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Effect of Eriodictyol on Glucose Uptake and Insulin Resistance in Vitro

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ABSTRACT: Eriodictyol [2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2,3-dihydrochromen-4-one] is a flavonoid with anti-inflammatory and antioxidant activities. Because inflammation and oxidative stress play critical roles in the pathogenesis of diabetes mellitus, the present study was designed to explore whether eriodictyol has therapeutic potential for the treatment of type 2 diabetes. The results show that eriodictyol increased insulin-stimulated glucose uptake in both human hepatocellular liver carcinoma cells (HepG2) and differentiated 3T3-L1 adipocytes under high-glucose conditions. Eriodictyol also up-regulated the mRNA expression of peroxisome proliferator-activated receptor γ2 (PPARγ2) and adipocyte-specific fatty acid-binding protein (aP2) as well as the protein levels of PPARγ2 in differentiated 3T3-L1 adipocytes. Furthermore, it reactivated Akt in HepG2 cells with high-glucose-induced insulin resistance. This response was strongly inhibited by pretreatment with the phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002, indicating that eriodictyol increased Akt phosphorylation by activating the PI3K/Akt pathway. These results imply that eriodictyol can increase glucose uptake and improve insulin resistance, suggesting that it may possess antidiabetic properties.

KEYWORDS: *eriodictyol, diabetes, glucose uptake, insulin resistance, PPARγ2, Akt/PKB*

INTRODUCTION

Diabetes is caused by the impaired production of insulin by pancreatic β-cells and/or by diminished tissue responses to insulin, resulting in high circulating blood glucose levels.[1] Key strategies to manage diabetes are to stimulate glucose uptake by the liver, skeletal muscles, and adipocytes and to improve insulin sensitivity and insulin resistance. Type 2 diabetes represents 90−95% of all diagnosed cases of diabetes.[2] Insulin resistance, a specific feature of type 2 diabetes, is the condition whereby major organs such as muscle and liver become resistant to the action of insulin, leading to increased glucose output from the liver and reduced uptake and metabolism of glucose by other organs.[3] Because insulin resistance is the major abnormality in type 2 diabetes, there has been considerable interest in identifying insulin-sensitizing agents to counteract insulin resistance for the treatment of type 2 diabetes.[4]

Thiazolidinedione (TZD) mediates activation of peroxisome proliferator-activated receptor γ (PPARγ), resulting in marked improvement in insulin sensitivity.[5−7] PPARγ belongs to the nuclear receptor superfamily and has key roles in glucose regulation, lipid metabolism, and inflammation.[8,9] PPARγ was previously reported to be important for insulin-responsive glucose uptake and enhancement of glucose transporter (GLUT) 1 and GLUT4 expression in adipocytes.[10,11] PPARγ2 is selectively expressed in adipocytes, and its expression is required for adipocyte differentiation.[12] Adipocyte-specific fatty acid-binding protein (aP2) also contributes to the adipocyte phenotype, as well as glucose and lipid metabolism.[13] Thus, agents that increase PPARγ2-mediated adipogenesis may improve insulin sensitivity and insulin-responsive glucose uptake.

According to current wisdom, insulin's positive effects, including maintenance of normoglycemia, vasodilation, and anti-inflammatory effects, are mediated by the canonical phosphoinositide 3-kinase (PI3K)/Akt pathway.[14] Failure of insulin treatment to induce phosphorylation of Akt under experimental conditions indicates insulin resistance. For example, high-glucose treatment (30 mM) attenuated the insulin-induced phosphorylation of Akt by PI3K, which is indicative of insulin resistance.[15,16] Cellular models can serve as experimental tools to screen for candidates to ameliorate insulin resistance.[17−19]

Inflammation and oxidative stress play crucial roles in the development of diabetes.[20,21] Eriodictyol [2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2,3-dihydrochromen-4-one; Figure 1] has been reported to be a flavonoid with both anti-

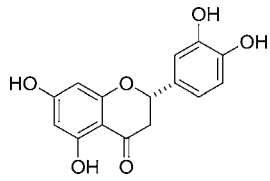

Figure 1. Chemical structure of eriodictyol.

