Antidiabetic Effect of Oleanolic Acid: A Promising Use of a Traditional Pharmacological Agent

Xin Wang, Yu-Liang Li, Hao Wu, Jiang-Zheng Liu, Jun-Xia Hu, Nai Liao, Jie Peng, Pei-Pei Cao, Xin Liang and Chun-Xu Hai*
Department of Toxicology, Faculty of Preventive Medicine, the Fourth Military Medical University, Xi’an, 710032, China

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia. Although the clear mechanisms of DM and insulin resistance are still to be cleared, it has been well documented that reactive oxygen species (ROS) play a pivotal role in DM and multiple types of insulin resistance. For the past few years, natural substances have been shown to have the potential to treat DM. Attention has been especially focused on plants rich in triterpenoids, which generally show antioxidant and antiglycation effect. In previous studies, it was shown that oleanolic acid (OA), a natural triterpenoid and an aglycone of many saponins, is a potent antioxidant acting as not only a free radical-scorcher through direct chemical reactions but also as a biological molecule, which may enhance the antioxidant defenses. The present study aimed to investigate the potential antidiabetic effect of OA. Oleanolic acid showed a significant blood glucose-lowering and weight-losing effect in diabetic animals induced by streptozotocin (STZ). In the insulin resistant model, it was also shown that OA may promote insulin signal transduction and inhibit oxidative stress-induced hepatic insulin resistance and gluconeogenesis, in which process the phosphorylation of ERK and the protective effect on mitochondrial function may be involved. These findings may significantly better the understanding of the pharmacological actions of OA and advance therapeutic approaches to DM. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: oleanolic acid; diabetes; antidiabetic effect; insulin resistance; hepatic gluconeogenesis.

Supporting information may be found in the online version of this article (Supplementary Material)

INTRODUCTION

The American Diabetes Association (ADA) estimates that there has been an explosive increase in the prevalence of diabetes mellitus (DM) (Leeelayuwat et al., 2008; Muin et al., 2008) and suggests that by the year 2030, over 350 million people worldwide will be afflicted with this disease and its complications (Seghrouchni et al., 2002). Hyperglycemia, insulin resistance (defects of insulin sensitivity) and subsequent pancreatic β-cell dysfunction (defects of insulin secretion) are the hallmarks of DM. Although the clear mechanism of DM is still to be determined, it has been well documented that reactive oxygen species (ROS) is closely connected with the genesis and development of DM. Moreover, ROS plays a causal role in multiple types of insulin resistance, which is the initial and essential attribute of type 2 diabetes mellitus (T2DM) (Ritchie, 2009), representing 80–90% of all DM incidences. Therapies attributing to lessen oxidative stress contribute to improve glycemic control and to prevent the complications of DM. At present, four types of chemical drugs, including sulphonylureas, biguanides, α-glucosidase inhibitors and euglycemic agents, are used clinically for the treatment of T2DM. Patients with type 1 diabetes mellitus (T1DM) mainly rely on the use of insulin. But the side effects of these drugs and the requirement of perpetually taking medicine significantly lower the quality of life of diabetic patients.

In the past few years, natural substances have been shown to have the potential to treat DM (Xi et al., 2007). Attention has been notably focused on plants rich in triterpenoid, which generally have shown antioxidant and antiglycosylative effects in our previous research (Xi et al., 2007). Oleanolic acid (OA) is a natural triterpenoid and a common aglycone of many saponins (Perez-Camino and Cert, 1999). It has been shown to protect mice from various hepatotoxicants, including carbon tetrachloride, acetaminophen, bromobenzene and thioacetamide, which cause oxidative and electrophilic stress. Administration of OA predominately leads to its distribution and metabolism in the liver. In our previous studies (Wang et al., 2010b), OA has been proved to be a potent antioxidant through enhancing the antioxidant defenses. OA ameliorates oxidative injury induced by tert-butyl hydroperoxide (tBHP) through stimulating the production of antioxidant and the expression of key antioxidant enzymes which may be regulated by nuclear factor erythroid 2 p45-related factor 2 (Nrf2) (Wang et al., 2010b). Activation of JNK and ERK are involved in the antioxidant activity of OA (Wang et al., 2010b). The effectiveness of OA in the treatment of liver disorders (such as hepatitis) in the past several decades proves that OA is an excellent pharmacological agent. The clinical use of OA has shown certain side effects of lowering blood glucose. Therefore, we suppose that OA has the potential to play a role in the therapy of DM. The present study verified the blood
glucose-lowering effect of OA in a T1DM animal model induced by streptozotocin (STZ). To determine whether OA could ameliorate insulin resistance, which is a fundamental characteristic of T2DM, the effect of OA was studied on insulin signal transduction in hepatocytes. This research is expected to provide a new insight into the treatment of hyperglycemia and insulin resistance related diseases.

