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Daidzein protects endothelial cells against high glucose-induced injury through the dual-activation of PPARα and PPARγ

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Abstract. Endothelial damage caused by persistent glucose and lipid metabolism disorders is the main reason of diabetic vascular diseases. Daidzein exerts positive effects on vascular dysfunction. Peroxisome proliferator-activated receptors (PPARs) regulate critically glucose and lipid metabolism. However, the interaction of daidzein to PPARs is still insufficiently explored. In this study, the cell proliferation was detected by EdU. The intrinsic activity and binding affinity of daidzein for human PPARs (hPPARs) were estimated by transactivation reporter gene test and HPLC-UV method, respectively. Daidzein significantly reversed high glucose (HG, at 30 mmol/l)-induced injury in HUVECs, which was inhibited by both PPARα and PPARγ antagonist, but no PPARβ antagonist. Daidzein selectively activated hPPARα and hPPARγ_1, but weakly hPPARβ. Additionally, daidzein also bound to both hPPARα and hPPARγ_1. The findings suggested that daidzein may be a PPARα and PPARγ dual-agonist. The amelioration of daidzein on HUVECs from hyperglycemia may be mediated by the activation of PPARα and PPARγ receptors.

Key words: Daidzein — Eendothelial cells — PPARs — Intrinsic activity — Binding affinity

Abbreviations: DMEM, Dulbecco's modified eagle medium; EdU, 5-ethynyl-2-deoxyuridine; HG, high glucose; HO-1, heme oxygenase-1; hPPARs, human PPARs; HUVECs, human umbilical vein endothelial cells; iNOS, inducible nitric oxide synthase; MEC, molecular exclusion chromatography; NB, non-specific binding; PPARs, peroxisome proliferator-activated receptors; pRL-TK, p renilla luciferase-thymidine kinase; SB, specific binding; TB, total binding.

Highlights

- Daidzein protected HUVECs from hyperglycemia.
- Daidzein activated hPPARα and hPPARγ_1, but weakly hPPARβ.
- Daidzein had the binding affinity to hPPARα and hPPARγ_1.
- Daidzein is a potential PPARα/γ dual-agonist.

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Introduction

Diabetes is recognized as an emerging global epidemic, representing one of the leading causes of morbidity and mortality worldwide (Lovic et al. 2020). Hyperglycemia and lipid metabolism disorders, the common characteristic of both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), have the potential to cause serious complications due to its insidious and chronic nature (Liang et al. 2021). In particular, endothelial dysfunction is an important cardiovascular risk factor and plays a key role in the occurrence and development of diabetes and its complications (Shi and Vanhoutte 2017). Studies have shown that vascular endothelial injury caused by persistent glucose and lipid metabolism disorders may be the main cause of diabetic vascular diseases, but there is still a lack of effective prevention and treatment targets.

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. The PPARs subfamily consists of three isotypes, PPARα, PPARβ (*i.e.* PPARδ) and PPARγ (Wagner and Wagner 2020). PPARs are critically involved in the regulation of a large number of genes about glucose, lipid, energy metabolism, cell proliferation, inflammation and vascular function (Gross et al. 2017; Shi et al. 2020). Therefore, it is considered as an important therapeutic target for diabetes mellitus, obesity and cardiovascular diseases. Activation of PPARα alleviates vascular endothelial dysfunction by reducing endoplasmic reticulum stress and activating endothelial nitric oxide synthase in streptozotocin-induced diabetic rats (Yao et al. 2021). PPARβ activation is conferred vascular protection against hyperglycemia-induced oxidative stress by suppressing the levels of reactive oxygen species and directly inducing the expression of heme oxygenase-1 (HO-1) (Jimenez et al. 2018). PPARγ has at least 4 subtypes, γ_1, γ_2, γ_3, and γ_4, among which subtype γ_1 is the main in humans (Li et al. 2016). Existing research has shown that PPARγ modulates oxidative/nitrative stress to improve endothelial function in diabetes (Dong et al. 2021). So, PPARs may regulate endothelial function in the environment of glucose and lipid metabolism disorder and may have a vascular protective effect in diabetic conditions. At present, the PPARα agonist, fibrates, has been used in dyslipidemia, and the PPARγ agonist, thiazolidinediones, also have been used in diabetes in medical practice. However, fibrates have been associated with the risk of myopathy, cholelithiasis, venous thrombosis, and decreased renal function (Davidson et al. 2007). Thiazolidinediones have been associated with weight gain and peripheral edema (Peters 2009). There are no PPARβ agonists to be approved in clinical due to the adverse reactions. Therefore, the search for safety and efficacy of PPARs agonists is still the focus in the field of diabetes and its complications.