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inflammatory and antioxidant activities.[22,23] These observations provide a compelling reason to perform a study to evaluate the potential of this flavonoid as a therapeutic agent for the management of type 2 diabetes. The overall aim of this study was to investigate the antidiabetic properties of eriodictyol. To this end, we assessed the ability of eriodictyol to stimulate the uptake of glucose by human hepatocellular liver carcinoma (HepG2) cells and 3T3-L1 adipocytes, to enhance insulin sensitivity and PPARγ2 and aP2 expression in adipocytes, and to restore Akt signaling in HepG2 cells with high-glucose-induced insulin resistance. Rosiglitazone, a potent TZD, was used as a positive control in the present study.

MATERIALS AND METHODS

Materials. Eriodictyol (CAS Registry No. 552-58-9; formula $C_{15}H_{12}O_6$; mol wt 288.25; purity 95%) was purchased from J&K Chemical Ltd. (Beijing, China). The fluorescent D-glucose analogue and glucose tracer 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) was acquired from Molecular Probes (Eugene, OR, USA). Rosiglitazone was purchased from Masung & Co., Ltd. (Seoul, Korea). Oil Red O, Accustain Harris hematoxylin solution, and insulin were obtained from Sigma-Aldrich (St. Louis, MO, USA). LY294002 [2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one] was purchased from Cayman Chemical (Ann Arbor, MI, USA). Antibodies against glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phospho-Akt1/2/3 (Ser 473)-R, and Akt were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). An antibody against PPARγ was purchased from Cell Signaling Technology (Danvers, MA, USA). Horseradish peroxidase-conjugated secondary antibodies (anti-mouse and anti-rabbit) were acquired from Vector Laboratories Inc. (Burlingame, CA, USA).

Cell Culture. Mouse 3T3-L1 preadipocytes and HepG2 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS) at 37 °C in a 5% CO_2 humidified atmosphere.

Flow Cytometric Analysis of Glucose Uptake. A glucose uptake assay was carried out as described previously.[17,19] Briefly, HepG2 cells or differentiated 3T3-L1 adipocytes were seeded into 96-well plates (1×10^4 cells/well) for 24 h, maintained in serum-free DMEM with 1 μM insulin in the presence of high (30 mM) concentrations of D-glucose, and treated with or without the indicated concentration of eriodictyol or rosiglitazone in the absence or presence of 10 μM 2-NBDG for 1 h. After collection and resuspension in 500 μL of precold fresh serum-free medium, cells were maintained at 4 °C for subsequent flow cytometric analysis. The fluorescence intensity of 2-NBDG was recorded on the FL1 channel using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Data from 1000 single-cell events were collected. The FCS (Flow Cytometry Standard) files were opened in WinMDI software (version 2.9) as dot plots, converted to text files, and then imported into SPSS 13.0 software (SPSS Software, Chicago, IL, USA). To rule out false positives, cells treated with either eriodictyol or rosiglitazone in the absence of 2-NBDG were measured to give background values. Values of relative fluorescence intensity minus background were used for subsequent data analysis.

Oil Red O Staining and Measurement of Lipid Accumulation. 3T3-L1 cells were induced to differentiate into adipocytes as previously described.[17,19] Briefly, 3T3-L1 cells were seeded into 96-well plates (4×10^3 cells/well) for 24 h. Then, the cells were cultured with a given concentration of eriodictyol or rosiglitazone in DMEM containing 10% FCS and 1 μM insulin for 3 days. Cells in the control group were treated with 1 μM insulin. After 12 days, the cells were stained and lipid accumulation was measured. Briefly, the cells were fixed through incubation in 10% formalin for 1 h and then stained through incubation at room temperature with Oil Red O (in 60% isopropanol, 40% water) for 2 h and Accustain Harris hematoxylin solution for 15 min. The cells were then washed three times with 60% isopropanol to remove unbound dye. The extent of lipid accumulation was quantified by measuring the optical absorbance at 510 nm. Cells were photographed under a light microscope at 400× magnification.

