Int J Clin Exp Med 2021;14(2):800-812
www.ijcem.com /ISSN:1940-5901/IJCEM0121332

Original Article

Gypenoside XLIX as a novel potential therapeutic agent against insulin resistance

Shulong Shi[1], Ning Jia[2], Mingliang Sun[3], Zhenhua Dong[4], Ling Gao[5], Wenbin Chen[5], Jiajun Zhao[5]

[1]Shandong University of Traditional Chinese Medicine, Jinan 250000, China; [2]Department of Traditional Chinese Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250000, China; [3]Department of Endocrinology, Hospital Affiliated to Shandong University of Traditional Chinese Medicine, Jinan 250000, China; [4]Department of Endocrinology, Jinan Central Hospital Affiliated to Shandong University, Jinan 250000, China; [5]Department of Endocrinology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250000, China

Received August 29, 2020; Accepted December 11, 2020; Epub February 15, 2021; Published February 28, 2021

Abstract: Objective: To investigate whether Gypenoside XLIX (Gyp-XLIX) can attenuate insulin resistance *in vivo*, and explore the possible molecular mechanism. Methods: We performed hyperinsulinemic-euglycemic clamp in overnight-fasted Sprague-Dawley rats infused with saline, or lipid with or without Gyp-XLIX. Steady-state glucose infusion rate (SSGIR) was analyzed. Plasma free fatty acid (FFA) levels were measured by using a colorimetric kit. IRS1/PI3K/Akt and IκBα/NF-κB signaling pathway in the liver, gastrocnemius muscle, and epididymis fat were analyzed by western blot assay. Expressions of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and IL-1β mRNA were determined by real-time fluorescence quantitative PCR. AutoDock Vina software was used to perform molecular docking so as to evaluate the binding affinity between Gyp-XLIX and IKKβ. Results: Lipid infusion remarkably decreased SSGIR compared with saline infusion ($P < 0.001$), which implicated a systemic insulin resistance. However, Gyp-XLIX pretreatment partially prevented the decrease of SSGIR caused by lipid infusion ($P < 0.01$), suggesting that insulin resistance was alleviated by Gyp-XLIX. Additionally, Gyp-XLIX attenuated lipid-induced impairment of IRS1/PI3K/Akt insulin signaling pathway in both liver and gastrocnemius muscle. Concomitantly, Gyp-XLIX also exerted an inhibitory potency on lipid-stimulated NF-κB activation and mRNA expression of proinflammatory genes (TNF-α, IL-1β, and IL-6). Molecular docking showed that Gyp-XLIX hold great potential to target IKKβ protein. Conclusion: Gyp-XLIX improved insulin sensitivity in lipid-infused rats, and the possible mechanism may involve inhibition of liver and muscle inflammation by suppressing IKKβ/NF-κB signaling pathway.

Keywords: Gypenoside XLIX, insulin resistance, lipid, inflammation

Introduction

According to prediction of the World Health Organization (WHO), by the year 2025, 300 million people or more will be affected by type 2 diabetes (T2D) [1, 2], which indicates that T2D will continue to place a heavy economic burden on society. Insulin resistance, a defining feature of T2D, is a state in which physiological concentrations of insulin produce a less than normal response, namely the decline of insulin's ability to stimulate glucose utilization. Mounting evidences show that insulin resistance is a key factor in the natural history of T2D during the progression from pre-diabetes to diabetes [3], and also plays a major role in the occurrence and development of many metabolic diseases, such as non-alcoholic fatty liver disease [4], hypertension [5], atherosclerosis [6], dyslipidemia and other metabolic syndrome [7, 8].

Since the precise molecular mechanism of insulin resistance has not been fully elucidated, the first emphasis on its treatment is lifestyle modification, such as strengthening exercise, controlling diet, and losing weight [9]. However, these medical recommendations often lead to poor patient compliance. Although some pharmacologic agents have been documented to have the effects of improving insulin sensitivity, potential side effects greatly limit their widespread use in clinical practice. Therefore, it is

urgent to find new agents with less side effects and more efficacious potential to treat insulin resistance. Fortunately, nature medicine monomers (NMMs) isolated from plants have been recognized for their prominent roles in counteracting many diseases, and thus more and more attention has been paid to the research of NMMs which can improve insulin sensitivity [10, 11].

Gynostemma pentaphyllum (GP), a trailing plant that belongs to Cucurbitaceae family, has been widely used in the prevention and treatment of multiple diseases in China. Numerous researches revealed that GP features many pharmacological properties including anti-hyperglycemia [12], anti-oxidation [13], anti-inflammation [14], anticancer [15], lipid-lowering as well as hepatoprotective effect [16, 17]. Notably, no toxicity and side effect were found at conventional dosage of GP [18]. Our previous study suggested that GP displayed beneficial effects in improving insulin sensitivity and ameliorating hepatic steatosis in C57BL/6 mice fed with high fat diets [19]. Gypenoside XLIX (Gyp-XLIX), a naturally occurring dammarane-type glycoside, is one of major active components in GP [20]. However, whether insulin resistance can be attenuated by Gyp-XLIX remains unknown.

In current study, we used a hyperinsulinemic-euglycemic clamp technique, the gold standard for assessing whole-body insulin sensitivity. We first demonstrated that Gyp-XLIX was effective in improving insulin resistance, and revealed a possible novel mechanism that its beneficial pharmacological action was related to the suppression of IKKβ/NF-κB pathway.

Materials and methods

Animals

For all experiments, adult male Sprague-Dawley rats (Vital River Laboratory Animal Technology Co Ltd Beijing, China) weighing 200-300 g were used, and housed in sterilized cages under the condition with 12 h light/dark cycles, 50% humidity, controlled temperature (22-24°C). They were given standard laboratory chow and water ad libitum.