**METHODS AND MATERIALS**

### Chemicals and reagents

Oleanolic acid was purchased from Xi’an Xiaocao Plant Technology Company. P-Akt, P-p38 MAPK, P-ERK, Akt, ERK and mTOR antibodies were purchased from Epitomics. P-mTOR and p38 MAPK antibodies were purchased from Bioworld Technology (Santa Cruz, CA). Insulin, rosiglitazone (RSG), streptozotocin (STZ), t-butyldihydroperoxide (tBHP), DMSO, rhodamine 123 and α-amylase were purchased from Sigma. A mitochondria isolation kit was obtained from Beyotime Institute of Biotechnology, China. All other chemicals and solvents were of analytical grade with the highest purity commercially available.

### Animal treatment

Sprague–Dawley rats (male, weighing 180–220 g) were obtained from the Experimental Animal Research Center at the Fourth Military Medical University. The animals were housed in stainless steel cages in a room kept at 22 ± 1 °C with a 12-h light/12-h dark cycle and fed with a standard pellet diet and water *ad libitum*. The animals were treated according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication #85-23, revised in 1985) and the experimental use of the animals was approved by the Animal Ethics Committee of the University. In brief, 36 Sprague-Dawley rats were randomly divided into six groups: control; STZ: OA pretreatment (POA); metformin pretreatment (PM); OA treatment (TOA); metformin treatment (TM). In the first week, the animals in the POA and PM groups were dosed with OA (100 mg/kg, i.p.) or metformin (100 mg/kg, i.p.) once daily for a week, respectively, while the other groups were given vehicle (2% Tween 80 in sterile saline, 10 mL/kg, i.p.). Fasting blood glucose (FBG) was measured at the end of the first week. In the second week, the animals in the STZ, POA, PM, TOA and TM groups were administered STZ (50 mg/kg, i.p.) twice on the first and the third days of the week, while the animals in the control group were given vehicle (citrate buffer, 10 mL/kg, i.p.). Then 5 days after the last injection of STZ, FBG was measured. In the last week, the animals in the TOA and TM groups were dosed with OA (100 mg/kg, i.p.) or metformin (100 mg/kg, i.p.) once daily for a week, respectively, while all the other animals were administered vehicle (2% Tween 80 in sterile saline, 10 mL/kg, i.p.). The FBG was also measured at the end of the experiment. After all treatments, the rats were killed by paralysis and the blood samples and livers were collected. Part of the livers was excised from the animals for observation of pathological histology. For RT-PCR and western blotting analysis, part of livers from rats was quickly frozen and then stored at −80 °C until use.

### Pathological histology

Immediately after removal from the animals, hepatic tissues were fixed in 10% buffered formaldehyde, processed for histological examination according to the conventional methods and stained with hematoxylin and eosin (H&E). The morphology of any lesions observed was classified and registered.

### Determination of advanced glycation end products (AGES)

50 μL of serum was diluted to 3 mL with physiological saline and then vortexed for 15 s. Fluorescence at 440 nm was determined with excitation at 370 nm (Munch et al., 1997).

### Cell culture

The human normal hepatocyte line (QZG) was purchased from Shanghai Cell Biology, Chinese Academy of Sciences. The cells were cultured in RPMI 1640 supplemented with 10% FBS (Gibco BRL, Rockville, MD, USA). The cell numbers were assessed using a hemocytometer. The experiments were conducted using serum-free RPMI 1640. The cells were seeded in 6-well plates at a density of 2 × 10⁴ cells per dish in 2 mL of culture medium. For some experiments, the cells were incubated with different concentrations of OA (dissolved in DMSO) for 4 h and then stimulated with 10 ng/mL insulin for 10–15 min. For other experiments, the cells were pre-cultured with or without 10 μmol/L OA or 10 μmol/L rosiglitazone (RSG) for 4 h and then treated with 200 μmol/L tBHP in the presence or absence of 10 μmol/L OA or 10 μmol/L RSG for another 4 h. Finally, the cells were stimulated with 10 ng/mL insulin for 10–15 min. OA and RSG were dissolved in DMSO at a concentration of 100 mM, and then freshly diluted with culture medium to the appropriate concentration. Culture medium with DMSO served as the control in each OA-treatment experiment.

