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Endothelium-dependent and -independent vasorelaxant actions and mechanisms induced by total flavonoids of *Elsholtzia splendens* in rat aortas

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ABSTRACT

Elsholtzia splendens (ES) is, rich in flavonoids, used to repair copper contaminated soil in China, which has been reported to benefit cardiovascular systems as folk medicine. However, few direct evidences have been found to clarify the vasorelaxation effect of total flavonoids of ES (TFES). The vasoactive effect of TFES and its underlying mechanisms in rat thoracic aortas were investigated using the organ bath system. TFES (5–200 mg/L) caused a concentration-dependent vasorelaxation in endothelium-intact rings, which was not abolished but significantly reduced by the removal of endothelium. The nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine methyl ester (100 μM) and the guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,2-α]quinoxalin-1-one (30 μM) significantly blocked the endothelium-dependent vasorelaxation of TFES. Meanwhile, NOS activity in endothelium-intact aortas was concentration-dependently elevated by TFES. However, indomethacin (10 μM) did not affect TFES-induced vasorelaxation. Endothelium-independent vasorelaxation of TFES was significantly attenuated by KATP channel blocker glibenclamide. The accumulative Ca^{2+}-induced contraction in endothelium-denuded aortic rings primed with KCl or phenylephrine was markedly weakened by TFES. These results revealed that the NOS/NO/cGMP pathway is likely involved in the endothelium-dependent vasorelaxation induced by TFES, while activating KATP channel, inhibiting intracellular Ca^{2+} release, blocking Ca^{2+} channels and decreasing Ca^{2+} influx into vascular smooth muscle cells might contribute to the endothelium-independent vasorelaxation conferred by TFES.

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1. Introduction

Hypertension affects an estimated billion people worldwide, especially in developing countries (Ibrahim and Damasceno, 2012), which is the first contributor to the Global Burden of Disease (Murray and Lopez, 2013). In China, hypertension is becoming the second leading risk factor, which accounted for 12.0% of disability-adjusted life-years and 24.6% of deaths in 2010 (Yang et al., 2013). It is known that lowering blood pressure by relaxing arteries greatly reduces the main risk for developing cerebrovascular, cardiovascular, and renal disease (Vernooij et al., 2013). Unfortunately, the common clinical strategies and drugs used to manage high blood pressure remain inadequate, and even show multiple side effects (Grassi et al., 2012; Veglio et al., 2013). Therefore, seeking new effective blood pressure-lowering drugs with minimal side effects is urgent for human populations.

Flavonoids, rich in many dietary plants and medicinal herbs, are important plant-derived polyphenolic compounds and beneficial to reduce morbidity and mortality of cardiovascular disease (Graf et al., 2005). This fact increasingly attracts the general public to treat diseases using traditional medicinal herbs or their active ingredients.

Elsholtzia splendens (ES), a perennial aromatic herb, widely survives in copper mining wastes and copper in China and is commonly used to repair soil that is contaminated by copper (Guo et al., 2012). As folk medicine, ES also has safely been used to treat fever, pain, inflammation, and infection of microorganism for a long history (Guo et al., 2012; Youn, 1992). The ingredient of ES is complex and it at least contains elsholtzidiol, sterol, and flavonoids (Shim et al., 2008; Song et al., 2010). Accordingly, modern pharmacological studies have also identified broad biological activities of ES, including anti-oxidative, anti-inflammatory, antiviral, and antibacterial actions (Guo et al., 2012; Shim et al., 2008). It is noteworthy that the beneficial actions of ES, such as inhibiting inflammation, progressively relaxing coronary artery, and reducing myocardial ischemic injuries, might be mainly attributed to its abundant flavonoids (Kim et al., 2003; Ling and Lou, 2005). These findings suggest that ES, especially its flavonoids, might be potential to treat hypertension and further cardiovascular diseases. However, there is too poor background information on cardiovascular effects of ES to give an assurance of its efficacy and safety in preventing cardiovascular injuries. In addition, few direct evidences have been supplied to clarify the vasorelaxation of total flavonoids of *Elsholtzia splendens* (TFES). Therefore, the aim of the current work was to investigate the vasorelaxation and underlying mechanisms of TFES in isolated rat thoracic aortas.

2. Materials and methods

2.1. Reagents and chemicals

Nitric oxide synthase (NOS) kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, PR China). Phenylephrine (PE), acetylcholine (ACh), N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ), indomethacin (INDO), 4-aminopyridine (4-AP), tetraethylammonium chloride (TEA), glibenclamide, $BaCl_2$ and EGTA were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). The other reagents were of analytical purity. Stock solution of INDO was prepared in distilled water containing 0.7% (wt/vol) sodium carbonate. Stock solutions of ODQ and glibenclamide were prepared in ethanol and diluted in Krebs-Henseleit (K-H) buffer. The final concentration of ethanol was less than 0.3% (v/v), which was ascertained no effect on the tension of rat thoracic aorta by our preliminary experiments. The other reagents were directly dissolved in K-H buffer.

2.2. Preparation of TFES

E. splendens were collected form Fuyang, Zhejiang Province, PR China on October, 2010. The species of this medicinal herb was confirmed by Associate Professor Guolin Zhang, Institute of Chinese Traditional Medicine, Zhejiang University, PR China. A voucher specimen (220709) has been deposited in College of Pharmacy, Zhejiang University. TFES was prepared as the previous study (Ling and Lou, 2005). Briefly, the air-dried inflorescences of *E. splendens* were extracted by heated water, ethyl acetate and n-butyl alcohol in turn. Finally, the extract was centrifuged gently at $850 \times g$ for 15 min, and the supernatant was collected as the crude TFES. Nitrogen gas was gently applied to evaporate organic solvents in TFES. The yield was 4.87% (w/w). Further analysis from our laboratory showed that the content of TFES in the resultant extract was 91.8% detected by colorimetric method using luteolin as the control. Stock solution of TFES (40 g/L) was prepared in distilled water containing 50% (v/v) ethanol and diluted in K-H buffer. The same volume of ethanol was added in all control groups. The final concentration of ethanol in all groups was less than 0.3% (v/v).

2.3. Preparation of rat aortic rings

Adult male Sprague-Dawley rats (230–250 g) were obtained from the Experimental Animal Center of Zhejiang Academy of Medical Sciences. All procedures were approved by the Ethics Committee for the Use of Experimental Animals in Zhejiang University, and were performed according to the guidelines on laboratory animal use and care founded by the US National Institutes of Health (NIH publication #85-23, revised in 1985). Rat aortic rings were prepared as our previously works (Fu et al., 2011; Xia et al., 2008). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg) and killed by cervical dislocation, and the thoracic aorta was immediately dissected out and immersed in chilled modified K-H buffer (in mM): 118.0 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 1.25 $CaCl_2$, 11.0 glucose, pH 7.4. After the perivascular tissue was carefully removed, the aorta was cut into 3 mm wide rings. Care was taken to avoid abrading the intimal surface in order to maintain the integrity of the endothelial layer. When required, endothelium was removed by gently rubbing the internal space with a cotton swab. Aortic rings were suspended in organ chambers containing 10 ml K-H buffer at 37 °C, aerated with 95% O_2 + 5% CO_2. After equilibration under no tension for 15 min, the vessel segments were allowed to equilibrate for 1 h at a resting tension of 2 g. During the equilibration period,