Surgery

All experimental procedures were approved by the Animal Ethics Committee of Shandong Provincial Hospital. The rats were fasted overnight prior to the experiment. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/Kg) and placed on a surgical platform in a supine position. A heating pad was used to ensure euthermia. A tracheotomy was performed to facilitate respiration. The carotid artery and the jugular vein were cannulated with polyethylene tubing (PE-50; Becton Dickinson, USA) for arterial blood sampling and various infusions. After a 30 min baseline period to assure hemodynamic stability and a stable level of anesthesia, rats were studied under the following protocol.

Experimental design

The rats were divided randomly into three groups (n=7 per group): SAL group, received an intravenous (i.v.) infusion of saline (SAL) (5 μl/min) for 3 h; IL group, received a 3-h i.v. infusion of 6.6% intralipid (IL) (5 μl/min); IL+Gyp-XLIX group, received a bolus i.v. injection of Gyp-XLIX (4 mg/Kg) prior to the onset of a 3-h intralipid infusion. Hyperinsulinemic-euglycemic clamp (3 $mU \cdot Kg^{-1} \cdot min^{-1}$) was performed in the last 2 h. During an insulin clamp, arterial blood glucose was determined every 10 min using Accu-Check Advantage glucometer (Roche Diagnostics, Indianapolis, IN), and 30% dextrose was intravenously infused at a variable rate to maintain blood glucose within 10% of basal value [21]. At the beginning and end of the infusion ("basal state" and "final state"), blood samples were taken for measurements of plasma FFA concentration. Upon completion of the insulin clamp, the rats were euthanized, immediately after which liver, gastrocnemius muscle and epididymis fat tissue samples were quickly collected for further study.

Gyp-XLIX (purity 99.8%) was purchased from Must Bio-Technology Co. Ltd (Chengdu, China). Throughout the entire study, close attention was paid to the physiological state of rats, and pentobarbital sodium was infused at a variable rate to maintain a steady state of anesthesia.

Plasma FFA assay

Plasma FFA level was measured by using a colorimetric kit from Jiancheng Bioengineering Institute (Nanjing, China), and the operating steps were in strict accordance with the manufacturer's instructions.

Table 1. Rat primers for quantitative PCR

Gene	Forward primer	Reverse primer
TNF-α	TACTGAACTTCGGGGTGATCG	CTCCTCCGCTTGGTGGTTT
IL-6	ATGATGACGACCTGCTAG	CTTCTTTGGGTATTGTTTGG
IL-1β	GAGGCTGACAGACCCCAAAA	GCTCCACGGGCAAGACATA
β-actin	CTAAGGCCAACCGTGAAAAGA	CCAGAGGCATACAGGGACAAC

Quantitative real-time PCR

Total RNA from liver and gastrocnemius muscle were respectively extracted using Trizol reagent (Takara, Japan) as per the protocols provided by the manufacturer. RNA concentration was determined via a NanoDrop1000 (NanoDrop, USA). Complimentary DNA was synthesized by using PrimeScript™ RT reagent kit (Takara, Japan) according to the manufacturer's instructions. Q-PCR was performed using SYBR green mix (Bestar qPCR Mastermix, DBI, Germany) and LighCycler 480 (Roche, Mannheim, Germany) according to the protocols. Primer sequences of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β) and β-actin were listed in **Table 1**. The cycle threshold (Ct) of each gene was normalized to β-actin mRNA and the fold change was calculated by $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Liver, gastrocnemius muscle, and epididymis fat samples were respectively lysed in RIPA lysis buffer with protease inhibitors and phosphatase inhibitors (Bimake, Houston, USA) for total proteins according to the manufacturer's instructions (Shenergy Biocolor Bioscience & technology CO., Shanghai, China). The cytosolic and nuclear proteins were obtained by the Nuclear and Cytoplasmic Protein Extraction kit (CWBIO). Protein concentration was measured using BCA protein Quantitative Assay kit (Shenergy Biocolor Bioscience & technology CO., Shanghai, China). Equal amounts of proteins were loaded onto 10% SDS-Polyacrylamide gel for electrophoresis and then transferred to polyvinyl difluoride membranes. The membranes were blocked with 5% non-fat milk, and then incubated with primary antibodies overnight at 4°C. The primary antibodies against IRS1, phospho-IRS1 (Ser307), Akt, phospho-Akt (Ser473), phospho-IκBα, mTOR, phospho-mTOR, JNK, phospho-JNK, ERK1/2, and phosoho-ERK1/2 were purchased from Cell Signaling Technology (Beverly, MA, USA), and anti-PI3K from Wuhan BOSTER Biotechnology Co., Ltd (Wuhan, Hubei, China). The primary antibodies against p-PI3K p85, IκBα, and NF-κB p65 were purchased from Abcam (Cambridge, MA, USA), and antibodies against GAPDH, LaminB, Tubulin, and β-actin were obtained from Proteintech Corporation (Chicago, IL, USA). The second antibody was horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse IgG (1:7500 dilution). The membrane was visualized by enhanced chemiluminescence (ECL) western blot detection system. The signal intensity was quantified using Image J software.

Molecular docking

AutoDock Vina software was used to perform in silico docking studies to evaluate the binding ability between Gyp-XLIX (ligand) and IKKβ protein (receptor). The structure of Gyp-XLIX was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the three-dimensional structure of IKKβ (PDB ID: 4KIK) was obtained from the PDB database (http://www.rcsb.org/). AutoDock Vina could evaluate the affinity of the receptor-ligand complex by calculating the spatial effect, repulsion, hydrogen bond, hydrophobic interaction and molecular flexibility, and finally gave the affinity score. PyMOL software was applied for visualization.

Statistical analysis

All data were analyzed by IBM SPSS Statistics 22.0 software. The results were presented as mean ± SEM and the statistical analysis was carried out by one-way ANOVA for multiple groups. *P*-value < 0.05 was considered that differences were significant.