### Reverse transcriptase-polymerase chain reaction analysis (RT-PCR)

RT-PCR was conducted as described previously (Wang et al., 2010a). Briefly, the liver tissue and hepatocyte mRNA were isolated using Trizol-reagent (Invitrogen, USA) and the quality of isolated RNA was checked by 1.2% formaldehyde agarose gel electrophoresis. For the reverse transcriptase-polymerase chain reaction, primers for rat Gpx1, Sod1 and β-actin and human PGC1α, PCK2 and GAPDH were designed with the aid of Clone Manager software. The RT-PCR reaction used a template cDNA followed by PCR amplification with Taq DNA polymerase in the same tube. And then PCR products were analysed by 1.5% agarose gel electrophoresis, stained with ethidium bromide, then photographed under ultraviolet light. Densitometric analysis of three different observations was performed using band ‘n’ map software (Bio-Rad, USA). The quantity of each transcript was calculated according to the instrument manual, and normalized to the amount of β-actin or GAPDH, housekeeping genes. The sequences of each primer used in this study are shown in Tables 1 and 2.

### Western blotting

Western blotting analysis was performed as follows. Briefly, cell lysates were prepared by incubation on ice with lysis buffer (50 mM Tris-Cl (pH 7.5), 20 mM NaCl, 5 mM EDTA, 1% TX-100, 0.1% SDS, 5% glycerol + protease inhibitors), and then centrifuged at 20000 × g for 20 min. The supernatant...
was collected and protein concentration was determined using the Pierce BCA protein assay kit (Thermo) with bovine serum albumin as a standard control. The supernatant was mixed with an equal volume of sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 5% mercaptoethanol, 1% bromophenol blue and 25% glycerol). Then the mixture was boiled for 5 min and centrifuged at 10000 × g for 10 min. The supernatant was used for immunoblotting. 20 μg protein extractions were separated by using SDS-PAGE on 10% polyacrylamide gels, and then transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). After blocking for 1 h with 8% skimmed milk in TBS buffer (10 mM Tris, 150 mM NaCl), the membrane was incubated with primary antibodies against P-Akt, P-mTOR, P-p38 MAPK, P-ERK, p38 MAPK, Akt, mTOR and ERK overnight at 4 °C. After the membrane was washed four times for 15 min each with TBST buffer (10 mM Tris, 150 mM NaCl), the membrane was incubated with primary antibodies against P-Akt, P-mTOR, P-p38 MAPK, P-ERK, p38 MAPK, Akt, mTOR and ERK overnight at 4 °C. After the membrane was washed four times for 15 min each with TBST buffer (10 mM Tris, 150 mM NaCl), the membrane was incubated with primary antibodies against P-Akt, P-mTOR, P-p38 MAPK, P-ERK, p38 MAPK, Akt, mTOR and ERK overnight at 4 °C. After the membrane was washed four times for 15 min each with TBST buffer (10 mM Tris, 150 mM NaCl), the membrane was incubated with primary antibodies against P-Akt, P-mTOR, P-p38 MAPK, P-ERK, p38 MAPK, Akt, mTOR and ERK overnight at 4 °C. After the membrane was washed four times for 15 min each with TBST buffer (10 mM Tris, 150 mM NaCl), the membrane was incubated with primary antibodies against P-Akt, P-mTOR, P-p38 MAPK, P-ERK, p38 MAPK, Akt, mTOR and ERK overnight at 4 °C. After the membrane was washed four times for 15 min each with TBST buffer (10 mM Tris, 150 mM NaCl and 0.1% Tween-20), it was incubated in the appropriate HRP-conjugated secondary antibody (diluted 1:1000 in TBST) at 37 °C for 30 min. The protein bands were visualized using chemiluminescent reagents according to the manufacturer’s instructions and quantified from three different observations using an image analyser Quantity One System (BIO-RAD, USA).

**Determination of mitochondrial membrane potential (Δψm).** The mitochondrial membrane potential (Δψm) was detected on the basis of cell retention of the fluorescent cationic probe rhodamine 123 (Manna et al., 2009). After experimental treatment, fresh mitochondria were isolated from the cells using a mitochondria isolation kit. The mitochondrial cell suspension was then incubated with 1 μmol/mL rhodamine 123 for 10 min, centrifuged at 50 × g for 5 min at 4 °C, washed and re-suspended in 1 mL of 0.1% Triton X-100. After centrifugation at 2000 × g for 5 min, rhodamine 123 in the supernatant was determined with a fluorescence spectrophotometer at the 505/535 nm excitation/emission wavelength pair. The results are expressed as percentage of the fluorescence values of the control.

