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Berberine Inhibits Inflammatory Response and Ameliorates Insulin Resistance in Hepatocytes

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Abstract—Berberine, a major isoquinoline alkaloid present in Chinese herb *Rhizoma coptidis*, is a potent inhibitor of inflammation and has anti-diabetic activity. This study aims to investigate effects of berberine on ameliorating insulin resistance and molecular mechanisms involved in HepG2 cells. Inflammatory responses and insulin resistance were induced by palmitate (PA) stimulation for 24 h. Treatment of berberine enhanced insulin-mediated glycogen synthesis and restored insulin inhibition of triglyceride secretion. Stimulation of PA resulted in IL-6 and TNF-α production in HepG2 cells, and antibody-neutralizing assay further confirmed that IL-6 and TNF-α were involved in the development of insulin resistance. Berberine effectively inhibited IL-6 and TNF-α production in a concentration-dependent manner, demonstrating its anti-inflammatory activity in hepatocytes. Meanwhile, PA-evoked inflammation impaired insulin signaling cascade and berberine improved insulin signaling cascade by modification of Ser/Thr phosphorylation of insulin receptor substrate-1 (IRS-1) and downstream Akt (T308). Above results suggest that berberine improved insulin sensitivity in PA-stimulated hepatocytes and this regulation might relative with its anti-inflammatory activity.

KEY WORDS: berberine; insulin resistance; inflammation; hepatocytes.

INTRODUCTION

Type 2 diabetes and obesity are rapidly becoming a worldwide epidemic and they are increasingly prevalent and strongly associated with the development of insulin resistance [1]. Insulin resistance is believed to be an underlying feature of type 2 diabetes and metabolic syndrome. Since Hotamisligil and Karasik first showed that the proinflammatory cytokine TNF-α was able to induce insulin resistance, more attention has been focused on the link between inflammation and insulin resistance [2, 3]. Proinflammatory cytokines can cause insulin resistance [4, 5] and anti-inflammatory medications may reverse it [6, 7], suggesting that inflammation may be directly involved in the pathogenesis. Increased adiposity, high-fat diet is associated with lipid accumulation in other tissues such as liver, resulting in increased circulating free fatty acids (FFA) which impair insulin signaling and glucose uptake. There is also elevated inflammatory gene expression in liver with increasing adiposity [8–10]. These evidences suggest that fat accumulation in the liver leads to subacute hepatic "inflammation" and causes insulin resistance in liver [11, 12], indicating that the liver is a major site for the initiation of insulin resistance.

Berberine is a major isoquinoline alkaloid present in Chinese herb *Rhizoma coptidis*, and has a wide range of pharmacologic actions, such as antidiarrheic, anticancer, and anti-inflammation [13]. It has been used for the treatment of infective and inflammatory disorders. Recently, there is a growing interest in its beneficial modulation in sugar and lipid metabolic disorders [14]. Some published studies reported that berberine

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improved insulin resistance, lowered blood sugar, and cured lipid metabolism disorders by activating the AMP-activated protein kinase (AMPK) pathway [15–18]. Jeong and his co-workers showed that berberine inhibited gene expression of proinflammatory cytokines in adipose tissue of obese db/db mice and suppressed inflammatory response through AMPK activation in macrophages, well demonstrating its anti-inflammatory potency [19].

Berberine is a potent inhibitor of inflammation and has anti-diabetic activity [20], but whether its anti-inflammatory activity contributes to the improvement of insulin resistance remains unclear. Considering the significance of inflammation in the progression of insulin resistance, this study aimed to evaluate its anti-inflammatory activity involved in the modulation towards insulin sensitivity in hepatocytes. Verification and confirmation of its anti-inflammation-related improvement of insulin sensitivity should be beneficial for the possible application of berberine in the clinical treatment of type 2 diabetes and metabolic syndrome.

