Research Paper

NO-1886 ameliorates glycogen metabolism in insulin-resistant HepG2 cells by GSK-3β signalling

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Abstract

Objectives The aim of the study was to elucidate the possible role and mechanism of NO-1886 (ibrolipim, a lipoprotein lipase activator) in ameliorating insulin resistance induced by high palmitate.

Methods HepG2 cells were cultured in RPMI 1640 medium and were treated with palmitate to induce insulin resistance. Free fatty acids (FFAs), glucose, glycogen, cell viability and mRNA and protein levels were analysed separately.

Key findings We found that HepG2 cells treated with 0.5 mM palmitate for 48 h led to a significant decrease of insulin-induced glucose consumption (from 2.89 ± 0.85 mM in the control to 0.57 ± 0.44 mM in palmitate). Insulin resistance (IR) of HepG2 cells was induced by 0.5 mM palmitate for 48 h. NO-1886 stimulated glucose consumption, glycogen synthesis and FFA absorption in insulin-resistant HepG2 cells. Maximum stimulation effects were observed with 10 μM NO-1886 for 24 h. Compared with the dimethyl sulfoxide-treated group, 2.5 μM NO-1886 or higher could induce the mRNA expression of lipoprotein lipase. Meanwhile, NO-1886 increased the protein content of P-GSK-3βser[9] and decreased the protein level of GSK-3β in insulin-resistant HepG2 cells, but NO-1886 didn't change the protein levels of PI3-Kp85 and Akt2.

Conclusion Lipoprotein lipase activator NO-1886 could increase glycogen synthesis in HepG2 cells and could ameliorate the insulin resistance, which was associated with GSK-3 signalling.

Introduction

Recent studies indicated that high levels of free fatty acids (FFAs) might be a main cause of insulin resistance. Palmitic acid, one of the most abundant FFAs, representing about 30% of the total FFA in human plasma, has been shown to induce insulin resistance in cultured cells.[1] However, the molecular mechanism by which FFAs contribute to the inhibition of insulin action and glycogen synthesis is still not completely understood. A number of studies have shown deleterious effects of FFAs, such as promotion of triglyceride accumulation,[2] activation of pro-inflammatory responses[2] and protein kinase C,[3] and impairment of glucose metabolism.[4]

Glucose homoeostasis depends on insulin responsiveness in target tissues, especially muscle and liver.[5] Insulin is the most potent hormone and is essential for appropriate tissue development, growth, and maintenance of whole-body glucose homoeostasis. The acute action of insulin results in stimulation of glucose uptake and glycogen synthesis.[6,7] Although the precise mechanisms by which insulin activates glycogen synthase remain unknown, numerous studies have suggested that the activation of phosphatidylinositol-3 kinase (PI3-K) by insulin may be a critical step.[8–10]

The latest developments in the study of the insulin signalling cascade show that glucose metabolism stimulated by insulin is mainly involved in PI3-K.[11,12] Activated protein kinase B (PKB) phosphorylates and inactivates glycogen synthase kinase-3 (GSK-3) at Ser[9/21], which prevents the phosphorylation of the GSK-3 substrate glycogen synthase. The end result of this phosphorylation cascade, initiated by insulin, is the dephosphorylation of glycogen synthase, leading to the activation of the glycogen synthase.[13]

GSK-3 is a serine/threonine kinase with an important role in the regulation of glycogen synthesis, protein synthesis and
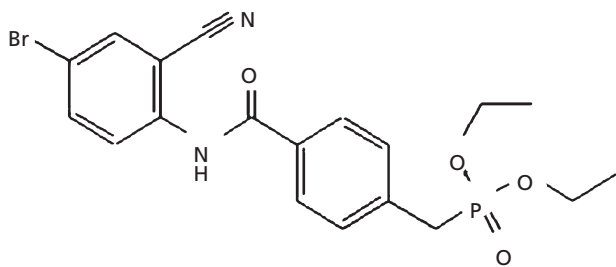
Figure 1 The chemical structure of ibrolipim (NO-1886), [4-(4-bromo-2-cyano-phenylcarbamoyl)-benzyl]-phosphonicacid diethyl ester, CAS 133208-93-2, Lot.No.C00C99H74SM. NO-1886 was synthesized in the new drug research laboratory of Otsuka Pharmaceutical Factory Inc. (Tokushima, Japan).

