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Bis(α-furancarboxylato)oxovanadium(IV) prevents and improves dexamethasone-induced insulin resistance in 3T3-L1 adipocytes

Yi-Qing Zuo, Wei-Ping Liu, Yan-Fen Niu, Chang-Fu Tian, Ming-Jin Xie, Xi-Zhu Chen and Ling Li

Abstract

Previous studies showed that bis(α-furancarboxylato)oxovanadium(IV) (BFOV), an orally active anti-diabetic organic vanadium complex, could improve insulin resistance in animals with type 2 diabetes. The present study has been carried out to evaluate the effects of BFOV on insulin-resistant glucose metabolism using dexamethasone-treated 3T3-L1 adipocytes as an in-vitro model of insulin resistance. The results showed that BFOV, similar to vanadyl sulfate and rosiglitazone, caused a concentration-dependent increase in glucose consumption by insulin-resistant adipocytes. Moreover, BFOV enhanced the action of insulin and completely prevented the development of insulin resistance induced by dexamethasone, leading to glucose consumption equal to that by normal cells. In addition, dexamethasone reduced the mRNA expression of insulin receptor substrate 1 (IRS-1) and glucose transporter 4 (GLUT4) in 3T3-L1 adipocytes, while BFOV normalized the expression of IRS-1 and GLUT4. These findings suggest that BFOV prevents and improves dexamethasone-induced insulin resistance in 3T3-L1 adipocytes by enhancing expression of IRS-1 and GLUT4 mRNA.

Yunnan Pharmacological Laboratories of Natural Products, Kunming Medical College, Kunming, PR China

Yi-Qing Zuo, Yan-Fen Niu, Chang-Fu Tian, Ling Li

Kunming Institute of Precious Metals, Kunming, PR China

Wei-Ping Liu, Xi-Zhu Chen

Department of Chemistry, Yunnan University, Kunming, PR China

Ming-Jin Xie

Correspondence: L. Li, Yunnan Pharmacological Laboratories of Natural Products, Kunming Medical College, 191 Renmin West Road, Kunming 650031, PR China.
E-mail: kmli62@yahoo.com.cn

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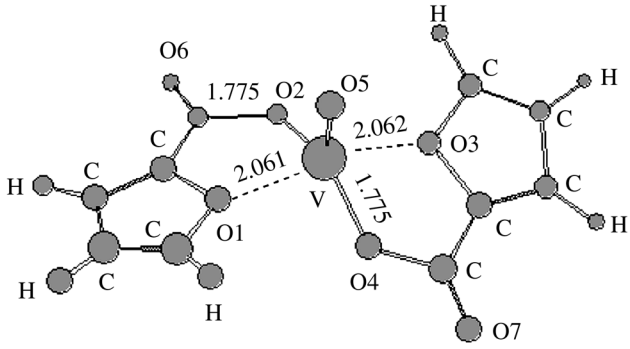
Introduction

Insulin resistance is a major pathophysiological abnormality in patients with type 2 diabetes mellitus and obesity. Resistance to insulin action develops in skeletal muscle, adipose tissue and liver, contributing to hyperglycaemia (DeFronzo 2004). These impairments in insulin action play an important role not only in the development of hyperglycaemia of type 2 diabetes mellitus but also in the pathogenesis of complications. Therefore, treatments that improve peripheral insulin resistance would be beneficial in the long-term management of these patients.

Vanadium compounds, as one of the new approaches for the treatment of both type 1 and type 2 diabetes, have been shown to possess insulin-mimetic and/or enhancing effects both in-vitro and in-vivo (Crans et al 2004; Srivastava & Mehdi 2005; Scior et al 2005). Treatment by this method improved diabetes as well as hepatic, peripheral and muscle insulin sensitivity (Cusi et al 2001). The functional mechanisms of vanadium complexes have been demonstrated to involve stimulation of glucose uptake, glycogen synthesis and lipogenesis, and inhibition of lipolysis and gluconeogenesis (Goldfine et al 1995; Nakai et al 1995; Sekar et al 1999). Oral administration of vanadium complexes restored the expression of the glucose transporter 4 (GLUT4) gene in skeletal muscle (Mohammad et al 2002) and suppressed the expression of the glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) genes in the livers of model animals with streptozotocin (STZ)-induced type-1-like diabetes (Marzban et al 2002).