**α-Amylase inhibitory activity.** The α-amylase inhibitory assay was performed as previously (Ali et al., 2006) with moderate modifications. Porcine pancreatic α-amylase (type VI, Sigma) was dissolved in ice-cold distilled water to 4 U/mL solution. Potato starch (0.5%, w/v) dissolved in 20 mM phosphate buffer (pH 6.9, 6.7 mM sodium chloride) was used as a substrate solution. Briefly, 40 μL of different concentrations of OA (dissolved in DMSO (I) or DMSO (I0)), 160 μL of distilled water and 400 μL of starch solution were mixed in a plastic tube. The reaction was started by the addition of 200 μL of the α-amylase solution. Then the mixture were incubated at 25 °C for 3 min. After that, 300 μL DNS colour reagent solution (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M NaOH) was added into the tubes and then the mixture was placed into a 100 °C waterbath for 15 min. At the end, the mixture was diluted with 1.9 mL distilled water and the final amylase activity was determined by measuring the absorbance of the mixture at 540 nm. The inhibitory effect on α-amylase activity was calculated as (%)

\[
\text{Inhibitory ratio} = \left( \frac{I_0 - I}{I_0} \right) \times 100\%.
\]

**Statistical analysis.** Data are expressed as the mean ± SD. The results were analysed by one-way ANOVA followed by a SNK-q test for multiple comparisons. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software. Data were considered statistically significant at p < 0.05.

**RESULTS**

OA significantly lowered fasting blood glucose of diabetic rats induced by STZ

In order to evaluate the influence of OA on FBG, type 1 diabetic animal models were established through the injection of STZ, which was a classic chemical used to induce specific damage to the pancreas. As shown in Fig. 1A, after 1 week of administration of OA and metformin, the FBG of the animals in the POA and PM groups were lowered significantly, compared with that of the other animals. Moreover, the FBG of the animals in the POA group (mean = 3.125 mmol/L) was lower than that of the PM group (mean = 3.45 mmol/L), although this difference was not statistically significant (p > 0.05). During the second week of the experimental period, the animals in the STZ, POA, PM, TOA and TM groups were dosed with STZ twice and the FBG of the animals in the STZ, TOA and TM groups (mean > 16 mmol/L)

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**Table 1. Sequences of primers for the genes studied in the animal experiment**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primer (5’ to 3’)</th>
<th>Anti-sense primer (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gpx1</td>
<td>ACATCGAGAAATGGCAAGGA</td>
<td>CCGCAGGAAGTAAAGAAGC</td>
</tr>
<tr>
<td>Sod1</td>
<td>GTCCTCAAGGAATAACAAAGA</td>
<td>AGACCTGACACCACATAGGGA</td>
</tr>
<tr>
<td>βactin</td>
<td>GACATCCGTAAGACCTCTATGCC</td>
<td>ATAGAGCCACCAATCCACACAGAG</td>
</tr>
</tbody>
</table>

**Table 2. Sequences of primers for the genes studied in the human cell experiment**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primer (5’ to 3’)</th>
<th>Anti-sense primer (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCK2</td>
<td>CCTGGCAGTGTCTTTAGGG</td>
<td>AAGAGTTCAATCCACGGTCTCT</td>
</tr>
<tr>
<td>PGC1a</td>
<td>TTTTGTAAACGGAACCTGG</td>
<td>CTCAACTAATCGCTCCT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GGATTTGCTGTATTTGGG</td>
<td>GGAAGATGGGTGATGGGATT</td>
</tr>
</tbody>
</table>
and the PM group (mean > 10 mmol/L) were increased significantly (Fig. 1B), compared with that of the control. In contrast, the FBG of the animals in the POA group were not significantly different from that of the control (Fig. 1B). At the end of the experiment, the treatment of OA in the TOA group decreased the FBG markedly to the level of the control (Fig. 1C). In addition, the FBG of the animals in the POA and PM groups were still at a normal level (Fig. 1C). In contrast, metformin (TM group) did not show as good FBG-lowering effects as did OA in the present experiment (Fig. 1C). Figure 1D showed the time-curve of the changes of the FBG in the different groups. The results showed that pre-treatment of OA maintained the FBG of the animals in the POA group at a relatively low level throughout the experimental period. Treatment with OA significantly reduced the high FBG induced by STZ. These results indicated that OA had the potential to reduce blood glucose and to treat hyperglycemia related diseases.