gene transcription in various cell types. An emerging body of evidence has implicated GSK-3 in the multifactorial aetiology of insulin resistance in obese animal models and in obese human type 2 diabetic subjects. Overexpression and overactivity of GSK-3 in skeletal muscle of rodent models of obesity and in obese type 2 diabetic humans are associated with an impaired ability of insulin to activate glucose disposal and glycogen synthase. There have been recent reports that selective inhibition of GSK-3 in insulin-resistant skeletal muscle causes improvements in insulin-stimulated glucose transport activity that are likely due to enhanced post-insulin receptor insulin signalling and glucose transporter type 4 (GLUT-4) translocation.[10]

On the other hand, it has been reported that the novel compound NO-1886 ([4-(4-bromo-2-cyanophenyl-carbamoyl) benzyl]-phosphoric acid diethyl ester, Figure 1) increases lipoprotein lipase (LPL) activity, resulting in a reduction in plasma triglycerides and concomitant increase in high-density lipoprotein cholesterol levels in experimental animals, including rats, hamsters, rabbits and minipigs.[14] In addition, NO-1886 decreases triglyceride and cholesterol concentrations and increases glycogen content in the liver of high-fat-fed rats.[15]

In view of these reports, we propose palmitate treatment can induce insulin resistance in cells of hepatic origin, and used HepG2, highly differentiated human derived hepatoma cells that represent a well-characterized model for metabolic studies, to establish insulin resistance model.[16] The aims of this study were to ascertain the relationship between lipid and glycogen content in insulin-resistant HepG2 cells induced by a high concentration of palmitate, and to evaluate the effects of the LPL activator NO-1886 on lipid and glycogen contents.

Materials and Methods

Agents

Ibrolipim (NO-1886) was synthesized in the new drug research laboratory of Otsuka Pharmaceutical Factory Inc.

(Tokushima, Japan). RPMI-1640 medium and fetal bovine serum were purchased from Gibco/BRL(Gibco BRL, Grant Island, USA). Glucose assay kit and free fatty acid quantification kit were obtained from BioVision, Inc. (San Francisco, USA). RevertAid First Strand cDNA Synthesis Kit was purchased from Fermentas Inc. (Ontario, Canada). Antibodies against PI3Kp85, Akt2 and GSK-3β were purchased from Biosynthesis Biotechnology Co. Ltd (Beijing, China). Antibody against P-GSK-3βser[9] was purchased from cell signaling technology (Danvers, USA). Enhanced chemiluminescence (ECL) detection kit was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, USA).

Palmitate solution preparation

The palmitate solution used for incubation with the HepG2 cells was prepared by conjugating palmitic acid (P5585; Sigma-Aldrich, St Louis, USA) with fatty acid-free bovine serum albumin (BSA) according to the method reported by Cousin.[17] Control group cells were incubated with identical concentration of fatty acid-free BSA without palmitate.

Cell culture

HepG2 cells were obtained from Soochow University (Suzhou, China). These cells were cultured in RPMI 1640 medium (Gibco BRL, Grant Island, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 units/ml penicillin and 100 μg/ml streptomycin, at 37°C in a humidified incubator containing 5% CO_2. In all experiments the medium was changed 24 h after plating, unless otherwise stated. Two days after seeding, the maintenance medium was removed and the monolayers were maintained with RPMI-1640 containing 1% FBS for 12 h. If the effect of fatty acid was to be assessed, the medium was supplemented with 0.5 mм palmitate bound to 1% FBS. Then, lipoprotein lipase activator NO-1886 was added as indicated for each experiments. At the end of incubation, the cells were treated with RPMI 1640 medium containing 10% FBS and 1×10^{-4} mм insulin for 1 h. The medium was centrifuged at 5000 rpm for 10 min at 4°C to removed small amounts of cell and debris. The supernatant fraction was concentrated and stored at −20°C. Medium glucose content was assayed by glucose assay kit to identified insulin resistance in HepG2 cells.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay

Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl) K2, 5-diphenyl tetrazolium bromide (MTT) assay. Briefly, cells seeded on 96-well microplates at 2000 cells/well were incubated with the test compounds for indicated period. After exposure, 20 μl of the MTT solution (5 mg/ml) was

added into each well, and the plates were incubated for an additional 4 h. After removing the medium, dimethyl sulfoxide (DMSO, 150 μl) was added into each well. The plates were read on an enzyme immunoassay analyser (ELx-800; Bio-Tek Inc., Winooski, USA) at 490 nm.

