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Berberine improves free-fatty-acid–induced insulin resistance in L6 myotubes through inhibiting peroxisome proliferator–activated receptor γ and fatty acid transferase expressions

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Abstract

The plant alkaloid berberine (BBR) has been reported to have antidiabetic effect in humans and animals. However, the mechanism of action is not well understood. The present study was conducted to determine the effect and mechanism of action of BBR on the free-fatty-acid (FFA)–induced insulin resistance in muscle cells. The FFA-induced insulin-resistant cell model was established in L6 myotubes by treating them with 250 μmol/L of palmitic acid. The inclusion of FFA in the medium increased peroxisome proliferator–activated receptor γ (PPARγ) and fatty acid transferase (FAT/CD36) expressions by 26% and 50% and decreased glucose consumption by 43% and insulin-mediated glucose uptake by 63%, respectively. Berberine treatment increased the glucose consumption and insulin-stimulated glucose uptake in normal cells and improved glucose uptake in the FFA-induced insulin-resistant cells. The improved glucose uptake by BBR was accompanied with a dose-dependent decrease in PPARγ and FAT/CD36 protein expressions. In insulin-resistant myotubes, BBR (5 μmol/L) decreased PPARγ and FAT/CD36 proteins by 31% and 24%, whereas PPARγ antagonist GW9662 reduced both proteins by 56% and 46%, respectively. In contrast, PPARγ agonist rosiglitazone increased the expression of PPARγ and FAT/CD36 by 34% and 21%, respectively. Our results suggest that BBR improves the FFA-induced insulin resistance in myotubes through inhibiting fatty acid uptake at least in part by reducing PPARγ and FAT/CD36 expressions.

1. Introduction

The common feature of obesity, type 2 diabetes mellitus, and metabolic syndrome is insulin resistance [1], which is characterized by a reduced ability of insulin to regulate glucose transport and utilization in peripheral tissues including liver, skeletal muscle, and adipose tissue [2,3]. A large body of evidence suggests that insulin resistance is closely associated with elevated levels of free fatty acids (FFA) in the circulating system [4-6]. In type 2 diabetes mellitus patients, a decreased capacity of fatty acid oxidation and elevated levels of circulating FFA are observed [7]. In addition, several studies have shown that a lipid infusion or high-fat feeding promotes insulin resistance in both rodents and humans [8-10]. Accordingly, the role of FFA in the pathogenesis of insulin resistance has received increasing attention in research and clinical practice, as well as in product development targeting the prevention and treatment of type 2 diabetes mellitus.

It is reported that peroxisome proliferator–activated receptors (PPARs) regulate gene expressions involved in fat metabolism and energy homeostasis [11]. Peroxisome proliferator–activated receptor α and PPARγ are involved in the regulation of the expression of genes related to β-oxidation and adipogenic processes. Thus, a number of synthetic PPAR ligands have been developed; and some are used clinically to manage diabetes [12,13]. For instance, hypolipidemic fibric acids are PPARα activators; and hypoglycemic thiazolidinediones (TZDs) are PPARγ activators [11]. They mediate the expression and activity of fatty acid translocase (FAT) (also known as CD36, a cellular

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"scavenger" receptor for atherogenic low-density lipoprotein) and carnitine palmitoyltransferase I (CPT-I) [14-16]. The expression and activity of FAT/CD36 change in the same direction with the rates of long-chain fatty acid (LCFA) transport and metabolism [17-20], including CPT-I–regulated LCFA transport and oxidation in mitochondria [16]. These findings suggest that PPARα and PPARγ signaling pathways play important roles in FFA uptake and oxidation, which are important in the onset and progression of type 2 diabetes mellitus.

The plant alkaloid berberine (BBR) is present in many medicinal plants including Chinese golden thread (*Coptis chinensis*), a species of golden thread native to China, and goldenseal (*Hydrastis canadensis*) in North America. Recent studies have shown that dietary supplementation of BBR improves the conditions of diabetes [21,22] by increasing insulin secretion [23,24] and glucose uptake [23,25,26]. It is suggested that BBR improves insulin sensitivity through different pathways [25,27,28], one of which is the PPAR signaling pathway.

As mentioned earlier, FFA is an important factor in the onset and progression of insulin resistance and type 2 diabetes mellitus. However, it is unknown whether and how BBR affects FFA-induced insulin resistance in skeletal muscle. Therefore, in the present study, we examined the effect of BBR on glucose and fatty acid transport in L6 skeletal muscle cells, which have been widely used to study in vitro insulin resistance [29-31]. We have also studied the mechanism of action by looking into the regulatory effect of BBR on PPARγ and PPARα expressions and the downstream targets, FAT/CD36 and CPT-I, which are important in the regulation of fatty acid transport, uptake, and oxidation in mitochondria.

