BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2009), 158, 1865–1873

0007-1188/09
www.brjpharmacol.org

RESEARCH PAPER

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 (PPARγ) 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 μg·mL^{-1}), phosphatidylinositol-3-kinase (PI3K) inhibitor (LY294002, 5 μM), nitric oxide (NO) synthase inhibitor (L-N^{G}-nitro-arginine methyl ester (L-NAME), 100 μM) or sodium nitroprusside (SNP, 25 μM). 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 PPARγ agonist rosiglitazone increased EPC function and attenuated EPC dysfunction induced by AGEs via upregulating the Akt-eNOS signal pathways of EPCs.
British Journal of Pharmacology (2009) **158,** 1865–1873; doi:10.1111/j.1476-5381.2009.00450.x; published online 16 November 2009

Keywords: endothelial progenitor cells; advanced glycation end products; receptor for advanced glycation end products; apoptosis; migration; peroxisome proliferator-activated receptor-γ 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; PPARγ, peroxisome proliferator-activated receptor-γ; 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; Safciuc *et al.*, 2007) and abundantly increase in the diabetic milieu (Schleicher and Friess, 2007; Csiszar 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

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Received 25 February 2009; revised 7 May 2009; accepted 10 June 2009

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 (Pistrosch *et al.*, 2005) and prevented H_2O_2-induced EPC apoptosis in a PI3K-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 (10^7 per well) at 37°C and 5% CO_2. 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 μL mixture of protease inhibitors, 5 μL phenylmethyl sulphonylfluoride, 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 Biotechnology, Santa Cruz, CA, USA) or antimouse secondary antibody (1:5000, Santa Cruz) for 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 plus6.0 (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 $\mu g \cdot mL^{-1}$ propidium iodide in the presence of 100 $\mu g \cdot mL^{-1}$ 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 $\mu g \cdot mL^{-1}$) or rosiglitazone (10 nM) for 24 h. EPCs (4×10^4 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 $ng \cdot mL^{-1}$) in medium was placed in the lower chamber. In some cases, cells were incubated with antibody to RAGE (50 $\mu g \cdot mL^{-1}$, R&D System, Minneapolis, MN, USA), PI3K inhibitor (LY294002, 5 μM dissolved in dimethyl sulphoxide), NOS inhibitor L-N^G-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% CO_2. 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 mM) derived from $NaNO_2$ (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′-tetramethy lindocarbocyanine-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-1 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.

