Rosiglitazone via upregulation of Akt/eNOS pathways attenuates dysfunction of endothelial progenitor cells, induced by advanced glycation end products

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Background and purpose: Advanced glycation end products (AGEs) and endothelial progenitor cells (EPCs) play key roles in pathogenesis of diabetes-related vascular complications. AGEs can induce dysfunction in EPCs. The peroxisome proliferator-activated receptor-gamma (PPAR\(\gamma\)) agonists are widely used in the treatment of type 2 diabetes, and it remains unknown if they could attenuate EPC dysfunction induced by AGEs.

Experimental approach: EPCs isolated from healthy adults were cultured with various concentrations of AGEs (0, 50, 100 and 200 mg·L\(^{-1}\)) with or without rosiglitazone (10 nM), antibody for the receptors for AGE-human serum albumin (anti-receptor for advanced glycation end products (RAGE); 50 \(\mu\)g·mL\(^{-1}\)), phosphatidylinositol-3-kinase (PI3K) inhibitor (LY294002, 5 \(\mu\)M), nitric oxide (NO) synthase inhibitor (L-N\(^{G}\)-nitro-arginine methyl ester (L-NAME), 100 \(\mu\)M) or sodium nitroprusside (SNP, 25 \(\mu\)M). Proliferation, apoptosis, cell adhesion, migration and NO production in EPCs were assessed, and expressions of endothelial NO synthase (eNOS) and Akt were determined.

Key results: Number, proliferation/migration capacities, eNOS and Akt phosphorylation as well as NO synthesized by EPCs were increased by rosiglitazone and reduced by AGEs. AGEs promoted while rosiglitazone reduced EPC apoptosis. The AGE-induced effects were significantly ameliorated by pre-incubation with rosiglitazone, RAGE antibody and SNP. The beneficial effects of rosiglitazone could be blocked by pretreatment with L-NAME and LY294002.

Conclusions and implications: The PPAR\(\gamma\) agonist rosiglitazone increased EPC function and attenuated EPC dysfunction induced by AGEs via upregulating the Akt-eNOS signal pathways of EPCs.

Keywords: endothelial progenitor cells; advanced glycation end products; receptor for advanced glycation end products; apoptosis; migration; peroxisome proliferator-activated receptor-\(\gamma\) agonists

Abbreviations: AGEs, advanced glycation end products; bFGF, basic fibroblast growth factor; Dil-ac-LDL, 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine-labelled acetylated low density lipoprotein; EPC, endothelial progenitor cell; FITC-UEA-I, fluorescein isothiocyanate-labelled Ulex europaeus agglutinin-I; PE, phycoerythrobilin; PI3K, phosphatidylinositol-3-kinase; PPAR\(\gamma\), peroxisome proliferator-activated receptor-\(\gamma\); RAGE, receptor for advanced glycation end products; SNP, sodium nitroprusside; VEGF, vascular endothelial growth factor

Introduction

Advanced glycation end products (AGEs), which accumulate with ageing (Ramasamy et al., 2005; Salciuc et al., 2007) and abundantly increase in the diabetic milieu (Schleicher and Fries, 2007; Csizsar and Ungvari, 2008; Unoki and Yamagishi, 2008), have been shown to promote diabetes-accelerated atherosclerosis (Jandeleit-Dahm et al., 2008) and induce dysfunction in endothelial progenitor cells (EPCs) via the activation of p38 and ERK 1/2 mitogen-activated protein kinase (MAPK) pathways (Sun et al., 2009). Increased formation of AGEs is generally regarded as one of the main mechanisms responsible for vascular damage in patients with diabetes (Brownlee, 2000). AGEs are the result of a non-enzymatic reaction...
between the amino groups of a protein with glucose. The mechanisms of AGEs linked to cellular stress and tissue dysfunction include receptor-independent and receptor-dependent processes. In the former mode, AGEs influence the structural integrity of tissues and molecules through direct cross-linking; in the latter mode, the receptor-dependent action is mediated by binding to specific cell surface molecules such as the receptor for AGE (RAGE) and others (Scheubel et al., 2006).