OA reduced the rate of the increase of animal body weight

Under normal conditions, the body weight of the animals gradually increased, as reflected by the body weight changes of the animals in the control group (Fig. 2). However, animals pretreated with OA in the beginning of the experiment had a very slight body weight increase, that indicated that the FBG-lowering effect of OA may be related to its effect on body weight.

The protective effects of OA on STZ-induced liver damage

Histopathological studies showed that STZ, compared with that of the control, induced a disordered arrangement of hepatocytes, degeneration of hepatocytes and hepatic cords, dissolved cytoplasm, punctiform necrosis, congestion in central vein and infiltration of lymphocytes (Fig. 3). According to microscopic examinations,
severe hepatic lesions induced by STZ were markedly reduced by the administration of OA and metformin. The livers of the rats pretreated with OA (POA group) showed the most trivial damage.

Antioxidant activity of OA was involved in its FBG-lowering effect

The previous results indicate that OA is a potent antioxidant activating a series of antioxidant enzymes regulated by Nrf2. In order to assess whether the antioxidant activity of OA was involved in the protective effects against diabetes, mRNA levels of glutathione peroxidase 1 (Gpx1) and superoxide dismutase 1 (Sod1) in livers, two main antioxidant enzymes, were measured using RT-PCR. As shown in Fig. 4, the mRNA expressions of Gpx1 and Sod1 were decreased significantly by STZ administration. Both of the preventive and therapeutic use of OA increased the mRNA levels of Gpx1 and Sod1, which denoted that the antioxidant activity of OA may play a role in its FBG-lowering effect, at least partly. However, the decrease of the mRNA expressions of the two enzymes were not ameliorated, in some degree even aggravated, in the PM and TM groups. In addition, the serum level of AGEs were also detected, which was a product of hyperglycemia (supplemental Fig. 1). However, there were no significant changes of serum AGEs levels.

OA promoted insulin signal transduction in hepatocytes

The effects of OA on insulin signal transduction in hepatocytes were further examined. As shown in Fig. 5, insulin-stimulated tyrosine phosphorylation of protein kinase B (Akt) in QZG hepatocyte was enhanced by OA in a dose-dependent manner. 10 µmol/L OA significantly increased the phosphorylation of Akt (> 4-folds) stimulated by insulin. In addition, a concentration of 5 µmol/L and 10 µmol/L, OA showed an inhibitory effect on insulin-stimulated phosphorylation of the mammalian target of rapamycin (mTOR), which signal pathway had been reported to impose a negative feedback program to attenuate insulin signal transduction (Shah et al., 2004). These results indicated that OA could stimulate the hepatic insulin signal through enhancing the phosphorylation of Akt and inhibiting the phosphorylation of mTOR.

OA inhibited ROS-induced insulin resistance in hepatocytes

A cellular insulin resistance model was established to investigate whether OA plays a role in protecting cells against insulin resistance induced by oxidative stress. Cells were cultured with 200 µmol/L tBHP to disturb the normal insulin signal in hepatocytes. As shown in Fig. 6, the insulin-stimulated phosphorylation of Akt
shown in Fig. 7, tBHP significantly decreased the phosphorylation of Akt under the insulin-stimulating condition. Pretreatment and co-treatment with OA and RSG, especially OA treatment, inhibited the decreased phosphorylation of ERK induced by tBHP (Fig. 7), demonstrating that increased phosphorylation of ERK may be involved in the protective role of OA in hepatic insulin signal transduction. The phosphorylation of p38 was not influenced significantly by tBHP under the insulin-stimulating condition (Fig. 7). Pretreatment of RSG and co-treatment with OA significantly stimulated the phosphorylation of p38.

Involvement of MAP kinases in the protective role of OA in insulin signal transduction

The phosphorylation of oxidative stress sensitive MAP kinases, including p38 and ERK, was also detected. As shown in Fig. 7, tBHP significantly decreased the phosphorylation of ERK under the insulin-stimulating condition. Pretreatment and co-treatment with OA and RSG, especially OA treatment, inhibited the decreased phosphorylation of ERK induced by tBHP (Fig. 7), demonstrating that increased phosphorylation of ERK may be involved in the protective role of OA in hepatic insulin signal transduction. The phosphorylation of p38 was not influenced significantly by tBHP under the insulin-stimulating condition (Fig. 7). Pretreatment of RSG and co-treatment with OA significantly stimulated the phosphorylation of p38.