The bioavailability of inorganic vanadium salts such as vanadyl sulfate is very low (Fugono et al 2001). An important recent advance has been the development of vanadium complexes with organic ligands, which are considered to be more active and less toxic than inorganic vanadium salts.

Bis(α-furancarboxylato)oxovanadium(IV) (BFOV; Figure 1) is a new organic vanadium complex which has been shown to be orally active and has hypoglycaemic effects in mice with type-1-like diabetes induced by alloxan and in rats with STZ-induced diabetes



O1-O2-O3-O4: 14.2°

Figure 1 Structure of bis(α-furancarboxylato)oxovanadium(IV).

(Xie et al 2002, 2005; Gao et al 2006). Further studies indicated that BFOV could control hyperglycaemia, decrease serum insulin level, increase insulin sensitivity index, improve glucose intolerance and inhibit gluconeogenesis in fat-fed rats with STZ-induced type-2-like diabetes (Niu et al 2007), showing that BFOV improved insulin resistance and type 2 diabetes. In-vitro, the compound markedly enhanced the uptake of 2-deoxy-D-[^{3}H]-glucose and inhibited lipolysis triggered by epinephrine (adrenaline) in isolated rat adipocytes (Gao et al 2008; Li et al 2008).

The present study evaluates the effects of BFOV on insulin-resistant glucose metabolism using dexamethasone-treated 3T3-L1 adipocytes as an in-vitro model of insulin resistance.

Materials and Methods

Chemicals

BFOV (C 41.52%, H 2.08%, V 17.61%; purity > 98.5% by HPLC) was synthesized according to the method described previously (Gao et al 2006). Vanadyl sulfate was the product of Acros Organics. Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco. Insulin, dexamethasone and 3-isobutyl-3-methyl-xanthine (IBMX) were purchased from Sigma. Fetal calf serum (FCS) was from Ming Hai Co (China). All other regents were of analytical grade commercially available.

Cell culture

3T3-L1 pre-adipocytes, obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China), were cultured in DMEM with high glucose concentration (25 mM) containing 10% FCS in a humidified atmosphere of 5% CO_2 at 37°C in a CO_2 incubator. Differentiation was induced by the method of Anli Kumar & Marita (2000) and Basuki et al (2006) with modification. Briefly, 2 days after confluence, differentiation of cells was induced by 0.5 mM IBMX, 0.25 mM dexamethasone and 10 mg L^{-1} insulin in DMEM containing 10% FCS. After 48 h, dexamethasone and IBMX were withdrawn and only insulin-containing medium was continued for the next 2 days. Thereafter, cells were maintained in insulin-free DMEM with 10% FCS until the cells completely differentiated into adipocytes (9–12 days after initiation).

Effects of BFOV on dexamethasone-induced insulin resistance

Insulin resistance was induced in differentiated 3T3-L1 adipocytes using the method reported by Grunfeld et al (1981) and Anli Kumar & Marita (2000). Fully differentiated adipocytes (in 96-well plates) were exposed to 100 nM dexamethasone in standard medium for a maximum of 6 days. BFOV, vanadyl sulfate or rosiglitazone (2.5–20 μM) were added 4 days after dexamethasone exposure and the drugs were incubated together with dexamethasone for 2 days. In some experiments, BFOV (2.5–20 μM) was added simultaneously with dexamethasone for 6 days. In another experiment, BFOV (20 μM) was added along with insulin (100 nM) and dexamethasone (100 nM) for 2 days to cells that had been pretreated with dexamethasone for 4 days. The culture medium was routinely changed every 2 days. The effects of BFOV on insulin resistance were estimated by the change in the concentration of glucose in the medium measured by the glucose oxidase method (Mueller et al 2000; Yin et al 2002). The MTT assay was used to monitor cell viability, and glucose consumption values were corrected accordingly.

