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Effect of propane-2-sulfonic acid octadec-9-enyl-amide on the expression of adhesion molecules in human umbilical vein endothelial cells

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A B S T R A C T

Oleoylethanolamide (OEA), an endogenous agonist of PPARα, has been reported to have anti-atherosclerotic properties. However, OEA can be enzymatically hydrolyzed to oleic acid and ethanolamine and, thus, is not expected to be orally active. In the present study, we designed and synthesized an OEA analog, propane-2-sulfonic acid octadec-9-enyl-amide (N15), which is resistant to enzymatic hydrolysis. The purpose of this study was to investigate the effects of N15 on the expression of adhesion molecules in human umbilical vein endothelial cells (HUVECs). The results showed that N15 inhibited TNFα-induced production of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 and the adhesion of monocytes to TNFα-induced HUVECs. Furthermore, the protective effect of N15 on inflammation is dependent upon a PPAR-α/γ-mediated mechanism. In conclusion, N15 protects against TNFα-induced vascular endothelial inflammation. This anti-inflammatory effect of N15 is dependent on PPAR-α/γ dual targets.

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1. Introduction

Atherosclerosis is one of the most important factors contributing to cardiovascular disease and, therefore, represents a serious threat to public health. Atherosclerosis is characterized by chronic inflammation of the vascular wall, with adhesion of circulating leukocytes to the endothelium a critical early step in its development (Tsoyi et al., 2009; Zandbergen and Plutzky, 2007). This process depends on interactions between adhesion molecules on the endothelial cell surface and their cognate ligands on leukocytes. The role of adhesion molecules on the endothelial cell surface, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin and P-selectin, is an important factor in the development and progression of atherosclerosis (Epstein and Ross, 1999; Hwang et al., 1997).

Peroxisome proliferator-activated receptors (PPARs) are members of a family of nuclear receptor proteins. The PPAR family, comprised of three members, α, γ and β/δ, plays a crucial role not only in improving glucose and lipid homeostasis, but also in the inhibition of vascular inflammation, oxidative stress and atherosclerosis (Kumagai et al., 2003; Lee et al., 2003). PPARα activation interferes with early steps in atherosclerosis by reducing leukocyte adhesion to activated endothelial cells in the arterial vessel wall and by inhibiting subsequent trans-endothelial leukocyte migration (Altman et al., 2008; Chen et al., 2011). PPARγ plays an important role in the regulation of inflammatory and immune reactions (Altman et al., 2008). PPARα and PPARγ have emerged as two popular drug targets for hyperlipidemia and inflammation in recent years (Bocher et al., 2001; Fan et al., 2014). PPARα and PPARγ agonists also have been reported to exert negative effects on hyperlipidemia and inflammation (Gosset et al., 2001; Wu et al., 2010). Therefore, finding new compounds directed at increasing activation of PPARα and PPARγ may have potential therapeutic advantages for inflammatory diseases.

Oleoylethanolamide (OEA) is an endogenous agonist of PPARα. Previous studies have shown that OEA can modulate lipid metabolism through a PPARα-mediated mechanism (Fan et al., 2014) and that it may be an effective anti-atherosclerotic agent acting through the inhibition of oxidation, inflammation and hyperlipidemia (Chen et al., 2011; Fu et al., 2007). OEA can be enzymatically hydrolyzed to oleic acid and ethanolamine and, thus, is not expected to be orally active (Fu et al., 2008). In the present study, we developed a novel analog of OEA, propane-2-sulfonic acid octadec-9-enyl-amide (N15), which is resistant to enzymatic hydrolysis but retains the ability to activate PPARα. The aim of the present study was to elucidate the role of N15 in the

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regulation of endothelial adhesion molecules induced by TNFα in human umbilical vein endothelial cells (HUVECs).

2. Materials and methods

2.1. Materials

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle Medium (DMEM) with L-glutamine and RPMI Medium 1640 were purchased from Hyclone Laboratories (Logan, Utah, USA). DMEM with L-glutamine was from GIBCO (Gaithersburg, MD, USA). Dual-Luciferase Reporter Assay System was from Promega (Madison, WI, USA). Mouse anti-human VCAM-1 monoclonal antibody was from Cell Signal Technology (1:1000; Boston, MA, USA). Mouse anti-human ICAM-1 monoclonal antibody was from Cell Signal Technology (1:1000; Boston, MA, USA). E-selectin monoclonal antibody was from Cell Signal Technology (1:1000; Boston, MA, USA). MK886, T0070907, TMB (3,3′,5,5′-tetramethyl-benzidine), and BCECF (2,7-bis-(2-carboxyethyl)-5-(nd-6)-carboxyfluorescein) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Chemicals

We synthesized N15 (Fig. 1) as follows (Scheme 1): 2.4 mmol (0.340 g) 2-propanesulfonyl, which was suspended in 1 ml dichloromethane, was added drop-wise into a solution of dichloromethane (10 ml) that included 2.0 mol (0.530 mg) oleic acid, a drop of N,N-dimethylformamide (DMF) and 3 mmol (0.305 g, 0.4 ml) triethylamine. The temperature was maintained below 0 °C for 30 min followed by stirring for 3 h at room temperature. The reaction mixture was washed with 1 mol HCl until the pH was 3, and then extracted three times with dichloromethane. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ and twice with a saturated aqueous solution of NaCl. The combined organic phases were then dried over $MgSO_4$ and concentrated to give the crude product. Flash chromatographic purification on silica gel (petroleum ether/ethyl acetate=20:1, 15:1, 10:1) yielded the product (N15) as a faint yellow solid. The chemical structure of the compound was verified by comparison of spectral properties (MS, ^{1}H-, and ^{13}C NMR).

