment with sinapic acid at the indicated concentration, glucose uptake was measured using [^{14}C] 2-DG.. Data from eight experiments are indicated as the mean ± SEM *, p < 0.05 and **, p < 0.01 vs the untreated control.

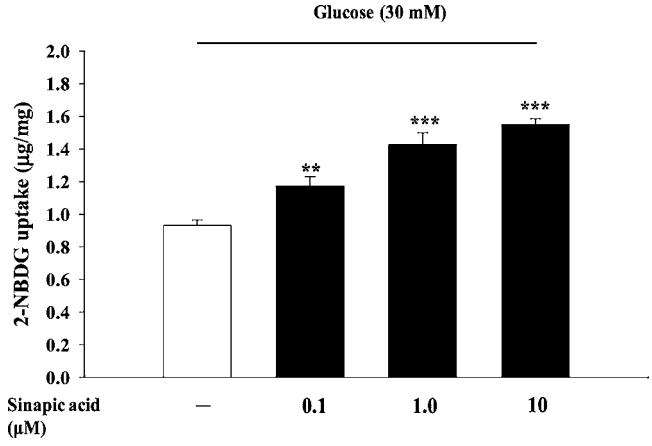

Figure 5. Increase of glucose uptake by sinapic acid in cultured L6 cells. After incubation with sinapic acid at the indicated concentration, cells were used to assay glucose uptake using 2-NBDG as the indicator. Data from eight experiments are showed as the mean ± SEM **, p < 0.01 and ***, p < 0.001 vs the untreated control.

Table 1. Inhibitory Effects of U73122 and Chelerythrine on Sinapic Acid-Induced Increase of Glucose Uptake in L6 Cells[a]

	2-NBDG in cells (μg/mg protein)
control	0.96 ± 0.02
sinapic acid (10 μM)	1.63 ± 0.02***
+ U73122	
0.01 μM	1.45 ± 0.03***
0.1 μM	1.23 ± 0.02***
1.0 μM	1.00 ± 0.02
+ chelerythrine	
0.01 μM	1.41 ± 0.01***
0.1 μM	1.18 ± 0.01***
1.0 μM	0.97 ± 0.02

[a]U73122 or chelerythrine at the indicated concentration was treated for 30 min before the application of sinapic acid. Cells suspended in 0.2 mM 2-NBDG were incubated with sinapic acid (indicated concentration) or the same volume of vehicle (as control) for 30 min. Data showing means ± SEM were grouped from eight experiments; ***p < 0.001 vs the control.

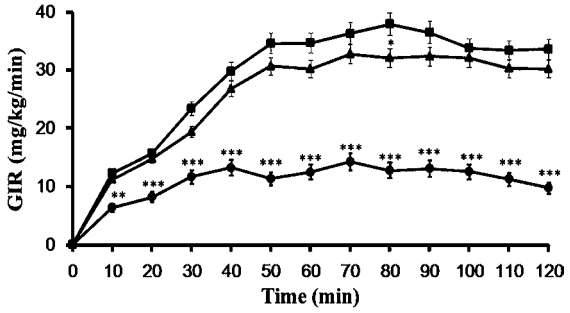

Figure 6. Measurement of insulin sensitivity, assessed by the glucose infusion rate during the last 20 min of a 2 h hyperinsulinemic–euglycemic clamp. The glucose clamp was performed for 120 min in each group. Group of the normal rats is indicated as the control (■). The fructose chow-fed rats received an oral intake of sinapic acid (25 mg/kg) (▲) were used to compare the vehicle-treated group (●) that received vehicle at same volume. Data showing mean ± SE were grouped from eight animals. * $p < 0.05$ and.*** $p < 0.001$ vs the control.

ally, sinapic acid has been documented to show cerebral protective and cognition-improving effects.[27] This is the first study to demonstrate the blood glucose-lowering action of sinapic acid in type-1-like diabetic rats (Figure 1).

Postprandial glucose is commonly applied to investigate the glucose homeostasis. In healthy subjects, blood glucose is usually raised after a meal. Glucose can stimulate the pancreas to secrete insulin, which increases the utilization of blood glucose. However, it is difficult for diabetic patients to produce sufficient insulin or proper response to insulin and their blood glucose remains higher. Glucose homeostasis reduced in diabetic patients may result in a marked postprandial hyperglycemia.[28] Thus, postprandial blood glucose is widely used to investigate the changes in glucose levels in normal conditions and evaluate the postprandial hyperglycemia.[29] However, this test is not suitable for diabetic animals because various factors may influence changes in glucose levels, including hyperglycemia and insulin resistance. Thus, normal rats were used to investigate the changes in blood glucose by sinapic acid in the present study. Actually, sinapic acid significantly lowered the postprandial glucose without altering the plasma insulin level (Figure 2). Thus, the effect of sinapic acid on postprandial glucose seems to be unrelated with insulin secretion and a direct influence of sinapic acid on glucose utilization seems more likely.

