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β-elemene inhibits monocyte–endothelial cells interactions via reactive oxygen species/MAPK/NF-κB signaling pathway *in vitro*

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ABSTRACT

The recruitment of monocytes to the active endothelial cells is an early step in the formation of atherosclerotic lesions; therefore, the inhibition of monocyte–endothelial cells interactions may serve as a potential therapeutic strategy for atherosclerosis. Recent studies suggest that β-elemene can protect against atherosclerosis *in vivo* and *vitro*; however, the mechanism underlying the anti-atherosclerotic effect by β-elemene is not clear yet. In this study, we aimed to investigate the effects of β-elemene on the monocyte–endothelial cells interactions in the initiation of atherosclerosis *in vitro*. Our results showed that β-elemene protects human umbilical vein endothelial cells (HUVECs) from hydrogen peroxide-induced endothelial cells injury *in vitro*. Besides, this molecule inhibits monocyte adhesion and transendothelial migration across inflamed endothelium through the suppression of the nuclear factor-kappa B-dependent expression of cell adhesion molecules. Further, β-elemene decreases generation of reactive oxygen species (ROS) and prevents the activation of mitogen-activated protein kinase (MAPK) signaling pathway in HUVECs. In conclusion, this study would provide a new pharmacological evidence of the significance of β-elemene as a future drug for prevention and treatment of atherosclerosis.

1. Introduction

The recruitment of circulating monocytes to the sites of arterial injuries and adhere to endothelium and transmigrate into the arterial wall are an initial step of the progression of atherosclerosis (Clapp et al., 2004; Erdogan et al., 2007; Lee et al., 2013; Mestas and Ley, 2008). Accumulated evidence demonstrates that monocyte infiltration plays a crucial role in the pathophysiology of coronary artery diseases including atherosclerosis, which can not only initiate and propagate the accumulation of monocyte-derived macrophages but also produce inflammatory mediators that destabilize atherosclerotic plaques (Kartikasari et al., 2009; Zhu et al., 2013). The monocyte–endothelium cells interactions consist of consecutive processes of monocytes adhesion and infiltration through the endothelium. During early stages of atherosclerosis, endothelial cells elicit the up-regulation of vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 levels, which mediate the monocyte adhesion to activated endothelial cells and finally induce the progression of atherosclerosis (Kim et al., 2008). Many of stimulators including H_2O_2, oxidized low-density lipoprotein (ox-LDL) and homocysteine have been indicated to promote the expression levels of CAMs in endothelial cells resulting in endothelial cells activation, dysfunction and injury (Boulden et al., 2006; Cernuda-Morollon and Ridley, 2006; Coyle et al., 2006; Wilson, 2003). Hence, the inhibition of monocyte–endothelial cells interactions may represent a therapeutic approach against atherosclerosis and other related disorders (Fuentes and Palomo, 2014).

Oxidative stress-induced disruption of redox states could lead to the activation of several redox-sensitive pathways including MAPK and NF-κB. These can result in the elevation of adhesion molecules, chemokines and cytokines expression and ultimately contribute to endothelial cells activation and chronic inflammation that induces leukocytes recruitment and infiltration (Kyaw et al., 2004). *In vitro* experiments have sufficiently proved that oxidative

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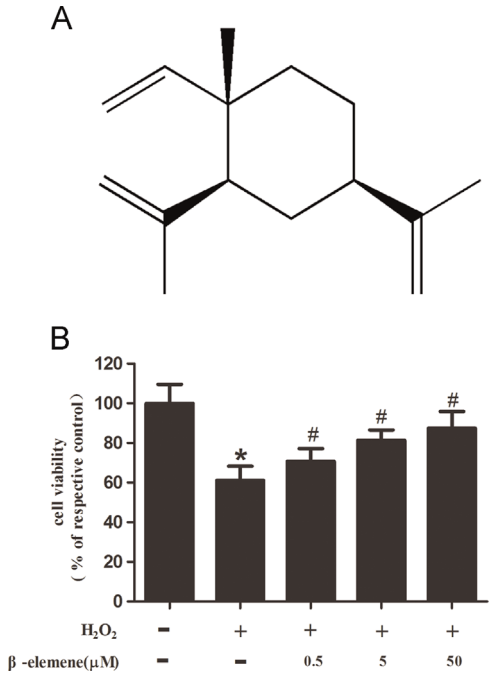

Fig. 1. Effect of β-elemene on H_2O_2-induced cytotoxicity in HUVECs. (A) Chemical structure of β-elemene. (B) After treatment with different concentrations of β-elemene for 24 h, HUVECs were incubated with 0.5 mM H_2O_2 for 2 h. MTT assay was used to analyze cells viability ($n=3$). $^{*}P<0.01$ versus controls, $^{\#}P<0.01$ versus H_2O_2 groups.

stressors (*e.g.* H_2O_2 and ox-LDL) could mimic the oxidative environment and lead to endothelial cells activation and elevation of adhesion molecule and chemokine expression (Song et al., 2014). H_2O_2 can up-regulate CAMs' expression leading to monocytes adhesion to endothelial cells through the activation of NF-κB and MAPK signaling pathways and ultimately induce endothelial dysfunction (Jin et al., 2014). Accordingly, studying the molecular recognition and signaling pathway might help us understand the mechanisms underlying pharmacological inhibition of monocyte–endothelial cells interactions implicated in atherosclerosis. In conclusion, the suppression of reactive oxygen species (ROS) generation, NF-κB translocation and MAPK signal pathways can be a very useful strategy to inhibit CAMs' expression and monocyte–endothelial cells interactions.