Measurement of PPARγ2 and aP2 mRNA Expression by Reverse Transcription−Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from differentiated 3T3-L1 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and dissolved in DEPC-treated water. RNA (2 μg) was reverse transcribed into cDNA using oligo(dT) primers and Moloney murine leukemia virus (MMLV) reverse transcriptase (Invitrogen). cDNA (1 μL) was subjected PCR amplification of PPARγ2 through 30 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 40 s, and extension at 72 °C for 60 s. To amplify aP2, an annealing temperature of 54 °C was used. The sense and antisense primers for β-actin (NM_007393) were 5′-ATC TGG CAC CAC ACC TTC TAC-3′ and 5′-GAC AGC ACT GTG TTG GCA TAG-3′, respectively; the sense and antisense primers for PPARγ2 (NM_011146) were 5′-TCT GGG AGA TTC TCC TGT TGA-3′ and 5′-TGC AGG TTC TAC TTT GAT CGC-3′, respectively; and the sense and antisense primers for aP2 (NM_024406) were 5′-AAA GTG GCA GGC ATG GCC AAG C-3′ and 5′-GCC TTT CAT AAC ACA TTC CAC C-3′, respectively. After RT-PCR, amplification products were separated on a 1.2% agarose gel, and the DNA was visualized by ethidium bromide staining. The relative expression of PPARγ2 and aP2 was calculated using the β-actin gene as an endogenous control.

Western Blotting. PPARγ2 protein levels in differentiated 3T3-L1 cells were measured by Western blotting as described previously.[24,25] Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed in buffer containing 20 mM Tris-HCl (pH 8.0), 1% Nonidet P-40, 1 mM EDTA, 1 mM EGTA, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM phenylmethanesulfonyl fluoride, 2 μg/mL aprotinin, 2 μg/mL leupeptin, and 1 μg/mL pepstatin and then sonicated. The homogenate was centrifuged and denatured at 95 °C for 5 min. The expression of PPARγ2 in each sample was normalized to that of GAPDH and expressed as a percentage of the normalized expression in the control group.

Insulin resistance was induced in HepG2 cells using a previously described method with minor modifications.[17−19] Cells were seeded into 6-well plates (3×10^5 cells/well) for 24 h and then serum-starved for 24 h. After pretreatment for 24 h with serum-free DMEM containing normal (5.5 mM) and high (30 mM) concentrations of D-glucose in the absence or presence of eriodictyol, the response to insulin (100 nM for 10 min) was measured. The ratio of phospho-Akt to GAPDH was expressed as a percentage of that in insulin-treated cells exposed to a normal concentration of glucose. After pretreatment with the specific PI3K inhibitor LY294002 (20 μM), the effect of eriodictyol on Akt phosphorylation in the presence of a high concentration of glucose was examined. The ratio of phospho-Akt to GAPDH was expressed as a percentage of that in insulin-treated cells exposed to a high concentration of glucose.

Protein samples (50 μg) from insulin-resistant HepG2 cells were separated by 10% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon-P; Millipore, Burlington, MA, USA). The membrane was blocked and then incubated at room temperature with primary antibody for 3 h, followed by secondary antibody for 1 h. Immunoreactivity for Akt, phospho-Akt, and GAPDH was detected using an enhanced chemiluminescence (ECL) Western blot detection system (Western Blotting Luminol Reagent; Santa Cruz Biotechnology), visualized, and measured by densitometry using a ChemiDoc XRS digital imaging system and Multi-Analyst software (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The phosphor-Akt signal was normalized to that of GAPDH and expressed as a percentage of the normalized signal in insulin-treated cells exposed to a normal concentration of glucose.

Statistical Analysis. All data were expressed as the mean ± the standard error of the mean (SEM) of three independent experiments. Statistical significance was determined by one-way analysis of variance (ANOVA), followed by Dunnett's or least significant difference (LSD)