The protective effects of OA on mitochondrial membrane potential against oxidative stress

Since there was evidence that mitochondrial dysfunction was associated with DM and age-related insulin resistance (Kim et al., 2008), mitochondrial membrane potential (Δψm) was also detected. As shown in Fig. 8, 200 μmol/L tBHP decreased Δψm seriously, leading to mitochondrial dysfunction, which may be involved in the onset of tBHP-induced insulin resistance. OA significantly inhibited the decrease of Δψm induced by tBHP and increased the decreased Δψm to control levels, and thus protected mitochondrial function, which confirmed the insulin resistance-inhibiting results of OA (Fig. 8). The protective effect of OA on mitochondria was more effective than that of RSG (Fig. 8).

The inhibitory effect of OA on ROS-induced gluconeogenesis in hepatocytes

Hepatic gluconeogenesis is tightly controlled by several transcription factors that regulate gene expression of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PCK) (Yoon et al., 2001). As shown in Fig. 9, the mRNA expression of phosphoenolpyruvate carboxykinase 2 (PCK2) increased significantly when the cells were exposed to tBHP, compared with that of
When the cells were exposed to OA, especially during tBHP treatment, the mRNA expression of PCK2 was significantly decreased, compared with that of the tBHP treatment group (Fig. 9). The mRNA expression of PGC-1α, a regulator of gluconeogenic enzymes, was also examined. However, both tBHP and OA had no marked effect on the expression of PGC-1α, indicating that PGC-1α was not involved in tBHP-induced gluconeogenesis (Fig. 9). These results confirmed that excessive ROS induced hepatic gluconeogenesis which may be inhibited by OA.

**α-amylase inhibitory effect may not be involved in the antidiabetic effect of OA**

An α-amylase-inhibiting assay in vitro was also performed to evaluate whether OA could influence the absorption of glucose from the intestinal tract. As shown in supplemental Fig. 2, when the concentration was in the range 0.0625–1 mg/mL, OA showed a weak α-amylase inhibitory effect. Oleanolic acid at 0.0625 mg/mL even increased the activity of α-amylase to some degree.

**DISCUSSION**

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin action and/or insulin secretion.
Streptozotocin, a commonly used agent in experimental diabetes, is an antibiotic produced by streptomyces achromogenes (Rakieten et al., 1963). The mechanism by which STZ destroys β cells of the pancreas and induces hyperglycemia is still unclear. However, oxidative stress has been implicated in the etiology and pathology of STZ-induced diabetes (Baynes and Thorpe, 1996). Chemicals with antioxidant properties have been shown to prevent pancreatic islets against cytotoxic effects of STZ.

Our previous studies (Wang et al., 2010b) have shown the potent antioxidant activity of OA, which is a natural triterpenoid and an aglycone of many saponins, indicating a potential use of OA in the therapy of diabetes. In the present study, diabetic animals were established using STZ to evaluate the antidiabetic effect of OA administration. Pretreatment of OA maintained the FBG of the animals at a relatively low level throughout the experimental period, despite the injection of STZ. Treatment of OA reduced the increased FBG induced by STZ to a normal level. Surprisingly, the effect of OA on controlling FBG was better than that of metformin, a commercial drug for the treatment of diabetic disease. In accordance with previous results (Kim et al., 2006), our results showed that after exposure to STZ, the body weight of animals decreased significantly compared with that of the control (Fig. 2). The change of body weight showed that OA treatment slowed down the speed of the body weight gain remarkably, which was consistent with the results of de Melo et al. (2010). The animals pretreated with OA had a significantly lower body weight when compared with the control, even before the injection of STZ (Fig. 2). Until the end of the experiment, the animals pretreated with OA had the lowest body weight compared with the other groups. The results of that investigation (de Melo et al., 2010) show that OA ameliorates visceral adiposity and improves glucose tolerance in mice and thus has an antiobese potential through modulation of carbohydrate and fat metabolism. So OA appeared to be an effective agent to promote weight loss. Consistent with the effect on FBG, OA protected the livers of animals against STZ-induced damage. In addition, OA also enhanced the antioxidant defenses through up-regulation of two main antioxidant enzymes, Gpx1 and Sod1. The results indicated that the antioxidant activity of OA may play a role in the FBG-lowering effect of OA, at least partly. OA had no effect on the generation of AGEs in this study. However, another experiment has shown the antiglycative effects of OA in the kidneys of diabetic mice (Wang et al., 2010c). The difference may be due to a different exposure time to OA. In that study, the test period was 15 weeks, which is much longer than our experimental period of 3 weeks.