Results

Animal characteristics

As depicted in **Table 2**, there were no significant differences in body weight among all groups. In addition, no significant difference was observed in the levels of blood glucose and plasma FFA among groups in the basal state. However, owing to lipid infusion, plasma FFA levels of IL and IL+Gyp-XLIX groups in the final

Table 2. Blood glucose and plasma FFA level in the basal and final states

Group	Body weight (g)	Glucose (mmol/L)		FFA (μmol/L)	
		Basal state	Final state	Basal state	Final state
SAL	240±14	5.58±0.12	5.72±0.15	492±22	220±14*
IL	257±13	5.43±0.11	5.45±0.13	481±33	912±61*
IL+Gyp-XLIX	238±12	5.63±0.14	5.82±0.18	506±30	938±53*

Notes: SAL, saline; IL, intralipid; IL+Gyp-XLIX, intralipid with Gyp-XLIX, n=7/group. Data are means ± SEM. *P < 0.05 versus basal state.

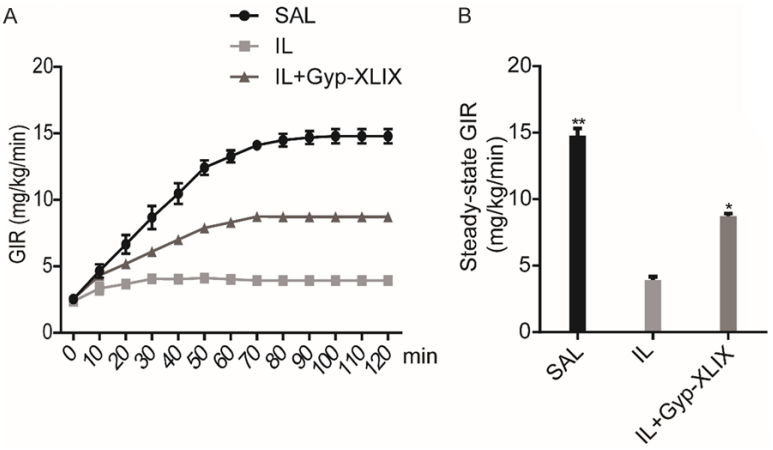

Figure 1. Effect of Gyp-XLIX pretreatment on the glucose infusion rate (GIR) during a hyperinsulinemic-euglycemic clamp. A. Time course of GIR; B. Steady-state GIR, n=7/group. Data were means ± SEM. SAL, saline; IL, intralipid; IL+Gyp-XLIX, intralipid with Gyp-XLIX. *P < 0.01, **P < 0.001 versus IL group.

state were significantly higher than the values in the corresponding basal state (P < 0.05). Conversely, in SAL group, a significant decrease in plasma FFA level was found during the experiment (P < 0.05), presumably due to the fat esterification effects of insulin.

Gyp-XLIX alleviated lipid-induced systemic insulin resistance

We next examined whether Gyp-XLIX had an attenuating effect on lipid-induced insulin resistance. Admittedly, steady-state glucose infusion rate (SSGIR) during a hyperinsulinemic-euglycemic clamp is an indicator of whole-body insulin sensitivity [21]. Compared with saline infusion, lipid infusion reduced SSGIR by 70% (14.79±0.54 *vs* 3.95±0.23 mg/Kg/min, P < 0.001) (**Figure 1A**, **1B**), indicating the establishment of insulin-resistant model. However, a significant increase (2.2-fold) of SSGIR was observed in the IL+Gyp-XLIX group (8.72±0.21 mg/Kg/min) relative to IL group (P < 0.01) (**Figure 1A**, **1B**), suggesting that Gyp-XLIX ameliorated lipid-induced systemic insulin resistance.

Gyp-XLIX attenuated lipid-induced impairment of insulin signaling

In order to explore possible molecular mechanism, we observed the effects of the acutely elevated plasma FFA levels on insulin signaling. We focused on insulin signaling pathway in liver and gastrocnemius muscle and measured the protein expression of key signals. Phosphorylated IRS1 at serine 307 may hinder the delivery of the insulin signaling, and our results showed that lipid infusion significantly enhanced the expression of phosphorylated IRS1 at serine 307 in both liver and gastrocnemius muscle compared with saline infusion. Gyp-XLIX pretreatment, however, remarkably reduced its expression (**Figure 2A**, **2D**, **2H**, **2I**).

PI3K/Akt signaling was regarded as the classical regulatory pathway in insulin resistance [22], and we found that lipid infusion markedly decreased their phosphorylated levels in both liver and gastrocnemius muscle compared with saline infusion. Notably, Gyp-XLIX showed a significant inhibition on these alternations induced by lipid, as manifested by up-regulated expression of p-PI3K p85 and p-Akt (Ser473) (**Figure 2B**, **2C**, **2E-I**).

In adipose tissue, lipid infusion did not alter the level of phosphorylated IRS1 at serine 307 (**Figure 2G**, **2J**). Based on this observation, we did not further detect the phosphorylated levels of PI3K and Akt which are downstream signaling molecules of IRS1.

Gyp-XLIX was effective in inhibiting lipid-induced NF-κB activation

Inflammation is directly interlinked with insulin resistance [23], and IKKβ is a central coordinator of inflammatory reactions via activation of

