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New thiazolidinediones affect endothelial cell activation and angiogenesis

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ABSTRACT

Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor-γ (PPARγ) agonists used in treating type 2 diabetes that may exhibit beneficial pleiotropic effects on endothelial cells. In this study, we characterized the effects of three new TZDs [GQ-32 (3-biphenyl-4-ylmethyl-5-(4-nitro-benzylidene)-thiazolidine-2,4-dione), GQ-169 (5-(4-chloro-benzylidene)-3-(2,6-dichloro-benzyl)-thiazolidine-2,4-dione), and LYSO-7 (5-(5-bromo-1*H*-indol-3-ylmethylene)-3-(4-chlorobenzyl)-thiazolidine-2,4-dione)] on endothelial cells. The effects of the new TZDs were evaluated on the production of nitric oxide (NO) and reactive oxygen species (ROS), cell migration, tube formation and the gene expression of adhesion molecules and angiogenic mediators in human umbilical vein endothelial cells (HUVECs). PPARγ activation by new TZDs was addressed with a reporter gene assay. The three new TZDs activated PPARγ and suppressed the tumor necrosis factor α-induced expression of vascular cell adhesion molecule 1 and intercellular adhesion molecule 1. GQ-169 and LYSO-7 also inhibited the glucose-induced ROS production. Although NO production assessed with 4-amino-5-methylamino-2′,7′-difluorofluorescein-FM probe indicated that all tested TZDs enhanced intracellular levels of NO, only LYSO-7 treatment significantly increased the release of NO from HUVEC measured by chemiluminescence analysis of culture media. Additionally, GQ-32 and GQ-169 induced endothelial cell migration and tube formation by the up-regulation of angiogenic molecules expression, such as vascular endothelial growth factor A and interleukin 8. GQ-169 also increased the mRNA levels of basic fibroblast growth factor, and GQ-32 enhanced transforming growth factor-β expression. Together, the results of this study reveal that these new TZDs act as partial agonists of PPARγ and modulate endothelial cell activation and endothelial dysfunction besides to stimulate migration and tube formation.

1. Introduction

Vascular complications are a leading cause of diabetes-related morbidity and mortality (Rask-Madsen and King, 2013). Macrovascular complications, which mainly result from atherosclerosis, compromise arteries and augment the chance of critical limb ischemia, myocardial infarction, and stroke (Laakso and Kuusisto, 2014). In turn, microvascular complications have deleterious effects on eyes (Antonetti et al., 2012), kidneys (Zent and Pozzi, 2007), and nerves (Boulton et al., 2004) leading to blindness, end-stage renal disease, and lower leg amputations.

Endothelial cells modulate several vascular functions and represent an important target for prevention and treatment of diabetes-induced vascular complications (Kolluru et al., 2012). In fact, endothelial dysfunction, which manifests itself by decreased bioavailability of endothelium-derived nitric oxide (NO), and endothelial cell activation, characterized by increased expression of cell-surface adhesion molecules, are defining features of vascular pathologies (Rask-Madsen and King, 2007). Moreover, endothelial cells are the main cells involved in the formation of new blood vessels from pre-existing ones, a process termed angiogenesis, and the abnormalities in this process also contribute to the development of vascular complications (Antonetti et al., 2012; Falanga, 2005; Zent and Pozzi, 2007). While deficient angiogenesis is associated with critical limb ischemia (Falanga, 2005), excessive angiogenesis is implicated in the pathogenesis of retinopathy (Antonetti et al., 2012) and nephropathy (Zent and Pozzi, 2007).