Free fatty acids and glucose assay

Base on the method reported by Yin,[18] glucose levels in the medium were detected by a commercially available kit (K606-100; BioVision, Inc, San Francisco, USA). The amount of glucose consumed was calculated by measuring glucose concentrations of blank wells and subtracting the remaining glucose in cell-plated wells. The supernatant fraction was used for detecting the FFAs with free fatty acid quantification kit (K612-100; BioVision, Inc, San Francisco, USA) according to the manufacturer's instructions. First, twenty microlitres of the supernatant fraction was added to a 96-well plate, then 2 μl ACS Reagent was added into all the standard and sample wells, mixed well, and incubated at 37°C for 30 min. Subsequently, 50 μl of the Reaction Mix was added to each well containing the Standard or test samples, and the the reaction was incubated for 30 min at 37°C. OD at 570 nm was measured for colorimetric assay in a micro-plate reader, and the FFA content was calculated according to the standard curve.

Glycogen synthesis assays

Anthrone colorimetric assay

The accumulation of glycogen in intact cells was determined by an adaptation of the method of Anthrone colorimetric assay kit (Institute of Jiancheng biological and engineering, Nanjing, China). After serum deprivation and pretreatment with or without palmitate, cells in 6-well plates were incubated in the presence or absence of NO-1886 for an additional 24 h. Subsequently, cells were incubated with insulin for 30 min at 37°C. Cells were then washed three times with ice-cold phosphate-buffered saline (PBS) and were collected into Eppendorf tubes. Glycogen detection liquid was added into the tubes for 20 min according to the assay instruction, subsequently 2 ml colour liquid was added for 5 min; the absorbance measured at 620 nm was used to calculate the glycogen content.

Periodic acid Schiff staining

HepG2 cells were seeded in 6-well plates at a density of 1.0×10^6 cells/ml. After treatment the cells were fixed with 10% neutral buffered formalin for 45 min. According to the protocol provided with the detection kit, the cells were washed twice with PBS for 2 min, incubated with periodic acid for 10 min, washed 3 times with de-ionized water for 3 min, incubated with Schiff for 20 min in the dark, treated with sodium metabisulfite for 1 min, and washed with distilled water for 10 min.

Reverse transcription-polymerase chain reaction

For reverse transcription-polymerase chain reaction (RT-PCR), total RNA was extracted from the cells using Trizol reagent (Gibco BRL) according to the manufacturer's protocol. Three micrograms of total RNA were used for reverse transcription in a total volume of 20 μl with the RevertAid First Strand cDNA synthesis Kit (Fermentas, Vilnius, Lithuania). Volumes of 2 μl cDNA were subsequently amplified in a total volume of 20 μl using the MasterMix PCR kit (TIANGEN, Beijing, China) following conditions recommended by the manufacturer. The sense and antisense primer sequences and PCR conditions are listed in Table 1. PCR products were separated on a 1.5% agarose gel and stained by ethidium bromide. These data were acquired with Alpha Imager 2200 software.

Western blot analysis

The protein levels of PI3-Kp85, Akt2, GSK-3β and p-GSK-3βser^9 were measured by Western blot analysis. Briefly, confluent HepG2 cells were rendered quiescent by incubation for 24 h in serum-free medium. After stimulation with palmitate for 48 h, the cells were treated with NO-1886 for 24 h. The cells were rapidly washed with ice-cold PBS and lysed in sample buffer. Lysates were boiled for 5 min and equal amounts of total cell protein were separated with SDS-PAGE under denaturing conditions. After the proteins were electrotransferred to nitrocellulose, the membranes were incubated overnight with a 1 : 1000 dilution of antibody recognizing PI3-Kp85, Akt2, GSK-3β

Table 1 Reverse transcriptase-polymerase chain reaction (RT-PCR) information: primer sequences and PCR conditions

genes	Primer sequences	Annealing	Products bp	Genebank number
LPL	sense: 5′-GAGATTTCTCTGTATGGCACC-3′ anti-sense: 5′-CTGCAAATGAGACACTTTCTC-3′	56°C	276 bp	NM 000237
GAPDH	sense: 5′-TCACCATCTTCCAGGAGCGAG-3′ anti-sense: 5′-TGTCGCTGTTGAAGTCAGAG-3′	60°C	697 bp	NM 002046

and p-GSK-3βser[9]. Bands of interest were visualized with enhanced chemiluminescence using an ECL detection kit (Santa Cruz Biotechnology, Inc., Santa Cruz, USA), and resulting digital images were quantified using Alpha Imager 2200 software.

Statistical analysis

All data were presented as mean ± SD, and statistical analysis was performed with one-way analysis of variance followed by

LSD and Dunnett's T3 test using SPSS 12.0. $P < 0.05$ was deemed statistically significant in all experiments.

Results

The effect of palmitate on cell viability

To determine the effect of palmitate on HepG2 cells, we first performed an MTT assay to measure cell viability. As shown in Figure 2, compared with control, exposure of HepG2 cells to 1.5 mM palmitate for 72 h resulted in a significant decrease of cell viability, to approximately 75.7%, while the cell viability showed no difference between control and 0.5 mM palmitate group.