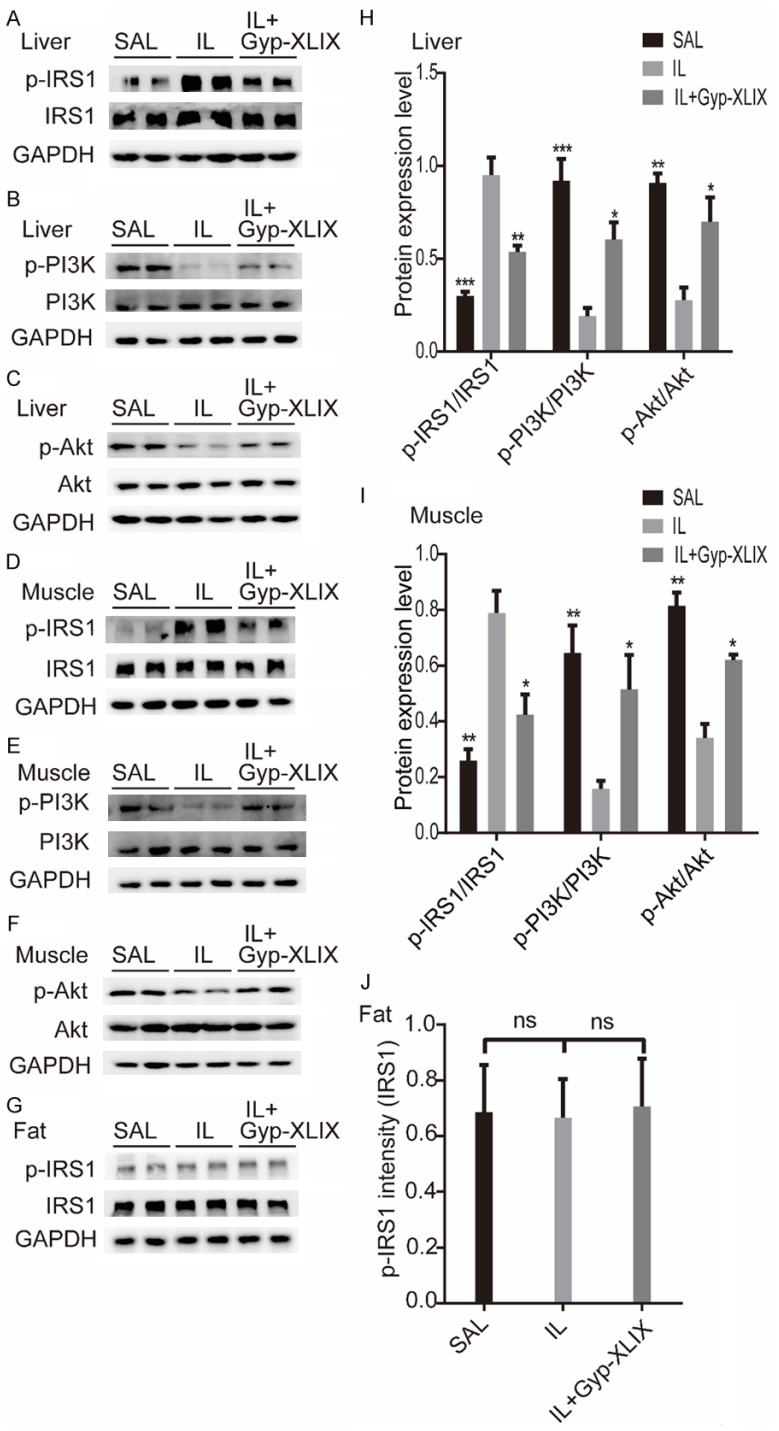

Figure 2. Effects of Gyp-XLIX pretreatment on serine 307 phosphorylated and total IRS1, phosphorylated and total PI3K p85 and Akt (Ser473). Representative images of immunoblots of related proteins in liver (A-C), gastrocnemius muscle (D-F) and fat (G) were shown. Bar graphs depicting the expression levels of related proteins in liver (H), gastrocnemius muscle (I) and fat (J) were shown. Independent experiments were run in triplicate. Data were means ± SEM. SAL, saline; IL, intralipid; IL+Gyp-XLIX, intralipid with Gyp-XLIX. *P < 0.05, **P < 0.01, ***P < 0.001 versus IL group; ns, not significant.

NF-κB. To evaluate the effects of Gyp-XLIX on IKKβ activity, we measured the IκBα content, which is a marker of IKKβ activation. Activated IKKβ phosphorylates IκBα, leading to its ubiquitination and subsequent degradation [24, 25]. Consistent with previous reports [24, 26], lipid infusion significantly increased IκBα phosphorylation and degradation in liver and gastrocnemius muscle, however, these increases were potently suppressed by Gyp-XLIX (**Figure 3A**, **3D**, **3H**, **3I**). Moreover, in adipose tissue, total and phosphorylated levels of IκBα were not changed by any treatment (**Figure 3G**, **3J**).

NF-κB is a ubiquitous nuclear transcription factor and functions prominently in the initiation of inflammation. During a resting-state condition, NF-κB is normally sequestered in the cytoplasm in an inactive complex with IκBα. Upon stimulation, IκBα is rapidly phosphorylated and degraded, leading to the translocation of free NF-κB into the nucleus. To analyze the nuclear translocation of NF-κB, we extracted nuclear and cytoplasmic protein from liver and gastrocnemius muscle. The results showed that lipid infusion significantly stimulated NF-κB p65 nuclear translocation, as evidenced by increased nuclear NF-κB levels and decreased cytosolic NF-κB levels compared with saline infusion (**Figure 3B**, **3C**, **3E**, **3F**, **3H**, **3I**). Importantly, Gyp-XLIX effectively suppressed lipid-triggered NF-κB p65 nuclear translocation.

Gyp-XLIX regulated lipid-induced proinflammatory gene expression

Once activated, NF-κB transfers into the nucleus and binds to target gene, which enhances