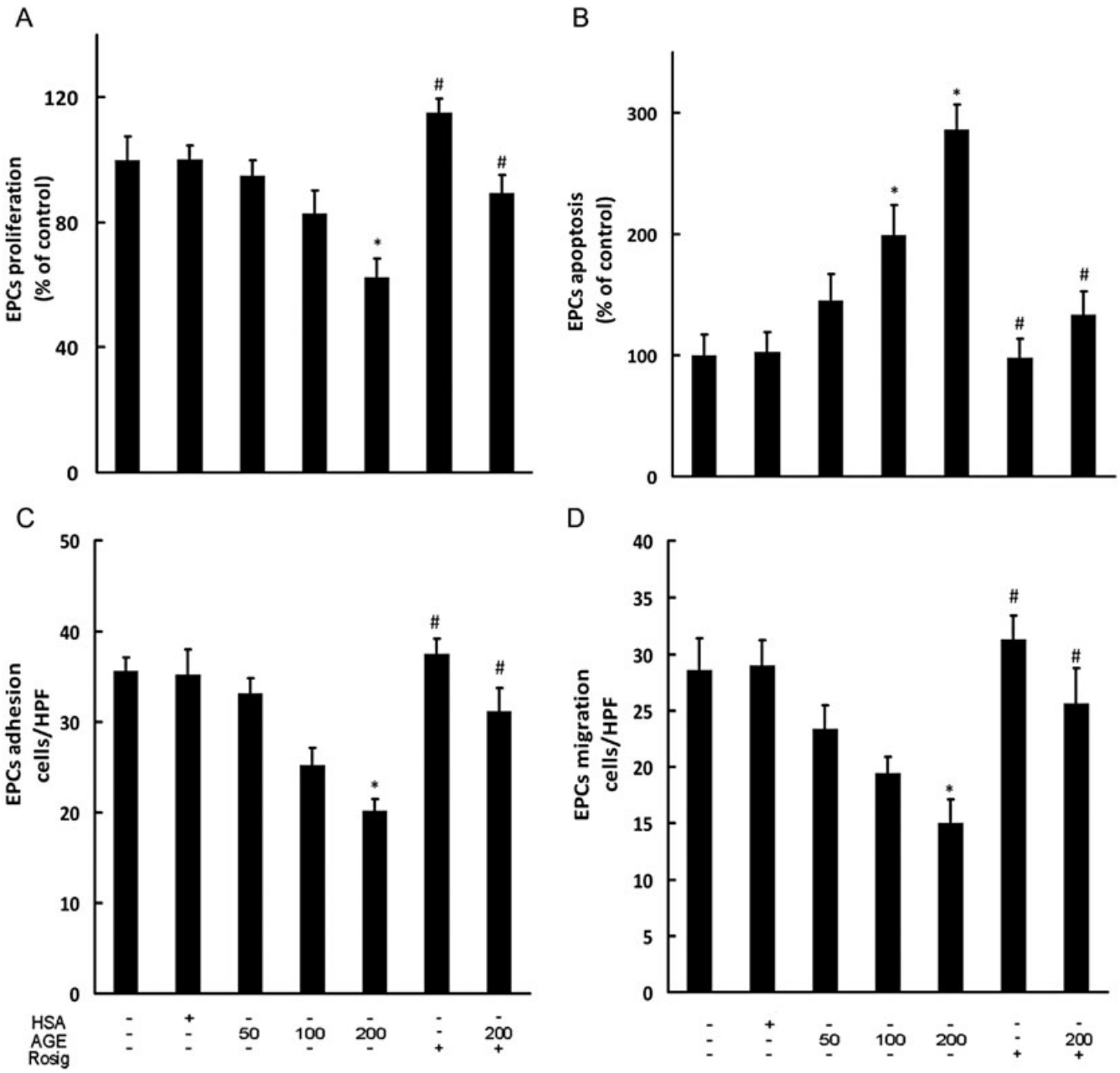

Figure 1 Effect of AGE-HSA and rosiglitazone on the proliferation (A), apoptosis (B), adhesion (C) and migration (D) capacities of EPCs. (A) Rosiglitazone (Rosig) increased EPC proliferation and reversed the inhibited proliferation induced by AGE-HSA: *P < 0.05 versus control, HSA (200 μg·mL^{-1}), AGE-HSA (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.

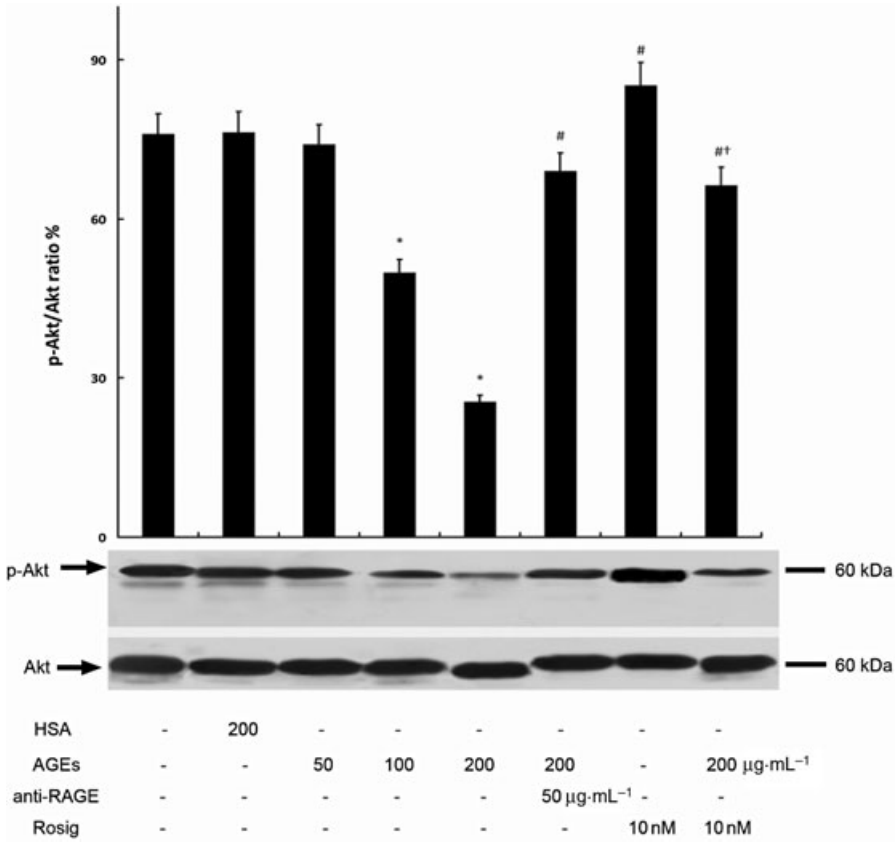

Figure 2 Effect of AGE-HSA, the anti-RAGE antibody and rosiglitazone on expression of phosphorylated-Akt (p-Akt) in EPCs. *$P < 0.05$ versus control and HSA (200 μg·mL^{-1}); #$P < 0.05$ versus AGE-HSA (AGEs; 200 μg·mL^{-1}). †$P < 0.05$ versus rosiglitazone (Rosig; 10 nM). Data are mean ± SEM for three independent experiments. AGE, advanced glycation end product; EPC, endothelial progenitor cell; HAS, human serum albumin; RAGE, receptor for advanced glycation end products.