Type 2 diabetes mellitus and the metabolic syndrome are characterized by resistance to the action of insulin in peripheral tissues, including skeletal muscle, liver and adipose deposits. A widely used therapy for the treatment of type 2 diabetes mellitus consists of peroxisome proliferator-activated receptor-γ (PPARγ) agonists (thiazolidinediones (TZDs)), treatments that are shown to reduce atherosclerosis progression in patients at high risk of type 2 diabetes (Xiang et al., 2005) and premenopausal women with insulin-requiring type 2 diabetes (Hodis et al., 2006; Mazzone et al., 2006). Previous studies have shown that PPARγ agonist rosiglitazone increased the number and migratory activity of cultured EPCs (Pistrosc et al., 2005) and prevented H2O2-induced EPC apoptosis in a P13K-dependent but nitric oxide (NO)-independent manner (Gensch et al., 2007). However, the effect of TZDs on EPC dysfunction induced by AGEs remains unknown. We therefore investigated the effect of TZDs on EPC dysfunction induced by AGEs and the possible molecular mechanisms involved.

Methods

Preparation of human EPCs

This study had been reviewed and approved by the ethics committee of Second Military Medical University and performed in accordance with the principles of the Declaration of Helsinki as revised in 2000. Informed consents were obtained from all volunteers. EPCs were prepared as previously described (Imanishi et al., 2008; Sun et al., 2009). Briefly, blood obtained from healthy volunteers was diluted 1: 2 in phosphate buffered saline (PBS) layered over Histopaque 1077 (Amersham Biosciences, Piscataway, NJ, USA) and centrifuged for 30 min at 400 g at room temperature. Peripheral blood mononuclear cells were incubated in M199 (GIBCO, Los Angeles, CA, USA) medium supplemented with recombinant human vascular endothelial growth factor (30 ng·mL⁻¹, from Pepro Tech, London) and recombinant human basic fibroblast growth factor (10 ng·mL⁻¹, Biosource, Camarillo, CA, USA) in fibronectin-coated (Sigma Aldrich, St. Louis, MO, USA) six-well tissue culture plates (10° per well) at 37°C and 5% CO₂. The medium was replaced every 3 days, and cells not adhering to the bottom of the culture plates were washed away. On day 7, the cells were changed to serum-free medium for another 24 h (Sun et al., 2009) and then challenged with AGEs and various interventions, according to experimental protocols.

Preparations of the AGE with human serum albumin (AGE-HSA)

AGE-HSA was prepared as described previously (Ge et al., 2005). Briefly, 2.0 g HSA was dissolved in 10 mL of 0.5 M sodium phosphate buffer (pH 7.4) with 3.0 g of D-glucose. Each sample was sterilized by ultrafiltration, incubated at 37°C for 8 weeks and dialysed against phosphate-buffered saline (pH 7.4). Control non-glycated HSA was incubated in the same conditions except for the absence of reducing sugars. The absence of endotoxin was confirmed (measured as <0.015 endotoxin U·mL⁻¹) by using the Limulus amoebocyte lysate assay (Charles River Laboratories, Wilmington, MA, USA). Protein concentrations were determined with bicinchoninic acid protein assay kit (Boston BioProducts, Inc, Worcester, MA, USA) using bovine serum albumin as a standard. The degree of glycation of the HSA was measured by spectrophotometric assay of pentosidine formation (excitation: 335 nm; emission: 385 nm), and the fluorescence ratio of AGE-HSA to HSA was 2.4. The AGE-HSA was stored under –80°C and protected from light until used.