The insulin-sensitizing thiazolidinedione (TZD) drugs are agonists of the peroxisome proliferator-activated receptor-γ (PPARγ),

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a ligand-activated transcription factor of the nuclear hormone receptor superfamily – and are used clinically for the treatment of type 2 diabetes. PPARγ is a master regulator of energy homeostasis, and in addition to its role in adipogenesis, strong evidence supports that PPARγ activation also regulates endothelial biology (Bishop-Bailey and Swales, 2008). Consistently, TZDs affect endothelial cell functions and activation. For instance, TZDs enhance NO production in endothelial cells (Calnek et al., 2003; Polikandriotis et al., 2005), suppress gene expression of adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and E-selectin (Kurebayashi et al., 2005; Pasceri et al., 2000; Wang et al., 2002), and inhibit the production of reactive oxygen species (ROS) (Ceolotto et al., 2007; Fujisawa et al., 2009). Furthermore, TZDs also regulate angiogenesis in both *in vitro* and *in vivo* models (Biscetti et al., 2008; Panigrahy et al., 2002; Scoditti et al., 2010; Xin et al., 1999). Nevertheless, despite the beneficial effects of TZDs on endothelial cells homeostasis, these drugs had restricted prescription or have been withdrawn from the market because of their association with important adverse effects, including weight gain, liver injury, and increased risk to bone fracture and heart failure (Cariou et al., 2012). Such safety concerns have highlighted the need to identify and characterize new drug candidates from the TZD class of drugs. In the current study, we sought to investigate the effects of three new TZDs on endothelial cells, with a special emphasis on the production of NO and ROS, gene expression of adhesion molecules, and modulation of cell migration and tube formation and compare the results to those obtained with rosiglitazone (RSG), which was used as a TZD control.

2. Materials and methods

2.1. Chemicals and reagents

The compounds GQ-32 [3-biphenyl-4-ylmethyl-5-(4-nitro-benzylidene)-thiazolidine-2,4-dione], GQ-169 [5-(4-chloro-benzylidene)-3-(2,6-dichloro-benzyl)-thiazolidine-2,4-dione], and LYSO-7 [5-(5-bromo-1*H*-indol-3-ylmethylene)-3-(4-chlorobenzyl)-thiazolidine-2,4-dione] (Fig. 1) were synthesized in the Laboratory of Design and Drug Synthesis of the Federal University of Pernambuco (Recife, Pernambuco, Brazil). Rosiglitazone (RSG) was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Tumor necrosis factor α (TNF-α) was obtained from Sigma-Aldrich (St. Louis, MO, USA). 4-Amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM diacetate) and 5-(and-6) chloromethyl-2′,7′ dichlorodihydrofluorescein diacetate acetyl ester (CM-H_2DCFDA) were obtained from Molecular Probes Inc. (Eugene, OR, USA). Tissue culture media, serum, and supplements were purchased from Life Technologies (Carlsbad, CA, USA) unless otherwise stated.

