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β-Elemene reduces the progression of atherosclerosis in rabbits

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[ABSTRACT] The present study aimed at investigating the possible effects of *β*-elemene on the progression of atherosclerosis in a rabbit model. The rabbit atherosclerosis model was established by the combination of balloon angioplasty-induced endothelial injury and an atherogenic diet fed to the rabbits. New Zealand White rabbits were randomly divided into four groups (8/group): the normal control group (fed with normal chow diet) , and three experimental groups, placebo group, atorvastatin group, and *β*-elemene group (received the atherogenic diet). After two weeks on the diet, the three experimental groups underwent balloon injury at right common carotid artery and were treated with drugs or placebo for five weeks. Serum lipids were measured. Carotid artery lesions were isolated for histological and immunohistochemical analysis. *In vitro,* RAW264.7 macrophages were pretreated with *β*-elemene and ox-LDL for 24 h and the viability of macrophages was assayed using the MTT method. TNF-*α* and IL-6 were also determined. Compared with the control group, the thickness of the atherosclerosis lesion in the placebo group was significantly increased; The thickness the drug treatment groups were significantly decreased, compared with that of the placebo group. The infiltration of macrophage was markedly reduced in the *β*-elemene group compared with that of the placebo group. *β*-Elemene treatment also reduced the levels of TC, TG, and LDL-C, compared with the placebo group. *β*-elemene decreased the TNF-*α* and IL-6 levels *in vitro*. In conclusion, our results demonstrated that *β*-elemene retarded the progression of atherosclerosis *in vivo* and *in vitro*, which may be related to the capacity of *β*-elemene to reduce the infiltration of macrophages and suppress inflammatory factors.

[KEY WORDS] *β*-Elemene; Atherosclerosis; Inflammation; Macrophage; TNF-*α*

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Introduction

Atherosclerosis is a complex disease which contributes considerably to the morbidity and mortalityworldwide. Chronic inflammation plays a crucial role in atherogenesis [1], suggesting that anti-inflammatory therapy may be effective in the treatment of atherosclerosis. Differences in the conventional risk factors do not account fully for the temporal and geographical variations in the prevalence of this disease [2]. Early lesions are characterized by the adhesion of leukocytes, particularly monocytes, to the vascular endothelium at sites of injury. After migration into lesion-prone areas of the arterial vasculature, monocytes ingest lipids to become foam cells, which is a hallmark of atherosclerotic plaque formation [3]. Inflammatory factors play a major role in the early lesions of atherosclerosis; the prevention and treatment of atherosclerosis have turned conventional lipid-lowering therapy into decreasing the level of the inflammatory cytokines in the early lesion stage. Statins and aspirin are thought to inhibit inflammatory process beyond the lipid lowing effect and platelet inhibition [4-5].

β-Elemene, extracted from the essential oils of *Curcuma aromatica* Salisb. (Zingiberaceae), is widely used as an anticancer agent in China [6-7]. There are many proposed mechanisms for *β*-elemene' anticancer, effects, such as inducing cell apoptosis and cell cycle arrest and inhibiting vascular endothelial cell proliferation and tumor angiogenesis [8-10]. These functions may be applicable to the inhibition of angiogenesis in atherosclerotic plaques, producing a positive effect in the treatment of the atherosclerotic disease. However, its anti-inflammatory role has not been well studied and the underlying mechanisms are unclear. The aim of the present

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study was to investigate the effects of *β*-elemene on the progression of atherosclerosis using a rabbit model.

Materials and Methods

Test materials

β-Elemene was purchased from CSPC Yuanda (Dalian) Pharmaceuticals Co., Ltd. (Dalian, LiaoNing Province, China). *β*-elemene, a natural anticancer plant-derived drug, has been approved by the Chinese Food and Drug Administration for the treatment of human cancers [11-13]. *β*-elemene has low toxicity and is well tolerated in treated patients [14-16] Atorvastatin was purchased from Merro Pharmaceutical, Co., Ltd. (Dalian, Liaoning Province, China). Atorvastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, is used to treat hyperlipidemia, with its anti-inflammatory and plaque-stabilizing effects in atherosclerosis being reported [17]. Therefore, Atorvastatin was used as a positive control in the present study.