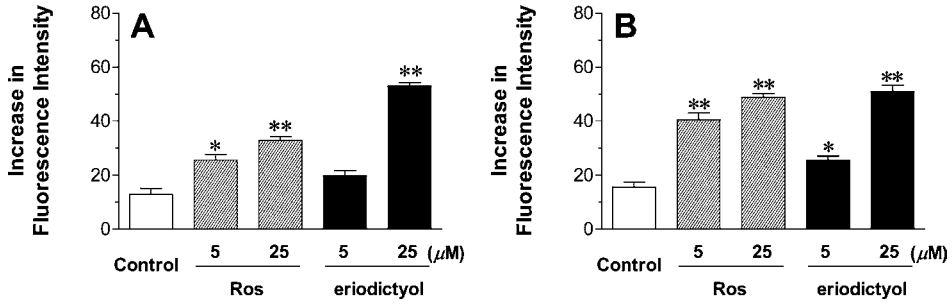

Figure 2. Effect of eriodictyol on insulin-stimulated glucose uptake in (A) HepG2 cells and (B) differentiated 3T3-L1 adipocytes under the high concentration glucose condition. A glucose uptake assay was performed using the fluorescent D-glucose analogue 2-NBDG. HepG2 cells or differentiated 3T3-L1 adipocytes at the concentration of 30 mM glucose were treated with 1 μM insulin in the absence or presence of the indicated concentrations of rosiglitazone or eriodictyol for 1 h, and insulin-stimulated 2-NBDG uptake was measured as described under Materials and Methods. Relative fluorescence intensity minus background was used for subsequent statistical analyses. Values are the mean ± SEM of three independent experiments. (*) $p < 0.05$ or (**) $p < 0.01$ versus control (2-NBDG and insulin cotreated high-concentration glucose group).

post hoc testing, performed using SPSS 13.0 software. $p < 0.05$ was considered to be statistically significant.

RESULTS

Effect of Eriodictyol on Glucose Uptake. To investigate the ability of eriodictyol to increase glucose uptake under high-glucose conditions, a 2-NBDG uptake assay was performed using both HepG2 cells and differentiated 3T3-L1 adipocytes. Rosiglitazone (at concentrations of 5 and 25 μM) significantly increased insulin-stimulated glucose uptake in both HepG2 cells (Figure 2A) and 3T3-L1 adipocytes (Figure 2B) under high-glucose conditions. At a concentration of 25 μM, eriodictyol significantly enhanced the insulin-stimulated uptake of 2-NBDG in HepG2 cells compared to the control (30 mM glucose and 1 μM insulin), with the effect being greater than that for rosiglitazone (Figure 2A). In 3T3-L1 adipocytes, eriodictyol significantly improved insulin-stimulated 2-NBDG uptake at concentrations of both 5 and 25 μM. At a concentration of 25 μM, the stimulatory effect of eriodictyol on glucose uptake in adipocytes was similar to that of rosiglitazone (Figure 2B). Thus, eriodictyol concentration-dependently enhanced insulin-stimulated glucose uptake in both HepG2 cells and differentiated 3T3-L1 adipocytes under high-glucose conditions.

Effect of Eriodictyol on Adipogenesis and Expression of PPARγ2 and aP2. As shown in Figure 3A, eriodictyol and rosiglitazone increased the accumulation of lipid droplets during the differentiation of cells into adipocytes. Statistical analysis (Figure 3B) showed that rosiglitazone (at concentrations of 5 and 25 μM) greatly enhanced lipid accumulation compared to the control and that eriodictyol induced a similar increase. Eriodictyol, at concentrations of 5 and 25 μM, increased lipid accumulation in adipocytes 2.3- and 2.7-fold, respectively. Moreover, eriodictyol at concentrations of 5 and 25 μM increased PPARγ2 gene expression by 52.9 and 111.2%, respectively (Figure 3C) and aP2 expression by 45 and 124%, respectively (Figure 3D). PPARγ2 protein levels were increased by 88% by 25 μM rosiglitazone and by 130% by 25 μM eriodictyol (Figure 3E). Thus, eriodictyol stimulated adipogenesis, increased mRNA expression of PPARγ2 and aP2, and increased PPARγ2 protein levels.