Induction of insulin resistance induced by high palmitate

To develop a model of insulin resistance *in vitro*, HepG2 cells were incubated in serum-free RPMI-1640 containing different concentrations of palmitate (0, 0.1 mM, 0.25 mM, 0.5 mM) for 24 h or 0.5 mM palmitate for different times (12 h, 24 h, 48 h, 60 h). After palmitate treatment, the medium was replaced with 5 ml serum-free RPMI-1640 and incubated for 12 h, and then the cells were stimulated with 1×10^{-4} mM insulin for 1 h. The glucose consumption levels showed no difference between control group and 0.1 mM palmitate group, but there were a significant decrease in 0.25 mM palmitate and 0.5 mM palmitate, compared with control group (Figure 3a). The glucose consumption levels showed a significant decrease in the 24 h, 48 h and 60 h groups, compared with control group (Figure 3b); the greatest decrease was observed in the 48 h group.
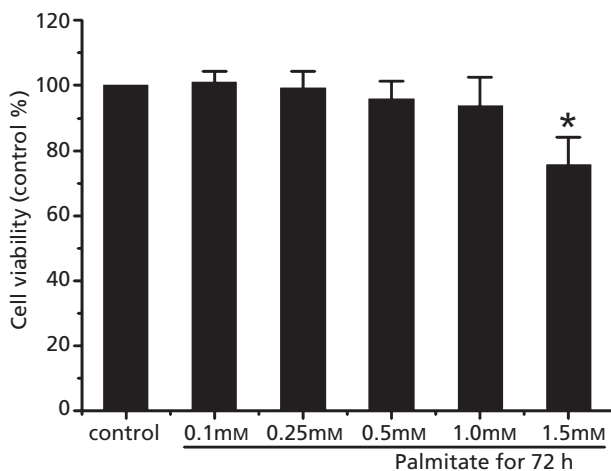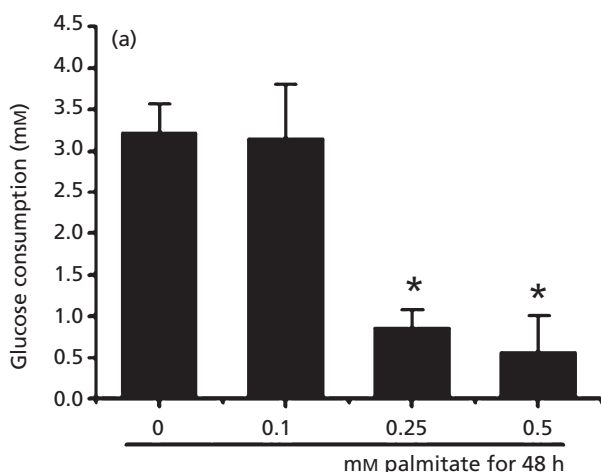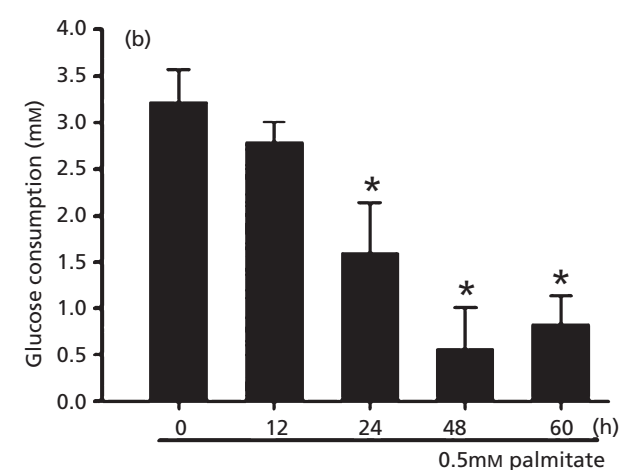

Figure 2 The effect of palmitate on HepG2 cells viability. The HepG2 cells were treated with different concentration palmitate for 72 h. The cell viability showed a significant decrease in the 1.5 mM group. Values represent the means ± SD, $n = 5$. *$P < 0.05$, compared with control group.

Figure 3 Induction of insulin resistance induced by high palmitate. (a) Dose-dependent effect of palmitate on glucose consumption in HepG2 cells. (b) Time-dependent effect of 10 μM palmitate on glucose consumption in HepG2 cells. Values represent the means ± SD, $n = 5$. *$P < 0.05$, compared with control group.