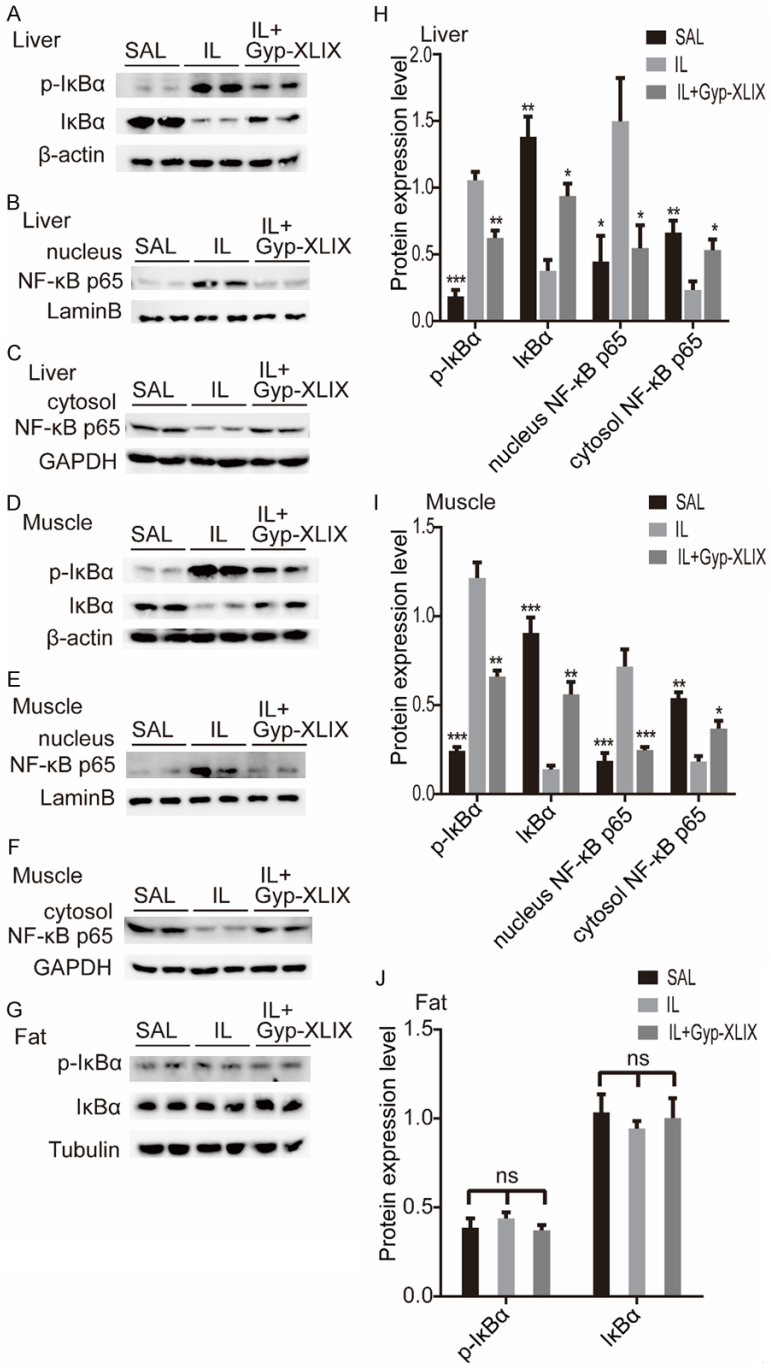

Figure 3. Effects of Gyp-XLIX pretreatment on the phosphorylation and degradation of IκBα, and protein expression of cytosolic and nuclear NF-κB p65. Representative images of immunoblots of related proteins in liver (A-C), gastrocnemius muscle (D-F) and fat (G) were shown. Bar graphs depicting the expression levels of related proteins in liver (H), gastrocnemius muscle (I) and fat (J) were shown. Independent experiments were run in triplicate. Data were means ± SEM. SAL, saline; IL, intralipid; IL+Gyp-XLIX, intralipid with Gyp-XLIX. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus IL group; ns, not significant.

the expression of proinflammatory cytokines. To investigate whether there was a change in the expression of proinflammatory gene at the transcriptional level, RT-PCR analysis was carried out. Compared with saline infusion, mRNA expression of TNF-α and IL-6 had a 6-fold and 4-fold increase in liver owing to lipid infusion, respectively (**Figure 4A**). Similarly, in gastrocnemius muscle, lipid infusion enhanced TNF-α and IL-1β gene expression by 6-fold and 3-fold, respectively (**Figure 4B**). Due to lipid infusion, mRNA expression of IL-1β in liver and IL-6 in gastrocnemius muscle both tended to increase, however, there were no statistical significance when compared with saline infusion. Importantly, in the IL+Gyp-XLIX group, mRNA expression of TNF-α and IL-6 in liver reduced by 58% and 41% relative to IL group, respectively (**Figure 4A**). Concomitantly, TNF-α and IL-1β mRNA expression in gastrocnemius muscle reduced by 47% and 38%, respectively (**Figure 4B**).

mTOR, JNK and ERK were not involved in lipid-induced acute insulin resistance

Additionally, we also examined other signaling molecules that have been proposed to play critical roles in insulin signaling transduction, including mammalian target of rapamycin (mTOR), c-Jun N-terminal kinase (JNK) and extracellular signal regulated kinase (ERK). However, results from our study showed that total and phosphorylated levels of these proteins in liver and gastrocnemius muscle were not altered by any treatment (**Figures 5A-D**), which was consistent with previous studies [26, 27], and suggested they were not involved in an

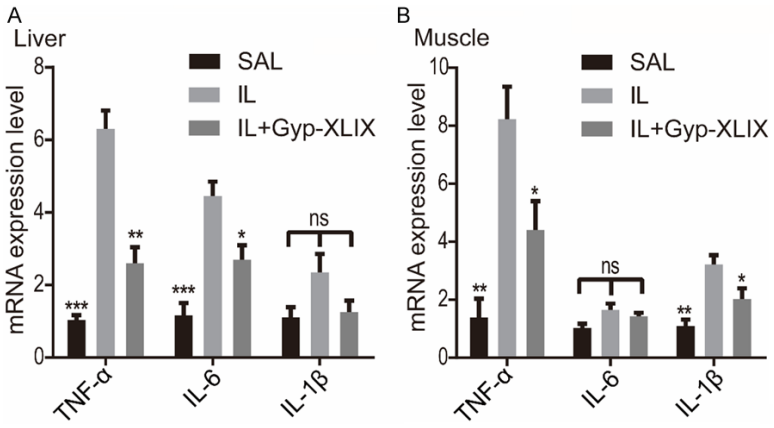

Figure 4. Effects of Gyp-XLIX pretreatment on mRNA expression of TNF-α, IL-6, and IL-1β. (A) in the liver, (B) in the gastrocnemius muscle. Independent experiments were run in triplicate. Data were means ± SEM. SAL, saline; IL, intralipid; IL+Gyp-XLIX, intralipid with Gyp-XLIX. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus IL group; ns, not significant.