2. Materials and methods

2.1. Reagents

Dulbecco modified Eagle medium (DMEM) was purchased from GIBCO (Grand Island, NY). Fetal bovine serum was purchased from the Institutes of Biotechnology, Chinese Academy of Sciences (Tianjin, China). Horse serum was bought from Hyclone (Logan, UT). Berberine chloride was purchased from Sigma-Aldrich (Mississauga, Ontario, Canada). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), palmitic acid, cytochalasin B, and GW9662 were purchased from Sigma-Aldrich (St Louis, MO). Rosiglitazone was purchased from Cayman Chemical (Ann Arbor, MI). 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxyglucose probes were obtained from Invitrogen (Eugene, OR). Rabbit polyclonal antibodies specific to β-actin (sc-1616-R) and PPARγ (sc-7196) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal antibodies specific to CD36 were purchased from Cayman Chemical. Mouse polyclonal antibody specific to PPARα (ab-24509) was obtained from Abcam (Cambridge, United Kingdom). Goat polyclonal antibodies specific to CPT-I, goat anti-rabbit (sc-2007), and rabbit anti-goat (sc-2768) secondary antibodies were purchased from Santa Cruz Biotechnology.

2.2. Cell culture and treatment

L6 myoblast cell line was purchased from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The cells were maintained in DMEM with 100 U/mL penicillin and 100 μg/mL streptomycin supplemented with 10% (vol/vol) fetal bovine serum. The cells were cultured in an incubator with 5% CO_2 at 37°C. To induce differentiation, L6 cells were transferred to DMEM containing 2% horse serum for 8 to 10 days until the myoblasts reached approximately 80% to 90% confluence. Differentiated L6 myotubes were cultured for 4 hours in serum-free DMEM supplemented with 0.5% bovine serum albumin (BSA). The cells were then treated for 24 hours with 250 μmol/L palmitic acid to induce insulin resistance [32-34]. The cells were subjected to the treatment of different concentrations of BBR or the combination of BBR and palmitic acid. Berberine was dissolved in dimethyl sulfoxide (DMSO), and palmitic acid was provided in a form of fatty acid–albumin complex [29,35]. The control cells were treated with equal amount of the vehicle. The PPARγ agonist rosiglitazone and antagonist GW9662 were also dissolved in DMSO and added to the medium at the specified concentrations.

2.3. Cytotoxicity assay

The cytotoxicity of BBR was determined by the MTT assay [36]. Briefly, L6 myoblasts were seeded in 96-well microtiter plates. After 24 hours, 100 μL complete culture medium containing different concentrations of BBR (1.56, 3.13, 6.25, 12.5, 25, 50, 100, or 200 μmol/L) was added. The same amount of DMSO was added to the control cells. The concentrations of BBR used were tested through preliminary studies. After 24 hours of treatment, 10 μL of 0.5% MTT in phosphate-buffered saline (PBS) was added to each well. After 4 hours of incubation, medium was aspirated; and 100 μL of DMSO was added to dissolve the purple crystal dye. The optical absorbance was read on a microplate reader (ELX 800; Bio-Tek Instruments, Winooski, VT) at 570 nm.

2.4. Glucose consumption

L6 cells were cultured in 6-well culture plates. After differentiation, culture medium was replaced with DMEM supplemented with 0.5% BSA and cultured for 4 hours. The myotubes were then treated with BBR (5 μmol/L) and palmitic acid (250 μmol/L) alone and the combination of both for 24 hours. Insulin at 100 nmol/L was used as the positive control. The glucose concentration in medium was determined by the glucose oxidase method. The intracellular

glucose was calculated by subtracting the glucose in the medium from the total glucose in the blank wells [22,37,38].

2.5. Glucose uptake

Glucose uptake was quantified as previously described with some modifications [39-41]. After 4-hour serum starvation and 24-hour treatment, L6 myotubes were washed with Krebs–Ringer phosphate–HEPES buffer (KRBH, containing 118 mmol/L NaCl, 5 mmol/L KCl, 1.3 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, and 30 mmol/L HEPES, pH 7.4) and incubated in KRHB containing 0.5% BSA with or without 100 nmol/L insulin for 10 minutes. The cells were then incubated with 10 mmol/L 2-NBD-glucose in PBS for 10 minutes. Cytochalasin B at a dose of 20 μmol/L was added to measure nonspecific glucose uptake. The assay was terminated by washing the cells 3 times with ice-cold PBS. The cells were fixed with 10% formalin, and the density of fluorescence was measured by a confocal laser scanning microscopy (Nikon EZ-C1, Tokyo, Japan). The background of glucose uptake was corrected by subtracting the glucose uptake in the presence of cytochalasin B. To avoid the yellow fluorescence contamination from BBR, glucose uptake after BBR treatment was corrected by subtracting the glucose uptake in the presence of BBR and cytochalasin B. The assay was performed in 2 wells for each treatment/concentration and repeated 3 times.