Effect of Eriodictyol on the Insulin-Sensitive Phosphorylation of Akt in Insulin-Resistant HepG2 Cells. To evaluate the ability of eriodictyol to restore insulin-mediated Akt activation in an insulin-resistant state, levels of phosphorylated Akt were measured in HepG2 cells with high-glucose-induced insulin resistance. Insulin significantly increased the phosphorylation of Akt in cells exposed to a normal concentration of glucose (control) (Figure 4A, lanes 1 and 2). In cells exposed to a high glucose concentration, insulin-sensitive Akt phosphorylation significantly decreased compared to control cells, suggesting an insulin-resistant state (Figure 4A, lanes 2 and 4). Rosiglitazone at concentrations of both 5 and 25 μM restored insulin-stimulated Akt phosphorylation in HepG2 cells with high-glucose-induced insulin resistance (Figure 4A, lanes 5 and 6). Similarly, both 5 and 25 μM eriodictyol significantly reactivated insulin-sensitive Akt phosphorylation under the same conditions (Figure 4A, lanes 7 and 8). To assess whether the eriodictyol-mediated amelioration of insulin resistance was attributable to PI3K-induced Akt phosphorylation, HepG2 cells with high-glucose-induced insulin resistance were pretreated with the specific PI3K inhibitor LY294002 for 2 h and then treated with 25 μM eriodictyol or rosiglitazone for 24 h. Insulin-treated cells exposed to a high glucose concentration were used as a control (Figure 4B, lane 2). Pretreatment of cells exposed to a high glucose concentration with LY294002 suppressed insulin-stimulated Akt phosphorylation compared to control cells (Figure 4B, lane 3). In HepG2 cells with high-glucose-induced insulin resistance that were pretreated with LY294002, 25 μM rosiglitazone and eriodictyol did not restore insulin-stimulated Akt phosphorylation (Figure 4B, lanes 4 and 5). These results indicate that the ability of eriodictyol to reverse the inhibition of Akt phosphorylation under conditions of high glucose is mediated by a PI3K signaling pathway.

DISCUSSION

This study has two major findings. First, eriodictyol significantly increased insulin-stimulated glucose uptake in both HepG2 cells and differentiated 3T3-L1 adipocytes. Second, eriodictyol reactivated Akt in HepG2 cells with high-glucose-induced insulin resistance, indicating the improvement of insulin resistance. Although further study is needed to thoroughly explore the mechanisms of action of eriodictyol, this study is the first to link eriodictyol to an increase in glucose uptake and amelioration of insulin resistance, which suggests that eriodictyol may be a useful therapeutic candidate for the management of type 2 diabetes.

The anti-inflammatory properties of flavonoids have been studied to establish and characterize their potential utility as