NO-1886 treatment increased glucose consumption and glycogen synthesis, and decreased free fatty acid content in HepG2 cells with insulin resistance induced by palmitate

Insulin-resistant HepG2 cells were treated with different concentrations of NO-1886 for 24 h or 10 mM NO-1886 for different times, and then the cells were stimulated with 1×10^{-4} mM insulin for 1 h. The glucose, FFA and glycogen were determined, respectively. The results are shown in Tables 2 and 3. The glycogen content and glucose consumption were significantly increased by 5 μM, 10 μM and 25 μM NO-1886 treatment for 24 h. In addition, FFA content was significantly decreased in 5 μM, 10 μM and 25 μM NO-1886 groups, compared with DMSO group. As shown in Table 3, compared with DMSO group, the increase of glucose consumption and glycogen, and the decrease of FFA content

showed a significant difference in 12 h, 24 h and 48 h groups. Figure 4 showed that NO-1886 treatment increased the glycogen deposition in insulin-resistant HepG2 cells.

Effect of NO-1886 on the lipoprotein lipase mRNA expression

Using RT-PCR, we examined the time course and concentration dependence effects of NO-1886 on LPL mRNA levels. Figure 5a and 5b showed the mRNA levels of LPL in HepG2 cells after 24 h exposure to NO-1886 at different concentrations (1.0, 2.5, 5, 10, 25 μM). The mRNA levels of LPL were gradually increased in a concentration-dependent manner, compared with DMSO group. Figure 5c and 5d showed the time course of changes in the expression levels of LPL mRNA after exposure to moderate concentrations of NO-1886 (10 μM). Compared with DMSO group, LPL mRNA significantly increased in the 16 h and 24 h groups.

Table 2   Effect of NO-1886 on glucose consumption, free fatty acid and glycogen content in insulin-resistant HepG2 cells

Concentration (μM)	Glucose consumption (mM)	Free fatty acid (mM)	Glycogen (mg/g)
DMSO	0.62 ± 0.50	1.07 ± 0.24	0.22 ± 0.09
2.5	1.44 ± 0.29	0.70 ± 0.25	0.28 ± 0.11*
5	1.90 ± 0.43*	0.57 ± 0.17*	0.36 ± 0.07*
10	2.25 ± 0.69*	0.41 ± 0.16*	0.42 ± 0.11*
25	2.23 ± 0.37*	0.40 ± 0.09*	0.43 ± 0.13*

The insulin-resistant HepG2 cells were treated with different concentrations of NO-1886 for 24 h, and then stimulated with 1×10^{-4} mM insulin for 1 h. Values represent the means ± SD, $n = 5$. *$P < 0.05$, compared with DMSO group.

Table 3   Effect of 10 μM NO-1886 on glucose consumption, free fatty acid and glycogen content in insulin-resistant HepG2 cells

Time(h)	Glucose consumption (mM)	Free fatty acid (mM)	Glycogen (mg/g)
0	0.51 ± 0.19	1.10 ± 0.34	0.24 ± 0.06
6	1.08 ± 0.19*	0.80 ± 0.28	0.30 ± 0.10
12	2.23 ± 0.20*	0.58 ± 0.09*	0.37 ± 0.07*
24	2.25 ± 0.69*	0.41 ± 0.16*	0.42 ± 0.11*
48	2.52 ± 0.21*	0.33 ± 0.23*	0.40 ± 0.10*

The insulin-resistant HepG2 cells were treated with NO-1886 (10 μM) for different times, and then stimulated with 1×10^{-4} mM insulin for 1 h. Values represent the means ± SD, $n = 5$. *$P < 0.05$, compared with DMSO group.
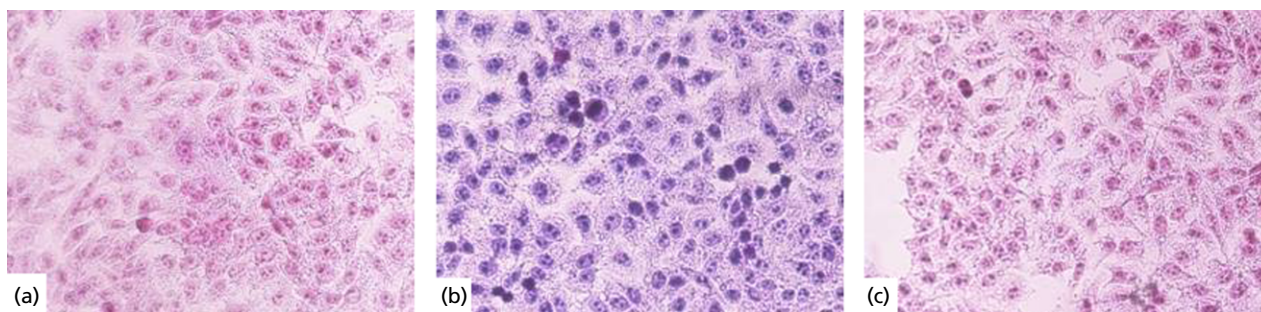