Effects of BFOV on insulin receptor substrate 1 (IRS-1) and glucose transporter 4 (GLUT4) mRNA expression

Fully differentiated adipocytes (in 75 cm^2 flasks) were exposed to 100 nM dexamethasone in standard medium for a maximum of 6 days. BFOV, vanadyl sulfate or rosiglitazone (20 μM) were added 4 days after dexamethasone exposure and the drugs were incubated together with dexamethasone (100 nM) for 2 more days. Subsequently, total RNA was extracted from 3T3-L1 adipocytes using an RNA kit (Tiangen, Beijing, China), in accordance with the manufacturer's instructions. The cDNA, synthesized by the reverse transcription of RNA, was used for amplification by PCR. Samples (2.5 μL) of cDNA were subjected to PCR with Taq polymerase and primers specific for mouse IRS-1 (forward 5′-TGAGCCT-CACATGAGAACTAGA-3′; reverse 5′-CAGTCAACTTTCG GACTCGTC-3′), or GLUT4 (forward 5′-TTTGCACT-CAGTGGTCAGGG-3′; reverse 5′-GGTTCGTCATTGAGC-GAGGAC-3′). The PCR amplifications were performed in 25 μL reaction mixture. The reaction mixtures were subjected to 30 cycles of PCR amplification (verified to be within the linear range of amplification by a preliminary experiment) consisting of denaturation for 60 s at 94°C, annealing for 40 s at 45°C (IRS-1) or 53°C (GLUT4) and elongation for 30 s at 72°C. The final extension was completed at 72°C for 7 min. IRS-1 and GLUT4 mRNA expression levels were normalized to mouse GAPDH expression. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide and gels were photographed with Gel Doc (Bio-Rad, USA). The amplified products of PCR were analysed with Quantity One software (Bio-Rad).

Statistical analysis

All data are expressed as mean ± s.e.m. Statistical analysis was performed by the Mann–Whitney test or Kruskal–Wallis test followed by Dunn's multiple comparison test using Prism version 4.0 software (GraphPad Software, Inc. La Jolla, CA, USA). A P value below 0.05 was considered significant.

Results

As shown in Figure 2, fully differentiated 3T3-L1 adipocytes exhibited highly insulin-sensitive glucose consumption. Insulin caused a concentration-dependent stimulation of glucose consumption, leading to a 25.4% increase from the baseline level at the maximum concentration of 200 nM. Six days' incubation with dexamethasone caused a reduction in glucose consumption which was significant at high insulin concentrations. The highest insulin concentration of 200 nM caused only a 15.6% increase from baseline and a 38.6% reduction compared with control adipocytes. In addition, dexamethasone treatment decreased basal glucose consumption of adipocytes. The decreased glucose consumption by adipocytes indicates that dexamethasone exposure leads to a state of cellular insulin resistance.

To determine whether BFOV is able to reverse or prevent the insulin resistance caused by dexamethasone, BFOV was added to 3T3-L1 adipocytes either 4 days after dexamethasone or simultaneously with dexamethasone. The experiments demonstrated that BFOV caused a concentration-dependent increase in the glucose consumption by insulin-resistant 3T3-L1 adipocytes induced by dexamethasone, similar to vanadyl sulfate and rosiglitazone (Figure 3A). Compared with untreated adipocytes, glucose consumption was increased by 41.2% with BFOV, 35.9% by vanadyl sulfate and and 50.7% by rosiglitazone (all at 20 μM) (Figure 3B).

BFOV added simultaneously with dexamethasone completely prevented the decrease in glucose consumption induced by dexamethasone, resulting in glucose consumption equal to normal cells with 2.5 μM BFOV (Figure 4A). When insulin-resistant adipocytes were incubated with 20 μM BFOV and 100 nM insulin during the last 2 days, glucose consumption was significantly increased compared with insulin-resistant cells treated with insulin or BFOV only (Figure 4B).