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

Rosiglitazone and anti-RAGE antibody attenuated downregulation of p-Akt and p-eNOS induced by AGE-HSA in EPCs

Expressions of p-Akt and p-eNOS in EPCs were increased by rosiglitazone (10 nM) and reduced by AGE-HSA (200 μg·mL^{-1}), and the AGE-induced effects were significantly attenuated by pretreatment with rosiglitazone (10 nM) or RAGE antibody (Figures 2,3).

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 EPCs 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

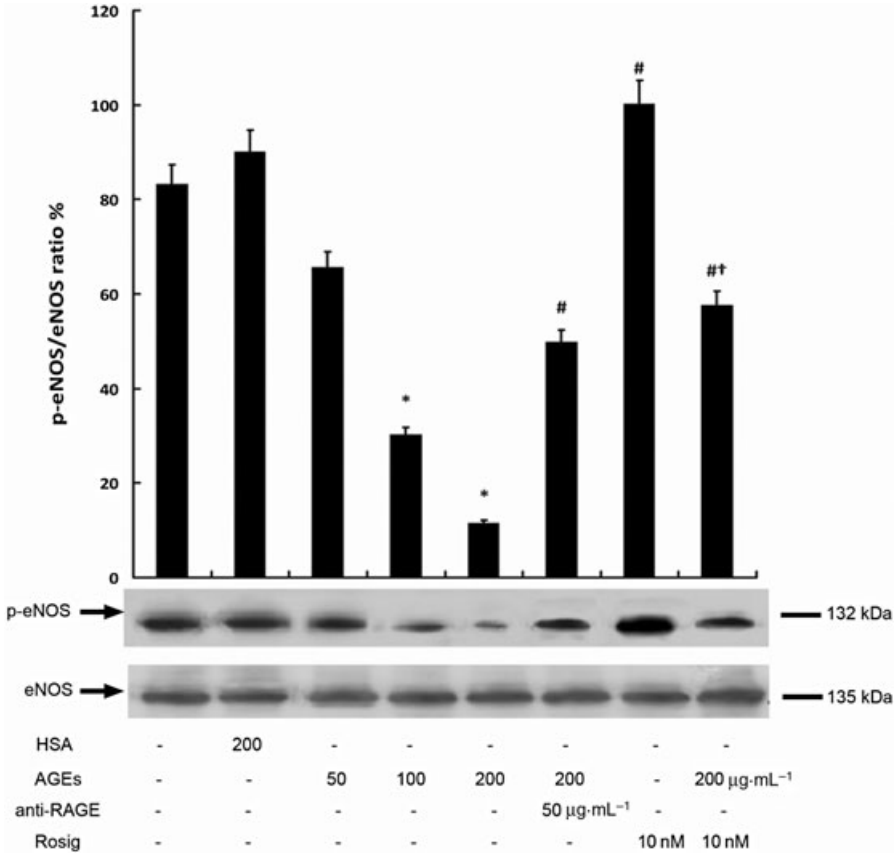

Figure 3 Effect of AGE-HSA, the anti-RAGE antibody and rosiglitazone on expression of phosphorylated-eNOS (p-eNOS) in EPCs. *$P < 0.05$ versus control and HSA (200 μg·mL^{-1}); #$P < 0.05$ versus AGE-HSA (AGEs; 200 μg·mL^{-1}); †$P < 0.05$ versus rosiglitazone (Rosig; 10 nM). Data are mean ± SEM for four independent experiments. AGE, advanced glycation end product; EPC, endothelial progenitor cell; HSA, human serum albumin; RAGE, receptor for advanced glycation end products.