Figure 4   Effect of NO-1886 on glycogen deposition in HepG2 cells with insulin resistance. Periodic acid Schiff (PAS) staining of HepG2 cells. (a) Control group. (b) Palmitate + DMSO group. (c) Palmitate + 10 μM NO-1886 group.
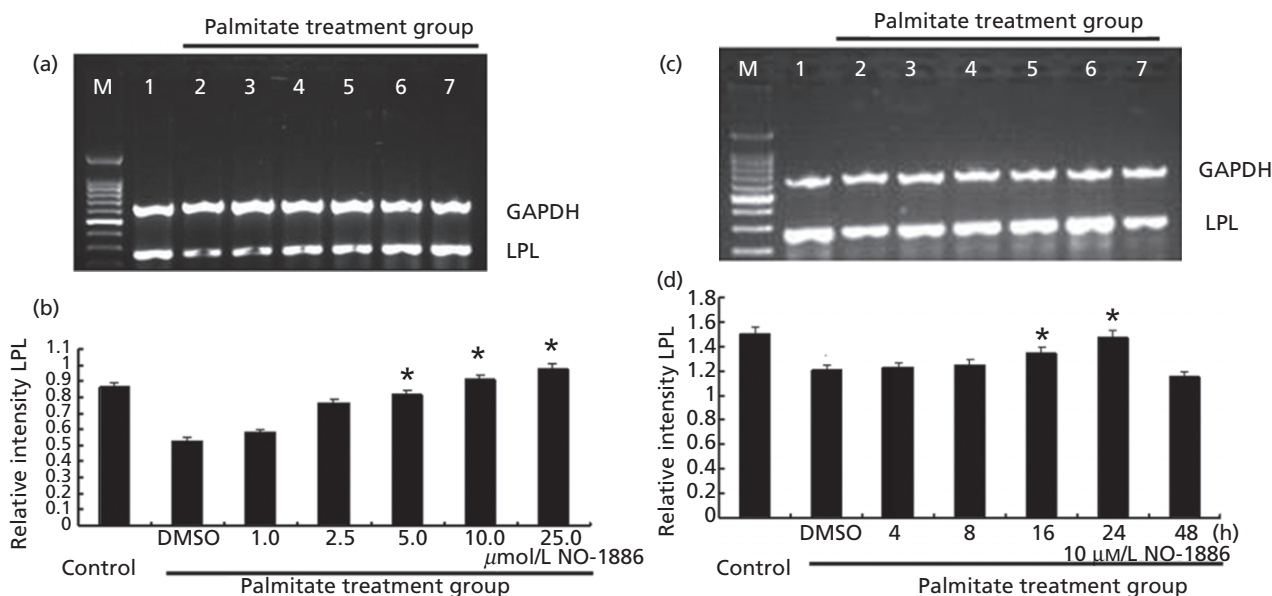
Figure 5 NO-1886 induced gene expression of LPL in insulin-resistant HepG2 cells. (a, b) Dose dependence. 1, Control group; 2, palmitate + DMSO group; 3, palmitate + 1 μM NO-1886 group; 4, palmitate + 2.5 μM NO-1886 group; 5, palmitate + 5 μM NO-1886 group; 6, palmitate + 10 μM NO-1886 group; 7, palmitate + 25 μM NO-1886 group. (c, d) Time dependence. 1, Control group; 2, palmitate + DMSO; 3 palmitate + 10 μM NO-1886 for 4 h group; 4, palmitate + 10 μM NO-1886 for 8 h group; 5 palmitate + 10 μM NO-1886 for 16 h group; 6 palmitate + 10 μM NO-1886 for 24 h group; 7, palmitate + 10 μM NO-1886 for 48 h group. Values represent the means ± SD, *n* = 5. *$P < 0.05$, compared with DMSO group.

Effect of NO-1886 on the protein expression of PI3K signal pathway

Insulin-resistant HepG2 cells were incubated with different concentrations of NO-1886 (2.5, 5.0, 10 and 25 μM). Protein levels of GSK-3β showed a significant decrease in the 10 and 25 μM NO-1886 groups, compared with the DMSO group (Figure 6a and 6b). Protein levels of Akt and PI3-kp85 showed no significant difference between the NO-1886 groups and DMSO group. In addition, as shown in Figure 6c and 6d, the expression of P-GSK-3βser[9] significantly increased in the 10 and 25 μM NO-1886 groups, compared with the DMSO group.