The MTT test showed that BFOV, vanadyl sulfate and rosiglitazone did not affect cell viability (data not shown). Correction of glucose consumption values according to the MTT optical density did not affect the results significantly.

As illustrated in Figure 5, IRS-1 and GLUT4 mRNA levels were significantly decreased in dexamethasone-treated

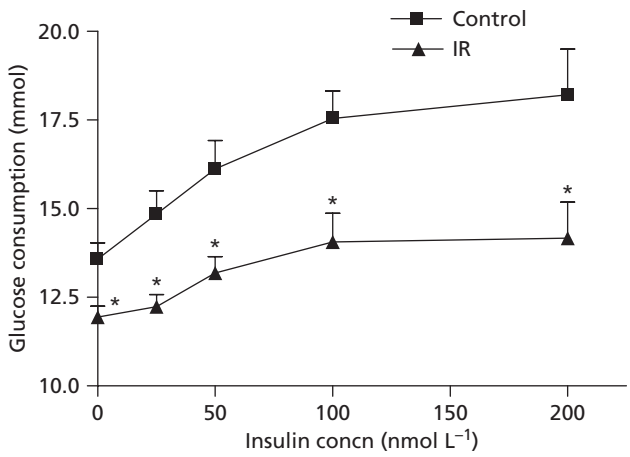


Figure 2 Effect of dexamethasone on glucose consumption in 3T3-L1 adipocytes. Fully differentiated 3T3-L1 adipocytes were incubated for 6 days with 100 nM dexamethasone or vehicle. Glucose consumption was assayed by the glucose oxidase method in the presence of insulin. Control: fully differentiated 3T3-L1 adipocytes; IR, dexamethasone-treated insulin-resistant adipocytes. Data are mean ± s.e.m. for four independent experiments. $^{*}P < 0.01$ vs normal control cells (Mann–Whitney test).

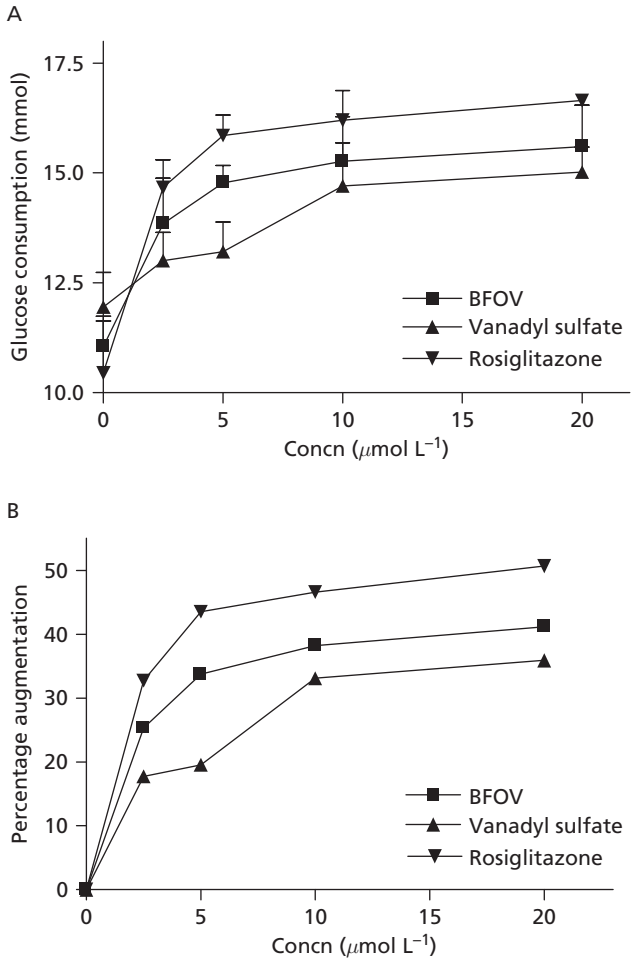


Figure 3 Effect of BFOV on glucose consumption in dexamethasone-induced insulin-resistant 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were incubated with 100 nM dexamethasone for 6 days. After 4 days, BFOV, vanadyl sulfate or rosiglitazone were added to 96-well plates and incubated with 100 nM dexamethasone for the next 2 days. At the end of 6 days, the concentration of glucose in medium was measured by the glucose oxidase method. Data are mean ± s.e.m. for four independent experiments.