2.6. Western blot analysis

At the end of BBR incubation, cells were harvested, washed with PBS, and pelleted by centrifugation. Proteins were isolated by adding RIPA lysing buffer (150 mmol/L NaCl, 50 mmol/L Tris, pH 8.0, 5 mmol/L EDTA, 10 mmol/L NaF, 10 mmol/L sodium pyrophosphate, 1% Nonidet P-40, 0.5% deoxycholate) with 1 mmol/L phenylmethylsulfonyl fluoride on ice. The protein concentration of each sample was determined using the nucleic acid and protein analyzer (DUw 640; Beckman Coulter, Fullerton, CA) according to the manufacturer's instructions. An aliquot of 50 to 100 μg protein was loaded onto 10% polyacrylamide gels. After electrophoretic separation, proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membrane was blocked in a blocking buffer (3% BSA and 0.05% Tween 20 in 20 mmol/L Tris-buffered saline [TBST], pH 7.6) for 1 hour at room temperature. The membrane was incubated with appropriate monoclonal or polyclonal primary antibodies in blocking buffer overnight at 4°C. The membrane was then washed with TBST 3 times (6 minutes each), followed by incubation with anti-mouse or anti-rabbit secondary antibodies for 1 hour at room temperature. After washing 3 times with TBST and once with TBS, the target proteins and β-actin were semiquantified by scanning the corresponding bands using a FluorChem Q Imaging System (Alpha Innotech, San Leandro, CA). Protein expression is presented as the ratios of target proteins to β-actin.

2.7. Statistical analysis

Data were analyzed by 1-way analysis of variance followed by a post hoc test using Dunnett t test for separate comparisons with the control group or Student–Newman–Keuls-q test for multiple-group comparisons. Significance level was set at $P < .05$. Results are presented as means ± SEM (n ≥3).

3. Results

3.1. The cytotoxicity of BBR in L6 cells

The cytotoxicity of BBR was tested in L6 cells using the MTT assay, and results are presented in Fig. 1. Berberine was not cytotoxic at concentrations lower than 50 μmol/L but became toxic when the concentrations were 50 μmol/L and higher. No changes in cell morphology and attachment properties due to BBR supplementation were observed at concentrations up to 200 μmol/L.

3.2. BBR enhances glucose consumption in normal and insulin-resistant L6 myotubes

To investigate the mechanism of action of BBR on glucose metabolism, glucose consumption was measured in L6 myotubes. The inclusion of insulin (100 nmol/L) in the culture medium resulted in an 80% increase ($P < .05$) in glucose consumption compared with the normal control group after 24 hours of treatment. By contrast, the addition of palmitic acid at a dose of 250 μmol/L reduced ($P < .05$) glucose consumption by 43% relative to the control group. The glucose consumption in BBR-treated (5 μmol/L) cells was 36% higher ($P < .05$) than that in the normal controls. Cells treated with the combination of BBR and palmitic acid showed similar glucose consumptions with the normal cells,

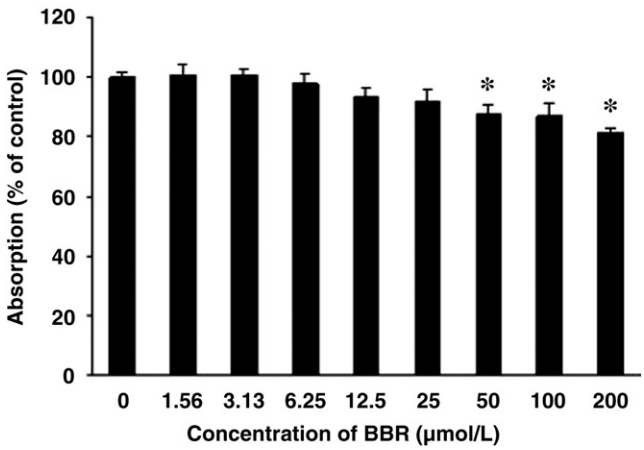

Fig. 1. Cytotoxicity of BBR in L6 cells. L6 myoblasts in 96-well plates were treated with BBR at different concentrations or with vehicle for 24 hours. There were 6 parallel wells in each concentration. Ten microliters of 0.5% MTT was added to each well, and the cells were incubated for 4 hours at 37°C. The absorbance was measured at 570 nm by a microplate reader. *$P <$ .05 compared with the normal control.

but higher consumptions ($P < .05$) than the FFA-induced insulin-resistant cells, indicating that BBR has potential to reverse the FFA-induced insulin resistance in myotubes.

3.3. Berberine enhances glucose transport in normal and insulin-resistant L6 myotubes

The effect of BBR on glucose uptake activity was tested in differentiated L6 myotubes. Berberine at 5 μmol/L increased the acute insulin-induced glucose uptake by 60% as compared with the normal control (Fig. 2). To examine whether BBR affects the insulin-mediated glucose uptake in FFA-induced insulin-resistant cells, BBR at 5 μmol/L was incubated with the addition of palmitic acid at 250 μmol/L for 24 hours. As shown in Fig. 2, the conclusion of FFA in the medium resulted in approximately 60% reduction of insulin-stimulated glucose uptake ($P < .05$). However, BBR treatment at 5 μmol/L increased the acute insulin-stimulated glucose uptake by 160% as compared with that in the insulin-resistant myotubes, which was close to the levels observed in the normal myotubes.