Western blot analysis

Cells were lysed in protein extract buffer (1 mL protein extract buffer with 5 mL mixture of protease inhibitors, 5 mL phenylmethyl sulphonyl fluoride, Sigma Aldrich) at 4°C with sonication for 30 min. The lysates were centrifuged at 14 000 × g and 4°C for 30 min. Loading buffer was added to each volume and boiled for 10 min. Samples were resolved on 12% (sodium dodecyl sulphate-polyacrylamide gel electrophoresis)-HSA and electrotransferred onto a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk. Blots were incubated with mouse anti-phospho-(Ser473) Akt antibody (IgG) rabbit anti-Akt antibody (IgG) (both from Cell Signaling, Danvers, MA, USA), rabbit anti-phospho-(Ser1177) expressions of endothelial NO synthase (eNOS) antibody (IgG) (Millipore, Billerica, MA, USA), and rabbit anti-eNOS antibody (IgG) (Millipore) each at a dilution of 1:1000 for 12 h at 4°C. The blots were washed in Tris-buffered saline containing 0.2% Tween 20(TBS/T) and exposed to horseradish peroxidase-conjugated antirabbit IgG (Santa Cruz Biotechnolog, Santa Cruz, CA, USA) or antimouse secondary antibody (1:5000, Santa Cruz) at 1 h respectively and then visualized by enhanced chemiluminescence detection reagents (Santa Cruz). The signal intensity of blotting was normalized to the signal of the corresponding total protein. Relative intensities of protein bands were analysed by Image-pro plus 6.0 (Media Cybernetics, Silver Spring, MD, USA).

EPC proliferation assay

Proliferation of EPC cultured for 7 days were measured with a Cell Counting Kit-8 (Takeuchi et al., 2003) (Dojindo Molecular Technologies, Gaithersburg, MD, USA) and bromodeoxyuridine (BrdU) assay separately. The tetrazolium compound WST-8 [2- (2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulphophenyl)-2H-tetrazolium, monosodium salt] and an electron coupling reagent were added to the medium to determine the number of viable EPCs (Moriya et al., 2007; Shatos et al., 2008). The tetrazolium compound is bioreduced by cells into a coloured formazan product that is soluble in the tissue culture medium. Four hours later, cells were collected and removed to 96-well plates. Thereafter, absorbance of formazan product, directly proportional to the number of
living cells in culture, was measured at 450 nm by using a microplate reader (Labsystems, Santa Fe, NM, USA). The BrdU assay was performed according to the manufacturer's protocol. Briefly, cells were incubated with 10 μM BrdU (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) then fixed and stained with anti-BrdU monoclonal antibody (Becton Dickinson), stained with 20 μg·mL⁻¹ propidium iodide in the presence of 100 μg·mL⁻¹ DNase-free RNase A (Roche, Nutley, NJ, USA). Measurements were performed by using a fluorescent-activated cell sorting (FACS) Calibur and analysed with CellQuestPro software (Becton Dickinson). Cell doublets were discriminated from G2 cells based on the difference in pulse shape. At least 20,000 cells were analysed per sample.

**EPC apoptosis assay**

The effects of AGE-HSA and other drugs on EPC apoptosis were investigated with the Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection Kit (BioVision Inc., Mountain View, CA, USA) (Chu et al., 2007; Caporali et al., 2008). After treatment, the adherent and non-adherent cells were harvested with trypsin. Trypsinization was stopped by serum medium, and cells were resuspended in PBS. Then, the cells were stained with annexin-V-FITC and propidium iodide in 1× binding buffer for 15 min at room temperature. Flow cytometric analyses were performed on a flow cytometer (Beckman Coulter 500, Fullerton, CA, USA), and the data were analysed by using the Cell Quest analysis program.

**EPC adhesion assay**

Adhesion of EPCs was determined as described before (Fadini et al., 2008). EPCs were washed with PBS and gently detached with 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA). After centrifugation and resuspension in M199 with 5% fetal bovine serum, equal cell numbers were seeded on fibronectin-coated culture dishes and incubated for 30 min at 37°C. Adherent cells were counted independently in six random high-power (£100) microscope fields (HPF)/well by three observers unaware of the treatments.