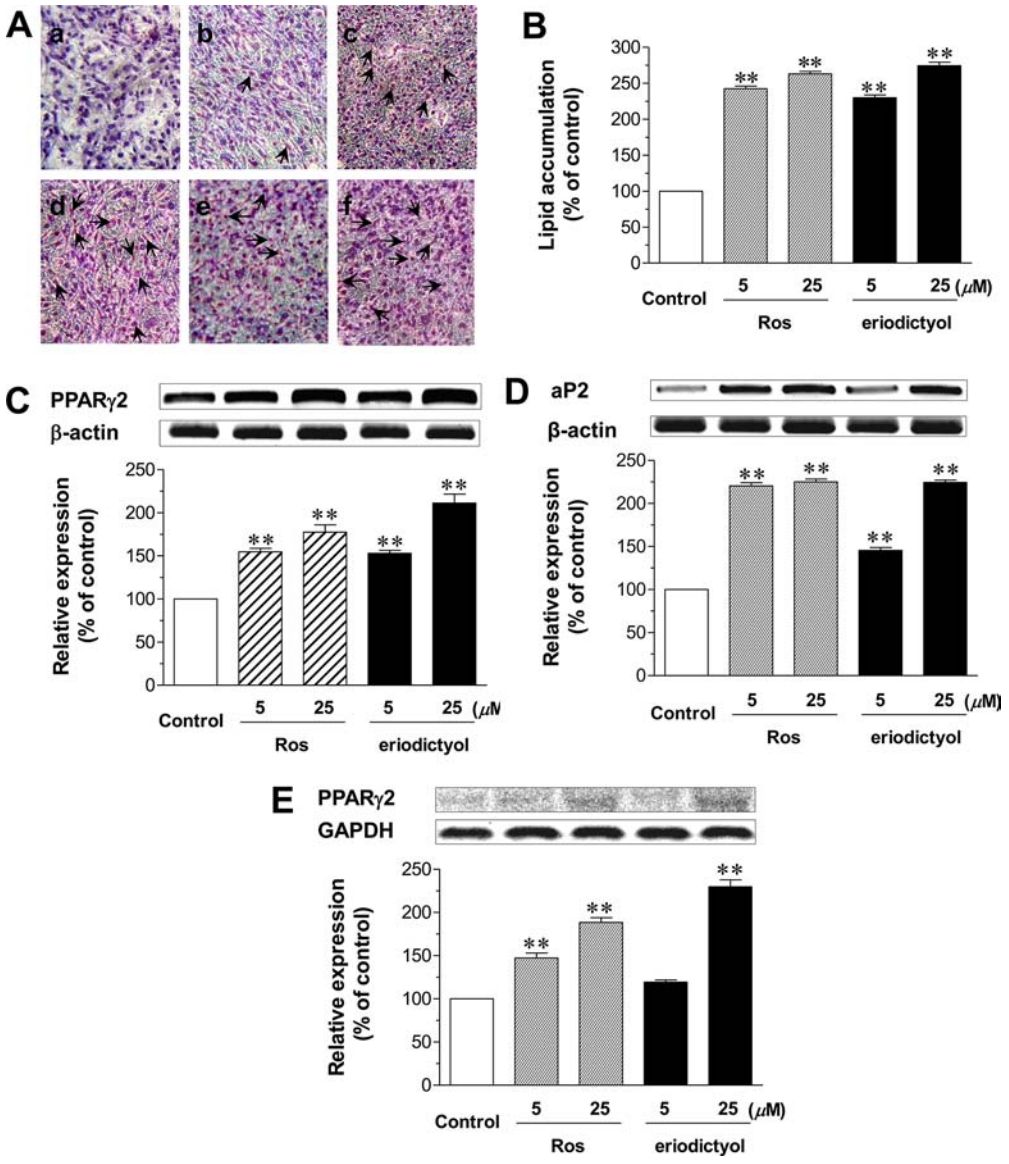

Figure 3. Effect of eriodictyol on adipogenesis and expression of PPARγ2 and aP2 in differentiated 3T3-L1 adipocytes. (A) Representative images of Oil Red O-stained (a) preadipocytes, (b) control cells (differentiated 3T3-L1 adipocytes), and cells treated with (c) 5 μM rosiglitazone (Ros), (d) 25 μM Ros, (e) 5 μM eriodictyol, or (f) 25 μM eriodictyol. (B) Effect of eriodictyol on lipid accumulation in differentiated 3T3-L1 adipocytes. 3T3-L1 preadipocytes were induced to differentiate into adipocytes, and lipid accumulation was measured as described under Materials and Methods. The optical density at 510 nm, a measure of lipid accumulation, was set to 100% in the control group. (C, D) Effects of eriodictyol on the mRNA expression of (C) PPARγ2 and (D) aP2 in differentiated 3T3-L1 adipocytes. Total RNA was extracted from differentiated adipocytes, and RT-PCR was performed as described under Materials and Methods. Bands corresponding to the expected PCR products were analyzed by densitometry, and the expression levels of PPARγ2 and aP2 were normalized to that of β-actin. Results are expressed as a percentage of control normalized expression. Upper panels display gel profiles that are representative of three independent experiments. (E) Effects of eriodictyol on PPARγ2 protein levels in differentiated 3T3-L1 adipocytes. To assess PPARγ2 protein expression, cell lysates from differentiated adipocytes were prepared, and Western blotting was performed as described under Materials and Methods. Immunoreactive bands were analyzed by densitometry. The PPARγ2 protein level was normalized to that of GAPDH and expressed as a percentage of the control group. Upper panels display blots that are representative of three independent experiments. Data shown in the bar graph are the mean ± SEM. (**) $p < 0.01$ versus control.

therapeutic agents in the treatment of diabetes.[20] Antioxidants could prevent the development of diabetes and be important in its treatment.[26,27] These observations provide a positive association between eriodictyol and management of diabetes. Previous studies reported that the flavonoid aromadendrin, which has anti-inflammatory[22] and antioxidant properties,[28] had a stimulatory effect on insulin-induced glucose uptake and ameliorated insulin resistance.[19] Considering that eriodictyol has potent anti-inflammatory[22] and antioxidant activity,[23] it may possess antidiabetic properties. In this study, eriodictyol enhanced insulin-stimulated glucose uptake in HepG2 cells and differentiated 3T3-L1 adipocytes under high-glucose conditions (Figure 2), which is a reflection of its glucose-lowering effect.