3.4. Berberine altered PPARγ but not PPARα protein expression in L6 myotubes

To investigate whether BBR exerted its effect against the FFA-induced insulin resistance in myotubes via regulating PPARγ and PPARα expressions, Western blot analysis was carried out in L6 myotubes. As shown in Fig. 3A, PPARγ protein levels were reduced ($P < .05$) by 23% and 49%, respectively, by BBR treatment at 5 and 25 μmol/L in normal cells. Compared with normal myotubes, the FFA-induced insulin-resistant myotubes showed a significant increase of PPARγ protein levels ($P < .05$). When insulin-resistant cells were treated with BBR combined with FFA, the expression of PPARγ was decreased by 31% and 47% at doses of 5 and 25 μmol/L, respectively (Fig. 3B). It was demonstrated that BBR reversed the increase of PPARγ caused by FFA in myotubes. Nevertheless, the protein levels of PPARα were not affected by BBR or FFA in either normal or insulin-resistant myotubes (Fig. 3A, B).

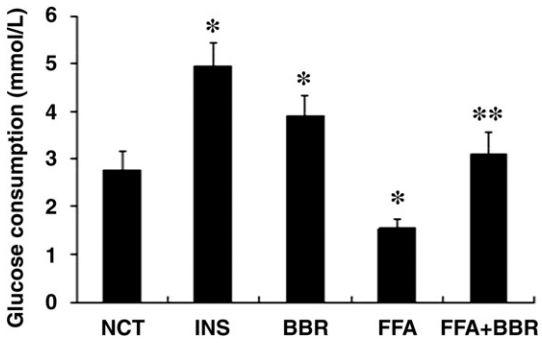

Fig. 2. Effect of BBR on glucose consumption in L6 myotubes. Differentiated L6 myotubes were incubated for 24 hours with BBR (5 μmol/L), palmitic acid (250 μmol/L), combination of both, and vehicle, respectively. Insulin at 100 nmol/L was used as the positive control. The glucose concentration in the medium after the incubation was measured by glucose oxidase method. The intracellular glucose was calculated by subtracting the glucose in the medium from the total glucose in the blank wells. The experiments were repeated once. *$P < .05$ compared with the normal control. **$P < .05$ compared with the FFA-induced insulin-resistant control. NCT indicates normal control; INS, 100-nmol/L insulin treatment (positive control); BBR, 5-μmol/L BBR treatment; FFA, 250-μmol/L palmitate treatment (negative control); FFA + BBR, 5-μmol/L BBR plus 250-μmol/L palmitate treatment.

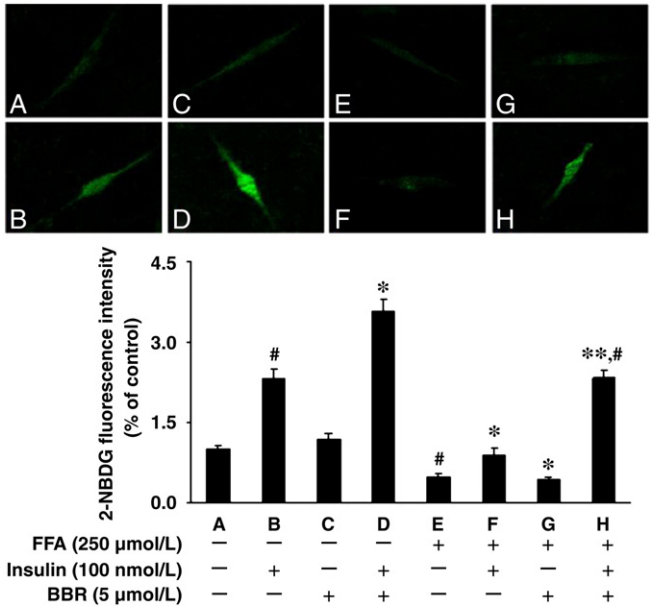

Fig. 3. Effect of BBR on glucose uptake in normal and insulin-resistant L6 myotubes. After starvation in 0.5% BSA for 4 hours, L6 myotubes were exposed to different treatments for 24 hours and then washed 3 times with KRHB containing 0.5% BSA at 40-minute intervals over 2 hours at 37°C. After 10 minutes of incubation with insulin at 100 nmol/L, 10 mmol/L 2-NBD-glucose in PBS was added; and the cells were incubated for an additional 10 minutes. Glucose uptake was stopped with 3 washes of ice-cold PBS. The cells were fixed with 10% formalin and observed by confocal laser scanning fluorescence microscope (400×). Glucose uptake with BBR treatment was corrected by subtracting the glucose uptake in cells treated with BBR and cytochalasin B to exclude the yellow fluorescence background of BBR. Data are presented as means ± SD (n = 3). #$P < .05$ compared with the normal cells without insulin stimulation. *$P < .05$ compared with the normal cells with insulin stimulation. **$P < .05$ compared with the insulin-resistant cells with insulin stimulation.