**EPC migration assay**

The migratory function of EPCs was evaluated by a modified Boyden chamber (Costar, Cambridge, MA, USA) assay (Li Calzi et al., 2008). In brief, after incubation with serum-free medium for 24 h, isolated EPCs were detached with trypsin/EDTA and then incubated with AGE-HSA (200 μg·mL⁻¹) or rosiglitazone (10 nM) for 24 h. EPCs (4 × 10⁶ cells) were placed in the upper chamber of 24-well transwell plates with polycarbonate membrane (8 μm pores) with serum-free M199 medium; vascular endothelial growth factor (VEGF) (50 ng·mL⁻¹) in medium was placed in the lower chamber. In some cases, cells were incubated with antibody to RAGE (50 μg·mL⁻¹, R&D System, Minneapolis, MN, USA), PI3K inhibitor (LY294002, 5 μM dissolved in dimethyl sulphoxide), NOS inhibitor L-N’-nitro-arginine methyl ester (L-NAME, 100 μM dissolved in medium) or NO donor sodium nitroprusside (SNP, 25 μM dissolved in medium) for 1 h before the treatment with AGE-HSA and rosiglitazone. The assays were conducted over a 24-h incubation period at 37°C in an incubator equilibrated with 5% CO₂. Then the membrane was washed with PBS and fixed with 4% paraformaldehyde. Non-migrating cells were gently removed with cotton balls from the upper side of the transwell, and the membrane of transwell filter was stained by using haematoxylin solution and washed away. The magnitude of migration of EPCs was evaluated by counting the migrated cells in six random HPF (£100). At least four experiments were performed per study group.

**NO production in EPCs**

NO production in EPC culture medium was determined as previously reported (Murphy et al., 2007). Culture medium (30 μL) and 50 μL Griess reagent (equal volume of 1% sulphanilamide in HCl 0.1 M and 0.1% N-[1-naphthyl-ethylenediamine dihydrochloride) were mixed in a 96-well plate. After incubation at 30°C for 30 min, nitrite concentration was determined by using a microplate reader (560 nm, Well Scan) from a standard curve (0–100 nM) derived from NaNO₂ (Sigma Aldrich).

**Data analysis**

Data analysis was performed with SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). All data are presented as mean ± SEM and analysed by using one- or two-way ANOVA with Bonferroni post hoc comparison analysis, and a value of \( P < 0.05 \) was considered as statistically significant.

**Materials**

Rosiglitazone, the PI3K inhibitor (LY294002) and the NOS inhibitor L-NAME were from Cayman Chemical, Ann Arbor, MI, USA. The NO donor SNP was from Calbiochem (San Diego, CA, USA). 1,1’-Dioctadecyl-3,3’,3’’,3’’-tetramethyl indocarbocyanine-labelled-acetylated, low-density lipoprotein (Dil-ac-LDL) was from Molecular Probe, Eugene, OR, USA, and fluorescein isothiocyanate-labelled *Ulex europaeus* agglutinin-I (FITC-UEA-I) was from Sigma Aldrich. The sources of endothelial antibodies were as follows: phycoerythrobilin (PE)-mouse antihuman CD133 monoclonal antibody (Miltenyi Biotec, Bergisch Gladbach, Germany), FITC-mouse antihuman CD34 monoclonal antibody (Southern Biotech, Birmingham, AL, USA), PE-Mouse antihuman VEGF-R2 monoclonal antibody (R&D System, Minneapolis, MN, USA).

All drug and molecular target nomenclature follows the guidelines in the work of Alexander et al. (2008).

**Results**

**Characterization of EPCs**

Cells began to adhere to the bottom of the well after 24 h culture. Colonies were observed at 48 h and reached a peak on the seventh day. More than 90% of the cells took up Dil-ac-LDL and were positively stained with FITC-UEA-I at
the eighth culture day. Endothelial progenitor identity was confirmed by FACS, with antibodies recognizing PE-mouse antihuman CD133 monoclonal antibody, FITC-mouse antihuman CD34 monoclonal antibody and PE-mouse antihuman VEGF-R2 monoclonal antibody. The expression profile of cultured EPCs included CD133 (83.5% ± 5.3%), CD34 (86.2% ± 6.2%), VEGF-R2 (83.2% ± 8.2%).