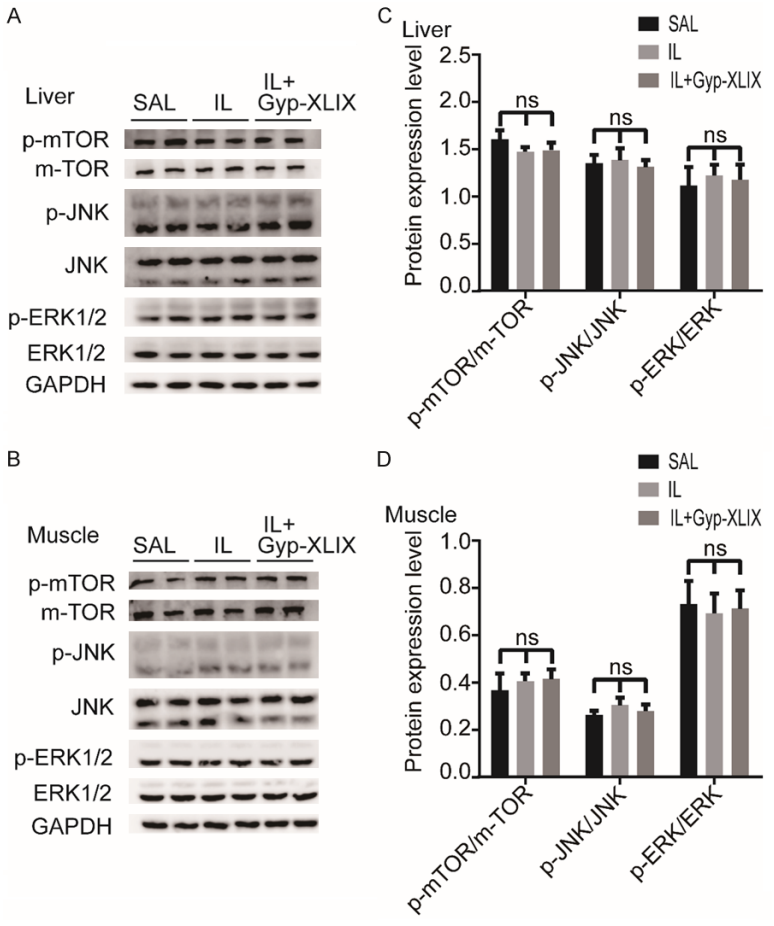

Figure 5. Effects of Gyp-XLIX pretreatment on total and phosphorylated levels of mTOR, JNK, and ERK1/2. Representative images of immunoblots of related proteins in liver (A) and gastrocnemius muscle (B) were shown. Bar graphs depicting the expression levels of related proteins in liver (C) and gastrocnemius muscle (D) were shown. Independent experiments were run in triplicate. Data were means ± SEM. SAL, saline; IL, intralipid; IL+Gyp-XLIX, intralipid with Gyp-XLIX. ns, not significant.

acute model of insulin resistance we established.

Validation of molecular docking

AutoDock Vina is a frequently-used, open-source molecular docking program to evaluate the binding capability between the ligand and receptor. The binding energy represents their binding ability, and the lower the energy level, the stronger the binding affinity. Generally, if the docking calculation score (kcal/mol) is less than -7 in AutoDock Vina, the binding affinity is considered to be strong. In our study, the binding energy between Gyp-XLIX and IKKβ protein was -9.9 kcal/mol (**Figure 6**), indicating that Gyp-XLIX had a great potential to bind to IKKβ.

Discussion

The current study demonstrated for the first time that Gyp-XLIX could attenuate lipid-induced insulin resistance *in vivo*, and this prominent pharmacological action was associated with improved insulin signaling in liver and gastrocnemius muscle. Furthermore, Gyp-XLIX potently suppressed lipid-stimulated activation of the canonical proinflammatory IKKβ/NF-κB pathway, contributing to alleviation of the damage to insulin signaling pathway by inflammation.

Plenty of evidences have shown that increased plasma free fatty acids (FFAs) play a crucial role in promoting loss of insulin sensitivity, thereby causing insulin resistance [28]. Conventionally, insulin-resistant rat model was often induced by chronic high-fat