Dietary soy, an important source of fiber, phytosterols, isoflavone, lecithin and proteins, has been shown to improve glycemic control, serum lipid levels and atherosclerosis in human and animal studies (Umeno et al. 2016; Mirzai and Laffin 2023). Isoflavones, one of the constituents of soy, are a group of polyphenolic compounds that have a variety of biological actions, such as in the reduction of serum cholesterol levels, reduction in the risk for breast cancer and osteoporosis in women, and alleviation of the disturbances caused by menopause (Křížová et al. 2019). Daidzein, one of the primary isoflavones, has a wide range of health benefits. Research reported that daidzein has a positive effect on T2DM-related dyslipidemia and vascular inflammation (Das et al. 2018). Chronic administration of daidzein, improved endothelial dysfunction in streptozotocin-induced diabetic rats (Roghani et al. 2013). Noteworthily, daidzein has a promising therapeutic potential in managing T2DM pathophysiology, including on the improvement of hyperglycemia, insulin resistance, dyslipidemia, obesity and inflammation (Zang et al. 2015; Das et al. 2018), which is similar to the effects of PPAR subtypes activation. However, whether the effect of improving endothelial dysfunction in diabetes is related to the activation of PPARs remains to be further explored, especially, the investigation of the receptor-ligand interaction between daidzein and PPARs is still lacking so far.

Materials and Methods

Chemicals and reagents

Daidzein ($C_{15}H_{10}O_4$; MW: 254.24; purity ≥ 98%, HPLC-grade) was purchased from National Institutes for Food and Drug Control (Beijing, China). Human umbilical vein endothelial cells (HUVECs) were from Sevier Biotechnology Co. Ltd (Hubei, China). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum were from Beyotime Biotechnology Co. Ltd. (Shanghai, China). Hoechst 33342 was from Invitrogen (CA, USA). GW6471 and GSK0660 were purchased from Abcam (Cambridge, UK), GW9662 and 5-ethynyl-2-deoxyuridine (EdU) were purchased from Sigma Aldrich (Santa Clara, CA, USA). p human PPARs-internal ribosome entry site 2-enhanced green fluorescent protein (phPPARs-IRES2-EGFP) was constructed by Dr. Zhang T (Chongqing Medical University, China), p thymidine kinase-peroxisome proliferator response element × 3-luciferase (pTK-PPRE×3-Luc) was presented by Prof. He T kindly (University of Chicago, USA) and p renilla luciferase-thymidine kinase (pRL-TK) was purchased from Promega (WI, USA). Purified maltose binding protein-tag human PPARs ligand binding domain (MBP-hPPARs-LBD) was constructed by Ms. Tian M (Chongqing Medical Uni-

versity, China). CV-1 cells were purchased from Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). LIPOFECTAMINE 2000 was purchased from Thermo Fisher Scientific (Shanghai, China). Fenofibrate, rosiglitazone and arachidonic acid were from Cayman Chemical (MI, USA). The remaining reagents were analytical grade.

Cell cultures

HUVECs were prepared and cultured in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin in a humid atmosphere of 95% O_2 and 5% CO_2 at 37°C. Cells were used between passages 3 and 5 for the experiments and were serum-starved before treatment with reagents for 24 h. The cells in the logarithmic growth stage were cultured in normal medium (glucose at 5 mmol/l with mannitol at 25 mmol/l) or the cells were stimulated with high glucose (HG, glucose at 30 mmol/l) medium with or without daidzein (0.1, 1, 10 μmol/l) for another 24 h. GW6471 (PPARα antagonist), GSK0660 (PPARβ antagonist), or GW9662 (PPARγ antagonist) at 10 μmol/l, respectively, were administered 0.5 h prior to high glucose exposure for further investigating the relationship of PPARs on the effects of daidzein.

Proliferation assays by EdU

The cells were treated with EdU (200 ng/ml) for 2 h as the manufacturer's instructions. The cells were fixed by 4% paraformaldehyde at 25°C for 10 min, and then incubated in Click-iT reaction buffer, $CuSO_4$, Alexa Fluor 555, and reaction buffer additive for 30 min. All the cell samples were incubated with Hoechst 33342 for 10 min at 37°C to stain cell nuclei. Then they were observed and imaged using immunofluorescent confocal microscopy (Nikon, Japan). EdU staining images were captured to quantify the percentage of cell proliferation. The number of EdU and Hoechst 33342 positive cells were counted by the ImageJ software cell count tool. The percentage of positive cells = (the number of EdU positive cells)/(the number of Hoechst 33342 positive cells) × 100%.

Intrinsic activity of daidzein to hPPARs by transactivation reporter gene assays

The activity of daidzein to hPPAR subtypes was determined by transactivation activity based on cell reporter-gene assay as previously described (Kliewer et al. 1992; Zhang et al. 2007; Yu et al. 2016) with minor modification. Briefly, the expression vectors, phPPARs-IRES2-EGFP, pTK-PPRE×3-Luc and pRL-TK, were co-transfected into CV-1 cells with LIPOFECTAMINE 2000 according to the manufacturer's instruction and our pre-experiment (Luo et al. 2012). The transfected cells were cultured with or without drugs [fenofibrate (PPARα agonist, as a positive control to PPARα and negative control to PPARγ), rosiglitazone (PPARγ agonist, as a positive control to PPARγ and negative control to PPARα) or daidzein from 0.1 to 100 μmol/l, respectively] for 48 h and then the luciferase activity of the cell lysate was measured with a Gloma 20/20 Luminometer (Promega, 2030-101) using the dual luciferase assay system.