The expression of PPARγ in adipocytes has been reported to be associated with adipogenesis and activation of insulin-induced glucose transport.[10] Rosiglitazone increased the expression of PPARγ2 and aP2 mRNAs and PPARγ2 protein in differentiated adipocytes concurrent with adipogenesis (Figure 3). This observation suggests that the increase in PPARγ2 expression and adipogenesis may contribute to the

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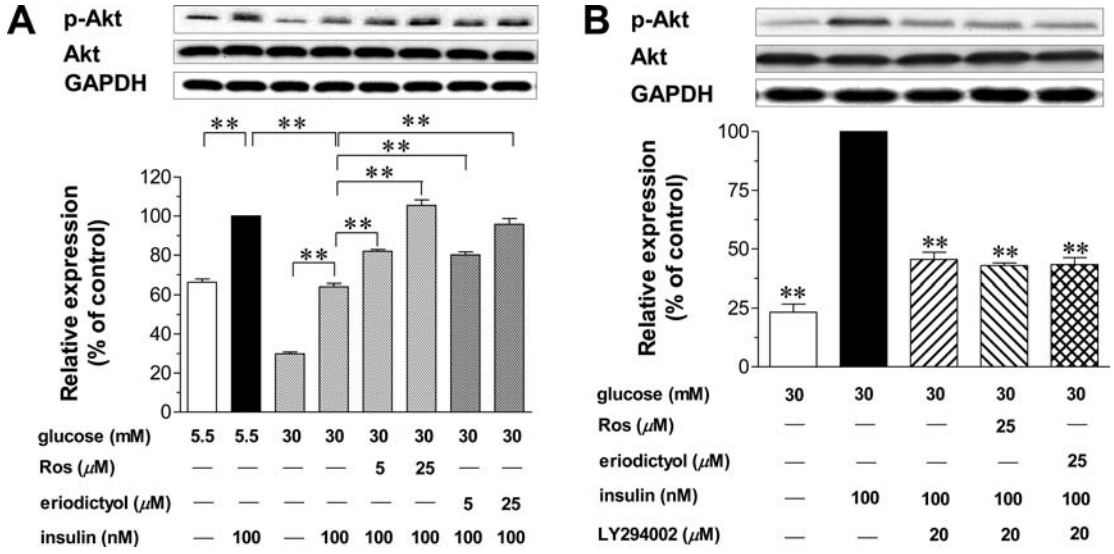

Figure 4. Eriodictyol reactivates insulin-stimulated Akt phosphorylation in HepG2 cells with high-glucose-induced insulin resistance. (A) Effect of eriodictyol on Akt phosphorylation in HepG2 cells with high-glucose-induced insulin resistance. Insulin resistance was induced in HepG2 cells by treatment with 30 mM glucose as described under Materials and Methods. Insulin-resistant HepG2 cells were treated with the indicated concentrations of rosiglitazone (Ros) or eriodictyol for 24 h, and the effect of insulin (100 nM for 10 min) on Akt phosphorylation was measured by Western blotting. The relative level of phosphorylated Akt was calculated as a percentage of the level in control cells (exposed to 5.5 mM glucose plus 100 nM insulin). (**) $p < 0.01$. (B) Effect of LY294002 on eriodictyol-mediated reactivation of insulin-stimulated Akt phosphorylation in HepG2 cells with high-glucose-induced insulin resistance. HepG2 cells were pretreated with 20 μM LY294002 for 2 h prior to treatment for 24 h with 25 μM eriodictyol or Ros in the presence of 30 mM glucose. Whole-cell lysates were subjected to Western blot analysis of Akt and phospho-Akt. The density ratio of phospho-Akt to GAPDH in control cells (exposed to 30 mM glucose plus 100 nM insulin) was set to 100%. Data are expressed as the mean ± SEM of three independent experiments. Upper panels show representative blots. (**) $p < 0.01$ versus the control.