Rosiglitazone reversed the inhibition by AGE-HSA of EPC functions
As shown in Figure 1, rosiglitazone reduced EPC apoptosis and increased EPC proliferation, adhesion and migration capacities, while AGES (200 μg·mL⁻¹) promoted EPC apoptosis and impaired EPC proliferation, adhesion and migration capacities.

Figure 1 Effect of AGE-HSA and rosiglitazone on the proliferation (A), apoptosis (B), adhesion (C) and migration (D) capacities of EPCs. (A) Rosiglitazone (Rosig) increased EPC proliferation and reversed the inhibited proliferation induced by AGE-HSA: *P < 0.05 versus control, HSA (200 μg·mL⁻¹), AGE-HSA (50 μg·mL⁻¹); #P < 0.05 versus AGE-HSA (200 μg·mL⁻¹); (B) Effect of AGE-HSA and rosiglitazone on apoptosis of EPCs: *P < 0.05 versus control and HSA (200 μg·mL⁻¹); #P < 0.05 versus AGE-HSA (200 μg·mL⁻¹); (C) Effect of AGE-HSA and rosiglitazone on adhesion of EPCs. *P < 0.05 versus control and HSA (200 μg·mL⁻¹); #P < 0.05 versus AGE-HSA (200 μg·mL⁻¹); (D) Effect of AGE-HSA and rosiglitazone on migration of EPCs: *P < 0.05 versus control and HSA (200 μg·mL⁻¹); #P < 0.05 versus AGE-HSA (200 μg·mL⁻¹). Data are mean ± SEM for four independent experiments. AGE, advanced glycation end product; EPC, endothelial progenitor cell; HSA, human serum albumin; HPF, high-power (×100) microscope fields.

These AGE-induced effects on EPCs were significantly attenuated by pretreatment with rosiglitazone (10 nM).

**Role of PI3K-Akt/p-Akt-eNOS pathways in EPC dysfunction induced by AGEs**

The role of the PI3K-Akt-eNOS pathway in the changes in EPC function induced by rosiglitazone or AGEs was investigated by pre-incubation with RAGE antibody, PI3K inhibitor (LY294002), L-NAME or SNP. Rosiglitazone promoted EPC function and increased NO production, while AGEs resulted in EPC dysfunction and reduced NO production. The effects induced by AGEs on EPCs were reversed by rosiglitazone. RAGE antibody and SNP enhanced the beneficial effect of rosiglitazone, while LY294002 and L-NAME pretreatment partly abolished the beneficial effects of rosiglitazone (Figure 4).

**Discussion and conclusions**

We showed that the PPARγ agonist rosiglitazone increased EPCs function and reduced apoptosis of cultured EPCs, as already described (Pistrosch et al., 2005). Additionally, our results demonstrated that (i) AGEs (200 μg·mL⁻¹) induced EPC dysfunction and promoted apoptosis; and (ii) the detrimental effect of AGEs on EPCs was significantly attenuated by rosiglitazone (10 nM) via the activation of the PI3K-Akt-eNOS pathway.