The activities of fenofibrate, rosiglitazone and daidzein for hPPARs were indicated by the median effective dose (half-maximal effect concentration, EC_{50}) and the maximum activity multiple (E_{max}) according to the classic occupation theory of Clark. EC_{50} was calculated by the concentration-response curve of the drug. E_{max} was determined by the normalized luciferase activity as fold-activation based on that of untreated cells. All points were performed in triplicate and varied in less than 10% range.

Binding of daidzein to hPPARs by receptor-ligand HPLC-UV assays

As previously described (Li et al. 2008; Tian et al. 2008), purified MBP-hPPARs-LBD 40 μg was incubated with 0.4 or 1.0 μmol/l of fenofibrate, rosiglitazone in the absence (non-specific binding, NB) or presence (total binding, TB) of 500 μmol/l arachidonic acid, or daidzein in the absence or presence of 500 μmol/l fenofibrate or rosiglitazone for 60 min at 25°C in a total volume of 0.3 ml. The receptor-ligand reaction was terminated by bathing on ice for 5 min. Each sample of 100 μl was used for HPLC-UV analysis (Jiang and Zhou 2010).

The HPLC chromatographic conditions were performed on a HiTrap desalting column (Sephadex G-25, 16×25 mm, Amers, Swiss) in an HPLC system (Waters Technologies Co. Ltd., USA) equipped with a 600 pump, 2487 double-channel UV detector, Rheodyne7725i injection valve, 100 μl sample loop and Alltech ChromStation V5.3 (Alltech Co. Ltd., USA), phosphate-buffered saline (PBS (g/l): NaCl 8.0, Na_2HPO_4 1.15, KCl 0.2, KH_2PO_4 0.2, pH 8.4) as mobile phase at a flow rate of 1.0 ml/min with a column temperature of 8°C and injection volume 20 μl, detection wavelength 286 nm for fenofibrate, 247 nm for rosiglitazone, 254 nm for daidzein, respectively, and the total run time for each sample analysis was 35 min. The first peak may be interfered by the signal absorption from the receptor and the dissociation of the receptor-ligand complex. So, the second peak was adopted to calculate the specific binding (SB), which was subtracted the peak area of NB from TB. Each assay was carried out in triplicate.

Statistical analysis

Results are expressed as mean ± SE. Statistical analyses were performed using the SPSS program (SPPS, Inc.,

Chicago, IL, USA). The experimental data were initially analyzed for normal distribution by Shapiro-Wilk, followed by one-way analysis of variance and post hoc further analysis by Bonferroni. Unless otherwise stated, all other experimental data passed Levene's test for homogeneity of variances.