Animals

The animal experiments were reviewed and approved by the Animal Committee of the Institute of Science and Technology (Jiangsu, China) Adult male New Zealand White rabbits (weighing 2.0−2.5 kg) were obtained from Nanjing Jinling Rabbit Breeding Farm (Nanjing, China). A total of 32 rabbits were used in these experiments. The rabbits were housed individually in stainless-steel, wire-bottomed cages in a room with a 12 : 12 h light : dark cycle at an ambient temperature of 25 °C and 60% humidity. The eight rabbits in the negative control group were fed with a normal diet for 12 weeks, while the rabbits in three experimental groups were fed with an atherogenic diet (containing 1% cholesterol, 5% axungia porci, and 7.5% yolk powder) for 12 weeks. Water was provided to all anumals *ad libitum*.

Experimental procedures

After 1-week acclimatization, the animals were divided into four groups: negative control group (n = 8) and three experimental groups (n = 8). The animals in the experimental grousp were anesthetized with an intramuscular injection of urethane (25 mg·kg^{-1}) and 1% lidocaine. A deflated 2.0 French Fogarty balloon catheter was inserted into the right carotid artery and then was inflated at 8 atm and passed three times along a 3 cm-long segment of the artery. After the model was established (6 weeks after induction of the injury), the rabbits in the experimental groups were randomly divided into a placebo group (saline intraperitoneal, n = 8), an atorvastatin group (oral, 2.5 mg·kg^{-1}·d^{-1}, Pfizer, n = 8), and a *β*-elemene group (sublingual, 50 mg·kg^{-1}·d^{-1}, n = 8) and received the specified treatment once a day for 5 weeks as shown diagrammatically in Fig. 1. At the end of treatment, the animals were anesthetized with an intramuscular injection of urethane (25 mg·kg^{-1}), and then sacrificed with air injected into the ear veins. The right (injured) carotid arteries were removed and washed in icy saline. Each artery was cut into two segments. The proximal segment was cut into pieces at 5-mm intervals, fixed with 4% paraformaldehyde, and embedded in paraffin, while the distal segment was immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

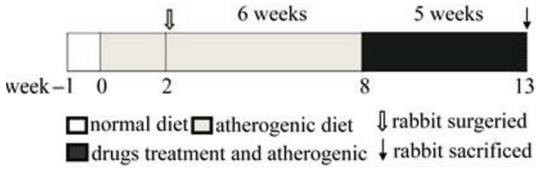

Fig. 1 Experimental Protocol for the experimental groups

Measurement of plasma lipids

Blood samples were drawn from the central ear artery at Week 0 (baseline) and at the end of week 8 (before drug treatment) and Week 13 (one week before the end of treatment). The blood samples were centrifuged at 3 000 r·min^{-1} for 10 min at 4 °C, and the serum samples were stored at −80 °C until analysis. Total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were analyzed by the enzymatic endpoint method (RANDOX, Hitachi, Japan) using an analyzer (Type AU2700, Olympus Co., Tokyo, Japan) [18].

Histological analysis of lesions

Paraffin-embedded arteries were cross-sectioned into 3 μm sections and were examined through hematoxylin and eosin (H&E) staining. The sections were stained with the following antibodies: monoclonal mouse anti-rabbit macrophage RAM-11 (DAKO, 1 : 200, Shanghai Limin Industrial Co., Ltd.) and counter-stained with hematoxylin. Digital micrographs of sections were captured under an Olympus DP70 microscope (Nanjing Ology Instrument Co., Ltd.).

Cell culture

The mouse macrophage cell line, RAW264.7 (Ruilu Biotechnology Co., Ltd.), was cultured in Dulbecco's Modified Eagle Medium (DMEM, Sunshine Biotechnology Nanjing Co., Ltd.) supplemented with 10% fetal bovine serum (FBS), 100 U·mL^{-1} penicillin, and 100 mg·mL^{-1} streptomycin (Sigma) at 37 °C in an atmosphere of 5% CO_2 and at 95% relative humidity.

Cell proliferation assay

The viability of cells was measured using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The cells were seeded in a 96-well plate at a density of 5 × 10^3 cells/well, in triplicate per experiment. The plates were placed in 5% CO_2 in an air incubator at 37 °C for 24 h, followed by treatment with *β*-elemene (10^{-4} mol·L^{-1}) or oxidized low density lipoprotein (ox-LDL, 40 μg·mL^{-1}; Yiyuan Biotechnology Co., Ltd., Guangzhou, China) for 24 h. MTT (100 μg) was added to each well for 4 h. After removing the supernatant, the formed insoluble formazan product was dissolved in 200 μL of DMSO. Finally, the absorbance of each well was detected at 570 nm in a microplate reader (Tecan, Männedorff, Switzerland).

Measurement of TNF-α and IL-6

To stimulate the expression of pro-inflammatory factors from the RAW264.7, the cells were pre-incubated with