MATERIALS AND METHODS

Materials

Berberine (Kunming Fengshanjian Medical Research Ltd, China; purity>98%) and PD98059 (Beyotime Institute of Biotechnology, China; purity> 98%), was dissolved in DMSO and stored at −20°C. Final concentration of DMSO in the medium was <0.1% either in control or treated cells. BAY11-7082 was purchased from Beyotime Institute of Biotechnology; sodium salicylate (Sal) was the product of Tianjin Kemiou Chemical Agent Center, China. Bovine serum albumin (BSA) was purchased from Nanjing Sunshine Biotechnology Co. Ltd., China. Palmitic acid (PA) was obtained from Sinopharm Chemical Reagent Co., Ltd., China. PA was first dissolved in ethanol at 200 mmol/L and then combined with 10% FFA-free low endotoxin BSA to obtain the final concentration of 5 mmol/L. Antibodies against phospho-ERK1/2 and GAPDH, HRP-conjugated goat anti-rabbit or anti-mouse secondary antibody were the products of Shanghai KangChen Bio-tech (China); anti-phospho-insulin receptor substrate-1 (IRS-1; Ser1101) and anti-IRS-1 were purchased from Bioworld Technology, MN, USA. Mouse anti-human IL-6 monoclonal antibody (CBL2117, 1.0 mg/mL) was the product of Millipore Co., and Mouse anti-human TNF-α monoclonal antibody (MAB1021, 1.0 mg/mL) was purchased from Chemicon International, Inc.

Cell Culture

HepG2 cells (a human hepatoma cell line, purchased from Shanghai Institutes for Biological Sciences, China) was cultured in Dulbecco's Minimum Essential Medium (DMEM). The DMEM was supplemented with 10% fetal bovine serum under endotoxin-free conditions at 37°C in a 5% CO_2 atmosphere.

Glycogen Content Assay

To measure the content of glycogen, HepG2 cells were seeded on 24-well cell culture plates at a density of 2×10^5 cells/well. Following 24 h of stabilization, the cells were pretreated with or without berberine at concentrations of 0.1, 1, and 10 μM, PD98059 (ERK inhibitor, 30 μM or Sodium salicylate (5 mM) for 30 min, followed by stimulation with PA (0.5 mM) for another 24 h. Then, cells were washed twice with phosphate-buffered saline (PBS) and cultured in DMEM containing100 nM insulin for 3 h. Incubation media was discarded and cells were collected and washed to remove extracellular glucose. Glycogen content of cells was determined by the anthrone reagent [21]. The amount of blue compound generated by the reaction was assayed at 620 nm. The protein content of the collected HepG2 cells was quantified with Bradford reagent [22]. Values were presented in the ratio of glycogen/protein (milligrams per milligram).

Extracellular Triglyceride Assay

HepG2 cells were seeded on 24-well dishes at a density of 2×10^5 cells/well. Following starvation in serum-free DMDM for 24 h, cells were pretreated with berberine (0.1, 1, and 10 μM), PD98059 (30 μM) or sodium salicylate (5 mM) for 30 min, then PA (0.5 mM) was added to the medium and cultured with cells for 24 h. Following this step, cells were washed twice with phosphate-buffered saline and cultured in Krebs–Henseleit solution (in grams per liter (g/L); NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$1.2, $MgSO_4{\cdot}7H_2O$ 1.2, $NaHCO_3$ 25, Glucose10.1, pH 7.4) with 100 nM insulin for 3 h. The amount of triglyceride (TG) in culture medium was measured by Triglyceride Assay kit (Nanjing Jiancheng Bioengineering Institute, China). The assay was initiated with the enzymatic hydrolysis

of the triglycerides by lipase to produce glycerol and free fatty acids. The glycerol released was subsequently measured by a coupled enzymatic reaction system with a colorimetric readout at 540 nm.

Enzyme-Linked Immunosorbent Assay

Cells were plated in 24-well cell culture plates at a density of 2×10^5 cells/well and allowed to grow for 24 h. Then the cells were treated with PA (0.5 mM) after incubation with or without berberine (0.1, 1 and 10 μM), PD98059 (30 μM) or sodium salicylate (5 mM). In some cases, cells were exposed to anti-IL-6 (1 or 0.5 μg/mL) or anti-TNF-α (1 or 0.5 μg/mL) for 0.5 h. After 24 h-stimulation with PA, the amounts of TNF-α or IL-6 in the culture media were quantified by using enzyme-linked immunosorbent assay (ELISA) kit (eBioscience).

Western Blotting

Cells in 6-well plates were treated with berberine (0.1, 1, and 10 μM) and sodium salicylate (3 mM), PD98059 (30 μM) for 30 min, then incubation with PA (0.5 mM) for another 24 h. Cells were collected using a cell scraper after washing with ice-cold PBS and lyzed with sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate, 10% glycerol, 50 mM dithiothreitol, 0.1% bromophenol blue). The protein concentration was determined by BCA Protein Assay Reagent. Total proteins (30–50 μg) were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred electrophoretically to PVDF membranes. Membranes were blocked in blocking buffer (20 mM Tris-HCl, pH 7.6, 140 mM NaCl, 0.1% Tween-20, 5% skim milk powder) and immunoblotted with primary and secondary antibody. The bound antibody was then detected with peroxidase-conjugated antibody followed by chemiluminescence (ECL system, Amersham, Buckinghamshire, UK) and exposed by autoradiography.