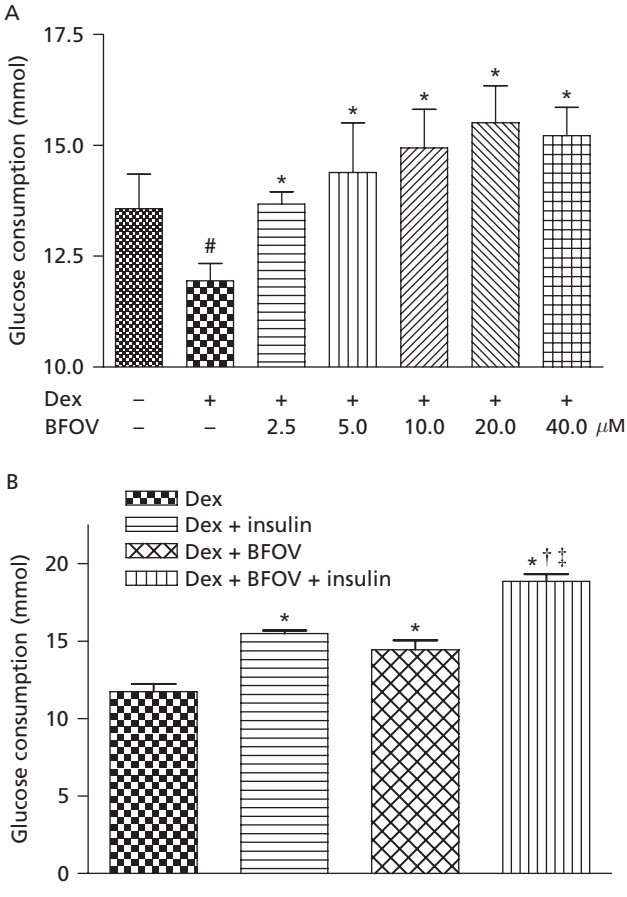


Figure 4 Effects of BFOV incubated simultaneously with dexamethasone (Dex) (A) or dexamethasone and insulin (B). In (A) differentiated 3T3-L1 adipocytes were incubated with BFOV (2.5–40 μM) together with 100 nM dexamethasone for 6 days. In (B) differentiated 3T3-L1 adipocytes were incubated for 4 days with 100 nM dexamethasone. After 4 days, insulin (100 nM), BFOV (20 μM) or both were added and incubated along with 100 nM dexamethasone for a further 2 days. At the end of 6 days, the concentration of glucose in medium was measured. Data are mean ± s.e.m. for four independent experiments. $^{\#}P < 0.05$ vs normal cells; $^{*}P < 0.05$ vs dexamethasone-treated cells; $^{\dagger}P < 0.05$ vs cells treated with dexamethasone and insulin; $^{\ddagger}P < 0.05$ vs cells treated with dexamethasone and BFOV (Dunn's test).