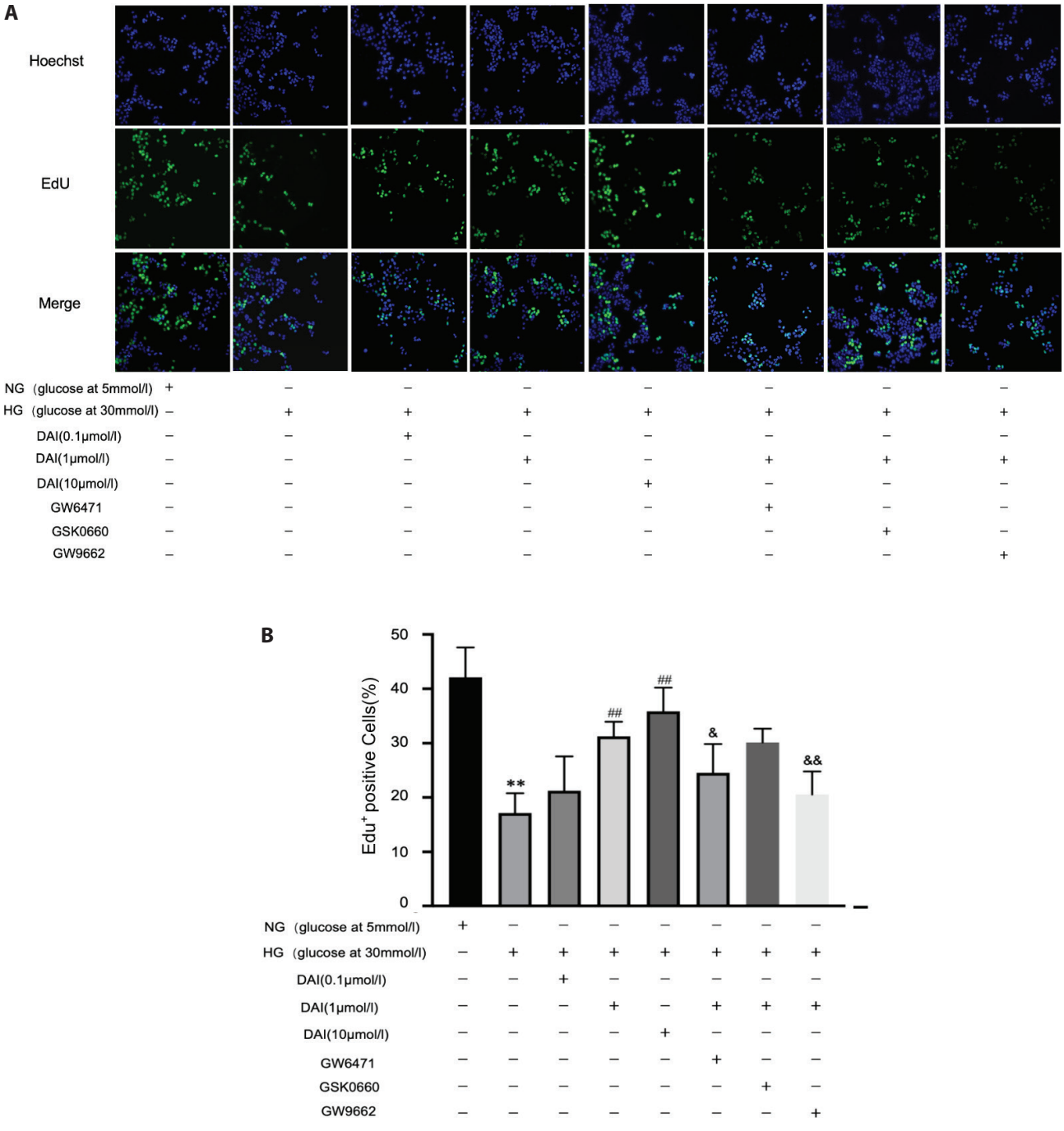

Figure 1. Effect of daidzein (DAI) on the proliferation of HUVECs incduced by glucose at 30 mmol/l (HG). **A.** The images of EdU^+ positive cells were captured by fluorescent microscope (magnification 20×). **B.** The percentage of positive cells is equal to (the number of EdU^+ positive cells)/(the number of Hoechst 33342 positive cells) × 100%. NG, glucose at 5 mmol/l with mannitol at 25 mmol/l; HG, glucose at 30 mmol/l. All data are means ± SE, $n = 3$. ** $p < 0.01$ *vs.* NG; $^{\#\#}$ $p < 0.01$ *vs.* HG; $^{\&}$ $p < 0.05$, $^{\&\&}$ $p < 0.01$ *vs.* DAI (1 μmol/l) treatment group.

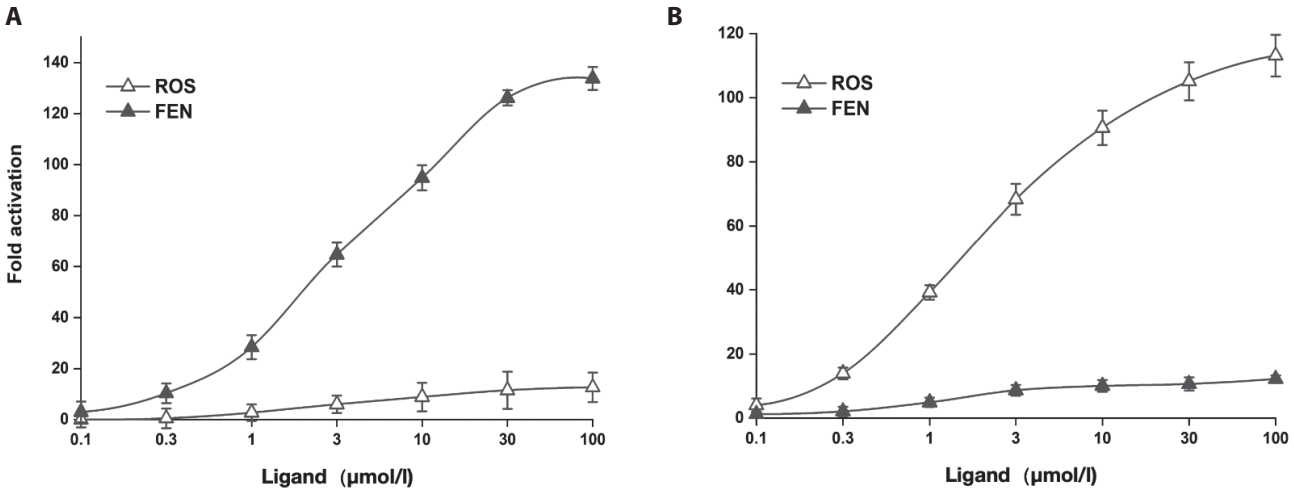

Figure 2. Concentration-activity curves of fenofibrate (FEN) and rosiglitazone (ROS) in transactivation assays for hPPARα (**A**) or hPPARγ$_1$ (**B**). The EC_{50} and E_{max} values were 3.4 μmol/l and 135.2 on fenofibrate to hPPARα, and 2.5 μmol/l and 123.7 on rosiglitazone to hPPARγ$_1$, respectively. All data are means ± SE, $n = 3$.