Statistical Analysis

The statistical analysis was performed by analysis of variance (one-way ANOVA) followed by Student Newman–Keuls test. $P<0.05$ was considered statistically significant. Results were expressed as mean±S.D.

RESULTS

Berberine Enhanced Glycogen Synthesis in HepG2 Cells

Insulin can promote glycogen synthesis, which was demonstrated by higher level of glycogen content in hepatocytes. We observed that PA (0.5 mM) treatment significantly decreased glycogen content in control. As show in Fig. 1, in the presence of berberine, PD98059 or high concentration of sodium salicylate, glycogen in HepG2 cells increased significantly.

Effect of Berberine on Extracellular TG in HepG2 Cells

Figure 2 shows the extracellular TG concentrations observed in blank, control, and different drug-pretreated groups after 3-h incubation with insulin. Exposure to 0.5 mM PA for 24 h resulted in a significant elevation of extracellular TG. Treatment of 10 μM berberine, 30 μM ERK1/2 inhibitor PD98059 and 5 mM sodium salicylate decreased the amount of TG, respectively, in the medium.

Berberine Reduced IL-6 and TNF-α Release from HepG2 Cells Stimulated with PA

As shown in Fig. 3, treatment of PA (0.5 mM) significantly increased IL-6 and TNF-α production, which were reduced by berberine in a dose-dependent

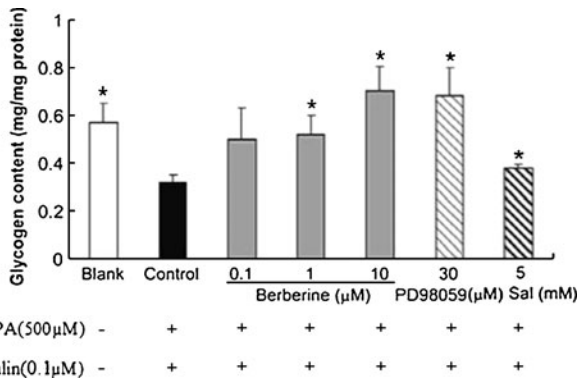

Fig. 1. Berberine enhanced insulin-mediated glycogen synthesis in HepG2 cells. Cells (2×10^5 cells/well) were incubation with PA (0.5 mM) for 24 h and then stimulated with insulin (100 nM) for 3 h. Glycogen content in cells was determined by the anthrone reagent. PD98059 and sodium salicylate (*Sal*) was taken as positive controls. Data are expressed as the mean±S.D. (n=4). *Asterisk* (*), $p<0.05$ (compared with the control).

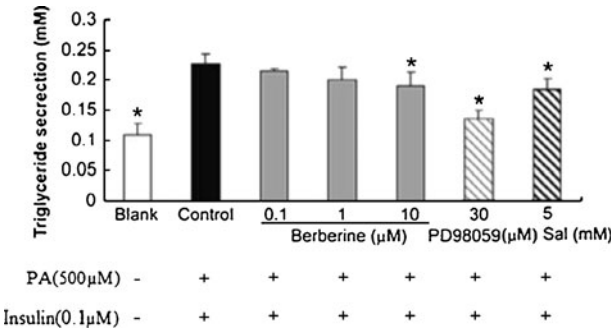

Fig. 2. Berberine restored insulin-mediated inhibition of lypolisis in HepG2 cells. Cells (2×10^5 cells/well) were incubation with PA (0.5 mM) for 24 h and then stimulated with insulin (100 nM) for 3 h. TG in the medium was assayed as the extracellular TG. PD98059 and sodium salicylate (*Sal*) was taken as positive controls. Data are expressed as the mean±S.D. (n=4). *Asterisk* (*), $p<0.05$ (compared with the control).

manner. PD98059 and sodium salicylate also showed the inhibitory effects.