enhancement of insulin-stimulated glucose uptake and subsequently improve insulin sensitivity. At a concentration of 25 μM, eriodictyol promoted adipogenesis and increased PPARγ2 and aP2 mRNA expression and PPARγ2 protein levels, with its pharmacological efficacy being comparable to that of rosiglitazone. These results suggest that the eriodictyol-induced changes in gene expression and adipogenesis may activate insulin-stimulated glucose uptake in adipocytes.

The binding of insulin to its receptor induced the activation of a complex network of downstream molecules, including PI3K and the serine/threonine kinase Akt.[29,30] In an insulin-resistant state, the PI3K/Akt pathway is not activated by insulin as much as under normal conditions. Treatment of insulin-treated HepG2 cells with a high concentration of glucose (30 mM) induced less activation of Akt than treatment with a normal concentration of glucose (5.5 mM), which indicates an insulin-resistant state (Figure 4A, lanes 2 and 4). At a concentration of 25 μM, eriodictyol restored insulin-stimulated Akt phosphorylation in insulin-resistant HepG2 cells. Pretreatment with LY294002 prevented the increase in Akt phosphorylation mediated by eriodictyol in insulin-resistant HepG2 cells. These results demonstrate the ability of eriodictyol to improve insulin resistance by reactivating the insulin-induced Akt phosphorylation under insulin-resistant conditions. Previous studies reported that PI3K activation stimulates glucose uptake.[31,32] Our results thus suggest that eriodictyol-induced improvement of insulin resistance via the PI3K/Akt pathway may be associated with the stimulation of insulin-sensitive glucose uptake by eriodictyol.

It was reported that eriodictyol activated Nrf2 and enhanced the expression of the phase 2 proteins in human ARPE-19 cells.[33] Recently, rosiglitazone was revealed to activate the Nrf2 pathway in a PPARγ-dependent manner,[34] which might protect human normal hepatocyte cell line (QZG) from high glucose. Much further study is needed to elucidate whether eriodictyol activates Nrf2 in a PPARγ-dependent manner to reduce high-glucose-induced oxidative injury and then contribute to increasing glucose uptake and ameliorating insulin resistance.

It was indicated that one important cellular pathway affected by polyphenols was the activation of the transcription factor Nrf2 via the electrophile response element, which mediates generation of phase 2 detoxifying enzymes.[35] However, phenolic compounds including flavonoids are readily oxidized in cell culture media to generate hydrogen peroxide.[36−38] Therefore, generation of hydrogen peroxide must be taken into account when the effects of phenolic compounds on cells in culture are interpreted.[36]

In summary, the data presented in this paper demonstrate that eriodictyol promoted insulin-stimulated glucose uptake in HepG2 cells and differentiated 3T3-L1 adipocytes and increased PPARγ2 expression in differentiated 3T3-L1 adipocytes. Furthermore, it prevented the inhibition of insulin-induced PI3K-dependent Akt phosphorylation in HepG2 cells with high-glucose-induced insulin resistance, which may, at least in part, be responsible for the ability of eriodictyol to stimulate insulin-induced glucose uptake. Therefore, our observations suggest that eriodictyol may be an attractive candidate as a glucose-lowering and insulin resistance-improving agent for the treatment of diabetes.

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Notes

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■ ABBREVIATIONS USED

2-NBDG, 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose; ANOVA, analysis of variance; aP2, adipocyte-specific fatty acid-binding protein; DMEM, Dulbecco's modified Eagle's medium; ECL, enhanced chemiluminescence; FCS, fetal calf serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LSD, least significant difference; PI3K, phosphoinositide 3-kinase; PBS, phosphate-buffered saline; PPARγ2, peroxisome proliferator-activated receptor γ2; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; RT-PCR, reverse transcription−polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; SEM, standard error of the mean.

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