3.5. Effects of BBR on protein expressions of FAT/CD36 and CPT-I in L6 myotubes

Compared with the normal cells, BBR showed a negative effect on the FAT/CD36 protein level, which was reduced ($P < .05$) by 30% and 52% after BBR treatment at 5 and 25 μmol/L for 24 hours, respectively (Fig. 4A). Free fatty acid infusion stimulated the protein expression of both FAT/CD36 and CPT-I as compared with the normal control. However, BBR treatment at 5 or 25 μmol/L decreased ($P < .05$) FAT/CD36 protein expression by 23% or 41% in the FFA-induced insulin-resistant cells; and the expression

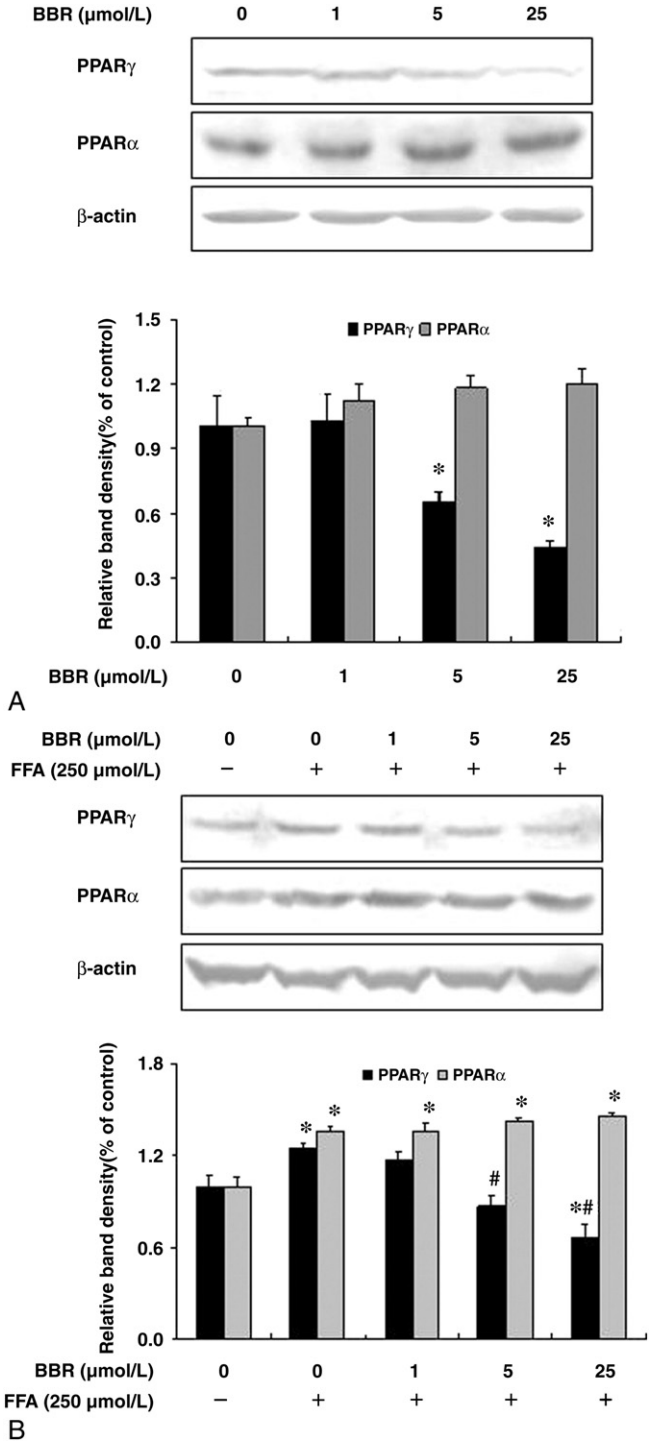

Fig. 4. The effect of BBR on PPARγ and PPARα expression in L6 myotubes. A, L6 myotubes were treated with 1, 5, and 25 μmol/L of BBR or vehicle for 24 hours. B, L6 myotubes were treated with 250 μmol/L palmitic acid; palmitic acid with 1, 5, and 25 μmol/L BBR; or vehicle for 24 hours. Proteins were isolated from the cell lysates and analyzed by Western blotting for PPARγ and PPARα expressions. The experiments were repeated 3 times, and all 3 experiments showed similar results. *$P < .05$ compared with the normal control. #$P < .05$ compared with the insulin-resistant control.