Effect of Neutralizing Antibody on Glycogen Synthesis in PA-Stimulated HepG2 Cells

To evaluate the effect of proinflammatory cytokines on the insulin sensitivity in PA-treated HepG2 cells, anti-IL-6 and anti-TNF-α were used in this experiment. Treatment of anti-IL-6 antibody increased glycogen content in HepG2 (compared with the control groups), but anti-TNF-α antibody was less effective, as well as the combination of semi-dose of anti-IL-6 plus anti-TNF-α. (Fig. 4)

Effect of Anti-TNF-α Antibody on IL-6 Production in PA-Stimulated HepG2 Cells

It appeared that IL-6 plays a key role in affecting glycogen content/synthesis in hepatocytes, but berberine reduced not only the production of IL-6, but also that of TNF-α in HepG2 cells significantly. Figure 5 shows that anti-TNF-α down-regulated the secretion of IL-6 from PA-stimulated HepG2 cells, which suggested TNF-α might positively regulate IL-6 production in PA-stimulated hepatocytes.

Effect of Berberine on PA-Induced ERK1/2 Activation

Data above have indicated that PD98059 could reduce proinflammatory cytokines release and improve insulin sensitivity in HepG2 cells, suggested MEK/ERK activation may be involved. Results in Fig. 6 shows PA-stimulation activated the phosphorylation of ERK1/2, which was down-regulated by the treatment of 10 μM berberine or PD98059.

PA-Induced Serine Phosphorylation of Insulin Receptor Substrate-1 (S1101) Were Attenuated by Berberine

PA stimulation resulted in serine phosphorylation of IRS-1. As shown in Fig. 7, the augmented phosphorylation of IRS-1 (S1101) was effectively reduced by berberine at the concentration range from 0.1 to 10 μM. Sodium salicylate also significantly attenuated serine phosphorylation of IRS-1.

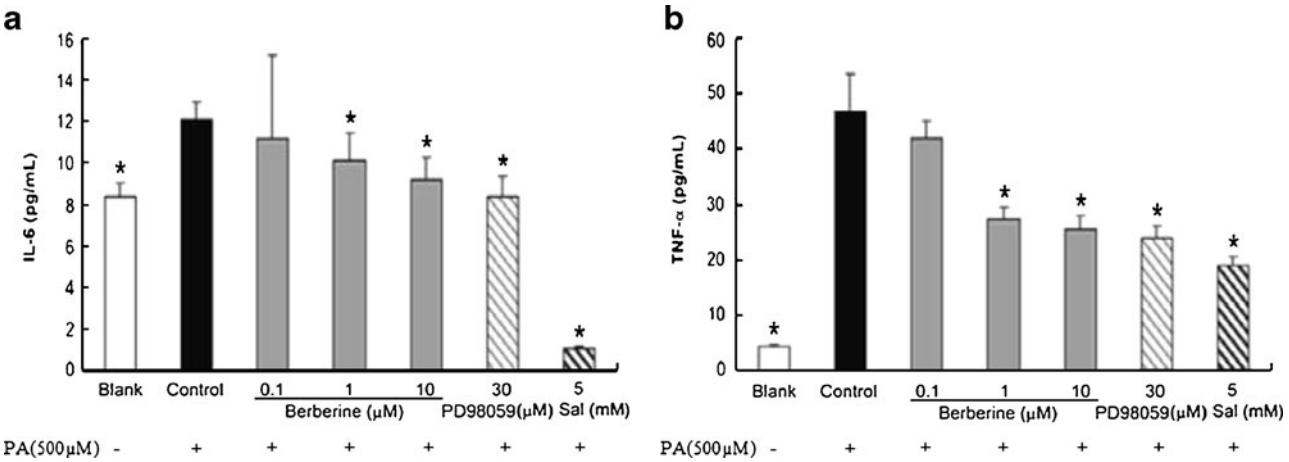

Fig. 3. Berberine reduced IL-6 (**a**) and TNF-α (**b**) production in HepG2 cells stimulated by PA. Cells (2×10^5 cells/well) were incubation with PA (0.5 mM) for 24 h. Amounts of the cytokines in the culture medium were quantified by ELISA. PD98059 and sodium salycylate (*Sal*) was taken as positive controls. Data are expressed as the mean±S.D. (n=4). *Asterisk* (*), $p<0.05$ (compared with the control).

