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In vitro vasorelaxation mechanisms of Isoapiole extracted from Lemonfragrant Angelica Root on rat thoracic aorta

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ABSTRACT

Ethnopharmcological relevance: Lemonfragrant Angelica (*Ostericum citriodorum* (Hance) C. Q. Yuan & Shan) is a traditional Chinese herb for treatment of angina pectoris, stomach pain and abdominal pain. However, its active components and mechanisms of action were not well understood.
Aims of the study: In this study, we investigated whether the isoapiole extracted from Lemonfragrant Angelica Root (LAR) could directly stimulate the production of nitric oxide (NO) in vascular endothelial cells (VECs) and lead to the vascular relaxation
Materials and methods: Vascular activity experiments were performed in aortic rings isolated from Wistar rats using standard muscle bath procedures. Isoapiole was added with different concentrations (0.75, 2.5, 5 μg/mL), and vessel relaxation of rat aortic rings pre-contracted with norepinephrine (NE) or potassium chloride was recorded. NO release from aortic rings exposed to isoapiole (5 μg/mL) was measured by Griess method. The endothelial nitric oxide synthase (eNOS) expression in primary human umbilical vein endothelial cells (HUVECs) incubated with isoapiole was determined using Western blot and microplate reader assay. Classical receptor antagonists, channel and enzymatic inhibitors were used to check the mechanisms involved.
Results: Isoapiole (0.75, 2.5, 5 μg/mL) inhibited norepinephrine-induced contraction in endothelium-intact rat aortic rings. However, a very weak relaxation of aortic rings was obtained in endothelium-denuded preparations. Isoapiole (0.75, 2.5, 5 μg/mL) did not have vascular relaxative effect on neither endothelium-intact nor endothelium-denuded aortas pre-contracted with KCl (60 mmol/L). The vasorelaxation effect of isoapiole on rat aortic rings was attenuated by the eNOS inhibitor, N^G-nitro-$_L$-arginine methyl ester (L-NAME). This result suggested that suggested that the isoapiole action was at least partially mediated by promoting eNOS expression. It was further found that isoapiole (5 μg/mL) increased NO production in isolated rat thoracic aorta rings. Isoapiole increased eNOS expression leading to NO production in HUVECs.
Conclusion: Isoapiole stimulates NO production in the endothelium, leading to vascular dilatation.

1. Introduction

Lemonfragrant Angelica (*Ostericum citriodorum* (Hance) C. Q. Yuan & Shan) is one of endemic traditional herbs in China for the treatment of angina pectoris, coronary disease, stomach pain, cough, and etc. It was first recorded that the whole plant and its root could promote "qi" circulation and relieving pain in *An Illustrated Book on Plants* in Qing dynasty. Its effects have been reported in many traditional Chinese recordings (*The national assembly of Chinese herbal medicine* (The national assembly of Chinese herbal medicine Editorial, 1975), *Chinese Materia Medica* (State Administration of TCM of PR China, 1999), *Chinese Materia Medica dictionary* (Najing University of Traditional Chinese Medicine, 2006)) and many local literature (*common herbal medicine manual from* Guangzhou troops (1969), *Zhejiang folk commonly used herbs* (Zhejiang Provincial Health Bureau, 1972), *Standard of Chinese herbal medicine in Hunan Province* (Hunan Food and drug administration, 2010)). For example, Huxinling and Huxin Capsules, both used *Lemonfragrant Angelica* Root (LAR) as basic remedy, have beneficial therapeutic effect on cardiovascular disease

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in experiment and clinic settings (Ye et al., 2001; Pan and Liu, 2011; Wang and Wei, 2006, Liu et al., 2015). LAR contains numerous components including essential oil, phenylpropanoids, triterpenes, sterols, fatty acids, and so on (Zhang, 2008; Zhang et al., 2009; Su et al., 2011) Isoapiole was one of the main compounds of LAR (about 1%) (Zhang, 2008), In this work, we presented the effects of isoapiole on relaxation of arterial vessels and investigated its possible mechanisms of action.

2. Materials and methods

2.1. Animals

Healthy Wistar rats weighting 150–180 g (42 – 56 days old) with SPF grades were used. The rats were obtained from the Experimental Animal Center of Guangzhou University of Chinese Medicine (No: SCXK-2013–0020). The animals were kept under standard laboratory conditions, with a constant temperature (22 ± 1 °C), and a 12 h light/dark cycle with free access to food (SCXK ®, China) and water. The rats were also allowed to acclimatize to the animal facility for at least seven days before the start of the experiments. The animal care and use protocol was reviewed and was approved by the Ethics Committee of Guangzhou University of Chinese Medicine.