EPCs, mobilized from bone marrow and other sites (Feng et al., 2008), restore endothelial dysfunction and increase angiogenesis in injured tissues (Oh et al., 2007; Surdacki et al., 2008). In addition, the function of EPCs can be significantly influenced by common risk factors for atherosclerosis, such as hypertension (Diller et al., 2008), smoking (Kondo et al., 2004), hyperlipaemia (Di Santo et al., 2008; Tang et al., 2008) and hyperglycaemia (Ingram et al., 2008). AGEs are mainly generated in diabetic patients (Nogueira-Machado and Chaves, 2008) as a result of chronic hyperglycaemia (Ahmed et al., 2005), and their pathological effects have recently been emphasized by the increasing evidence for a pivotal role of the AGES/RAGE axis in diabetes-accelerated atherosclerosis (Bro et al., 2008). The interaction of AGE with its receptor RAGE induced oxidative stress (Su et al., 2008), increased...
inflammation via the activation of NF-κB (Tikellis et al., 2008) and enhanced extracellular matrix accumulation (Thallas-Bonke et al., 2004). EPCs were shown to be depressed by AGEs in earlier studies (Scheubel et al., 2006; Chen et al., 2009; Sun et al., 2009). Sorrentino et al. (2007) reported that EPCs isolated from diabetic individuals had a substantially increased superoxide production and impaired NO biosynthesis and that these biological effects were reversed by rosiglitazone treatment. In addition, they showed that, in a mouse model of carotid injury, rosiglitazone improved in vivo re-endothelialization. Here, we report complementary data that show that the detrimental effect of AGEs on EPCs not only is limited to increased oxidative stress but also results in increased apoptosis and impaired function (reduced adhesion, migration and proliferation) effects that could be significantly reversed by rosiglitazone via the activation of the PI3K-Akt-eNOS pathway. This is the first report, to our knowledge, about the involvement of the activation of the PI3K-Akt-eNOS phosphorylation pathway on the effects of rosiglitazone in limiting the EPC dysfunction induced by AGEs. The PI3K-Akt pathway plays a pivotal role in the regulation of EPCs (Chen et al., 2007; Xia et al., 2008). Indeed, PI3K−/− EPCs showed defects in proliferation, survival, integration into endothelial networks and migration towards SDF-1 (Madeddu et al., 2008). In an ex vivo study, the migration and tube formation ability of early and late EPCs were related to NO (Chen et al., 2007). In line with these findings, our results showed that the PI3K-Akt-eNOS pathway plays an important role for the rosiglitazone-mediated beneficial effect on EPC dysfunction induced by AGEs (Xia et al., 2008). On the basis of these results, it is possible that rosiglitazone could also induce down-regulation of RAGE in smooth-muscle cells derived from diabetic rats (Wang et al., 2006), which might be one of the mechanisms that mediated the crosstalk between PPARγ agonists and PI3K-Akt signal pathway.

Many drugs, such as oestrogens (Fadini et al., 2008), statins (Li and Xu, 2009), erythropoietin (Westenbrink et al., 2007) and TZDs (Sorrentino et al., 2007; Werner et al., 2007), improve the quantity and quality of EPCs. Although one of the TZDs has been reported to increase the risk of myocardial infarction (Nissen and Wolski, 2007), TZDs increased EPC numbers and migration in patients with coronary disease or type 2 diabetes (Werner et al., 2007). Thus, new clinical studies are needed to elucidate the net efficacy of TZDs in diabetic patients in terms of improvement of EPC function and regression of atherosclerosis.