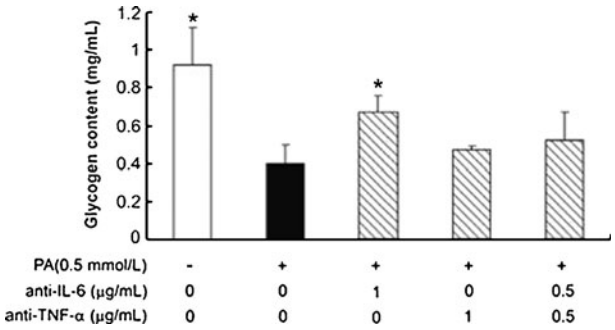

Fig. 4. Effects of anti-IL-6 and anti-TNF-α antibodies on insulin-mediated glycogen synthesis in HepG2 Cells. Cells (2×10^5 cells/well) were incubation with PA (0.5 mM) for 24 h and then stimulated with insulin (100 nM) for 3 h. Glycogen content in cells was determined by the anthrone reagent. Data are expressed as the mean±S.D. (n=4). *Asterisk* (*), $p<0.05$ (compared with the control).

Effect of Berberine on Insulin-Mediated Tyrosine Phosphorylation of IRS-1 and Akt Activation in the Presence of PA

Stimulation of insulin resulted in tyrosine phosphorylation of IRS-1(detected by antibody of PY99) and downstream Akt phosphorylation at T308. Long-term PA pretreatment impaired insulin signaling cascade, as tyrosine phosphorylation of IRS-1 and downstream Akt phosphorylation were greatly attenuated. As shown in Figs. 8 and 9, berberine, at concentrations of 0.1 and 1 μM, successfully restored insulin-mediated tyrosine phosphorylation of IRS-1 and subsequent Akt activation. Sodium salicylate, PD98059, and BAY11-7082 demonstrated a similar regulation of phosphorylation modification in IRS-1 and downstream Akt as berberine.

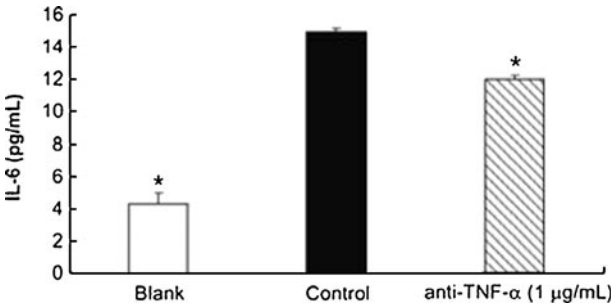

Fig. 5. Effect of anti-TNF-α antibody on IL-6 production in PA-stimulated HepG2 cells. Cells (2×10^5 cells/well) were stimulated with PA (0.5 mM) for 24 h. Amounts of the IL-6 in the medium were quantified by ELISA. Data are expressed as the the means±S.D. n=4. *Asterisk* (*), $p<0.05$ (compared with the control).

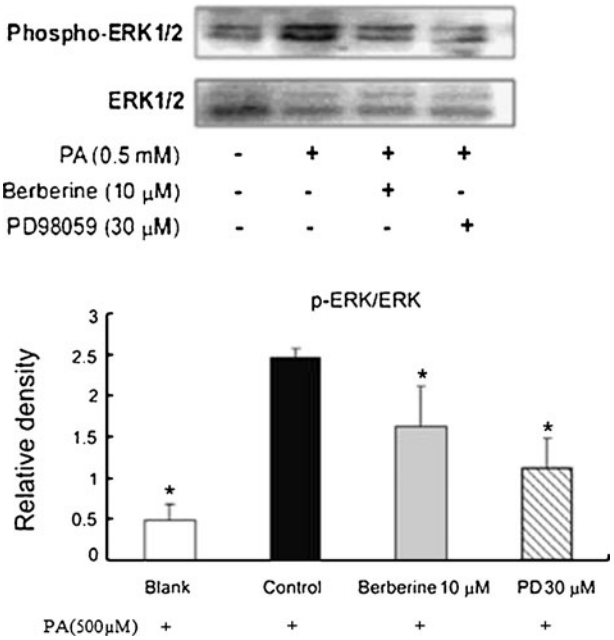

Fig. 6. Berberine inhibited ERK1/2 activation in HepG2 cells. Cells (2×10^5cells/well) were stimulated with PA (0.5 mM) for 30 min. The phosphorylation of ERK1/2 was determined by Western blot. PD 98059 (PD) was taken as a positive control. Data were expressed as the mean±S.D. (n=3). *Asterisk* (*), $p<0.05$ (compared with the control).