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

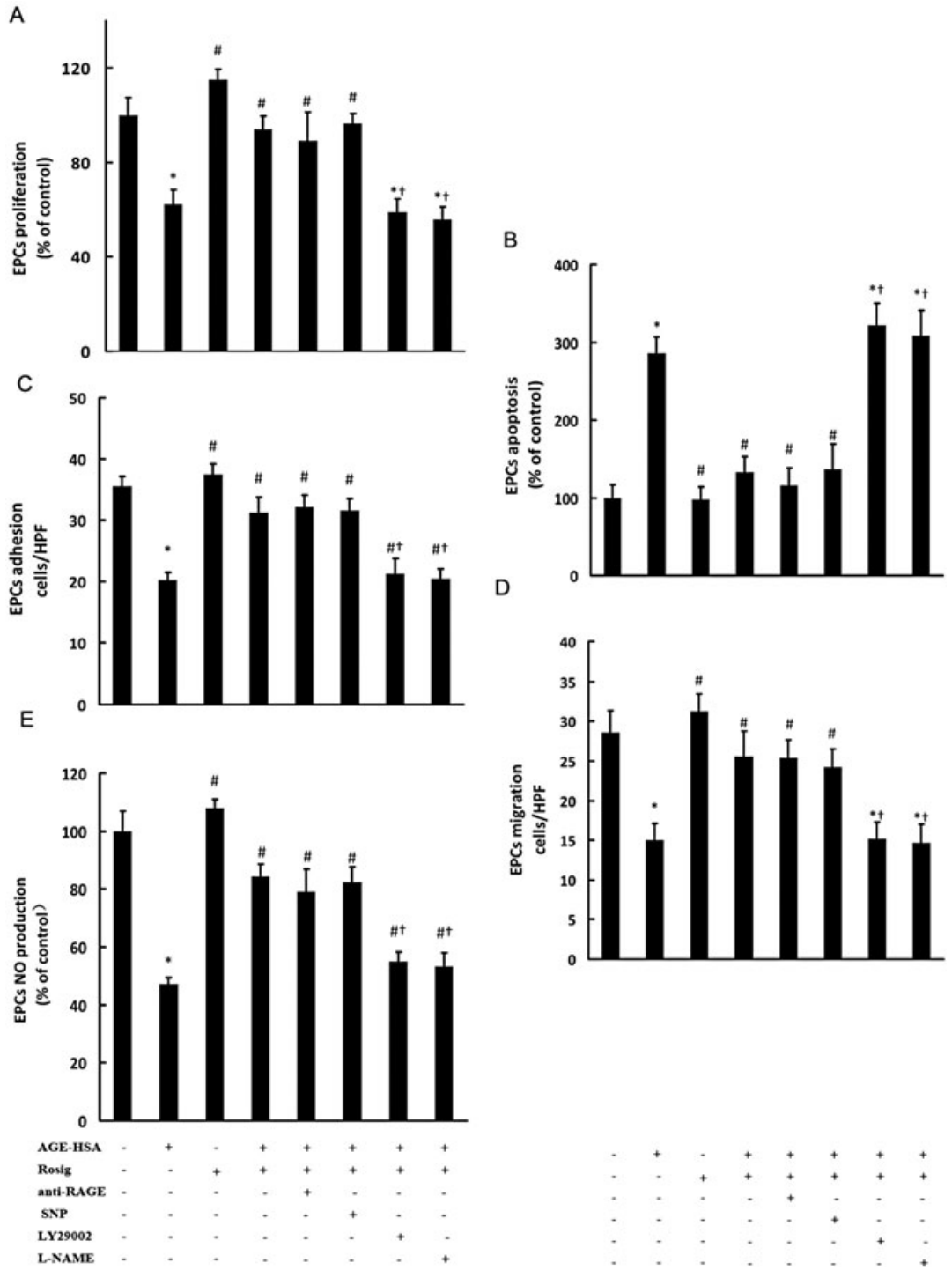

Figure 4 Effect of AGE-HSA, the anti-RAGE antibody, Rosiglitazone, SNP, Ly29002 and L-NAME on the proliferation, apoptosis, adhesion, migration capacities, and NO production of EPCs. (A) Rosiglitazone, anti RAGE antibody and SNP significantly reversed the inhibition by AGE-HSA of proliferation of EPCs, 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-N^{G}-nitro-arginine methyl ester; RAGE, receptor for advanced glycation end products; SNP, sodium nitroprusside.

PPARγ agonists in diabetic patients, and the potential therapeutic role of PPARγ agonists on chronic vascular complications induced by AGEs in diabetic patients warrants new clinical studies.

Acknowledgements

This work was supported by the Major National Basic Research Development Program of People's Republic of China (2005CB523309), the Key Basic Research Grants from Science & Technology Commission of Shanghai Municipality under grant number 08JC1407300, National Natural Science Foundation of China Project (No.30971101) and the Military Medical Research Fund of 'the Eleventh 5-year plan' (2006171003). We thank Dr Paula Alias for the help in the preparation of this manuscript.

Conflict of interest

None.

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