In conclusion, the PPARγ agonist rosiglitazone increased EPC function and ameliorated AGE-induced dysfunction of EPCs via activation of the PI3K-Akt-eNOS signal pathway. These findings provide a rationale for the pleiotropic effects of
Figure 4 Effect of AGE-HSA, the anti-RAGE antibody, Rosiglitazone, SNP, Ly29002 and L-NAME on the proliferation, apoptosis, adhesion, migration capacities, and NO production of EPCs. (A) Rosiglitazone, anti RAGE antibody and SNP significantly reversed the inhibition by AGE-HSA of proliferation of EPCs, which could be blocked by LY29002 and L-NAME: *P < 0.05 versus control; †P < 0.05 versus AGE-HSA (AGES; 200 µg·mL⁻¹); ‡P < 0.05 versus rosiglitazone (Rosig; 10 nM). (B) Rosiglitazone, anti RAGE antibody and SNP significantly attenuated AGE-HSA induced EPCs apoptosis, and this effect could be blocked by LY29002 and L-NAME: *P < 0.05 versus control; †P < 0.05 versus AGE-HSA (200 µg·mL⁻¹); ‡P < 0.05 versus rosiglitazone (10 nM). (C) Rosiglitazone, anti RAGE antibody and SNP significantly attenuated the inhibition effect of AGE-HSA on adhesion capacity of EPCs, which could be blocked by LY29002 and L-NAME: *P < 0.05 versus control; †P < 0.05 versus AGE-HSA (200 µg·mL⁻¹); ‡P < 0.05 versus rosiglitazone (10 nM). (D) Rosiglitazone, anti RAGE antibody and SNP significantly reversed the inhibition by AGE-HSA of migration of EPCs, addition of LY29002 and L-NAME significantly blocked this effect: *P < 0.05 versus control; †P < 0.05 versus AGE-HSA (200 µg·mL⁻¹); ‡P < 0.05 versus rosiglitazone (10 nM). (E) Rosiglitazone, anti RAGE antibody and SNP significantly reversed the inhibitory effect of AGE-HSA on NO production of EPCs, and LY29002 and L-NAME blocked this effect: *P < 0.05 versus control; †P < 0.05 versus AGE-HSA (200 µg·mL⁻¹); ‡P < 0.05 versus rosiglitazone (10 nM). Data are mean ± SEM for three independent experiments. AGE, advanced glycation end product; EPC, endothelial progenitor cell; HAS, human serum albumin; HPF, high-power (×100) microscope fields; L-NAME, L-N⁵-nitro-arginine methyl ester; RAGE, receptor for advanced glycation end products; SNP, sodium nitroprusside.
PPARγ agonists in diabetic patients, and the potential therapeu-
tic role of PPARγ agonists on chronic vascular complications
induced by AGEs in diabetic patients warrants new
clinical studies.

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Conflict of interest

None.

References

Ahmed N, Babaei-Jadidi R, Howell SK, Thornalley PJ, Beisswenger PJ
(2005). Glycated and oxidized protein degradation products are
indicators of fasting and postprandial hyperglycemia. Br J Pharmacol

Alexander SP, Mathie A, Peters JA (2008). Guide to receptors and
S209.

Bro S, Flyvbjerg A, Binder CJ, Bang CA, Denner L, Olgaard K
et al. (2006). Negative consequences of glycation.
Diabetes 55: 2157–2163.

Brownlee M (2000). Advanced glycation end products (RAGE) reduces

Caporali A, Panelli E, Horrevoets AJ, Kraenkel N, Oikawa A, Sala-Newby
GB et al. (2008). Neurotrophin p75 receptor (p75NTR) promotes
endothelial cell apoptosis and inhibits angiogenesis: Implications for
diabetes-induced impaired neovascularization in ischemic limb

end products impair function of late endothelial progenitor cells
through effects on protein kinase Akt and cyclooxygenase-2.

High glucose impairs early and late endothelial progenitor cells by modi-
fying nitric oxide-related but not oxidative stress-mediated mecha-

Chu QD, Sun L, Li J, Byrnes K, Chervenak D, DeBenedetti A et al.
(2007). Rat adenocarcinoma cell line infected with an adenovirus
 carrying a novel herpes-simpex virus thymidine kinase suicide gene
 construct dies by apoptosis upon treatment with ganciclovir.

Csizsar A, Ungvari ZI (2008). Endothelial dysfunction and vascular
inflammation in type II diabetes: interaction of AGE/RAGE and
H476.

(2008). Oxidized low density liprotein impairs endothelial
progenitor cell function by downregulation of E-selectin and inte-

Eisenmenger syndrome and idiopathic pulmonary arterial hyper-

Fadini GP, de Kreutzbenberg S, Aliiero M, Coracina A, Pagnin E, Baeso
I et al. (2008). Gender differences in endothelial progenitor cells and
cardiovascular risk profile: the role of female estrogens. Aterio-

Feng Y, Jacobs F, Van Craeyveld E, Brauncaud N, Snoeys J, Tywa M et al.
(2008). Human Apo-A1 transfer attenuates transplant arteriosclero-
sis via enhanced incorporation of bone marrow-derived endothelial

glycosylation end products might promote atherosclerosis through
inducing the immune maturation of dendritic cells. Aterioscler

Gensch C, Clever YP, Werner C, Hanhoun M, Bohm M, Laufs U
(2007). The PPAR-gamma agonist pioglitazone increases neangi-
genesis and prevents apoptosis of endothelial progenitor cells.
Atherosclerosis 192: 67–74.

