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In vitro inhibitory effects of ethanol extract of Danshen (*Salvia miltiorrhiza*) and its components on the catalytic activity of soluble epoxide hydrolase

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ABSTRACT

Background: Soluble epoxide hydrolase (sEH) has been demonstrated to be a key enzyme involved in the pathologic development of several cardiovascular diseases and inflammation, and inhibition of sEH is therefore very helpful or crucial for the treatment of ischemia-reperfusion injury, cardiac hypertrophy, hypertension and inflammation. Danshen, the dried root of *Salvia miltiorrhiza* (Fam. *Labiatae*), has been used for the treatment of cardiovascular and cerebrovascular diseases in China and other countries for hundreds of years. Recent studies indicated that Danshen and its preparations also have potential for the management of inflammation. However, little information is available about the possibility of Danshen and its components on sEH inhibition.

Purpose and methods: Danshen extracts and its constituents were tested for sEH inhibition using its physiological substrate, 8,9-EET, based on a LC–MS/MS assay in this study.

Results: Among the tested 15 compounds, tanshinone IIA and cryptotanshinone were found to be the potent ($K_i = 0.87$ μM) and medium ($K_i = 6.7$ μM) mixed-type inhibitors of sEH, respectively. Salvianolic acid C ($K_i = 8.6$ μM) was proved to be a moderate noncompetitive sEH inhibitor. In consistent with the inhibition results of the pure compounds, the 75% ethanol extract of Danshen (EE, $IC_{50} = 86.5$ μg/ml) which contained more tanshinone IIA and cryptotanshinone exhibited more potent inhibition on sEH than the water extract (WE, $IC_{50} > 200$ μg/ml) or 1 M $NaHCO_3$ (BE, $IC_{50} > 200$ μg/ml) extract.

Conclusion: These data indicated that using the ethanol fraction of Danshen and increasing the amounts of tanshinone IIA, cryptotanshinone and salvianolic acid C, especially the contents of tanshinone IIA in Danshen extract or preparations to enhance the inhibitory effects on sEH might be efficient ways to improve its cardiovascular protective and anti-inflammatory effects, and that herbal medicines could be an untapped reservoir for sEH-inhibition agents and developing sEH inhibitors from the cardiovascular protective and anti-inflammatory herbs is a promising approach.

Introduction

Soluble epoxide hydrolase (sEH), a member of the epoxide hydrolase family, is found mostly in cytosol but also in the peroxisomes of some mammalian tissues including liver, kidney, intestine and vascular tissues (Morisseau and Hammock 2013). Mammalian sEH converts epoxides or three membered cyclic ethers to their corresponding diols through the addition of a water molecule (Morisseau and Hammock 2013). Epoxyeicosatrienoic acids (EETs), the epoxidized lipids from arachidonic acid (AA) by the action of cytochrome P450s, are the most-studied endogenous substrates of sEH (Morisseau and Hammock 2013; Ni et al. 2011; Shen et al. 2012). Early studies have indicated that EETs are endogenous vasodilative, anti-inflammatory, antiarrhythmic, pro-angiogenesis, anti-atherosclerosis, pro-fibrinolytic and anti-migratory eicosanoids, which therefore contributes to the regulation of vascular tone, angiogenesis, inflammation and other pathologic response (Kaspera and Totah 2009; Roman 2002). Conversion of EETs to the less active dihydroxyeicosatrienoic acids (DHETs) by sEH, however, diminishes the beneficial cardiovascular properties of these epoxyeicosanoids. Because of the central physiological role of sEH in disease states such as ischemical reperfusion

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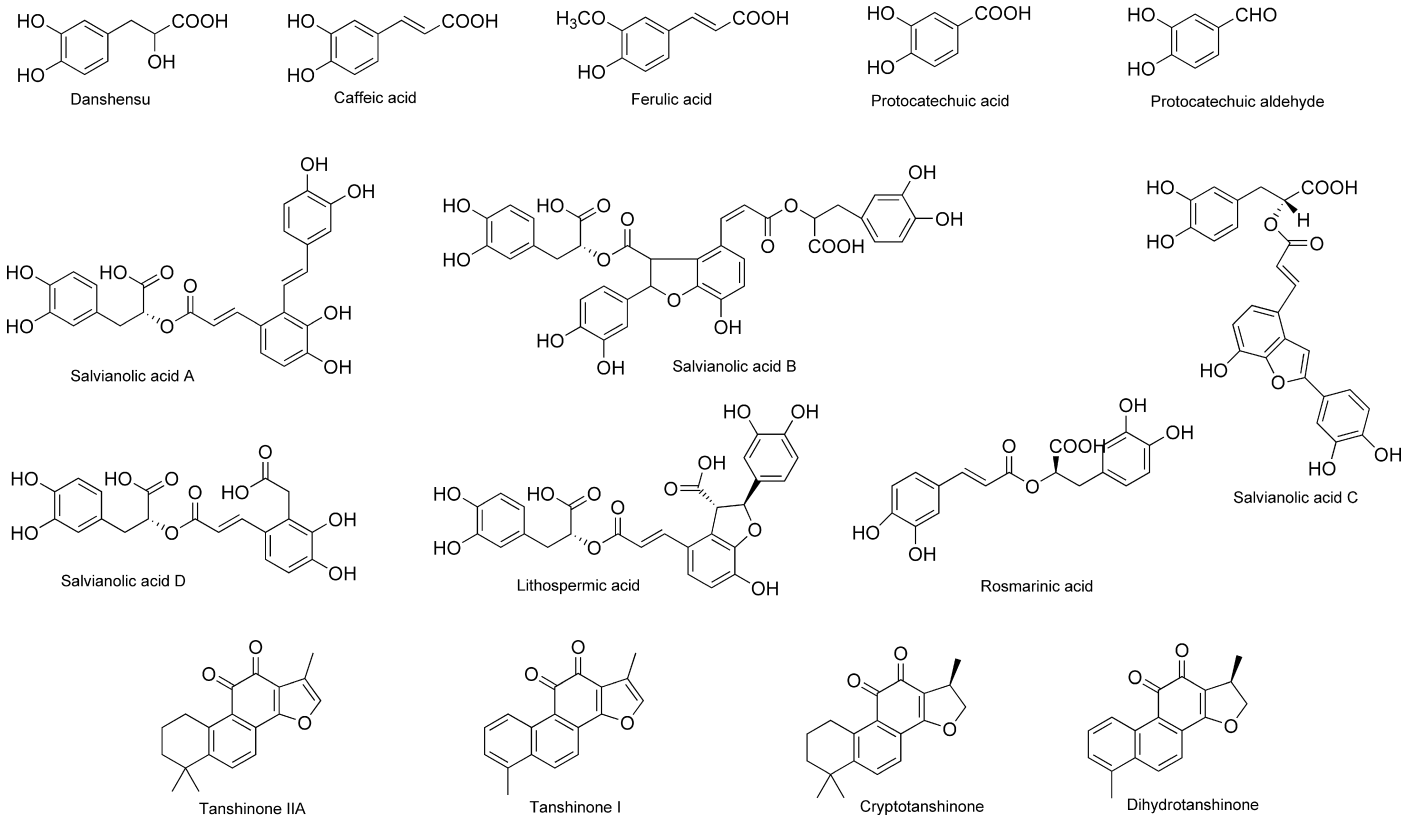