Results

Effect of daidzein on the HUVECs proliferation damaged by HG

HG (glucose at 30 mmol/l) significantly reduced the HUVEC proliferation compared with that of the normal control (glucose at 5 mmol/l) ($p < 0.01$). Supplementation with daidzein (0.1, 1, and 10 μmol/l) ameliorated HG-decreased cell proliferation in a concentration-dependent manner. Compared with HG group, daidzein (0.1 μmol/l) improved HUVEC proliferation, but there was no significant difference. Exposure to daidzein 1 μmol/l and 10 μmol/l significantly increased HUVEC proliferation ($p < 0.01$). The effect of daidzein (1 μmol/l) was reversed by both PPARα antagonist GW6471 and PPARγ antagonist GW9662 ($p < 0.05$). However, PPARβ antagonist GSK0660 had little effect on the protection of daidzein in HUVEC proliferation (Fig. 1).

Selective activation of daidzein to hPPARs

Fenofibrate activated hPPARα, but not hPPARγ$_1$, in a concentration-dependent manner from 0.1 to 100 μmol/l (Fig. 2A), which EC_{50} was 3.4 μmol/l and E_{max} was 135.2 (Table 1). In contrast, rosiglitazone activated hPPARγ$_1$, but not hPPARα (Fig. 2B), in which EC_{50} was 2.5 μmol/l and E_{max} was 123.7 (Table 1). Daidzein activated all hPPARs in a concentration-dependent manner (Fig. 3). The EC_{50} and E_{max} values were 3.7 μmol/l and 120.3 to hPPARα, 2.7 μmol/l and 102.1 to hPPARγ$_1$, respectively (Table 1). However, daidzein activated weakly hPPARβ (EC_{50} = 13.5 μmol/l, E_{max} = 16.4) (Table 1). The differences among hPPARα, hPPARγ$_1$ and hPPARβ revealed that daidzein could activate PPARα and PPARγ$_1$, but not PPARβ.

Binding ability of daidzein to hPPARS

Fenofibrate was used to evaluate the reliability of HPLC-UV under optimal chromatographic conditions. The wide linear ranges (from 0.2 μmol/l to 100 μmol/l, *i.e.*

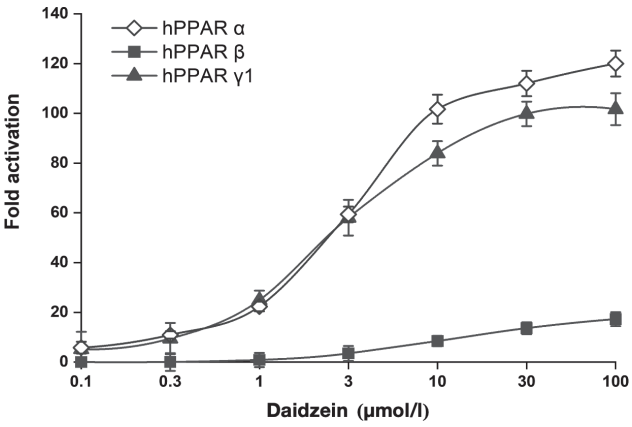

Figure 3. Concentration-activity curves of daidzein in transactivation assays for hPPARs. Daidzein activated hPPARs in a concentration-dependent manner from 0.1 to 100 μmol/l. The EC_{50} and E_{max} values were 3.7 μmol/l and 120.3 to hPPARα, 2.7 μmol/l and 102.1 to hPPARγ$_1$, respectively. However, the activation of daidzein to hPPARβ was weak, the EC_{50} was 13.5 μmol/l, and E_{max} was 16.4. All data are means ± SE, $n = 3$.

Table 1. The activity of daidzein to hPPARs

	hPPARα		hPPARβ		hPPARγ$_1$	
	EC_{50} (μmol/l)	E_{max} (Fold activation)	EC_{50} (μmol/l)	E_{max} (Fold activation)	EC_{50} (μmol/l)	E_{max} (Fold activation)
Fenofibrate	3.4	135.2	NSA	NSA	NSA	NSA
Rosiglitazone	NSA	NSA	NSA	NSA	2.5	123.7
Daidzein	3.7	120.3	13.5	16.4	2.7	102.1

E_{max}, the maximum activity multiple; NSA, no significant activity.

from 1.4 ng to 720 ng) was obtained with correlation coefficients (r^2) at 0.9998. The retention times were 5.6, 13.9, 23.7 min for fenofibrate, rosiglitazone and daidzein, respectively. The analytical method was highly specific to entirely separate the ligands and receptors. No apparent interference was observed in the samples. The limits of detection, calculated on the base of signal/nose (S/N) of 3, were 72 ng/ml and the limits of quantification (S/N = 10) were 144 ng/ml for all analytes. The developed methods are sensitive enough to measure these analytes. The precision was based on peak areas expressed as relative standard deviations in %. Intra-day precision was ≤ 5.34%, whereas inter-day precision was ≤ 6.92%. The accuracy expressed as relative recovery was ranged from 88.12% to 110.55% for all analytes. All the compounds were stable for 24 h as relative standard deviations of peak area within 8.21%, the processed samples stored at 4°C should be analyzed within 24 h to guarantee a high level of stability and reliable calculations. These values showed that the method is responsive and suitable for the determination of the selected analytes.

