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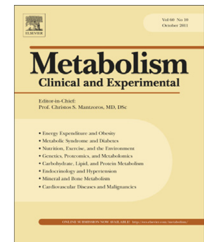

Dehydroepiandrosterone prevents linoleic acid-induced endothelial cell senescence by increasing autophagy

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ABSTRACT

Background. Autophagy has emerged as a potentially important factor in the pathogenesis of atherosclerosis. Dehydroepiandrosterone (DHEA) is an adrenal steroid of great recent interest due to its anti-aging and anti-atherogenic effects; however, little is known about its role in autophagy and endothelial senescence.

Objective. The aim of this study was to investigate whether DHEA prevents linoleic acid (LA)-induced endothelial senescence by enhancing autophagy.

Materials/methods. After pre-treatement with or without DHEA prior to LA treatment in human aortic endothelial cells (HAECs), the level of senescence was compared by senescence-associated acidic β-galactosidase (SA-β-Gal) staining and hyperphosphorylated pRB (ppRB) protein level. Autophagy was detected by LC3 conversion and measuring the level of p62/SQSTM1 (sequestosome 1), a protein degraded by autophagy. The fusion of autophagosome and lysosome was confirmed by fluorescence microscopy.

Results. Pre-treatment with DHEA inhibited LA-induced endothelial senescence. DHEA increased the conversion of LC3-I to LC3-II and decreased the level of p62 in a time- and dose-dependent manner. Although both DHEA and LA treatment increased the conversion of LC3-I to LC3-II, treatment of LA increased p62 and decreased fusion of autophagosome and lysosome, which reflected decreased autophagic flux. However, pre-treatment with DHEA restored autophagic flux inhibited by LA. When we evaluated signaling pathways, we found that JNK activation involved in LC3 conversion induced by DHEA.

Abbreviations: ANOVA, one-way analysis of variance; AMPK, AMP-activated protein kinase; ATG, autophagy related genes; CVD, cardiovascular disease; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; DMSO, dimethyl sulfoxide; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; FFA, free fatty acid; GFP-LC3, green fluorescent protein fused to the N-terminus of LC3; HAEC, human aortic endothelial cells; ICAM-1, intercellular adhesion molecule 1; IDL, intermediate-density lipoprotein; JNK, c-Jun N-terminal kinase; LA, linoleic acid; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; mTORC1, mammalian target of rapamycin complex 1; NO, nitric oxide; PAI-1, type 1 plasminogen activator inhibitor; PI3K, phosphatidylinositide 3-kinases; pRB, retinoblastoma gene product; RFP-LAMP, lysosome-associated membrane protein 1 tagged with red fluorescent protein; ROS, reactive oxygen species; SA-β-Gal, senescence-associated acidic β-galactosidase; siRNA, small interfering RNA; SQSTM1, sequestosome 1; VLDL, very low-density lipoprotein.

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Conclusion. DHEA prevents LA-induced endothelial senescence by restoring autophagy and autophagic flux through JNK activation.

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1. Introduction

Aging is the greatest risk factor for the development of atherosclerosis, which can often result in fatal cardiovascular diseases (CVD) such as myocardial infarction, stroke, and ischemic heart failure [1]. One important mechanism that links aging and the development of vascular pathology is cellular senescence [2]. In endothelial cells, senescence impairs the capacity of the vascular endothelial lining to repair, angiogenesis, and nitric oxide (NO) bioavailability [2]. In addition, endothelial senescence also promotes increased expression of proinflammatory and prothrombotic molecules such as interleukin-1α [3], intercellular adhesion molecule 1 (ICAM-1) [4] and type 1 plasminogen activator inhibitor (PAI-1) [5]. Endothelial cell senescence is induced by a number of factors including oxidative stress [2], endothelial cell mitogens [2], elevated serum glucose [6], and mitochondrial dysfunction [2]. Among the various factors affecting endothelial senescence, lipotoxicity from free fatty acid (FFA) is known to cause endothelial senescence by increasing the production of reactive oxygen species (ROS) and endoplasmic reticulum stress [6]. In a previous study, high-saturated fat diet caused premature endothelial senescence [7], and treatment of linoleic acid (LA) increased IKKβ activity in bovine aortic endothelial cells [8], which is the major signaling pathway stimulating senescence-associated secretory phenotype [9].