2.2. Drugs and reagents

Norepinephrine (NE) and acetylcholine (Ach) were purchased from Sigma (St. Louis, MO, USA). N^G-nitro-$_L$-arginine methyl (L-NAME) was obtained from Beyotime Institute of Biotechnology (Shanghai, China). Lemonfragrant Angelica Root (LAR) was purchased from Chinese medicine port of Guangxi Yulin, and was identified as genuine by Professor Haibo Huang at Guangzhou University of Chinese Medicine. Herbarium voucher specimens of LAR (XZ 08) were prepared and were deposited in Development & Research Center for new Chinese drugs, Guangzhou University of Chinese Medicine. The purity of isoapiole was greater than 98% (HPLC, Fig. 1(b)). The bath concentration of DMSO did not exceed 0.5%, which was shown to have no effect per se on the basal tonus of the preparations or on the agonist-mediated contraction or relaxation. Because isoapiole was practically insoluble in water, so it was firstly dissolved in 100% dimethyl sulfoxide (DMSO) as a stock solution (2 mg/mL). All other reagents were of analytical grade.

2.3. Preparation of aortic rings and tension measurement

The animals were sacrificed by cervical dislocation. The thoracic aorta was removed immediately and placed in ice-cold modified krebs' solution (composition, mM: NaCl 119, KCl 4.7, KH_2PO_4 1.2, $NaHCO_3$ 25, D-Glucose 11, $MgSO_4$ 1.2, $CaCl_2$ 1.6; pH 7.4) and adhesive connective tissues were carefully cleared away. The tissues were then cut into segments of approximately 3–4 mm length ring. The endothelium-removed aortic rings were prepared by cotton pipe-cleaners. This procedure removed the endothelium but did not affect the ability of the vascular smooth muscle to contract or relax. The isolated aorta ring was mounted with two wire hooks and suspended in organ baths containing 8 mL Krebs' solution filled with continuously gas mixture of 95% O_2 and 5% CO_2 at 37 °C. One hook was fixed at the bottom of the organ bath, while the other was connected via a micrometric manupulator to a force displacement transducer for measurement of the isometric force. Isometric tension change was recorded with a BL-420E$^+$ Biological Signal Collection System (Thai Union Chengdu Science and Technology, Sichuan, China). Before starting the experiment, the rings were initially stretched to a basal tension of 1.5 g and allowed to equilibrate for 90 min The Krebs' bicarbonate solution was changed every 20 min The endothelium was considered to be intact when the Ach-induced relaxation was at least 80% after pre-contracted with NE (10 μM). Endothelium was deemed removed completely when the relaxation response did not occur. Sequentially, each ring was washed and re-equilibrated for 30 min

2.4. Experimental protocols

2.4.1. Extraction of isoapiole and HPLC analysis

A dried powder of LAR (1 kg) was extracted with 80% ethanol (5000 mL) for three times at room temperature. The combined extracts were filtered, and solvent was removed in vacuo until the alcohol smell disappeared. The residue was successively partitioned with petroleum ether (1000 mL), ethyl acetate (1000 mL), *n*-butanol (1000 mL saturated with water). Isoapiole was

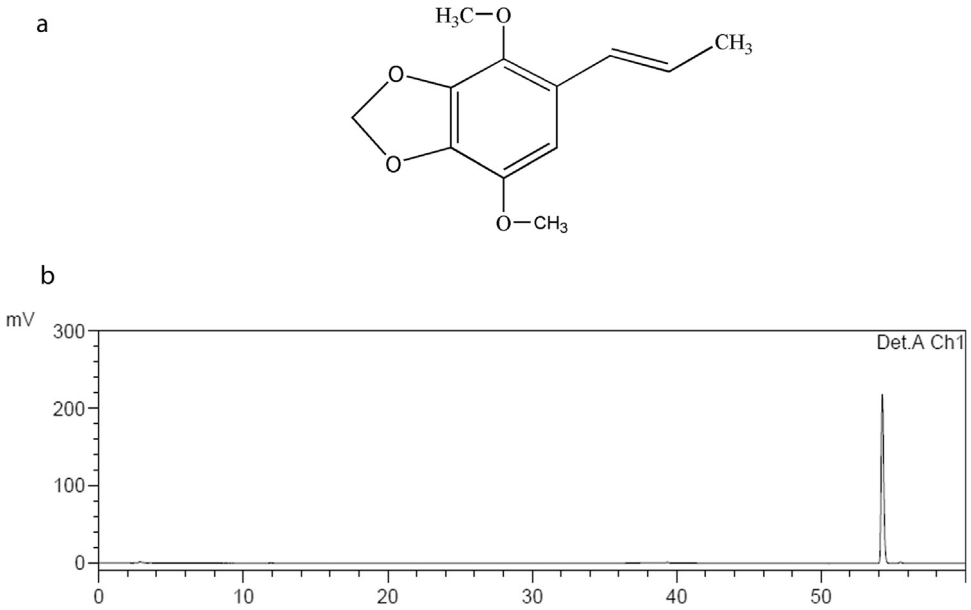

Fig. 1. Chemical structure (a) and HPLC Chromatographic analysis (b) of isoapiole.