Discussion

The mechanism by which FFAs induce peripheral insulin resistance remains unknown.[19] Increased FFA availability results in decreased rates of glucose oxidation and inhibits liver and muscle glycogen deposition stimulated by insulin and whole body glucose uptake.[8] Liver plays a critical role in glycogen metabolism.[20] In diabetes, hyperglycaemia is partly caused by a decrease in glycogen synthesis and increase in glucogenesis. Recent studies have shown that stimulation of hepatic glycogen deposition might be the chief basis for the use of antidiabetic drugs.[21,22] In this study, we used a high concentration of palmitate (0.5 mM) to induce insulin resistance in HepG2 cells. The glucose consumption of HepG2

cells was suppressed by treatment with palmitate. With increasing palmitate concentration, the glucose consumption significantly decreased. NO-1886 could increase the glycogen synthesis initiated by insulin in insulin-resistant HepG2 cells.

HepG2 cells have been used to study enzyme regulation, insulin receptor phosphorylation and hormone-sensitive tyrosine kinase activity and second messenger generation.[23–25] Moreover, these cells are very sensitive to insulin concentrations as low as 1.0 pM and have been shown to regulate enzyme activity.[26–28] In this study, we investigated the regulation of glycogen metabolism in insulin-resistant HepG2 cells by NO-1886, a lipoprotein lipase activator. In HepG2 cells, we found NO-1886 could improve the decreased glycogen deposition induced by palmitate and could stimulate glucose consumption in insulin-resistant HepG2 cells. In addition, NO-1886 decreased the FFA content in insulin-resistant HepG2 cells induced by high palmitate, which could be related to NO-1886 accelerating the mRNA expression of fatty acid oxidation-related enzymes.[29]

Signal transduction mediated by PI3-K/Akt is one of the signalling cascades known to play an important role in the development and progression of several types of cancer and metabolic disorders.[30–32] Acute and chronic inhibition of GSK-3 by either insulin or cell permeable chemical inhibitors can result in activation of glycogen synthase activity and glycogen deposition in a variety of cell and tissues.[33,34] GSK-3 expression and activity are elevated in diabetic rodents and
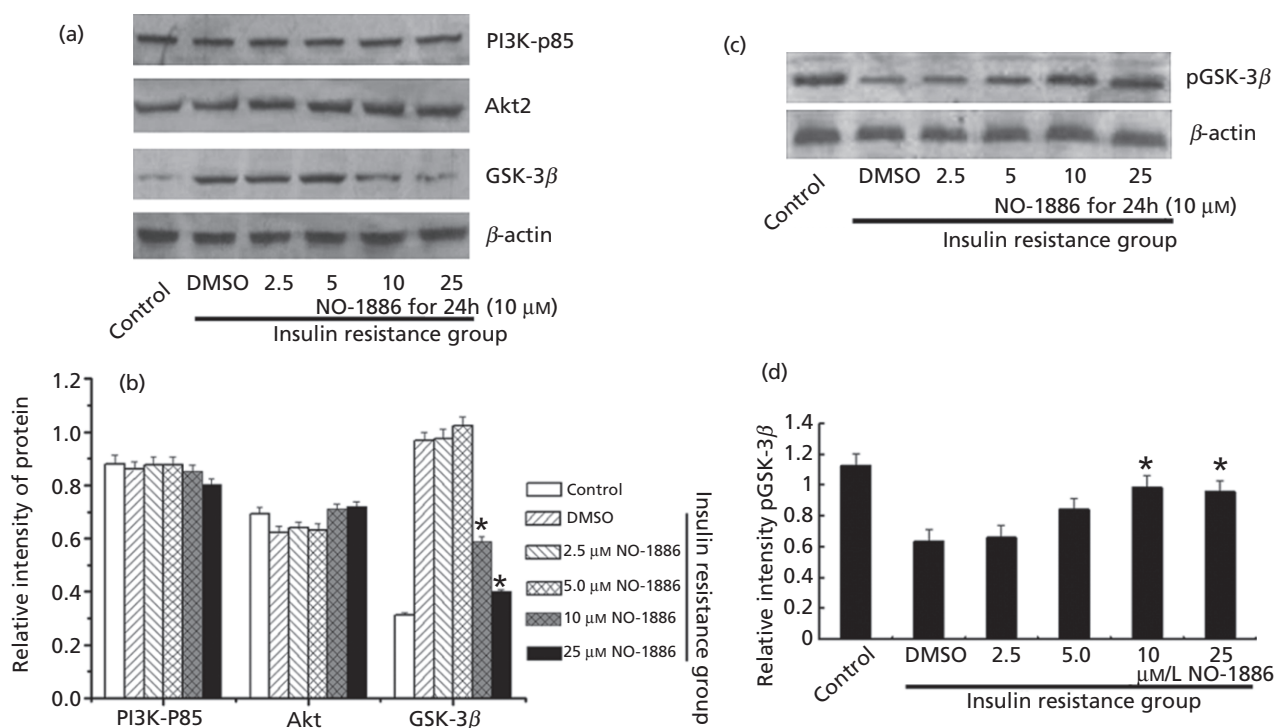
Figure 6 Effect of NO-1886 on protein expression of PI3Kp85, Akt2 and GSK-3β in insulin-resistant HepG2 cells. Insulin-resistant HepG2 cells were incubated with different concentrations of NO-1886 for 24 h, or 10 μM NO-1886 for different times, and then the cells were treated with 1×10^{-4} mM insulin for 1 h. (a, c) Lane 1, control group; lane 2, 0.1%DMSO group; lane 3, 2.5 μM NO-1886 group; lane 4, 5 μM NO-1886 group; lane 5, 10 μM NO-1886 group; lane 6, 25 μM NO-1886 group. (b) Semiquantified data for the levels of PI3Kp85, Akt2 and GSK-3β proteins. (d) Semiquantified data for the levels of pGSK-3β proteins. Values represent the means $\pm$ SD, $n = 5$. *$P < 0.05$, compared with DMSO group.