Hyperglycemia is often considered to be the result of a dysfunction in peripheral glucose utilization during diabetic disorders.[30] Many proteins are involved in glucose metabolism, but GLUT4 is commonly investigated in diabetic condition.[31] In diabetic disorders, the reduced GLUT4 expression in skeletal muscle has been introduced to lower insulin-mediated glucose uptake into skeletal muscle.[32] It is interesting to determine that action of sinapic acid is produced through reversing the lowered GLUT4 expression. Thus, STZ-diabetic rats were employed to receive the repeated sinapic acid treatments for 3 days. The lower expression of GLUT4 in soleus muscle from STZ-diabetic rats was significantly raised by sinapic acid (Figure 3). Thus, sinapic acid is able to increase GLUT4 expression in soleus muscle. We also employed a radioactive tracer to identify the elevation of glucose uptake into soleus muscle induced by sinapic acid (Figure 4). The changes in the GLUT4 levels in skeletal muscle caused by sinapic acid seem to be related to the regulation of glucose homeostasis, which is possible linked to decrease insulin resistance.[33] Thus, the elevation of GLUT4 seems responsible for the antihyperglycemic action of sinapic acid.

Isolated preparations of skeletal muscle are hard to maintain in experimental manipulations and shall be isolated from a sacrificed animal. Thus, L6 cells were applied in the assay of glucose uptake as an alternative way. Also, 2-NBDG was used as a tracer in this assay of glucose uptake in L6 cells.[18] Sinapic acid concentration-dependently enhanced the glucose uptake into L6 cells, indicating sinapic acid has the ability to stimulate glucose uptake directly. However, the mechanisms for this action of sinapic acid are still unclear. Increased glucose uptake is known to link with an activation of the PLC-PKC signals.[34] Thus, mediation of the PLC-PKC pathway was investigated in the action of sinapic acid. Sinapic acid-induced glucose uptake into L6 cells was markedly reduced by pretreatment with U73122 at an effective concentration for blockade of PLC[23] and by chelerythrine at a concentration sufficient to inhibit PKC[23] (Table 1). Thus, the mediation of PLC-PKC signals in the action of sinapic acid is identified. It has been documented that an activation of PKC may increase glucose uptake through the transportation of GLUT4.[32] Thus, depending on these signals, sinapic acid can enhance glucose uptake into skeletal muscle.

In addition, type-2 diabetes is known to associate with insulin resistance. Fructose chow-fed rat is widely used to induce insulin resistance as a type-2 diabetic animal model. We employed it to study the relations of sinapic acid and insulin resistance. Insulin resistance was characterized using a hyperinsulinemic euglycemic clamp, the established useful method as described previously.[15] Generally, the rate of glucose infusion (GIR) is employed to indicate the insulin resistance from hyperinsulinemic euglycemic clamp. A high GIR value shows that the patient is insulin-sensitive, while a low GIR value indicates the insulin resistant.[35] Actually, sinapic acid induced a higher GIR value in fructose chow-fed rats (Figure 6). This result seems associated with the enhanced glucose uptake in skeletal muscle. Thus, sinapic acid is helpful to improve the insulin resistance. Basically, increased glucose utilization in skeletal muscle induced by sinapic acid seems related to the changes in GLUT4. However, it is possible that actions of sinapic acid on glucose homeostasis are also produced through the effects on other metabolic tissues, including liver and adipose tissues. Thus, the influence of sinapic acid on other tissues relating to glucose homeostasis need to be further investigated in the future.

Many natural products and/or Chinese medicines have been documented to have blood glucose-lowering action through increased GLUT4 expression in STZ-diabetic rats, such as, Huanglian Jiedu decoction,[36] isoferulic acid, and plumbagin.[37] It has also been demonstrated that the clinical drug, telmisartan, has a blood glucose-lowering effect through induction of increased GLUT4 expression in STZ-diabetic rats.[38] The secretion of β-endorphin[39] and the administration of hydrogen rich water[40] showed similar abilities to lower blood glucose. In this study, our results indicate that sinapic acid can ameliorate hyperglycemia through inducing higher expression of GLUT4 in type-1 like diabetic rats.

In conclusion, the amelioration of hyperglycemia by sinapic acid is mainly related to higher GLUT4 expression and/or increased glucose uptake via the PLC-PKC pathway in diabetic

rats. Thus, sinapic acid is suitable to develop as new agent for the handling of diabetic hyperglycemia in advance.

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Notes

The authors declare no competing financial interest.

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