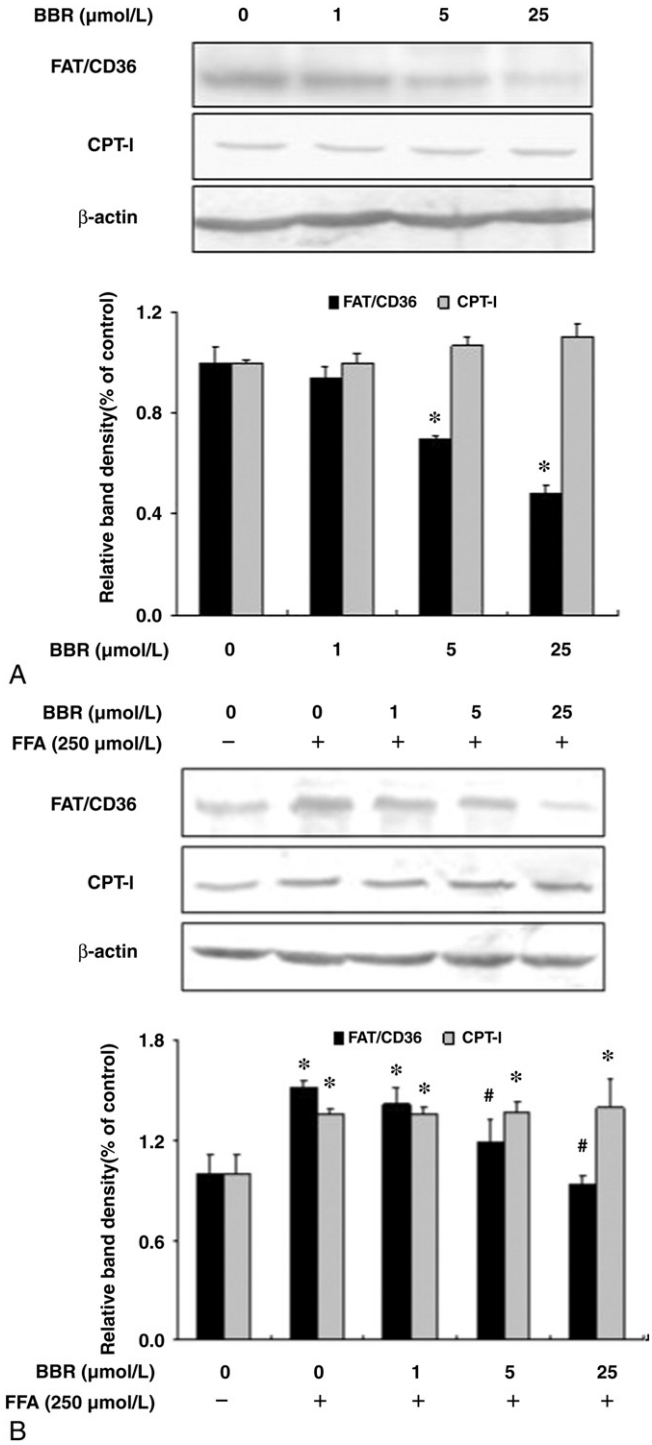

Fig. 5. Effect of BBR on FAT/CD36 and CPT-I protein expressions in L6 myotubes. A, L6 myotubes were treated with 1, 5, and 25 μmol/L of BBR or vehicle for 24 hours. B, L6 myotubes were treated with 250 μmol/L palmitic acid; palmitic acid with 1, 5, and 25 μmol/L BBR; or vehicle for 24 hours. Cell lysates were prepared and used to determine the expression of FAT/CD36 and CPT-I proteins by Western blot. *$P < .05$ compared with the normal control. **$P < .05$ compared with the insulin-resistant control.

became similar with that in the normal control (Fig. 4B). The protein expression of CPT-I was not altered by BBR, FFA, or the combination of BBR and FFA.