humans, with consequential suppression of glycogen synthase activity and severely impaired glycogen deposition.[35,36] In this study, we observed that palmitate significantly increased the protein expression of GSK-3β stimulated by insulin and induced insulin resistance in HepG2 cells.

It has previously been reported that NO-1886 increases the mRNA and protein levels of LPL in tissues, and produces a reduction in plasma triglyceride levels with concomitant elevation of HDL-c levels in animals with lipid disorders.[37–39] Kusunoki also reported that NO-1886 inhibited fat accumulation and reduced insulin resistance in obesity diabetes type 2 rat model induced by high fat.[15] Yin discovered that NO-1886 also lowered plasma glucose in rabbit and minipig diabetes mellitus induced by high fat/high sucrose diet.[40–42] Kusunoki reported NO-1886 decreased lipid content and increased glycogen content in liver of high-fat-fed rats, an insulin-resistant animal model, by improving insulin resistance.[43]

In this study, we observed that NO-1886 decreased FFA levels, and increased glucose consumption and glycogen deposition in HepG2 cells in which insulin resistance had been induced by high-concentration palmitate treatment. However, the mechanisms of NO-1886 on anti-insulin resistance or the involved glycogen metabolism remained unclear. To clarify the mechanism, we studied the effects of NO-1886 on the expression of PI3K/Akt/GSK-3 signalling pathway in insulin-resistant HepG2 cells. High concentration of palmitate increased the protein levels of GSK-3β and induced insulin resistance in HepG2 cells, while the protein levels of PI3K and Akt showed no significant difference between HepG2 cells and insulin-resistant HepG2 cells induced by palmitate. NO-1886 treatment significantly decreased protein expression of GSK-3β and pGSK-3β initiated by insulin in insulin-resistant HepG2 cells. However, compared with the DMSO group, NO-1886 treatment did not change the protein levels of PI3-K and Akt in insulin-resistant HepG2 cells.

These results showed that expression of GSK-3β in HepG2 cells was suppressed by high-concentration palmitate. NO-1886 treatment decreased the protein expression of GSK-3β, and increased the levels of pGSK-3β in insulin-resistant HepG2 cells. These results probably explained the reason that NO-1886 could accelerate glycogen synthesis, increase glucose consumption and decrease FFA content. The above results showed that NO-1886 could ameliorate insulin resistance.

In summary, this study provides strong biochemical evidence that the effect of NO-1886 on glycogen synthesis in HepG2 cells depends on regulation of GSK-3β. Despite the fact that the mechanism by which NO-1886 stimulates GSK-3β remains controversial, this study demonstrates that GSK-3β may be the principal mediator of the effects of NO-1886 on glycogen synthesis and glucose consumption in insulin-resistant HepG2 cells. Therefore, lipoprotein lipase activator NO-1886 could increase glycogen synthesis in HepG2 cells and reverse the insulin resistance, which was associated with GSK-3 signalling.

Declarations

Conflict of interest.

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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