2.2. GQ-32, GQ-169, and LYSO-7 synthesis

Thiazolidine-2,4-dione was N-(3)-alkylated in the presence of sodium hydroxide, which allows the thiazolidine sodium salt to react with the benzyl halide in a hot ethanol medium, yielding the first group of intermediates. The 5-arylidene-3-benzyl-thiazolidine-2,4-diones were prepared by a nucleophilic Michael addition of the 3-benzyl-thiazolidine-2,4-dione and the respective aryl-substituted ethyl-(2-cyano-3-phenyl)-acrylates to obtain the arylidene-thiazolidine-2,4-diones 3-substituted. After cooling, the precipitates were purified by column chromatography or crystallized in suitable solvents. The ethyl-(2-cyano-3-phenyl)-acrylates were prepared by Knoevenagel condensation of ethyl cyanoacetic ester and substituted benzaldehydes in the presence of piperidine (Pitta et al., 2007). All of the synthesized compounds were analyzed with multiple analytical procedures. Melting points were determined in a capillary tube using a Quimis apparatus. Infrared spectra (IR) were recorded on a Bruker IFS66 spectrometer (Bruker Daltronics, Billerica, MA, USA). ^{1}H NMR spectra were recorded on a Varian Plus 300 MHz spectrometer using dimethyl sulfoxide (DMSO)-d_6 as a solvent and tetramethylsilane as an internal standard. Mass spectra were recorded by a high capacity ion trap mass spectrometer (HCT Ultra; Bruker Daltonics, Billerica, MA, USA) and were performed by electrospray ionization in negative mode or by electronic impact. The following results were obtained: GQ-32—$C_{23}H_{16}N_2O_4S$. Mp: 152–153 °C. Yield: 52%. IR (KBr, cm^{-1}): 1738, 1691, 1614, 1410, 1343, 1149, 843. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (d, 2*H*, J=9.0 Hz, 5-Ar-H, 3,5 pos.), 8.09 (s, 1*H*,=CH), 7.91 (d, 2*H*, J=9.0 Hz, 5-Ar-H, 2,6 pos.), 7.66–7.62 (m, 4*H*, 3-Ar-H, 3,5 pos. and 2′,6′ pos.), 7.48–7.33 (m, 5*H*, 3-Ar-H, 2,6 pos. and 3′,4′,5′ pos.). MS *m/z* (%), observed: 167 (100.0), 416 (77.3), 32 (73.8), calculated 416.08; GQ-169–$C_{17}H_{10}Cl_3NO_2S$. Mp: 208–210 °C. Yield: 57%. IR (KBr, cm^{-1}): 1744, 1686, 1611, 1488, 1438, 1378, 1339, 1138, 1075, 939, 783, 568, 521. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (s, 1*H*,=CH), 7.62 (d, 4*H*, J=2.1 Hz, 5-Ar-H, 2,3,5,6 pos.), 7.50–7.47 (m, 2*H*, 3-Ar-H, 3,5 pos.), 7.37 (t, 1*H*, J=16.2 Hz, J=7.2 Hz, 3-Ar-H, 4 pos.), 5.09 (s, 2*H*,-CH_2). MS *m/z* [M−H], observed: 397.04, calculated 398.69. The analytical results of LYSO-7 have already been reported (Santin et al., 2013b).

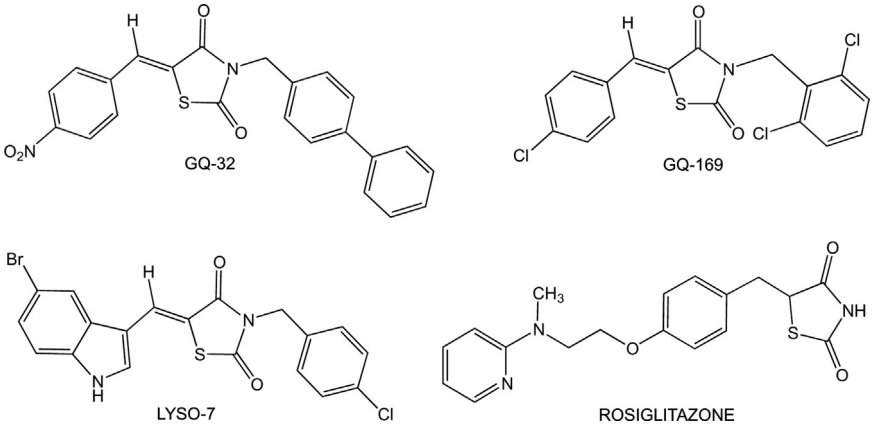

Fig. 1. Chemical structures of GQ-32, GQ-169, LYSO-7, and rosiglitazone. GQ-32 [3-biphenyl-4-ylmethyl-5-(4-nitro-benzylidene)-thiazolidine-2,4-dione], GQ-169 [5-(4-chloro-benzylidene)-3-(2,6-dichloro-benzyl)-thiazolidine-2,4-dione], and LYSO-7 [5-(5-bromo-1*H*-indol-3-ylmethylene)-3-(4-chlorobenzyl)-thiazolidine-2,4-dione] were synthesized as indicated under Materials and Methods.