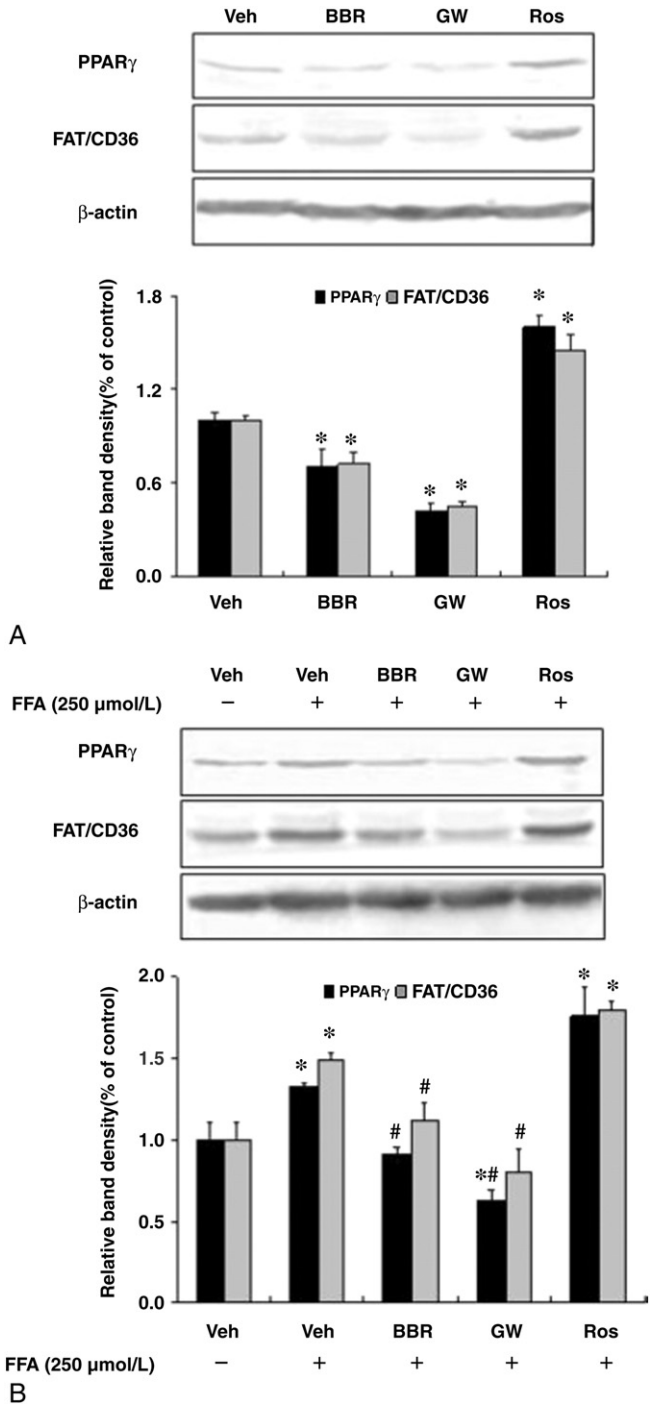

Fig. 6. Comparisons of BBR with PPARγ antagonist and agonist for regulating PPARγ and FAT/CD36 protein expressions in L6 myotubes. A, Cells were treated with BBR, GW9662, or rosiglitazone for 24 hours. After washing and lysis, total cell lysates was tested by Western blot to measure PPARγ and FAT/CD36. B, Cells were pretreated with 250 μmol/L FFA without and with 5 μmol/L BBR or 1 μmol/L GW9662, or 10 μmol/L rosiglitazone for 24 hours. *$P < .05$ compared with the normal control. #$P < .05$ compared with the insulin-resistant control. Veh indicates vehicle-treated normal control; BBR, 5-μmol/L BBR treatment; GW, 1-μmol/L GW9662 treatment; Ros, 10-μmol/L rosiglitazone treatment.

3.6. Comparisons of BBR with PPARγ antagonist GW9662 and agonist rosiglitazone for regulating PPARγ and FAT/CD36 in L6 myotubes

Because BBR decreased FAT/CD36 but not CPT-I protein expression in myotubes, we used GW9662, an irreversible antagonist of PPARγ, and rosiglitazone, an agonist of PPARγ, to further investigate the role of the PPARγ signaling pathway in the regulatory effect of BBR on FAT/CD36. We compared the expression levels of PPARγ among BBR, GW9662, and rosiglitazone without FFA (Fig. 5A) and with FFA (Fig. 5B), respectively. In the normal cells, PPARγ protein levels were inhibited ($P < .05$) by 29% and 56%, respectively, by BBR and GW9662, whereas levels were increased ($P < .05$) by 62% after rosiglitazone treatment. In the FFA-treated myotubes, the PPARγ protein expression was decreased ($P < .05$) by BBR and GW9662 at 31% and 53%, respectively, and up-regulated by rosiglitazone by 34% ($P < .05$). There was a similar tendency of FAT/CD36 changes with the expression of PPARγ not only in the normal myotubes but in the FFA-induced insulin-resistant myotubes as well (Fig. 6).

4. Discussion

Elevation of FFA levels in the blood was reported for the first time in 1963 as an important feature of diabetes and other disorders of carbohydrate metabolism [42]. Since then, several studies in humans confirmed the association of increased plasma FFA levels and reduced carbohydrate, especially glucose, oxidation in skeletal muscle [4,43-45]. Furthermore, it is reported that fatty acid transporter FAT/CD36 expression is closely linked with FFA transport and that FAT/CD36 deficiency increases insulin sensitivity in muscle [46,47], indicating that increases of FFA uptake by myoblasts cause muscle insulin resistance. In this study, we used a well-established insulin-resistant cell model [29,32] to test the effect of BBR on FFA-induced insulin resistance in myotubes. The results demonstrated that BBR improved the glucose consumption and glucose uptake in both normal and FFA-induced insulin-resistant cells. It is suggestive that BBR is beneficial to FFA-induced insulin resistance in skeletal muscle. The effective dose (<50 μmol/L) of BBR observed in the present study in L6 myotubes was similar with that reported in 3T3-L1 adipocytes [48].