DISCUSSION

Plasma levels of FFAs are usually increased in obese subjects, and FFAs are considered as a causative link between obesity and type 2 diabetes [21, 23–26]. Fat accumulation in the visceral depot and liver are strongly correlated, and both are highly correlated with the development and severity of insulin resistance [10, 12, 27]. Berberine has been shown to have antidiabetic properties and can reduce the production of inflammatory molecules in adipocytes [20], suggesting that its anti-inflammatory activity perhaps contributes to the improvement of insulin sensitivity. In this study, we stimulated hepatocytes with PA to induced insulin resistance. Here we showed that berberine inhibited inflammatory response and effectively ameliorated insulin sensitivity in hepatocytes. These results further confirmed that its anti-inflammatory activities were involved in the regulation of insulin sensitivity.

The main insulin action in liver is to increase glycogen synthesis, and hepatic insulin resistance is characterized by a reduced capacity of insulin to increase glycogen synthesis. Stimulation of palmitate resulted in a decrease in glycogen content and berberine effectively

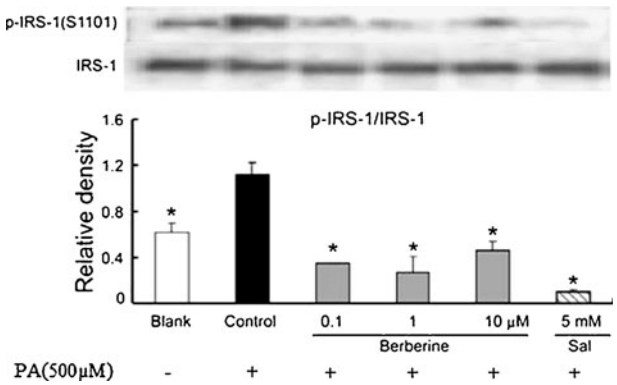

Fig. 7. Berberine inhibited serine IRS-1 phosphorylation (S1109) induced by PA in HepG2 cells. Cells (2×10^5cells/well) were stimulated with PA (0.5 mM) for 24 h. The serine phosphorylation of IRS-1 (S1109) was determined by Western blot. Sodium salicylate (*Sal*) was taken a positive control. Data were expressed as the mean±S.D. (n=3). *Asterisk* (*), $p<0.05$ (compared with the control).

increased insulin-mediated glycogen synthesis in hepatocytes. Insulin also acts in liver to inhibit lipolysis. TG is the main component of very low-density lipoprotein and increased extracellular TG suggested insulin-mediated inhibition of lipolysis was attenuated. We also observed that berberine reduced extracellular TG accumulation after the stimulation of palmitate. Above evidences well demonstrated that berberine successfully attenuated PA-induced insulin resistance in hepatocytes.

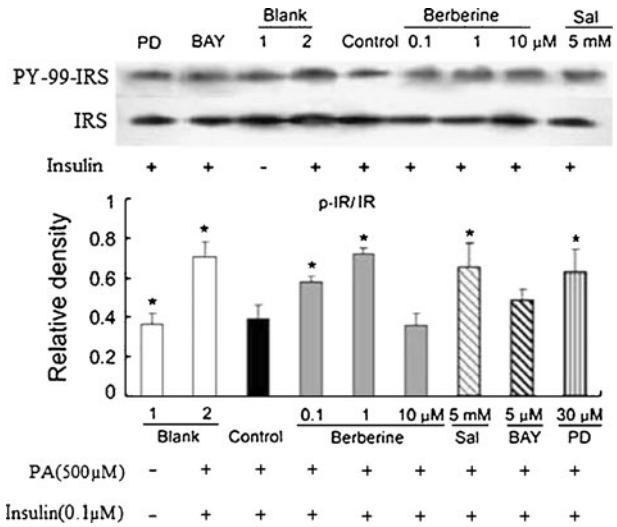

Fig. 8. Berberine enhanced insulin-mediated tyrosine phosphorylation of IRS-1(PY99) in HepG2 cells. Cells (2×10^5cells/well) were incubated with PA (0.5 mM) for 24 h and then stimulated with insulin (100 nM) for 20 min. The tyrosine phosphorylation of IRS-1 (PY99) was determined by Western. Sodium salicylate (*Sal*), BAY11-7082 (*BAY*), and PD98059 (*PD*) were taken as positive controls. Data were expressed as the mean±S.D. (n=3). *Asterisk* (*), $p<0.05$ (compared with the control).