Typical overlay chromatograms of fenofibrate or rosiglitazone with same concentrations (0.4 and 1.0 μmol/l) binding to MBP-hPPARα were shown in Figure 4 and Table 2. The SB of fenofibrate binding to hPPARα was elevated significantly with the increasing concentrations ($p < 0.01$), however, the SB of rosiglitazone binding to hPPARα remained stable. In contrast, the SB of rosiglitazone binding to hPPARγ$_1$ was elevated with the increase of drug ($p < 0.01$), but not of fenofibrate. Similar to the effect of fenofibrate to hPPARα and rosiglitazone to hPPARγ$_1$, the SBs of daidzein were increased with the accumulated concentrations, from 6445 to 10933 for hPPARα ($p < 0.01$), and from 1905 to 28246 for hPPARγ$_1$ ($p < 0.01$), respectively, which suggested that daidzein can bind to both hPPARα and hPPARγ$_1$ (Fig. 5, Table 2).

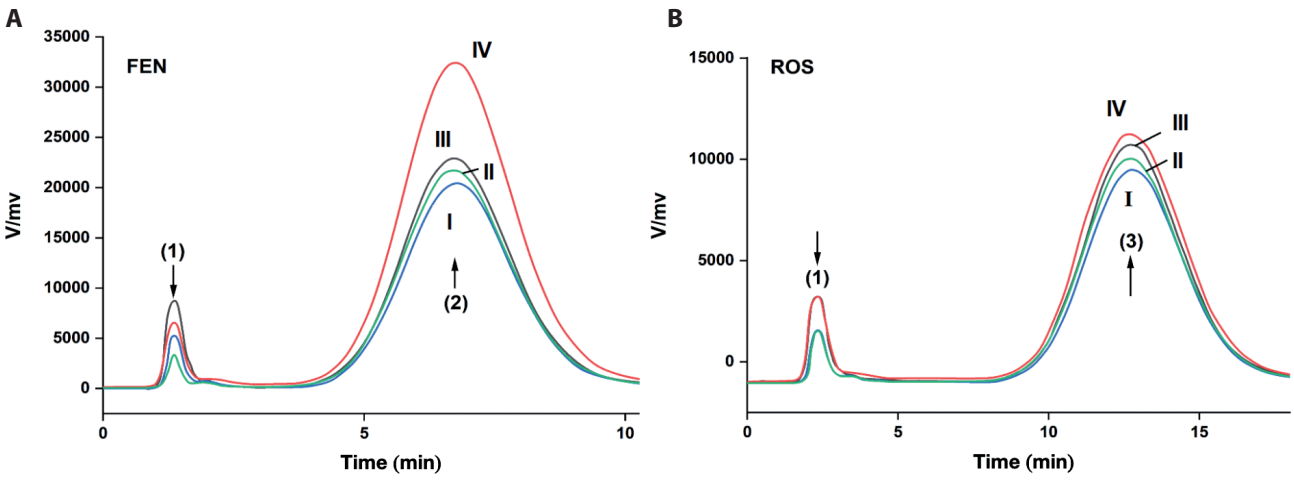

Figure 4. Typical overlay chromatograms of fenofibrate (FEN, **A**) and rosiglitazone (ROS, **B**) bound with MBP-hPPARα-LBD. Purified MBP-hPPARα-LBD 40 μg was incubated with 0.4 μmol/l (I, II) or 1.0 μmol/l (III, IV) of FEN or ROS in the absence (I, III) or presence (II, IV) of 500 μmol/l arachidonic acid (AA). The receptor protein linking FEN or ROS was eluted first (1), free FEN second (2), and free ROS last (3) at 1.5, 5.6, and 13.9 min, respectively. The specific binding (SB) for 0.4 μmol/l FEN or ROS to hPPARα could be calculated by subtracting the peak area of I (absence of AA, non-specific binding) from II (presence of AA, total binding), and the SB for 1.0 μmol/l FEN or ROS also could be obtained by subtracting III peak area (absence of AA) from IV (presence of AA).