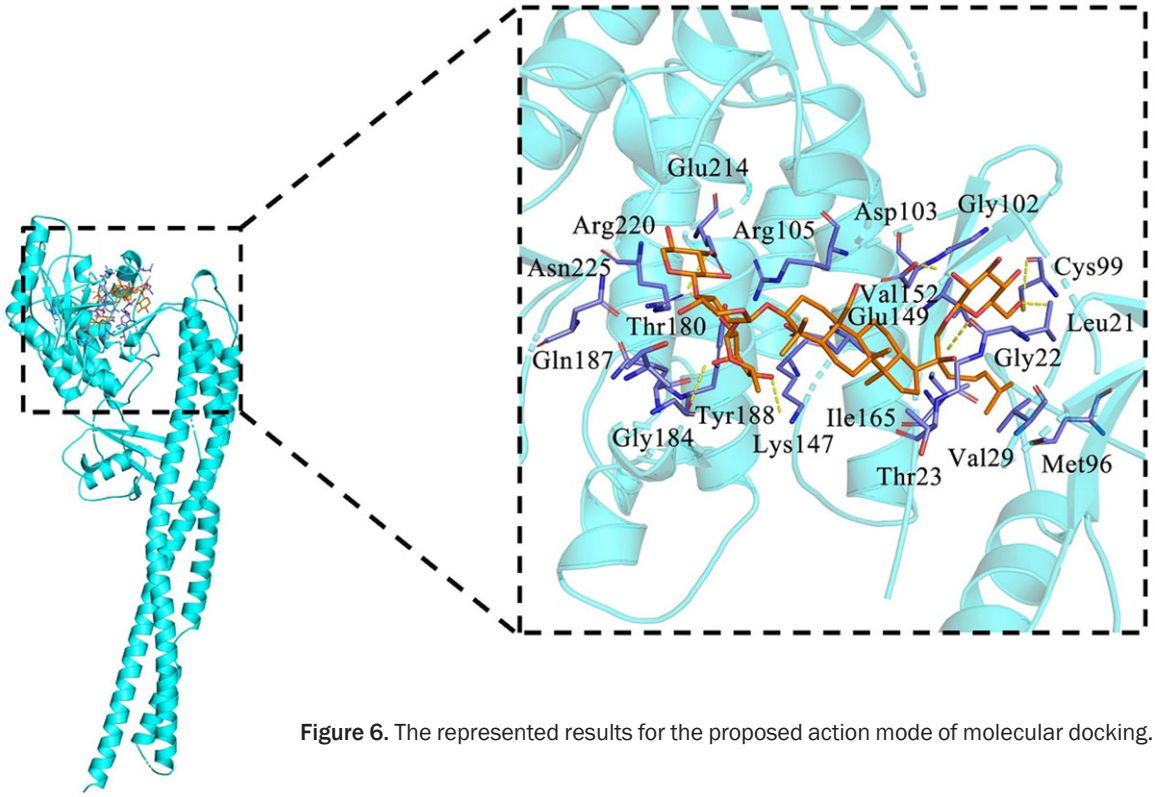

Figure 6. The represented results for the proposed action mode of molecular docking.

diets feeding, however, some defects have been found in this method. If the rats were fed high-fat diets for a long time, they could compensatorily secrete some gastrointestinal hormones, such as gastric inhibitory polypeptide, which could cause the fatty acids to enter adipose tissue and thus led to a spontaneous attenuation of insulin resistance [29, 30]. As has been demonstrated repeatedly, short-term lipid infusion could induce severe insulin resistance in rats and humans, accompanied by a sharp increase in plasma FFA concentration, and this insulin-resistant model was increasingly used [31, 32]. In agreement with previous studies [33, 34], our results showed that lipid infusion raised the plasma FFA level and caused a significant decrease in SSGIR, indicating the establishment of insulin-resistant model.

Gypenosides, the major active constituents in *Gynostemma pentaphyllum* (GP), are mainly responsible for the pharmacological action of GP. Since gypenosides are structurally similar to the ginseng saponins, which are the well-known biologically active ingredients in ginseng, gypenosides have attracted much attention. It has been shown that gypenosides can counter many metabolic diseases including insulin resistance [35]. However, little is known about which types of monomer saponins in gypenosides are responsible for this beneficial pharmacological role. Gyp-XLIX is a pure compound isolated from GP and has been reported to inhibit cytokine-induced vascular cell adhesion molecule-1 and lipopolysaccharide (LPS)-induced tissue factor overexpression *in vitro* [20, 36]. In the present study, Gyp-XLIX exerted a potent role in alleviating insulin resistance *in vivo*. Based on this finding, we speculated that the ability of Gyp-XLIX to improve insulin sensitivity may contribute to insulin-sensitizing action of GP and gypenosides.

In the beginning, we conducted a pilot experiment about dose of Gyp-XLIX and applied 1, 2 and 4 mg/kg Gyp-XLIX to lipid-infused rats respectively. Among the 3 doses, insulin resistance was significantly reduced when we applied 4 mg/kg. For this reason, we chose this dose in the following research. Additionally, Gyp-XLIX was given as a single-dose i.v. injection because of its long plasma half-life (3 to 4 hours) [37]. Insulin resistance was not com-