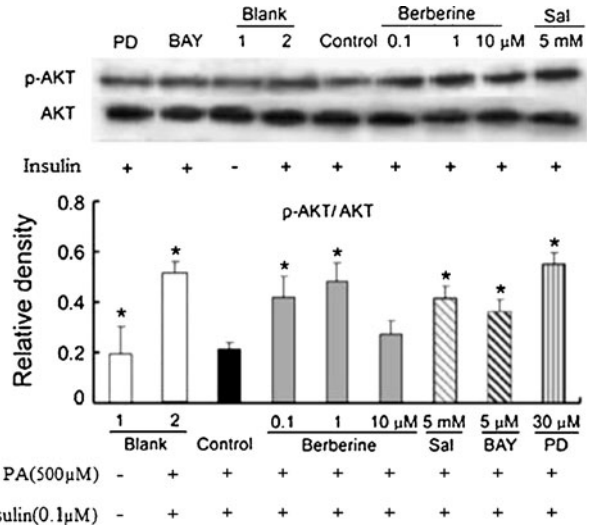

Fig. 9. Berberine restored insulin-mediated Akt activation. in HepG2 cells. Cells (2×10^5cells/well) were incubated with PA (0.5 mM) for 24 h and then stimulated with insulin (100 nM) for 20 min. The phosphorylation of Akt was determined by Western. Sodium salicylate (*Sal*), BAY11-7082 (*BAY*), and PD98059 (*PD*) were taken as positive controls. Data were expressed as the mean±S.D. (n=3). *Asterisk* (*), $p<0.05$ (compared with the control).

Inflammation plays an important role in the development of insulin resistance. PD 98059 is a specific inhibitor of extracellular receptor-activated kinase (ERK) and high dose of salicylate is an inhibitor of NF-κB. PD 98059 and sodium salicylate improved insulin sensitivity, suggesting ERK/NF-κB—dependent proinflammatory pathway was involved in the initiation of insulin resistance. TLR4 is the receptor for LPS and plays a critical role in innate immunity. Up binding to TLR4, LPS can activate proinflammatory pathway and induce cytokine expression in a variety of cell types. Han Shi and his colleagues showed that FFAs were capable of utilizing the innate immune receptor TLR4 to induce inflammatory response and insulin resistance in macrophages and adipocytes [28]. We stimulated hepatocytes with PA and induced release of TNF-α and IL-6. Treatment with berberine reduced over-production of TNF-α and IL-6 in hepatocytes, well demonstrating its anti-inflammation potency. Meanwhile, we also observed that berberine inhibited PA-induced ERK1/2activation. ERK is a subfamily of mitogen-activated protein kinases, displays in a wide range of cellular functions, including inflammation. Berberine inhibited

ERK1/2 activation and this action should be responsible for its suppression of inflammation in this study. This anti-inflammatory activity was similar to the data of a recent study reporting berberin inhibited LPS-induced inflammatory response in macrophages [29].

Berberine inhibited the production of TNF-α and IL-6, whether its inhibition contributed to improve insulin resistance, in this study remained to be determined. We then investigated the possible involvement of proinflammatory cytokines in insulin resistance by the use of relative antibodies to neutralize TNF-α and IL-6 respectively. Treatment of anti-IL-6 antibody significantly increased the insulin-mediated glycogen synthesis, which revealed by the increased glycogen content in cells, but the same tendency was not observed in the treatment of anti-TNF-α antibody, and we did not find any synergistic action between TNF-α and IL-6 antibodies. These evidences strongly suggested that IL-6, not TNF-α, was directly responsible for the insulin resistance induced by PA in hepatocytes. This result is in accordance with a publishing report, which showed that IL-6 exerted an inhibitory effect on both insulin receptor signal transduction and downstream insulin action, especially glycogen synthesis [30].

Many studies have investigated the role of TNF-α-induced insulin resistance in cell lines, animal models, and humans. Hotamisligil *et al*. provided evidence that TNF-α increase serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1) and thereby blocked insulin receptor signaling pathway in obese tissue [31]. But the validity of this action in the liver is less certain. In both cell line and animal studies, the effect of TNF-α on hepatic insulin receptor signal transduction is insignificant or absent [32–35]. Interestingly, in this work, we observed that the inhibition of berberine (10 μM) on the production of TNF-α was much stronger than that of IL-6, as the inhibitory rate was about 44% and 25% separately. Although antibody neutralizing assay indicated that TNF-α is not directly involved in the PA-induced insulin resistance, as shown in the glycogen synthesis (Fig. 4), we couldn't simply exclude its possible involvement. Recent evidence showed that some of the observed effects of TNF-α may be mediated by its ability to induce IL-6 and IL-6 receptor expression in tissues such as the liver and muscle [36, 37]. In view of this connection between TNF-α and IL-6, we investigated PA-induced IL-6 production in the presence of anti-TNF-α antibody. The result indicated that TNF-α could effectively induce IL-6 production and then raised the possibility that inhibition of berberine on TNF-α production should be partially responsible for its downregulation of IL-6 production. Based on above evidences, it is reasonable for us to believe that berberine inhibited proinflammatory cytokine production, including both of IL-6 and TNF-α, and this action should be responsible for its beneficial modulation of insulin sensitivity in hepatocytes.