Insulin resistance is considered to be the primary defect of T2DM and insulin resistance syndrome (Kasuga, 2006). Dysfunction of liver, skeletal muscle and adipose tissue, which are the body’s three main insulin-sensitive tissues, are responsible for the development of insulin resistance and T2DM (Choukem et al., 2008). Hepatic insulin resistance is of particular interest because it is a major determinant of fasting hyperglycemia and is consequently the major dysfunction in impaired fasting glucose, a prediabetic state (Abdul-Ghani et al., 2006). In the insulin resistant state, impaired insulin action allows the enhancement of glucose production in the liver, resulting in systemic hyperglycemia and contributing to the development of T2DM. Although the clear mechanism of insulin resistance is still to be elucidated, many studies have confirmed that ROS play a causal role in multiple types of insulin resistance (Ritchie, 2009). The present study investigated whether OA could affect insulin signal transduction. Akt is an important kinase in insulin signaling. The decreased insulin-stimulated phosphorylation of Akt is usually considered to be a symbol of insulin resistance. tBHP, an oxidant which is used widely to induce oxidative stress (Lin et al., 2000), was used to establish a hepatic insulin resistant model and the influence of OA on Akt signal transduction stimulated by insulin under oxidative stress condition was evaluated. Our results showed that tBHP significantly decreased insulin-stimulated phosphorylation of Akt in hepatocyte in a dose-dependent manner, indicating that tBHP induced hepatic insulin resistance through the generation of ROS. As expected, certain concentrations of OA promote Akt signal transduction stimulated by insulin, which means that OA could enhance the insulin signal in hepatocytes. The results showed that OA caused a conspicuous improvement of tBHP-induced insulin resistance. Consistent with previous results (Chu et al., 2010), OA decreased the phosphorylation of mTOR, which is a feedback inhibiting signal. These results suggest that OA may improve insulin resistance induced by oxidative stress.

Decreased phosphorylation of ERK may play an important role in oxidative stress-induced hepatic insulin resistance. Consistent with our previous results (Wang et al., 2010b), OA promoted the phosphorylation of ERK, which process was related to its antioxidant activity and may be involved in its inhibitory effect on insulin resistance. In addition, defects in mitochondrial function are probably involved in the development and/or maintenance of the insulin resistant state (Turner and Heilbronn, 2008). Mitochondria are the main ROS-generating sites, and so have the closest relationship with ROS. The present study also explored whether mitochondrial dysfunction was involved in the ROS-induced insulin resistance, and the effect of OA on mitochondrial membrane potential. The results showed that OA had a mitochondria-protecting effect, which may be involved in its inhibition of insulin resistance.

Increased hepatic gluconeogenesis is a hallmark of insulin resistance. The level of PCK, a regulating gluconegenic enzyme, is very tightly controlled at the transcriptional level by key hormones, including insulin and glucagon (Lim et al., 2009). Also, many transcription factors and coactivators are involved in the regulation of PCK. Among them, PGC-1α, for example, is highly enriched in the liver and is a well-known coactivator of PCK expression (Lim et al., 2009). The present study also showed that ROS stimulated the mRNA expression of PCK2 which could contribute to increase hepatic gluconeogenesis. Treatment with OA, especially when co-cultured with tBHP, reduced the tBHP-induced increase of the expression of PCK2, indicating an inhibitory role of OA in oxidative stress-induced hepatic gluconeogenesis. However, the mRNA expression of PGClα was not affected, indicating that OA regulated the expression of PCK2 in a PGClα-independent way. The carbohydrate hydrolysing enzymes, α-amylase and α-glucosidase, are the main glucose production in the liver, resulting in systemic hyperglycemia and contributing to the development of T2DM. Although the clear mechanism of insulin resistance is still to be elucidated, many studies have confirmed that ROS play a causal role in multiple types of insulin resistance (Ritchie, 2009). The present study investigated whether OA could affect insulin signal transduction. Akt is an important kinase in insulin signaling. The decreased insulin-stimulated phosphorylation of Akt is usually considered to be a symbol of insulin resistance. tBHP, an oxidant which is used widely to induce oxidative stress (Lin et al., 2000), was used to establish a hepatic insulin resistant model and the influence of OA on Akt signal transduction stimulated by insulin under oxidative stress condition was evaluated. Our results showed that tBHP significantly decreased insulin-stimulated phosphorylation of Akt in hepatocyte in a dose-dependent manner, indicating that tBHP induced hepatic insulin resistance through the generation of ROS. As expected, certain concentrations of OA promote Akt signal transduction stimulated by insulin, which means that OA could enhance the insulin signal in hepatocytes. The results showed that OA caused a conspicuous improvement of tBHP-induced insulin resistance. Consistent with previous results (Chu et al., 2010), OA decreased the phosphorylation of mTOR, which is a feedback inhibiting signal. These results suggest that OA may improve insulin resistance induced by oxidative stress.