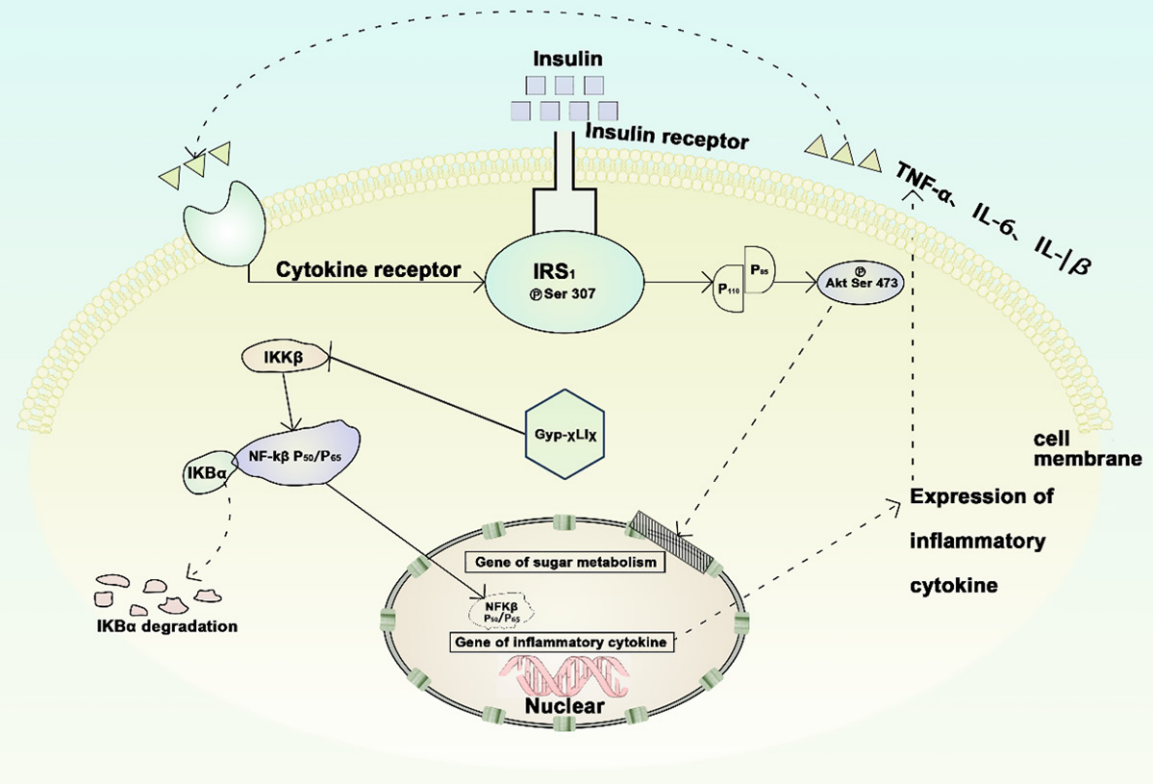

Figure 7. Possible mechanism of the effects of Gyp-XLIX on attenuating insulin resistance caused by lipid infusion.

pletely reversed by Gyp-XLIX, which may be associated with the fact that concentration of Gyp-XLIX in the blood decreased gradually, and further research remained to be done.

We first investigated the effects of Gyp-XLIX on insulin signaling pathway of insulin-responsive tissues (liver, gastrocnemius muscle, adipose tissue). Although the precise molecular mechanism of insulin resistance caused by lipid infusion remained elusive, a consensus existed that insulin signaling transduction was interrupted, with phosphorylation of IRS1 at serine 307 being a crucial element [34, 38]. In this study, lipid infusion augmented the phosphorylation of IRS1 at serine 307 in liver and gastrocnemius muscle, which implied that insulin signaling was impaired and accounted for the decreased phosphorylation levels of downstream signaling molecules PI3K and Akt. However, Gyp-XLIX played a beneficial role in protecting against lipid-induced damage to insulin signaling pathway, as proved by down-regulated serine 307 phosphorylation of IRS1 and up-regulated phosphorylation of PI3K p85 and Akt (Ser473) compared with those of IL group. Taken together, these results suggested that the effect of Gyp-XLIX to restore insulin sensitivity was closely correlated with its reversal of lipid-induced impairment of insulin signaling.

Increasing evidences show that inflammation serves as a vital role in the occurrence of insulin resistance, and anti-inflammatory medications may reverse insulin resistance [39]. In fact, many anti-inflammatory drugs such as Aspirin [40], Sodium Salicylate and Indomethacin [41, 42], have been proved to be effective in improving insulin sensitivity. Additionally, many nature medicine monomers (NMMs) which have anti-inflammatory activity, such as Berberine [43], Kaempferol [44], and Tanshinone IIA [45], could also ameliorate insulin resistance by reducing inflammation. Lately, it was shown that Ginsenoside Rg1 (a compound exacted from *panax notoginseng*) could correct high-fat induced insulin resistance through inhibiting inflammation and further promoting PI3K/Akt signaling pathway [46]. Interestingly, Ginsenoside Rg1 and Gyp-XLIX both belong to dammarane-type tetracyclic triterpenoid saponin.

Recently, several kinds of monomer saponins in GP, such as Gypenoside III, Gypenoside IV, Gypenoside UL4, and Gypenoside IX, have been demonstrated to resist inflammatory-associated diseases, such as obesity, myocardial ischemia-reperfusion injury, non-alcoholic fatty liver, and neuroinflammatory disorder [47-50]. Of note, their anti-inflammatory properties were mainly responsible for these observed beneficial effects. In our study, Gyp-XLIX exerted an inhibitory effect on lipid-induced NF-κB activation and thus efficiently attenuated the inflammatory response, as manifested by reduction of IκBα phosphorylation and degradation, suppression of NF-κB p65 nuclear translocation, and down-regulation of proinflammatory gene expression. Moreover, virtual molecular docking further verified the great potential of Gyp-XLIX targeting IKKβ protein. Based on our own experimental results and other researchers' related studies, it can be said that the effect of Gyp-XLIX to improve insulin resistance may be connected with its anti-inflammatory activity (**Figure 7**). Nevertheless, our data were still correlative and did not prove causality.

Interestingly, in adipose tissue, the phosphorylation of IRS1 at serine 307 was unchanged, which suggested that insulin signaling transduction of adipose tissue remained normal under the condition of lipid infusion. Similarly, in Kim's study, short-term lipid infusion did not affect insulin-stimulated glucose uptake in adipose tissue [41], which also supported that lipid infusion did not alter the biological effects of insulin on adipose tissue. Moreover, lipid infusion did not change total and phosphorylated levels of IκBα either, which corresponded with unaffected insulin signaling. This may also reflect a close interaction between inflammatory signaling and insulin signaling pathway from the side.

Although mTOR and JNK have been shown to phosphorylate the serine 307 site of IRS1 [51], they were not involved in our model of insulin resistance induced by acute FFA elevation. In this study, expression of ERK was not influenced by lipid infusion as well, which suggested that MAPK branch of insulin signaling pathway was not relevant to lipid-induced insulin resistance.

In conclusion, Gyp-XLIX attenuated lipid-induced insulin resistance in the rats, with favorable effects on inhibiting NF-κB activation. To our knowledge, this is a first study to demonstrate insulin-sensitizing effects of this compound in an acute model of insulin resistance. The novel finding might highlight a promising therapeutic option for overcoming FFA-mediated insulin resistance.

Acknowledgements

The study was supported by National Natural Science Foundation of China (grant number: 91957209, 81670796).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jiajun Zhao, Department of Endocrinology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, 324 Jing-Wu-Wei-Qi Road, Huaiyin District, Jinan 250000, Shandong, China. Tel: +86-0531-68776910; Fax: +86-0531-68776910; E-mail: jjzhao@sdu.edu.cn

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