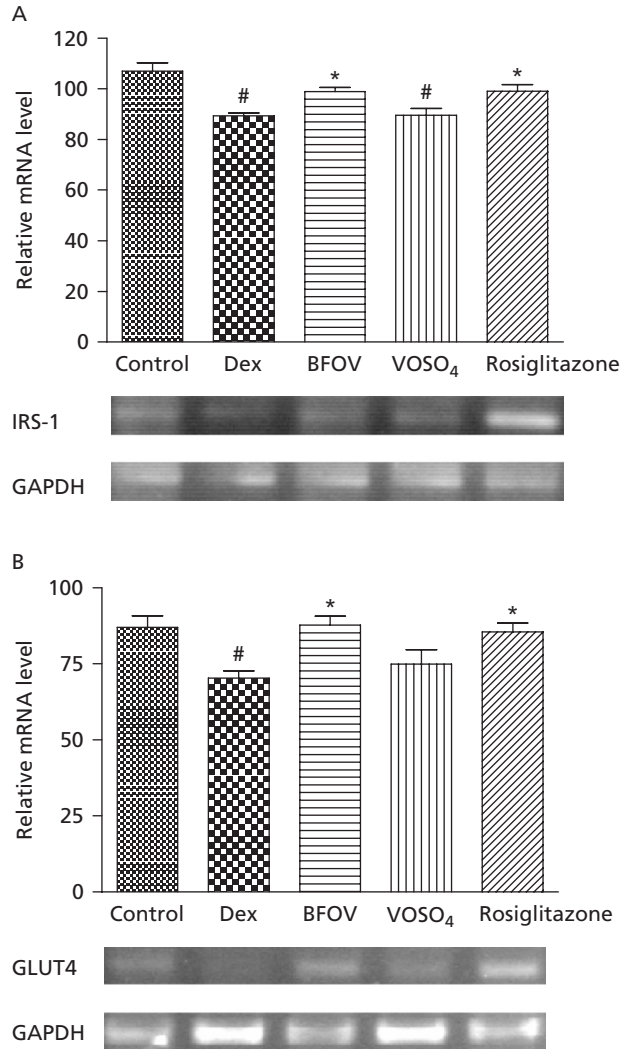


Figure 5 Effects of BFOV on IRS-1 (A) and GLUT4 (B) mRNA levels in 3T3-L1 adipocytes incubated with dexamethasone. Differentiated 3T3-L1 adipocytes were incubated for 4 days with 100 nM dexamethasone. After 4 days, BFOV, vanadyl sulfate ($VOSO_4$) or rosiglitazone (20 μM) was added and incubated along with 100 nM dexamethasone (Dex) for a further 2 days. IRS-1 and GLUT4 mRNA levels were normalized against GAPDH levels. Values are mean ± s.e.m. of the relative optical densities of scanned images (IRS-1 or GLUT4/GAPDH internal control) in four independent experiments. $^{\#}P < 0.05$ vs normal control adipocytes; $^{*}P < 0.05$ vs cells treated with dexamethasone (Dunn's test). The lower panels show representative analyses of PCR products from different treatment groups following electrophoresis on 2% agarose gel.

cells compared with normal cells ($P < 0.05$). Two days' treatment with BFOV partly or completely restored IRS-1 and GLUT4 mRNA expression, which was similar to rosiglitazone. However, no improvement was observed in insulin-resistant cells treated with vanadyl sulfate.

Discussion

3T3-L1 cells are a well-established cell line that respond to physiological doses of insulin with increases in glucose uptake, glucose oxidation, glycogen synthesis and lipogenesis in-vitro (Knutson & Balba 1997). Dexamethasone-induced insulin resistance in 3T3-L1 adipocytes (Grunfeld 1981; Turnbow et al 1994) has been well characterized. In the current experiment, glucose utilization in 3T3-L1 adipocytes treated with dexamethasone was markedly reduced both in the basal situation and under insulin stimulation, suggesting that cells were insulin resistant.

In the previous study, BFOV has been shown to counteract insulin resistance in fat-fed rats with STZ-induced diabetes (Niu et al 2007). To further understand the effect of BFOV on insulin-resistant glucose metabolism, we used dexamethasone-treated 3T3-L1 adipocytes as an in-vitro model of insulin resistance to evaluate the action of BFOV through the following pathways: (i) reversing the already

existing insulin resistance, similar to the pathophysiology of type 2 diabetes mellitus; (ii) preventing the development of insulin resistance; (iii) enhancing the insulin action in 3T3-L1 adipocytes treated with dexamethasone. The results showed that BFOV significantly improved the glucose consumption of cells with dexamethasone-induced insulin resistance, even in the absence of insulin. Moreover, BFOV completely prevented the development of insulin resistance induced by dexamethasone, resulting in glucose consumption equal to that of normal cells. However, in another experiment, BFOV only exhibited the tendency to increase glucose consumption in normal 3T3-L1 adipocytes (data not shown). These findings indicated that BFOV could improve and prevent the dexamethasone-induced insulin resistance independent of insulin.