It is well known that PPARγ regulates fatty acid uptake and promotes adipogenesis and fat deposition [49,50]. Berberine markedly inhibited PPARγ protein expression in normal and FFA-induced insulin-resistant myotubes. The decreased PPARγ protein expression by BBR in muscle cells may protect the skeletal muscle from intracellular lipid deposition/accumulation, which is the main adverse effect of the current antidiabetic drugs and insulin sensitizers, such as rosiglitazone [51,52], which up-regulates PPARγ expression. Peroxisome proliferator–activated receptor α acts as a

molecular sensor of fatty acids and their derivatives and regulates the expression of genes encoding enzymes and transport proteins that are involved in lipid homeostasis. In contrast, PPARα stimulates fatty acid β-oxidation in liver, heart, skeletal muscle, and kidney [53,54]. The ineffectiveness of BBR on PPARα in myotubes with and without chronic incubation of FFA indicates that BBR does not affect fatty acid catabolism or β-oxidation in these organs or tissues. Berberine may improve insulin resistance in myotubes by inhibiting fatty acid uptake and accumulation rather than enhancing fatty acid oxidation in muscle cells.

Fatty acid transferase/CD36 is a key protein involved in regulating the uptake of LCFA across the plasma membrane in heart and skeletal muscle [55,56]. It promotes triglyceride breakdown and fatty acid metabolism, so the increased fatty acid uptake did not result in lipid accumulation because triglyceride turnover is enhanced [57]. Insulin and contractions induce the translocation of intracellular FAT/CD36 to the plasma membrane and consequently increase the intracellular uptake of LCFA [58]. Carnitine palmitoyltransferase I is an important protein involved in the movement of LCFA across the mitochondrial membrane, an essential first step in the β-oxidation of LCFA [59,60]. Peroxisome proliferator–activated receptor γ regulates FAT/CD36 expression [61-63], whereas PPARα mediates CPT-I expression [53,64,65]. In line with the effects on PPARγ and PPARα, BBR decreased FFA-induced increase of FAT/CD36 protein expression but had no effect on CPT-I protein levels. The differential effects of BBR on FAT/CD36 and CPT-I expressions imply that BBR down-regulates FAT/CD36-mediated fatty acid transport instead of stimulating fatty acid oxidation in FFA-induced insulin-resistant myotubes. Consequently, myotubes shifted the energy metabolism from fatty acid oxidation to glucose oxidation. This change in energy homeostasis results in the increases of glucose uptake and utilization by muscle cells, and is thus beneficial to glucose control and diabetes. It is well known that, in the glucose–fatty acid dynamic cycle, glucose reciprocally affects fatty acid metabolism [66-68]. We postulate that stimulation of glucose uptake and utilization by BBR at the expense of fatty acid uptake may protect the skeletal muscle from the ectopic deposition of triglycerides and improve insulin sensitivity.

Thiazolidinediones are a family of antidiabetic drugs, which improve insulin resistance and diabetic complications by activating PPARγ. Adipose tissue is the major target of TZD-mediated improvement of hyperlipidemia and insulin sensitization [69]. The activation of PPARγ results in the enhancement of adipogenesis in adipose tissues. It is reported that, in skeletal muscle, TZD increases glucose uptake and oxidation by improving insulin signaling, up-regulating glucose transporter 4 and phosphoinositide-dependent kinase (PDK), and relocating FAT/CD36 to mitochondria instead of increasing CPT-I [69-73]. However, the mechanism of action of TZD on glucose uptake and utilization in skeletal muscle is not as clear as in adipose tissues. There are increased concerns about the adverse effects associated with the use of antidiabetic drugs such as TZD. The main adverse effects of TZD include weight gain, fluid retention, hepatotoxicity, and increased risk of heart failure and bone loss [74-78]. Berberine, different from TZD, is a natural product that has been thought to act through multiple targets to improve insulin resistance and diabetes. Except for activating AMP-activated kinase (AMPK), which leads to the reductions of adiposity and improvement of insulin sensitivity [79], BBR also inhibits the differentiation of 3T3-L1 preadipocytes [27,80] and stimulates glucose uptake through a mechanism distinct from that of TZD. Several PPARγ antagonists have shown similar effects as demonstrated by BBR, for example, tannic acid that stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 Cells [81], and a selective PPARγ antagonist, SR-202, that shows antiobesity and antidiabetic activity in transfected Hela cell and diabetic *ob*/*ob* mice [82]. Therefore, BBR is potentially able to prevent obesity and enhances glucose uptake and insulin sensitivity without causing adverse effects associated with TZD drugs.

In conclusion, BBR improved the FFA-induced insulin resistance in skeletal muscle cells through improving glucose uptake and utilization and inhibiting fatty acid uptake. Our findings have provided additional evidence to support the beneficial effects of BBR on diabetes. Berberine is a promising natural agent that may be used for the prevention of and cotherapy for insulin resistance and type 2 diabetes mellitus.

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