Effect of peroxisome proliferator-activated receptor gamma agonist
treatment on subclinical atherosclerosis in patients with insulin-

Pioglitazone inhibits angiostatin II-induced senescence of endothelial

Ingram DA, Lien IZ, Mead LE, Estes M, Prater DN, Derr-Yellin E et al.
(2008). In vitro hyperglycemia or a diabetic intraverteine environ-
ment reduces neonatal endothelial colony-forming cell numbers and

RAGE axis in diabetes-accelerated atherosclerosis. Clin Exp Pharma-

Kondo T, Hayashi M, Takeshita K, Numaguchi Y, Kobayashi K, Imoto
S et al. (2004). Smoking cessation rapidly increases circulating
progenitor cells in peripheral blood in chronic smokers. Aterioscler

Li Calzi S, Purich DL, Hee Chang K, Afzal A, Nakagawa T, Busk JV et al.
(2008). Carbon monoxide and nitric oxide mediate cytoskeletal
reorganization in microvascular cells via vasodilator-stimulated
phosphoprotein (VASP) phosphorylation: evidence for blunted

Li X, Xu B (2009). HMG-CoA reductase inhibitor regulates endothelial
progenitor function through the phosphatidylinositol 3
′-Kinase/ AKT signal transduction pathway. Appl Biochem Biotechnol
157: 545–553.

Madeddu P, Kraenkel N, Barcelos LS, Siragusa M, Campagnolo P,
Oikawa A et al. (2008). Phosphoinositide 3-kinase gamma gene
knockout impairs postschismic neovascularization and endothelial
progenitor cell functions. Atherioscler Thromb Vasc Biol 28:
68–76.

Mazzone T, Meyer PM, Feinstein SB, Davidson MH, Kondos GT,
D’Agostino RB Sr et al. (2006). Effect of pioglitazone compared with
glimeride on carotid intima-media thickness in type 2 diabetes: a
randomized trial. JAMA 296: 2572–2581.

Correlation between circulating endothelial progenitor cells.

Mysliwiec C, Kanagayaagym GS, Jiang B, Chowiencyzk PJ, Zbinden R,
Saha M et al. (2007). Vascular dysfunction and reduced circulating
endothelial progenitor cells in young healthy UK South Asian men.

Nissen SE, Wolski K (2007). Effect of rosiglitazone on the risk of
Su J, Lucchesi PA, Gonzalez-Villalobos RA, Palen DI, Rezk BM, Suzuki Y et al. (2008). Role of advanced glycation end products with oxi-
Tang D, Lu J, Walterscheid JP, Chen HH, Engler DA, Sawamura T et al. (2008). Electronegative LDL circulating in smokers impairs endo-
Thallas-Bonke V, Lindschau C, Rizkalla B, Bach LA, Boner G, Meier M et al. (2004). Attenuation of extracellular matrix accumulation in diabetic nephropathy by the advanced glycation end product cross-
Westenbrink BD, Lipscie E, van der Meer P, van der Harst P, Oeseburg H, Marchie Sarvaas D et al. (2007). Erythropoietin improves cardiac function through endothelial progenitor cell and vascular endo-
Xia I, Wang XX, Hu XS, Guo XG, Shang YP, Chen HJ et al. (2008). Resveratrol reduces endothelial progenitor cells senescence through augmentation of telomerase activity by Akt-dependent mecha-
Xiang AH, Peters RK, Kjos SL, Ochoa C, Marroquin A, Goico J et al. (2005). Effect of thiazolidinedione treatment on progression of sub-