Fig. 1. Chemical structures of compounds from Danshen (*Salvia miltiorrhiza*).

injury, artherosclerosis, cardiac hypertrophy, diabetes, hypertension, pain, and inflammation, sEH and its inhibitors are being investigated as a promising therapeutic target to treat numerous ailments (Chiamvimonvat et al. 2007; Kaspera and Totah 2009; Kim et al. 2014; Ni et al. 2011; Shen et al. 2012).

Danshen, the dried root of *Salvia miltiorrhiza* (Fam. *Labiatae*), has been widely used in China and, to a less extent, in Japan, the United States and other European countries for the treatment of cardio-cerebrovascular diseases (Zhou et al. 2005). In China, the specific clinical use of Danshen and its preparations include angina pectoris, hyperlipidemia, artherosclerosis, cerebrovascular disease and acute ischemic stroke (Cheng 2007; Wu et al. 2007; Zhou et al. 2005). Increasing studies have exhibited that Danshen and its preparations also have promising therapeutic applications in hypertension and inflammatory diseases for its properties of improving microcirculation, causing coronary vasodilatation, and suppressing the formation of inflammatory factors (Cheng 2007; Li et al. 2012; Yang et al. 2012). However, little information is available about the link between the cardio-cerebrovascular protective effects of Danshen and sEH inhibition. In this study, we chose 15 components from Danshen and three kinds of Danshen extract to examine their inhibitory effects on the catalytic activity of sEH using a cDNA-expressed recombinase system, and the mechanism, kinetics, and the type of inhibition of Danshen components were also determined.

Materials and methods

Chemicals and reagents

Recombinant human sEH, 8,9-EET, 8,9-DHET, 11,12-EET, 14,15-EET-d11 were obtained from Cayman Chemicals (Ann Arbor, MI, USA). Danshensu, protocatechuic aldehyde, protocatechuic acid, salvianolic acid A, salvianolic acid B, salvianolic acid C, salvianolic acid D, lithospermic acid, rosmarinci acid, caffeic acid, ferulaic acid, tanshinone I, tanshinone IIA, cryptotanshinone and dihydrotanshinone I were bought from Chengdu Must Bio-technology Co., Ltd (Chengdu, China). The purity of all chemicals was above 98%, and the structures of the components of Danshen are shown in Fig. 1. HPLC-grade formic acid and acetonitrile were obtained from Merck (Darmstadt, Germany). Water was produced by a Milli-Q water system (Millipore, Bedford, MA, USA). All the other chemicals were the best grade that was commercially available.

Danshen extract preparation

Danshen, the dried roots of *Salvia miltiorrhiza* (Fam. *Labiatae*), was authenticated according to the Chinese Pharmacopoeia (2010) and then crushed to powders. The dried powder of Danshen (40 g) was extracted at room temperature with 1000 ml of water, 75% ethanol or 1 M $NaHCO_3$ aqueous solution thrice at 2 h intervals. The incorporated extracts of water, 75% ethanol and 1 M $NaHCO_3$ aqueous solution were then concentrated and freezing-dried to yield WE, EE and BE crude products, respectively. The products were dissolved and diluted with 50% methanol aqueous solution, and the contents of Danshen constituents in the extracts were then determined using a LC–MS assay.

LC–MS analysis of Danshen extract

LC–MS analysis was carried out on a LC–MS/MS system consisting of an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled to an API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) using a C_{18} column (2.6 μm, 100 × 2.1 mm; Kinetex, Phenomenex, USA) at 45 °C. The mobile phase consisted of acetonitrile (A) and 0.1% formic acid aqueous solution (B) with gradient elution as follows: 0–3 min, 10% A; 3–7 min, 10–15% A; 7–14 min, 15–23% A; 14–22 min, 23–70% A; 22–30 min, 70–78% A; 30–40 min, 78–10% A. The flow rate was set at 0.4 ml/min and the