Decreased phosphorylation of ERK may play an important role in oxidative stress-induced hepatic insulin resistance. Consistent with our previous results (Wang et al., 2010b), OA promoted the phosphorylation of ERK, which process was related to its antioxidant activity and may be involved in its inhibitory effect on insulin resistance. In addition, defects in mitochondrial function are probably involved in the development and/or maintenance of the insulin resistant state (Turner and Heilbronn, 2008). Mitochondria are the main ROS-generating sites, and so have the closest relationship with ROS. The present study also explored whether mitochondrial dysfunction was involved in the ROS-induced insulin resistance, and the effect of OA on mitochondrial membrane potential. The results showed that OA had a mitochondria-protecting effect, which may be involved in its inhibition of insulin resistance.

Increased hepatic gluconeogenesis is a hallmark of insulin resistance. The level of PCK, a regulating gluconegenic enzyme, is very tightly controlled at the transcriptional level by key hormones, including insulin and glucagon (Lim et al., 2009). Also, many transcription factors and coactivators are involved in the regulation of PCK. Among them, PGC-1α, for example, is highly enriched in the liver and is a well-known coactivator of PCK expression (Lim et al., 2009). The present study also showed that ROS stimulated the mRNA expression of PCK2 which could contribute to increase hepatic gluconeogenesis. Treatment with OA, especially when co-cultured with tBHP, reduced the tBHP-induced increase of the expression of PCK2, indicating an inhibitory role of OA in oxidative stress-induced hepatic gluconeogenesis. However, the mRNA expression of PGClα was not affected, indicating that OA regulated the expression of PCK2 in a PGClα-independent way. The carbohydrate hydrolysing enzymes, α-amylase and α-glucosidase, are the main
enzymes responsible for absorption of glucose in the digestive tract. So, inhibition of these enzymes is considered to be a therapeutic approach to decrease post-prandial hyperglycemia. The α-amylase inhibitory effect of OA was examined in our current study. However, the inhibitory effect of OA on α-amylase activity was so weak that it is probably not involved in its antidiabetic activity.

In addition to our results, there are also a few experiments that tried to study the therapeutic effect of OA, or its derivatives. For example, the OA derivative, NPLC441, potently stimulates glucose transport in 3 T3-L1 adipocytes (Lin et al., 2008). A recent report showed that OA inhibited protein tyrosine phosphatase 1B, a key factor in the negative regulation of the insulin pathway (Zhang et al., 2008). Oleolic acid also enhanced insulin secretion at basal and stimulatory glucose concentrations in INS-1 832/13 cells and enhanced acute glucose-stimulated insulin secretion in isolated rat islets (Teodoro et al., 2008). There is also research on the synthesis of gluco-conjugates of OA acting as inhibitors of glycogen phosphorylase (Cheng et al., 2009, 2010). OA may also have beneficial effects on some processes associated with renal derangement of STZ-induced diabetic rats (Mapanga et al., 2009).

Despite the above, the present study, for the first time, investigated the effect of OA on FBG in animals and the effect of OA on insulin signal transduction in hepatocytes. In conclusion, our findings indicate that OA could effectively lower FBG, improve hepatic insulin resistance and inhibit hepatic gluconeogenesis. Moreover, the weight loss-promoting effect, enhancement of antioxidative defense, activation of the MAP kinases (mainly ERK), and mitochondria-protecting effect were involved in the antidiabetic effect of OA. In company with the emerging amount of literature, we believe that OA or its liver protectant, acting via a multi-target mechanism, may become a potential pharmacological agent for the treatment of DM.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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