MS (ESI, *m*/*z*): 374.2 (MNa^+)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, *J*=6.8 Hz, 3H, H-18), 1.27–1.30 (m, 22H, H3-7, H12-17), 1.37 (s, 3H, H-1′), 1.39 (s, 3H, H-3′), 1.52–1.59 (m, 2H, H-2), 1.95–2.04 (m, 4H, H-8,-11), 3.07–3.12 (m, H, H-1), 3.16–3.21 (m, H, H-2′), 4.30 (t, *J*=6.0 Hz, H, NH), 5.33–5.39 (m, 2H, H-9,-10).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.11, 16.64, 22.68, 26.59, 27.17, 27.21, 29.21, 29.31, 29.41, 29.51, 29.52, 29.65, 29.71, 29.76, 30.71, 31.90, 43.72, 53.26, 129.75, 129.99 ppm.

2.3. Cell culture

Human umbilical vein endothelial cells (HUVECs) were harvested from human umbilical vein as previously described (Khan et al., 1995), and grown on plastic culture plates in DMEM containing 10% FBS, 5 ng/ml bFGF, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate. Cells at passages 3–9 were used for the experiments. HeLa cells expressing both the pFR-luc plasmid and a plasmid containing the ligand-binding domain of either human PPARα (nucleotides 499–1407), human PPAR β/δ (412–1323), or human PPAR γ (610–1518) fused to the DNA binding domain of the GAL4 yeast regulatory protein and a neomycin resistance gene under the control of the human cytomegalovirus promoter, were donated by the department of Pharmacology and Center for Drug Discovery, University of California, Irvine, CA, USA. Cells were cultured in DMEM containing 10% FBS, 250 μg/ml G418, and 200 μg/ml hygromycin. Human peripheral blood monocyte (THP-1) cells, which were donated by the College of Life Sciences, Suzhou University, were grown in RPMI-1640 medium with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate.

2.4. Cell viability assay

Cell viability was determined using the CCK-8 assay. HUVECs were grown in 96-well plates at a density of 4×10^4cells/well until confluence, then incubated with N15 (0–400 μM) for 12 h. CCK-8 was added to each well and incubated for 2 h at 37 °C. The absorbance was read at 450 nm by a spectrophotometer (Molecular Devices) and this was used as a measurement of cell viability. Cells incubated with control medium were considered 100% viable and were used for normalization.

2.5. Trans-activation assay

The stably transfected HeLa cells were seeded in six-well plates and incubated with N15 (10 nM to 150 μM) for 7 h. The Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) and a Mix Microtiter plate luminometer (Berthold, Pforzheim, Germany) were used to determine luciferase activity in cell lysates.

2.6. Real-time PCR assay

Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions. Reverse transcription was performed in a 20-μL mixture with 1 μg of total RNA. Real time-PCR was conducted using a 7300 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) and SYBR Green (Applied Biosystems) to measure gene expression. Specific primers for glyceraldenhyde-3-phosphate dehydrogenase (GAPDH), VCAM-1, ICAM-1, and E-selectin mRNAs were designed using Primer Express 3.0 software (Table 1).

2.7. Enzyme-linked immunosorbent assay (ELISA) of HUVECs

ELISA was performed as previously described in detail (Verme et al., 2005). Briefly, HUVECs were cultured in 96-well plates at a density of 1×10^4 cells/well until confluence, then incubated with

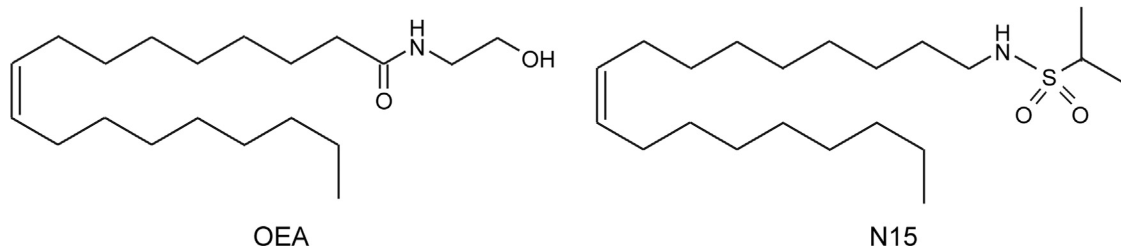

Fig. 1. Chemical structures of OEA (left) and N15 (right).

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