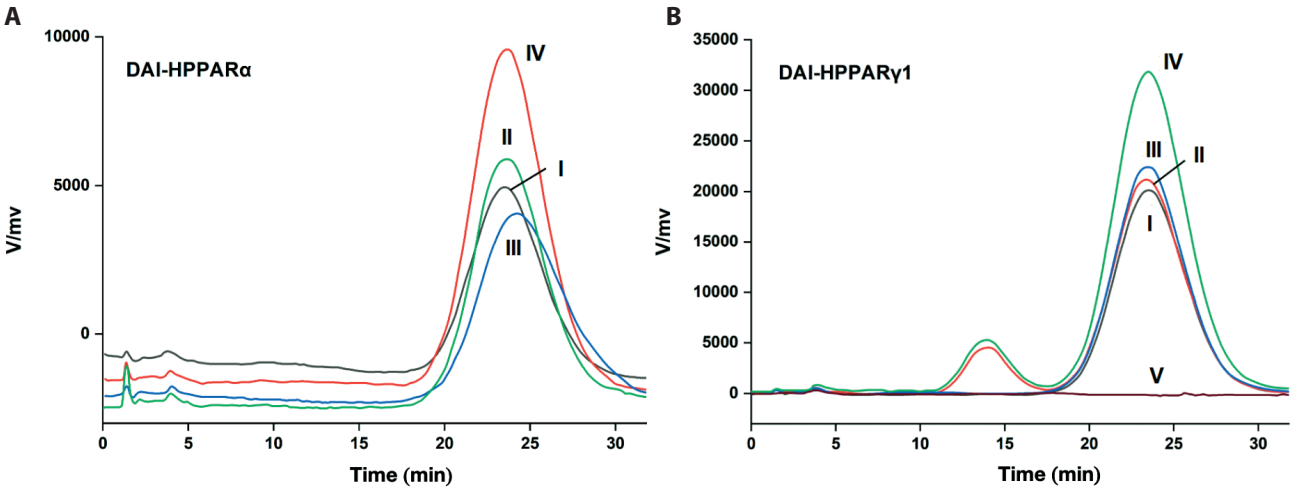

Figure 5. Typical overlay chromatograms of daidzein (DAI) bound with MBP-hPPARα/γ_1-LBD. **A.** DAI bound with MBP-hPPARα-LBD. **B.** DAI bound with MBP-hPPARγ_1-LBD. Purified MBP-hPPARα/γ_1-LBD 40 μg was incubated with 0.4 μmol/l or 1.0 μmol/l of DAI in the absence (I, III) or presence (II, IV) of 500 μmol/l fenofibrate (A) or rosiglitazone (B). Free DAI was eluted at 23.7 min. The specific binding (SB) for 0.4 μmol/l DAI to hPPARα was calculated by subtracting the peak area of I (absence of 500 μmol/l fenofibrate, non-specific binding) from II (presence of 500 μmol/l fenofibrate, total binding), and SB for 1.0 μmol/l DAI also could be obtained by subtracting III peak area (absence of 500 μmol/l fenofibrate) from IV (presence of 500 μmol/l fenofibrate). A similar procedure was done when fenofibrate was replaced by rosiglitazone, then the SBs of DAI at 0.4 and 1.0 μmol/l to hPPARγ_1 were obtained. The V line was produced by the sample only containing rosiglitazone.

Discussion

This study explored the efficacy of daidzein on HG-induced HUVECs, and identified the receptor-ligand interaction of daidzein to PPARs for the first time. Daidzein increased HG-damaged cell proliferation, which was inhibited by both PPARα and PPARγ antagonist, but no PPARβ antagonist. Daidzein selectively activated hPPARα and hPPARγ_1, but weakly hPPARβ. Additionally, daidzein also bound to both hPPARα and hPPARγ_1. Our findings suggested that daidzein may be a PPARα and PPARγ dual-agonist. The amelioration of daidzein on HUVECs from hyperglycemia may be mediated by the activation of PPARα and PPARγ receptors.

Diabetes has become one of the most prevalent public health problems (Sun et al. 2022). Vascular injury plays a crucial role in the appearance and deterioration of macro- and micro-vascular complications in diabetes patients (Peng et al. 2020). The damage of the endothelium caused by continuous hyperglycemia may be the essential pathological basis of diabetic vascular diseases. Some studies have confirmed daidzein has helpful effects on improving hyperglycemia, insulin resistance, dyslipidemia, and obesity, which indicated that daidzein may have good prevention and treatment effects on diabetes and its complications (Das et al. 2018). This study showed that daidzein could ameliorate the decrease of HUVECs proliferation under high glucose conditions in a dose-dependent manner, suggesting that daidzein may have a protective effect on endothelial cells damaged by hyperglycemia.

A large number of studies have shown that activation of PPARs is closely related to improving blood glucose, lipid metabolism and endothelial cell function in diabetes mellitus (Li et al. 2016; Jimenez et al. 2018; Yao et al. 2021). The effects of each subtype of PPAR receptor can complement with each

Table 2. The peak area of specific binding of daidzein bound with MBP-hPPARs-LBD

Analytes	Receptor			
	hPPARα		hPPARγ_1	
	0.4 μmol/l	1.0 μmol/l	0.4 μmol/l	1.0 μmol/l
Fenofibrate	2671 ± 15	3233 ± 20**	620 ± 28	704 ± 23
Rosiglitazone	1542 ± 49	1570 ± 35	2984 ± 62	6662 ± 60**
Daidzein	6445 ± 85	10933 ± 94**	1905 ± 45	28246 ± 52**