The insulin receptor (IR), a ligand-activated tyrosine kinase, phosphorylates intracellular substrates including IR substrate (IRS) family members and Shc that serve as docking proteins for downstream signaling molecules. IRS-1 plays a critical role in insulin signaling transduction. In target cells, insulin activates IR and subsequently phosphorylates tyrosine residues on intracellular substrates leading downstream signaling cascade. However, phosphorylation of certain serine residues on IRS-1 is an important negative regulation of insulin signaling, as it impedes the normal association of IRS-1 with the insulin receptor. Serine phosphorylation of IRS-1 links with proinflammatory pathways. Some proinflammatory cytokines, including IL-6 and TNF-α have been well reported to be associated with the initiation of insulin resistance by disturbing insulin-mediated tyrosine phosphorylation of IRS-1 [3, 38]. Sodium salicylate is potent inhibitor of inflammation, and effectively improved PA—induced insulin resistance, suggesting that inflammation was involved in the development of insulin resistance. Moreover, sodium salycylate attenuated PA-induced serine phosphorylation of IRS-1 (S1101), indicating that serine phosphorylation of IRS-1 was a key event linking insulin resistance with inflammatory response. Ser/Thr phosphorylation reduces IRS-1's ability to undergo tyrosine phosphorylation by the insulin receptor kinase and might serve as a physiological negative feedback control mechanism to turn off insulin signaling [39, 40]. Berberine attenuated PA-induced serine phosphorylation of IRS-1 and subsequently restored insulin-mediated tyrosine phosphorylation of IRS-1, well demonstrating its positive modulation against inflammatory impact. IRS-2 is also an essential component in insulin signaling in liver. Edward Park *et al*. demonstrated that salicylate decreased free fatty acid-induced serine phosphorylation of IRS-1 (Ser 307) and IRS-2 (Ser 233) in liver, strongly suggesting that serine phosphorylation of IRS-2 was also associated with insulin resistance inflammation involved [41]. Shu Shi *et al*. have reported that berberine could upregulate IRS-2 mRNA and protein expression in the liver from 2-type diabetic rats [42]. We have investigated the modulation of berberine on IRS-1phosphorylation, but whether it affects IRS-2 remains to be determined.

Verifying the effect of berberine on IRS-2 phosphorylation should be beneficial for us to get further information to confirm its improvement on insulin signaling by its anti-inflammatory activity.

Berberine reversed inflammation-induced changes of IRS-1 phosphorylation and this action should be directly responsible for the downstream Akt activation. ERK1/2-specific inhibitor PD98059, NF-κB-specific inhibitor BAY11-7082 and sodium salicylate also demonstrating positive phosphorylation modification of IRS-1 and downstream Akt, suggesting inhibition of ERK/NF-κB dependent inflammatory pathway should be beneficial for the amelioration of impaired insulin signaling progression. In the present study, we demonstrated that berberine modulated insulin signal transduction by an IRS-1/Akt-dependent pathway, and the resulting improvement should be responsible for its positive modulation of insulin-mediated glycogen synthesis and lipolysis inhibition in the presence of PA. PI3K/Akt activation is also essential for the insulin stimulation of glucose transporter-4 (Glut-4) translocation [43]. Kim SH *et al*. reported berberine enhanced Glut-1 protein expression and stimulate Glut-1- mediated glucose uptake in 3T3–L1 adipocytes [44], but whether berberine modulates Gluts activity in hepotacytes still needs to be clarified.

In conclusion, stimulation of PA evoked inflammatory response and impaired insulin signaling transduction, resulting in insulin resistance in hepatocytes. Berberine inhibited proinflammatory cytokine production and effectively ameliorated insulin resistance. Berberine inhibited inflammation-related phosphorylation of IRS-1 at serine residues and effectively improved insulin signaling transduction. These results bring us further information about its anti-inflammatory activity involved in the treatment of metabolic disorders associated with insulin resistance.

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