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S) are the most abundant adrenal steroids that also have been of great interest due to their anti-aging roles [10] and anti-atherosclerotic effect [11–13]. In human studies, plasma and serum DHEA-S levels are negatively associated with the incidence of CVD [12]. In addition, treatment with DHEA inhibited the development of atherosclerosis in hypercholesterolemic rabbits [13] and apolipoprotein E-deficient mice [14]. Despite these anti-atherogenic effects of DHEA, the underlying mechanism has not been fully explored. In previous studies, DHEA treatment improved endothelial dysfunction [13] and decreased proliferation of vascular smooth muscle cells [11]. However, to date, its biological role in endothelial senescence has not been reported.

Autophagy is a reparative, life-sustaining process involved in the degradation of long-lived proteins and dysfunctional organelles, and this process also provides cells with an alternate source of nutrients generated by the recycling of cellular proteins [15]. The age-dependent decline in autophagic activity and accumulation of intracellular damage has been postulated to determine the level of cellular senescence [16,17]. In line with this hypothesis, decreased autophagic function has been reported to be closely associated with the senescence of vascular endothelial cells and the pathogenesis of CVD [18].

Based on previous reports on the anti-aging and anti-atherosclerotic effects of DHEA [10–12], we hypothesized that DHEA might prevent endothelial senescence induced by lipotoxicity. Considering that autophagy is an important defense mechanism against cellular senescence [16], we also examined whether increased autophagy and autophagic flux might mediate this inhibitory effect of DHEA on endothelial senescence.

2. Materials and Methods

2.1. Cell Culture and Treatment

Human aortic endothelial cells (HAECs) were obtained from Lonza and maintained at 37 °C in a humidified incubator supplemented with 5% CO_2 in an endothelial basal medium (EBM-2, Lonza Group, Basel, Switzerland, CC-3156) supplemented with 2% fetal bovine serum (FBS, Welgene, Republic of Korea, S001-01) and various growth factors required for the growth of endothelial cells. In all experiments, cells were used at six passages or fewer. Linoleic acid (LA), obtained from Sigma-Aldrich (St. Louis, MO, L5900), was used as the representative FFA. Dimethyl sulfoxide (DMSO, Duchefa, the Netherlands, D1370) was used as a vehicle. The cells were transferred to medium containing 0.5% FBS and incubated for four hours in media containing 100 μmol/L of LA. DHEA was obtained from Sigma-Aldrich (St. Louis, MO, D4000), and DMSO was used as vehicle for its delivery. Cells were transferred to a medium containing 1% FBS and incubated in media containing various concentrations of DHEA for the indicated time before treatment with LA. For assessing autophagic flux, HAECs were treated with 10 nmol/L of bafilomycin A1 (inhibitor of vacuolar H^+ adenosine triphosphatase, Sigma-Aldrich, St. Louis, MO, B1793).

To evaluate the possible signaling pathways involved in autophagic induction by DHEA, HAECs were treated with various signal transduction inhibitors, including 3-methyladenine (PI3K inhibitor, 5 mmol/L, Sigma-Aldrich, M9281), compound C (AMP-activated protein kinase inhibitor, 10 μmol/L, Sigma-Aldrich, P5499), FR180204 (extracellular signal-regulated kinase (ERK) inhibitor, 10 μmol/L, Santa Cruz Biotechnology, Dallas, TX, sc-203945), p38 MAP Kinase Inhibitor (p38 inhibitor, 10 μmol/L, Santa Cruz Biotechnology, sc-204157), and SP600125 (c-Jun N-terminal kinase (JNK) inhibitor, 10 μmol/L, Calbiochem, EMD Millipore, Billerica, MA, 420119).

2.2. Senescence-Associated Acidic β-Galactosidase (SA-β-GAL) Staining

Senescence was determined by SA-β-GAL staining with cellular senescence staining kit (Cell Signaling Technology, Danvers, MA, 9860) in accordance with the manufacturer's protocol [19].

2.3. Western Blot Analysis

After lysing the cells, protein samples (20 μg/lane) were resolved by electrophoresis on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels and then transferred to nitrocellulose

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