** $p < 0.001$ *vs.* 0.4 μmol/l hPPARα/hPPARγ_1. Data are means ± SE, $n = 3$.

other. Therefore, dual and three PPARs agonists are also hot issues in metabolic diseases. However, many dual or three PPARs agonists failed during the preclinical stage or the clinical development stage due to the lack of efficacy or safety (Cheng et al. 2019). The search for safe and effective PPARs agonists, especially dual or three, is still the focus in the field of diabetes and its complications. Daidzein is one of the main bioactive substances isolated from leguminous plants, such as *Pueraria lobata (Willd.) Ohwi, Trifolium pretense L., Medicago sativa L., and Glycine max L. Merr*, which has multiple pharmacological actions. In humans, daidzein has been used in medicine for menopausal relief, osteoporosis, and lowering the risk of some hormone-related cancers due to its phytoestrogenic structure (Zaheer and Humayown Akhtar 2017; Zhang et al. 2017; Křížová et al. 2019). It was reported that daidzein attenuates lipopolysaccharide-induced inflammation *via* activation of the PPARα in macrophages (Mueller et al. 2010). Meanwhile, the regulatory effect of daidzein on proinflammatory adipokines was through PPARγ in 3T3-L1 adipocytes and diet-induced obese C57BL/6J mice (Sakamoto et al. 2014). There were a few studies on the effects of daidzein in diabetic vascular dysfunction. Park et al. found that the protection of daidzein in HUVECs damaged by high glucose is mediated by the down-regulation of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and NF-κB proteins (Park et al. 2016). Daidzein also protects the vascular endothelial cells in H_2O_2-induced oxidative stress injury, which is related to the regulation of Bcl-2/Bax expression, PI_3K and Rho/ROCK signaling pathways, and estrogen receptor β expression (Xu et al. 2009). However, the relationship between daidzein and PPARs in diabetic endothelial cells remained largely unknown. In this study, the protective effects of daidzein were blocked by both PPARα antagonist GW6471 and PPARγ antagonist GW9662, but excluding PPARβ antagonist GSK0660. The results suggested that the improvement of daidzein on the decrease of HUVECs proliferation induced by high glucose may be related to the activation of PPARα and PPARγ, but not PPARβ. Interestingly, Sakamoto et al. also have made similar findings in adipocyte and macrophage co-cultures (Sakamoto et al. 2016). To directly observe the receptor-ligand interaction, the activating and binding potency of daidzein to PPARα and PPARγ were detected.

The transactivation reporter gene method is designed founded on the characteristics of the target nuclear receptors to be detected (Peekhaus et al. 2003). Till date, the method is more appropriate to test the functional activity of receptor-ligand. The results showed that daidzein strongly activated hPPARα and hPPARγ$_1$ in a concentration-dependent manner. The EC_{50} and E_{max} values were 3.7 μmol/l and 120.3 to hPPARα, 2.7 μmol/l and 102.1 to hPPARγ$_1$, respectively. However, the EC_{50} was 13.5 μmol/l, and the E_{max} was only 16.4 to hPPARβ. The differences among hPPARα, hPPARγ$_1$ and hPPARβ revealed that daidzein has the intrinsic activity to PPARα and PPARγ, but not to PPARβ. Daidzein may be a PPARα/γ dual agonist.

Besides the activating activity, the binding activity is essential for receptor-ligand interaction. The key of affinity detection is the separation of receptor-ligand complexes and free ligands. Size exclusion chromatography (molecular exclusion chromatography, MEC) can purify samples according to different molecular sizes. In the drug-receptor interaction, the protein molecules (receptors) are directly eluted because they neither enter the stationary phase nor are absorbed onto the external surface; then the small molecules (free drugs) are separated from the large molecules (receptor-ligand compounds) because they enter the porous stationary phase (Bustos-Valdes and Dounce 1970). Therefore, the receptor-ligand complexes and the free ligands were separated according to the MEC theory, then the absorbance of the ligand was detected with a UV detector, and the SB was calculated by subtracting the peak area of NB from TB. The results showed that daidzein could bind directly to hPPARα and hPPARγ$_1$ with increasing concentrations, which was similar to that of fenofibrate and rosiglitazone. The results suggested that daidzein has an affinity to bind to hPPARα and hPPARγ$_1$. Because of the weak agonist activation, the binding ability of hPPARβ was not detected.

Our finding that the protection of daidzein on high glucose-damaged HUVECs proliferative may be related to the activation of PPARα and PPARγ, but no PPARβ. Then the receptor-ligand interaction also showed that daidzein not only activates but also binds to hPPARα and hPPARγ$_1$, which means daidzein has simultaneously intrinsic activity and affinity to hPPARα and hPPARγ$_1$. The results suggested that daidzein may be a PPARα/γ double activator. As one of the main components of dietary soy, daidzein is well tolerated with low levels of toxicity in adults. Therefore, daidzein may be a potential medicine to prevent and treat diabetes, especially for diabetic vascular complications. However, due to the complexity of the pathogenesis of endothelial dysfunction in diabetes, as well as the complex pharmacokinetic features of daidzein, the therapeutic effect of daidzein needs to be confirmed in further studies on animal models and clinical trials.

Conclusion

Daidzein ameliorates HUVECs proliferative damage induced by high glucose which is mediated by the activation of PPARα and PPARγ. Daidzein is a potential PPARα/γ double activator.

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