β-elemene (Fig. 1A), a sesquiterpenes compound extracted from the *Curcuma Wenyujin* (Guo, 1983), is a traditional Chinese herbal medicine that has been shown to inhibit tumor cell growth *in vivo* and *in vitro*. Because of its proven safety, this compound has been applied in clinics for the treatment of malignant effusions and some solid tumors (Li et al., 2005; Wang et al., 2005a, 2005b). Previous studies have shown that β-elemene derivatives have a significant antioxidant activity and cytoprotective effects against oxidative damage in HUVECs (Chen et al., 2014). In addition, another study indicated that β-elemene can inhibit smooth muscle proliferation/migration and inhibit neointima formation *in vivo* (Wu et al., 2011). However the effect of β-elemene on H_2O_2-induced monocyte–endothelial cells interactions and the underlying molecular mechanisms remain unknown. Therefore, the aim of this study is to investigate the potential inhibition effect of β-elemene on monocyte–endothelial cells interactions and unveil the possible mechanism for preventing atherosclerosis.

2. Materials and methods

2.1. Materials

β-elemene [99.3% purity, CSPC Yuanda (Dalian) Pharmaceutical Co., Ltd. Dalian Liaoning China,1 mg/l]. DAPI were from Life Technologies Corporation (USA), Hydrogen peroxide (H_2O_2), Pyrrolidinedithiocarbamate (PDTC), Dimethylsulfoxide (DMSO), 3-(4,5-dimethyl– thiazol-2-yl)- 2,5-diphenyl tetrazolium bromide (MTT), β-actin antibody, and horseradish peroxidase- conjugated secondary antibodies were purchased from Sigma-Aldrich (MO, USA). The Takara quantitative RT-PCR kit and SYBR Green Premix Ex Taq were products of Takara Biomedical Inc. (Shiga, Japan). Antibodies against p-p38, p38, p-ERK1/2, ERK1/2, p-JNK (Thr183/Tyr185), JNK, p-Iκβα (Ser32), Iκβα, NF-κB p65, Histone H3 were from Cell Signaling Technology (MA, USA), Anti-Cytochrome b245 Light Chain antibody was from Abcam (Cambridge, UK). ICAM-1(G-5): sc-8439, VCAM-1(E-10): sc-13160 was from Santa Cruz Biotechnology (Santa Cruz, CA). BCA protein assay kit, phenylmethylsulfonyl fluoride (PMSF) and cell lysis buffer for Western and IP, 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF/AM), Reactive oxygen species and Superoxide dismutase (SOD) Assay Kit were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Other reagents were of the highest obtainable quality.

2.2. Cell culture

Human umbilical vein endothelial cells (HUVECs) were from Nanjing Key Gen Biotech Co. Ltd. (Nanjing, China). HUVECs were cultured in DMEM (Invitrogen) containing 10% heat-inactivated fetal bovine serum (FBS) at 37 °C in 5% CO_2 humid incubator. Human peripheral blood monocyte (THP-1) cells were grown in RPMI-1640 medium with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin sulfate.

2.3. Cell viability assay

HUVECs were seeded in 96-well plates at a density of 5×10^3 cells/well. After 24 h growth, HUVECs were pre-treated with β-elemene (0.5, 5, 50 μM) for 24 h at 37 °C in 5% CO_2 incubator. Then, HUVECs were treated with 0.5 mM H_2O_2 for 2 h. After that MTT (0.5%, 20 μl) was added to the medium and cells were further incubated for 4 h. The supernatant was removed and 100 μl DMSO was added to dissolve the precipitate. Finally, the absorbance was measured spectrophotometrically at 570 nm.

2.4. Detection of $OH^{\bullet}$ and H_2O_2 in HUVECs

The effects of β-elemene on H_2O_2-induced hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) production were measured with commercially available kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. Briefly, $OH^{\bullet}$ was generated by the Fenton reaction and treated with a chromogenic substrate nitrotetrazolium blue chloride (NBT) to yield a stable colored substance, which was measured at 450 nm. The reaction product of H_2O_2 and molybdic acid was determined at 405 nm using the fluorescence microplate reader (Xing et al., 2014) (TECAN Safire^{2TM}). The scavenging rate (%)=$[(O.D_{control}-O.D_{sample})/O.D_{control}]\times100$.

2.5. Reactive oxygen species contents analysis

The intracellular generation of reactive oxygen species was measured by the fluorescent probe, 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA). Briefly, HUVECs were incubated