The molecular basis of insulin resistance involves a defect in the insulin signalling pathway. Insulin exerts its effects by binding to the insulin receptor (IR), which activates an intrinsic IR tyrosine kinase, resulting in a trigger for mediating signalling transduction to the downstream targets of the IR, such as IRS, phosphatidylinositol 3-kinase (PI3-K), protein kinase B (PKB/Akt), glycogen synthase kinase-3β (GSK3β) and GLUT4. These signalling events lead to a series of responses such as glucose uptake and glycogen synthesis (Basuki et al 2006). Binding of protein tyrosine phosphatase 1B (PTP1B), a negative regulator of insulin signalling, with IRβ subunits and IRS stimulates the dephosphorylation of tyrosine residues in IRβ subunits and IRS, which in turn suppresses insulin signalling (Byon et al 1998). Vanadium is known to inhibit the activity of PTP1B, which mimics a number of metabolic actions of insulin, leading to the enhancement of tyrosine phosphorylation of IRβ and IRS; this in turn activates PI3K and Akt to regulate the activity of GSK3β and GLUT4 (Srivastava & Mehdi 2005; Basuki et al 2006; Hiromura et al 2007). Vanadium also directly inhibits protein kinase A (PKA) (Jelveh et al 2006) associated with the activity of a hormone-sensitive lipase, a rate-limiting enzyme, to release free fatty acids from triglycerides in a lipid droplet and regulates the expression of gluconeogenesis genes, including the PEPCK and G6Pase genes (Postic et al 2004).

Dexamethasone interferes with the insulin signalling pathway. Three mechanisms have been suggested for dexamethasone-induced insulin resistance: (i) reduced expression of IRS-1 (Bure'n et al 2002; Turnbow et al 1994); (ii) reduced phosphorylation of IRS-1 and decreased PI3K activity (Saad et al 1993; Bure'n et al 2002); and (iii) impaired GLUT4 translocation (Weinstein et al 1995; Sakoda et al 2000). Therefore, to understand the mechanism of BFOV on insulin-resistant glucose metabolism, we studied its effects on the expression of IRS-1 and GLUT4 mRNA in 3T3-L1 adipocytes with dexamethasone-induced insulin resistance. The expression of IRS-1 significantly decreased in dexamethasone-treated 3T3-L1 adipocytes. Moreover, we found that dexamethasone also reduced the expression of GLUT4, suggesting that dexamethasone could produce not only impairment of GLUT4 translocation but also down-regulation of GLUT4 expression. But treatment with BFOV, but not vanadyl sulfate, normalized IRS-1 and GLUT4 mRNA expression in insulin-resistant 3T3-L1 adipocytes, similar to rosiglitazone. These findings suggest that the actions of BFOV are at least partially mediated by recovering expression of IRS-1 and GLUT4 in 3T3-L1 adipocytes treated with dexamethasone. Ou et al (2005) also observed that bis (acetylacetonato)oxovanadium(IV) [VO(acac)2] restored the phosphorylation of IRβ and IRS1 in insulin-resistant 3T3-L1 adipocytes induced by low-dose insulin to levels observed in normal cells, even in the absence of added insulin. However, further investigation is needed to elucidate the effect of BFOV on the protein level and tyrosine phosphorylation of IRS-1, as well as on the protein level and translocation of GLUT4.

Conclusions

The present study shows that BFOV can improve and prevent dexamethasone-induced insulin resistance in 3T3-L1 adipocytes, which is related to recovery of impaired expression of IRS-1 and GLUT4 mRNA.

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